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Journal of Baghdad College of Dentistry

Contents

Journal of Baghdad College of Dentistry

Oral and Maxillofacial Surgery and Periodontology

Journal of Baghdad College of Dentistry

Orthodontics, Pedodontic, and Preventive Dentistry

Basic Sciences

Journal of Baghdad College of Dentistry

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Comparison of apical seal of four obturation techniques after delayed post space preparation.

Ali H. Al-Shimmary, B.D.S. [1]
Hussain F. Al-Huwaizi, B.D.S., M.Sc., Ph.D. [2]

ABSTRACT

Backgroound: This in vitro study was conducted to compare the apical seal of four obturation techniques after delayed post space preparation.

Materials and methods: Sixty simulated straight canals in clear resin blocks were used. The samples which had the same length, size of apical preparation and taper, were divided into four groups obturated with lateral condensation, warm vertical compaction, Thermafil, and Softcore obturators. Apexit was used as root canal sealer. Delayed post space preparation was carried out by peeso reamers after one week leaving 6 mm of gutta-percha apically. The coronal cavity was sealed and the samples immersed in 2 % methylene blue dye for 7 days after which the samples were examined by stereomicroscope and calibrated grid to measure apical dye leakage in mm.

Results: Vertical compaction leaked significantly less than lateral condensation and Softcore, and Thermafil leaked significantly less than Softcore. Both Thermafil and Softcore were comparable to lateral condensation; there was no significant difference between vertical compaction and Thermafil.

Conclusion: Thermafil and Softcore had no effect on the apical seal when delayed post preparation was considered and that the apical seal obtained by Thermafil and Softcore was comparable to lateral condensation technique.

Keywords: Apical microleakage, post preparation, Thermafil, Softcore. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):1-4).

INTRODUCTION

The restoration of endodontically treated teeth often requires the use of intracanal posts, which are fitted into the root canal following removal of a portion of the root canal filling material. The final preparation of the post space is usually achieved with rotary instruments, which are often introduced into the canal to refine the space created. During such mechanical preparation of the post space it is possible that the root filling may become twisted or vibrated in such a way as to break the apical seal [1]. Lateral compaction of gutta-percha is one of the most widely used techniques and often has been used as the standard to which the sealing ability of new filling techniques or materials are compared.

Disadvantages include the potential lack of homogeneity of the gutta-percha mass, a high percentage of sealer in the apical portion of the canal, and poor adaptation to root canal walls [2].

Warm vertical compaction of gutta-percha has been introduced, producing a more homogeneous mass of gutta-percha and a very thin layer of the dimensionally less stable sealer. This possibly reduces leakage along root fillings [3].

Vertical compaction technique may be more difficult and time consuming, especially for the incremental backfilling of the coronal part of the root canal.

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Thermafil obturators were introduced to make root canal filling easier and less time consumig, with a clinical outcome apparently similar to cold lateral condensation therefore becoming a clinical alternative to other techniques [4].

Softcore is comparable to Thermafil, which belongs to the carrier obturation systems and involves thermoplasticized gutta-percha as a coating on a flexible carrier [5], but the difference is in the core carrier. The carrier of the Softcre is thinner and less tapered than that of Thermafil, round and hollow which should make post preparation easier.

MATERIALS AND METHODS

Sixty simulated straight canals in clear resin blocks were used in this study with main canal of 17 mm length and apical end corresponding to 2 canals prepared to MAF size 40 from which the dye material can penetrate. The canals were divided into 4 groups, 15 samples for each group.

Simulated canals were used in this study to eliminate the variables of canal anatomy, canal preparation which may produce variables in depth of dye penetration (6), and to standardize the internal canal volume. The transparency of the clear resin blocks enabled visibility of gutta-percha and penetrated dye material clearly without samples sectioning.

Group 1 (Lateral condensation technique)

Simulated canals were obturated by lateral condensation technique and Apexit root canal sealer. The sealer which had a creamy

homogenous consistency was picked up by K reamer and distributed on the canal wall by counterclockwise rotation of the reamer. All resin blocks were held by a previously prepared plaster mold. Master gutta-percha cone size 40 was selected and its tip was coated with sealer, inserted in the canal and condensed with finger spreader size 25. Accessory gutta-percha cones which their tips coated with sealer were used to fill the space created by finger spreader, condensed and adapted to canal wall until the spreader could not be introduced more than 3 mm in the canal. Excess gutta-percha was removed with a hot instrument and the remainder mass was condensed with endodontic plugger.

Group 2 (Vertical compaction)

Schilder technique of warm vertical compaction of gutta-percha was used to obdurate simulated canals by a set of hand endodontic pluggers after being pre-fitted at different levels of the canal lengths. Root canal sealer was applied on the canal walls by K reamer rotated counterclockwise. Apical two millimeters of master gutta-percha cone size 40 was cut and its tip was dipped in the sealer and placed in the canal. Canal down packing was achieved by alternating heating and compaction waves. The spreader was used as a heat carrier to soften and remove gutta-percha.

Compaction waves were accomplished by endodontic pluggers to condense the softened gutta-percha in the canal starting with the largest plugger coronally and ending with the smallest one apically, filling the apical third of the canal. Back filling was achieved by condensing softened pre-cut gutta-percha segments with pluggers to the level of canal entrance.

Group 3 (Thermafil)

Thermafil cones with plastic carrier size 40 were used to fill the canals after their walls were coated with Apexit sealer by K reamer rotated counterclockwise. Thermafil cone was placed in the heating chamber of Thermaprep Plus oven to soften the guttapercha according to the manufacturer's instructions. The softened Thermafil cone was inserted in the canal in a single motion without twisting with firm apical pressure until the full canal length determined by stopper on Thermafil shaft was reached. The handle of Thermafil cone was removed after 4-5 minutes when gutta-percha cooled by inverted cone bur in high speed handpiece.

Group 4 (Softcore)

Softcore obturators sizes 40 were used to fill the canals. Apexit sealer was picked up by K reamer which was rotated counterclockwise to distribute the sealer on the canal walls. Softcore obturators were softened in Softcore cordless oven according to the manufacturer's instructions then picked up and inserted in the canal in a manner similar to that of Thermafil. The handle and the insertion pin were removed after gutta-percha was cooled by twisting motion.

Sample storage

Samples were stored in normal saline solution, in an incubator at 37 °C for 7 days.

Dowel space preparation

All the obturated canals received dowel space preparation after 7 days storage period. Peeso reamers were used for post preparation because they are commonly used and have minimal influence on apical seal (7). Peeso reamers sizes 2 and 3 were used to 3 remove gutta-percha in straight slow speed handpiece rotating at 5000 rpm. The handpiece was attached to the swiveling arm of a modified surveyor to align Peeso reamers with the long axis of the canals which were placed in the plaster mold that was fixed to the surveyor base. Six millimeters of gutta-percha were left apically by removing 11 mm from the total canal length of 17 mm to obtain the best apical seal and decrease microleakage after post preparation (8, 9).

The post length was determined by silicon stops placed on Peeso shafts. The post space was filled by temporary filling material and the coronal orifice of the canal was sealed with sticky wax.

Leakage study

All the samples were placed in 2% methylene blue dye for a period of 7 days after which the samples were removed from the dye and washed with distilled water. Samples were examined for the apical dye penetration by light stereomicroscope under 40 X magnification with calibrated grid to measure the level of apical dye penetration in millimeters.

RESULTS

Mean values of apical dye penetration are shown in table 1.

Vertical compaction had the least leakage followed by Thermafil and lateral condensation, while Softcore had the highest mean leakage value. One way ANOVA test showed statistically significant difference among four obturation groups, P value < 0.05 (table 2).

LSD test was employed to identify the significant difference between pairs representing each two groups; the results of LSD test are shown in table 3.

Table 1: Mean values of apical dye penetration in millimeters.

Obturation Techniques	Sample No.	Mean	Std. Deviation
Lateral	15	0.61	±0.087
Vertical	15	0.55	±0.081
Thermafil	15	0.57	±0.072
Softcore	15	0.63	±0.070

Table 2: Analysis of variance of means (ANOVA) test

Source of variation	SS	df	MS	F	P value
Between Groups	0.060	3	0.020	3.26	0.028
Within Groups	0.343	56	0.006		
Total	0.403	59			

Table 3: Least significant difference (LSD) test

ObturationTechniques	P value	Sig.
Lateral-Vertical	0.040	S
Lateral-Thermafil	0.167	NS
Lateral-Softcore	0.487	NS
Vertical-Thermafil	0.487	NS
Vertical-Softcore	0.007	S
Thermafil-Softcore	0.040	S

DISCUSSION

Vertically compacted gutta-percha showed significantly less apical dye penetration than the lateral condensation. This comes in agreement with Pommel and Camps [10], and Jarrett et al. [11].

Lateral condensation produces many irregularities in the final mass of gutta-percha, higher percentage and inadequate dispersion of sealer, voids around gutta-percha due to the repeated procedure of addition of accessory gutta-percha cones and spreader insertion. Vertical compaction technique is conductive to better canal adaptation. The canals are densely filled with 4 homogenous mass of gutta-percha.

No significant difference was found between lateral condensation and Thermafil. The results of the dye leakage study confirmed further the overall impression that Thermafil obturators were as effective as lateral condensation. These findings are in agreement with those of Saunders et al. [12], Dalat and Spangberg [13], Rybicki and Zillich [14]. Al-Shimmary [15] found that lateral condensation had a better adaptation apically to canal wall than Thermafil. Contradictory results have been reported between Thermafil obturation technique and lateral condensation which can exhibit less; Ricci and Kessler [16], or more apical leakage; Mattison et al. [17] than Thermafil while using passive dye penetration. These discrepancies in literature may be related to some variations in the root canal preparation. These variations are eliminated by using standardized simulated canals.

No significant difference in dye penetration was found between lateral condensation and Softcore. There is disagreement with DeMoor and Martins [18], DeMoor and De Boever [19], De Moor and Hommez [20], who found that Softcore had higher leakage than lateral condensation, and with Boussetta et al. [5], in which Softcore leaked less than lateral condensation. The different characteristics of resin used in this study and dentin walls used in previous studies may affect the distribution of the sealer and the adaptation of gutta-percha and may result in differences in the apical leakage.

Vertical compaction was not significantly different from Thermafil in apical dye penetration. This agrees with the findings of Bhambhani and Sperchman in [21], Qiong et al. [22]. Both techniques achieved good adaptation of gutta-percha to canal walls. The use of Peeso reamers in post preparation with parallel sides design may provide vertically directed condensing force against apical root filling which is softened by frictional heat; this may reduce the disruption of apical seal.

Vertically compacted gutta-percha and Thermafil leaked significantly less than Softcore which agrees with DeMoor and DeBoever [19], DeMoor and Hommez [20]. They found that the pre-heated gutta-percha of Softcore appeared to be porous when viewed under the microscope which may explain the higher leakage in Softcore. The carrier of Softcore is thinner than that of Thermafil which means more gutta-percha and more volumetric shrinkage in Softcore. When Softcore gutta-percha was removed it stacked to Peeso reamer more than Thermafil, which upon Peeso removal from the canal may cause coronal dislodgement of apical filling, accounting for higher leakage scores in Softcore.

REFERENCES

1. Jeffrey LWM, Saunders WP. An investigation into the bond strength between a root canal sealer and root filling points. Intern Endod J 1987; 20:217-21.
2. Dalat DM, Spangberg LS. Comparison of apical leakage in root canals obturated with various gutta-percha techniques using a dye vacuum tracing method. J Endod 1994; 20:315-9.
3. Wu MK, Wesselink PR, Boersma J. A 1-year follow-up study on leakage of four root canal sealers at different thicknesses. Intern Endod J 1995; 28:185-9.

4. Fan B, Wu MK, Wesselink PR. Leakage along warm gutta-percha fillings in the apical canals of curved roots. J of Endod and Dent Traumat 2000; 16, 29-33.
5. Boussetta F, Bal S, Romeas A, Boivin G, Magloire H, Farge P. In vitro evaluation of apical microleakage following canal filling with a coated carrier system compared with lateral and thermomechanical gutta-percha condensation techniques. Intern Endod J 2003; 36:367-71.
6. Pathomvanich and Edmunds. The sealing ability of Thermafil obturators assessed by four different microleakage techniques. Intern Endod J 1996; 29:327-34.
7. Camps LR, Todd MJ. The effect of dowel preparation on the apical seal of three common obturation techniques. J Prosthet Dent 1983; 50:664-6.
8. Mattison GD, Delivanis PD, Thacker RW and Hasssell KJ. The effect of post preparation on the apical seal. J Prosthet Dent 1984; 51(6): 785-9.
9. Nixon C, Vertucci FJ, Swindle R. The effect of post space preparation on the apical seal of root canal obturated teeth. Todays FDA 1991; 3(8): 1C, 6C.
10. Pommel L, Camps J. In vitro apical leakage of System B compared with other filling techniques. J of Endod 2001; 27:449-51.
11. Jarrett S, Marx D, Covey D, Karmazin M, Lavin M, Gound T. Percentage of canals filled in apical cross sections, an in vitro study of seven obturation techniques. Intern Endod J 2004; 37:392.
12. Saunders WP, Saunders EM, Gutmann JL, Gutmann ML. An assessment of the plastic Thermafil obturation technique, part 3. The effect of post-space preparation on the apical seal. Intern Endod J 1993; 26:184-9.
13. Dalat DM, Spangberg LS. Effect of post preparation on the apical seal of teeth obturated with plastic Thermafil obturators. Oral Surgery 1993; 76:760-5.
14. Rybicki R, Zillich R. Apical sealing ability of Thermafil following immediate and delayed post-space preparations. J of Endod 1994; 20:64-7.
15. Al-Shimmary S. The adaptability of three different gutta-percha obturation techniques. A master thesis, Department of conservative Dentistry, Baghdad University, 2007.
16. Ricci ER, Kessler JR. Apical seal of teeth obturated by the laterally condensed gutta-percha, the Thermafil plastic, and Thermafil metal obturator techniques after post-space preparation. J Endod 1994; 20:123-126.
17. Mattison CD, Hwang CL, Cunningham C, Pink FE. The effect of the post preparation on the apical seal in teeth filled with Thermafil. J Dent Res 1992; 72:600 Abstract.
18. DeMoor RJ, Martens LC. Apical microleakage after lateral condensation, hybrid gutta-percha condensation and Softcore obturation: an in vitro evaluation. Endod Dent and Traumatol 1999; 15:239-43.
19. De Moor RJG, De Boever JG. The sealing ability of an epoxy resin root canal sealer used with five gutta-percha obturation techniques. J of Endod and Dent Traumatol 2000; 16:291-7.
20. De Moor RJG, Hommez GMG. The long-term sealing ability of an epoxy resin root canal sealer used with five gutta-percha obturation techniques. Intren Endod J 2002; 35:275-82.
21. Bhambhani SM, Sprechman K. Microleakagc comparison of Thermafil vs. vertical condensation using two different sealers. Oral Surgery 1994; 78:105-8.
22. Qiong Xu, Ling J, GaryCheung, Yan Hu, Guangzhou, Guangdong. A quantitative evaluation of sealing ability of four obturation techniques by using a glucose leakage test. Oral Surgery Oral Medicine Oral Pathology Oral Radiololgy and Endodontics 2007; 104:109-13

Evaluation of the tensile bond strengths of heat cure acrylic and Valplast with silicone self cure soft liner

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ABSTRACT

Background: Soft lining materials have a key role in modern prosthodontics because of their capability of restoring health of inflamed and distorted mucosa. Gradual changes of oral tissues require that complete or partial dentures be relined to improve their adaptation to the supporting tissue. This study aimed to evaluate the tensile bond strength of heat cure acrylic and Valplast denture base materials to silicone self-cure soft lining material stored in artificial saliva.

Materials and method: Two types of self cured silicone soft lining material (one with prime the other without prime or adhesive) applied to polymethylmethacrylate and injection-molded nylon denture base materials for tensile bond strength testing using Instron machine.

Results: The comparison between all test groups after (48) hours immersion in artificial saliva were highly significantly different from each other except for the comparison between groups PSP and VSP in which their means were non-significant. After (12) weeks, the comparison between all test groups were highly significantly different from each other when compared statistically.

Conclusion: This study indicated that prime (adhesive) increase the bond strength of the silicone soft lining materials with denture base materials. Silicone soft lining materials are affected by artificial saliva storage.

Key words: Self-cure silicone soft lining material, acrylic denture base, injection-molded nylon, tensile bond strength. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):5-9).

INTRODUCTOIN

Relining is defined as "the procedures used to resurface the tissue side of the denture with new base material, thus producing an accurate adaptation to the denture foundation area" [(1)].

A major objective in construction of complete dentures is to attain a denture base that conforms to the supporting tissues with a high degree of accuracy.

The greater the accuracy of the base, the more stable is the prosthesis [(2-4)].

Polymethyl methacrylate resins have been preferred as denture base resins because of their physical and esthetic properties as well as the material's availability, reasonable cost, and ease of manipulation[(5)].

The introduction of injection molding, which allows directional control of the polymerization process through the flask design. A constant flow of new material from the sprue compensates for the polymerization shrinkage.

Gradual changes of oral tissues require that complete or partial dentures be relined to improve their adaptation to the supporting tissue [(4)]. Soft liners provide comfort to patients who cannot tolerate occlusal pressure [(15)]. Soft liners are often used for management of painful or atrophied mucosa or traumatic ulceration associated with wearing dentures. The soft liner provides comfort for the patient and may it reduce residual ridge resorption by reducing the impact force in the load –bearing areas in the supporting structures during function [(16)].

During the use of soft liner, the materials are in continuous contact with saliva and during denture storage they are soaked in water or an aqueous cleaning solution [(11)]. The desirable properties of soft liner include long term elasticity; loss of elasticity could result in delivery of higher occlusal forces to the underling mucosa. Also the soft liner should be resistant to imbibing of the oral fluids or releasing compounds into the saliva, fluid imbibition would result in liner discoloration and swelling and the potential growth of microorganism [(26)].The relining material used my be classified as either hard or soft, the selection depends on oral circumstance and treatment planning [(24,10,21)]. Silicone soft lining materials have the advantage of being inherently soft over a long period, whilst the development of polyvinylsiloxanes similar to those used in dental impression materials allows simple application procedures to be used. However, the achilles heel of silicone products is often an inadequate bonding to the denture base. Several publication have focused on the factors which can affect bonding, including the nature and direction of the de-bonding force and liner thickness and. The tear strength of the soft liner itself, the nature of the adhesive agent and variations in the structure of the acrylic resin denture base [(29)].

Relining materials are classified to three groups: hard reline materials, tissue conditioning materials and soft lining materials. There are many types of soft lining materials like plasticized acrylic type soft liner and silicone soft liner. They

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basically consist of polydimethylsiloxane polymer to which filler is adding to give the correct consistency. The material harden by cross linking process, as the materials is already polymer this cross linking can be achieved either by heat, using benzoyl peroxide, or at room temperature, using tetraethyl silicate [9, 19] .

MATERIALS AND METHODS

Specimens were prepared from two chemically different denture base materials

1- Polymethylmethacrylate (Heat cure acrylic non cross linked ENTACRYL, ENTA B.V. Bergen op zoom the Netherlands ISO 9000, Holland).

2- Injection-molded nylon (Valplast INTERNATIONAL CORP., New York, USA) denture base materials.

Joined by two types of self-cure silicon soft lining material one with prime (bredent), the other one without prime or adhesive (Zhermack), they were evaluated for changes in tensile bond strength. (Immersion in artificial saliva at different time of immersion).

Specimens grouping:

Two major groups include (4) subgroups of specimens, (2) subgroup of polymethylmethacrylate and (2) subgroup of injection-molded nylon, with (2) types of self-cure silicone soft lining materials; the number of specimens in each subgroup was depending on the tests made. The specimens grouping for each major group were classified as follow:

Group1: Specimens immersed in artificial saliva for (48) hours for tensile bond strength test.

Group2: Specimens immersed in artificial saliva for (12) weeks for tensile bond strength test**.**

Each group was divided into (4) subgroup according to the material used**:**

(PS) heat cures acrylic denture base blocks with soft lining material without prime or adhesive.

(PSP) heat cures acrylic denture base blocks with soft lining material with prime.

(VS) Valplast denture base blocks with soft lining material without prime or adhesive.

(VSP) Valplast denture base blocks with soft lining material with prime.

Preparation of the artificial saliva:

Artificial saliva was prepared in the pedodontic department in the College of Dentistry /University of Baghdad, under supervisions of the seniors of the department **[14]**.

The composition was as follows: 6.8 mM NaCl, 5.4 mM $CaCl_2$, 5.4 mM KCL, and 5.0 mM NaH_2Po_4, 0.021 mM Na_2S, 16.7 mM urea and deionized distilled water. (mM=mg/m.wt.In 1 liter) (m.wt.= molecular weight**).**

Tensile bond strength test:

80 Specimens were prepared for tensile testing in (2) groups, each groups was divided into (4) subgroups each one contains (10) specimens aging in 250 cc. closed polyethylene containers containing artificial saliva in an incubator at 37.5^{o}C.

Specimens of each group were divided as follow:

1. (40) Specimens were tested after 48 hours immersion in artificial saliva
2. (40) Specimens were tested after 12 weeks immersion in artificial saliva.

Each specimens consisted of (2) heat-cure acrylic or Valplast blocks denture base with dimension of (6*6*30) mm width, depth, length respectively, and intermediate part of soft lining material with dimensions of (6*6*3) mm width, depth, length respectively joining the 2 pieces of denture base block so the total dimension of the specimen was (6*6*63) mm, using digital vernier for checking the dimension of the specimens [2,3] **.**

Preparation of denture base specimens:

For the ease of sample preparation, a metal mould was constructed **[14] .**Figure (1), it consisted of sample parts which consist of 2 pattern of samples, first pattern is (6*6*30) mm width, depth, length respectively indentations and the second pattern is (6*6*63) mm width, depth, length respectively indentation .The samples part contains a cover which is in intimate contact with samples part and held by screws. Before mixing of acrylic or soft liner the mould should be painted with separating medium [14, 28, 3]**.**Fig (1 and 2)**.**

Figure 1: Metal mould of denture base specimens for tensile testing

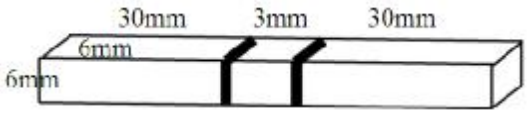

Figure 2: Dimensions of tensile test specimen

Test equipment and procedure:

The rectangular shaped specimens were tested using Instron testing machine with a suitable grips for the test specimens with cross head speed (5mm/min) using load cell with maximum load capacity (1000 N). Force at failure was recorded in Newton. The value of tensile bond strength were calculated for each test specimen as the force at the de-bonding divided by a cross-section area of interface according to the following formula:

$$Bond\ \ strength = \frac{F\ (N)}{A\ (mm^2)}$$

(ASTM Specification D-638 M, 1986)

Where: F = force of failure (N)

A = surface area of the cross section (mm^2)

RESULTS

The mean bond strength between the two types of soft lining materials and heat cured acrylic and Valplast after (48) hours and after (12) weeks in artificial saliva storage are listed in tables (1), and showed in figure (3).

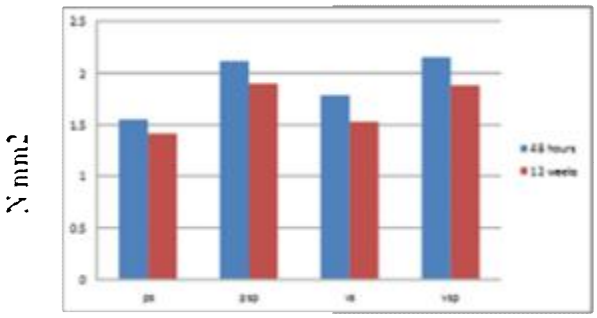

Figure 3: Bond strength for the four test group

Table 1: Description statistics for tensile strength (N/mm^2) in artificial saliva storage for different time intervals and t- test

Types of Surface	Time	Mean	S.D.	t-test	p-value
PS	48 hours	1.56	0.054	7.003	0.000 HS
	12 weeks	1.42	0.033		
PSP	48 hours	2.19	0.104	5.644	0.000 HS
	12 weeks	1.9	0.059		
VS	48 hours	1.79	0.119	6.607	0.000 HS
	12 weeks	1.52	0.043		
VSP	48 hours	2.15	0.043	11.851	0.000 HS
	12 weeks	1.88	0.057		

Table 2: LSD between groups of soft lining materials with different surfaces

Type of Surface	48h		12 weeks	
	P-value	Sig	P-value	Sig
PS-PSP	0.000	HS	0.000	HS
PS-VS	0.000	HS	0.39	HS
PS-VSP	0.000	HS	0.073	HS
PSP-VS	0.000	HS	0.000	HS
PSP-VSP	0.356	NS	0.373	NS
VS-VSP	0.000	HS	0.24	HS

***P<0.05 significant**
****P>0.05 Non significant**
*****P<0.0001 High significant**

The means of the bond strength of the heat cure acrylic with soft lining materials with prime gave rise to the greatest bond strength of all test groups ($2.19N/mm^2$). The lowest value ($1.42N/mm^2$) was for the acrylic with soft lining materials without prime, storage in artificial saliva for 12weeks. Statistically, as seen in tables (2), the comparison between all test groups after (48)hours immersion in artificial saliva were highly significantly ($p< .001$) different from each other except for the comparison between groups PSP and VSP in which their means were non-significant ($p> .05$).

After (12) weeks, the comparison between all test groups were highly significantly ($p< .001$) different from each other when compared statistically as shown in table(2).

DISCUSSION

The results of this study support the hypothesis that the chemical and physical properties of denture base resins, as well as surface treatments, affect the bond strength of the soft lining materials with denture base materials.

Tensile bond strength test is a good method of investigating the bond strength of lining materials, because it gives information on the bond strength of the material [7, 18].

It is important to measure the adhesive bond instead of the cohesive strength of resilient liners to assess interfacial separation under oral conditions. Otherwise, cohesive rupture of the resilient liner give only limited information on the strength of the liner material [18].

The tensile bond strength for the two types of denture base material were tested at (48 hours) and (12 weeks) after different types of soft lining materials application. The soft lining materials type and artificial saliva storage effects on tensile bond strength were tested.

In general the bond strength of the heat cure acrylic denture base material (2.19 N/mm2) was greater than that with Valplast thermoplastic

denture base material (2.15 N/mm2).This could be the result of the difference in the nature of the bond between the soft lining materials and the two types of denture base materials[27,20,22,16] .The results in the table (1), showed samples with PSP type of denture base obtained mean value higher than the other type of samples. Because there is no chemical reaction between soft liner and polymethylmethacrylate denture base resin [17], the bond level of the soft liner attributed to the acryloxyalkylsilicone which as well as improving the cross linking of the silicone soft liner, it intended to adhere to the (PMMA), besides; the adhesive contained a γ-Methacryloxypropyltrimethoxysialie which improved the adhesion and cross linking to the underlying (PMMA), this is according to [28].

The results in the table (1), showed samples with PS type of denture base obtained mean value lower than the other type of samples.

An adhesive is supplied to aid in bonding to denture base resin because silicone soft liner has little or no chemical adhesion to polymethylmethacrylate denture base resin [17].

While the sample with VS type of denture base obtained mean value higher than the PS type of samples.

Valplast denture base materials have a high level of roughness and sogginess of the surface than (PMMP), so there will be mechanical retention between Valplast denture base and soft lining materials [1].

Effect of Artificial Saliva Storage, Table (1) showed a decrease in mean values of bond strength of the all types of denture base, the pure silicone rubber has very low water sorption and solubility but it has been suggested that fillers and impurities presented besides inter molecular spaces are responsible for water sorption and solubility [8].

The sorption and solubility values which were very low due to the high cross linking nature of the soft lining materials, besides; according to[31] .silicone soft lining materials shows very low level of microleakage at the bond liner/denture base surface; so the material was highly affected by artificial saliva storage. But this will lead to stresses concentration at the sharp edges of materials in which the stresses were applied [6], this causing decrease in the mean values of bond strength of the soft lining materials. The most common reason for the failure of dentures lined with a silicone-based soft lining material is the failure of adhesion between the denture base and soft lining materials. In a clinical setting, adhesive failure is initially observed at the edge of the denture border region as cracks involving localized unhygienic debris, and it usually spreads inside a denture with time. The stress occurs between the bonding surfaces when the soft lining material absorbs water. Thus, it is conceivable the adhesive failure starts from the edge of the denture because the edge can be immersed in saliva more easily. In addition to these facts, the recorded failure strength value and the mode of specimen failure were affected by the type of the test method such as peel, tensile and shear tests [30].

REFERENCES

1. MacGregor AR, Graham J, Stafford GD, Huggett R. Recent experiences with denture polymers. J Dentistry 1984; 12:147-157.
2. Abdul – Rahman BA. Evaluation of water sorption, solubility, and bond strength of some soft lining materials. A master thesis, prosthetic Department, University of Baghdad, 2002.
3. Mohammed Ali B. Preparation and evaluation of some properties of heat-cured, acrylic-based soft denture liner. A Ph.D. thesis, Prosthetic Department, University of Baghdad, 2006.
4. Anil N, Canan H. Microleakage study of various soft denture liners by autoradiography Effect of accelerated aging. J Prosthet Dent 2000; 84(4): 394-9.
5. Anusavice KJ, Phillips RW. Phillip's science of dental materials. 10th ed. Philadelphia: W B Sauders Co.; 1996.
6. Aydin AK, Terzioglu H, Akinay AE, Ulubayram K, Hasirci N. Bond strength and failure analysis of lining materials to denture resin. Dent Mater 1999; 15(3): 211-8.
7. Rosen B. Fracture processes in plyometric solids. New York: John Wiley and sons Inc.; 1964.
8. Bates JF, Smith DC. Evolution of indirect resilient liners for dentures Laboratory and clinical tests. JADA 1965; 70: 344-53.
9. Braden M, Wright PS. Water absorption and water solubility of soft lining materials for acrylic dentures. J Dent Res 1983; 62(6): 764-8.
10. Combe EC. Notes on dental materials. 6th ed. London: Churchill Livingstone; 1992.
11. Domingnez NE, Thomas CJ Gerzina TM. Tissue conditions Protected by poly (Methyl Methacrylate) coating. Int J prosthodont 1996; 9: 137-41.
12. El-Hadary A, Drummond JL Comparative study of water sorption, solubility, and tensile bond strength of two soft lining materials. Prosthet Dent 2000; 83(3): 356-61.
13. The glossary of prosthodontic terms. 8th ed. J Prosthet Dent 2005; 94(1):1-92.
14. Han S, Quick Dc. Nickel-titanium spring properties in a simulated oral environment. Angle Orthod 1993; 63: 67-72.
15. Jacobsen NL, Mitchell DL, Johnson DL, Holt RA. Lased and sandblasted denture base surface preparations affecting resilient liner bonding. J Prosthet Dent 1997; 78(2): 153-8.

16. Kawano F, Dootz ER, Koran A, Carig RG. Sorption and solubility of 12 soft denture liners. J Prosthet Dent 1994; 72(4): 393-8.
17. Kawara M, Carter JM, Ogle RE, Johnson RR. Bonding of plastic teeth to denture base resins. J Prosthet Dent 1991; 66:566–71. Cited in: Marra J, Paleari AG, Pero A, de Souza RF, Barbosa DB, Compagnoni MA. Effect of methyl methacrylate monomer on bond strength of denture base resin to acrylic teeth. Int J Adhesion & Adhesives 2009; 29:391-5.
18. Kulak-Ozkan Y, Sertgos A, Gedik H. Effect of themocycling on tensile bond strength of six silicones – based, resilient denture liners. J Prosthet Dent 2003; 89(3): 303-10.
19. Kutay O. Comparison of tensile and peel bond strengths of resilient liner. J prosthet Dent 1994; 71: 525-31
20. McCabe JF. Soft lining materials: composition and structure. J Oral Rehabil 1976; 3: 273-8.
21. McCabrthy JF. Applied dental materials.7th ed. London: Oxford Blackwell Scientific Publications; 1990.
22. Meng GK, Chung K, Fletcher-Stark ML, Zhang H. Effect of surface treatments and cyclic loading on the bond strength of acrylic resin denture teeth with autopolymerized repair acrylic resin. J Prosthet Dent 2010; 103: 245-52.
23. Mitchel DA, Mitchel L. Oxford Handbook of Clinical Dentistry. 4th ed. New York: Oxford University Press; 2005.
24. Moffitt AR, Woody RD, Parel SM, Miller BH. Failure modes with point loading of three commercially available denture teeth. J Prosthodont 2008; 17:432-8.
25. Nagle RJ, Sears VH. Dental Prosthetics. 2nd ed. St. Louis: Mosby; 1962.
26. O'Brien WJ, Ryge G. An outline of denture materials their selection. Philadelphia: Saunders; 1978.
27. Parvizi A, Lindguist T, Schneider R, Williamson D, Boyer D, Dawson DV. Comparison of the dimensional accuracy of injection-molded denture base materials to that of conventional pressure-pack acrylic resin. J Prosthodontics 2004; 13(2):83-9.
28. Storer R. Resilient denture base materials. Part 2, clinical trial. Br Dent J 1962; 113(7): 231-9.
29. Vallittu PK. Bonding of resin teeth to the polymethyl methacrylate denture base material. Acta Odontol Scand 1995; 53(2): 99–104.
30. Wright PS. The success and failure of denture soft lining materials in clinical use. J Dent 1984; 12:319-27.
31. Carrick TE, Kamohara H, McCabe JF. Adhesive bond strength and compliance for denture soft lining materials. J Biomaterials 2002; 23:1347-1352. (IVSL).
32. Tanimoto Y, Saeki H, Kimoto S, Nishiwaki T, Nishiyama N. Evaluation of adhesive properties of three resilient denture liners by the modified peel test method. J Acta Biomaterials 2009; 2: 764-769. (IVSL).
33. Anil N, Canan H. Microleakage study of vatious soft denture liners by autoradiography: Effect of accelerated aging. J Prosthet Dent 2000; 84(4): 394-9.

The effect of plasma on transverse strength, surface roughness and Candida adhesion of two types of acrylic denture base materials (Heat cure and light cure)

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ABSTRACT

Background: Dental polymers have a great use in dental applications such as denture, temporary crowns....etc; this is due to their superior physical and chemical characteristics. At the same time some of these properties impose a limitation on applications in several new and high technology areas. Plasma treatment is one of the most widely used surface treatment techniques in which the composition and structure of a few molecular layers at or near the surface of the polymer are modified. The aims of this study were to evaluate the effect of plasma treatment by argon gas on transverse strength; surface roughness and Candida adherence to heat cure and light cure acrylic denture base materials. Also compare the effect of plasma treatment on heat and light cure denture base materials.

Materials and methods: A total number of 180 specimens were prepared in this study; they were divided into two main groups according to the type of the material used (heat cure acrylic resin and light cure acrylic resin). Each main group was subdivided into three subdivisions according to the type of the test used (transverse strength, surface roughness and Candida adherence), for each test 30 samples were divided into three groups according to the time of plasma treatment that were applied (control, 5 and 10 minutes). Plasma treatment process was performed for all the studied groups in two different periods (5 and 10 minutes) except for control group no plasma treatment were performed.

Results: Plasma treatment of heat cured acrylic specimens revealed a decrease in the transverse strength of the studied groups for 5 and 10 minutes. Similar results were obtained for light cure denture base material after treatment with argon gas plasma for the same periods of time used for heat cure. Plasma treatment of heat cure and light cure acrylic specimens showed decrease in surface roughness and Candida adherence for (5min and 10min).The correlation between surface roughness and Candida adherence in the present study showed a weak correlation for all tested groups for both types of materials except for 5 minutes plasma treated heat cure acrylic specimens which were moderate. Statistically, there was no significant difference between surface roughness and Candida adhesion for all groups of both types of materials except for 5min group of heat cure acrylic specimens.

Conclusion: Within the limitation of this study it can be concluded that argon plasma treatment to the surface of heat and light cure denture base materials can cause a decrease in transverse strength, surface roughness and Candida adherence for 5 and 10min treatment times.

Key words: Plasma, argon gas, candida albicans, heat and light cure acrylic. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):10-17).

INTRODUCTION

Dental polymer introduced in 1937 and since that time it has a great use in dental applications such as denture, temporary crowns….etc, this is due to their superior physical and chemical characteristics such as high strength, and chemical inert nature. They are also relatively inexpensive and easy to process. At the same time some of these properties impose a limitation on applications. Thus it is required that their surface properties be modified to suit a particular application without affecting their bulk properties [(1)].

Plasma treatment is one of the most widely used surface treatment techniques. Plasma can be defined as a mixture of charged and neutral species, such as electrons, positive ions, negative ions, radicals, neutral atoms and molecules. During plasma treatment, the composition and structure of a few molecular layers at or near the surface (approximately 10nm) is modified due to the action of the energetic particles [(2)].

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Plasma used to alter surface properties of polymers such as wettability and adhesion to metals or to other types of polymers, without changing their bulk properties [(3)].

A wide variety of parameters can greatly affect the physical characteristics of plasma and subsequently affect the surface chemistry obtained by plasma modification. Processing parameters, such as gas types, treatment power, treatment time and operating pressure, can be varied by the user; however system parameters, such as electrode location, reactor design, gas inlets and vacuum are set by the design of the plasma equipment [(4)].

The present study designed to evaluate the effect of glow discharge plasma by argon gas on some properties of heat cure and light cure acrylic denture base materials.

Aims of this study are to:

1. Evaluate the effect of plasma treatment (argon) on heat cure and light cure acrylic denture base materials related to the following properties:

A. Transverse strength.
B. Surface roughness.

C. Adherence of Candida albicans to the surface of two types of acrylic denture base materials.

2. Evaluate the effect of exposure time to plasma argon gas for the same properties and materials mentioned above.

3. Compare the differences between heat and light cure acrylic denture base materials (in regarding to the same properties mentioned above) after plasma treatment with argon gas.

MATERIALS AND METHODS

A total No. of 180 specimens were prepared in this study, they were divided into two main groups according to the type of the material used (heat cure acrylic resin and light cure acrylic resin). Each main group was subdivided into three subdivisions according to the type of the test used (transverse strength, surface roughness and Candida adherence), for each test 30 samples were divided into three groups according to the time of plasma treatment that were applied (control, 5min and 10min). For transverse strength and surface roughness tests the metal patterns were constructed with the dimensions (65×10×2.5mm) length , width and depth respectively, while the dimensions for the circular metal pattern used for the adherence of Candida albicans test were (50mm diameter × 2.4 mm depth). All those done according to ADA specification No.12[(5)].

After the specimens being conditioned in distilled water for 48 hours, all the specimens were cleaned for five minutes using ultrasonic cleaning device in Methanol.

Plasma treatment done using a device called Dc-glow discharge plasma which is a homemade manufactured system based on the following principle components: (1) the plasma chamber (a cylindrical stainless steel vacuumed chamber with (50 ×50 cm) length and diameter respectively (2) Vacuum pumps system (rotary and turbo pumps) , (3) High voltage Dc- power supply (4 kV), (4) gas source. (5) Multimeters for discharging voltage and current measurements (6) penning and pirani heads and readers.

Plasma treatment process were performed for all studied groups in two different periods (5 min and 10 min) except for control group no plasma treatment were performed.

After plasma treatment the specimens were tested for transverse strength using instron transverse testing mechine (model 1195 with digital display unit and chart drive) and for surface roughness using profilometer device (surface roughness tester, Tylor Hobson).

RESULTS

The effect of plasma treatment (argon gas) on transverse strength of heat cure and light cure acrylic specimens.

The mean distribution, standard deviation, minimum and maximum values of the transverse strength of two tested materials (heat-cure and light cure) observed in **table 1.** In general the results of the transverse strength test revealed that the highest mean value (60.48 N/mm²) was for L.C.A. control group specimens, also the control group of H.C.A. specimens had the highest mean value (24.53N/mm²), in this table two way (ANOVA) test revealed a highly significant difference (P>0.05) between all groups of the same material while one way (ANOVA) showed a highly significant difference (P<0.01) between the two tested groups of H.C.A. and L.C.A. specimens.

Table 1: Mean distribution and ANOVA tests for transverse strength (N/mm²) of H.C.A. and L.C.A. specimens

Studied groups		N	Mean	SD	Min	Max	Two- way ANOVA test P value between groups of same material	One-way ANOVA test P value of all groups
HCA	Control	10	24.53	4.15	21.1	30.7	0.0001*	0.0001*
	5min P.T.	10	14.22	4.96	9.4	19.2		
	10min P.T.	10	13.64	1.47	12.2	15.4		
LCA	Control	10	60.48	0.02	60.5	60.5	0.0001*	
	5min P.T.	10	42.48	2.56	40.3	46.1		
	10min P.T.	10	34.27	1.07	33.1	35.5		

The effect of plasma treatment (argon gas) on surface roughness of heat cure and light cure acrylic specimens.

The mean distribution, standard deviation, minimum and maximum values of the surface roughness for the experimental and control groups of two tested materials (heat-cure and light cure) were shown in **table 2.** In general the results of the surface roughness test for H.C.A. specimens showed that control group specimens had the

highest mean values (0.908μm) while the samples exposed to argon gas for 10 min had the lowest mean values of surface roughness (0.350 μm), on the other hand for light cure specimens, control group had the highest mean values (5.845 μm) compared to 10min light cure group specimens which had the lowest mean values (4.636 μm). Also from this table two way (ANOVA) revealed a highly significant difference (P>0.05) between all groups of the same material, and one way (ANOVA) showed a highly significant difference between two test groups (P<0.01) H.C.A. and L.C.A. specimens.

Table 2: Mean distribution and ANOVA tests for surface roughness (μm) for H.C.A. and L.C.A. specimens

Studied groups		N	Mean	SD	Min	Max	Two- way ANOVA test P value between groups of same material	One-way ANOVA test P value of all groups
HCA	Control	10	0.908	0.102	0.76	1.00	0.0001*	0.0001*
	5min P.T.	10	0.762	0.045	0.71	0.82		
	10min P.T.	10	0.350	0.075	0.23	0.43		
LCA	Control	10	5.845	0.006	5.83	5.85	0.0001*	
	5min P.T.	10	5.557	0.092	5.45	5.66		
	10min P.T.	10	4.636	0.319	4.20	4.96		

The effect of plasma treatment (argon gas) on Candida adherence of heat cure and light cure acrylic specimens.

The mean distribution, standard deviation, minimum and maximum values of the Candida adherence in experimental and control groups of two tested materials (heat-cure and light cure) were shown in **table 3.** Statistical analysis revealed that plasma treatment to H.C.A. and L.C.A. specimens have remarkable effect on the adhesion of Candida albicans to the surface of the samples. The highest mean values (0.259) was found for control samples while for H.C.A. specimens exposed for 10min to plasma argon gas had the lowest mean values of candida albicans adherence (0.124), on the other hand for light cure specimens, control group had the highest mean values (1.095). Also from this table two way (ANOVA) revealed a highly significant difference (P<0.01) between all groups of the same material, and one way (ANOVA) showed a highly significant difference between the test groups (P<0.01) for the H.C.A. and L.C.A. specimens.

Table 3: Mean values distribution and ANOVA tests for Candida adherence to H.C.A. and L.C.A. specimens

Studied groups		N	Mean	SD	Min	Max	Two- way ANOVA test P value between groups of same material	One-way ANOVA test P value of all groups
HCA	Control	10	0.259	0.020	0.22	0.28	0.0001*	0.0001*
	5min P.T.	10	0.183	0.027	0.15	0.23		
	10min P.T.	10	0.124	0.012	0.11	0.15		
LCA	Control	10	1.095	0.034	1.01	1.13	0.0001*	
	5min P.T.	10	0.946	0.014	0.92	0.97		
	10min P.T.	10	0.877	0.012	0.86	0.89		

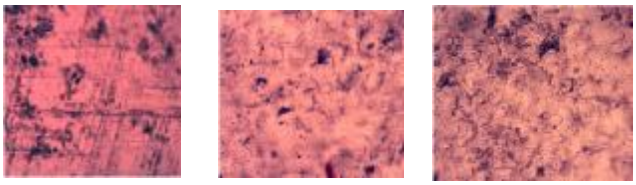
Microscopical examination

The results of Candida adherence to acrylic surfaces (heat and light cure acrylic) were obtained from examining the sample surfaces through optical light microscope and enumerating the numbers of Candida that adhere to the surface of the acrylic samples.

The number of Candida albicans cells on the surfaces of heat cure acrylic control specimens was higher than that on the surfaces of plasma treated specimens (P<0.005). Also the number of Candida albicans cells significantly decrease with increasing the time of plasma treatment from 5min to 10 min as shown in the microscopical **figures 1(A,B,C).**

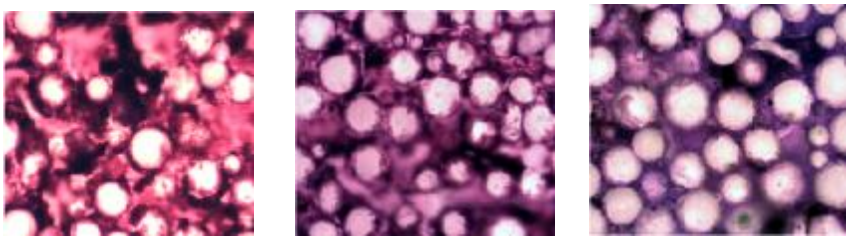
Also the number of Candida albicans cells on the surfaces of light cure acrylic control specimens was higher than that on the surfaces of plasma treated specimens (P<0.005). Also the number of Candida albicans cells significantly decrease with increasing the time of plasma treatment from 5min to 10 min as shown in the microscopical **figures 2 (A,B,C).**

While more Candida albicans cells were counted on the surface of light cure acrylic control specimens than that on the surfaces of heat cure acrylic control specimens (P<0.005).



A B C

Figure 1: Optical microscopical picture for heat cure acrylic specimens ((A)control, (B)5min,(C) 10min))



A B C

Figure 2: Optical microscopical picture for light cure acrylic specimens ((A) control, (B) 5min, (C) 10min))

Relation between surface roughness and Candida adherence

The result of this study showed that there was a correlation between surface roughness and Candida adherence in all studied groups, this correlation was either weak or moderate depending on the effect of surface roughness of the specimens on Candida adherence. Also this correlation was either direct or inverse depending on the amount of Candida adherence to the surfaces of the specimens. **Table 4** shows the correlation between surface roughness and Candida adherence in which there was a weak correlation between surface roughness and amount of Candida adherence for all tested groups for both types of materials except for 5 min plasma treated heat cure acrylic specimens which was moderate. Statistically there was no significant difference between surface roughness and Candida adhesion for all groups of both types of materials except for 5min group of heat cure acrylic specimens.

Table 4: Correlation between surface roughness and Candida adherence

		surface roughness X Candida adherence
Heat cure acrylic Control	R	-0.336
	P	0.342 (NS)
5 minutes	R	-0.673*
	P	0.033 (S)
10 minutes	R	0.044
	P	0.905 (NS)
Light cure acrylic Control	R	-0.079
	P	0.828 (NS)
5 minutes	R	-0.057
	P	0.876 (NS)
10 minutes	R	-0.078
	P	0.831 (NS)

DISCUSSION

The effect of glow-discharge plasma on the transverse (flexural) strength of two types of denture base materials.

Transverse strength or modulus of rupture is obtained when a load is applied in the middle of a beam supported at each end. Transverse strength measurements are used to greater extent than

either tensile or compressive strength; because, this test more closely representing the type of loading applied to a denture in the mouth since it reflects the loading arrangement in clinical practice [6].

The results of transverse strength test in this study showed that heat cured acrylic control specimens had lower transverse strength values than light cure acrylic control specimens (highly significant difference) and this result was in agreement with[7,8] who attributed their results to the difference in their structural formula (chemical composition),since the light cure acrylic consist of micro fine amorphous silica filler particles, the filler content is about 15% ,the presence of fillers are thought to increase mechanical properties of the material such as transverse strength. While the results of [9] showed that the transverse strength of light cure acrylic resin is lower than that of heat cure acrylic resin, the reason for this result might be due to the presence of the large number of porosities in this material which could not be kept under pressure during the polymerization process so common defects and internal voids often result. Several studies have been proposed that internal porosities concentrated stresses in the matrix and contributed to the formation of micro cracks under loading[10,11]

The results of the present study showed a decrease in the transverse strength value for both types of materials (H.C.A and L.C.A.) after plasma treatment i.e. there are significant differences between control and plasma treated specimens for 5min. and 10min., this decrease of the transverse strength of both materials since Ar gas might has a high etch rate and according to the study of **Zhang**[12] which showed that during the etching process by Ar gas, the plasma will generate volatile etch products at room temperature from the chemical reactions between the elements of the material etched and the reactive species generated by the plasma. Eventually the atoms of the shot element embed themselves at or just below the surface of the target, thus modifying the physical properties of the target.

The results of transverse strength test for heat cure acrylic specimens after 5 and 10 min treatment time to plasma appeared a non significant difference between 5 and 10 min groups of specimens, this might be due to that, at short exposure times Ar plasma seems to have little effect on transverse strength of heat cure acrylic specimens. While the results of transverse strength test for light cure acrylic specimens showed statistical significant differences between 5 and 10 min treatment times.

There were high significant differences between the specimens of two types of materials after plasma treatment; this is related to the main difference between the control groups of the two materials (heat cure and light cure).

The effect of glow-discharge plasma on the surface roughness of two types of denture base materials.

Achieving a smooth surface with extremely fine or no surface scratches has always been a prime objective for resin restoration. This is because of biological consequence of plaque accumulation on rough surfaces [13].

The digital profilometer is a suitable device for studying the surface roughness of restorative materials, it gives quantitative measurement in micron that can be evaluated and compared statistically.

The results revealed that there were highly significant differences between control specimens of heat cure acrylic and their contrast of light cure acrylic material, in which light cure specimens had higher values of surface roughness. This might be due to the chemical differences between the two materials since the light cure material chemically are more common with composite materials than with the denture base resins. From another point of view, when the fractured section of cured (VLC) material examined under scanning electron microscopy, spherical particles can be seen protruding in some area and in others spherical pits, which make it had rough surface as it compared with heat cure acrylic resin [14].

Regarding the results of the effects of plasma on the surface roughness of heat and light cure acrylic specimens, it appeared that there was decrease in the surface roughness of both materials after treatment with plasma, this might be due to, during the plasma treatment process; glow discharge plasma is created by evacuating a reaction chamber and then refilling it with a low-pressure gas. The gas is then energized; the energetic species in gas plasma include ions, electrons, radicals, metastables, and photons [15]. Surfaces in contact with the gas plasma are bombarded by these energetic species and their energy is transferred from the plasma to the solid leading to remove the peak and valley on the surface of the specimens result in slight removing of the surface particles which might lead to a significant decrease in surface roughness. The results of this study were in agreement with previous studies who showed that these species (ions, electrons, radicals, metastables, and photon) are involved in the process of plasma treatment;

they interact with the exposed surfaces causing some chemical changes at the surface of the material. If the applied energies are higher than the characteristic bonding energies of the polymers, some parts of the surface can undergo scission reactions and form new bonding configurations [16,17]

Statistically the results showed a significant difference between 5 and 10 min plasma treated time for both types of materials (heat cure and light cure) denture base materials, this might be attributed to that, argon plasma treatment decreases surface roughness at short treatment times and this reduction in surface roughness increases continuously after longer time of treatment **[18].**

There were high significant differences between the same groups of different materials (heat cure and light cure) after plasma treatment process. This might be due to the main differences between the control groups of the two materials.

The effect of glow-discharge plasma on Candida adherence.

The initial attachment of Candida albicans on the mucosal surface of the denture is essential in the colonization and development of denture stomatitis [19]. The development of methods that reduce the adherence of Candida to these surfaces could be a significant step toward treatment and prevention of denture stomatitis. Glow-discharge plasma, a type of cold plasma, has been often used as a method of surface modification; however, in dentistry it has received little attention. In this technique, gas temperature can remain as low as room temperature—preserving the integrity of polymer-based materials [20]. This is of particular importance for denture base acrylic resins, in which increase temperature might cause dimensional changes and hence the fitness of the denture bases to the supporting tissues will be affected [21]**.** Glow-discharge technique may affect the surfaces of acrylic resins in many ways, including cleaning of organic or inorganic debris, generating reactive and functional groups on the surface layers without affecting their bulk properties and making the surfaces more adherent to specific cells and proteins depending on the plasma atmosphere.

The results of this study revealed that there were significant differences between control specimens of heat cure acrylic and their contrast of light cure acrylic material, in which light cure specimens had a higher value of Candida adherence to the surface of the specimens, which might be due to their high value of surface roughness.

Adherence of Candida albicans to the surface of heat cure denture base materials decrease after plasma treatment, the same results obtained for light cure acrylic specimens in which there was a marked decrease in Candida adherence to the surface of light cure acrylic denture base material. Statistically high significant differences were found between different groups (control, 5 min, and 10 min) for both types of denture base materials.

The results of this study revealed that Ar plasma treatment significantly reduced the yeast adhesion. These results were in agreement with **Zamperini [22]** who found that Ar plasma treatment showed promising potential for reducing the adherence of Candida albicans to denture base resins (heat cure), this might be due to the movement of polar groups from the surface to the polymer bulk. It has been reported that surface-charged resins may alter the ionic interaction between the denture base and Candida spp**. [23,24]**. Negatively charged resin surfaces showed significantly lower levels of Candida than the untreated ones **[25].**

While the results of this study were in disagreement with the data reported by **Yildirim [26]** who have found higher counts of Candida albicans in plasma treated surfaces than in the unmodified control group.One possible reason for this disagreement could be due to the difference in the methodology in which oxygen gas were used for plasma surface treatment at 50 or 100 W, for 15 min**.**

There were significant differences between the same groups of different materials (heat cure and light cure) after plasma treatment process, this might be due to the main differences between the control groups of the two materials.

The correlation between surface roughness and Candida adherence.

Roughness has been considered as a factor that affects the adhesion of Candida albicans to acrylic denture base materials [27]**.** The results of the present study showed statistically there was no significant difference between surface roughness and Candida adherence however, there were various studies have found that an increase in surface roughness facilitated the yeast retention **[27-30]**.

In this study the results appeared that there were a weak correlation between surface roughness and Candida adhesion in all groups but no significant influence of roughness on adherence of Candida albicans was verified, and these results were in accordance with other studies [31-34]**.** This can be explained that specimens of acrylic denture base materials with more surface

roughness may serve as a reservoir, with surface irregularities providing increased microorganism retention and inducing adhesion of microorganism (Candida and bacteria). The superficial defect such as voids and micro cracks on surface were possible sites for Candida adhesion **(35).**

REFERENCES

1. Listen EM, Martinu L, Wertheimer MR. Plasma surface modification of polymers for improved adhesion: a critical review. J Adhesion Sci Technol 1993; 7: 1091-27.
2. Bailey AG. The science and technology of electrostatic powder spraying, transport and coating. J Electrostatics1998; 45: 85-120.
3. Garbassi F, Morra M, Occhielo E. Physical Modifications. In Polymer surfaces: from physics to technology. New York: John Wiley and Sons; 1996. pp. 223-237.
4. Fowler S. Packaging for the 21st Century 2001; Electro Static Journal.
5. American Dental Association specification No.12 for denture base polymer guide to dental materials and devices. 7th ed., Chicago Illinois; 1999.
6. Powers JM, Sakaguchi RL. Craig (Restorative Dental materials) 12th ed., Elsevier, Mosby, 2006,
7. Khan Z, Von Fraunhofer JA, Razavi R. The staining characteristics, transverse strength and microhardness of a visible light cured denture base. J Prosthet Dent 1987; 57(3): 384-6.
8. Noort RV. Introduction to dental materials. 2nd ed. Elsevier Science Limited, Mosby; 2002.
9. Ozlem Gurbuz, Fatma Unalan, Idil Dikbas. Comparison of the transverse strength of six acrylic denture resins. J Oral Health 2010; Den Manag 9(1).
10. Tan HK, Brudvik JS, Nicholls JL, Smith DE. Adaptation of a visible light cured denture base material. J Prosthet Dent 1989; 61: 326-30.
11. Machado C, Sanchez E, Azer SS, Uribe JM. Comparative study of the transverse strength of three denture base materials. J Dent 2007; 35(12): 930-3.
12. Zhang DA. Surface reaction mechanism in plasma etching process. A thesis, College of the University of Illinois at Urbana-Champaign, Urbana, Illinois, 2000
13. Bassiouny MA, Grant AA. The surface finish of a visible light-cured composite resin. J Prosthet Dent 1980; 44(2): 175-82.
14. McCabe JF, Walls AWG. Applied dental material. 8th ed. London: Blackwell Scientific publication; 1998.
15. Shimizu RN, Demarquaette RN. Evaluation of Surface Energy of Solid Polymers Using Different Models. J Applied Polymer Science 2000; 76: 1831-45.
16. Kolluri OS. Plasma surface engineering of plastics. In ASM Handbook, Vol.5 (edsCotell CM. Sprague JA; Smidt FA) surface engineering, materials park OH, ASM International. The material information society 1996; 892-9.
17. Lee C, Kim HW, Kim S. Organic contaminent removal by oxygen ESR plasma. Appl Surf Sci 2007; 253: 3658-63.
18. Bismarck J, Springer. Wettability of Materials: Plasma Treatment Effects, in Encyclopedia of Surface and Colloid Science, P Somasundaran, Ed., Taylor & Francis, New York, 6592 (2006).
19. Webb BC, Thomas CJ, Willcox MD, Harty DW, Knox KW. Candida associated denture stomatitis. Etiology and management: a review. Part 2. Oral diseases caused by Candida species. Aust Dent J 1998; 43(3):160-6.
20. Liu Y, Kuai P, Huo P, Liu C. Fabrication of CuO nanofibers via the plasma decomposition of $Cu(OH)_2$. Mater Lett 2009; 63: 188–90.
21. Polukoshko K.M, JS Brudvik, JI Nicholls and DE Smith. Evaluation of heat-cured resin bases following the addition of denture teeth using a second heat cure. J Prosthet Dent 1992; 67(4): 556–62.
22. Camila Andrade Zamperini, Ana Lucia Machado, Carlos Eduardo Vergani, Ana Claudia Pavarina, Eunice Terezinha Giampaolo, NilsonCristino da Cruz. Adherence in vitro of Candida albicans to plasma treated acrylic resin. Effect of plasma parameters, surface roughness and salivary pellicle. AST Technical Journal 2010.
23. Park SE, Periathamby AR, Loza JC. Effect of surface-charged poly (methylmethacrylate) on the adhesion of Candida albicans. J Prosth 2003; 12: 249–54
24. Puri G, Berzins DW, Dhuru VB, Raj PA, Rambhia SK, Dhir G, et al. Effect of phosphate group addition on the properties of denture base resins. J Prosthet Dent 2008; 100: 302–8.
25. Park SE, R Blissett, SM Susarla and HP Weber. Candida albicans adherence to surface-modified denture resin surfaces. J Prosth 2008; 17(5): 365–9.
26. Yildirim MS, Hasanreisoglu U, Hasirci N, Sultan N. Adherence of Candida albicans to glow-discharge modified acrylic denture base polymers. J Oral Rehabil 2005; 32: 518–25.
27. Verran J, Maryan CJ. Retention of Candida albicans on acrylic resin and silicon of different surface topography. J Prosthet Dent 1997; 77(5): 535-9.
28. Radford DR, Sweet SP, Challacomb SJ, Walter JD. Adherence of Candida albican to denture base material with different surface finishes. J Dent 1998; 26(7): 577- 83.
29. Taylor R, Maryan C, Verran J. Retention of oral microorganisms on cobalt-chromium alloy and dental acrylic resin with different surface finishes, J Prosthet Dent 1998; 80: 592–7.
30. Lamfon H, Porter SR, McCullough M, Pratten J. Formation of Candida albicans biofilms on non-shedding oral surfaces. Eur J Oral Sci 2003; 111: 465–71.
31. Nikawa H, Jin C, Makihira S, Egusa H, Hamada T Kumagai H. Biofilm formation of Candida albicans on the surfaces of deteriorated soft denture lining materials caused by denture cleansers in vitro. J Oral Rehabil 2003; 30: 243–50.
32. Nevzatoglu EU, Özcan M, Ozkan YK, Kadir T. Adherence of Candida albicans to denture base acrylics and silicone-based resilient liner materials with different surface finishes. Clin Oral Investig 2007; 11(3): 231–6.

33. Burgers R, Schneider-Brachert W, Rosentritt M, Handel G, Hahnel S. Candida albicans adhesion to composite resin materials. Clin Oral Investig 2009; 13(3): 292–9.
34. Ferreira MAF, Pereira-Cenci T, Rodrigues de Vasconcelos LM, Rodrigues-Garcia RCM, Del BelCury AA. Efficacy of denture cleansers on denture liners contaminated with Candida species, Clin Oral Investig 2009; 13: 237–42.
35. Kawai K, Ebisus U. Effect of surface roughness of porcelain on adhesion of bacteria arc their synthesis glucan. J Prosthet Dent 2000; 83(6): 664-7.

The effect of different acidic environments on the apical microleakage of different obturation techniques (An in vitro study)

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ABSTRACT

Background: Pulpal and apical inflammation or infection decreases tissue pH in the region surrounding the involved tooth which might affect the sealing ability of different obturation systems. This study evaluate the apical microleakage of three obturation techniques (lateral condensation of Gutta-percha/AH 26, Soft-Core gutta-percha/AH 26 and lateral condensation of Resilon/Real Seal SE), when exposed to 7.3 6.5, 6.0 and 5.5 pH values.

Materials and method: One hundred and thirty two roots of freshly extracted teeth were selected. Teeth were decoronated, working length was established and the roots were instrumented using a crown down technique with ProTaper rotary files (SX-F3). The specimens were divided into three groups of 44 samples each. Group A: obturated using lateral condensation of gutta percha and AH 26. Group B: obturated using soft-core and AH 26. Group C: obturated using lateral condensation of Resilon and Real Seal SE. Each group was further subdivided into four subgroups, 10 samples each, which were exposed to pH values of 7.2, 6.5, 6.0 and 5.5 respectively. Microleakage was evaluated by longitudinal sectioning, and measurement of liner dye penetration.

Results: There was a non significant difference within each group regarding the different pH media. Both Soft-Core and Resilon showed less apical microleakage than lateral condensation of gutta percha with a highly significant difference in all the tested acidic media.

Conclusion: Resilon/Real Seal SE subgroups showed the least apical microleakage, however, it didn't provide the complete sealing claimed by the manufacturer.

Key words: Soft-Core. Resilon, Real Seal SE, pH of the periapical area, apical dye penetration. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):18-24).

INTRODUCTION

Complete obturation of the root canal with an inert filling material and creation of a fluid-tight seal are among the major goals of successful endodontic treatment. Among numerous obturation techniques and filling materials available, gutta-percha continues to be the material of choice, owing to its unique chemical and physical properties [(1)]. Lateral condensation technique has proven to be a very popular technique utilizing gutta-percha filling material; however its ability to conform to the internal surfaces of the root canal has been questioned [(2)].

Thermoplasticized obturation techniques were introduced to improve the homogeneity and surface adaptation of gutta-percha. One of these techniques involves the use of a metal or plastic carrier coated with a layer of gutta-percha that is heated, to permit thermoplasticized canal obturation. The Soft-Core System (CMS-Dental, Copenhagen, Denmark) uses a similar strategy to achieve root canal obturation. The Soft-Core obturator consists of a plastic core which is coated with thermoplastic alpha phase gutta-percha. It offers many advantages such as the reduction in chair-side time and rapid set of the gutta percha [(3)].

Many root canal sealers are currently being used in combination with gutta-percha to fill the root canal system. Epoxy resin sealers such as AH 26 and AH plus have been used because of their reduced solubility, apical seal and micro-retention to root dentin [(4)]. Self-etch primers have been used for bonding to the root canal dentin, and as the epoxy resin sealers do not copolymerize with methacrylate resin-based adhesives, a dual-curable methacrylate resin sealer (Epiphany, Pentron, Wallingford, CT) or (Real Seal, SybronEndo, Orange, CA), was developed with a self-etch primer, and a new thermoplastic filled polymer (Resilon, Resilon Research LLC, USA), as an alternative to gutta-percha. Resilon contains dimethacrylates, which can bond to methacrylate-based resin sealers, such as Epiphany [(5)].

A new self-etch sealer (epiphany SE, Pentron, Wallingford, CT) or (RealSeal SE, SybronEndo, Orange, CA) has evolved which eliminates the priming step and has been claimed to have similar sealing abilities as the original system. It has been claimed that this system creates a mono-block effect with the canal wall. Such a mono-block eliminates the gaps associated with the core material and sealer, resists shrinkage and strengthens the root [(6)].

Epidemiological studies of root-filled teeth in various countries and different populations have

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demonstrated the presence of apical periodontitis in a relatively high proportion of these teeth, which most of them are symptom-free [7]. The pH of the aspirated periapical periodontitis was found to be acidic (6 to 7.3) in a study done by Nekoofar et al in 2009[8]. Acidic pH may have an effect on the properties of the dental materials, which are routinely placed in environments that may be inflamed or infected [8].

MATERIALS AND METHOD

Sample selection and preparation

One hundred and thirty two freshly extracted upper and lower first molar teeth with mature apices and semi straight roots were selected. The criteria for teeth selection included a straight root canal and a mature, centrally located patent apical foramen, and roots that devoid of any resorption, cracks and fractures by using an Illuminated magnifying lens (X10) [9].

External soft tissue and debris were removed using periodontal curette, then the teeth were decoronated and the distal roots of the lower 1st molar and the palatal roots of the upper 1st molars were detached using a diamond disk bur. In order to get a flat reference point for measurement and to eliminate any variable in access preparation and to facilitate a straight line access in the coronal portion of the canal for both instrumentation and obturation, roots were sectioned through a line drown perpendicular to the long axis of the tooth at the cemento-enamel junction [10].

All roots were stored in normal saline. In order to standardize the length of the root canals involved in each experimental group, the length of all roots were measured and root segments ranging from 13-16 mm were equally distributed to the groups [11]. The exact working length was established by passing a size 10 or 15 stainless steel file until it's tip was just out of the apical foramen and then by subtracting 1 mm from the measured length.

The roots were placed in an assembly made of an acrylic block and a light body impression material as seen in Figure (1.1 A). This procedure would allow maximum simulation of the practiced clinical condition in a normal endodontic treatment especially regarding the bony socket with its periodontal ligament.

Instrumentation and irrigation

The canals were instrumented using a crown-down technique with NiTi rotary ProTaper files (Figure 1.1 B). Apical patency was maintained throughout instrumentation using a size 15 file [12]. The sequence of ProTaper instruments used in the present investigation is summarized in Table (1.1).

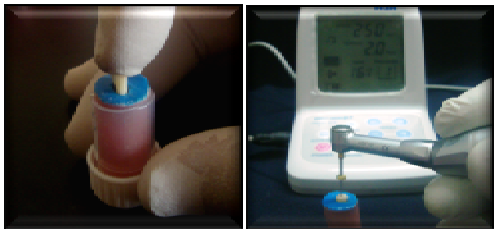

Figure 1.1: Roots placed in an assembly made of an acrylic block and a light body impression material (A), instrumentation with rotary NiTi ProTaper system (B).

Table 1.1: Instrument sequence.

Sequence	File name	Depth of insertion
1.	S1	-6 mm of the working length
2.	Sx	-4 mm of the working length
3.	S1	Full working length
4.	S2	Full working length
5.	F1	Full working length
6.	F2	Full working length
7.	F3	Full working length

All instruments were used in a low-torque motor with torque control and a constant speed of 250 rpm. The instruments were inserted into the root canal in a continuous in-and-out movement and were never forced apically. Maximum effort was made to take the files to length only one time for no more than 1 second [12].

Irrigation was performed with 2 ml NaOCl (2.5%) after each change in instrument size. The smear layer was removed by rinsing with 2.0 ml of 17% ethylene diamine tetraacetic acid (EDTA) solution for 60 seconds [13]. The root canal was then flushed with 5 ml of distilled water and dried with ProTaper F3 paper points.

Canal obturation

The specimens were divided into three experimental groups of 44 samples each. Each group had two positive and two negative control samples.

Group A: 40 roots obturated using lateral condensation of gutta percha and AH 26 sealer.
A master gutta-percha cone size 30 taper .06 was fitted to the working length with tug back.

The sealer was introduced to the full working length using a ProTaper F3 paper point with simultaneous rotary movement in a counter clock wise direction to coat the canal with a thin layer of sealer. The master cone was coated with the AH 26 sealer and gently seated to the working

length. Lateral condensation was carried out using accessory gutta-percha cones ISO size 20 taper 0.02 with an endodontic finger spreader size 20 by applying apical pressure (1-3 Kg) placed in the first instance to within 1 mm of the working length [14]. The gutta-percha cones coated with sealer were laterally condensed until they could not be introduced more than 3 mm into the root canal. Following obturation, the excess gutta-percha was removed from the coronal cavity with a warm instrument (excavator) and vertically condensed with a straight endodontic plugger. The remaining cavity was filled with a temporary filling [15, 16].

Group B: 40 roots obturated using Soft-Core and AH 26 (carrier system). The size 30 Soft-Core obturator was verified using the 'Size Verifier'. At first the oven was adjusted on the button H (for regular heat obturators) and then heated (Soft-Core regular needed 110˚C to melt). The stopper was adjusted to the premeasured working length and the obturator was placed in its specific hole in the oven. While the obturator was heated, AH 26 sealer was mixed according to manufacturer's instruction and the canal wall was lightly coated with the sealer using ProTaper F3 paper point. When the oven indicated that the obturator was ready with a beep sound and a green diode light, the obturator was carefully removed from one of the slots in the top of the oven. The plasticized Soft-Core was then inserted to the apical stop with apical pressure. After the gutta-percha had been cooled, the handle and insertion pin were removed by a twisting motion. Any excess plastic core was cut away with a small inverted cone bur and the extra gutta-percha was trimmed away. The remaining cavity was filled with a temporary filling [17, 18].

Group C: 40 roots obturated using lateral condensation of Resilon and Real Seal SE. A size 30 taper .06 Resilon cone was fitted to the working length with tug back. The dual syringe (with mixing tip) was used to express the self-etch root canal sealant onto the cement slab which was then introduced to the canal on ProTaper F3 paper point to coat the canal with a thin layer of sealer according to manufacturer's instruction. The lateral condensation was made in the same manner mentioned in the gutta percha group (group A). Following obturation, the excess Resilon was removed from the coronal cavity with a warm instrument and vertically condensed with finger plugger. The orifice was light cured for 40 seconds according to manufacturer's instruction. The remaining cavity was filled with a temporary filling [19, 16].

After obturation samples of all groups were stored in an incubator for 48 hours in a 37˚C and 100 % relative humidity to ensure complete setting of the sealer, then the roots were removed from their assembly and each group was further subdivided into four subgroups, 10 samples each (1, 2, 3 and 4) which were exposed to (7.2, 6.5, 6.0 and 5.5 pH solutions) respectively.

Each group had two roots acting as positive controls and two roots as negative controls. The negative control roots were obturated and completely coated with one layer of nails varnish and two layers of sticky wax, while positive controls where left uncoated and unobturated. All experimental roots were coated with one layer of nails varnish and two layers of sticky wax except for the apical 3mm.

An ELIZA test micro-plate was used to immerse about 7.5 mm of the root apically in the freshly prepared acidic solutions according to their subgroups, and the assembly was incubated for two days. Indian ink was used as the leakage indicator, and all samples including the positive and negative controls were immersed in the ink and then the assemblies were re-incubated for another one week. At the end of this period, the roots were removed from the ink and washed and by using lacron carver the coating layers were removed from the roots [15]. Longitudinal sectioning was made using chisel and mallet taking care to include the apical foramen in the fracture line [20]. The linear extent of dye penetration from the apical end coronally was measured by means of stereomicroscope at (X40) magnification with calibrated grid. Apical micro-leakage was measured independently by two evaluators one of them not aware of the obturation technique used and the average of the two measurement of each sample was considered for statistical analysis [21, 22].

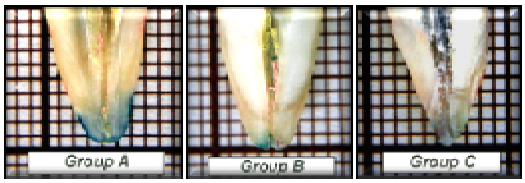

Figure 1.3: Dye penetration in samples of the experimental groups. (Group A) lateral condensation GP/AH 26, (Group B) Soft-Core/AH 26, (Group C) lateral condensation Resilon/Real Seal SE.

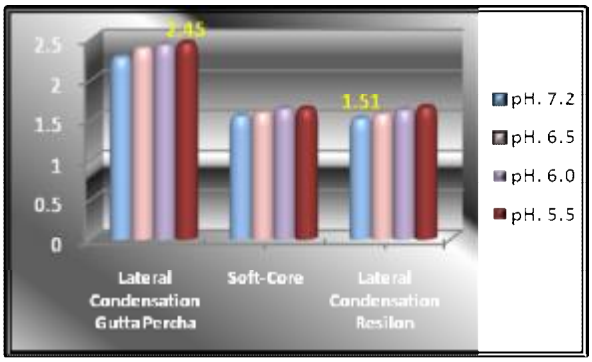

Figure 1.2: Mean values of apical microleakage in mm with the highest and lowest mean values in all tested subgroups.

Statistical analysis of the data was done using the analysis of variance (ANOVA). The results showed that there was no statistical significant difference within each group regarding different pH media (P 0.05). ANOVA test was also done between groups A, B and C to establish the difference in microleakage among the different obturation techniques. It showed a highly statistical significant difference ($p \leq 0.01$).

Student's t-test was performed between subgroups that were subjected to the same acidic environments. The results of this test revealed a highly significant difference between all subgroups of both Soft-Core and Resilon with all subgroups of gutta percha (lateral condensation); this result was constant in all the tested acidic media, while a non significant difference was observed between all sub groups of Soft-Core and Resilon in all the tested acidic media.

DISCUSSION

In the current study, sodium hypochlorite was not used as the final irrigation solution since it might result in reduced resin bond strengths [23]. EDTA (17%) was used as the final irrigation to remove the smear layer which may improve the apical seal [24]. The Soft-Core System (a carrier system that consists of a Thermoplasticized alpha phase gutta-percha coating a plastic carrier) was evaluated in this study since it was introduced to improve the homogeneity and surface adaptation of gutta-percha [2]. AH26, an epoxy resin sealer, was combined with gutta-percha obturated groups in this study, because it performed better than quite a lot of sealer types [20, 25] and AH 26 (an improved epoxy resin sealer in the AH series) [26]. The second generation of the Resilon/ Epiphany obturation system (an alternative system that offers the promise of adhesion to dentine) replaced the original sealer and primer with the self-etch Real Seal SE, that's why Resilon combined with Real Seal SE was used in this study to evaluate the benefit of this self-etch dual-cure, hydrophilic resin sealer that omitted the separate priming step [27].

However the canals were instrumented with the ProTaper system, obturation was not made with ProTaper gutta-percha points because the Resilon core material is available only in tapers 0.02, 0.04 and 0.06 [28]. And since the last instrumentation file used was F3 which have a D0 diameter and apical taper of 30/0.09, and a decreasing percentage taper from D4 to D14 [29]. In order to obtain maximum geometrical apical fitness, the greatest taper of Resilon cones available (size 30/0.06 taper) were used in this study, and for standardization reasons gutta-percha cones with the same geometry were utilized. Since the pH of the periapical periodontitis was found to be acidic ranging from 6 to 7.3[8], the obturated roots in the current study were subjected to solutions ranging from a neutral pH value of (7.2) to an increased acidity of (5.5).

Comparison between the different acidic media in each group

Statistical analysis of the data showed a non significant difference within each group regarding different pH media.

Since, to our knowledge, this is the first time to assess the effect of an increased acidity on the apical microleakage encountered in obturation, it is of benefit to compare our results with other studies that evaluated the effect of decreased pH on dental materials. The effect of an acidic environment (pH 5) was found to be of a non significant difference on the microleakage of various root-end filling materials in two in vitro studies [30, 31] which is the same conclusion drawn by the current study regarding the obturation materials.

However the statistics revealed a non significant difference, the data showed an increase in dye penetration with the decreased pH but to a mild extent. This mild effect can be hypothesized in two ways:

1. Short exposure time (48 hours) which might reflect the mild effect.
2. The use of resin sealers (AH26, Real Seal SE) which have a relatively short setting time and low solubility in aqueous solutions [11, 32] which might explain the mild effected of the acidic solutions on these resin sealers.

Lateral condensation of (Gutta-Percha + AH26) versus (Resilon + Real Seal SE)

Student's t-test revealed a highly significant difference between all subgroups of Resilon +

Real Seal SE with all subgroups of gutta percha + AH 26. These results were in agreement with many studies that compared the same materials using the same dye penetration methodology [33, 34] and the microbial leakage and fluid filtration methods [13, 35, 36, 37] and when comparing Resilon with gutta-percha and the other AH sealer (AH plus) [38].

Excellent sealing capability of Resilon may be attributed to the "mono-block" which is created by the Resilon filling material being closely adapted to the Epiphany sealer and in turn the Epiphany sealer adhering to the dentin walls. In contrast, the high-power SEM micrograph showed how the gutta-percha filling pulled away from the AH 26 sealer, whereas the resin tags held the sealer against the dentin wall which created a gap between gutta-percha and sealer forming an avenue for microleakage [35].

The obtained results could be related to the type of sealer used, since Epiphany performed better than both of the epoxy resin sealers (AH26, AH plus) which could be explained by the inferior adaptation and penetration ability of gutta-percha with AH26 and AH plus across the root canal [39]. In addition AH26 is hand-mixed and the formation of voids is a common finding which might explain the increased leakage [40].

The results of this study disagree with studies that either showed a non significant difference or proved better results in gutta-percha [19, 41, 42, 43].

Shemesh et al concluded that Resilon/Epiphany had more glucose penetration than gutta-percha and AH 26 [41]; while equivalent apical leakage of Resilon/Epiphany and gutta-percha/AH Plus sealed roots was also shown by Biggs et al using a silver nitrate microleakage study [42]; however the smear layer was not removed in their study compared to our study since Cobankara et al showed a significant decrease in leakage when the smear layer was removed [44].

Paque´ & Sirtes in 2007 showed that roots filled with Resilon/Epiphany allowed significantly more fluid movement when compared to gutta-percha/AH plus after 16 months (long-term) compared with the immediate measurement in which they allowed relatively less fluid movement [19]. However these results disagree with our study in long term but still it agrees with our results in the short term, since (in the long term) the susceptibility of a polycaprolactone-based root canal filling material to degradation can be an important factor [45]. Oliveira et al, in 2011, used bacterial leakage and found that Epiphany SE had intermediate results in contrast to AH Plus which had the least leakage [43]. The cause of disagreement in all of the previously mentioned studies could be attributed to the differing methodologies applied in instrumentation and obturation and to the microleakage testing methods that were not used in the present study.

Lateral condensation of (Gutta-percha + AH26) versus carrier coated gutta-percha (Soft-Core + AH26)

Soft-Core showed less apical microleakage than lateral condensation in all subgroups with high significant difference. This was in agreement with two in vitro studies [2, 17] that used different techniques to measure microleakage (computerized fluid filtration meter and dye infiltration ratio in horizontal cross sectioning respectively, while the same results was reached by Nema, who utilized the same dye penetration method [18].

These results can be explained either by the use of heat softened GP that created a better homogenous mass with less voids and better adaptation of the GP to the canal wall [46], or by the sealer content since Gençoğlu, in 2003, established that Soft Core technique had more gutta-percha content than lateral condensation technique and that higher sealer content might lead to higher leakage amounts, since endodontic sealers are soluble materials and the shrinkage may result in potential leakage pathways in root canal fillings [47].

The results of this study disagree with two in vitro studies [15, 20] that used dye penetration and fluid filtration respectively. This might be explained by the differing instrumentation and obturation techniques, since the root canals were prepared using Gates Glidden/step-back technique without removing the smear layer in contrast to NiTi ProTaper files and smear layer removal in the current study, also AH 26 was sparingly introduced into the coronal third of each canal using the rotary paste filler while in our study a thin layer of sealer was introduced to the full working length using a paper point according to manufacturer's instruction.

Lateral condensation of (Resilon + Real Seal SE) versus carrier coated gutta-percha (Soft-Core + AH26)

A non significant difference was observed between these two obturation techniques, still they showed a highly significant difference when compared to lateral condensation of gutta-percha.

It should be noticed that both of the obturation techniques didn't prevent apical microleakage completely, which can be attributed

in case of Resilon to the polymerization shrinkage and failure to generate a complete mono-block without gaps when examined under SEM [23], while in case of Soft-Core it could be attributed to the thermal shrinkage of the softened gutta-percha [18].

Within the circumstances of this in vitro study the following conclusions could be withdrawn:

. The increased pH did not affect the apical microleakage of the examined obturation techniques.
. Both lateral condensation of Resilon/Real Seal SE and Soft-Core/AH 26 provided less microleakage when compared to the lateral condensation of gutta-percha/AH 26.
. Lateral condensation of Resilon/Real Seal SE showed the least apical microleakage, however, it didn't provide the complete sealing claimed by the manufacturer.

REFERENCES

1. Gulsahi K, Cehreli ZC, Onay EO, Tasman-Dagli F, Ungor M. Comparison of the Area of Resin-based Sealer and Voids in Roots Obturated with Resilon and Gutta-Percha. JOE 2007; 33: 1338–1341.
2. Gençoğlu N, Oruçoğlu H, Helvacıoğlu D. Apical leakage of different gutta-percha techniques: thermafil, js quick-fill, soft core, microseal, system b and lateral condensation with a computerized fluid filtration meter. Eur J Dent 2007; 1: 97-103.
3. Özer SY, Aktener BO. Outcome of Root Canal Treatment Using Soft-Core™ and Cold Lateral Compaction Filling Techniques: A Randomized Clinical Trial. J Contemp Dent Pract 2009; 10: 74-81.
4. Sevimay S, Kalayci A. Evaluation of apical sealing ability and adaptation to dentine of two resin-based sealers. J Oral Rehabil 2005; 32: 105–10.
5. Garcia LFR, Naves LZ, Consani S, Correr-Sobrinho L, Pires-de-Souza FCP. Apical obturation quality of Epiphany/Resilon root canal filling system. Braz J Oral Sci 2009; 8(3): 132-136.
6. Metkari SS, Kamra AI, Banga KS. Evaluation of quality of apical seal in Resilon/Epiphany SE, gutta-percha/AH Plus and gutta-percha / tubli-seal EWT-filled by dye leakage approach - An in vitro study. Endodontology Journal 2011; 35(1): 61-67.
7. Haapasalo M, Udnæs T, Endal U. Persistent, recurrent, and acquired infection of the root canal system post-treatment. Endodontic Topics 2003; 6: 29–56.
8. Nekoofar MH, Namazikhah MS, Sheykhrezae MS, Mohammadi MM, Kazemi A, Aseeley Z, Dummer PMH. pH of pus collected from periapical abscesses. Int Endod J, 42, 534–538, 2009.
9. Al Hashimi MM. An evaluation of coronal microleakage in endodontically treated teeth using two different obturation techniques and two types of sealers at four different time periods. A master thesis, conservative department, university of Baghdad, 2005.
10. Gondim E, Zaia A, Gomes B, Ferraz C, Tixeeura F, Souza-Filho F. Investigation of marginal adaptation of root end filling materials in root end cavities prepared with ultrasonic tips. Int Endod J 2003; 36: 491–499.
11. Salz U, Poppe D, Sbicego S, Roulet J.-F. Sealing properties of a new root canal sealer. Int Endod J 2009; 42: 1084–1089.
12. Mahera F, Economides N, Gogos C, Beltes P. Fluid-transport evaluation of lateral condensation, ProTaper gutta-percha and warm vertical condensation obturation techniques Aust Endod J 2009; 35: 169–173.
13. Wedding JR, Brown CE, Legan JJ, Moore K, Vail MM. An in vitro comparison of microleakage between resilon and gutta-percha with a fluid filtration model. JOE 2007; 33:1447–1449.
14. Whitworth J. Methods of filling root canals: principles and practices Endodontic Topics 2005; 12: 2–24.
15. De Moor RJG, Martens LC. Apical microleakage after lateral condensation, hybrid gutta-percha condensation and Soft-Core obturation: an in vitro evaluation. Endod Dent Traumatol 1999; 15: 239-243
16. Abdul-rada NJ. An evaluation of apical microleakage in roots filled with thermoplastic synthetic polymer based root canal filling material (Real Seal 1 bonded obturation). A master thesis, conservative department, university of Baghdad, 2011.
17. Boussetta F, Bal S, Romeas A, Boivin G, Magloire H, Farge P. In vitro evaluation of apical microleakage following canal filling with a coated carrier system compared with lateral and thermomechanical gutta-percha condensation techniques. Int Endod J 2003; 36: 367-71.
18. Nema TG. An evaluation of the use of different sizes and techniques of thermoplasticized obturators on the apical seal (a comparative study). A master thesis, conservative department, university of Baghdad, 2010.
19. Paque´ F, Sirtes G. Apical sealing ability of Resilon/Epiphany versus gutta-percha/AH Plus: immediate and 16-months leakage. Int Endod J 2007; 40: 722–729.
20. De Moor RJ, De Boever JG. The sealing ability of an epoxy resin root canal sealer used with five gutta-percha filling techniques. Endod Dent Traumatol 2000; 16: 291–297.
21. Holland R, Murata S, Barbosa H, Garlippo, Sousa V. Apical seal of root canals with gutta-percha points with calcium hydroxide. Braz Dent J. 2004; 15(1): 26-29.
22. Al Bakri MM. Comparative study of apical microleakage by using different preparation and obturation techniques. A master thesis, conservative department, university of Baghdad, 2009.
23. Skidmore LJ, Berzins DW, Bahcall JK. An In Vitro Comparison of the Intraradicular Dentin Bond Strength of Resilon and Gutta-Percha. JOE 2006; 32: 963–966.
24. Dultra F, Barroso JM, Carrasco LD, Capelli A, Guerisoli DMZ, Pécora JD. Evaluation of apical microleakage of teeth sealed with four different root canal sealers. J Appl Oral Sci 2006; 14(5): 341-345.
25. Mutal L, Gani O. Presence of pores and vacuoles in set endodontic sealers. Int Endod J. 2005; 38: 690–696.
26. Saatchi M, Rabie H. The Apical Sealing Ability of AH26, AH Plus and ZOE Root Canal Sealers. Dent Res J 2005; 2: 12-16.
27. Pentron (manual) Epiphany. Soft Resin Endodontic Obturation System. Wallingford, CT: Pentron Clinical Technologies, LLC; 2007.
28. Teixeira FB, Trope M. Gutta-percha the end of era. Journal of alpha omegan 2004; 97(4): 66-72.

29. Ruddle CJ. The ProTaper technique. Endodontic Topics 2005; 10: 187–190.
30. Roy CO, Jeansonne BG, Gerrets TF. Effect of an acid environment on leakage of root-end filling materials. JOE 2001; 27: 7–8.
31. Muhammed redha HA. The effect of an acid environment on the microleakage of different root end filling materials. A master thesis, conservative department, university of Baghdad, 2008.
32. Resende LM, Rached-Junior FJA, Versiani MA, Souza-Gabriel AE, Miranda CES, Silva-Sousa YTC, Sousa Neto MD. A comparative study of physicochemical properties of AH Plus, Epiphany, and Epiphany SE root canal sealers. Int Endod J 2009; 42: 785–793.
33. Bodrumlu E, Tunga U. Apical leakage of Resilon obturation material. J Contemp Dent Pract. 2006; 7(4):45-52.
34. Aptekar A, Ginnan K. Comparative analysis of microleakage and seal for 2 obturation materials: Resilon/Epiphany and gutta-percha. J Can Dent Assoc 2006; 72(3):245-245d.
35. Shipper G, Ørstavik D, Teixeira FB, Trope M. An Evaluation of Microbial Leakage in Roots Filled with a thermoplastic Synthetic Polymer-Based Root Canal Filling Material (Resilon). JOE 2004; 30(5): 342-347.
36. Shipper G, Teixeira FB, Arnold RR, Trope M. Periapical inflammation after coronal microbial inoculation of dog roots filled with Gutta-percha or Resilon. JOE 2005; 31(2): 91–96.
37. Stratton RK, Apicella MJ, Mines P. A fluid filtration comparison of gutta-percha versus Resilon, a new soft resin endodontic obturation system. JOE 2006; 32(7): 642-645.
38. Kqiku L, Städtler P, Gruber HJ, Baraba A, Anic I, Miletic I. Active versus passive microleakage of Resilon/Epiphany andgutta-percha/AH Plusa. Aust Endod J 2010; 10: 1-6.
39. Bodrumlu E, Tunga U. Coronal sealing ability of a new root canal filling material. J Can Dent Assoc 2007; 73(7):623-623c
40. Bouillaguet S, Shaw L, Barthelemy J, Krejci I, Wataha JC. Long-term sealing ability of Pulp Canal Sealer, AH-Plus, GuttaFlow and Epiphany. Int Endod J 2008; 41: 219–226.
41. Shemesh H, Wu M-K, Wesselink PR. Leakage along apical root fillings with and without smear layer using two different leakage models: a two-month longitudinal ex vivo study. Int Endod J 2006; 39: 968-976.
42. Biggs, SG, Knowles KI, IbarrolaJL, Pashley DH. An In Vitro Assessment of the Sealing Ability of Resilon/Epiphany Using Fluid Filtration. JOE 2006; 32: 759-761.
43. Olivera ACM, Tanomaru JMG, Faria-Junior N, Tanomaru-Filho M. Bacterial leakage in root canals filled with conventional and MTA-based sealers. Int Endod J 2011; 44: 370–375.
44. Çobankara FK, Adanir N. Belli S. Evaluation of the influence of smear layer on the apical and coronal sealing ability of two sealers. JOE 2004; 30: 406-409.
45. Tay FR, Pashley DH, Williams MC, Raina R, Loushine RJ, Weller RM, Kimbrough F, King NM, Susceptibility of a Polycaprolactone-Based Root Canal Filling Material to Degradation. I. Alkaline Hydrolysis. JOE 2005b; 31(8): 593-595.
46. Anbu R, Nandini S. Velmurugan N. Volumetric analysis of root fillings using spiral computed tomography: an in vitro study. Inter Endod J 2010; 43: 64–68.
47. Gençoğlu N. Comparison of 6 different gutta-percha techniques (part II): Thermafil, JS Quick-Fill, Soft Core, Microseal, System B and lateral condensation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 9: 91-95.

Effect of thermocycling on some mechanical properties of polyamide hypoallergenic denture base material (comparative study)

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ABSTRACT

Background: Hypoallergenic denture base material became recently the most attractive option due to their use as alternative to poly methyl methacrylate in hypersensitive patients. The study of the effects of thermocycling on the mechanical properties is very important, as it is beneficial for clinical purposes.

Materials and methods: One hundred and sixty specimens were prepared according to manufacturer's instructions and they were divided into two groups: Valplast and Vertex as a control group (eighty specimens for each), twenty specimens from each material were used to test each of property. They were either thermocycled or not thermocycled (n = 10).

Results: There was significant difference between polyamide and conventional heat cured acrylic in the four tested properties. Furthermore, thermocycling significantly decreased the flexural strength of both polyamide and the heat cured acrylic and it significantly increased the tensile strength and hardness of both tested materials. Thermocycling did not significantly affect the impact strength of both materials.

Conclusions: Vertex showed higher values of flexural strength than Valplast, flexural strength of both materials decreased post-thermocycling. Although the flexural strength of valplast was relatively low, it demonstrated greater impact strength than Vertex, impact strength of both tested materials was not affected by thermocycling. The tensile strength of Vertex was more than Valplast, for both materials tensile strength increased after thermocycling. The hardness of Vertex was higher than that of Valplast, both materials' hardness increased after thermocycling.

Keywords: polyamide, thermocycling, flexural, impact, tensile strength, and hardness. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):25-30).

INTRODUCTION

Poly methyl methacrylate (PMMA) resin has been widely used as a denture base material due to its desirable properties of excellent esthetics, ability to repair and simple processing technique [1,2]

Poly methyl methacrylate denture base has dominated the market for more than 50 years [3]. Conversely some disadvantages have also been described, hypersensitivity to methyl methacrylate and allergic reaction to residual monomer have been reported [4,5]. Due to the general increase in patients with allergy, dentists are confronted with more patients with allergic reaction to the classic PMMA denture base material [6,7]. To overcome the allergy problem, other denture base materials, including methyl methacrylate (MMA) free materials, have been introduced. The PMMA has presumably been replaced by hypoallergenic denture base materials [8].

The recent developments in the field of science of dental materials and polymer technology enabled us to overcome some of the drawbacks of PMMA by improvement and development of newer and more novel forms of denture base resins. Hypoallergenic denture resin is one such invention [9].

Some new types of hypoallergenic denture base resin, such as polyamide (Pa) which is composed of monomers of higher molecular weight than methyl methacrylate or oligomers [10]. These monomers are trapped into the polymer structure and their release should be minimal [11].

Since the Pa is still at an experimental stage it was felt that an evaluation of its mechanical properties may facilitate its further refinement. With regard to PA denture base resins, previous researchers such as [2,10-15] studied some physical and mechanical properties (like flexural strength, modulus of elasticity, colour stability, solubility, sorption, surface roughness and impact strength). Despite these studies, little is known about the mechanical properties of Pa after thermocycling as one of artificial ageing process. The effect of thermal shock on the mechanical properties of injection molded thermoplastic denture base resins is beneficial for clinical purposes, but presently, there is insufficient information about it.

During mastication, the oral cavity gets in contact with food at different temperatures. The most critical effect of temperature is due to chewing of hot food and drinking of cold fluid, this temperature changes may affect the mechanical properties of denture base, so Dootz et al [16] and Hekimoglu and Anil [17] have shown

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that material ageing can dramatically affect the physical and mechanical properties.

Mechanical properties are very important to be measured after ageing process because acrylic resin removable dentures are susceptible to fracture after periods of clinical use. In a survey prevalence of fractured dentures found that 68% of dentures were broken within 3 years of their fabrication [18]. Information about the mechanical properties of acrylic materials could help in the understanding and improvement of denture fractures [19].

MATERIALS AND METHODS

One hundred and sixty specimens (eighty specimens for each material) were prepared. Forty specimens from each material were subjected to thermocycling as an artificial ageing process, and forty specimens from each material were not subjected to thermocycling as control group. Forty specimens used for the following tests (flexural strength, impact strength, tensile strength and surface hardness) ten specimens for Pa and ten specimens for heat cured acrylic resin (Hca) were evaluated for differences in means for each test with and without thermocycling.

Plastic patterns were prepared with the following dimensions:

A- Specimens for flexural strength test: Rectangular shape, (65x10x2.5mm), the flexural strength test is a part of ANSI/ADA specification no.12 (ISO 1567) for denture base resin.

B- Specimens for impact strength test: Rectangular shape, (80x10x4mm), for charpy impact strength, ISO standard 179-1:2000 were followed.

C- Specimens for tensile strength test: Flat dumbbell shape, (16±1mm length, 3±0.2mm width, and 2±0.2mm thickness at the parallel segments). The tensile strength test was conducted according to ISO 527:1993.

D- Specimens for surface hardness: Disc shape, 25mm in diameter and 2mm in thickness according to ANSI/ADA specification no.17.

Preparation for Hca Specimens

The plastic patterns were inserted in the lower half of flask, care was taken that only one half of the pattern thickness was embedded in the stone, where the plastic patterns were placed, sufficient distance between them and also from the walls of the flask was kept, after setting of the stone the patterns and the stone were painted with separating medium and the counter part of the flask was then assembled and another mix of dental stone was poured to complete flasking, the flask was opened and the plastic patterns were removed carefully, and the stone molds were ready for packing. The polymer/monomer in ratio of 2.3g/1ml according to manufacturer's recommendation was thoroughly mixed. Once the mixture reached the dough stage, it was kneaded thoroughly to make homogeneous dough.

The dough was then packed into the mold with slow pressure, final closure was done under a hydraulic press at (100 KPs/cm²) to ensure even flow of the material within the mold. After the final closure, the flask was left in the clamp for 30 minutes at room temperature to allow proper penetration of the monomer into the polymer beads, even flow of the material, and outward flow of excess material **[20].**

Curing was carried out by placing the clamped flask in the water bath and processed by short curing cycle (90 minutes at 74ºC followed by 30 minutes at 100ºC) according to (ADA specification No.12: 1999) for curing acrylic denture base material. Then the flask was left on bench to cool slowly before deflasking, and then the specimens were removed from the mold. Any specimens had faults or defects should be discarded, then finishing and polishing of HCA specimens as usual manner.

Preparation for Pa Specimens

The lower half of flask was prepared as for heat cured acrylic specimens, but a wax sprues were fabricated onto the plastic patterns before filling the upper half with stone. After setting of stone of upper half, wax elimination was done in the boiling water bath for 5 minutes, according to manufacturer's recommendations for Pa specimens. The flask was opened for removing the plastic patterns and let the stone mold to dry to get rid of moisture.

Capsule was placed in cylindrical metal sleeve and this sleeve was placed in the metal ring heater of the injection machine for 12 min., and when the temperature reached 288ºC the ring alarmed that the Pa was ready to be injected under pressure 1.0 MPa**. [14]**. Meanwhile, the Pa flask was closed and screwed tightly in its specially designed clamp and placed in hot oven at 75ºC for 12 min. All these steps carried out according to manufacturer's recommendations. Once the ring was heard, screwing of the press arm quickly till the spring was completely compressed, the Pa flowed into the mold, the pressure was kept for 15 seconds, then relieved and clamped flask was taken out of the machine and left to cool at the bench before deflasking, then the samples finished and polished as manufacturer recommended.

Thermocycling of the Specimens

Thermocycling was carried out by soaking the specimens alternatively into (5ºC and 55ºC) ±2ºC water bath chambers with 14 sec. dwelling time at each temperature and 1 sec. transition time.

The specimens were submitted to thermocycling for continuous 30 hrs, in 1hr the specimens were submitted to 120 cycles and this effectively ensured that each specimen was exposed to 3600 cycles.

Mechanical Tests

The specimens were tested for transverse strength with a three point bending test using the universal testing machine at 50mm span length.

Charpy impact tester machine (pendulum) was used. The bar of the material was supported as a beam and struck in the middle with weighted swinging pendulum.

An instron testing machine was used to measure the tensile strength of the specimens. All specimens were placed under tension until failure in a unilateral testing machine at a cross-head speed of 1mm/min. for acrylic and 25 mm/min. for polyamide until fracture [14].

Surface hardness testing was conducted by using stainless steel cone indenter of (5 mm in diameter) which attached to universal testing machine and subjected to 123 N, the indenter was remained in contact with each specimen tested for a fixed time of 30 seconds that made indentation [21], after that it was removed and the indentation diameter was immediately measured after each indentation by travelling microscope.

RESULTS

Flexural Strength Test

The results indicated very clear and highly significant difference between mean of the flexural strength for Hca and Pa both with and without thermocycling; the higher mean values were for Hca; both with and without thermocycling. The highest mean value was for Hca without thermocycling **(129.48 MPa.).** The lowest mean value was for Pa with thermocycling **(45.84 MPa.).** The flexural strength of Hca and Pa decreased after thermocycling. The t-test between different experimental groups indicated a highly significant difference between with and without thermocycling groups for both tested materials; table 1 and 2**.**

Table 1: t-test of flexural strength between with and without thermocycling groups for the same material

Mat	Thermo-cycling	Group difference		
		t-test	P-value	Sig.
Hca	without	11.49	P<0.01	HS
	with			
PA	without	4.71	P<0.01	HS
	with			

Table 2: t-test of flexural strength between Hca and Pa with and without thermocycling

Thermo-cycling	Mat	Group difference		
		t-test	P-value	Sig.
without	Hca	32.62	P<0.01	HS
	Pa			
with	Hca	24.98	P<0.01	HS
	Pa			

Impact Strength Test

The results indicated very clear and highly significant differences between means of the impact strength for Hca and Pa both with and without thermocycling; the higher mean value was for Pa; both with and without thermocycling. The highest mean value was for Pa with thermocycling **(41.00 KJ/m²).** The lowest mean value was for Hca without thermocycling **(9.33 KJ/m²)**. In both materials the impact strength was not significantly influenced by thermocycling. The t-test between different experimental groups indicated no significant difference between with and without thermocycling groups for both tested materials; table 3 and 4.

Table 3: t-test of impact strength between with and without thermocycling groups for the same material

Mat	Thermo-cycling	Group difference		
		t-test	P-value	Sig.
Hca	Without	-1.52	P>0.05	NS
	with			
Pa	without	-0.69	P>0.05	NS
	with			

Table 4: t-test of impact strength between Hca and Pa with and without thermocycling.

Thermo-cycling	Mat	Group difference		
		t-test	P-value	Sig.
Without	Hca	-17.20	P<0.01	HS
	Pa			
With	Hca	-22.73	P<0.01	HS
	Pa			

Tensile Strength Test

The results indicated very clear and highly significant differences between means of the tensile strength for Hca and Pa both with and without thermocycling; the higher mean value was for Hca; both with and without thermocycling. The highest mean value was for Hca with thermocycling **(67.06 MPa.)**. The lowest mean value was for Pa without thermocycling **(33.44 MPa.)**. In both materials the tensile strength increased after thermocycling.

The t-test between different experimental groups indicated a highly significant difference between with and without thermocycling group for both tested materials; table (5) and (6).

Table 5: t-test of tensile strength between with and without thermocycling groups for the same material

Mat	Thermo-cycling	Group difference		
		t-test	P-value	Sig.
Hca	without	-21.79	P<0.01	HS
	with			
Pa	without	-5.25	P<0.01	HS
	with			

Table 6: t-test of tensile strength between Hca and Pa with and without thermocycling.

Thermo-cycling	Mat	Group difference		
		t-test	P-value	Sig.
without	Hca	25.99	P<0.01	HS
	Pa			
with	Hca	36.31	P<0.01	HS
	Pa			

Brinell Hardness Test

The results indicated very clear and highly significant differences between means of the hardness for Hca and Pa both with and without thermocycling; the higher mean value was for Hca; both with and without thermocycling. The highest mean value was for Hca with thermocycling **(7.92 kg/mm²)**. The lowest mean value was for Pa without thermocycling **(3.35 kg/mm²)**. In both materials the hardness increased after thermocycling.

Table 7: t-test of Brinell hardness between with and without thermocycling groups for the same material

Mat	Thermo-cycling	Group difference		
		t-test	P-value	Sig.
Hca	Without	-8.79	P<0.01	HS
	With			
Pa	Without	-8.59	P<0.01	HS
	With			

The t-test between different experimental groups indicated a highly significant difference between with and without thermocycling groups for both tested materials (table 7, 8).

Table 8: t-test of Brinell hardness between Hca and Pa with and without thermocycling.

Thermo-cycling	Mat	Group difference		
		t-test	P-value	Sig.
without	Hca	20.79	P<0.01	HS
	Pa			
with	Hca	15.80	P<0.01	HS
	Pa			

DISCUSSION

Flexural Strength

The Hca flexural strength was significantly higher than that of Pa. The difference is related to the strength and numbers of primary and secondary bonds [22]. Furthermore, the low flexural strength of Pa might be related to its polymerization process during synthesis [22], which is condensation polymerization, while it is addition polymerization for heat cured acrylic [23,24]. O'Brien [22] explained the high flexural strength of Hca as it is cross linked, while Pa is not cross linked polymer, so the difference is related to the presence of cross linking agent in the polymer structure, although Powers and Sakaguchi [23] stated the cross linking agent has little effect on the transverse and hardness properties of polymers.

After thermocycling the flexural strength decreased in Hca, thermocycling and water immersion lead to leach out of plasticizers and acrylic material became brittle [25-27] this brittleness was clearly demonstrated in the fracture behavior of the specimens, all the specimens were broken with a sharp line fracture, exhibiting typical brittle fracture behavior that is characterized by lack of distortion of the broken parts [28].

Flexural strength of Pa was decreased after thermocycling. Thermal stress is created as a result of the varying amount of thermal expansions and contractions during thermocycling, this caused static fatigue which affected the Pa flexural properties [29].

Impact Strength

Pa showed significantly higher impact strength than Hca. Pa structure is based primarily on aliphatic chains [30]. The backbone of Pa is regular and symmetrical, so forms very good resistance to shock and repeating stress [31]. Porosities of Hca more than that of Pa denture base material, this comes from presence of residual monomer and its evaporation leads to formation of these porosities,

also the compression packing of Hca leads to more porosities than injection molding processing of Pa [32]. Pa contains flexible agents in its composition, so it absorbs more energy to fracture [10].

The impact strength of Hca was not significantly changed by thermocycling. Although thermocycling may lead to further polymerization, because the surface of specimens affected by this continuous polymerization more than the deep bulk of them, so this might explain why the hardness of Hca was increased but the impact strength was not affected [33].

The impact strength of Pa was not significantly affected by thermocycling; because the monomer of Pa is oligomer so it is trapped into the polymer structure, so its release into water would be minimal [10].

Tensile strength

The Hca tensile strength was significantly higher than that of Pa. This difference might be due to the difference between these two polymers in the process of polymerization [14,15]. The difference is also related to the strength and numbers of primary bonds [22]. the higher tensile strength of Hca could be attributed to its high molecular weight linear polymer molecules [34].

After thermocycling the tensile strength of Hca was significantly increased. The leaching out of the residual monomer, plasticizer could result in increasing tensile strength [35-38]. Further polymerization may occur in the acrylic specimens submitted to thermocycling [33].

After thermocycling the tensile strength of Pa was increased. The complete polymerization of Pa specimens submitted to heat energy during the experiment is probably the reason [10].

Indentation Hardness

The Hca hardness was significantly higher than that of Pa, Goiato et al [10] explained that as the conventional resin based on poly methyl methacrylate does not have flexible agents in its composition, so it has more resistance to penetration of the indenter.

Hardness was increased in Hca after thermocycling. This might be due to further polymerization, by thermocycling [33,34,38]. Leach out of monomer and other soluble components by diffusion from the polymer [33,38,39]. Leach out of external plasticizers that resulting in the hardening of the Hca [40].

After thermocycling the hardness of Pa was significantly increased, this might be related to further polymerization and their submission to heat energy during the experiment [10].

REFERENCES

1. Vojdani M, Khaledi AR. Transverse strength of reinforced denture base resin with metal wire and E-glass fibers. J Dent Tehran University of Medical Sciences 2006; 3(4): 167-72.
2. Menaka AA, Suman B, Nancy D, Billy BK, Priscilla LU, Nick P, Dharshan S, Vinh TT. Evaluating surface roughness of a polyamide denture base material in comparison with poly methyl methacrylate. J Oral Sci 2010; 52(4): 577-81.
3. Price CA. A history of dental polymers. Aust Prosthodont J 1994; 8: 47-54.
4. Jorge JH, Giampaolo ET, Machado AL, Vergani CE. Cytotoxicity of denture base acrylic resins: A literature review. J Prosthet Dent 2003; 90(2): 190-3.
5. Leggat PA, Kedjarune U. Toxicity of methyl methacrylate in dentistry. Int Dent J 2003; 53(3): 126-31.
6. Alanko K, Kanerva L, Jolanki R, Kannas L, Estlander T. Oral mucosal diseases investigated by patch testing with dental screening series. Contact Dermatitis 1996; 34: 263-7.
7. Radzi Z, Abu Kasim NH, Yahya NA, Gan SN. Impact strength of an experimental polyurethane-based polymer. Annal Dent Univ Malaya 2007; 14: 46-51.
8. Pfeiffer P, Rosenbauer EU. Residual methyl methacrylate monomer, water sorption and water solubility of hypoallergenic denture base materials. J Prosthet Dent 2004; 92(1): 72-8.
9. Sheeba G, Arun KG. A comparative evaluation of flexural properties of flexible denture base material and compression molded heat polymerized denture base material: An in vitro study. Kerala Dent J 2010; 33(4): 213-5.
10. Goiato MC, Santos DM, Haddad MF, Pesqueira AA. Effect of accelerated ageing on the microhardness and color stability of flexible resins for dentures. Braz Oral Res 2010; 24(1): 114-9.
11. Kurtulmus H, Ovul K, Rahime TA, Aylin K, Hayal B, Onur O, Atilla U. Effects of saliva and nasal secretion on some physical properties of four different resin materials. Med Oral Patol Oral Cir Bucal 2010; 15(6): 969-75.
12. Pfeiffer P., Chrisian R., Lamia S.: Flexural strength and moduli of hypoallergenic denture base materials. J Prosthet Dent 2005; 93(4): 372-7.
13. Pusz A, Szymiczek M, Michalik K. Ageing process influence on mechanical properties of polyamide-glass composites applied in dentistry. Journal of Achievements in materials and manufacturing engineering 2010; 38(1): 49-55.
14. Yota T. Characteristics of denture thermoplastic resins for non metal clasp dentures. Dent Mater J 2010; 29(4): 353-61.
15. Hamanaka I, Takahashi Y, Shimizu H. Mechanical properties of injection-molded thermoplastic denture base resins. Acta Odontol Scand 2011; 69(2): 75-9.
16. Dootz ER, Koran A, Craig RG. Physical property comparison of 11 soft denture lining materials as a function of accelerated ageing. J Prosthet Dent 1993; 69(1): 114-9.
17. Hekimoglu C, Anil N. Sorption and solubility of soft denture liners after accelerated ageing. Am J Dent 1999; 12(1): 44-6.

18. Shah FK. Effect of joint surface contours on the transverse strength and impact resistance of denture base resin repaired by various methods: An in vitro study. A master thesis, Rajiv Gandhi University of Health Sciences, Karnataka, Bangalore, 2006.
19. Gurbuz O, Unalan F, Dikbas I. Comparison of the transverse strength of six acrylic denture resins. OHDMBSC 2010; 9(1): 21-4.
20. Rahn AO, Heartwell CM. Textbook of complete denture. 5th edition. USA: Lippincott Williams & Wilkins, 1993. p 379.
21. Sakaguchi RL, Powers JM. Craig's restorative dental materials 13th edition. USA: Mosby Inc., an affiliate of Elsevier Inc., 2012. p. 39, 90, 140.
22. O'Brien WJ. Dental materials and their selection 4th edition. USA: Quintessence publishing Co. Inc., 2008.
23. Powers JM, Sakaguchi RL. Craig's restorative dental materials 12th edition. USA: Mosby Elsevier, 2006. p. 38, 84, 153, 516, 517.
24. Tieng C. Effect of denture cleanser on the surface roughness and hardness of denture base material. A master thesis, Mahidol University, 2008.
25. Garcia RM, Leon BT, Oliveira VB, Del Bel Cury AA. Effect of a denture cleanser on weight, surface roughness and tensile bond strength of two resilient denture liners. J Prosthet Dent 2003; 89(5): 489-94.
26. Meng TR, Latta MA. Physical properties of four acrylic denture base resins. J Contemp Dent Pract 2005; 6(4): 93-100.
27. Elias CN, Henriques FQ. Effect of thermocycling on the tensile and shear bond strength of three soft liners to a denture base resin. J Appl Oral Sci 2007; 15(1): 18-23.
28. Rahamneh A. Impact strength of acrylic resin denture base material after the addition of different fibers. Pakistan Oral & Dental Journal 2009; 29(1): 181-3.
29. Takahashi Y, Hamanaka I, Shimizu H. Effect of thermal shock on mechanical properties of injection-molded thermoplastic denture base resins. Acta Odontol Scand 2011; Downloaded from informahealthcare.com by Hinari on 24/08/2011:1-6.
30. John J, Shivaputrappa AG, Shah I. Flexural strength of heat polymerized poly methyl methacrylate denture resin reinforced with glass, aramid or nylon fibers. J Prosthet Dent 2001; 86(4): 424-7.
31. Dogan OM, Bolayir G, Keskin S, Dogan A, Bek B. The evaluation of some flexural properties of a denture base resin reinforced with various esthetic fibers. J Mater Sci: Mater Med 2008; 19(6): 2343-9.
32. Banerjee R, Banerjee S, Prabhudesai PS, Bhide SV. Influence of the processing technique on the flexural fatigue strength of denture base resins: An in vitro investigation. Indian J Dent Res 2010; 21(3): 391-5.
33. Seo RS, Vergani CE, Giampaolo ET, Pavarina AC, Machado AL. Effect of post-polymerization treatments on the flexural strength and Vicker's hardness of reline and acrylic denture base resins. J Appl Oral Sci 2007; 15(6): 506-11.
34. Azevedo A, Machado AL, Vergani CE, Giampaolo ET, Pavarina AC. Hardness of denture base and hard chairside reline acrylic resins. J Appl Oral Sci 2005; 13(3): 291-5.
35. Vallittu PK, Miettinen V, Alakuijala P. Residual monomer content and its release into water from denture base materials. Dent Mater 1995; 11(6): 338-42.
36. Mello JA, Braun KO, Rached RN, Del Bell Cury AA. Reducing the negative effects of chemical polishing in acrylic resins by use of an additional cycle of polymerization. J Prosthet Dent 2003; 89(6): 598-602.
37. Urban VM, Machado AL, Oliveira RV, Vergani CE, Pavarina AC, Cass QB. Residual monomer of reline acrylic resins: Effect of water-bath and microwave post-polymerization treatments. Dent Mater 2007; 23(3): 363-8.
38. Neppelenbroek KH, Pavarina AC, Vergani CE, Giampaolo ET. Hardness of heat polymerized acrylic resins after disinfection and long term water immersion. J Prosthet Dent 2005; 93(2): 171-6.
39. Arima T, Murata H, Hamad T. The effects of cross-linking agents on the water sorption and solubility characteristics of denture base resin. J Oral Rehabil 1996; 23(7): 476-80.
40. Dhanraj M, Ariga P, Reddy T, Philip JM, Anand S. Comparative evaluation of tensile bond strength of heat polymerized permanent acrylic soft liner with various surface pre-treatments of denture base: An in vitro study. The Internet J Dent Sci 2011; 9(2).

Influence of high expansion dental stone and teeth on the adaptation of maxillary complete denture base

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ABSTRACT

Background: The aim of this study was to verify the influence of high expansion dental stone and teeth on the adaptation of maxillary complete dentures.

Materials and Methods: Maxillary complete dentures/bases were processed on type III dental stone and high expansion dental stone casts. The gap-space between the acrylic denture base and the cast in the posterior palatal seal area was measured by using dino-lite digital microscope. A comparison was made between G1 and G2, G3 and G4 to evaluate the influence of dental stone on the adaptation accuracy and another comparison between G1 and G3, G2 and G4 was made to evaluate the influence of teeth on the adaptation accuracy of maxillary complete denture.

Results: Statistical analysis of the data revealed that the fitness of maxillary complete denture base was significantly improved in some points with high expansion dental stone compared to dental stone type III and the presence of teeth reduced the gap-space in the posterior palatal seal area when dentures with teeth were compared with denture bases without teeth ($p<0.05$).

Conclusions: Using the high expansion dental stone and presence of teeth would influence the adaptation accuracy of denture base which in turn would improve the quality of the dentures.

Key words: High expansion dental stone, Gap-space, Adaptation accuracy. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):31-35).

INTRODUCTION

Since the introduction of acrylic resin as a denture base material in 1937, its use has become almost universal [(1)]. The dimensional changes that occur during the processing of these materials, however, still continue to concern the practitioner seeking an accurate denture base [(2,3)]. Dimensional changes during processing have been reported as resulting from the resin itself as well as the accompanying manipulative procedures [(1-5)].

Upon polymerizing, these poly (methyl methacrylate) resins exhibit a 0.2% to 0.5% linear polymerization shrinkage. A similar degree of linear expansion should occur because of water sorption [(6)]. Therefore, it would appear that these two opposing processes would balance each other and would result in an accurately fitting base. However, clinical experience and research findings indicate that this does not occur. Linear shrinkage is actually greater than linear expansion [(7)]. The expansion of dentures after storage in water at room temperature for one week failed to compensate for the initial processing shrinkage [(8)]. The dentures remained dimensionally stable even after storage in water for eight months [(9)].

A long-established method for denture processing for acrylic polymer is a closed-flask compressing molding with heat activation in a water bath for resin polymerization [(10)].

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However, polymerization shrinkage of the resin and distortion of the denture base due to thermal stress is virtually unavoidable during the processing of dentures.

These adverse effects cause movement of the artificial teeth position and increase the gap between the denture base and underlying mucosa, resulting in an ill-fitting denture [(11)]. It was suggested that an accurate fitting is a key factor in the physical mechanisms of complete denture retention [(12)].

The current study was carried out to assess the effect of the high expansion dental stone (dental stone type V) and teeth on the adaptation accuracy of the maxillary denture base at the posterior palatal seal area.

MATERIAL AND METHODS

Silicon duplication material (16), dental stone type III, high expansion dental stone (dental stone type V), dental plaster, heat cure acrylic resin powder and liquid, bioacryl plates (2mm thickness), articulator (Hanau), biostar machine, electronic scale, flask, clamp, hydraulic press, vibrator, water bath, cutting saw device and Dino-Lite digital microscope, were some of the material used in this study.

Sample grouping:

The study involved 40 samples (maxillary complete denture/base) grouped as following:

Group 1 (G1): denture base without teeth processed on type III dental stone cast.

Group 2 (G2): denture base without teeth processed on type V dental stone cast.

Group 3 (G3): denture base with teeth processed on type III dental stone cast.

Group 4 (G4): denture base with teeth processed on type V dental stone cast.

Each group included 10 samples.

Maxillary cast mold preparation:

A maxillary edentulous cast of medium depth palate was sealed on the base of a container which was already placed on the vibrator. The silicon duplication material was mixed (1:1) and poured gradually in 45° angle into the container to avoid air bubble entrapment. After complete setting of the silicone, the mold was removed from the container. This mold was used to pour 40 casts; 20 casts poured with type III dental stone while the other 20 casts poured with type V dental stone.

Conventional denture mold preparation (denture base with teeth):

A cast with its record base which was made from a biostar sheet was used to construct the occlusion rim. Both width of the occluding surfaces and the contour of the arch form of the occlusion rim were established to facilitate the arrangement of the artificial teeth. The maxillary cast along with its occlusion rim was mounted on the articulator and a plate was used to assemble the lower occlusion rim. Once the cast was mounted on the articulator, the teeth were set on the occlusion rim which provided reliable guides for the placement of the teeth. After completing teeth arrangement, wax sprues were made and waxed on each side on the maxillary tuberosities. Then the cast was glued to the base of a container which was already on the vibrator; the biostar denture base was sealed to the cast by waxing. Silicone duplication material was mixed (1:1) and poured gradually in 45° angle into the container which was already placed on the vibrator. After complete set, the set mold was removed from the container, the cast and the wax sprues were removed as well and a cutter was used to remove the excess of the duplication material.

Duplication of maxillary complete waxed denture (with teeth):

Wax sheet was cut into pieces and heated by using burner; meanwhile, artificial teeth were inserted into their holes in the mold and the cast was adapted onto the mold. The wax was poured from one hole until the wax was spilled out from the other hole. After waiting for an hour, the cast was removed from the mold. The sprues were removed and carved. Group 3 and 4 were duplicated by using this mold.

Record base preparation:

The record base was prepared by using a biostar machine. This was done to all samples in both, group 1 and 2, to ensure even thickness for the samples.

Flask preparation and wax elimination:

The cast with the record base was flasked in the lower part of a traditional brass flask with (50/50) plaster/stone mixture [13]. A separating medium was applied to the investment and allowed to dry, and then the upper part of the flask was assembled and filled with the same plaster/stone mixture ratio. After 1 hour, the flask was placed in boiling water for 10 minutes for wax elimination. The flask parts were separated, the record bases were removed, and a layer of separating medium of fixed volume about 1 ml was applied on the surface of the investment material and cast.

Packing and curing the acrylic:

Poly (methyl methacrylate) dough was used for packing with a monomer/polymer ratio of (1:3) by volume according to the manufacturer instruction. Each two flasks were placed in a clamp after a final pressing in the hydraulic press under the load of 100 Bar for 5 minutes. The flasks were immersed in water at room temperature, heated up to 100 ºC, left in the boiling water for 30 minutes according to the manufacturer instructions.

De-flasking and Sectioning:

The flasks were removed from the water bath and allowed to bench cool for 3 hours before the casts were de-flasked with their corresponding dentures on and sectioned. While de-flasking, great deal of attention was made to avoid separating the denture from its corresponding cast because once the denture is removed from the gypsum cast on which it has been cured, the denture, when it is replaced on it, will not fit it. The denture/base casts were transversely sectioned 2 mm posterior to the fovia palatinae [14]. After determining the cutting line, each denture/base-cast set was positioned in the sawing device. The cutting was made on a fixer table under constant water cooling. Then five points were marked on the section (midline point “M.P”, right 5mm from the midline point “R5mm”, right 10mm from the midline point “R10mm”, right crest ridge “R.C.R”, right marginal ridge “R.M.R”) in order to measure the gap-space at these points. The midline of each maxillary cast was determined by drawing a line from labial frenum & incisive papilla along the mid-palatine raphe to the posterior border of the cast.

Measurements were made on the right side of the midline.

Measurements:

Measurements were made immediately after sectioning of the casts by using a digital microscope (Dino-Lite digital microscope) with magnification power of 200x. The software of the microscope allows the investigator to take the measurements while observing the magnified objects. Three measurements were taken for each point & their mean was estimated.

RESULTS

T-test and P-value were used to evaluate the influence of high expansion dental stone and teeth on the adaptation accuracy of denture base.

Influence of dental stone on the adaptation of maxillary denture bases:

Table (1) shows that there is a significant statistical difference between G1 and G2 at M.P as ($P < 0.05$) while there are no significant differences in the other four points. Table (2) shows that there are significant statistical differences between G3 and G4 at M.P, R5mm, R.C.R as ($P < 0.05$) while there are no significant differences in the other points.

Influence of teeth on the adaptation of maxillary complete denture base:

Table (3) shows that there is a significant statistical difference between G1 and G3 at the R10mm as ($P < 0.05$) while there are no significant differences in the other four points.

Table (4) shows that there is a significant statistical difference between G2 and G4 at the R5mm as ($P < 0.05$) while there are no significant differences in the other points.

Figure (3) shows the Mean distribution of the measured gap-space between G 1 and G 3 samples. It is obvious that there is a significant difference between the means at R10mm. Figure (4) shows the Mean distribution of the measured gap-space between G 2 and G 4 samples. It is obvious that there is a significant difference between the means at R5mm.

DISCUSSION

Influence of dental stone on the adaptation accuracy of maxillary complete denture:

Dentures processed on high expansion high strength dental stone casts (group 2 and group 4) produced more adaptive dentures than those processed on type III dental stone casts (group 1 and group 3). Type III dental stone which was used in this study has setting expansion of only (0.07%). In contrast, the high expansion dental stone which was used in this study has a setting expansion of (0.18-0.20%). This study demonstrated that the high setting expansion of dental stone can help compensate for shrinkage that occurs as a result of thermal contraction of the acrylic resin material. The addition of different chemicals affects the setting expansion of gypsum products and also may change other properties. As the addition of sodium Chloride (NaCl) in a small concentration increases the setting expansion of the mass yet shortens the setting time [15].

Influence of teeth on the adaptation accuracy of maxillary complete denture:

Dentures processed with teeth (group 3 and group 4) produced more adaptive dentures than those processed without teeth (group 1 and group 2). This is probably due to the thickness of the denture base; the denture base in group 3 and group 4 was thicker than that in group 1 and group 2 at the buccal flange and the ridge because of the extra acrylic needed to attach the teeth to the denture. The thicker the denture base, the more the polymerization shrinkage but the higher the adaptation in the palatal seal area because the strain will be higher in that area since de-casting of the dentures from their casts was not required in this study so there was no release for the stress. According to this, the thicker denture supposes to have higher internal stress. However, the adaptation was not improved in both the right crest of the ridge (R.C.R) and right marginal ridge (R.M.R); figures (3) and (3) show that the gap is increased but this increase was statistically non-significant. This may be related to the anatomical form of that area (topographic form); acrylic in the marginal ridge is not as confined as the palatal area so the internal stress will be somewhat released there which will somehow increase the gap between the acrylic denture base and the cast.

The position of the denture in the flask may affect the adaptation since the direction and the amount of force applied on the acrylic may vary. This could be explained as the acrylic in the palatal area is subjected to higher forces than the crest & the marginal ridge areas due to the higher the investment column (pillar) upon the palatal area compared to the crest and the marginal ridge areas. This means that more force is subjected on the palatal area in comparison to the other areas.

The discrepancies recorded on the cast that take place during denture base fabrication are not uniform and depend on its location inside the flask, this agrees with the results of this study [16].

Also in this study, rapid curing polymerization cycle was used. This means that acrylic was cured

up to 100°C for 30 min. which probably increased the amount of residual monomer contents in the final denture bases. Several studies showed that the polymerization temperature and time considerably affected the residual monomer content of the denture base polymers [17].

REFERENCES

1. Craig RG. Restorative Dental Materials, 8th ed. St. Louis: Mosby Inc; 1989. p.509-559.
2. Baemmert RJ, Lang BR, Barco MT, Billy EJ. The effect of denture teeth on the dimensional accuracy of acrylic resin denture bases. Int J Prosthodont 1990; 3:528-37.
3. Jackson AD, Grisius RJ, Fenster RK, Lang BR. The dimensional accuracy of two denture base processing methods. Int J Prosthodont 1989; 2:421-8.
4. Woelfel JB, Paffenbarger GC, Sweeney WT. Dimensional changes occurring in dentures during processing. J Am Dent Assoc 1960; 61:413-30.
5. Takamata T, Setcos JC, Phillips RW, Boone ME. Adaptation of acrylic resin dentures as influenced by the activation mode of polymerization. J Am Dent Assoc 1989; 119: 271-6.
6. Phillips RW. Skinner's Science of Dental Materials, 8th ed. Philadelphia: WB Sanders Co; 1982.p.177-215.
7. Woelfel JB, Paffenbarger GC. Method of evaluating the clinical effect of warping a denture: Report of a case. J Am Dent Assoc 1959; 59:250-60.
8. Polyzois GL, Karkazis HC, Zissis AJ, Demetriou PP. Dimensional stability of dentures processed in boilable acrylic resins: A comparative study. J Prosthet Dent 1987; 57:639-47.
9. Anthony D, Peyton F. Dimensional accuracy of various denture-base materials. J Prosthet Dent 1962; 12:67-81.
10. Takamata T, Setcos JC. Resin denture bases: Review of accuracy and methods of polymerization. Int J Prosthodont 1989; 2: 555-62.
11. Garfunkel E. Evaluation of dimensional changes in complete dentures processed by injection-pressing and the pack-and press technique. J Prosthet Dent 1983; 50: 757-61.
12. Chung-Jae Lee, Sung-Bem Bok, Ji-Young Bae, Hae-Hyoung Lee. Comparative adaptation accuracy of acrylic denture bases evaluated by two different methods, Dental Materials J 2010; 29(4): 411-7.
13. Lambe DJ, Samara R, Johnson A. Palatal discrepancies and Post dams. J Oral Rehab 2005; 32:188-92.
14. Sykora O, Sutow EJ. Improved fit of maxillary complete denture processed on high expansion stone casts. J Prosthet Dent 1997; 77:205-8.
15. Powers JM, Sakaguchi RL. Craig's restorative dental materials. 12th ed. St. Louis: CV Mosby; 2006. p. 315.
16. Wolfoardt JF, Jones PC, Fatti P. The influence of processing variables on dimensional changes of heat cured poly (methyl methacrylate). J Prosthet Dent 1986; 55 (4): 518-25.
17. Jorge JH, Giampaolo ET, Machado AL, Vergani CE. Cytotoxicity of denture base acrylic resin: A literature review. J Prosthet Dent 2003; 90(2): 190-3.

Table 1: t-test results between G1 & G2

G1 & G2	t-test	P-value	Sig.
M.PG1-M.PG2	2.787	0.021	S
R5mmG1-R5mmG2	2.123	0.063	NS
R10mmG1-R10mmG2	0.025	0.981	NS
R.C.RG1-R.C.RG2	2.045	0.071	NS
R.M.RG1-R.M.RG2	1.438	0.184	NS

Table 2: t-test results between G3 & G4

G3 & G4	t-test	P-value	Sig.
M.PG3-M.PG4	2.741	0.023	S
R5mmG3-R5mmG1	2.876	0.018	S
R10mmG3-R10mmG4	2.037	0.072	NS
R.C.RG3-R.C.RG4	2.429	0.038	S
R.M.RG3-R.M.RG4	1.909	0.089	NS

Table 3: t-test results of the influence of teeth on the adaptation of maxillary denture bases between G1 & G3

G1 & G3	t- test	P-value	Sig.
M.PG1-M.PG3	1.094	0.302	NS
R5mmG1-R5mmG3	0.765	0.464	NS
R10mmG1-R10mmG3	2.826	0.020	S
R.C.RG1-R.C.RG3	0.840	0.423	NS
R.M.RG1-R.M.RG3	0.817	0.435	NS

Table 4: t-test results of the influence of teeth on the adaptation of maxillary denture bases between G2 & G4

G2 & G4	t-test	P-value	Sig.
M.PG2-M.PG4	1.542	0.157	NS
R5mmG2-R5mmG4	2.317	0.046	S
R10mmG2-R10mmG4	1.470	0.176	NS
R.C.RG2-R.C.RG4	0.813	0.437	NS
R.M.RG2-R.M.RG4	1.158	0.276	NS

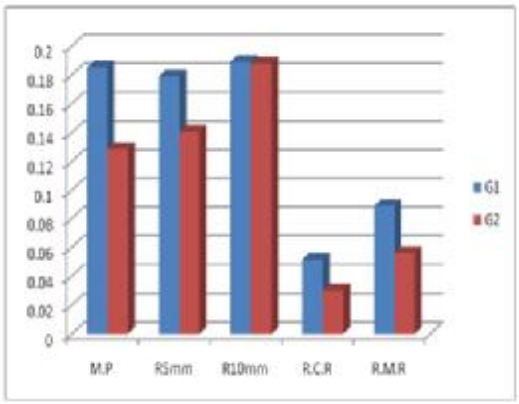

Figure 1: Mean distribution of the measured gap-space between G1 & G2

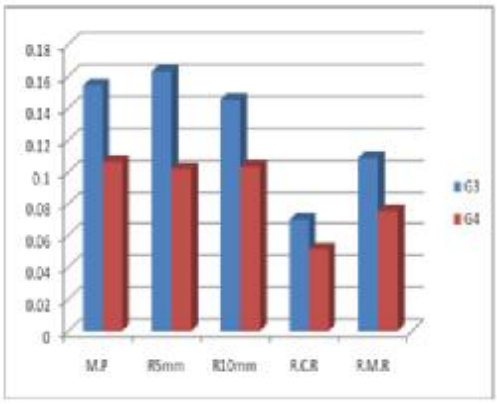

Figure 2: Mean distribution of the measured gap-space between G3 & G4

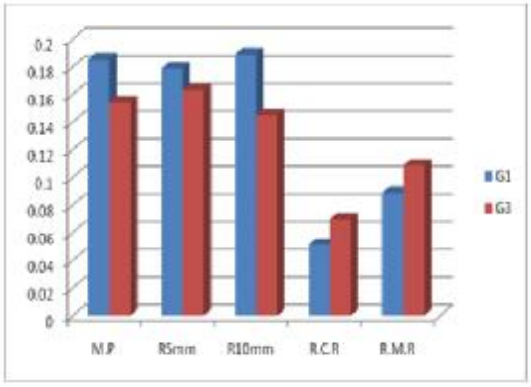

Figure 3: Mean distribution of the measured gap-space between G1 & G3

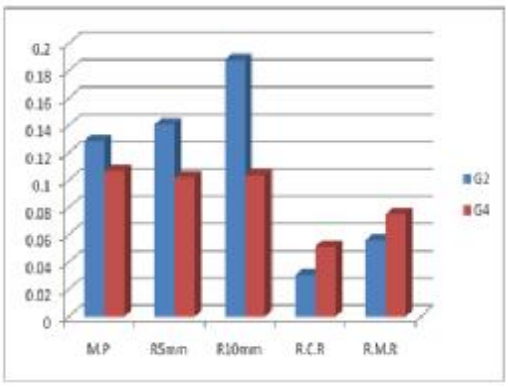

Figure 4: Mean distribution of the measured gap-space between G2 & G4

Assessment of zirconium oxide nano-fillers incorporation and silanation on impact, tensile strength and color alteration of heat polymerized acrylic resin

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ABSTRACT

Background: The mechanical strength and color stability of poly methylmethacrylate (PMMA) remains far from ideal for maintaining the longevity of denture. The purpose of this study was to assess impact, tensile strength and color stability of heat polymerized denture base after addition of salinated and non-salinated zirconium oxide (ZrO_2) nano-fillers.

Materials and methods: zirconium oxide nanofillers were incorporated into (PMMA) denture base by free radical bulk polymerization. The nano-particales were Silanated by coating with a layer of trimethoxysilypropylmethacrylate (TMSPM) before dispersed and sonicated in monomer (MMA) in two percentages of 3% and 5% by weight. Then it was mixed with acrylic powder as general conventional method. One hundred and seventy five specimens were prepared for this study. fifty bar shapes specimens (80mm x 10mm X 4mm) for impact strength test, another fifty dumbbell shaped specimens (75mm × 12.75mm × 2.5mm) were prepared for tensile strength test. On the other hand, seventy five disc shaped specimens (50mm diameter and 0.5mm thickness) were prepared for color stability test. They were divided into two groups according to the nanofillers used ; silanated and non-silanated ZrO_2 nanofillers, for each group Three subgroups were prepared (one control and two(3% and 5%) for the silanated and non-silanated ZrO_2 nano-fillers. Impact strength test was carried out with charpy type impact testing instrument. While tensile strength test were done by using Jain Qiao equipment system. . On the other hand, color stability was objectively assessed by using a spectrophotometer device after 48 hours immersion in three different solutions (distilled water, cola and tea) and the amount of light absorption was calculated. Impact and tensile strength means (in MPa) and color stability means (in nm) were analyzed statistically by analysis of variance and the comparative T-test and least significance test (LSD).

Result: Significant increase in impact strength occur in acrylic reinforced with 5% wt silanated (ZrO_2) nanofillers , but non significant increase was observed at 5% wt non-silanated (ZrO_2) nanofillers when compared with control group. On the other hand, there were non-significant improvements in the tensile strength for all groups of (ZrO_2) reinforcements. While for the color stability test data showed significant increase in the light absorptions for specimens reinforced with (ZrO_2) nanofillers when compared with control group. on the other hand, there were no significant difference observed between specimens reinforced with non-silanated and silanated (ZrO_2) nanofillers.

Conclusion: the findings of this study showed that Silanaized ZrO_2 nano-fillers is effective in improving impact strength while it was not effective in improving the tensile strength, the maximum increase in impact strength was observed in denture base nano composite containing 5% wt of silanated ZrO2 nano-fillers. On the other hand, significant color differences were detected between control group and specimens incorporated with zirconium oxide nano-fillers at different immersion solutions .

Key words: polymethylmethacrylate, nano composite, silanated, impact strength, color stability, spectrophotometers. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):36-42).

INTRODUCTION

Attention had been directed toward the incorporation of an inorganic nano-particle into PMMA to improve its properties. The properties of polymer nano-composites depend on the type of incorporated nano-particles, their size and shape, as well as the concentration and interaction with the polymer matrix.[(1)] Zirconium oxide nano-particles mechanically reinforce the polymers and allows for high impact strength, fracture toughness, hardness and density of the reinforced PMMA matrix.[(2)]

Surface modification of an inorganic particle with an organic substance is a useful way to reduce its surface energy and increase its compatibility with polymer matrix and dispersion homogeneity and thus improve the properties of the polymer/ inorganic particles. The non-competitive Nanoparticles were undergone surface treatment with saline coupling agent and embedded into PMMA.[(3)] Treating the surface of ZrO_2 nano-particles using trimethoxysilypropyl methacrylate (TMSPM) could eliminate aggregation of ZrO_2 particles and improve its compatibility with organic polymer.[(4)]

Dentures are subjected to a combination of compressive, tensile, shearing and tension loads. [(5)] but they also break due to sudden dropping of the prosthesis and as a result, fracture of the denture base can result.[(6)] The use of some kinds of

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inorganic fillers to reinforce the acrylic resin such as zirconium oxide could improve the impact strength.[7] Many attempts have been carried out to incorporate inorganic nano-particles into PMMA. For example, Alumina nano-particles which were coated with acryloxypropyldimethyl methoxy silane to get PMMA/alumina nano-composite with increased impact and tensile properties over pure PMMA. [8]

The change in the appearance and staining indicates reduction of the long term quality of the prosthesis.[9] The addition of non transparent fillers to the PMMA for the purpose of improving mechanical properties would induce increase in the amount of light absorption as measured by spectrophotometric device which detect the amount of staining on the prosthesis. [10] Studies have been carried out to confirm these results and to investigate whether color alteration which were not detected visually and detected by using apparatus because the human eyes is not sensitive like specific devices used.[11]

This study was conducted to evaluate effect of salinated and non-salinated (ZrO_2) nanofillers incorporation on impact, tensile strength and color alteration of heat cure PMMA.

MATERIALS AND METHOD

The materials that were used in the current study were illustrated in table 1.

Table 1: Some of the materials that were used in the study.

	Material	Trade	Manufacturer
1	**Zirconium oxide ZrO_2 (IV) nano-filler**	544760	Sigma-Aldrich Germany
2	**Trimethoxysilylpropyl methacrylate 98%**	Silane 440159	Sigma-Aldrich Germany
3	**Toluene**	solvents	GCC , U.K.
4	**Heat-curing resin for denture**	Super acryl plus	Spofa Dental Czechoslovakia

Silanation of (ZrO_2) fillers:

The introduction of reactive groups onto fillers surface was achieved by reaction of the 3-trimethoxysilyl propylmethacrylate (TMSPM) with zirconium oxide nano-fillers.

Addition procedure was done as follows:

30g of nano-fillers and 200 ml pure toluene solvent were placed into a glass flask then sonicated at ambient temperature of (37C°) for 20min. After that, the nano-filler and toluene were placed into a flask equipped with a magnetic stirrer (labinco, bv model l-81) at room temperature. Then, 1.5 gm of silane (5% wt to nano-filler) was added drop wisely by sterile syringe under rapid stirrer. The flask was covered by para-film and the slurry was left standing in the flask for 2 days. The toluene solvent was removed by rotary evaporator (RE 510, Yamato, Japan) under vacuum at 60°C and rotation of 150 rpm for 30 min. Then the silenated nano-filler was dried in vacuum oven (Gallen bamp, England) at 60C° for 20 hours. After that nano filler were stored at room temperature before use. [11]

Preparation of test specimens:

Mould preparation:

Three metal stainless steel patterns were used for the study. For impact strength test, a bar shaped specimen (80mm x 10mm X 4mm) length, width and thickness respectively was used. On the other hand, Dumbbell- Shaped specimens were prepared for tensile strength test with dimension given by **(ASTM specification D-638M, 1986)**. While for color alteration test, disc shaped specimens (50mm diameter and 0.5mm thickness) were also prepared as shown in figure1.

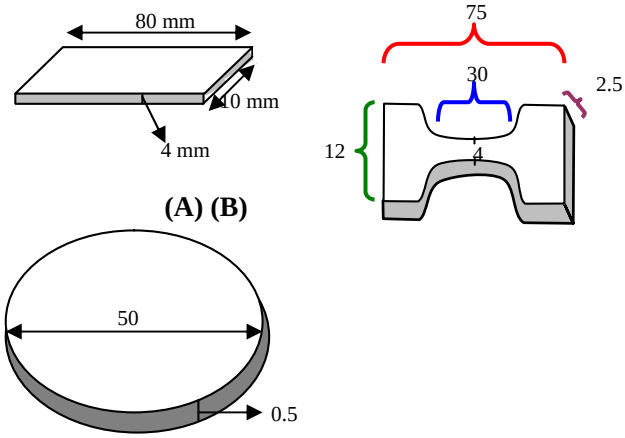


Figure 1: The test specimens and the dimension of each one: A and B for impact and tensile strength and C for color alteration test.

The metal patterns were coated with a thin layer of petroleum jelly before being invested. The flask base was prepared by using 120 gm of dental stone type III. Retention undercuts were placed in the stone for better retention of a second layer of dental stone type IV, where the metal patterns were invested. A new coat of petroleum jelly was applied before pouring the die stone and after that, a final pouring of 300 gm of dental stone type III was applied. After the complete set of the stone, the dental flasks were opened and the metal patterns were removed from the investing stone. The bar, dumbbell and disc cavities were used as a moulds for the packing of acrylic resin specimens.

Proportioning and mixing of the acrylic:

Addition of nano-fillers:

The incorporation of modified Zirconium oxide nano-filler powder to monomer was done by weight in two groups; 3% and 5% of silanated and Non silanated. An electronic balance (Sartorius BP 30155, Germany) with accuracy of (0.0001gm) was used. After the addition of ZrO_2 nano-filler to monomer, the fillers were well dispersed in the monomer by ultra sonication, using a probe sonication apparatus (Soniprep-150, England) at 120 W, 60 KHz for 3 minutes to break them into individual nano-crystals [12] as shown in the figure2.

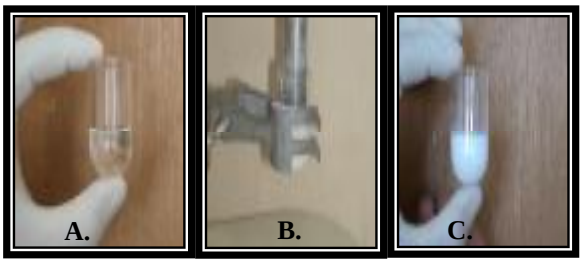


Figure 2: Nano-fillers were well dispersed in the monomer by ultrasonication as shown in A: before, B: during and C: after sonication.

The suspension of the monomer with ZrO_2 nano-filler was immediately mixed with acrylic powder to reduce the possibility of particle aggregation and phase separation. The proportion for mixing of acrylic resin was (12 gm: 6 ml) P/L ratio. All materials were mixed and manipulated according to manufacturer's instructions as illustrated in table 2. The mixing was carried out at once, in a clean and dry mixing vessel and mixed by a clean wax knife for 30 second. The mixture was then covered and left to stand until a dough stage was reached and then placed inside the mould.

Table 2: Percentages and amounts of polymer, monomer and zirconium oxide nano-filler powder.

ZrO_2 percentage	Amount of ZrO_2	Amountof polymer	Amount of monomer
0%	0	12g	6ml
3%	0.360g	11.640g	6ml
5%	0.600g	11.400g	6ml

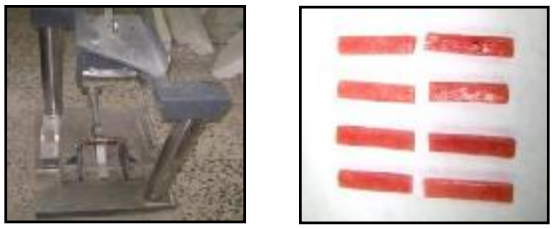
The flasks were immersed in water bath at 73C° for 90 minutes, raising the temperature to 100ºC and maintaining the boiling for 30 minute. Once the polymerization cycle was completed, the flasks were allowed to slow cooling in a water bath at room temperature before deflasking. The acrylic specimens were trimmed with a tungsten bur and ground wet to the final dimension with a silicon carbide abrasive papers. Pumice was used for final polishing. The specimens were immersed in water at 50ºC for 1 hour for excess residual monomer removal and stored in water at room temperature for 48 hours until the testing were performed[12].

A total of 175 specimens were fabricated for this study and they were subdivided as follows: 100 specimens were used for impact and tensile strength and these were subdivided into five subgroups in which each subgroup contains 10 specimens as follows: control, 3% & 5% silanated and 3% & 5% non silanated zirconium oxide nano-filler.

While for the assessment of color alteration 75 specimens were prepared in which the same subgroups contain 5 specimens and were subjected to the following soaking trials: 15 minutes for 3 times daily for 10 days (this trials simulates a 15 minutes daily soaking for 30 days) in three different solutions: distilled water, cola and tea and the immersion solutions were replaced every day[13].

Impact strength testing

Impact strength loading test was carried out using a charpy type impact testing instrument (Impact tester N. 43-1, INC. USA.). A pendulum of 2 joules testing capacity was used. The specimen was supported horizontally at its ends and struck by a free swinging pendulum which released from a fixed height in the middle. The charpy impact strength of unnotched specimen was calculated in KJ/m^2 using a formula of $IS=E/b.d\times10^3$ where IS the impact strength, E is the impact absorbed energy in joules, B and D is the width and thickness in millimeters of the test specimens respectively and this is shown in figure 3.



(A) (B)

Figure 3: A, the test specimens held horizontally, B. the test specimens after testing.

Tensile strength testing:

The tensile strength was estimated by using Jian Qiao testing equipment for measuring tensile strength. As illustrated in figure 4. The specimen was held at each two ends and the force at the failure was recorded in Newton (N) and the tensile strength values were calculated from the following equation:

$TS = \frac{F}{A}$ *where*

TS: tensile strength in Mpa.
F: Force at failure (Newton).
A: Minimum cross sectional area (mm2).

(A) **(B)**

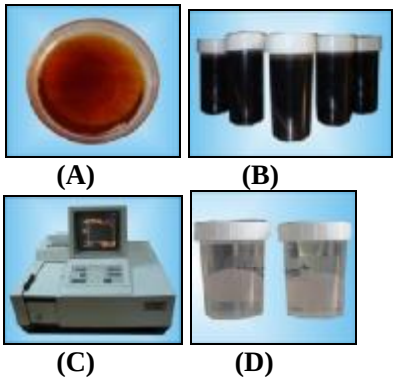
Figure 4: A, the testing device used to measure the tensile strength. B. the test specimens after failure.

Color alteration testing:

The control group of specimens and specimens of other subgroups were immersed in the following solutions: distilled water, cola and tea as shown in figure 5. After the completion of the immersion of the specimens of all groups and subgroups the light absorption of the discs were measured by using a spectrophotmetric device at the wave length of 500 nm and the amount of absorption of the light was measured in nm in order to detect the degree of specimens staining.

D. the spectrophotometric device.

Impact, tensile strength and color alteration results were analyzed statistically by analysis of variance (ANOVA), t-test and LSD test for multiple comparisons of the means. Significance level was set at a level of 5%.



(A) **(B)**

(C) **(D)**

Figure 4: test specimens immersed in A: tea, B: colla and C: distilled water.

For impact strength test:

The results of this study indicated that Silanaized ZrO_2 nano-fillers is effective in improving impact strength and the maximum improvements were showns in the subgroups which were reinforced with 5% silaned filler and this were illustrated in table 4.

RESULTS

Impact, tensile strength means in Mpa and color alteration mean in (nm) results were given in Table3.

Table 3: Means and standard deviations for the data for the impact, tensile and color alteration tests.

	Control	3%non silane	5% non silane	3% silane	5% silane
Impact strength					
Mean	8.7	8.775	9.2	9.2	**9.95**
SD	0.806	0.731	0.329	0.349	**0.329**
Tensile strength					
Mean	43.0	46.0	44.0	48.0	**47.0**
SD	2.15	2.3	2.2	2.4	**2.35**
Color alteration (distilled water)					
Mean	1.071	2.259	2.483	2.343	**2.444**
SD	0.083	0.048	0.003	0.042	**0.024**
Color alteration (cola)					
Mean	1.055	2.248	2.408	2.316	**2.331**
SD	0.102	0.018	0.045	0.021	**0.028**
Color alteration (tea)					
Mean	0.942	2.255	2.391	2.338	**2.381**
SD	**0.132**	**0.036**	**0.056**	**0.067**	**0.0007**

Table 4: t-test between control and experimental groups for impact strength test.

	t-test	P-value	Sig
Control&3%non silanated (ZrO_2)	**0.209**	**0.839**	**NS**
Control&5% non silanated (ZrO_2)	**1.957**	**0.083**	**NS**
Control&3% silanated (ZrO_2)	**1.917**	**0.088**	**NS**
Control&5% silanated (ZrO_2)	**4.841**	**0.001**	**S**

Table 5: t-test between experimental groups

	t-test	P-value	Sig
3%&5% non silanated (ZrO_2)	**2.08**	**0.049**	**S**
3%&5% silanated (ZrO_2)	**6.068**	**0.000**	**HS**
3% non silanated &3% silanated (ZrO_2)	**1.825**	**0.101**	**NS**
5% non silanated &5% silanated (ZrO_2)	**4.108**	**0.003**	**S**

P<0.001 high significant *P<0.05 Significant **P>0.05 Non significant

On the other hand the results indicated that significant improvements in the mount of impact strength for groups reinforced with silanated nanofillers when compared with same groups

without silane surface treatment as shown in table 5 and figure 5.

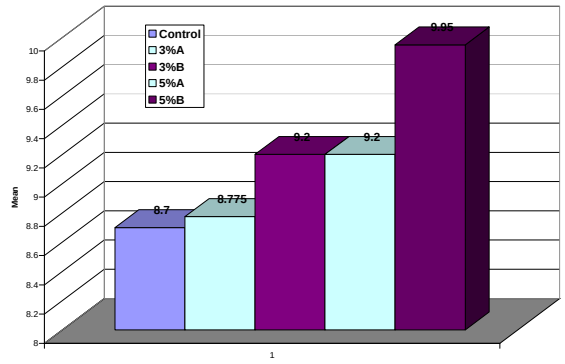


Figure 2: Bar chart of mean in Mpa of impact strength among studied groups.

For tensile strength test:

For the tensile strength data shows there were non significant improvement in the subgroups which were reinforced with both silanated and non silanated ZrO_2 nano fillers when compared with the control group.

On the other hand, there was non significant reduction in the tensile strength for groups reinforced with 5% ZrO_2 when compared with same groups incorporated with 3% ZrO_2 nanofillers. While the silane resulted in non significant improvements for the same groups as shown in table 6

Table 6: LSD test for groups and sub-groups tested for tensile strength.

Groups	Tensile strength	
	P	***Sig***
Control&3%	0.355	NS
Control&5%	0.098	NS
Contrl&3%S	0.125	NS
Control&5%S	0.520	NS
3%&5%	0.254	NS
3%&3%S	0.365	NS
3%&5%S	0.107	NS
5%&3%S	0.458	NS
5%&5%S	0.254	NS
3%S&5%S	0.089	NS

***P<0.05 Significant.**

For color alteration test:

The data indicated that there was significant increase in the light absorption for all groups and all immersion solutions when compared with the control group.

For the comparison between groups there was significant increase in the light absorption for groups with 5% ZrO_2 when compared with 3% for both silanated and non silanated groups and for all immersion solutions.

While for the effect of ZrO_2 salination there were significant increase for light absorption for 3% silanated and non silanated, on the other hand there was no significant reduction in the amount of light for 5% of the same groups as shown in table 7.

Table 7: LSD test for comparison of the control group and subgroups for color alteration test.

Groups	Distilled water		cola		tea	
	P	***Sig***	***P***	***Sig***	***P***	***Sig***
Control&3%	0.049	S	0.032	S	0.033	S
Control&5%	0.025	S	0.049	S	0.049	S
Contrl&3%S	0.049	S	0.044	S	0.040	S
Control&5%S	0.03	S	0.026	S	0.041	S
3%&5%	0.103	NS	0.176	NS	0.288	NS
3%&3%S	0.03	S	0.246	NS	0.160	NS
3%&5%S	0.049	S	0.049	S	0.127	NS
5%&3%S	0.145	NS	0.114	NS	0.682	NS
5%&5%S	0.302	NS	0.379	NS	0.858	NS
3%S&5%S	0.078	NS	0.742	NS	0.528	Ns

***P<0.05 Significant**
****P>0.05 Non significant**

The analysis of variance (ANOVA) table shows that there were no significant difference between the tested groups for the different immersion solutions except for the control and 5% silanated group as shown in table 8.

Table 8: ANOVA for comparison of control, distilled water, cola and tea for color alteration.

	F-test	P-value	Sig
Control	15.9	0.049	S
3%	0.031	0.969	NS
5%	4.69	0.175	NS
3%S	0.123	0.889	NS
5%S	9.19	0.049	S

***P<0.05 Significant**
****P>0.05 Non significant**

Table 9: ANOVA table for comparison of the different immersion solutions of tea, Pepsi and distilled water.

	F-test	P-value	Sig
Distilled water	30.64	P<0.01	HS
Cola	22.99	P<0.01	HS
Tea	14.25	P<0.01	HS

On the other hand, analysis of variance illustrated that there were high significant difference between the sub groups for the different immersion solutions as shown in table 9.

DISCUSSION

Zirconium oxide fillers (ZrO_2) were used because of their excellent biocompatibility and also for being white; so they are less likely to alter esthetic. Salinization of the nano-filler particles yields a better dispersion, eliminate aggregation and improve the compatibility with organic polymer. The addition of salinated ZrO_2 powder increased the value of the impact strength more than non-salinated ZrO_2 powder when compared with control group. 5% wt salinated nano-ZrO_2 group had the highest impact strength, the increase in impact strength of salinated groups (3%,5%wt) were due to the high interfacial shear strength between the nanofiller and resin matrix as a result of formation of cross-links or supra molecular bonding which cover or shield the nanofillers which in turn prevent propagation of crack. Also the crack propagation can be reduced by good bonding between nano-fillers and resin matrix. [14] so the salinated group 3%wt and non-salinated group 5%wt have the same impact strength, on the other side the slightly increase in impact strength that occur with addition of non-salinated fillers (3% and 5%wt) ZrO_2 particles may be due to good distribution of the very fine size of nano-particles which enable them to enter between the linear macromolecular chains of the polymer, so the segmental motions of the macromolecular chains are restricted lead to slightly improve impact strength [15].

Results demonstrated a non significant increase in tensile strength as the percentage of ZrO_2 fillers increased. There is slight reduction in tensile strength as the percentage of ZrO_2 nano fillers increased from 3% to 5% and could be due to a decrease in the cross section of load bearing polymer matrix, also incomplete wetting of the filler by resin and the presence of filler particles will form stress concentration and affecting the mode of crack propagating lead to reduction in tensile strength as volume of filler increased. [16, 17]

The data showed that the increase in light absorption is statistically significant; there was an increase in the relative amount of light absorption with the increasing of modified nano-ZrO_2 concentration. This is obviously due to the presence of opaque nano-ZrO_2 powder in the polymer matrix which absorbs more light energy than polymer matrix and appears more opaque. These findings were due to the high atomic number of Zr compared to the chemical constituent of acrylic which has low atomic number. The absorption of light energy by an element is dependent chiefly on the cube of its atomic number. [18]

On the other hand, the silane surface treatment of the groups incorporated with 5% ZrO_2 nano-fillers leads to non-significant reduction in light absorption when compared with the same groups without silanation which was attributed to that the surface area and dispersion of such nano-particles is much higher than that of non silanated particles particles. [19]

Color alterations were objectively measured using a spectrophotometer. However, the tested acrylic specimens did not show any noticeable color change with the addition of ZrO_2 nano fillers and the values which were measured by the spectrophotometer were not correspond to any clinical detectable color alteration. [20,21]

REFERENCES

1. Jordan J, Jacob KL, Shart MA. Experimental trends in polymer Nan composites-A review. Mater Sci Eng 2005; 393:1: 1-11.
2. Skukla S. and Seal S. Phase stabilization in nanocrystalline zirconia. Rev Adv Mater 2003; 5:117-20.
3. Shi J, Bao Y, Huang Z. Preparation of PMMA-nanomater calcium carbonate composites by in-situ emulsion polymerization. J zhejiang University Sci 2004; 56:709-13.
4. Erjun T, cheng G, pang X, Ma X, and Xing F. Synthesis of nano-ZnO2/PMMA composite micros sphere through emulsion polymerization and UV-Shielding property. J colloid and polymer science 2005; 284:4: 422-8.
5. Dhuru VB. Contemporary dental materials. Oxford University UK: 2003.
6. Zappini A, Kammann, Wachter W. Comparison of fracture tests of denture base materials. J Prosthet Dent 2003; 90: 578-85.
7. Chen SY, Liang W.M. Effects of fillers on fiber reinforced acrylic denture base resins. Mid Taiwan J 2004; 9: 203-10.
8. Singh D, Jayasimha T, Rai KN, Kumar A. Preparation of PMMA nanocomposite with superior impact strength. J Appl Polym Sci 2007; 105(6) 3183-94.
9. Sazbo' G, Valderhaug J, Ruyter I E. Some properties of a denture acrylic coating. Acta Odontol Scand 1985; 43:249-56.
10. Claussen A. Director Global Sc. And Information Management Intermediates". BASF, BTC specialty chemical distribution. 2010. [UK active member of the chemical business association].
11. Ayad NM, Badawi MF, Fatah AA .Effect of reinforcement of high impact acrylic resin with micro-Zirconia on some physical and mechanical properties. Rev Clin Pesq Odontol 2008;4(3):145-51.
12. Mohammed A, Solhi l, Nodehi A, Mirabedini SA, Akbari K, Babanzadeh S. PMMA-grafted nano clay as novel filler for dental adhesives. Dent Mater 2009; 25:339-47.
13. Sandra Sato, Marcia RS, Iara AO, Helena OP, Osvaldo Z. Assessment of flexural strength and color alteration of heat polymerized acrylic resins after simulated use of denture cleansers. Braz Dent J 2005; 16(2): 124-8.
14. Sun L, Ronald FG, Suhr J. Energy absorption capability of nano composites: A review. Compo Sci Tech 2009; 69: 2392-409.
15. Katsikis N, Franz Z, Anne H, Helmut M. Thermal stability of PMMA/ Silica nano- and micro composites as investigated by dynamic-mechanical experiments.

Polym Degrad Stabil 2007; 22:1966.
16. Craig RG, Power JM. Restorative dental material. 11th ed. St. Louis: Mosby; 2002. pp.50, 185-95.
17. Sehajpal SB, Sood VK. Effect of metal filler on some physical properties of acrylic resin, J Prosthet Dent 1989; (61) 4: 746-51.
18. Stafford GD, MacCulloch WT. Radiopaque Denture base material. Br Dent J 1971; 131:22.
19. Ricker A, Liu-Suyder P, Webster TJ. The influence of nano- MgO and nano-BaSo4 particle size additives on properties of PMMA bone cement. Inter J Nanomedicine 2008; 3(1) 125-32.
20. Cook WD, Chong MP. Color stability and visual perception of dimethacrylate based dental composite resins. Biomaterials 1985; 6: 257-64.
21. Kiat amnuay S, Bar bower M, Pera vina RD. The influence of pigments and opacifiers on color stability of silicone maxillofacial elastomers. J Dent 2009; 37: 45-50. **(IVSL).**

Evaluation of epidermal growth factor receptor (EGFR), proliferation (Ki-67) and apoptosis (P53) in salivary mucoepidermoid carcinoma in relation to tumor grade

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ABSTRACT

Background: Mucoepidermoid carcinoma (MEC) is the most common salivary gland malignancy. They display a variety of biological behaviours, and several systems have, therefore, been proposed to grade this neoplasm. Today, the most popular grading systems are Auclair et al, (1992) and Brandwein et al,(2001) grading systems. Assessment of cellular proliferation, apoptosis and growth molecules are important factors in tumor kinetic which may reflect tumor biological behaviour.

Materials and methods: Immunohistochemical analyses of seventeen cases of -fixed paraffin-embedded tissue blocks of MEC of salivary gland origin using (Ki-67, P53 and EGFR) monoclonal antibodies.

Results: The samples comprised of ten males and seven females to give male to female ratio (1.4:1). The mean age was (47.06±8.5) years. The submandibular salivary gland was the most predominant affected site (5 cases). 100% of cases were EGFR immunopositive. Only 47% of MEC cases showed Ki-67 immunopositivity, while P53 immunopositivity were shown in 94% of MEC cases. There was no statistically significant correlation regarding P53 or EGFR markers in relation with grading systems. There was a statistically significant correlation between the expression of Ki-67 marker and Auclair grading system. There were no significant statistical correlation among markers except between Ki-67 expression and P53.

Conclusions: Assessment of tumor biology in term of apoptosis (p53), proliferation (Ki-67) and EGFR are not reflected on tumor grade.

Key words: Mucoepidermoid carcinoma, EGFR. Ki-67, p53. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):43-47).

INTRODUCTION

Mucoepidermoid carcinoma (MEC) is one of the most common salivary gland malignancies. It demonstrates a wide age distribution with a mean of 45 years [1]. Histologically MEC is composed of a mixture of mucous, intermediate, and epidermoid cells, with columnar, clear cell and oncocytoid features [2].

Mucoepidermoid carcinoma grading systems have a long history of controversy over the best grading system use and which facilitates inferences in the prognosis of this neoplasm [3]. Different systems have been proposed with special characteristics for establishing tumor grade, which then require different types of treatment [4,5]. In their original report, Stewart and colleagues in 1945 defined benign and malignant varieties of mucoepidermoid tumors. Nonetheless, subsequent metastases of a few of the previously benign tumors has led to all mucoepidermoid tumors being considered carcinoma [6].

A three-level system of dividing tumors into low, intermediate, and high grades has widely been used [2]. Today, the most popular grading systems are Auclair et al. [4] and Brandwein et al. [5] grading systems. Both are point based, assigning point values to each adverse histologic parameters and with ascending point scores equating to a higher grade. However, the way in which each system correlates with outcome varies. The Auclair system appears to 'down grade' tumors while the Brandwein system appears to 'upgrade' tumors [7].

Epidermal growth factor receptor is the cell surface receptor for members of the epidermal growth factor family [8]; it is a member of the ErbB family of receptors. It plays an important role in the differentiation and morphogenesis of many organs and proliferation and survival in mammalian cells [9]. Mutations affecting EGFR expression or activity could lead to its constant activation which could result in uncontrolled cell division and cancer [10].

Antigen Ki-67 is a nuclear protein that is associated with and may be necessary for cellular proliferation [11]. The cellular appearance and location of this protein throughout the cell cycle is not homogeneous, during interphase, the Ki-67 antigen can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes [12].

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Apoptosis is a specific mode of cell death by which deletion of cells occurs [13]. P53 tumor suppressor gene is a transcription factor which regulates cell proliferation and apoptosis to prevent division of potentially malignant cells [14]. An alteration of the P53 gene is often observed in various human cancers [15].

MATERIALS AND METHOD

Seventeen formalin-fixed paraffin-embedded tissue blocks of mucoepidermoid carcinoma of salivary gland origin which were collected from laboratory archive of college of dentistry from the period between 1972 to 2011, in addition to cases from Al-Shaheed Ghazi Hospital/ Medical City/ Baghdad and private laboratories/ Baghdad included in this study.

Diagnosis was performed through examination of hematoxylin and eosin sections. Based on the criteria of the Auclair et al, and the criteria of Brandwein et al,. All MEC tissue samples were scored and graded using a quantitative grading systems based on their histological features, and samples were separated into three grades; low, intermediate and high according with these grading systems.

Immunostaining

Five micrometer thick sections were cut and mounted on (Bio care, USA) positively charged slides, then deparaffinized and rehydrated. For p53 and EGFR (US biological); the sections were immersed in 0.3% hydrogen peroxide (H2O2) to block the endogenous peroxidase activity, washed in phosphate-buffered saline (PBS), and then incubated in 10% normal serum to block any non-specific binding of antibodies. The tissue sections were incubated with monoclonal mouse anti-human p53 (diluted 1:60) and EGFR (diluted 1:50) antibodies over night at 37 ºC. The bounded antibodies were detected by the streptavidin-biotin complex method, after an immunoreaction, the sections were counterstained with Hematoxylin. For Ki-67 monoclonal antibody (Abcam) the same steps were done in addition to epiotope retrieving by citrate buffer solution pH 6.0 after blocking the endogenous peroxidase activity, the dilution of Ki-67 was 1:40.

Scoring system

The scoring of all markers was done by examining of at least 1000 cells per section in five different representative fields. The intensity of staining was not taken into consideration. For EGFR: (0) point for negative staining of the considered cells, (+) <10%, (++) 10-50%, (+++) 51-80%, and (++++) ≥81% positive staining of the considered cells [16]. For Ki-67 and p53: nuclear expression in ≤5% of tumor cells was scored as negative, 6-25% (+), 26-50% (++), and 51-100% (+++) [17].

Statistical analysis

The data were compiled into statistical software, statistical package of social sciences (SPSS) version 18. All variables were compared using Chi- square test. While Pearson correlation coefficient was applied to plot a correlation matrix among the different immunohistochemical markers expression values altogether. P values of less than 0.05 were considered significant.

RESULTS

The sample comprised of ten males and seven females to give males to females ratio (1.4:1). The mean age was (47.06±8.5) years (Table 1).

Table 1: Case distribution according to age groups

Age groups years	No.	%
30-39	3	17.65%
40-49	8	47.06%
50-59	4	23.53%
60-69	2	11.76%

According to site, the submandibular salivary gland was the most predominant affected site (5 cases) followed by parotid gland and buccal mucosa (4 cases for each), then palate (3 cases) and the lowest site was tongue (1 case).

According to Auclair grading system, 14 cases were found as low grade, non were intermediate and 3 cases were high grade. Brandwein grading system revealed 4 cases being as low grade, 7 intermediate and 6 cases were high grade. Concerning the site, sex and predominant cells, none of them had a significant statistical relationship with Auclair or Brandwein grading systems.

All cases (100%) were EGFR immunopositive in different scores (Figure 1). No statistical significant relationship was seen between this marker and both Auclair or Brandwein grading systems.

Only 47% of MEC cases showed Ki-67 immunopositivity (Figure 2). A significant statistical relationship with Auclair grading system in which all high grade cases were positive for this marker.

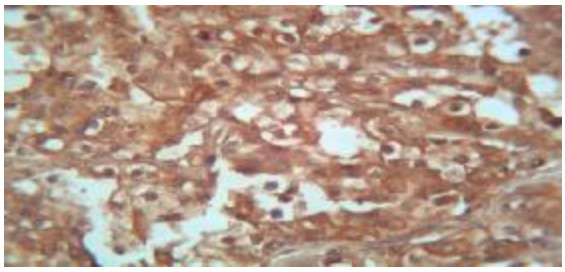

Figure 1: Photomicrograph revealed membranous EGFR expression (original magnification X400).

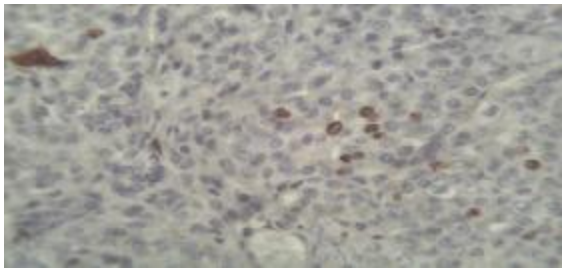

Figure 2: Photomicrograph revealed nuclear Ki-67 expression (original magnification X400).

P53 immunopositivity was seen in 94% of MEC cases (Figure 3), 75% of these positive cases showed low expression, it had no significant statistical relationship with the tumor grading systems.

Table 2: Description of statistics concerning the results of immunohistochemical findings in relation with Auclair grading system

Marker		Auclair grading system		
		low	high	Total
EGFR	_			
	+	1		**1**
	++	6	2	**8**
	+++	5	1	**6**
	++++	2		**2**
Chi square test P value=0.815 NS				
Ki-67	_	9		**9**
	+	4	1	**5**
	++	1	1	**2**
	+++		1	**1**
Chi square test P value=0.045 S				
P53	_	1		**1**
	+	10	2	**12**
	++	3		**3**
	+++		1	**1**
Chi square test P value=0.137 NS				

NS non significant S significant

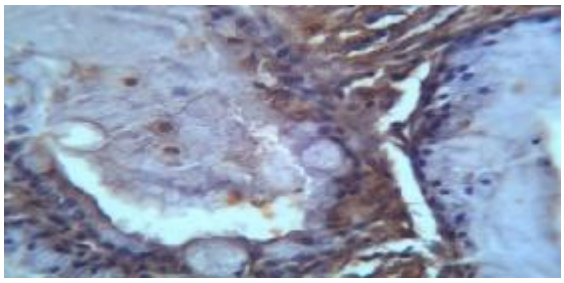

Figure 3: Photomicrograph revealed nuclear and focal cytoplasmic P53 expression (original magnification X400)

Table 3: Description of statistics concerning the results of immunohistochemical findings in relation with Brandwein grading system

Marker		Brandwein grading system			
		Low	Inter	High	Total
EGFR	_				
	+		1		**1**
	++	2	3	3	**8**
	+++	2	3	1	**6**
	++++			2	**2**
Chi square test P value=0.413 NS					
Ki-67	_	3	5	1	**9**
	+	1	2	2	**5**
	++			2	**2**
	+++			1	**1**
Chi square test P value=0.248 NS					
P53	_		1		**1**
	+	3	5	4	**12**
	++	1	1	1	**3**
	+++			1	**1**
Chi square test P value=0.750 NS					

NS non significant

There was no significant statistical correlation among markers except between Ki-67 expression and P53 expression.

Table 2 and 3 shows the relation between markers and Auclair and Brandwein grading systems respectively.

DISCUSSION

Mucoepidermoid carcinoma (MEC) is a malignant glandular epithelial neoplasm characterized by mucous, intermediate, and epidermoid cells. Grading of MEC is subjective with different criteria used in various series, and there is no universally accepted grading system [(2)].

Apoptosis is a pathway of cell death. P53 is a tumor suppressor and nuclear transcription factor [(8)]. An alteration of the p53 tumor suppressor gene is often observed in various human cancers [(15)]. In this study, 94% of cases were p53 positive and 75% of this positive cases were low expression, this finding is near the finding of Ehab et al, [(18)]

whom found that 80% of primary parotid MEC was p53 positive and 100% of recurrent cases were p53 positive, Jeanine et al, [19] study found p53 expressed in 75% of the MEC cases and had a weak expression, while Kiyoshima et al, [15] observed expression of p53 corresponding to 85% in MEC. The variation in the expression of p53 in this study and the aforementioned studies may be due to the use of different antibodies, different scoring systems, fixation times and concentrations of antibodies, and the sensitivity of the technique used. Only nuclear positive P53 immunoreactivity was considered in this study because p53 function depends on nuclear localization [14]. Occasionally in some cases nuclear and cytoplasmic staining was observed at the same time. No statistical significance found between P53 expression and either Auclair or Brandwein grading systems. This sign that the parameters used for tumor grading don't revealed the actual biological behavior of the MEC.

Ki-67 is a nuclear Ag, it is present throughout the complete cell cycle with the exception of early G1 phase [20]. In this study; (47%) of cases were Ki-67 positive. This finding is in agreement with the results of Brandwein et al, [5] who found no nuclear staining in 62% of the MECs evaluated, Alves et al, [17] study on 15 cases of MEC of the submandibular glands found that Ki-67 expression was 47% and was related to bad prognosis. Ki-67 positivity and negativity found in all grades. Because mucoepidermoid carcinomas are usually slow-growing tumors with proliferative rates lower than those observed in more aggressive carcinomas such as head and neck squamous-cell carcinomas. Therefore, it is not unexpected that low levels of Ki-67 may be observed. The relation of Ki-67 expression and Auclair grading system was statistically significant, this improves that Auclair grading system is down grading of MEC so only highly aggressive tumors are considered high grade.

The epidermal growth factor receptor is overexpressed in 80-100% of epithelial tumors of the head and neck [8]. In this study; all cases were immunopositive for EGFR, this result is disagreed with the results of Jeanine et al, [19] whom found 75% of MEC cases showed score 2 & 3 positivity. This variation may be due to using of another scoring system, for instance Jeanine et al, [19] considered score 1 as a negative. Only membranous positive EGFR immunoreactivity was considered in this study because it indicates the receptor site. Occasionally membranous and cytoplasmic staining was observed at the same time but membranous staining was always stronger. Similarly Sarkis et al, [9] observed membranous and cytoplasmic EGFR staining in oral squamous cell carcinoma. The cytoplasmic staining may represent cytoplasmic synthesis or breakdown of the protein [9]. There was no statistical significance found between EGFR expression and either Auclair or Brandwein grading systems. This sign that the parameters used for tumor grading don't revealed the actual biological behavior of the MEC. The assessment of tumor growth using growth fraction and apoptosis as biological markers of tumorigenesis in MEC namely EGFR and Ki-67 proliferative index and P53 apoptotic marker, were irrelevant to tumor grade, as the results were insignificant except Auclair grading system which was significantly correlated with proliferation. This is in accordance to the conclusion drown from a previous study of MEC done by Taher, 2011 [21] which assessed the metastatic behavior of the tumor, proven to be not significantly related to tumor grade.

REFERENCES

1. Cardesa A, Slootweg PJ Palma SD, Simpson RH, Skalova A, Leivo I. Major and Minor Salivary Glands. Pathology of the Head and Neck Springer-Verlag Berlin Heidelberg, 2006;132-64.
2. Gnepp D, Allen CM, Bouquot JE, Eveson J. iagnostic surgical pathology. 2nd edition, Saunders, an imprint of Elsevier Inc. USA, 2009.
3. Luna M, Batsakis A, El-Naggar JG. Salivary gland tumors in children. Ann Otol Rhinol Laryngol 1991; 100:869–71.
4. Auclair PL, Goode RK, GL. Mucoepidermoid carcinoma of intraoral salivary glands. Evaluation and application of grading criteria in 143 cases. Cancer 1992; 69:2021-30.
5. Brandwein MS, Ivanov K, Wallace DI. Mucoepidermoid carcinoma: A clinicopathologic study of 80 patients with special reference to histologic grading. Am J Surg Pathol 2001; 25:835–45.
6. Goode RK, Auclair PL, Ellis GL. Mucoepidermoid carcinoma of the major salivary glands: clinical and histopathologic analysis of 234 cases with evaluation of grading criteria. Cancer. 1998; 82(12):17-24.
7. Seethala RR. An Update on Grading of Salivary Gland Carcinomas. Head and Neck Pathol 2009; 3: 69–77.
8. Kumar V, Abbas A, FAusto N, Mitchell R. Robbins Basic Pathology. 8th edition, elsevir, 2007.
9. Sarkis S, Abdullah B, Abdul Majeed B and Talabani N. Immunohistochemical expression of epidermal growth factor receptor (EGFR) in oral squamous cell carcinoma in relation to proliferation, apoptosis, angiogenesis and lymphangiogenesis. Head & Neck Oncology 2010; 2:13.
10. Zhang H, Berezov A, Wang Q, Zhang G, Drebin J, Murali R, Greene MI. ErbB receptors: from oncogenes to targeted cancer therapies. Clin Invest 2007; 117 (8):2051–8.
11. Rahmanzadeh R, Hüttmann G, Gerdes J, Scholzen T. Chromophore-assisted light inactivation of pKi-67 leads to inhibition of ribosomal RNA synthesis. Cell Proliferation 2007; 40(3):422-30.

12. Gonçalves CF, Morais MO, Alencar RCG, Mota ED, Silva TA, Batista AC, Mendonça EF. Expression of Ki-67 and MUC1 In mucoepidermoid carcinomas of young and adult patients: Prognostic implications. Experimental and Molecular Pathology 2011; 90:271–5.
13. Krecicki T, Jelen M, Krecicka MZ, Rak, Szkuldlarek T, Krzeszewska JJ. Epidermal growth factor receptor (EGFR) proliferating cell nuclear antigen (PCNA) and Ki-67 antigen in laryngeal epithelial lesions. Oral Oncol 1999; 35:180-6.
14. Ryan KM, Phillips AC and Vousden KH. Regulation and function of the p53 tumor suppressor protein. Current Opinion in Cell Biology 2001; 13: 332–337.
15. Kiyoshima T, Shima K, Kobayashi, K. Matsuo, K. Okamura, Komatsu S. Expression of p53 suppressor gene in adenoid cystic carcinoma and mucoepidermoid carcinomas of the salivary glands. Oral Oncol 2001; 37:315-22.
16. Laimer K, Spizzo G, Gastl G, Obrist P, Brunhuber Th, Fong D, Barbieri V, Jank S, Doppler W, Rasse M, Norer B. High EGFR expression predicts poor prognosis in patients with squamous cell carcinoma of the oral cavity and oropharynx: A TMA-based immunohistochemical analysis. Oral Oncology 2007; 43:193-8.
17. Alves FA, Pires FR, de Almeida OP, Lopes MA, Kowalski LP. PCNA, Ki-67 and p53 expressions in submandibular salivary gland tumours. Int J Oral Maxillofac Surg 2004; 33 (6):593-7.
18. Ehab AS, Mohamed H Elmalahy. Image cytometric analysis of p53 and mdm-2 expression in primary and recurrent mucoepidermoid carcinoma of parotid gland: immunohistochemical study. Diagnostic Pathology 2010; 5:72.
19. Jeanine HG, Nehmé E, Georges A, Sader G, Zouka S, Haddad A. prognostic significance of EGFR, p53 and e-cadherin in mucoepidermoid cancer of the salivary glands a retrospective case series. Lebanese Medical J 2007; 55 (2).
20. Torres RA, Roy S, Craig G T and Speight P M. Expression of Mcm2, geminin and Ki67 in normal oral mucosa, oral epithelial dysplasias and their corresponding squamous-cell carcinomas. Brit J Cancer 2009; 100:1128–34.
21. Taher MT. Immunohistochemical expression of D2-40m Cd34 and MMP2 as a Biological Markers of Lymphangiogenesis, Angiogenesis and Invasion Potential in Mucoepidermoid Carcinoma of the Oral and Maxillofacial Region. A master thesis, University of Baghdad, department of oral pathology, 2011.

Histopathological evaluation of oral lichen planus

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ABSTRACT

Background: Oral lichen planus(OLP) is a chronic inflammatory disorder affecting mucosal surfaces , which can cause an important discomfort to the patients . To highlight the most characteristic histopathological findings of OLP which are useful in making a diagnosis of OLP. In addition, by studying the association of these findings it was hoped that information about pathogenic mechanism would be obtained.

Material and Methods : In this study a retrospective analyses of 194 cases of OLP being diagnosed at Oral and Maxillofacial Pathology Department, College of Dentistry , were obtained over a period of 26 years , spanning from 1985- 2010 . We analyzed the age and sex of the patients, clinical type of lichen planus, site and different histopathological finding, comparing them with each others.

Results: (61%) of the patients are female and (39 %) are males, with an average age for both sexes (49.75 years).

The most frequent clinical form is reticular, presented in (78%) of cases, and the most common location is buccal mucosa, presented in (60%) of the patients. The mononuclear infiltration beneath and adjacent to the epithelium, parakeratosis and degeneration of the basal layer of the epithelium were consistent features. Linear regression analysis revealed a positive correlation between basal degeneration and mononuclear infiltration and an inverse correlation between the mononuclear infiltrate and the parakeratosis.

Conclusion: Linear regression analysis of the parameters studied provided partial support for a cell- mediated immune mechanism.

Key words: Oral lichen planus, histopathological finding. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):48-54).

INTRODUCTION

Lichen planus, a chronic autoimmune mucocutaneous disease affects the oral mucosa beside the skin, genital mucosa, scalp and nails. An immune mediated pathogenesis is recognized in Lichen planus although the exact etiology is unknown [1]. OLP is a chronic inflammatory disease with the reported prevalence rate varies from 0.5% to 2.2% of the population. The typical age of presentation is between 30-60 years and the disease is more frequently seen in women [2].

Clinically, OLP has specific and clearly identifiable features [3], usually presenting in one of two main forms the reticular and the erosive forms, although other forms are not rare [4]. In fact, according to Mollaoglu [5], four other forms were originally described the popular, plate – like, bullous and atrophic forms.

The reticular form occurs more frequently and is characterized by white Lacy streaks known as Wickham's striae, which generally are surrounded by discrete erythematous borders. Fig. 1 .Such features may not be evident in certain sites, such as the dorsum of the tongue, where lesions presented as keratotic plagues. The reticular form usually causes no symptoms; it involves the posterior jugal mucosa bilaterally. Other sites may be simultaneously involved, such as the upper and Lateral surfaces of the tongue, the gums and the palate [4-6]. In its characteristic reticular form, OLP can be diagnosed clinically in most instances as well [7].

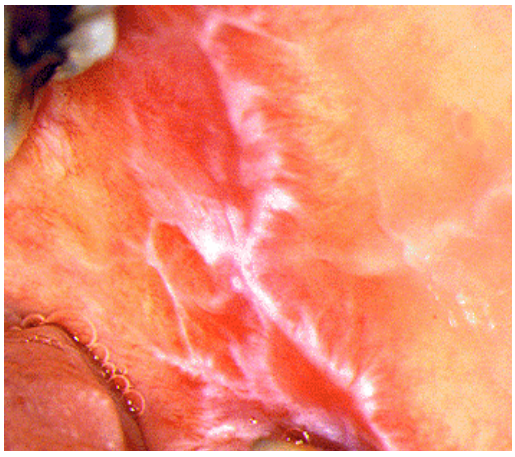

Figure 1: Oral lichen planus lesion with reticular and erythematous manifestations [4].

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In the absence of typical reticular LP manifestation elsewhere in the mouth, the non-reticular types may be difficult to diagnose clinically with confidence. In such event, the taking of a biopsy should be considered. [7].

It is well accepted that OLP is a chronic possibly life- long, disease that is characterized by remission and exacerbation. Several suggestions for monitoring the severity of OLP have been reported based on clinical aspect, number of involved oral subsites and severity of symptoms [8].

Histopathological aspect

In 1978 a set of histopathological criteria for OLP has been provided by the WHO that probably is still regarded as authoritative source [9].

The histological criteria include the existence of a band of lymphocytic inflammatory infiltrated in the subepithelial connective tissue, hydropic degeneration of the basal layer and the absence of the epithelial dysplasia. If the above three criteria are met, the lesion is considered a typical LP from a histological perspective and as for those that do not meet one of the histological criteria, they are considered to be lesions that histologically compatible with LP [10].

The histological feature of OLP was first described by Dubreuill in 1906 and later by Shklar (1996). Shklar described three classic histological features which are overlaying keratinization, a dense band- like layer of lymphocytic infiltrate within the underlying connective tissue and liquefaction degeneration of based cell layer [1].

Pindborg et al. [11] have further described the histological feature of OLP which have similar features to that described by Shklar above. Within the based layer degenerating basal keratinocytes form colloid (civatte, hyaline, or cytoid) bodies that appear as homogenous eosinophilic globules. The ultrastructure of colloid suggests that they are apoptotic keratinocytes. An eosinophilic band which represents thickened basement membrane may also be presented.

The essential histological feature of OLP are liquefactive degeneration of basal epithelial cells, dense, band-like inflammatory infiltrate consisting of lymphocyte, normal maturation epithelium , saw- tooth appearance of rate ridges, civatte bodies and hyperkeratosis [1] (Figure 2)

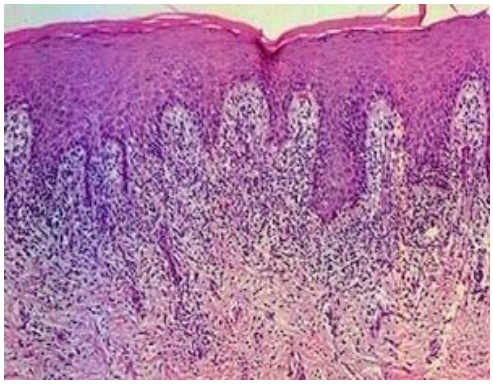

Figure 2: Histopathological features of oral lichen planus [1].

The aim of this study is to highlight the most characteristic histopathological finding of OLP in an attempt to describe more precisely the parameters which are useful in making a histopathological diagnosis of OLP. Rather, it was hoped that a set of parameters could be defined which would be diagnostic of the disease despite the variability of its presentation. In addition, by studying the associated of these parameters it was hoped that information about pathogenic mechanism would be obtained.

MATERIALS AND METHODS

A retrospective study was carried out on a sample of 194 biopsy specimens of patients diagnosed with OLP, the tissue were fixed in neutral – buffered formalin (10%) embedded in paraffin and section were cut at 6µ. They were stained with hemotoxylin and eosin stain, by examine case sheets in Oral and Maxillofacial Pathology Department, College of Dentistry , Baghdad University from the 1985 to 2010.

The cases selected for study showed at least 3 of the histological parameters listed in table 1. in addition to a mononuclear infiltrate which closely apposed the epithelium . The group comprised 118 female (61%) and 76 males (39%) with age ranging from 20-82 years and with mean age (49.75years) (Figure 3).

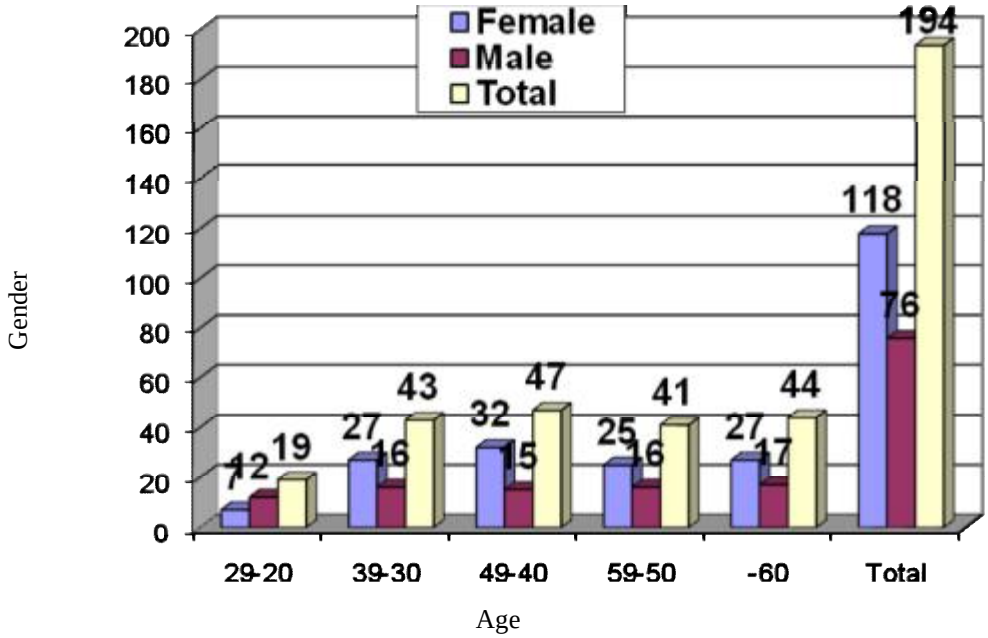

Figure 3: Age and gender distribution of oral lichen planus patients

The parameters listed in table 1 were scored according to the method of assessment described. Scoring was carried out by only one observer with frequent referral to a reference slide in order to optimize the reproducibility of the assessment made. All sections were scored along their length. The scoring of the parameters were subjectively done from+ to++++ as seen in table (1) and according to width of keratin, severity of edema, severity of basal layer degeneration and density of mononuclear infiltrate.

The statistical analysis of the data was performed using the statistical Package for Social Sciences (SPSS) version. The linear regression analysis was used to show the relationship between the different parameters and the results obtained are considered significant at $r < 0.5$.

RESULTS

The biopsies were taken from various sites including buccal mucosa 161 causes (60%) , tongue 21 cases (11%) , alveolar mucosa 10 cases(5%) , palate 4 cases (2%), lip 11 cases (6%), gingiva 7 cases (4%) and there were 27 cases (14%) showed involvement of more than one site in the oral cavity by the disease.

Clinical Picture:

Of the case selected for study, 152 cases (78%) were described clinically as being reticular, 23 cases (12%) as erosive, 6 cases (3%) as atrophic only 4 cases (2%) as anular , with 5 cases (2.5%) as bullous and 4 cases as erthroplakia (2%).

The results of this study show that mononuclear infiltration beneath and adjacent to the epithelium and basal layer degeneration were consistent findings in OLP. Parakeratinization , acanthosis and a prominent granular layer were also frequent findings (table 1).

Table 1: Incidence of parameters studied and the method of their assessment

Parameter	% of cases	Method of assessment of parameter
Keratin	18%	assessed as being present or absent and graded + → ++++ according to width
Parakeratin	92%	cell layers counted
Granular layer	80%	cell layers counted
Acanthosis	68%	cell layers counted
Intercellular edema	62%	assessed as being present or absent and grading + → ++++ according to severity
Intracellular edema	71%	assessed as being present or absent and grading + → ++++ according to severity
Basal layer degeneration	82%	assessed as being present or absent and grading + → ++++ according to severity
Mononuclear infiltrate	100%	assessed as being present or absent and grading + → ++++ according to density of the infiltrate
Bandlike distribution of infiltrate	56%	assessed as being present or absent
Civatte bodies	35%	counted
Focal separation of epithelium and connective tissue	30%	assessed as being present or absent
Saw – tooth rete ridges	65%	assessed as being present or absent
Widening of the basement membrane zone	70%	assessed as being present or absent
Lymphocytic predominance in infiltrate	93%	assessed as being present or absent
Dilated vessels in connective tissue	59%	assessed as being present or absent
Atrophy	15%	assessed as being present or absent
Hyperplasia	62%	assessed as being present or absent
Area of atrophy and hyperplasia	23%	assessed as being present or absent

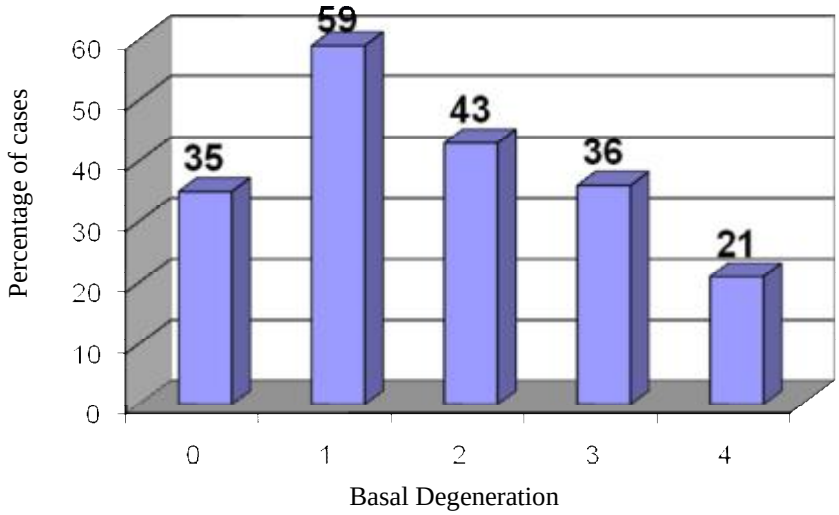

Fig 4: Quantitative assessment of severity of basal degeneration versus percentage of cases

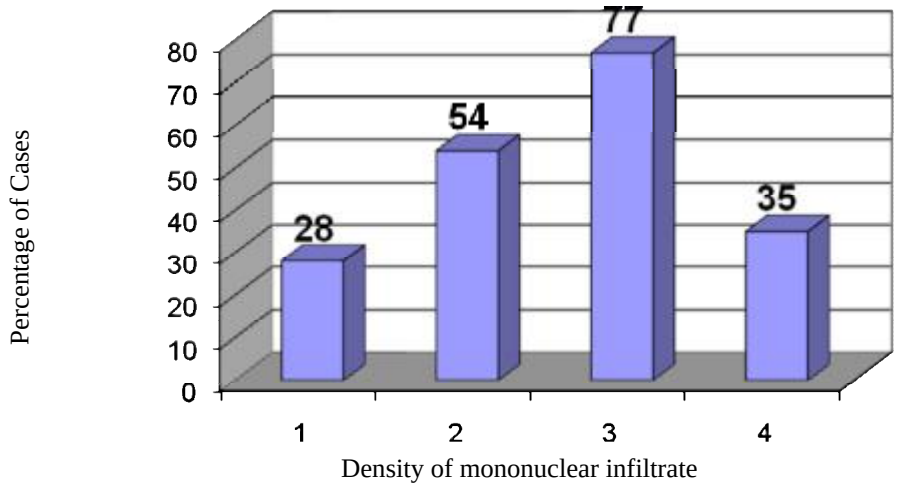

Fig 5: Quantitative assessment of density of mononuclear infiltrate versus percentage of cases

The relationship of basal degeneration and mononuclear infiltration was studied and linear regression analysis revealed a positive correlation between these two parameters. (Fig 6).

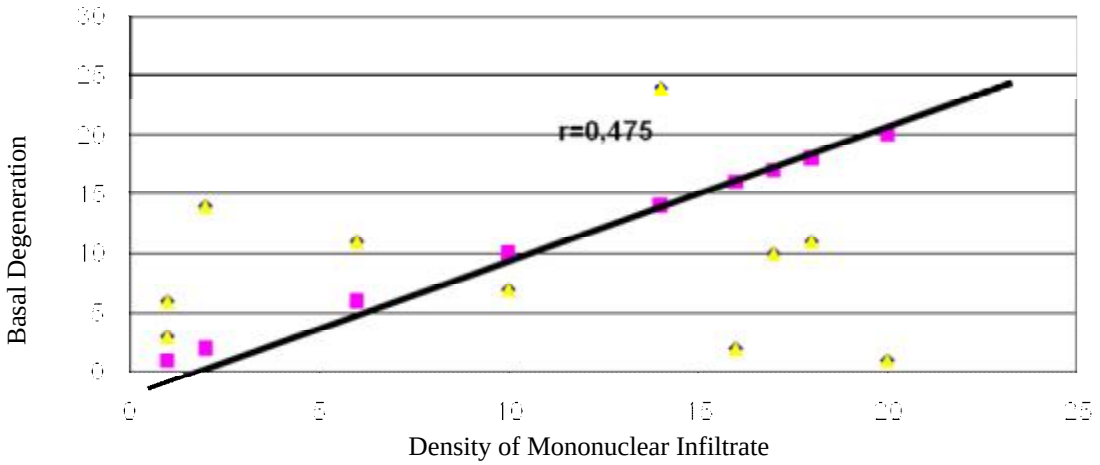

Fig 6: Scattergram indicating a positive linear correlation between density of mononuclear infiltrate and severity of basal degeneration.

The density of the mononuclear infiltrate and the severity of the basal degeneration were studied more closely (Figure 4, 5)

As parakeratosis was such a frequent finding, it's relationship to both mononuclear infiltration and basal layer degeneration was studied. Linear regression analysis of mononuclear infiltration versus the number of layers of parakeratin revealed an inverse correlation between these two parameters (Figure 7).

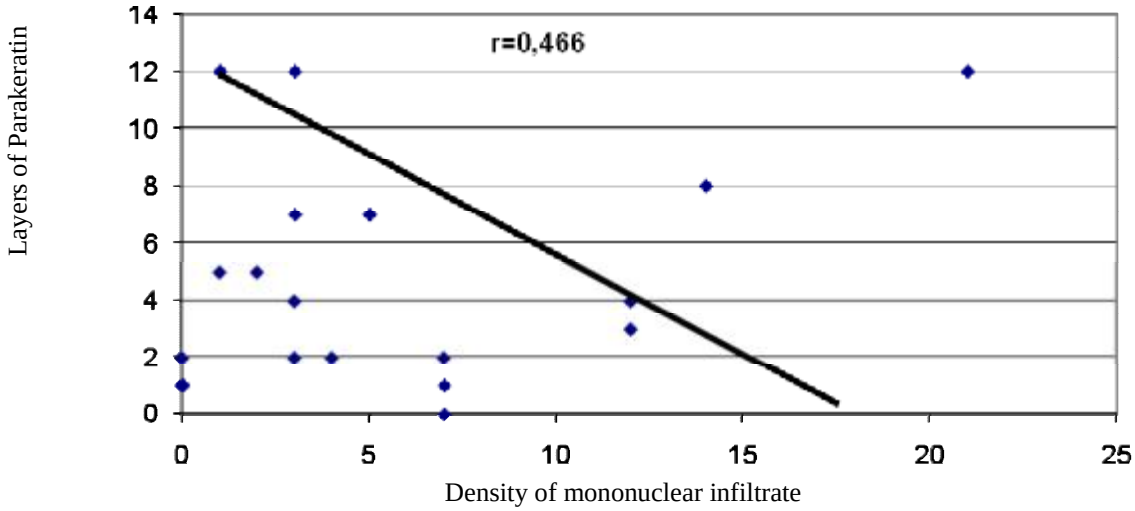

Figure 7: Scatter gram indicating an inverse correlation between the density of the mononuclear infiltrate and the number of layers of parakeratin.

There was no linear correlation between parakeratinization and basal layer degeneration. Linear regression analysis of the amount of intracellular edema versus density of the mononuclear infiltrate, intercellular edema versus density of the mononuclear infiltrate and the number of civatte bodies versus the infiltrate were performed. There was no linear relationship between any of these parameters.

DISCUSSION

A predominace of OLP among female patients was observed in the present study, in agreement with other report's [10,12-17] .

A predominace of OLP in the fourth, fifth and sixth decade of life was observed in the present study , in agreement with Ingafou et al. [9,17] , although other studies did not show the expressive involvement of patients in their seventh decade [13 , 18] . The cheek mucosa was the site most affected , followed by the tongue and the other locations being less common in agreement with other reports [10,13,15,16]

The reticular form was the most frequent, followed by erosive form, these two forms were found to be associated or not with other form, as also reported by other investigators. [10,13,16]

Focusing on the histopathological findings, degeneration of the basal layer of the epithelium and the subepithelial lymphocytic inflammatory infiltrate is identified in 100% of the patients , a finding that is corroborated by other authors [10,12] and which , along with the absence of epithelial dysplasia, constitutes the three typical histological criteria of oral lichen planus.

It was found that parakeratosis was more consistent findings than basal degeneration, being present in 92% of cases studied. This result was in agreement with Hedberg et al [12] and higher than the incidence of 66% previously reported by Fernańdez [10]. Acanthosis and the present of a prominent granular layer (80%) were also found more often than other parameter in this study. As regard acanthosis, our data was in agreement with Hedberg et al. [12] and higher than that found in literature [10,19]

The present of saw tooth rete ridge (65%) is another histological finding of OLP observed in our sample and described by other authors [10,19] .

Epithelial erosion is a finding that is relatively more common in atrophic and erosive form, observed in only (15%) of the cases. This could be because, as some authors claim [20], the thickness of the epithelium is greater in the reticular forms, with a thining observed in the atrophic and erosive forms, therefore making them more prone to erosion.

Although is it generally accepted that the pathology of lichen planus represents tissue damage as a result of some form of immune response, this study make an attempt to evaluate the association of these parameters in relation to possible pathogenic mechanisms for the disease.

It has been proposed that the tissue response represents a cell – mediated attack against the basal layer of the epithelium [21]. OLP is a T- cell mediated inflammatory disease [22,23] . The basal keratinocytes appear to be the primary site of immunological injury in OLP and molecular biological changes in the basal cell compartment have been a matter of particular interest in later research in OLP [23] of current interest is presence of cytotoxic T cells in OLP with the potential of targeting basal keratinocytes [24], based on that T-cell lines cultured from LP skin lesions have

proven to lyse autologous lesional keratinocyte in vitro [25]. The present data show a significant and positive correlation between the severity of basal degeneration and density of mononuclear infiltration which is compatible with a cell-mediated type of response. The association of several other parameters studied, however, showed no correlation.

Dvorak et al [26] listed necrosis and hyperplasia of epithelium and the presence of intercellular edema as being forms of epithelial changes found in contact hypersensitivity reactions in man. These parameters were examined , but were not found to correlate with the density of the mononuclear infiltrate in this study as well as in Hedberg et al study [12] .

Sarkany and Gaylarde [27] observed liquefaction degeneration of basal cells even in the absence of an inflammatory response in lichen planus. This may present an early stage in the disease process.

REFERENCES

1. Ismail SB, Kumar SK and Zain RB. Oral lichen planus and lichenoid reactions: etiopathogenesis, diagnosis, management and malignant transformation. J Oral Sci 2007; 49:89-106.
2. Nagao T, Ikeda N, Fukano H, Hashimoto S, Shimozato K, Warnakulasuriya S. Incidence rates for oral leukoplakia and lichen planus in Japanese population. J Oral Pathol Med 2005; 34: 532-9.
3. Eisen D, Carrozzo M, Sebastian J-VB, Thongprasom K. Oral lichen planus: clinical features and management. Number V. Oral Dis 2005; 11(6): 338-49.
4. Edwards PC, Kelsch R. Oral lichen planus: clinical presentation and management. J Can Dent Assoc 2002; 68(8): 494-9.
5. Mollaoglu N. Oral lichen planus: a review. Br J Oral Maxillofac Surg 2000; 38(4): 370-7.
6. Neville BW, Dann DD, Allen CM, Bouquet JE. Pathologia oral and maxillofacial. 2nd ed. Rio de Janeiro: Guanabara koogani; 2004 .784p.
7. Van der Waal I. Oral lichen planus and oral lichenoid lesions; a criteria appraisal with emphesis on the diagnostic aspects. Med Oral Pathol Oral Cir Bucal 2009; 14(7): E 310-4.
8. Escudier M, Ahmed N, Shirlaw P, Setterfield J, Tappuni A, Black MM, et al . A scoring system for mucosal disease severity with special reference to oral lichen planus. Br J Dermato 2007; 157:765-70.
9. Kramer IR, Lucas RB , Pindborg JJ, Sobin LH. Definition of leukoplakia and related lesions: an aid to studies on oral precancer . Oral Surg Oral Med Oral Pathol 1978; 46: 518-39.
10. Fernández – González F, Vaźquez- Álvarez R, Reboiras- López D, Gándara – Vila P, Garcia – Garcia A,Gándara Rey JM. Histopathological findings in oral lichen planus and their correlation with the clinical manifestations. Med Oral Patol Oral Cir Bucal 2011;16(5): e 641-6.
11. Pindborg JJ, Reichart PA , Smith CJ , Vander Waal I. Histological typing of cancer and precancer of oral mucosa. 2nd ed. New York: Springer; 1997. p.30.
12. Hedberg N, Ng A, Hunter N. A semiquantitative assessment of the histopathology of oral lichen planus. J Oral Pathol 1986; 15: 268-272.
13. Xue JL, Fan MW, Waag SZ, Chen XM , Li Y, Wang′ L: A clinical study of 674 patients with oral lichen planus in China. J Oral Patho Med 2005; 34: 467-72.
14. Bermejo-Fenolla , Sanchez –Siles M, López-Jomet P, Camacho- Alonsof , Salazar N: Premalignant nature of oral lichen planus. A retrospective study of 550 oral lichen planus patients from South-eastern Spain. Oral Oncol 2009; 45: e 54-6.
15. Pakfetrat A, Javadzaden –Bolouri A, Basir – Shabestari S, Falaki F: Oral lichen planus : a retrospective study of 420 Iranian patients . Med Oral Pathol Oral Cir Bucal 2009; 14: e 315-8.
16. Ingafou M, Leao JC, Porter SR, Scully C: Oral lichen planus : a retrospective study of 690 British patients . Oral Dis 2006; 12: 463-8.
17. Aves MGO, Almeida JD, Balducci I and Cabral LA: Oral lichen planus : A retrospective study of 110 Brazilian patients. BMC Research Notes 2010; 3:157.
18. Carbone M, Arduino PG, Carrozzo M, Gandolfo S, Argiolas MR, Bertolusso G, Conratta D, Pentenero M, Broccoletti R: Course of oral lichen planus: a retrospective study of 808 Northern Italian patients. Oral Dis 2009; 15: 235-43.
19. Batra P, Wang N, Kaminott, Possick P. Linear lichen plannus. Dermatol Online J 2008; 14:16.
20. Bagam Sebastian JV, Aguirre Vrizar JM, Milian Masanet A, Peńarrocha Diago M, Garcia Pola Vallejo MJ: A morphometric study of 74 cases of oral lichen planus. Rev Stomatol Chir Maxillofac 1991; 92: 265-8.
21. Sloberg K, Jonsson R, Jontell M. Assessment of the langerhans cells in oral lichen planus using monoclonal antibodies. J Oral Pathol 1984; 13: 516.
22. Gilhar A, Pillar T, Winterstein G, Etzioni A. The Pathogenesis of lichen planus. Br J Dermatol 1989; 120(4): 541-4.
23. Sugerman PB, Savage NW, Walsh LJ, Zhao ZZ, Zhou XJ, Khan A, Seymour GJ, Bigby M. The pathogenesis of oral lichen planus. Crit Rev Oral Biol Med 2002; 13(4): 350-65.
24. Santoro A, Majorana A, Bardellini E, Gentili F, Festa S, Sapelli P, Facchetti F. Cytotoxic molecule expression and epithelial cell apoptosis in oral and cutaneous lichen planus. Am .J Clin Pathol 2004; 121(5): 758-64.
25. Sugerman PB, Satter White K, Bigby M. Autocytotoxic T-cell clones in lichen planus. Br J Dermatol 2000; 142(3):449-56.
26. Dvorak HF, MihmJr MC, Dvorak AM et al. Morphology of delayed hypersensitivity reactions in man. Quantitative description of inflammatory response. Lab Invest 1976; 31:11.
27. Sarkany I, Gaylarde PM. Ultrastructural and light microscope changes of the epidermo-dermal junction. Trans St. John's Hosp Dermatol Soc 1971; 57:139.

Evaluation the effect of autologous bone marrow – derived mesenchymal stem cells as a treatment in diabetic rabbits

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ABSTRACT

Back ground: Type 1 diabetes is the result of an autoimmune attack against the insulin-producing beta cells of the pancreas. Current treatment for patients with type 1 diabetes typically involves a rigorous and invasive regimen of testing blood glucose levels many times a day along with injections of recombinant insulin. Many recent researches have shown that stem cell therapy can be the best choice for treatment of this disease. The aims of this research were investigating regeneration of pancreatic beta cells of type 1 diabetic rabbits after stem cell transplantation.

Materials and Methods: 32 rabbits weighting an average of (2.5 - 3 kg) were used in this experimental study, and divided into 2 groups as follows; group A (contains 16 controlled diabetic rabbits received insulin as a treatment) and group B (contains 16 diabetic rabbits received autologous mesenchymal stem cells as a treatment).The induction of diabetes was achieved by a single dose of intravenous injection of the Alloxan, which was administered to the rabbits via the marginal ear vein, mesenchymal stem cells were differentiated into insulin – producing cells and reimplanted into the rabbits of group B with daily monitoring of blood glucose level and body weight.

Results: The insulin – producing cells regulated the hyperglycemia resulted from diabetic rabbits , 7 to 9 days after reimplantation the blood glucose level were decreased from about(400 mg/dl into 180 mg/dl).

Conclusions: Islet-like functional cells can be differentiated from bone-marrow mesenchymal stem cells (MSCs), which may be a new procedure for clinical diabetes stem -cell therapy, these cells controlled blood glucose level in diabetic rabbits as the effect of insulin. MSCs play an important role in diabetes therapy by islet differentiation and transplantation.

Key words: Type 1 diabetes rabbits, bone marrow mesenchymal stem cell, stem cell therapy. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):55-60).

INTRODUCTION

Diabetes mellitus is a chronic disease in which the body either does not produce enough, or does not properly respond to, insulin, a hormone produced in the pancreas. Hyperglycemia or high blood sugar, a common condition caused by the uncontrolled diabetes, damage the body system seriously, especially the nervous system and blood vessels. Current diabetes treatments just aim to lower the blood sugar through diet, exercise, medication with tablets and insulin. Therefore, researchers have been carrying out new diabetes treatments such as artificial CD3 antibody, pancreas graft and pancreatic islet cell graft in order to restore the insulin production of the body. However, these therapies are expensive, low compatibility, and easy to be rejected by the receiver's immune system [(1,2)]**.** Diabetic mellitus (DM), one of the leading causes of morbidity and mortality in many countries, is caused by an absolute insulin deficiency due to the destruction of insulin secreting pancreatic cells (type 1 DM) or by a relative insulin deficiency due to decreased insulin sensitivity, usually observed in overweight individuals (type 2 DM).

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In both types of the disease, an inadequate mass of functional islet cells is the major determinant for the onset of hyperglycemia and the development of overt diabetes. Islet transplantation has recently been shown to restore normoglycemia in type 1 DM [(3)]**.**

Multipotent stem cells have been described within pancreatic islets and in nonendocrine compartments of the pancreas, and these cells have the capacity of differentiating into pancreatic islet-like structures. Furthermore, cells that do not reside within the pancreas, such as mesenchymal stem cells, hepatic oval cells and cells within spleen, have been differentiated into pancreatic endocrine hormone-producing cells *in vitro* and *in vivo* [(4-12)] **.** Mesenchymal stem cells (MSC) have received widespread attention because of their potential use in tissue engineering applications [(13)]

MSC are defined as self-renewable, multipotent progenitor cells with the capacity to differentiate into several distinct mesenchymal lineages**.** Also MSCs are defined as non-hematopoietic cells that are able to replicate for a long time while maintaining heir multilineage differentiation potential. These cells were first recognized with the capacity to generate three osteoblastic, chondroblastic and adipocytic lineages [(14,15)] **.** Many recent research studies have demonstrated that MSCs may possess more extensive differentiation potentials than expected. These cells have been shown that are able to

differentiate into many other specialized phenotypes other than the skeletal lineages (osteocytes, chondrocytes and adipocytes) including neural cell, pancreatic cell, cardiomyocyte, renal epithelial cell, intestinal cell and keratinocyte [15,16]. MSCs were first isolated using their plastic adherent properties and till now this property is utilized as current method for MSCs isolation from variety of species including human, mouse, rat, cat, rabbit, pig and baboon by many researchers [17] .

MATERIALS AND METHODS

In this experimental study **32** adult rabbits weighting an average of (2.5 - 3 kg) were used and maintained under control conditions of temperature, drinking and food consumption. The experimental animals were divided into two groups as follows:

Group A : contains 16 controlled diabetic rabbits received daily insulin as a treatment in a dose of 0.1 mg/ kg of body weight.

Group B : contains 16 diabetic rabbits received autologous bone marrow-derived stem cells as a treatment.

Induction of Diabetes Mellitus in Rabbits

All animals were weighted to calculate the dose of anesthesia and alloxan which were given to them **(Figure 1).**The general anesthesia was induced by intramuscular injection of xylazine (0.4 mg/kg of body weight) plus ketamine hydrochloride (40 mg/kg of body weight). After 10 to 15 minutes the animals were anesthetized, the skin covering the ears of the rabbits was shaved carefully from the outer surface to expose the external auricular vein. The rabbits were injected by a single dose (120 mg/kg) intravenous injection of the pancreatic beta-cells toxin monohydrate (Alloxan), which was administered to the rabbits via the marginal ear vein **(Figure 2).** Severity of the induced diabetic state was assessed by daily monitoring of blood glucose levels with a calibrated glucose meter (few drops from the ear) and daily estimation of the body weight **(Figure 3)**. For determination of blood glucose level, the animals whose blood glucose level was greater than 200mg / dl were indicated as hyperglycemic**.** Five to seven days after injection, Alloxan induced diabetes by destroying the beta cells of the pancreas; the blood glucose level was elevated above the 200mg/dl **(Figure 4).** Animals of group A were treated by daily injection of insulin to control the hyperglycemia.

Figure 1: Rabbits weighting

Figure 2: Alloxan injection via marginal ear vein

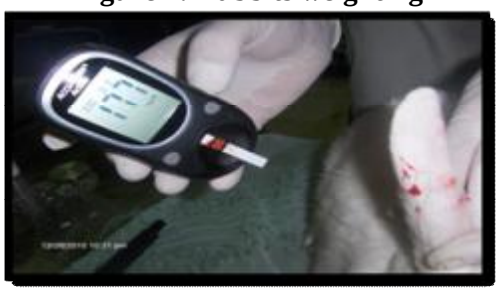

Figure 3: Monitoring of blood glucose level

Figure 4: Elevation of blood glucose level

Isolation of MSCs from the Bone marrow

The surgery was performed under well sterilized condition and gentle surgical technique. The surgical towels were placed around the site of operation; the site chosen for operation was the proximal tibia metaphysis of the right limb (Figure5). Skin incision was done by using a sharp blade to expose the muscle (Fig.6). Then the muscle was dissected to expose the tibia (Figure 7). By intermittent drilling with (1 mm surgical drill) and continuous, vigorous irrigation with sterile normal saline, a guide hole was made (Figure 8). By using sterile syringe (5ml) that contains few drops of heparin (to prevent blood clotting) the bone marrow was aspirated as soon as possible (Figure 9). After that the area was

washed very well with a sterile normal saline, the muscle was sutured with 3/0 absorbable (catgut) suture (Figure 10). The skin was sutured with interrupted 3/0 silk suture (Figure 11).

Figure 5: The site of operation

Figure 6: Skin incision

Figure 7: Dissection of the muscle

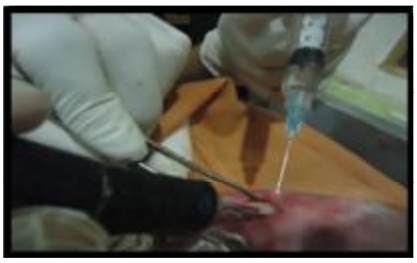

Figure 8: 1mm guide hole was made

Figure 9: Aspiration of bone marrow

Figure 10: Muscle sutured with cat gut suture

Figure 11: Skin sutured with silk suture

Inside the hood (previously sterilized by UV Rays over night), the bone marrow was inserted into two test tubes (t.t.), equal volume of phosphate buffer saline(PBS) was added to (t.t.) and shake very well until the solutions became homogenous .Then the two t.t. was put inside the centrifuge (2000 RPM) for 10 minutes. Inside the hood the top two thirds of the solution were removed (that contains non adherent cells). RPMI-culture media was added to the precipitate 1/3 of the t.t. & shake very well Until the media was became homogenous, then the media was added into a well sterilized plastic falcons & covered very well by a parafilm, finally the media was incubated at (37 °C, 5% Co2 & 95% air).The cells were checked periodically under inverted microscope, the culture media was changed twice a week for two weeks. With the medium changes, almost all the non adherent cells were washed away.

Differentiation of MSCs into Insulin producing cells

1- Inside the hood about 2/3 of the medium in the falcons was removed and pre-inducing medium was added to the remaining 1/3 of the falcons, the pre-inducing medium containing low glucose–RPMI (L-RPMI) supplemented with 10 mM nicotinamide, plus 1 mM beta-

mercaptoethanol and 10% of fetal bovine serum(FBS) , then covered by a parafilm and incubated at (37 °C, 5% CO2 & 95% air) (for 24 hours).

2- The medium was changed with fresh inducing medium; containing serum free high glucose–RPMI (H-RPMI) , supplemented with 10 mM nicotinamide , plus 1 mM beta-mercaptoethanol , then covered by a parafilm and incubated at (37 °C, 5% CO2 & 95% air) (for 10-12 days).

Detection of Insulin producing cells

The insulin producing cells can be detected by dithiazone (DTZ) stain. DTZ is a zinc-chelating agent known to selectively stain pancreatic beta cells because of their high zinc content [18].

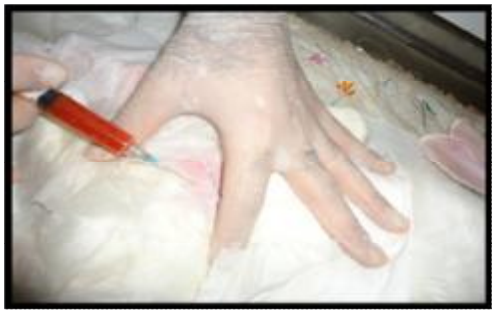

Figure 12: Reimplantation of MSCs

Inside the hood about 2/3 of the medium was removed from the falcon, then 2 ml of DTZ solution was added for the remaining 1/3 of the medium in the falcon that containing the MSCs, the cells were incubated at (37 °C, 5% CO2 & 95% air) for 30 minutes and examined under inverted microscope.

Reimplantation of MSCs

5 ml of the medium was reimplanted to the rabbits by subcutaneous injection **(Figure12).**

RESULTS

About 4 to 5 days after incubation the stem cells adhered to the base of the sterilized falcons and started to be elongated and became spindle in shape just like a fibroblast cells, some of them became growing to have a neuron- like shaped **(Figure 13).** 14 to 16 days after incubation, the cells grew as a monolayer which were completely attached with each other by a network like connection and filled all the base of the sterilized plastic falcons **(Figure 14).** Ten to 12 days after adding the differentiated medium the undifferentiated MSCs were typical of adherent spindle and fibroblasts- like. However, under differentiation condition changed from spindle-like cells into round or oval types, these cells were morphologically similar to pancreatic islet cells **(Figure 15).**

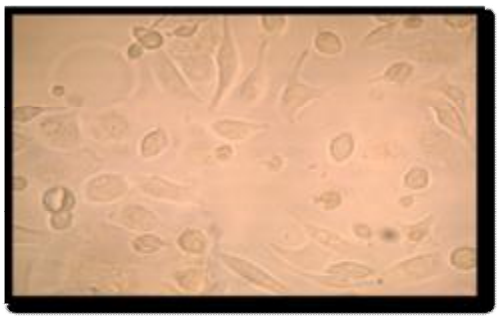

Figure 13: Colonies of MSCs 4 days after incubation, the cells became neurons-like shaped, inverted

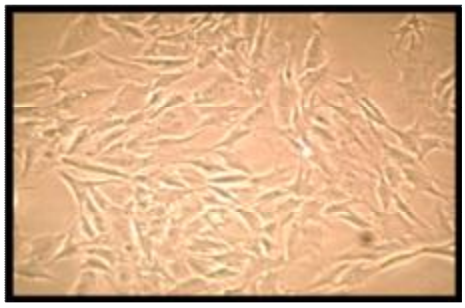

Figure 14: The monolayer of MSCs, 2 weeks after incubation, inverted microscope 40X.

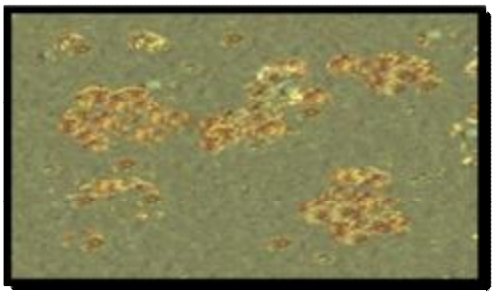

Figure.15: The colonies of pancreatic islet cells, inverted microscope 40X.

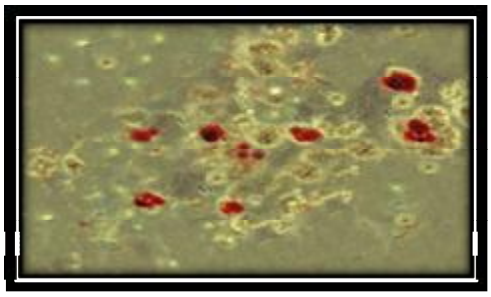

Figure.16: Insulin- producing cells stained positive by DTZ stain, inverted microscope 40X.

Detection of Insulin producing cells

Almost a lot of insulin-like cells were stained positive to DTZ stain and they appeared like crimson-red appearance under inverted microscope **(Figure 16).**

DISCUSSION

In pancreas, insulin is produced and secreted by specialized structures, islets of Langerhans. Diabetes, which affects thousands of million people in the world, results from abnormal function of pancreatic islets. The main obstacle to successful islet transplantation for diabetes is the limitation of available insulin-producing tissue [(19)]. The present study introduced to generate cells expressing insulin from rabbit's bone marrow – derived mesenchymal stem cells. This approach may provide a potential new source of pancreatic islet cells for transplantation. Bone marrow is an important source of easily procurable adult stem cells, in addition to the ability of bone marrow-derived stem cells to reconstitute the hematopoietic system [(20)]. In the present study MSCs was successfully isolated from the bone marrow, this finding was in agreement with [(21)] , who found extra pancreatic proinsulin-producing cells present in the liver, BM, spleen, adipose tissue, and thymus in hyperglycemic animals and that the majority of these proinsulin producing cells were derived from the donor BM, as evidenced by BM transplantation experiments. The routine method for MSCs isolation from bone marrow samples is to plate the bone marrow cells in plastic dishes in the presence of appropriate medium and to incubate the cultures in an atmosphere of 5% CO2 and 37C° temperature. The next step is to discard the non adherent cells by medium replacement, keep and expand the adherent population which mainly possesses a fibroblastic morphology [(22)] . The cells are cultured in glucose-rich medium. Glucose is a growth factor for beta-cell replication in vitro and in vivo. Glucose has been shown to increase the insulin content in cells derived from adult stem cells. In the proliferation phase, the high glucose content may support the extra energy needed for cell division. In the differentiation stage, it could modulate specific gene programs linked to glucose sensing and insulin secretion [(23)] . Several in vitro studies have shown that bone-marrow–derived stem cells could be reprogrammed to become functionally insulin-producing cells under certain culture conditions [(24,25)] . In this experimental study The MSCs were successfully differentiated into pancreatic islet β-like cells. These cells were morphologically similar to pancreatic islet cells and have the ability for regulating rabbit's blood glucose level. High glucose concentration was considered as a potent inducer for pancreatic islet differentiation. Nicotinamide was used to preserve islet viability and function [(26)], β-mercaptoethanol was commonly used as a neurocyte inducer. In the present experiment, high glucose alone could not effectively induce MSC to differentiate into islet-like cells. After adding nicotinamide, they effectively transformed MSCs into islet-like cells. This implies that nicotinamide has been an effective inducer, or it protected the differentiated cells from dying or transforming into other cell types. β-mercaptoethanol increased the potency of nicotinamide . DTZ, a zinc-chelating agent, is known to selectively stain pancreatic beta cells crimson red. As they contain a large amount of zinc. Using this characteristic of DTZ, insulin-producing cells derived from rabbit's bone marrow mesenchymal stem cells was identified as well as cellular clusters. DTZ is a zinc-binding substance, and pancreatic islets from such animal species as mouse, rabbit, rat, dog, pig, and human are known to be stained crimson red by its treatment, because of their higher zinc contents compared with other tissues [(27 , 28)] .

REFERENCES

1. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet

transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoidfree immunosuppressive regimen. N Engl J Med 2000; 343: 230–8.
2. Ramiya VK, Maraist M, Arfors KE, Schatz DA, Peck AB, Cornelius JG. Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. Nat Med 2000; 6: 278–82.
3. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose- competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest 2003; 111: 843-50.
4. Schmied BM, Ulrich A, Matsuzaki H, Ding X, Ricordi C, Weide L, Moyer MP, Batra SK, Adrian TE, Pour PM. Transdifferentiation of human islet cells in a long-term culture. Pancreas 2001; 23: 157-71.
5. Lipsett M, Finegood DT. Beta-cell neogenesis during prolonged hyperglycemia in rats. Diabetes 2002; 51: 1834–41.
6. Schwitzgebel VM, Scheel DW, Conners JR, Kalamaras J, Lee JE, Anderson DJ, Sussel L, Johnson JD, German MS. Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. Development 2000; 127: 3533-42.
7. Jensen J, Heller RS, Funder-Nielsen T, Pedersen EE, Lindsell C, Weinmaster G, Madsen OD, Serup P. Independent development of pancreatic alpha- and beta-cells from neurogenin3-expressing precursors: a role for the notch pathway in repression of premature differentiation. Diabetes 2000; 49: 163-76.
8. Guz Y, Nasir I, Teitelman G. Regeneration of pancreatic beta cells from intra-islet precursor cells in an experimental model of diabetes. Endocrinology 2001; 142: 4956–68.
9. Zulewski H, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W, Muller B, Vallejo M, Thomas MK, Habener JF. Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. Diabetes 2001; 50: 521-33.
10. Soria B, Roche E, Berna G, Leon-Quinto T, Reig JA, Martin F. Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. Diabetes 2000; 49: 157–62.
11. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 2001; 292: 1389-94.
12. Assady S, Maor G, Amit M, Itskovitz-Eldor J, Skorecki KL, Tzukerman M. Insulin production by human embryonic stem cells. Diabetes 2001; 50: 1691-7.
13. Nambu M, Ishihara M, Nakamura S, Mizuno H, Yanagibayashi S, Kanatani Y, Hattori H, Takase B, Ishizuka T, Kishimoto S, Amano Y, Yamamoto N, Azuma R, Kiyosawa T. Enhansed healing of mitomycin C-treated wounds in rats using inbred adipose tissue-derived stromal cells within an atelocollagen matrix. Wound Repair. Regen. 2007 ;15: 505-10.
14. Fuchs E, Segre JA. Stem cells: A new lease on life. Cell 2001 ; 100: 143-55.
15. Woodbury D, Schwaz E, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. J Neuro Scien Res, 2000; 61:364-70.
16. Chapel A, Bertho JM, Bensidhoum M, Fouillard L, Young RG, Frick J. Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multiorgan failure syndrome. J Gene Med, 2003; 5: 1028- 38.
17. Eslaminejad MB, Nikmahzar A, Taghiyar L, Nadri S, Massumi M. Murine mesenchymal stem cells isolated by low density primary culture system. Dev Growth Differ, 2006 ; 48: 361-70.
18. Shiroi A, Yoshikawa M, Yokota H, Fukui H, Ishizaka S, Tatsumi K, Takahashi Y. Identification of insulin-producing cells derived from embryonic stem cells by zincchelating dithizone. Stem Cells 2002 ; 20: 284-92.
19. Path G, Seufert J. Current status and perspectives of stem cell therapy for the treatment of diabetes mellitus. Med Klin 2003; 98: 277-82.
20. Welssman IL,,Sader,M, German MS. Stem cells: units of development. Cell 2000; 100:157-68 .
21. Kojima H, Fujimiya M, Matsumura K, Nakahara T, Hara M, Chan L . Extrapancreatic insulin-producing cells in multiple organs in diabetes. *Proc Natl Acad Sci U S A* 2004 ; 101:2458–63.
22. Lagasse E, Connors H, Al-Dhalimy M et al. Purified hematopoietic stem cell can differentiate into hepatocytes in vivo. Nat Med. 2000; 6:1229-34.
23. D'Amour KA, Bang AG, Eliazer S. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol. 2006; 24:1392-401.
24. Jahr H, Bretzel RG. Insulin-positive cells in vitro generated from rat bone marrow stromal cells. Transplant Proc. 2003; 35(6):2140-1.
25. Oh SH, Muzzonigro TM, Bae SH, LaPlante JM, Hatch HM, Petersen BE. Adult bone marrow-derived cells trans-differentiating into insulin-producing cells for the treatment of type I diabetes. Lab Invest. 2004; 84(5):607-17.
26. Kolb H, Burkart V. Nicotinamide in type 1 diabetes. Mechanism of action revisited. Diabetes Care 1999; 22(Suppl 2): B16-20.
27. Miyamoto M, Inoue K, Gu Y. Improved large-scale isolation of breeder porcine islets: possibility of harvesting from non heart-beating donor. Cell Transplant 1998; 7:397-402.
28. Shewade YM, Umrani M, Bhonde RR. Large-scale isolation of islets by tissue culture of adult mouse pancreas. Transplant Proc 1999; 31:1721-3.

Immunohistochemical expression of Cyclooxygenase 2 and Caspase 7 in oral lichen planus

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ABSTRACT

Background: Oral lichen planus (OLP) is one of the most common dermatological diseases presenting in the oral cavity. Although relatively frequent, OLP is the target of much controversy, especially in relation to its potential for malignancy. This study was conducted to find biological changes in the expression of caspase 7 and cyclooxygenase 2(cox2) in OLP by immuohistochemistry and to explore the correlation between them.

Materials and Methods: Fifteen cases of randomly chosen paraffin embedded tissue blocks of OLP with 5 normal oral mucosa cases were included in this study. Immunohistochemistry was performed to evaluate Cox2 and caspase 7 proteins expression.

Results: The expression of cox2 was positive in all studied cases of OLP with negative expression in normal oral mucosa. Caspase 7 expression was positive in (73%) of the cases of which (36.5%) showed strong positive expression score. Non-significant positive correlation was found between the two markers.

Conclusion: This study provided further evidence that epithelial cells in OLP undergo apoptotic death, on the other hand they develop high rate of inflammation which may create a good environment for malignant transformation.

Key words: oral lichen planus, cox2, caspase 7, immunohistochemistry. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):61-64).

INTRODUCTION

Oral lichen planus is a chronic inflammatory disease of oral mucosa. The world health organization has defined it as potentially precancerous disorder, representing a generalized state associated with a significantly increased risk of cancer [(1, 2)].

Cyclooxygenase-2 which is an inducible enzyme in most cell types including keratinocytes, fibroblast and Tcell, catalyzes the synthesis of prostaglandins [(3)].Several processes in cancer may be influenced by Cox2 including cell proliferation ,apoptosis, and angiogenesis.Cyclooxygense2 may inhibit apoptosis via different pathways like down- regulation of arachidonic,up regulation of proto-oncogene Bcl2 and down-regulation of Bax, thus contributing to increased survival [(4)].

Caspase 7 is a member of the caspase family and has been shown to be an executioner protein of apoptosis. The precursor of this caspase is cleaved by caspase-3,9and 10.It is activated upon cell death stimuli and induces apoptosis [(5)].It is a303-amino acid protein with high Similarity to caspase-3.Caspase -3 and caspase-7 are functionally similar substrate specificities [(6)].Caspase -7 is important to caspase -3 in apoptosis execution, especially in the cells with deficient or under expressed caspase-3[(7)].

MATERIALS AND METHODS

Fifteen retrospective tissue samples of paraffin embedded blocks histologically verified as oral lichen planus were randomly chosen from the archives of oral pathology department, College of Dentistry, Baghdad University.

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Immunohistochemistry staining procedure

Immunohistochemical staining technique was performed to examine Cox2 and caspase 7 protein expressions .Four um thickness sections of each case were cut and mounted on positively charged slides for immunohistochemical staining with monoclonal antibodies Cox2 and caspase 7(Abcam).

Positive and negative tissue controls were obtained according to antibodies manufacturer data sheets and included in each run. Normal oral mucosa (5 cases) from voluntary healthy individuals was also included in the study.

Assessment of immunohistochemical results:-

The immunoreactivity of cox2 was evaluated according to (8).Immunostained regions at 400 magnification were scored as follows :positive expression was evaluated by taking three fields per case and staining intensity was scored as 0(negative),1(weak),2(weak),and 3(strong).Staining extent was scored as o(0%) ,1(1-25%) ,2(26-50 %),3(51-75 %),and 4(76-100%)according to the percentage of positively stained cells. The sum of intensity and extent scores was used as the final staining score. All cases were divided into four groups as follows:-0=(negative) ,1-3(low) 4-5(moderate),and 6-7(high).If the scores were moderate or high, cases were classified as cox2 over expressed.

Regarding Caspase 7 immunostaining evaluation, the staining extent was scored as follow:-0<5%, 1:5-25,%,2:26-50%,3:51-75%and 4:>75% and the Staining intensity was graded as follow:0:negative ,1:weak ,2:moderate ,3:strong.The final score was achieved by multiplication of the two scores above and scores of 0-4 were defined as negative expression (-

),scores of 5-8 as weakly positive expression (+)and scores of 9-12 were defined as strongly positive expression (++) (9).

Statistical analysis; Numerical values were used to describe variables which include, No, Mean, SD for age , cox2 and caspase 7.Pearson correlation coefficient of correlation (r) was used to find the relation between the two markers .The statistical analysis achieved by using SPSS (statistical package for social sciences).

RESULTS

The mean age of the study sample was (44) years +stDv (13.8), ranged from (22-68) years, eight of them were males and seven were females. The most predominant site was the buccal mucosa (66.7%) followed by the tongue (20%).

Cox-2 expression was indicated as brown granular cytoplasmic and membranous staining in both basal and parabasal epithelial cells,Fig.(1). The results of this study showed positive expression of cox2 enzyme in all OLP cases with strong positive expression in 5(33 %) cases, moderate positive expression in 4(27 %) cases and weak positive in the remaining 6(40 %) cases (Table 1).

Table 1: Cox2 expression in 15 cases of OLP

Cox2 expression	No.	%
Low	6	40
Moderate	4	27
High	5	33

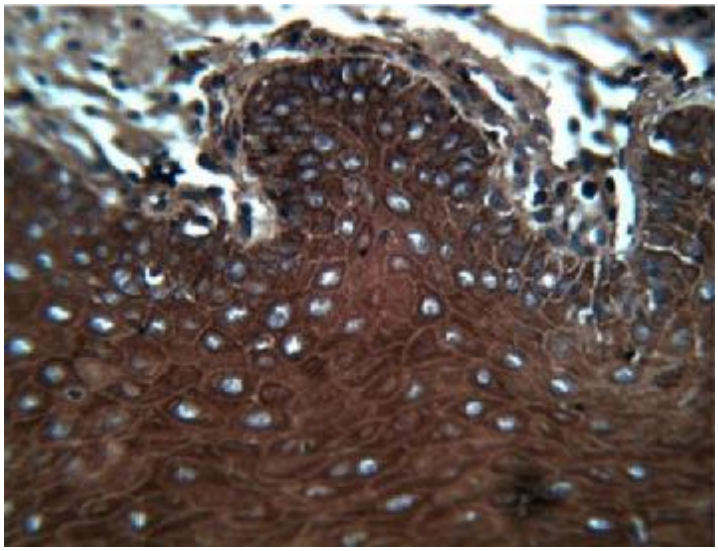

Figure 1: Strong positive cox2 immunohistaining in olp (brown granular cytoplasmic and membranous staining in both basal and parabasal epithelial cells). X400.

Caspase 7 expression was detected as brown granular mostly cytoplasmic immunohistaining of the basal and parabasal cells, Fig. (2). Positive expression was found in 11(73%) of the cases. Of them, 4(36%) cases showed strong positive expression and 7(64%) with weak positive expression as clarified in table (2).

Table 2: Caspase 7 expression in 15 cases of OLP

Caspase 7 expression	No.	%
Negative(0-4)	4	27
Weak positive(5-8)	7	64
Strong positive (9-12)	4	27

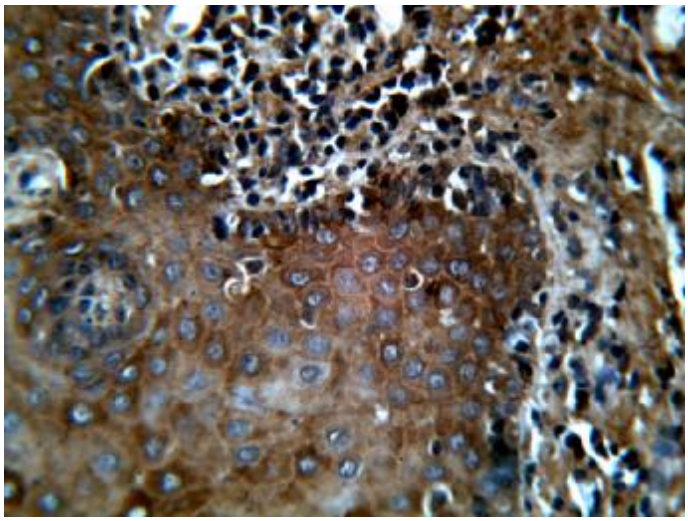

Figure 2: Strong positive caspase7 immunohistaining in olp (brown granular cytoplasmic staining of basal and parabasal epithelial cells). X400.

Regarding the correlation between either markers expression, the present study revealed a statistically non significant positive correlation.

DISCUSSION

In order to investigate potential biological objective predictive marker, the expression of cox2 and caspase 7 were evaluated on biopsies of oral lichen planus by immunohistochemistry.

The results of cox2 immunohistochemistry revealed positive expression in all investigated olp cases in basal and parbasal cell layers with negative expression in normal oral mucosa. This finding is in agreement with a previous study [10].

Similarly, a varying degree of cox2 expression was observed in sub epithelial infiltrate of olp. In previous studies on oral sequamous cell carcinoma, results revealed positive expression of cox2 in all studied cases with negative expression in normal oral mucosa [12]. These findings supported the suggested link between chronic inflammation and the development of oral squamous cell carcinoma in olp.

Regarding caspase 7 expressions, results showed increased expression in olp cases in comparison to normal oral mucosa with varying degree of expression among different OLP lesions. Up to our knowledge there are no earlier reports on caspase 7 expressions in OLP, however caspase cascade pathway had been investigated in olp [13]. Similarly caspase 3 expression in OLP was studied that revealed high expression in olp lesions compared with normal oral mucosa with co-localization in basal and supra basal epithelial layers suggesting that proliferating epithelial cells may be targeted for destruction in OLP. [14].There are several studies on Bcl-2 expression in OLP lesions, which have all shown only weak Bcl-2 expression in OLP keratinocytes,supporting the role of apoptosis in OLP [14-16]. These findings supported the present finding since caspase 3 and caspase 7 are functionally similar in substrate specificities and caspase 7 is important for caspase 3 in apoptosis. Similarly, previous study on capase 7 expression in OSCC showed positive expression in74% o f cases. [17].This study confirmed the view that apoptosis may play a role in olp tumor genesis.

Regarding the correlation between cox2 and caspase 7 expressions, the present finding showed a non significant positive correlation, however further studies with larger samples are needed to find out the relation between these two markers.

As a conclusion, this study provided further evidence that epithelial cells in olp die by apoptosis, on other hand they develop high rate of inflammation which may create a good environment for malignant transformation.

REFERENCES

1. Abdel-Latif AM, Abuel-Ela HA, El-Shourbagy SH. Increased caspase-3 and altered expression of apoptosis-associated proteins, Bcl-2 and Bax in lichen planus. Clin Exp Dermatol; 2009; 34(3):390-95.
2. Bascones C, Gonzalez-Moles MA, Esparza G, Bravo M, Acevedo A, Gil-Montoya JA, Bascones A. Apoptosis and cell cycle arrest in oral lichen planus Hypothesis on their possible influence on its malignant transformation. Arch Oral Biol 2005; 50(10):873-81.
3. Cortés-Ramírez DA, Rodríguez-Tojo MJ, Gainza-Cirauqui ML, Martínez-Conde R, Aguirre-Urizar JM. Overexpression of cyclooxygenase-2 as a biomarker in different subtypes of the oral lichenoid disease. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010 110(6):738-43.
4. Fernades AT, Armstrong RC, Krebs J, Srinivasula

SM, Wang L, Bllrich F, et al. Proc Natl Acad Sci U.S.A. 1996; 93: 7464-9.
5. Jabur AS, Alkaisi OR. Immunohistochemical Expression of Caspase 7 and Annexin V as Apoptosis Markers in Oral Squamous Cell Carcinoma (A clinicopathological study). J Bagh College Dentistry; 2011; 23(2): 74-7.
6. Lakhani S A,Masud A,kuida K,Porter G A,Jr et al .Caspase 3 and 7:key mediators of mitochodrial events of apoptosis. Science 2006; 311: 847-51.
7. Lin DT, Subbaramaiah K, Shah JP, Dannenberg AJ, Boyle JO Cyclooxygenase-2: a novel molecular target for the prevention and treatment of head and neck cancer. Head Neck 2002; 24(8):792-9.
8. Maglott D,Pruitt K, and Tatusova T:caspase -7 apoptosis-related cysteine peptide, Entrez Gene :A Directory of genes; 2005
9. Merza SM, Ali RD. Immnohistochemical expression of cyclooxygenase-2 and heparanase enzymes in oral squamous cell carcinoma. Egyptian Dent J 2011; 57(1) 225-9.
10. Mattila R, Syrjänen S. Caspase cascade pathways in apoptosis of oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010; 110(5): 618-23.
11. Neppelberg E, Johannessen AC. DNA content, Cyclooxygenase-2 expression and loss of E-cadherin expression do not predict risk of malignant transformation in oral lichen planus. Eur Arch Otorhinolaryngol 2007; 264(10): 1223-30.
12. Se Min Jang, Young Jin Jun, Woong Na Clinicopathologic significance of cyclooxygenase-2 over expression in colorectal adenocarcinoma. Basic and applied Pathology 2010; 3: 14-20
13. Smith WL, DeWitt DL, Garavito RM .Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000; 69: 145-82.
14. Sousa FA, Paradella TC, Carvalho YR, Rosa LE. Immunohistochemical expression of PCNA, p53, bax and bcl-2 in oral lichen planus and epithelial dysplasia. J Oral Sci 2009; 51(1):117-21.
15. Van der Waal I. Potentially malignantdisorders of the oral and oropharyngeal mucosa;terminology, classification and present concepts ofmanagement. Oral Oncol 2009; 45(4-5):317-23.
16. WHO. World health organization collaborating centre for oral precancerous lesions, Definition of leukoplakia and related lesions; an aid to study precancer. Oral Surg Oral Med Oral Pathol 1978; 46: 518-539.
17. Xue L,Hu N,Song Y,Zou SH,Shou JZ et alTissue microarray analysis reveals a tight correlation between protein expression pattern and progression of esophageal squamous cell carcinoma. BMC Cancer 2006; 6: 296.

Oral manifestations, oral health status and saliva composition changes in a sample of Iraqi systemic lupus erythematosus patients

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease associated with significant morbidity and mortality. Sicca symptoms are frequent in SLE which may be related to concomitant occurrence of Sjögren's syndrome (SS). The aims of study were to determine prevalence of oral manifestations and tempromandibular joint disorders, and to find a correlation between the changes in saliva flow rate, pH and composition with the incidence of dental caries in SLE patients.

Subjects, materials and methods: One hundred and two individuals were enrolled in this study; 52 of them were SLE patients; and 50 were healthy control individuals matched in age and gender. The assessment of dental status was made according to the decay missing filling teeth (DMFT) index; the gingival inflammation was assessed using the criteria of gingival index; Clinical pocket depth was measured with periodontal probe type William, and whole unstimulated saliva samples have been collected from each subject for biochemical analysis. Also, salivary flow rate and pH were measured. After centrifugation, the supernatant of saliva was aspirated for biochemical analysis.

Results: Oral ulceration was the most prominent orofacial manifestations of SLE patients followed by Tempromandibular joint (TMJ) disorders and facial skin rash then oral vesicles& bullae, oral lichen planus and finally oral petechiae & purpura. Salivary flow rate and salivary pH were significantly lower in SLE patients than in the control subjects. Oral hygiene index (DMFT index, gingival index, Clinical pocket depth) were significantly higher in SLE patients than in the control subjects .Salivary calcium, sodium, chloride, and total protein were significantly higher among SLE patients than in the control subjects. While salivary potassium and inorganic phosphorus were significantly lower among SLE patients than in the control subjects. In addition, there was a highly significant positive linear correlation between age of SLE patients and DMFT, and between age and clinical pocket depth; and a highly significant negative linear correlation between salivary flow rate and salivary calcium in SLE patients. Also there was highly significant positive linear correlation between DMFT and salivary calcium, and between DMFT and salivary chloride.

Conclusions: Oral manifestations are common in Iraqi SLE patients. Changes in salivary flow rate, pH, salivary composition, and increased dental caries may serve as potential markers of the extent of autoimmune mediated salivary gland dysfunction which is similar to Sjogren's syndrome.

Keywords: systemic lupus erythematosus, Oral manifestations, saliva. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):65-69).

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a clinical heterogeneous disease which is autoimmune in origin, and characterized by the presence of auto antibodies directed against nuclear antigens. It is by definition, a multi-system disease and patients can present in vastly different ways [(1)].

The clinical heterogeneity of this disease is mirrored by its complex aetiopathogenesis reviewed [(2)].

Dysfunction in immune regulation plays the principal role in the pathogenesis of SLE. Hyper reactivity of B-cells, producing a spectrum of autoantibodies, is primarily responsive for the immune dysregulation, although T-cells are involved in the pathogenesis as well. The tissue injury is caused by immune complexes, deposition of which induces cell infiltration and damage to the tissue by proteolytic and collagenolytic enzymes [(3)].

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The American College of Rheumatology established eleven criteria in 1982 and revised in 1997 [(4)] as a classificatory instrument to operationalise the definition of SLE in clinical trials.

Oral manifestations like recurrent infections or mouth ulcers, severe gingivitis, or excessive dental caries have been found frequently in SLE [(5)]. Many patients with SLE suffer from tempromandibular joint disorders, which are a painful complication of the mandible joint that can impair the ability to speak and chew; Osteonecrosis of the mandible frequently leads to articular collapse, bone destruction and loss of function with varying clinical mandibular dysfunction. This manifestation can also be associated with poor oral hygiene, increasing the risk of oral infections and tooth extraction [(6)].

SLE is closely associated with excretory gland involvement. Thus, oral and ocular symptoms are frequent findings. Minor salivary gland lymphocytic infiltrates are found in 50-75% of the patients; whether they are complaining of dry mouth or not [(7)].

Unstimulated salivary flow rate is decreased in many of the SLE patients; also SLE is a diagnostic component of secondary Sjogren's syndrome (sSS) [8].

MATERIALS AND METHODS

This study was carried out during the period from the middle of November 2010 till the end of March 2011 in Baghdad city The total sample examined in this study consisted of 102 subjects; they were divided into 2 groups; 52 patients (SLE group); 50 patients were females (96.1%) and two patients were males (3.8%); 50 healthy control females (healthy control group). Informed patients consent and ethical approval were obtained for this study; all patients were diagnosed by a Rheumatologist as SLE patients depending on the criteria of the ACR, 1982. All the subjects answered a written questionnaire regarding their name, age, gender, occupation, dental and medical histories, feeling of dry mouth, any oral, and systemic diseases, SLE duration, SLE activity using SLE disease activity index (SLEDAI) [9] and drugs used in the management of the disease, further complications associated with SLE and all investigations (hematological and immunological) were recorded. Examination of the tempromandibular joint was done and all clinically evident changes (clicking, limitation, dislocation…etc) was determined and recorded. Intraoral examination was done for each individual [10] All clinically evidences of mucosal alteration (redness, swelling, ulcer, etc.) was determined and recorded, to find any oral manifestations. The assessment of dental status was made according to the (Decayed, Missed, and Filled Teeth index); the gingival inflammation was assessed using the criteria of gingival index; clinical pocket depth was measured with periodontal probe type William; whole unstimulated saliva samples have been collected from each subject for biochemical analysis. Salivary samples were collected by spitting method. Saliva pH was measured immediately by digital pH meter; salivary flow rate was measured by collection of saliva through 10 minutes, the volume of saliva is recorded in order to give the salivary flow rate in ml/min; after centrifugation the supernatant of saliva was aspirated for biochemical analysis. Calcium, sodium and potassium were measured by using Atomic absorption spectrophotometer; while inorganic phosphorus, chloride and total protein were measured by colorimetric method. Oral manifestations, saliva flow rate, pH, oral health indices, salivary (Calcium Sodium and Potassium Inorganic phosphorus, Chloride and Total protein) of SLE patients were recorded according to (age, duration of disease, disease activity, and treatment) subgroups.

RESULTS

The mean age of SLE patients was 32.24± 9.26 years, with age range of 16-53 years old, while the mean age of healthy control subjects was 29.24± 7.87years, and range of 17-54 years old, according to the age, SLE patients were divided into four sub-groups (15-24, 25-34, 35-44, 45-54) years. It has been shown that the highest percentage of SLE patients and control subjects was in the age group of 25-34 years. Patients with SLE have been divided into four sub-groups according to disease duration periods (< 1, 1-4, 5-9, 10-14) years; it has been shown that the number of SLE patients of (1-4 years) duration period was significantly higher (P< 0.01).SLE patients have been divided according to treatment into three sub-groups: patients on corticosteroid therapy (Predinisolone) (34%), patients on antimalarial drugs (Hydroxychloroquine) (26%) and patients under combination of medications which include: Predinisolone, Hydroxychloroquine and Immunosuppressive drugs (Azathioprine) (40%) with no significant differences in the percentage of SLE patients according to their treatment.

It has been shown that the number of systemic lupus erythematosus patients with active disease in this study was significantly higher than the number of systemic lupus erythematosus patients with inactive (remission) disease according to SLEDAI.

Table 1: Orofacial manifestations in SLE patients

Orofacial manifestations	N	%
Oral ulceration	36	72
Lichen planus	4	8
TMJ Disorders	27	54
Facial skin rash	27	54
Petechiae and purpura	2	4
Vesicles and Bullai	5	10

Oral ulceration (72%) was the most prominent orofacial manifestations of SLE patients followed by tempromandibular joint (TMJ) disorders (54%) and facial skin rash (54%) then oral vesicles& bullae (10%), oral lichen planus (8%)and finally oral petechiae & purpura (4%). Salivary flow rate and salivary pH were significantly lower in SLE patients than in the control subjects (0.36±0.21 versus 0.85±0.29 ml/min, p <0.001; 6.34±0.60 versus 6.74 ± 0.51, p=0.001 respectively).Oral hygiene index (DMFT index, gingival index, Clinical pocket depth) were significantly higher in

SLE patients than in the control subjects (p <0.001).

Table 2: The mean values of different salivary elements in both SLE patients and control subjects:

Salivary elements	SLE (N=50)		Control (N=50)		t	P
	Mean	SD	Mean	SD		
Calcium (µmol/L)	2.35	0.26	1.60	0.23	15.112	0.000
Potassium (µmol/L)	7.77	1.38	21.58	6.68	-14.318	0.000
Sodium (µmol/L)	10.60	2.54	8.76	2.06	3.985	0.000
Chloride (µmol/L)	40.23	5.86	36.36	4.76	3.626	0.000
Inorganic Phosphorus (µmol/L)	4.47	0.85	7.15	1.74	-9.747	0.000
Total Protein (g/100ml)	0.21	0.06	0.13	0.03	7.249	0.000

Significant using t-test, p <0.001

Salivary calcium, sodium, chloride, and total protein were significantly higher among SLE patients than in the control subjects (P<0.001).While salivary potassium and inorganic phosphorus were significantly lower among SLE patients than in the control subjects (P<0.001).In addition, there was …a highly significant positive linear correlation between age of SLE patients and DMFT(r=0.434,p= 0.002**)**, and between age and clinical pocket depth (r=0.355, p=0.012**)**; and a highly significant negative linear correlation between salivary flow rate and salivary calcium in SLE patients(r= -0.396, p=0.004). Also there was highly significant positive linear correlation between DMFT and salivary calcium(r=0.323, p=0.022**),** and between DMFT and salivary chloride (r=0.325, p=0.021**)**.

DISCUSSION

In the present study 50 SLE patients were females; only 2 patients were males and were statistically excluded. The female to male ratio was 25:1, this was agree with other studies who found that the number of SLE female patients was higher than SLE male patients [11,12,13], found that the female to male ratio was 27:1,37:2 and 17:1 respectively. Oral ulceration was the important oral manifestation of SLE patients in the present study which was present in 72% of those patients. Oral ulceration is commonly found in patients with SLE and represents one of the 1982 revised ACR criteria for the classification of SLE. Oral ulcers are present in roughly 25% to 45% of SLE patients. Oral ulcerations in SLE patients have for a long time been considered as a sign of "vasculitis" and predictors of severe systemic flares of the disease [14]. In the present study 8% of SLE patients were with oral lichen planus which agreed with other study [15] who found that Classic lesions of lichen planus (LP) was uncommon and the chances of conversion of the syndrome into systemic lupus erythematosus are 5-10%.

Facial malar rash was found in 54% of SLE patients in the present study, this agrees with other study [16] who found that 58% of SLE patients have malar rash. Tempromandibulare joint disorders were found in 54% of SLE patients in the present study, other study documented the prevalence of TMJ disorders was 60% in SLE patients [17]. Autoimmune disorders, such as rheumatoid arthritis and lupus erythematosus can cause significant inflammation and destruction within the TMJ, Joint disease associated with lupus, is associated with high concentrations of inflammatory mediators within the joint but sometimes is triggered by system-wide immune dysregulation; Pathophysiology of autoimmune-based arthritis of the TMJ is the same as that found in other joints [18].

In the present study oral petechiae and purpura found in 4% of SLE patients, Thrombotic thrombocytopenic purpura (TTP) in patients with SLE is extremely rare. The overall incidence of TTP in SLE patients is unclear and has been reported to be as low as 0.5% [19].Patients with TTP have a severe deficiency of Von Willebrand Factor (VWF) cleaving metalloproteinase (ADAMTS-13), which normally cleaves the unusually large VWF into smaller and less adhesive VWF resulting in micro vascular thrombosis and thrombocytopenia when deficient, Connective tissue disorders like SLE have low levels of ADAMTS-13 suggesting a possible common Pathophysiology for this disease association [20].

In this study it has been shown that oral vesicles and bullai were found in 10 % of SLE patients, bullous lesions can occur in SLE as a subepidermal blistering disease [21], or when severe edema and hydropic degeneration occur in the basal layer. The latter condition is considered a lupus erythematosus (LE)-specific lesion [22].The former condition is a rare disorder characterized by tense fluid-filled vesicles and bulla, with an erythematous or urticarial background. Bullous SLE is a rare, transient autoimmune bullous disease that occurs in established cases of SLE [23].It appears in less than 5% of patients with SLE, either in isolation or in

addition to other cutaneous manifestations, this condition usually affects young females [24].

The reduction in salivary gland function as measured by saliva flow rate in SLE patients result from that the salivary gland are major target organs of SLE. Reduced salivary flow rate and the concomitant reduction of oral defense systems may cause severe caries and mucosal inflammations [25].

One study found that there is association between dental caries and pneumonia in patients with systemic lupus erythematosus (SLE), also found that there is an impaired salivary flow rate in SLE patients, which is considered a risk factor for dental caries [26]

Other study found that the medians of the PI and the GI were higher in JSLE patients than in controls (61.5 versus 38.10, P = 0.003 and 26.0 versus 15.95, P = 0.014; respectively) [27]. another study reported an incidence of periodontitis in 94% of patients with SLE [5], and another case showed 18 of the 30 patients (60%) had periodontitis in their SLE group [28]. to understand the reasons for the observed sialochemical changes in SLE, the process of saliva production needs to be studied closely. Under normal circumstances, primary saliva is secreted into the acinar lumen and subsequently transported to the oral cavity through the salivary ducts by contraction of epimyoepithelial cells and other hydrostatic forces. As primary saliva traverses the striated ducts, salivary composition is modified considerably: phosphate is thought to be slightly concentrated, whereas sodium and chloride are extensively reabsorbed at low flow rate [29].

Duct cells may impair in their function by the periductal lymphocytic infiltration that is present in the major salivary glands affected by the autoimmune disorder; perhaps, locally produced autoantibodies directed against duct cells cause impairment of electrolyte transport in duct cells [30].

The salivary changes observed in systemic lupus erythematosus patients reflect impaired ductal salt re-absorption; the results of this study suggest that changes in salivary flow rate, pH and salivary composition as well as increase dental caries experience in those patients may serve as potential markers of the extent of auto immune mediated salivary gland dysfunction which is similar to Sjögren's syndrome.

REFERENCES

1. Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, and others. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. Medicine (Baltimore) 2003; 82(5): 299–308.
2. Manson JJ, Isenberg DA. The pathogenesis of systemic lupus erythematosus. Neth J Med 2003; 61:343-6.
3. Nguyen QD, CS Foster: Systemic lupus erythematosus and the eye. Int Ophthalmol Clin 1998; 38:33-60.
4. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of Systemic Lupus Erythematosus.ArthritisRheum 1997; 40:1725.
5. Rhodus N, Johnson D. The prevalence of oral manifestations of systemic lupus erythematosus. Oral Pathol 1990; 21:461–5.
6. Merigo E, Manfredi M, Meleti M, Guidotti R, Ripasarti A, Zanzucchi E et al. Bone necrosis of the jaws associated with bisphosphonate treatment: a report of twenty-nine cases. Acta Biomed 2006; 77:109-17.
7. Orteu CH, Buchanan JA, Hutchison I, Leigh IM and Bull RH.Systemic lupus erythematosus presenting with oral mucosal lesions: easily missed Br J Dermatol 2001; 144:1219-23.
8. Patel P, Werth V Cutaneous lupus erythematosus: a review. Dermatol Clin 2002; 20:373-85.
9. Gladman DD, Ibanez D, Urowitz MB. SLE disease activity index 2000. J Rheumatol 2002; 29: 288-91.
10. W.H.O, 1987. Oral health survey, Basic methods 4th edition, World Health Organization, Geneva, Switzerland.
11. Al-Attia HM, George S. Characterization of systemic lupus erythematosus in patients in U.A.E. Clin Rheumatol 1995; 14(2):171-5.
12. Al-Maini MH, Mountz JD, Al-Mohri HA, El-Ageb EM, Al-Riyami BM, Svenson KL, Zhou T, Richens ER. Serum levels of soluble Fas correlate with indices of organ damage in systemic lupus erythematosus. Lupus 2000; 9(2):132-9.
13. Ebrahim RA, Farid EMA, Greally JF. SLE in Bahrain: A review of clinical and laboratory data in 50 Bahraini patients. Emirates Med J 2002; 20(2):147-52.
14. Parodi A, Massone C, Cacciapuoti M, et al. Measuring the activity of the disease in patients with cutaneous lupus erythematosus. Br J Dermatol 2000; 142: 457-60.
15. Jablonska S, Blaszozyk M. Lupus erythematosus. What's new? J EurAcad Dermatol Venereol 2000; 15: 103-5.
16. Jimenez S, Cervera R, Font J, et al. The epidemiology of Systemic lupus erythematosus. Clin Rev Allergy Immunol 2003; 25:3-12.
17. Hellman DB. Arthritis and musculoskeletal disorders. In: Tierney LM, McPhee SJ, Papadakis MA, eds. Current medical diagnosis and treatment. Stamford, Conn.: Appleton &Lange 1996:719-67.
18. Dao TT, Reynolds WJ, Tenenbaum HC. Comorbidity between myofascial pain of the masticatory muscles and fibromyalgia. J Orofac Pain 1997; 11:232–41.
19. Musio F, Bohen EM, Yuan CM, Welch PG. Review of thrombotic thrombocytopenic purpura in the setting of systemic lupus erythematosus. Semin Arthritis Rheum 1998; 28:1-19.
20. Maoke JL. Von Willebrand factor, ADAMTS-13, and thrombotic thrombocytopenic purpura. Semin Hematol 2004; 41:4-14.
21. Yell JA, Wojnarowska F: Bullous skin disease in lupus erythematosus. Lupus 1997; 6:112-21.

22. Vassileva S: Bullous systemic lupus erythematosus. Clin Dermatol 2004; 22: 129-38.
23. Sirka CS, Padhi T, Mohanty P, Patel DK, Parida PR, Kar CR: Bullous systemic lupus erythematosus: response to dapsone in two patients. Indian J Dermatol Venereol Leprol 2005; 71:54-56.
24. Dhir R, Desylva PLK, Gehi N, Malik A, Singh YD, Jagannayakulu H, Tampi PS,Ramasethu R: Pericardial effusion with vesiculobullous lesions in ayoung female. Bullous systemic lupus erythematosus (bullous SLE).Indian J Dermatol Venereol Leprol 2006; 72:175-77.
25. Van der Reijden WA, van der Kwaak JS, Veerman ECI, Nieuw .Amerongen AV. Analysis of the concentration andoutput of whole salivary constituents in patients' with Sjogren's syndrome. Eur J Oral Sci 1996; 104:335-340.
26. Pascual-Ramos V., C. Hernández, A.E. Soto-Rojas, E. Celis-Aguilar, J. Sánchez-Guerrero,. Association between Dental Caries and Pneumonia in Patients with Systemic Lupus Erythematosus. J Rheumatol 2006; 33:1996-2002.
27. Fernandes EG, Savioli C, Siqueira JT, Silva CA. Oral health and the masticatory system in juvenile systemic lupus erythematosus. Lupus 2007; 16:713-9.
28. Novo, E.; Garcia-MacGregor, E.; Viera, N.; Chaparro, N.; Crozzoli, Y. "Periodontitis and anti-neutrophil cytoplasmic antibodies in systemic lupus erythematosus and rheumatoid arthritis: a comparative study." J Periodontol 1999; 70(2): 185-8.
29. Michels LF. Sialometry and sialochemistry. In: GraamansK, Akker van den HP, eds. Diagnosis of salivary gland disorders.Dordrecht: Kluwer Academic Publishers 1991; 139–62.
30. Atkinson JC, Travis WD, Pillemer SR, Bermudez D, Wolff A, Fox PC.Major salivary gland function in primary Sjögren's syndrome and its relationship to clinical features.J Rheumatol 1990; 17:318–22.

Etiology of the oral burning pain and its relationship to sex, age and anatomical sites (Clinical study among a sample of Iraqi patients in Baghdad)

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ABSTRACT

Background: The studies about oral burning pain are few in Iraq in spite of this disease is a significant common among numbers of Iraqi patients, so more information were required in order to avoid its effect and occurrence. The aim of the current study is to determine the actual causes for the disease by examining a sample of Iraqi patients in Baghdad, in order to evaluate its relationship to the age, sex and the anatomical sites.

Subjects and methods: Sixty patients were selected from two hospitals, several specialized dental clinics and public medical clinic, in east of Baghdad (Sadder, Jamella and Baladeyate cities). Nineteen patients were excluded because they could not continue in this study. The remaining forty one patients, 23 female patients, their ages ranged between 25 – 60 years, while the male patients were 18, their ages ranged between 20 – 60 years. The duration of symptoms of burning inside the oral cavity ranged from 6 months to 3 years . Each patient in this study was examined clinically to detect any oral lesion may have direct cause for the disease , also patients were asked about the types of drugs intake , in addition to their psychological conditions . The medical and dental histories were taken from all examined patients, also all medical and dental reports of the patients were determined. Few results of different investigations of the examined patients were replaced by new ones, and the others were taken in consideration for obtaining results for this study. Fasting blood sugar, thyroid function test, histopathological examination and others were examples for such investigations which had been done. The most important finding in this current study that the cause of oral burning pain in the examined Iraqi sample was mainly multi factorial causes and a few cases were caused by single etiology.

Results: This study revealed that the most common causes of the oral burning pain in this Iraqi sample was , hormonal changes , bad psychological conditions , the side effects of some drugs intake , chronic gastritis in addition to other factors. The most anatomical site affected inside the oral cavity was the dorsal portion of the tongue.

Conclusion: One conclusion for the current study in some examined cases was found that a hiding cause may play a role in oral burning pain occurrence beside other factors. This study appeared that female patients over 50 years were more susceptible to this disease than male patients; also older age groups for both sexes were mostly affected.

Key words: Oral burning pain, Menopause. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):70-77).

الخلاصة :

أن الدراسات حول مرض حرقة الفم قليلة في العراق رغم أن هذا المرض شائع جدا بين أعداد من المرضى العراقيين الذين يعانون من هذه المشكلة لذا فأن المزيد من المعلومات حول هذا المرض أو المتلازمة مطلوب لغرض التقليل من انتشاره وتأثيره .

الهدف من الدراسة الحالية هو لتحديد الأسباب الحقيقية والفعلية لمرض حرقة الفم من خلال فحص نموذج لمرضى عراقيين في بغداد وكذلك لتقييم العلاقة بين هذا المرض وجنس و عمر المريض إضافة إلى المناطق التشريحية الأكثر تعرض داخل الفم بسبب هذا المرض .

ستون مريضاً تم اختيارهم من مستشفيين وعدة عيادات اختصاصية لطب الأسنان إضافة إلى عيادة طبية شعبية في شرق بغداد ومن مناطق الصدر و جميلة و البلديات , تسعة عشر مريض تم أبعادهم من هذا النموذج لعدم إمكانيتهم من الاستمرار في هذه الدراسة , ما تبقى واحد وأربعون مريضاً , ثلاثة وعشرون مريضة من الإناث تتراوح أعمارهن من (25-60) سنة , بينما المرضى الذكور كانوا ثمانية عشر مريضاً وأعمارهم تتراوح بين (20-60) سنة , فترة جهور أعراض الحرقة داخل الفم تراوحت بين ستة أشهر وثلاث سنوات . كل مريض مشارك لغرض هذه الدراسة يفحص سريرياً لإيجاد أية بجرة مرضية قد تكون سبباً مباشراً لهذا المرض . وتم أيضا مسائلة المرضى حول أنواع الأدوية التي يتعاطوها إضافة إلى التساؤل عن حالتهم النفسية , كذلك تم اخذ التاريخ المرضي ولحالة الأسنان من جميع المرضى المفحوصين إضافة إلى إن جميع التقارير الطبية وما يتعلق بالأسنان تم أخذها بنظر الاعتبار , قليل من النتائج لمختلف الفحوصات تم استبدالها بأخرى جديدة بينما أخذت الأكثرية بنظر الاعتبار بهذه الدراسة , وكأمثلة لهذه الفحوصات هي , فحوصات الدم لغرض أيجاد نسبة السكر الصباحي (بدون فطور) وفحوصات الغدة الدرقية وكذلك الفحوصات المرضية النسيجية وغيرها . من أهم الأمور التي تم إيجادها خلال هذه الدراسة هو انه سبب مرض حرقة الفم ومن خلال النموذج المفحوص هو عامل متعدد الأسباب ومن أهم هذه العوامل هي التغيرات الهرمونية . سوء الحالة النفسية , التأثيرات الجانبية للأدوية التي يتعاطاها المرضى , التهاب الأمعاء المزمنة إضافة إلى أسباب أخرى . كذلك أظهرت هذه الدراسة بأن أكثر المناطق التشريحية تأثراً بهذا المرض هو السطح الظهري للسان . واحدة من استنتاجات الدراسة الحالية هي أن في بعض الحالات قد يكون هناك سبب خفي إلى جانب العوامل الأخرى قد تلعب دوراً في حدوث مرض حرقة الفم . كذلك بينت الدراسة الحالية بأن المرض يصيب كلا الجنسين ولكن النساء فوق الخمسين سنة تكون أكثر تعرضاً من الرجال والأعمار المتقدمة لكلا الجنسين غالباً ما تتأثر بهذا المرض .

INTRODUCTION

Oral burning pain is a burning symptom in the oral cavity or tongue that might be associated with clinical or laboratory abnormalities. The pain may coexist with other oral conditions; patients with oral burning pain may have a significant emotional impact which suspected of exaggerating their symptoms. The condition is seen most often in women, particularly those are post menopausal. Oral burning pain is often absent during the night but progressively increases throughout the day and into the evening [1]. Oral burning pain is common complex problem that causes the individual to experience burning pain on the lips, tongue and some times throughout the mouth. There are often no visible signs of irritation [2].

The main symptom of oral burning pain is moderate to severe burning in the mouth and can

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persist for months or years, in many people, the burning sensation begins in late morning, builds to a peak by evening, and even subsides at night. Anxiety and depression are common in people with oral burning pain and may result from chronic pain [3]. It is well known that symptoms of burning in the oral tissues are concomitant to certain oral diseases. However, burning symptoms might occur when oral mucosa has clinically healthy appearance. Additionally, in some patient underlying local and systemic condition which could lead to symptoms of burning might be found, finally, in patient where these clinical and laboratory finding are lacking, diagnosis of true oral burning pain is established and that all the other oral burning pain are due to a different pathologies representing with one symptom within the clinical spectrum of such a group of pathologies [4].

Several diseases of the oral mucosa may have burning as symptoms, such as herpes simplex virus, aphthous stomatitis, xerostomia and others. However, patients who refer a burning sensation if the oral mucosa or a chronic pain without any visible alteration of the oral tissues might be diagnosed as having burning mouth syndrome. It is very important for clinicians to be able to distinguish the oral burning pain caused by a specific disease beside burning mouth syndrome (BMS). Burning mouth syndrome (BMS) usually affects people over the age of sixty years and occurs more in older women [5].

The etiology of the burning mouth syndrome is still poorly understood, recent studies have proposed neurological background, trigeminal small – fibers sensory neuropathy, also interactions between taste and oral pain are responsible for oral burning pain and that the intensity of the peak oral pain correlated with the density of fungi form papillae [6]. Among the possible risk factors for oral burning pain are numerous psychopathological situations in which the microcirculatory mechanisms are involved in pain generation. A local microcirculatory disturbance in the areas affected by oral burning pain could contribute to the burning sensation descried by patients. Some authors found that patients with oral burning pain had Para functional habits such as tooth grinding, clenching (bruxism), or tongue thrusting that could lead to changes in the intra – oral blood flow. Oral burning pain may be caused by hormonal changes, vitamin deficiencies, systemic diseases such as diabetes, some drugs intake, sensitivity to some materials, food and other causes [7]. Therefore this study aimed to evaluate the causes of oral burning pain and its relationship to sex, age and anatomical sites among a sample of Iraqi patients in Baghdad.

PATIENTS AND METHODS

Patients

Sixty patients were studied in this study they were collected from:

a- Al-Imam Ali general Hospital in Sadder city.
b- Al-Sadder Hospital also in Sadder city.
c- Medical public health clinic in Baladeyate – Baghdad.
d- Several private special dental clinics in Jamella city.

Nineteen patients were excluded from this study because they could not continue to the end of this study. The remaining forty-one patients were used to achieve this study and actually had symptoms of oral burning pain and they suffered from the disease. Forty-one patients were 23 female patients, their ages ranged between 25 – 65 years, while the 18 male patients, their ages ranged between 20 – 65 years.

Methods

To detect the causes of the oral burning pain and its relationship to sex, age and anatomical sites for this study, the following procedures were done:

a. History taking: included knowledge about the medical, dental and psychological conditions for the examined patients.

1. Determine the previous medical reports for the examined patients from the Hospitals, special medical or dental centers and special private clinics.
2. Recording of the previous tests (investigations) for the examined sample of patients to avoid the repeating of the tests and to save time.
3. The old previous investigations were replaced with new ones.
4. Patients were asked about the medication intake specially the anxiolytic, angiotensin, diabetic drugs and antispasmolytic medication and others.

b. Clinical examinations: Dental and medical history was taken from each participant, also clinical examination of the oral cavity was performed, the quantity and the type of saliva, concerning xerostomia, the mouth dryness is primarily felt by the patient himself (subjective xerostomia) when there are insufficient amount of saliva inside his mouth. The examiner also can

identify the quantity of saliva clinically by lipstick method on the labial surface of the anterior maxillary teeth.

Also when the tongue is held by blade against the buccal mucosa, then the blade is lifted away; the tissue will adhere to the tongue; these signs suggest the mucosa is not sufficiently moisturized by saliva and that means hypo secretion of salivary glands. Measurement the quantity of saliva was done by assessment the salivary flow rate by using simple spitting method into calibrated tube during five minutes while participants were sitting; values lower than 0.2 ml/minutes were considered as an indicator of xerostomia. Altered taste sensation was described by the patient either with reduction in taste perception or the presence of a persistent unusual frequently bitter or metallic taste, or alteration in taste included complaints of changes in the intensity of taste perception. The type of saliva can be evaluated by examining the remaining of saliva inside the patient mouth or obtaining excessive saliva by stimulating the major salivary glands in order to determine the consistency of saliva whether it is watery, clear, viscous or scant in nature. The conditions of oral hygiene was assessed clinically by mirror and probe to detect any dental caries, pocket, calculus and any other oral lesions, also evaluation to the dentures and bridges that the patients wearing from any porosity and roughness, in addition to that special attention must be given to the dentures, since it has been demonstrated that there is a possible correlation between the adjustment and the design of denture both may cause changes in the sensory nerve function, causing atypical oral pain. Any sensitivity or allergies were detected to the denture material or foods depending on patient's experience or clinician observation (such tests not sent to the laboratory to investigate the sensitivity test).

c. Laboratory investigations:

1. Hematological screening for complete blood count, glucose level, thyroid function test, blood serum and nutritional factors.
Blood work was done to look for infection, nutritional deficiencies and disorders associated with oral burning sensation such as diabetes or thyroid gland problems. Complete blood picture was used to asses HB, PCV, ESR and the counts of white and red blood cells. Fasting blood sugar was done to estimate the level of glucose in the blood to prove the previous diagnosis; whether the examined patients were really diabetes (hyperglycemic) or not. Thyroid function test also was done to prove the previous diagnosis of hypothyroidism by using TSH, T3 and T4 tests to estimate the level of thyroxin in the blood. The mal nutrition patients were diagnosed according to referral forms by their physicians describing them that they suffered from, pale skin, brittle finger nails, weakness, loss of appetites and loss of hair in addition to oral sensation. Mal nutrition may be caused by chronic mal absorption which trigger a loss of appetite and abnormal metabolism. The laboratory investigations were described as follow: anemia typically estimated by HB of less than 11gm/dl, while B_{12}, iron deficiencies were detected by performing complete blood picture tests and finally minerals were investigated by biochemical test of blood serum, all these signs and investigations proved the presence of malnutrition and iron deficiency anemia.

2. Some examined patients were diagnosed by the Gastrointestinal physicians that they were affected with helicobacter pylori bacteria and developed gastro oesephagal infection according to their medical records and referral forms since the diagnosis was present in the referral forms, in Iraq, the test which required to identify the H. pylori bacteria called (H. pylori antigen rapid test) and it is described in brief as follow; H. pylori antigen in human stool specimen, the test results are intended to aid in the diagnosis of H. pylori infection, to monitor the effectiveness of therapeutic treatment and to confirm the eradication of H. pylori in peptic ulcer patients.

Other report related to patients with BMS and affected with gastritis by H. pylori bacteria, described the detection of this bacteria by using an endoscopy to go down the throat to collect a tissue sample from the stomach to be tested histopathologically, these procedures were conducted under anesthesia.

3. Oral candidiasis cases: Candidiasis species from oral mucosa can be scraped off easily (scraping test) to differentiate it from other oral white lesions leaving ulcerative painful surface, for the direct microscopic examination and detection of Candida-albicans hyphae, scraped specimen from the inflamed tissues was collected by using a sterile lancet, also it is possible to culture Candida using a sabauraued agar to aid in the definitive identification of the fungal organism. The procedures that were followed in the laboratory concerning Candida described as: The swab was cultured on sabauraued media for 24 hours in 37c by biochemical test; identification of Candida-albican will be confirmed.
   In brief words, positive cultures for samples from the mouth, Candida was observed and by biochemical test Candida-albican was the most species in the examined samples.
4. Psychiatric diagnosis were examined by reviewing patients medical records and referral forms, since a number of diagnoses were present in the referral forms, they were categorized according to the International Statistical Classification of Disease and related Health problems, into tenth revision (ICD-10).

RESULTS

Twenty three female patients and eighteen male patients , that was forty one males and females patients all of them suffered from oral burning pain, those patients were examined in this study to determine the actual causes of this disease among the tested samples and the relation of age , sex and oral sites affected. The examined patients were divided into female and male patients, the following procedures for obtaining the results were done as follow:

1. <u>The female patients</u>: Forming 56.1% from the total examined cases. Table 1 illustrated that female patients over 50 years were mostly involved by oral burning pain due to hormonal changes and psychological conditions, the tongue was the common anatomical site affected, and they were described as follow:

a- Seven females forming 30.6% from the total examined females and about 17% of the total included cases, aged over 50 years suffered from oral burning pain with duration about (1-1.5) years. Three of them complained from depression, they received sedative drugs, according to medical report one of them her psychiatrist physician described her; diminished ability to think or concentrate or indecisiveness nearly every day either by subjective account or as observed by others. The other two women with depression had medical reports from the Hospitals and physicians describing them that they are in a mild, chronic depression, they may not realize that they are depressed, anti-depressant or psychotherapy can help. The psychiatric conditions were classified according to the referral form into:
   F_6: Two female patients (disorders of adult personality and behavior).
   F_3: One female patient (mood affective disorders), also in this group there were other oral pathological conditions related to those female patients, such as dental pockets, poor oral hygiene, gingivitis, calculus, caries and finally mobile or missing teeth. The sensation was present in the tip, dorsum of the tongue and floor of the mouth.

b- Three females formed 13.1% from the total examined females, aged (35-50) years , had a history of hyperglycemia , according to previous medical reports , the diagnosis of diabetes was confirmed by doing new fasting blood sugar test, the symptoms duration of oral burning pain was about 2 years. The sensation was present in the lower lip and lower mucosa.

c- Two females formed 8.6% from the total females; aged (30-40) years respectively had a history of hypothyroidism, to confirm this diagnosis, the thyroid function test was done to estimate the level of thyroxin in the blood by doing TSH, T3 and T4 test and this test proved the previous diagnosis. The duration of the symptoms was about 9 months. The sensation was present in the lateral sides of the tongue and corner of the mouth.

d- Three females formed 13.1% from the total examined females, were under the medication of angiotensin drugs with cardiac problems according to medical reports from the Hospitals, their ages ranged between (55-65) years, and the duration of oral burning pain symptoms was about one years. Two of them received drugs for heart disease in addition to the antihypertensive drugs. The sensation was present in the ventral surface of the tongue, floor of the mouth and hard palate.

e- Three females formed 13.1% from the total examined females, with oral burning pain aged (25-40) years had a history of chronic gastrointestinal infection according to reports from private Gastroenterologist, revealed that those patients affected with H.pylori infection, in Iraq the presence of this type of bacteria is detected by H.pylori antigen rapid test, this test depending on detection of H.pylori antigen in human stool specimen, or by histopathological examination to the stomach biopsy by using an endoscopy to collect a tissue sample from the stomach to be tested. The duration of the oral sensation was about (3-4) years ago. The sensation was present through all over the mouth and soft palate.

f- Two females formed 8.6% from the total females, with oral burning pain aged (60, 65) years with long duration of symptoms sensation suffered from chronic vomiting with irritable colon according to their previous medical reports, this may cause acid reflux, also they were under medication. The pain sensation was present in tongue, lower lip and lower gingiva.

g- One female 28 years with oral burning pain, formed 4.3% from the total females had microglossia, sore tongue and congested oral soft tissues, the duration of the sensation was about 9 months. The sensation was present in the dorsum, ventral, lateral and tip of the tongue.

h- Two females formed 8.6% from the total females, aged (32 , 38) years respectively with oral burning pain and duration of 1 year , the previous and recent blood tests revealed the presence of Iron deficiency anemia for both of them, one female had pale face with fainting attack. The oral sites of sensation were the dorsum of the tongue, oro-pharangeal portion and buccal cheek.

2- <u>The male patients</u>: Forming 43.9% from the total examined patients. Table 2 showed that poor oral hygiene and bad dental condition male patients in different age groups were mostly affected, and the tongues, floor of the mouth were commonly involved by oral burning pain.

The eighteen male patients with oral burning pain, who involved in this study, were described as follow:

a- Four male patients with oral burning pain formed 22.3% from the total examined males, aged between (50-65) years with duration of burning sensation for about 1.5 years, had ill fitting dentures, and one of them had poorly constructed bridge, the oral sites affected were the mucosa of the lower lip, ventral site of the tongue and buccal cheek.

b- Five male patients formed 28.1% from the total examined males and about 12.2% of the total included cases, with different age groups had a history of very bad oral hygiene, with bad dental condition and multi retained roots in addition to bad oral odor, the oral sites affected were all the oral mucous tissues specially the floor and the corners of the mouth.

c- Three male patients formed 16.6% from the total examined males, aged (30-55) years, with duration of sensation for 6 months, they were treated from renal colic by anti spasmolytic drugs, which may cause dry mouth and the affected oral sites were lips, dorsum of the tongue and floor of the mouth.

d- One male patient formed 5.5% from the total examined males, aged 53 years, under modueretic drugs also suffered from oral burning pain since 4 months ago, the main affected oral sites were the tongue, buccal check and the floor of the mouth.

e- Two male patients with oral burning pain, formed 11% from the total examined males, one of them 39 years had fissure tongue with food lodging inside the fissures. The other male patient 57 years, suffered from recurrent ulcers mostly appeared in the tip and the ventral surface of the tongue, the duration of sensation appeared 1.5 years ago.

f- Two male patients formed 22.3% from the total examined males, aged (20, 35) years suffered from psychological disturbances and received anxiolytic and sedative drugs according to medical reports from psychiatric physicians, the duration of the sensation was about 1 year. All over the mouth was affected.

g- One male patient formed 5.5% from the total examined males, aged 45 years with oral burning pain, developed severe gingival hyperplasia with pus discharge and bad oral odor; the common oral sites affected were the gingiva and the palate. This study revealed that female patients with hormonal changes and psychological problems mostly suffered from BMS, while male patients with poor oral hygiene and

bad oral conditions mainly complained from BMS.

DISCUSSION

Instability of the social environment is considered an important factor which has a negative impact reflects on the health status of the society. Sanctions, wars and postwar pollutions, economical and political instability all are factors the Iraqi society has been exposed for years. The incidence of diseases and stress related conditions is usually increased in such circumstances. BMS is a condition usually of unknown reason but may be associated with stress or hormonal disturbances. So this study revealed that the causes of the oral burning pain were mostly multi-factorial because more than one etiology may share in causing this disease, so it appeared from this study that both males and females were affected but it was mostly more common in females especially those with older ages. This study revealed that the older ages were mostly involved and the tongue was the most common oral anatomical site affected. The hormonal changes in women during menopausal period, psychological disturbances and the side effects of the drugs intake were the most possible etiological factors for the oral burning pain. Females suffered from oral sensation may be explained due to hormonal changes during the transitional and post menopausal period, when the ovaries make much less estrogen and progesterone or may be due to diabetes disease.

The etiology in the studied sample was mainly multi factorial and a single cause for a few numbers of cases, but in other similar statistical studies population was found that the main causes of oral burning pain were a single etiology and a few cases of oral burning pain were multi factorial. The current study resembled other similar studies in respect that the tongue was the most common oral site affected and menopausal women also were more affected especially older ages. Examined patients with gastritis complained from BMS may be due to the reflex effect of the disease on the oral soft tissues, also patients under anti spasmolytic drugs, suffered from BMS may be because the dryness of the mouth which was one of the etiological factors in the occurrence of BMS.

In this study many BMS associating factors has been included to detect the most common factors related with this symptom condition. It was noticed that this condition among Iraqi population is multi factorial, but the current study revealed that females over 50 years were more affected than males, may be because of the hormonal changes in females. During this study it was found that depression and drugs intake for hypertension and hyperglycemia, gastritis and bad oral hygiene were the significant etiologies for oral burning pain, the cause may be due to the spreading of these diseases among Iraqi society, also old age groups were more affected than younger ages may be because of hormonal changes or due to immunity weakness.

This study also revealed that the most common oral anatomical site affected was the tongue; this may belong to the fact that the tongue contains the papillae which exaggerated the feeling of pain sensation. During this study it was found that most cases of oral burning pain in this Iraqi sample of patients was caused by multi factors , differed from other similar population studies which mostly the oral burning pain caused by single factor .

A study had been conducted in 2005 by Lauria et al [(8)] proposed neurological back ground and trigeminal small-fibers , sensory neuropathy as etiological factor in causing oral burning pain, in the current study there were emotional stress and depression as etiological factors for oral burning pain.

Lamey et al [(9)] applied in 2005 a study proved significantly higher prevalence of gastritis in patients with true oral burning pain, this study suggested that every patient with oral burning pain should be referred to the gastroenterologist; in the present study it was found that gastritis also was a cause for oral burning pain.

In 2007 Patton [(10)] conducted a study which revealed that the supertaster people who have the highest density of fungi form papillae, which are responsible for taste, in the anterior tongue and taste as intensely bitter, the current study, also appeared that the tongue was more sensation inside the oral cavity in the examined patients with oral burning pain.

The relation between the etiological factors of oral burning pain and anxiolytics intake was highly significant, so psychological examination and counseling should be offered to those patients [(11)], the present study agreed that anxiolytics drugs intake also played a role in causing this disease according to medical reports from Hospitals and the physicians of the private clinics.

Some authors have suggested that there is multi factorial etiology, local systemic and psychological factors [(12)], in the present study the findings agreed with these suggestions.

The oral burning pain effected Italian people and it was more in women particularly after they

experience menopause-than in men 1.6 percentage [13] , in the present studied sample also the women over 50 years were more affected with oral burning pain than men .

Other authors described a lower tongue temperature in patients with oral burning pain, which also could indicate alterations of the autonomic functions , still other researchers found Para functional habits in patients with oral burning pain, such as teeth grinding or habitual pressing of the tongue against the teeth , both of which could lead to changes in the intraoral blood flow [14] , the present results pointed out that the studied affected patients with oral burning pain had many symptoms in the tongue such as sore tongue , microglosia , irritation or ulcers in the tongue by carious teeth and other causes . But the current study did not reveal Para functional habits or thrusting of the tongue.

Al-Aswad[15] in 2009 applied a study on (83) elderly Iraqi patients, the study revealed that 72% of them complained from dry mouth and 42% of the examined patients suffered from burning mouth syndrome, that agreed with the findings of the current study in respect that oral burning pain affected mainly older age groups more than others.

From this study the following conclusions were obtained:

1. There are multi factorial etiologies played roles in causing oral burning pain.
2. Old age groups of patients were more affected than others.
3. The tongue especially the dorsal portion of it was the most anatomical site affected in the oral cavity.
4. Female patients in old ages were more susceptible to oral burning pain than male patients.
5. Hormonal changes, psychological conditions, poor oral hygiene, gastro-intestinal disorders and the effects of some drugs intake were the main etiologies in causing oral burning pain.

REFERENCES

1. Zakrzewska JM. The burning mouth syndrome remains an enigma. Oroface pain 1995; 62: 253 – 257.
2. Grushka M, Ebstein JB, Gorsky M. Burning mouth syndrome. Am Fam physician 2002; 65: 615 – 20.
3. List T, Leijon G, Svensson P. Soma to sensory abnormalities in atypical odontalgia. Oro face pain 2009; 139: 333 – 41.
4. Scala A, Checchi L, Montevecchi M, Marini I, Glamberardino MA. Update on burning mouth syndrome, overview and patient management. Crit Rev Oral Biol 2003; 14: 275 – 91.
5. Amenabr JM, Pawlowski J, Hilgert JB. Anxiety and salivary control levels in patients with burning mouth syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 105(4): 460 – 65.
6. Bartoshuk LM, Snyder DJ, Grushka M, Berger AM, Duffy VB, Kveton JF. Taste damage previously unsuspected consequences. Chem Senses 2005; 30: 218 – 19.
7. Heckmann SM, Heckmann JG, Hilz MJ. Oral mucosal blood in patients with burning mouth syndrome. Oroface pain 2001; 90(3): 281 – 86.
8. Lauria G, Majorana A, Lombardi R, Padovant A. Trigeminal small fibers sensory neuropathy causes burning mouth syndrome. Oroface pain 2005; 115: 332 – 7.
9. Lamey PJ, Freeman R, Eddle SA, Rees T. Vulner ability and presenting symptoms in burning mouth syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 99: 48 – 54.
10. Patton LL, Siegel MA, Benollel R. Management of burning mouth syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103:13-39.
11. Maresky LS, Vander Bigip, Gird I. Burning mouth syndrome. Oral Surg Oral Med Oral Pathol 1993; 75: 303 – 7.
12. Egusa Del valle A, Aguirre Urzar JM, Martinez Conde, Sagastes Pujana O. Burning mouth syndrome in Basque country. Oral Med 2003; 8: 84 – 90.
13. Bergdhl M, Bergdhl J. Burning mouth syndrome, prevalence and associated factors. J Oral Pathol 1999; 28(8): 350 – 54.
14. Zakrzewska JM, Forsell H, Glenny AM. Intervention for the treatment of burning mouth syndrome. Oroface pain 2003; 17: 293-300.
15. Al-Aswad F. Oral findings and health status among elderly Iraqi patients. J Bagh College dentistry 2009; 21(1): 53-6.

Table 1: Twenty three female patients with oral burning pain were divided according to the number of patients, possible etiology, age groups, the percentage of females among the total examined female cases and the common anatomical oral sites affected.

No. of patients	Possible etiology	Age groups	% of patients	The common anatomical oral sites affected
7	Hormonal changes and psychological condition	Over 50 years	30.6	Tip and dorsum of the tongue, floor of the mouth.
3	Hyperglycemia (diabetic patients)	35-50 years	13.1	Lower lip and labial lower mucosa.
2	Hypothyroidism	30 , 40 years	8.6	Lips, tongue and corners of the mouth.
3	Angiotensin and medication for cardiac disease	55-65 years	13.1	Floor of the mouth, ventral of the tongue and hard palate.
3	Chronic gastro-intestinal infection	25-40 years	13.1	All over and corners of the mouth.
2	Chronic vomiting and irritable colon	60-65 years	8.6	Lower lip, Floor of the mouth, tongue, and lower gingiva.
1	Microglossia , sore tongue	28 years	4.3	Ventral, tip and lateral sides of the tongue.
2	Iron deficiency anemia	32 , 38 years	8.6	Upper and lower lip, buccal mucosa, tongue and hard palate.
23			100	

Table 2: Eighteen male patients with oral burning pain were divided according to the number of patients, possible etiology, age groups, the percentage of males among the total examined male cases and the common anatomical oral sites affected

No. of patients	Possible etiology	Age groups	% of patients	The common anatomical oral sites affected
4	Ill fitting dentures and poorly constructed bridges	50-65 years	22.3	Mucosa of lower lip, tongue and buccal cheek.
5	Poor oral hygiene with bad dental condition	Different age groups	28.1	All over the mouth, dorsum of the tongue and floor of the mouth.
3	Anti spasmolytic medication	30-55 years	16.6	Lips, dorsum of the tongue and corner of the mouth.
1	Modeuretic drugs and dry mouth	53 years	5.5	Tongue, buccal mucosa and floor of the mouth.
2	fissure tongue and recurrent ulcers	39 ,57 years	11	Tip and ventral sides of the tongue and floor of the mouth.
2	Anexiolytic and sedative medication	20 , 35 years	11	All over the oral cavity and the oro-pharyngeal site.
1	Severe gingival hyperplasia with pus discharges.	45 years	5.5	Gingiva and palate.
18			100	

The influence of menopause on unstimulated salivary flow and subjective oral dryness inrelation to other oral symptoms and salivary gland hypofunction

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ABSTRACT

Background: The aim of the present study was to evaluate unstimulated salivary flow rate and oral symptoms in menopausal women.

Materials and Methods: A total of 200 individuals including 100 women in their menopause (case group) and 100 men in the same age range (control group) participated in this analytic descriptive investigation. None of the patients were being treated for any systemic disease or taking any medication. Unstimulated salivary flow rate was measured using the spitting method and the prevalence of oral symptoms was evaluated by filling out a questionnaire. The results were analyzed with ANOVA, chi-square and Student's t-test (P<0.05).

Results: The average of unstimulated salivary flow rate was 0.127 ml/min (SD=0.057) in women and 0.214 ml/min (SD=0.105) in men. The prevalence of dry mouth was 50% versus32%, difficulty in eating dry foods 31% versus 8%, burning sensation in oral mucosa 3% versus 0%, taste reduction, 4% versus 2% and bitter or metallic taste 16% versus 8% in female and male subjects, respectively.

Conclusion: A significant difference in salivary flow rate and prevalence of oral symptoms was found between the two groups (P<0.05). Reduced salivary flow rate and a high prevalence of oral symptoms in menopausal women may be related to the hormonal alterations that occur during this period.

Key words: menopause, salivary flow. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):78-80).

INTRODUCTION

Oral discomfort including dry mouth, altered taste and burning sensation are common chief complaints encountered in dental clinics. Most oral sensory complaints are caused by systemic diseases or are side effects of different medications, however this does not hold true for a considerable number of patients seeking oral care. Previous studies have shown that many of these patients are menopausal women. The probable etiology of oral discomfort in menopausal women has been related to alterations in the quantity and/or quality of saliva [1].

Menopause is defined as the permanent cessation of menstruation that occurs after loss of ovarian function and oocyte depletion. It has been suggested that the years immediately prior to and the decades following the initiation of menopause are of greater clinical significance. This process occurs at a median age of 51 years in western countries. Genetics appears to play a major role in the determination of menopausal age, but the effect of race and nutritional status seems to be limited [2]. Average life expectancy in females is estimated to be approximately 78.3 years; therefore it can be assumed that women generally live about one-third of their life beyond menopause [3].

Women at menopausal may repeatedly develop a number of oral mucosal disorders.

Burning mouth syndrome is considered as a common oral problem in these patients. A mean age of 50-60 years and a marked female predominance (3:1) has been reported for theonset of burning mouth syndrome. Gender difference demonstrates an increase with age suggesting that menopause may have an important part in the incidence of burning mouth syndrome [4]. Xerostomia is also a frequent finding among postmenopausal women. Other less common menopause- associated symptoms include bad or altered taste, viscous saliva and mucosal disorders such as lichen planus, benign mucosal pemphigoid and Sjogren's syndrome [4].

Saliva plays an essential role in maintaining oral health. Alterations in salivary function may lead to impairment of oral tissues andhave a large impact on the patient's quality of life [5]. A higher incidence of dental caries, oral mucositis, dysphagia, oral infections and altered taste has been reported in individuals with reduced salivary flow [6]. There is controversy regarding the effect of menopause on the quantity of saliva. A number of studies demonstrated reduction [7-10], while others have not found any changes in the saliva of menopausal women [1,3,11]. Our hypothesis suggests that menopause is associated with lower salivary flow rate and higher prevalence of oral symptoms. Accordingly, the aim of the present study was to investigate unstimulated salivary

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flow rate and oral symptoms among menopausal women.

MATERIALS AND METHODS

The study sample of this analytic- descriptive investigation consisted of 200 individuals including 100 menopausal women and 100 men. All subjects were in the same age range and one year had passed from the last menstruation of the female participants. The patients were selected from those referred to the Department of Oral Medicine, College of Dentistry, Baghdad University, from August to December 2011.

A questionnaire covering information on age, sex, systemic disease, daily medication and various oral symptoms was filled out for each individual by a trained interviewer.

Whole unstimulated salivary flow rate was determined by the spitting method. The individuals were told to refrain from eating and drinking at least one hour prior to the examination time (between 8:00 and 9:00 AM for all patients) and were asked to rinse their mouth with water. Each sample was obtained by havingthe patient expectorate into a disposable cup every 1 minute, for 5 minutes. The volume of saliva was measured by a 5cc syringe andthe flow rate was calculated in milliliters per minute.

Data were analyzed with the SPSS statistical analysis software and chi-square along withStudent's t-test was used for analysis of the differences between the groups. Two-wayANOVA was applied to determine the effect of age and gender on salivary flow rate. A probability value of $P<0.05$ was accepted as statistically significant for all tests.

RESULTS

The mean age of the female and male participants was 60.72 and 62.33, respectively; without a significant difference between the two groups (P=0.1). A total of 81 (40.5%) individuals, 42 women and 39 men, were using oral prosthesis, but the difference was not statisticallysignificant (P=0.66). The mean unstimulated salivary flow rate was 0.171 ml/min; with 0.127 ml/min (SD=0.057) recorded in females and 0.214 ml/min (SD=0.105) in males. Minimum and maximum salivary flow rates were respectively 0.04 and 0.28 ml/min in women and 0.04 and 0.6 ml/min in men. We determined the effect of age and gender on salivary flow rate and found no interaction between age and gender (P=0.362). According to Table 1, the impact of age on salivary flow rate was not significant (P=0.168), while it was significant for sex ($P<0.001$). The prevalence of oral symptoms in women was 50% for dry mouth, 31% for difficulty in eating dry foods, 3% for burning sensation, 4% for taste reduction and 16% for bitter or metallic taste. In men a prevalence of 32%, 8%, 0%, 2% and 8% were found for dry mouth, difficulty in eating dry foods, burning sensation, taste reduction and bitter or metallic taste, respectively. A significant difference in three of the symptoms including dry mouth, burning sensation and difficulty in eating dry foods was found between males and females ($P<0.05$) but the difference was not significantfor taste reduction (P=0.40) and bitter or metallic taste (P=0.082)(Table 2) .

DISCUSSION

The present study was designed to evaluate unstimulated salivary flow rate and the occurrence of oral symptoms in menopausal women. A significant difference in unstimulated salivary flow rate was found between males and females ($P<0.05$), indicating decreased flow rate in women. The prevalence of the studied oral symptoms in women and men was 50% vs. 32% for dry mouth, 31% vs. 8% for difficulty in eating dry foods, 3% vs. 0% for burning sensation, 4% vs. 2% for taste reduction and 16% vs. 8% for bitter or metallic taste. Only the first three symptoms showed a significant difference between male and female subjects. In the current investigation, the number of patients was similar in both male and female groups, which could be considered as an advantage compared to other studies like that conducted by Aghahosseini et al [1] who used 158 menopaused women and 83 men in order to evaluate stimulated whole salivary flow rate and composition in menopausal women. The age range of females and males (case and control groups) was similar in our sample which was in accordance with the work of Aghahosseiniet al [1] but in contrast to others who studied pre-menopausal women [3,11,12]. We determined unstimulated salivary flow rate, while similar studies assessed stimulated or both stimulated and unstimulated flow rates [1,3,8,9,11-13]. It has been suggested that unstimulatedwhole saliva collection is the most valuable method for evaluation of salivary gland function. Ideally, dentists should determine baseline values for unstimulated whole salivary output at an initial examination [14]. According to Navazesh et al [15] resting methods are preferable for the differentiation of individual salivary flow rates. In the present study the mean unstimulatedsalivary flow rate was significantly lower in menopausal

women as compared to male controls (P<0.05), which suggests a strong relation between unstimulated salivary flow rate andmenopause. Similar findings were also reportedby Dodds et al [8], and Laine and Leimola- Virtanen [9] who respectively showed an age-related decrease in salivary output and a higher salivary flow rate in pre-menopausal compared to post menopausal women. On the contrary Aghahosseini et al [1] and Ship et al [3] did not find a significant difference in major salivary gland flow rates between pre- and post-menopausal females. In the current investigation subjective complaints of oral symptoms were compared between the two groups.

Table 1: Unstimulated salivary flow rate (ml/min) between genders in the different age-subgroups.

	Mean (SD)	
Age-subgroup	**Women**	**Men**
50 – 53 (15)	0.142 (0.068)	0.262 (0.141)
54 – 57 (13)	0.135 (0.055)	0.212 (0.071)
58 – 61 (28)	0.143 (0.073)	0.203 (0.088)
62 – 65 (23)	0.112 (0.046)	0.204 (0.084)
66 – 69 (12)	0.127 (0.039)	0.180 (0.088)
70 – 73 (7)	0.092 (0.037)	0.227

Table 2: The prevalence of oral symptoms

Criteria	**Women**	**Men**
Dry mouth	50%	32%,
Difficulty in Eating dry foods	31%	8%
Burning sensation	3%	0%
Taste reduction	4%	2%
Bitter or metallic taste	16%	8%

A significantly higher prevalence of dry mouth, burning sensation and difficulty in eating dry foods was found among the female subjects, but the other studied oral symptoms were similar in both men and women. Likewise, Aghahosseini et al [1] and Equia et al [16] also indicated a high prevalence of oral symptoms among menopausal women. The later study showed that 82.9% of the patients with burning mouth syndrome were post-menopausal females.

This study demonstrates that unstimulated salivary flow rate and subsequently oral symptomsmay be influenced by menopause.

REFERENCES

1. Aghahosseini F, Akhavan Karbasi MH. Evaluation of stimulated salivary flow rate in menopausal and post-menopausal women. Journal of Dental Medicine Tehran University of Medical Sciences 2003; 16(2):39-45.
2. Berek Js, Adashi E, Hillard PA. Novaks Gynecology. 12th ed. Canada: Williams and Wilkins; 1996. p. 981-1011.
3. Ship JA, Patton LL, Tylenda CA. An assessment of salivary function in healthy premenopausal and postmenopausal females. J Gerontol 1991; 46 (1): M11-5.
4. Frutos R, Rodríguez S, Miralles L, Machuca G. Oral manifestation and dental treatment in menopause. Medicina Oral 2002; 7: 26-30.
5. Silverman S, Eversole LR, Truelove EL. Essentials of Oral Medicine. Canada: BC Decker Inc; 2002. p. 256, 260.
6. Fischer D, Ship JA. Effect of age on variability of parotid salivary gland flow rates over time. Age Ageing 1999; 28(6):557-61.
7. Bergdahl M, Bergdahl J. Low unstimulated salivary flow and subjective oral dryness: association with medication, anxiety, depression, and stress. J Dent Res 2000; 79(9):1652-8.
8. Dodds MW, Johnson DA, Yeh CK. Health benefits of saliva: a review. J Dent 2005; 33(3): 223-33.
9. Laine M, Leimola-Virtanen R. Effect of hormone replacement therapy on salivary flow rate, buffer effect and pH on perimenopausal and postmenopausal women. Arch Oral Biol 1996; 41(1):91-6.
10. Närhi TO. Prevalence of subjective feelings of dry mouth in the elderly. J Dent Res 1994; 73(1):20-5.
11. Ghezzi EM, Wagner-Lange LA, Schork MA, Metter EJ, Baum BJ, Streckfus CF et al. Longitudinal influence of age, menopause, hormone replacement therapy, and other medications on parotid flow rates in healthy women. J Gerontol A Biol Sci Med Sci 2000; 55(1):M34-42.
12. Streckfus CF, Baur U, Brown LJ, Bacal C, Metter J, Nick T. Effects of estrogen status and aging on salivary flow rates in healthy Caucasian women. Gerontology 1998; 44(1): 32-9.
13. Sreebny LM,Valdini A. Xerostomia. Part I: Relationship to other oral symptoms and salivary gland hypofunction. Oral Surg Oral Med Oral Pathol 1988; 66(4):451-8.
14. Greenberg MS,Glick M. Burkets Oral Medicine. 10th ed. Spain: BC Decker Inc; 2003. p. 238.
15. Navazesh M, Christensen CM. A comparison of whole mouth resting and stimulated salivary measurement procedures. J Dent Res 1982; 61(10): 1158-62.
16. Eguia Del Valle A, Aguirre-Urizar JM, Martinez-Conde R, Echebarria-Goikouria MA, Sagasta- Pujana O. Burning mouth syndrome in the Basque Country: a preliminary study of 30 cases. Med Oral 2003; 8(2):84-90.

Detection of genomic instability in oral squamous cell carcinoma using random amplified polymorphic DNA based on polymerase chain reaction method (RAPD-PCR)

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ABSTRACT

Background: Oral squamous cell carcinoma is an invasive epithelial neoplasm, occurred most commonly in alcoholic and tobacco using adults. The present study is aimed to identify the genomic instability in OSCC patients using random amplified polymorphic DNA (RAPD), a polymerase chain reaction (PCR) based technique.

Materials and methods: Twenty five blocks of formalin fixed paraffin embedded tissue was used as malignant DNA source and five sample of healthy DNA obtained from the oral tissue and blood. Using DNA extraction kit (Geneaid minikit) and eleven random sequencing primers to visualize the amplifications pattern under UV.

Results: The primer detectability of genomic instability ranged from 21% in well differentiated OSCC to 68% in poorly differentiated OSCC. CasesT8 and T13 showed highest genomic instability (75%). The results determined numbers of genomic instabilities among OSCC patients by comparing the pattern of amplifications of the primers in both malignant and healthy DNA.

Conclusions: High significance correlation between primers detection rate and histopathological grade of OSCC. Further larger studies are needed to: 1) Obtain RAPD markers useful for OSCC for early diagnosis; 2) investigate different genes directly involved in the etiology of OSCC; 3) analyze chromosomal instability among OSCC patients.

Keyword: Oral squamous cell carcinoma (OSCC), random amplified polymorphic DNA (RAPD), genomic instability. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):81-83).

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is associated with major mortality. It is the most common cancer worldwide and its incidence is either stable or slowly increasing in most populations in the world[(1)]. The etiology of oral cancers is complex due to the multigenic nature of the disease and the number of potential environmental agents to which individuals may be exposed[(2)]. Tobacco and alcohol are considered as the major etiological agents involved in development of oral tumors.

Measurement of genomic instability has been performed by techniques like flow cytometry, fluorescent insituhybridization, comparative genomic hybridization (CGH) and allelotyping, which, although informative, but are cumbersome to perform and hence, impractical in the assessment of clinical cases [(3)]. Random amplified polymorphic DNA (RAPD) is a polymerase chain reaction (PCR) based fingerprinting technique that amplifies random DNA fragments with short primers of arbitrary nucleotide sequence under low annealing stringency [(4,5)].

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The applications of RAPD technique have been found among several kinds of organism including bacteria, fungi, plants, animals, insects and humans [(6-11)].

MATERIALS AND METHODS

DNA extraction: Twenty five formalin fixed paraffin embedded tissues blocks of oral squamous cell carcinoma OSCC were included in this study collected from the archives of histopathological department in the college of dentistry/Baghdad University and from histopathological department in Gazi Al-Hariri hospital/Baghdad. While the control group was three samples of fresh blood as a source of healthy DNA and two samples of gingival mucosa. DNA extraction was done using (Geneaid Minikit) which contain Lysis buffers and DNA binding silica filters.

DNA amplification: Total reaction volume of 20 µl of 5µl PCR Master Mix(Bioneer) was used and contains 100 Pmol of each 11 different arbitrary 10-mer primers and 25 to 50 ng of genomic DNA. The names and sequences of these oligoprimers are listed in Table1. The RAPD-PCR amplification reactions were performed in Eppendorfthermal cycler using the following PCR program: one cycle of 95ºC for 4 min then amplification was carried out for 40

cycles of 92ºC for 1 minute, annealing for 1 minute at 36ºC, and 72ºC extension for 1 minute, followed by a 5 minute 72ºC for final extension. After the amplification, the PCR reaction products were electrophoresed on 10 x 14 cm 1.5%-agarose gel for 2 hours using Tris-borate- EDTA Buffer. The gel was stained with 0.5g/ml of ethidium bromide.

Table 1: RAPD Primers sequences and their GC ratio.

No.	Primers codes	Sequencing	GC Ratio
1	**OPI-01**	5′-GGTCTGAAC-3′	55.6%
2	**OPA-04**	5′-TACGGACAC-3′	55.6%
3	**OPA-07**	5′-ACGGTACACT-3′	60%
4	**OPC-08**	5′-ACGGCGCA-3′	75%
5	**OPA-09**	5′-GTCCTCAACG-3′	70%
6	**OPA-11**	5′-CAGGCCCTTC-3′	60%
7	**OPC-12**	5′-TAGGCTCACG-3′	60%
8	**OPA-13**	5′-CCGGCTACGG-3′	70%
9	**OPA-16**	5′-TACGGTTCGC-3′	60%
10	**OPA-20**	5′-AGCTTCAGGG-3′	60%
11	**GB8**	5′-AGGCATTCCC-3′	70%

Data analysis: The analysis is based on the DNA polymorphisms of the tumor comparing with healthy DNA using the same primer to detect deletion, addition, increasing or decreasing in intensity of bands.

RESULTS AND DISCUSSION

Results of genomic instability detected by RAPD-PCR analysis are shown in Table (2). Among all studied cases, genomic instability was demonstrated with at least one primer. Among all studied cases with all primers, the detectability of genomic instability ranged from 21% in well differentiated OSCC to 68% (± SD) in poorly differentiated OSCC. With all used primers, case T8 and T13 showed the highest genomic instability (75%), whereas, the lowest genomic instability was (12.5%) with cases T7, T10.

There are three grades of OSCC according to the histopathological differentiation (Well, Moderate, and Poor). The powerful technique that detects genomic alteration correlated with human tumor is microsatellites analysis[12].

Table 2: Showing percentage of detection rate in correlation with the grade of differentiation.

CASE ID	Grades	Primers Codes								Detections Rate %
		OPI 01	OPA-04	OPA-07	OPA-08	OPA-09	OPA-11	OPA-13	OPA-16	
T1	Moderate	+	-	-	+	-	+	-	+	50
T2	Moderate	+	-	+	-	+	+	-	+	62
T3	Well	-	-	+	-	-	-	-	+	25
T4	Moderate	-	+	+	-	+	+	-	-	50
T5	Moderate	+	+	-	-	-	+	-	-	37.5
T6	Poor	+	+	+	-	-	-	+	+	62
T7	Well	-	+	-	-	-	-	-	-	12.5
T8	Poor	-	-	+	+	+	+	+	+	75
T9	Well	-	-	-	-	-	+	+	+	37.5
T10	Well	-	-	+	-	-	-	-	-	12.5
T11	Poor	+	+	-	+	-	-	+	+	62
T12	Moderate	+	-	-	-	-	+	-	+	37.5
T13	Poor	-	+	+	+	-	+	+	+	75
T14	Well	-	-	-	-	-	+	-	-	12.5
T15	Moderate	-	+	-	+	+	-	-	+	50
T16	Moderate	-	-	-	+	-	+	-	+	37.5
T17	Moderate	-	-	+	+	+	-	-	+	50
T18	Well	-	-	+	-	-	-	-	+	25
T19	Well	-	-	+	-	-	+	-	-	25

However, this methodology is time consuming and can only detect base-pair expansion or contraction in specific microsatellite loci[4]. On the contrary, for genomic instability analysis, it is important to investigate genetic alterations in the entire genome besides microsatellite loci. In contrast, the RAPD method can simply and rapidly detect genetic alterations in the entire genome without knowledge of specific DNA sequence information[13,14]. In the RAPD method, genetic alterations appeared as either loss or gain of a band, shift of a band, or decrease or increase of intensities of a band of cancer tissue DNA relative to the corresponding normal tissue DNA[15]. Obtained results indicated that RAPD-PCR is an effective tool for identifying genetic alteration and genomic

instability which is in agreement with several various studies[14]. Figure1 shows the banding profiles of OSCC and corresponding normal DNAs and demonstrate the detected genetic alteration by RAPD technique among OSCC patients in comparison with normal control group. Banding shifts, missing bands and/or banding intensity changes, which indicate genomic instability, were demonstrated in this figure.

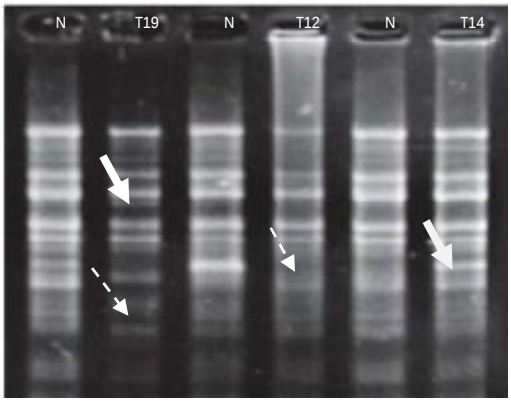

Figure 1: RAPD analysis with primer (OPA-11). Bold arrow refer addition of bands, dashed arrow refer to deletion of bands.

These results might be due to mutations that occurred at the primer - template interaction sites[15]. The summarized results which are illustrated in Table 2 indicated that, there are differences in genetic instability among the studied cases which ranged from 21% with case number T7 to 75% for case T8. These differences might be due to differences in studied OSCC grades (well, moderate and poor).

REFERENCES

1. Dobrossy L. Epidemiology of head and neck cancer, Magnitude of the problem. Cancer Metastasis Rev 2005; 24:9–17.
2. Scully C, Field JK and Tanzawa H. Genetic aberrations in oral or head and neck squamous cell carcinoma (SCCHN). Carcinogen metabolism, DNA repair and cell cycle control oral onco 2000;36:256-63.
3. Basik M, Stoler D, Kontzglou K, Rodriguez-Bigas, Petrelli N, Anderson G. Genomic instability in sporadic colorectal cancer quantitated by inter-simple sequence repeat PCR analysis. Gene Chrom Can 1997; 18: 19–29.
4. Ong T, Song B, Qian H, Wu L and Whong W: Detection of genomic instability in lung cancer tissues by random amplified polymorphic DNA analysis. Carcino 1998; 19: 233–5.
5. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA and Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic acids Res 1990; 18:6531-5.
6. Sahoo L, Das K, Parhi J and Mukherjee S. DNA fingerprinting of Flavobacteriumcolumnare using RAPD-PCR. Ind J Micro 2010; 50:10-16.
7. Motlagh R and Anvari M. Genetic variation in a population of Bipolarisoryzae based on RAPD-PCR in north of Irn Afri J Biotech 2010; 9: 5800-4.
8. El-Tarras A, Bazaid S and Munshi A. Genetic identification of KSA pomegranate using PAGE and RAPD techniques. Egypt J Genet Cytal 2004; 33: 433-41.
9. Güneren G, Akyüz B and Ertugrul O. Use of RAPD-PCR for genetic analyses on the native cattle breeds in Turkey. Ank.Üniv Vet FakDerg 2010; 57: 167-72.
10. Awad N, Sally M, Margueriet A, Morad H and Ayman Z. Fingerprinting and assessment of genetic variability of Varroa destructor in Egypt. J Apicu Res 2010; 49: 251-6.
11. Saleh N, Ibrahim A, Archoukieh E, Makkiya A, Al-Obaidi M and Alobydi H: Identification of genomic markers by RAPD-PCR primer in leukemia patients. Biotech 2010; 9: 170-5.
12. Odenthal M, Barta N, Lohfink D,Drebber U, Schulze F, Dienes P andBaldus E. Analysis of microsatellite instability in colorectal carcinoma by microfluidic-based chip electrophoresis J Clin Pathol 2009; 62:850–2.
13. Papadopoulos S, Benter T, Anastassiou G, Pape M, Gerhard S, Bornfeld N, Ludwig WD and Dörken B: Assessment of genomic instability in breast cancer and uveal melanoma by random amplified polymorphic DNA analysis. Int J Cancer 2002; 99: 193-200.
14. Ibrahim M, Saleh N, Archoukieh E, Al-Obaide H, Al-Obaidi M and Said H. Detection of novel genomic polymorphism in acute lymphoblastic leukemia by random amplified polymorphic DNA analysis. Int J can Res 2010; 6:19-26.
15. Maeda T, Jikko A, Hiranuma H and Fuchihata H. Analysis of genomic instability in squamous cell carcinoma of the head and neck using the random amplified polymorphic DNA method. Cancer Lett 1999; 138:183-8.

Prevalence of myofascial pain in students of selected secondary schools in Baghdad city

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ABSTRACT

Background: Myofascial face pain (MFP) is painful disorder of masticatory muscles thought to be the most common type of temporomandibular disorder (TMD).This study was done to evaluate the prevalence of MFP in students of secondary schools of Baghdad city

Materials and methods: The sample comprised 242 females' students and 222 males' students of secondary schools, aged 17-18 years. The MFP evaluated according to the specific screening questionnaire of research diagnostic criteria of temporomandibular disorders (RDC/TMD) axis I with clinical examination.

Results: the study revealed that (50.8%) of the students had history of pain where females reported higher percentage than males with statistical significant difference. The history of pain in muscles of mastication was higher than joint pain in both genders. After clinical examination this study also showed that (25.4%) of students with history of pain had MFP. The differences between both genders regarding the diagnosis of MFP were higher in females than males but statistically not significant.

Conclusion: high percentage of students reported a history of pain which could be attributed to MFP in (25.4%) of the students. The prevalence of pain history and MFP was higher in females' than males.

Keywords: Myofascial pain, temporomandibular disorders, orofacial pain. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):84-87).

INTRODUCTION

Myofascial pain (MFP) is very common. It is complex because of the singularities of the trigeminal nervous system, which often leads to spread and diffuse pain [(1).] TMD is characterized by functional abnormalities and/or musculo-skeletal pain at the masticatory muscles. Pain can be continuous or occasional and brief during mastication, and it is frequently associated with jaw restricted movements and joint sounds [(2).] It is present in 16–59% of the population [(3).]

Etiological factors of TMD areundefined and include anatomical, articular, neuromuscular and psychological factors [(3; 4).] Psychological aspects, coping and catastrophizing differ among orofacial pains. TMD is considered easily handled by patients when compared to neurovascular headaches, e.g. tension headaches that have similar symptoms and signs [(5).] Levels of anxiety, depression, and illness behavior change during time, depending on external factors (e.g. family, job) and the course of the disease (e.g. pain intensity, crises) [(5-6).]

Previous studies reported that over one-third of adolescents were under stress [(8; 9).] Many of these emotional disturbances seem to be caused by school-related stress such as inappropriate workloads or assignments, examinations, falling behind compared to others and inappropriate treatment by teachers [(9).] There are many studies about the psychological aspects of TMD, and in general they are similar to other chronic pain syndromes in many samples around the world [(10; 11).]

As there is no previous Iraqi study concerned in the prevalence of MFP in the school's student of Baghdad city, this study was done to evaluate the prevalence of MFP in students of secondary schools of Baghdad city in relation to gender by history and clinical examination.

MATERIALS AND METHODS

This study was carried out in selected secondary schools of Baghdad city for assessment of Myofascial pain (MFP) in students according to the research diagnostic criteria of TMD (RDC/TMD axis I) which isthe most successful diagnostic protocol for temporomandibular muscle and joint disorder[(12)].The RDC/TMD Axis I is standardized series of diagnostic tests based on clinical signs and symptoms. Diagnostic algorithms using different combination of clinical and questionnaire measures are used to

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differentiate eight RDC/TMD-defined Axis I diagnosis for TMD. These diagnoses include:

Ia- Myofascial pain.

Ib- Myofascial pain with limited opening.

IIa- Disc displacement with reduction.

IIb- Disc displacement without reduction with limited opening.

IIc- Disc displacement without reduction without limited opening.

IIIa- Arthralgia.

IIIb- Osteoarthritis.

IIIc- Osteoarthrosis[13]

The sample size was (464) of students in the fifth and sixth class(242 females and 222 males) in some secondary schools in Baghdad city subjected to specific screening questionnaire for Myofascial pain and TMD according to the RDC/TMD (Axis I)from December 2010 to April 2011. A questionnaire inquiring about the initial joint symptoms was filled by the students. Subjects gave their informed consent and the local ethical committee approval. The selected students whom subjected to clinical examination had no history of head injury and without orthodontic treatment, dental pain, muscle tenderness due to systemic diseases as fibromyalgia, neuralgia or local infection and had no more than 2 missing posterior teeth.

The students who had pain in the face, jaw, temple, priauricular or in the ear and headaches or migraine(is the most common of vascular headaches which cause pain of face and jaw, it start with prodromal aura that is usually visual includes flashing lights or localized area of depressed vision followed by increasingly severe unilateral throbbing headache that is frequently accompanied by nausea and vomiting[14] or pain that limit these activities: chewing, exercising, eating hard or soft food or drinking, smiling, oral hygiene, yawning and talkingdepending to specific screening questionnaire for Myofascial pain and TMD according to the RDC/TMD (Axis I)[15]were asked about the pain history with conformation of pain location plus palpation of masticatory muscle sites, results in report of familiar pain, then determination of masticatory muscles pain during active mouth opening (un-assisted mouth opening) and passive mouth opening (assisted mouth opening).This accomplish by palpation of masticatory muscles when the patient open his mouth as wide as he could and after application of downward pressure on the mandible by the second and third fingers of investigator respectivelyand determination of tender points by examination of masticatory muscles [16]

RESULTS

Table (1) revealed that 236(50.8%) of the students had history of pain when subjected to specific screening questionnaire for Myofascial pain and TMD (192, 41.3% females' students and 44, 9.5% males' students). The history of pain were in the face, jaw, temple, priauricular or in the ear and headaches or migraine or pain that limit these activities: chewing, exercising, eating hard or soft food or drinking, smiling, oral hygiene, yawning and talking.

Table (2) demonstrated that the females' students, who had jaw joint pain, were 22 (11%) while males' students recorded no jaw joint pain. Females with masticatory muscle pain were 158 (82%) and with both muscles and jaw joint pain were 12(6%). Males showed 38 (86%) masticatory muscle pain and 6 (14%) with both muscles and jaw joint pain. The differences between both genders were significantly higher in females than males regarding the total students with history of pain. The history of pain in muscles of mastication was higher than joint pain in both genders.

After clinical examination this study showed that (60, 25.4%) of students with history of pain had MFP according to the RDC/TMD (54, 22.8% females and 6, 2.5% males), table (1).

The students had pain in temporalis, masseter muscles or both muscles during palpation, and some students had pain during mouth opening with or without pain on palpation. The differences between both genders regarding these finding were higher in females than males but statistically not significant as listed in table (3).

Table 1: The percentage of students with pain history and MFP according to gender

Variables	Female		Males		Total	
	No	%	No	%	No	%
Pain history	192	41.3	44	9.5	236	50.8
MFP	54	22.8	6	2.5	60	25.4

Table 2: The differences in the frequency of pain history between both genders

Pain history	Female		Males		total	
	No	%	No	%	No	%
Jaw joint	22	11	0	0	22	11
muscle	158	82	38	86	196	83
Both	12	6	6	14	18	7.6

P value =0.022 (Significant by chi square test)

Table 3: the differences in the frequency of MFP between both genders

MFP	Female		Males		total	
	No	%	No	%	No	%
masseter	22	41	1	16	23	38.3
temporalis	10	18.5	2	33	12	20
both	18	33	2	33	20	33.3
Pain on opening	13	24	2	33	15	25

P value=0.78 (not significant by chi square test)

DISCUSSION

This study revealed that the percentage of students in the secondary schools (17-18) years old with pain history were relatively higher than the percentage reported by other studies [17,18]. The higher percentage of pain may be due to other causes (complaints of pain are often related with depression, migraine, stress and tension- type headaches) rather TMD pain. Complaints of pain are often related with depression and school related stress.Several authors have observed that the prevalence of psychological distress is higher among students than among working nonstudent populations of the same sex and age [19].

The history of pain was reported higher in females than males with statistical significant differences generally females have more signs and symptoms than males. This is in agreement with other reports in the literature [20, 17] .It has been stated that these sex differences could probably be explained by mental factors i.e. young females seem to present a lower pain threshold [20]. Other factors such as stress is well known from TMD studies in adults that women are more affected than men [20,21]. Sex difference may also be explained by some physiological changes seen at pubescence, as in the present study. The pattern of onset of TMD after puberty and lowered prevalence rates in the postmenopausal years suggest that female reproductive hormones may play an etiologic role in temporomandibular disorders [22]. This is also supported by the longitudinal data reported by Magnusson et al., 2005[23].They found that gender difference in signs and symptoms was small in childhood, but from late adolescence females reported more symptoms and exhibited more clinical signs than males did.

History of pain was recorded higher in the muscles of mastication than joint pain in both genders.Lobbezoo et al at 2004[24] revealed that between 50% and 70% of all patients with TMDs reported masticatory muscle pain, and in 25% of these patients, pain in masticatory muscle is the principle source of pain.

This study showed that (25.4%) of students with history of pain had MFP according to the RDC/TMD. This percentage agrees with the results that observed in previous studies [25, 17, 18] and higher than that observed in another's [26, 27], this disagreement may be related to different samples and different examining methods.

The higher prevalence of MFP in females than in males has been attributed to an interaction of a variety of factors ranging from biological and hormonal factors to psychological and social ones.

REFERENCES

1. Sessle BJ. Acute and chronic craniofacial pain: Brainstem mechanisms of nociceptive transmission and neuroplasticity, and their clinical correlates. Critical Reviews in Oral Biology and Medicine 2000; 11: 57–91
2. IASP—International Association for the Study of Pain.Classification of chronic pain. Seattle: IASP Press 1994.
3. Carlsson GE, DeBoever JA. Epidemiology. In Zarb G A, Carlsson G E, Sessle J B, Mohl N D (Eds.), Dysfunctions of TMJ and mastication muscles. Sa˜o Paulo: Livraria Santos; 2000. p.159-70.
4. Turp JC, Jokstad A, Motschall E, Schindler HJ, Widecker- Getaz I, Ettlin, DA. Is there a superiority of multimodal as opposed to simple therapy in patients with temporomandibular disorders? A qualitative systematic review of the literature. Clinical Oral Implants Research 2007; 18: 128–50.
5. Jerjes W, Madland G, Feinmann C, Hopper C, Kumar M., Upile T. A psychological comparison of temporomandibular disorder and chronic daily headache: Are there targets for therapeutic interventions? Oral Surg Oral Med Oral Pathol Oral Radiol and Endod 2007; 103: 367–73.
6. Litt MD, Shafer D, Napolitano C. Momentary mood and coping processes in TMD pain. Health Psychology 2004; 23: 354–62.
7. Turner JA, Mancl L, Aaron LA. Brief cognitive behavioral therapy for temporomandibular disorder pain: Effects on daily electronic outcome and process measures. Pain 2005; 117: 377–87.
8. Khalid SAG. Depression, anxiety and stress among Saudi adolescent school boys. J R Prom Health 2007; 127(1):33-7.
9. Yusoff MSB. Stress, stressors & coping strategies among secondary school students in a Malaysian government secondary school: Initial findings. ASEAN J Psychiatry 2010a; 11(2): 1-15.
10. Aaron LA, Turner JA, Mancl LA, Sawchuk, CN, Huggins K H, Truelove EL. Daily pain coping among patients with chronic temporomandibular disorder pain: An electronic diary study. J Orofac Pain 2006; 20: 125–37.
11. Turner JA, Holtzman S, Mancl, L. Mediators, moderators, and predictors of therapeutic change in

cognitive behavioral therapy for chronic pain. Pain 2007; 127: 276–86.
12. Dworkin SF, Le Resche L. Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. J Craniomandib Disord 1992; 6:301-55.
13. Smith TW. Measurement in health psychology research. In: Friedam HS, Silver RC, ed. Foundation of health psychology. New York: Oxford University Press; 2007. p. 19-51.
14. Martin S, Michael G, Jonathan A. Oral medicine, Diagnosis and treatment. 11th ed. BC Decker Inc; 2008. p. 286.
15. Look JO, John MT, Tai F, Huggins KH, Lenton PA, Truelove EL et al. Research diagnostic criteria for temporomandibular disorders: reliability of Axis I diagnosis and selected clinical measure. J Orofac Pain 2010; 24:25-34.
16. Okeson JP. Management of temporomandibular disorders and occlusion. J Prosthet Dent 2003; 69(1): 1-4.
17. Farsi NM. Symptoms and signs of temporomandibular disorders and oral parafunctions among Saudi children. J Oral Rehabil 2003; 30:1200-8.
18. Feteih RM. Signs and symptoms of temporomandibular disorders and oral parafunctions in urban Saudi Arabian adolescents: a research report. Head and face medicine 2006; 5:567-71.
19. Dyrbye LN, Thomas MR, Shanafelt TD. Systematic review of depression, anxiety, and other indicators of psychological distress among U.S. and Canadian medical students. Acad Med 2006; 81:354–3.
20. Dao TT, Le Resche L. Gender differences in pain. J Orofac Pain 2000; 14:169-84.
21. Nassif NJ, Al-Salleeh F, Al Admawi M. The prevalence and treatment needs of symptoms and signs of temporomandibular disorders among young adult males. J Oral Rehabil 2003; 30:944-50
22. Nekora-Azak A. Temporomandibular disorders in relation to female reproductive hormones a literature review. J Prosthet Dent 2004; 91:491-3.
23. Magnusson T, Egermarki I, Carlsson GE A prospective investigation over two decades on signs and symptoms of temporomandibular disorders and associated variables.A final summary. Acta Odontol Scand 2005; 63:99–109.
24. Lobbezoo MD, Shafer D, Napolitano C. Momentary mood and coping processes in TMD pain. Health Psychology 2004; 23, 354–62.
25. Thilander B, Rubio G, Pena L, Mayorga C. Prevalence of temporomandibular dysfunction and its association with malocclusion in children and adolescents: an epidemiologic study related to specified stages of dental development. Angle Orthod 2002; 72:146-54.
26. Deng Y, Fu MK, Hagg U. Prevalence of temporomandibular joint dysfunction (TMJD) in Chinese children and adolescents. A cross-sectional epidemiological study. Eur J Orthod 1995; 17:305-9.
27. Farsi N. Temporomandibular dysfunction and emotional status of 6–14 years old Saudi female children. Saudi Den J 1999; 11:114-9.

Trial usage of Iodoform powder as an adjunct in periodontal therapy

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ABSTRACT

Background: Tooth mobility refers to the movement of loose teeth within their sockets primarily caused by periodontal disease. Treatment involves identifying and addressing the cause of tooth mobility. If detected early enough, loose teeth can be made firm again. This study aimed to evaluate the changes in teeth mobility before and after using iodoform powder in periodontal Flap in chronic periodontitis patients.

Materials and methods: The study sample consisted of twenty chronic periodontitis patients of both gender (13 males and 7 females) with age ranged from 30 to 50 years. Periodontal parameters used in this study were plaque index (PLI), gingival index (GI), probing pocket depth (PPD) and tooth mobility grades. The patients were examined at base line, 2 months and 6 months after periodontal therapy. Treatment included oral hygiene instruction, scaling, root planing, internal splints, iodoform powder and periodontal surgery. Clinical parameters were evaluated.

Results: The present study showed that teeth mobility was decreased after using iodoform powder as an adjunct in periodontal therapy in chronic periodontitis patients.

Conclusions: Iodoform powder is aid in decrease tooth mobility with periodontal surgery.

Keyword: Tooth mobility, chronic periodontitis, iodoform powder, periodontal therapy. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):88-92).

INTRODUCTION

Periodontal disease is an inflammatory process that affects the protective and supportive tissues around the tooth (1). Chronic periodontitis is a common disease characterized by a painless, slow progression, loss of attachment, and bone resorption, eventually resulting in tooth mobility and loss (2). Chronic periodontitis is prevalent than the general population recognizes. Different methods for diagnosing chronic periodontitis exist, a full- mouth examination and recording is preferred so that initial stages of periodontal disease can be detected and treated. A complete examination should include pocket depth (PD), clinical attachment level (CAL), bleeding on probing (BOP), plaque index/score, furcation involvement, radiographic interpretation of bone levels, and mobility (3). Tooth mobility is an important part of a periodontal examination and represents a function of the persisting height of the alveolar bone and the width of the periodontal ligament (4). Measuring the degree of mobility is an important part of any thorough periodontal examination (5). Reduction or elimination of increasing tooth mobility is now utilized as one clinical sign indicative of successful periodontal therapy (6).

The goal for both patient and clinician would seem to stress patient comfort and a stable efficient occlusion compatible with periodontal health. Most would now agree that presence and degree of tooth mobility should be determined, and a functional evaluation of the occlusion should be performed during any periodontal therapy (6). Iodoform is organoiodine compound, with the formula CHI_3. A pale yellow, crystalline, volatile substance, it has a penetrating odor and, analogous to chloroform, sweetish taste. It is occasionally used as a disinfectant (7). Around the beginning of the 20th century it was used in medicine as a healing and antiseptic dressing for wounds and sores (7). This study aimed to evaluate the changes in teeth mobility after using iodoform powder with periodontal surgery in chronic periodontitis patients.

MATERIALS AND METHODS

Human Sample

Sample population consisted of twenty male and female, age ranged from 30 to 50 years. Samples collection was started at 10th of January 2012 till June 2012. Patients participating in the present study with chronic periodontitis (chronic periodontitis in patients was defined as the presence of teeth with periodontal pockets equal or greater than 4mm with clinical attachment level of 1-2mm) (no=20, 13 males and 7 females) were recruited from the Clinic of the Department of Periodontics / Faculty of Dentistry/ AL- Yarmouk University College.

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Inclusion criteria

Subjects who had not received any periodontal treatment during the past 3 months, those who had not taken any antibiotic therapy during the past 3 months. All patients participated in this study without any systemic conditions.

Clinical examination

Periodontal examination consisted of plaque index (PLI), gingival index (GI), probing pocket depth (PPD) at 4 sites for all teeth except 3rd molar on (mesial, mid-vestibular, distal, mid-lingual), using a calibrated periodontal probe (Michigan O probe) and tooth mobility grades. Grade I (GI: mobility of the crown of the tooth in horizontal direction 0.2-1mm), Grade II (GII: mobility of the crown of the tooth in horizontal direction exceeding 1mm), and Grade III (GIII: mobility of the crown of the tooth in vertical as well as horizontal direction) [8].

Patients with chronic periodontitis had periodontal pockets equal or greater than 5mm with clinical attachment loss. Periodontal therapy consisted of oral hygiene motivations and instructions, full-mouth scaling and root planing and modified Widman flap of all deep periodontal sites. After raising buccal gingival flap, a total of pocket epithelium, underlying connective tissue, and granulation tissue were removed by a periodontal curette from the periodontal lesion, then iodoform powder 0.3mg (LABTECHo chemicals iodoform, CASR No. 75-47-8. A.B. No. 170511 MFG. DT. May. 2011, Exp. DT. May.16.2014) applied on the root of the teeth, and then the flap was closed. After two months, the clinical periodontal parameters (PLI), (GI), (PPD), and tooth mobility were measured and after six months, the clinical periodontal parameters, (PLI), (GI), (PPD), and tooth mobility, were measured.

Statistical Analysis

The data were processed and analyzed using the statistics package for social sciences (SPSS Inc., version 17 for windows XP and excel 2007). Both descriptive and inferential statistics were used.

1. Descriptive Statistics; included means, standard deviations (SD), number (No.), and statistical tables.

2. Inferential Statistics; included Student t-test.

In the statistical evaluation, the following levels of significance are used:

$P > 0.05$	Non-significant (NS)
$0.05 \geq P > 0.01$	* Significant (S)
$P \leq 0.01$	** Highly significant (HS)

RESULTS

The study group consisted of twenty chronic periodontitis patients (thirteen male and seven female) age ranged (30-50 years) with mean and standard deviation of age in male were (41.9±8.0) and in female were (36.4±5.6). In this study the mean and standard deviation of plaque and gingival index in male before treatment were (1.3±0.3, 1.3±0.3) respectively and in female were (1.2±0.5, 1.4±0.2) respectively. The mean and standard deviation of plaque and gingival index in male after two months of treatment were (0.7±0.3, 0.8±0.3) respectively while in female were (0.6±0.3, 0.8±0.2) respectively. The mean and standard deviation of plaque and gingival index in male after six months of treatment were (0.5±0.2, 0.6±0.3) respectively and in female were (0.4±0.2, 0.6±0.2) respectively. There was highly significant difference in mean PLI & GI for both male and female ($P<0.01$) before and after 2 months and after 6 months of treatment as shown in table 3.The mean and standard deviation of probing pocket depth before and after two months of treatment are shown in table 4, the mean and standard deviation of PPD before treatment in male were (6.7±4.5) and after two months of treatment were (1.0±1.4) while in female were (5.9±3.6, 0.9±1.5) respectively. There was highly significant difference in mean PPD in male and female before and after two months of treatment ($P<0.01$). There was no PPD found after six months of treatment. Descriptive statistics of number of mobile teeth and teeth mobility grade before, after two months and after six months of treatment are shown in table 5. The number of mobile teeth in male before periodontal treatment was 45 with mean and standard deviation (3.5±2.2) while in female was 23 with mean and standard deviation (3.3±2.1). The number of mobile teeth GII in male before periodontal treatment was 31 with mean and standard deviation (3.1±2.0) and in female were 21 with mean and standard deviation (3.0±2.2). The number mobile teeth GIII in male before treatment were 14 with mean and standard deviation (2.3.1±0.8) and in female was 2 with mean and standard deviation (2.0±). The number of mobile teeth with GI after two months of periodontal treatment (with usage of iodoform powder) in male was 25 with mean and standard deviation (1.9±1.1) and in female was 15 with mean and standard deviation (2.1±0.9), while the number of teeth mobility GII in male was 20 with mean and standard deviation (2.9±1.3) and in female was 8 with mean and standard deviation (2.7±0.6). The number, mean and standard

deviation of teeth mobility GI in male after six months of treatment (with usage of iodoform powder in periodontal flap) were 45 (3.5±2.2) respectively, and in female were 23 (3.3±2.1) respectively. There was significant difference in GII tooth mobility before and after two months of periodontal treatment (with usage of iodoform powder in periodontal flap) in both male and female ($P<0.05$) and highly significant difference in GIII teeth mobility before and after six months of periodontal treatment (with usage of iodoform powder in periodontal flap) in male and female ($P<0.01$). There was no teeth mobility GII and GIII after six months of treatment in both male and female.

DICUSSION

In this study there was highly significant difference in PLI, GI and PPD in male and female before and after periodontal treatment which include; oral hygiene motivation and instruction scaling, root planing (SRP), combined with periodontal flap. The role of bacteria in the etiology of periodontal diseases has been established. Conventional periodontal treatment consists of mechanical debridement to eliminate the subgingival microbiota and infected tissue in the inflamed pocket, usually performed by SRP. However, achieving consistent success is demanding for both the patient and therapist. Deep periodontal pockets, especially with root concavities or furcation involvement prevent the effectiveness of SRP [(9)]. In the above-mentioned situations, it is necessary to perform a flap operation, in order to obtain access and visibility, to the underlying root surface and bone. One of the commonly performed procedures for this purpose is the modified Widman flap [(10)]. This procedure has shown to reduce pocket depths by removing the proliferating epithelium in the deep pocket and promote attachment gain. In the treatment of deep pockets open flap debridement results in greater PPD reduction, reduction in gingival inflammation and clinical attachment gain [(11)]. In this study there was significant difference in GII tooth mobility before and after two months of treatment and highly significant difference in GIII teeth mobility before and after treatment in male and female. After six months of treatment, all mobile teeth with GII and GIII become GI. There is a study reported in 2004 (Feller and Lemmer) where they found that there were statistically significant differences in mobility after surgery between tooth types [(12)]. Kerry *et al*, found that tooth mobility increased temporarily after pocket reduction surgery but was not altered following curettage, modified Widman flap or scaling and root planning. Two years post-treatment there was a trend toward further decrease in tooth mobility with professional tooth cleaning every 3 months [(13)]. In this study, iodoform powder was used with periodontal flap in decrease the tooth mobility by promoting high degree of fibrosis. There was highly significant difference in teeth mobility before and after six months of periodontal treatment and usage of iodoform powder with periodontal flap, fixation of the root by iodoform powder up and down provokes fibrosis. Iodoform powder resists the infection and also it is an antiseptic and analgesic. Iodoform powder cause irritation to the area that was applied on it and this will lead to increase in the fibrosis which increase the chance for bone deposition in the area and fixation of the root and finally decrease tooth mobility.

As a conclusion; the usage of iodoform powder as an adjunct in periodontal therapy may decrease tooth mobility in chronic periodontitis patients.

REFERENCES

1. Burt B. Position paper: Epidemiology of periodontal diseases. J Periodontol 2005; 76:1406–1409.
2. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. J Periodontol 2007; 78:1387–1399.
3. Luciana M. Shaddox, Clay B. Walker. Treating chronic periodontitis: current status, challenges, and future directions. Clinical, Cosmetic and Investigational Dentistry 2010; 2: 79-91.
4. Kim SY, Lim SB, Chung CH. Comparative study on the tooth mobility after Modified Widman Flap. J Korean Acad Periodontol 2003; 33(1): 103-111.
5. Charles R. Anderegg, David G. Metzler. Tooth mobility revisited. J Periodontol 2001; 72(2): 963-967.
6. The American Academy of Periodontology. Parameters on occlusal traumatism in patients with chronic periodontitis. J Periodontol 2000; 71:873-875.
7. Phyllis A. Lyday. Iodine and Iodine Compounds in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim, 2005.
8. Carranza FA, Takei HH. Clinical Diagnosis. In Newman MG, Takei HH, Carranza FA (ed). Carranza's clinical periodontology. 10th ed. Philadelphia: W.B. Saunders Company; 2009. pp. 546.
9. Listgarten MA, Hellden L. Relative distribution of bacteria at clinically healthy and periodontally diseased sites in humans. J Clin Periodontol 1978; 5:115–32.
10. Azmak N, Atilla G, Luoto H, Sorsa T. The effect of subgingival controlled release delivery of chlorhexidine chip on clinical parameters and MMP-8 levels in GCF. J Periodontol 2002; 73: 608–15.
11. Heitz-Mayfield LJ, Trombelli L, Needleman I, Moles D. A systemic review of the effect of surgical debridement vs non-surgical debridement for the treatment of chronic periodontitis. J Clin Periodontol 2002; 29(3): 92-102.

12. Feller L Lemmer J. Tooth mobility after periodontal surgery. SADJ 2004; 59(10): 407, 409-11.
13. Kerry GJ, Morrison EC, Ramfjord SP, Hill RW, Caffesse RG, Nissle RR, Appleberry EA. Effect of periodontal treatment on tooth mobility. J Periodontol. 1982; 53(10):635-8.

Table 1: Number, Means and SD of the age for males and females

Gender	Male	Female	Total
Age	41.9±8.0	36.4±5.6	40.0±7.6
No	13	7	20

Table 2: Means and SD of PLI and GI in male and female before, after two months and after six months of periodontal treatment (with usage of iodoform powder in periodontal flap)

	Gender	Male	Female	Total
Mean PLI	Before	1.3±0.3	1.2±0.5	1.2±0.3
	After 2 months	0.7±0.3	0.6±0.3	0.7±0.3
	After 6 months	0.5±0.2	0.4±0.2	0.5±0.2
Mean GI	Before	1.3±0.3	1.4±0.5	1.3±0.4
	After 2 months	0.8±0.3	0.8±0.2	0.8±0.3
	After 6 months	0.6±0.3	0.6±0.2	0.6±0.2

Table 3: P-value of PLI and GI in study group before and after periodontal treatment

		Male	Female	Total
Mean PLI	Before and after two months of treatment	0.0001**	0.0001**	0.0001**
	Before and after six months of treatment	0.0001**	0.001**	0.0001**
Mean GI	Before and after two months of treatment	0.0001**	0.011*	0.0001**
	Before and after six months of treatment	0.0001**	0.0001**	0.0001**

Table 4: Means and SD of PPD in study groups before and after two months of treatment

Gender	Male	Female	Total
PD≥ 5mm (Before)	6.7±4.5	5.9±3.6	6.4±4.1
(After2 months)	1.0±1.4	0.9±1.5	1.0±1.4

**HS (P<0.01)

Table 5: Number, mean and SD of mobile teeth in study group before, after two months and after six months of periodontal treatment (with usage of iodoform powder in periodontal flap).

		No	Mean mobile teeth/ Male	No	Mean mobile teeth/ Female	No	Mean mobile teeth/Total
No. of mobile teeth		45	3.5±2.2	23	3.3±2.1	68	3.4±2.1
Tooth mobility Grade							
Before	GI	-	-	-	-		-
	GII	31	3.1±2.0	21	3.0±2.2	52	3.1±2.0
	GIII	14	2.3±0.8	2	2.0±	16	2.3±0.8
After 2 months	GI	25	1.9±1.1	15	2.1±0.9	40	2.0±1.0
	GII	20	2.9±1.3	8	2.7±0.6	28	2.8±1.1
	GIII		-	-	-	-	-
After 6 months	GI	45	3.5±2.2	23	3.3±2.1	68	3.4±2.1
	GII	-	-	-	-	-	-
	GIII	-	-	-	-	-	-

Table 6: P value of tooth mobility GII and GIII before and after periodontal treatment (with usage of iodoform powder in periodontal flap)

Tooth mobility grade	Male	Female	Total
GII before & after two months of treatment	0.141	0.184	0.030*
GIII before & after two months of treatment	0.004*	0.001**	0.001**

*S (P<0.05), **HS (P<0.01)

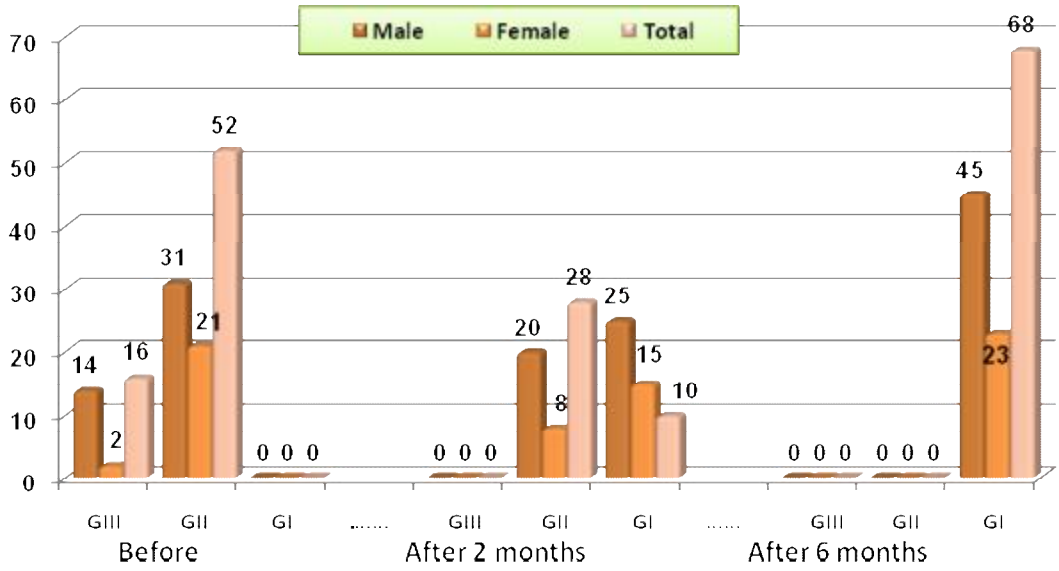

Figure 1: Number of mobile teeth with grade I, II and III mobility before, after 2 months and after 6 months of periodontal treatment in both genders and total sample

Comparing the effect of probiotic and chlorhexidine as a mouth rinses in bacterial plaque

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ABSTRACT

Background: Probiotic technology represents a breakthrough approach to maintaining oral health by utilizing natural beneficial bacteria commonly found in healthy mouth to provide a natural defense against those bacteria though to be harmful to periodontal tissue. Data are still sparse on the probiotic action in the oral cavity. The aim of study was the present study evaluated clinically the efficacy of probiotic type inarched yeast extract, and chlorhexidine mouth rinses on plaque.

Material &method: Four strain of probiotics bacteria were used, *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus & bifidubacterium actiregularis*, and grown in skim milk and plus 0.25, 0.5 % yeast extract individually, the cell free extract of each one of those treatments were add 1: 9 todiluted bacterial plaque taken from 5 subjects, to measure the effect of cell free extract on aerobic & anaerobic bacteria.

45 subjects with chronic periodontitis attend periodontal clinic in college of dentistry university of Baghdad (age 25-35) the trial design as 14 days comparative study between a probiotic and chlorhexidine.

Result: add yeast extract increased the total count of 4 probiotic bacteria 16.25 -29.22% , Cell free extract of *Lb. acidophilus* probiotic skim milk dairy product was the most powerful in reducing both aerobic and anaerobic plaque bacteria among other probiotic bacteria, it reduced 3.38 – 2.17 log cfu/gr aerobic bacteria while the same treatment reducing the anaerobic bacteria 2.5- 2.1 log cfu/gr , the probiotic and chlorhexidine groups had less plaque accumulations compared with the control group at the end of 14day (P<0.001and P<0.001)respectively .

Conclusion: the probiotic mouth rinse was found effective in reducing plague accumulation and gingival inflammation, probiotic mouth rinse obviously has a potential therapeutic value and further study is recommended to determine the efficacy.

Key words: chlorhexidine, probiotic mouth rinse. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):93-99).

INTRODUCTION

Probiotics were defined by FAO/WHO (The Food Agricultural Organization/World Health Organization) aslive microorganisms which when administered in adequateamounts (in food or as a dietary supplement) confer a health benefit on the host (improving microbiologicalbalance in intestinal tract) [(1)]

Probiotics manufacture B-vitamins, such as biotin, niacin (B3), pyridoxine (B6) and folic acid.

- They act as anti-carcinogenic (anti-cancer) factors, with powerful anti-tumourpotentials [(2)].

- They act as 'watchdogs' by keeping an eye on, and effective controlling, the spread of undesirable microorganisms (by altering the acidity of the region they inhabit and/or producing specific antibiotic substances, as well as by depriving rival unfriendly bacteria of their nutrients) [(3)].

The antibiotics some of the friendly bacteria produce are effective against many harmful bacteria, viruses and fungi.

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Not the least of the potentially harmful yeasts controlled by some lactobacilli is "*Candida albicans*," now implicated in many health problems in people who are malnourished or whose immune systems are depleted.[(4)]

- They effectively help to control high cholesterol levels, thereby affording us protection from the cardiovascular damagewhich excessive levels of this nevertheless important substancecan create.[(5)]

- They sometimes act to relieve the symptoms of anxiety.

- They play a role in protecting against the negative effects of radiation and toxic pollutants, enhancing immune function.[(6)]

- They help considerably to enhance bowel function. Where bowel bacteria are absent, the function of peristalsis is impaired, and the amount of time it take for food to pass completely through the system is much increased.[(7)]

Probiotics have been found to be beneficial to host health. Their primary use in medicine has been for the management of intestinal tract problems in recent years, probiotics have been used as a treatment to promote oral health, probiotics on oral health is relatively new with lots of research going on; the area of probiotics and periodontal disease is still in its infancy[(8)]

Essential requisites for microorganisms to exert probiotic properties in the oral cavity: Evidences are there which proves that oral cavity is a natural habitat for some probiotic species.[9]Have found that *Lactobacillus salivarius, Lactobacillus gasseri, Lactobacillus fermentum* and *Bifidobacterium* are among the mostprevalent species in the mouth and theirpresence may be associated withperiodontal health status. Studies haveshown that to be able to exert probioticproperties in the oral cavity it is essentialfor the micro-organisms [10].

_ To resist the oral environmental conditions and defense mechanisms
_ To adhere to the saliva coated surfaces
_ To colonize and grow in the mouth
_ To inhibit oral pathogens and
_ To be also safe for the host.[11]

In oral cavity, probiotics can create a biofilm, acting as a protective lining for oral tissues against oral diseases. Such a biofilm keeps bacterial pathogens off oral tissues by filling a space pathogens would invade in the absence of the biofilm, and competing with cariogenic bacteria and periodontal pathogens growth [12].

MATERIAL AND METHODS

Probiotics bacteria: four strain of probiotics bacteria were used, *Lactobacillus acidophilus, Lactobacillusrhamnosus, Lactobacillus bulgaricus&bifidubacteriumactiregularis (*from food science dep., collage of Agriculture, Baghdad university)

Starters preparation: skim milk 12% T.S was sterilized in autoclave " 121 c, 5 min , 15 atom " and cultured with 5% inoculum of these four strains individually and incubated in 37c till angulation this process repeated three time [13]

Dairy probiotic products: 12 different type of probiotics dairy products were prepared by adding 5% from the four strain of probiotic bacteria as starter culture individually to three type of 12% T.S skim milk , 12% T.S skim milk + 0.25 yeast extract & 12% T.S skim milk+0.5 yeast extract individually , yeast extract was added before sterilization treatment , starter total count bacteria were examined by using serial dilution , MRS agar " himedia com. ,India " was used and incubated in anaerobic conduction + 5% Co_2 for 48 hours. [14]

Antagonism examination:

plaque samples: plaque were collected from five subject (22- 25 years , healthy , none had antibiotic therapy for one month) individually, from three a proximal sites of sub gingival were taken the deepest pockets were selected , after removal of supra gingival plaque the sites were isolated from saliva by application of cotton rolls and were gently dried with compressed air ,three medium sterile papers were transferred into capped vial containing normal 5% saline and vortex mixed for 60 sec [15] then the samples added to Mueller Hinton broth & Mueller Hinton + 0.1Hcl cysteine (himedia , India) to cultivated aerobic and anaerobic plaque bacteria respectively in 35 c for 24 hr., total count for both type of bacteria was enumerated by used of Mueller Hinton agar , the anaerobic bacteria incubated in anaerobic conduction in 35 c for 24 hr. , the same time and period used for the aerobic bacteria [16]

Cell free extract: the supernatants of each dairy probiotic products was collected by centrifugation 50x g , for 4 minutes in 4 c, then by bacterial filter 0.22 micrometers the supernatants was sterilized , 10% v/v supernatants :aerobic or anaerobic plaque bacteria was add , the total count of each was numerated after 24hr in 20 c before & after adding cell free extract as log cfu/ gram plaque individually.[17]

In vivo examination:

This study consisted of 45 subject volunteers with chronic periodontitis in deferent severity ,age group between 25-35 years attending the department of periodontology college of dentistry ,university of Baghdad .

Information oral and written consent was obtained from the subject.

Inclusive criteria:-

*healthy subject without any known systemic illness.
*no recent history of use of antimicrobial agents or any other drugs (at least last four weeks)

Exclusive criteria:-

*subject using any other oral hygiene aids other than routine tooth brushing.

The participants were divided into three groups (A, B&C) with 15th subject in each group as.

Group A: control group (mint water).

Group B: probiotic dairy product group.

Group c: chlorhexidene group .

The mouth rinse were dispensed to the subject , base line scores of plaque index (PI) [18],and gingival index(GI), [19],were taken from all the participants by follow by full mouth prophylaxis.

The designed mouth rinse were dispended to the recipient groups, group A received mint water group B received the probiotic mouth rinse,group C received chlorhexiden mouth rinse .

Group C instructed to rinse two times daily about 30 mint after tooth brushing with 10 ml of the solution (0.02%) chlorhexidine) for 60

second , followed by expectoration to the residual mouth rinsed .

Group A they were instructed to rinse two times daily about 30 mint after tooth brushing with 10 ml of solution for 60 second.

Group B; they were instructed to rinse with probiotic two times daily about (10 ml)

For 60 seconds.

RESULTS AND DISCUSSION

The result in fig. 1 shown that the log cfu/ml for (*Lb . acidophilus, Lb.rhamnosus , Lb. bulgaricus&Bif. aciregularis)* probiotic products were 8.66, 7.91, 7.51 & 8.31 respectively, those values increased after added 0.25% yeast extract to skim milk 25.98 , 24.02 , 9.45 & 15.81% (calculated as log cfu/ ml) respectively , log cfu/ml for the same probiotic bacteria increased for 0.5 % yeast extract treatment and became 29.44 , 27.81 , 10.65 & 18.17 % respectively , the maximum increased was for *Lb . acidophilus* bacteria & it was 2.25 ,2.55 log cycle in 0.25 & 0.5 yeast extract respectively , the minimum increasing was for *Lb. bulgaricus*bacteria0.77, 0.80 55 log cycle in 0.25 & 0.5 yeast extract respectively .

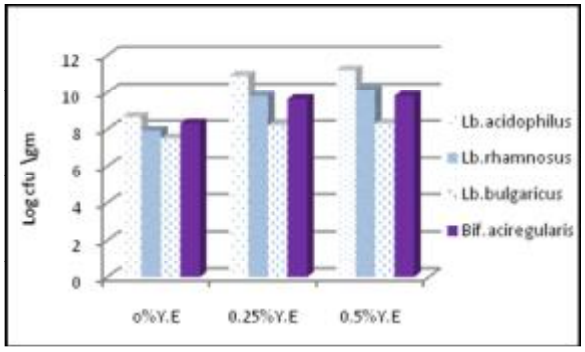

Figure 1: Effect of adding 0.2,0.5% yeast extract to skim milk on logcfu/ ml for probiotic bacteria

Probiotic bacteria grow weakly in skim milk as other dairy products compering with other culture media ex. MRS,Regosa& others [13]. It is well documented that various substances can stimulate the growth of certain strains of probiotic and yogurt cultures in milk [20],Significant increases in total numbers of *L. acidophilus* O16 were observed in milk containing WPH-1 (whey high protein) concentrations of 0.5%, 0.2%, 0.1%, and 0.05%. The growth of *L. acidophilus* L-1 was significantly increased at WPH-1 concentrations of 0.5% and 0.2%. This suggests that strain L-1 has a higher requirement for one or more components of WPH-1 than does strain O16. The growth of each of these cultures was increased over 1-log cycle when grown in milk supplemented with 0.5% WPH-1, compared with the control. Even though not compared in the same experiments, none of the cultures grown in milk containing 1% WPH-1 grew better than in milk containing 0.5% WPH. This indicates that using a WPH-1 concentration level of 0.5% would work just as well in effectively stimulating the growth of probiotic cultures in milk, compared with a concentration level of 1%. It would be more cost-effective to use the lower concentration level (0.5%) to achieve the same results. [21] 11 probiotic *Lactobacillus* strains were capable of utilizing all the 10 oligosaccharides examined but the growth varied among the species, strains and substrates.The three *L. reuteri* and two *L. gallinarum* strains showed strain differences ($P< 0.05$) within the same species based on their different growth activities on various oligosaccharide substrates . Differences were also shown among the four different species, (*L. reuteri*, *L. gallinarum*, *L. salivarius* and *L. brevis*) as demonstrated in the growth profiles of their representative strains on different oligosaccharides such as GOS, IMO, GTO and FOS, and found species-related fermentation behavior shown by the *Lactobacillus* species [6].

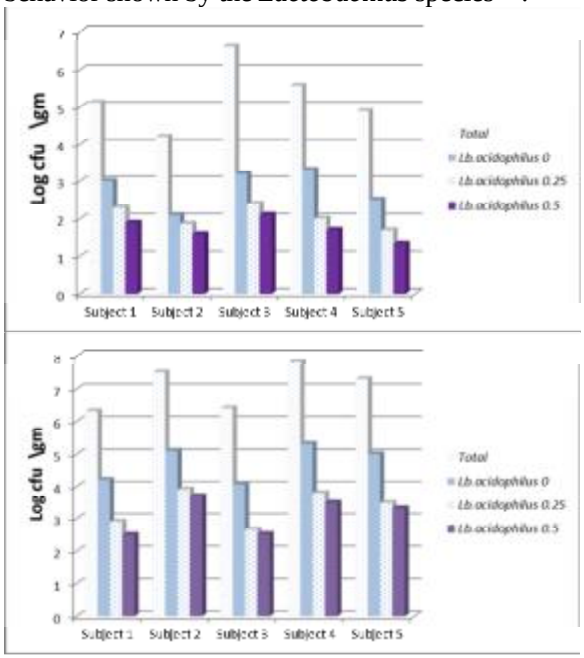

Figure 2: effect of the cell free extract of *Lb. acidophilus* on aerobic & anaerobic bacterial plaque

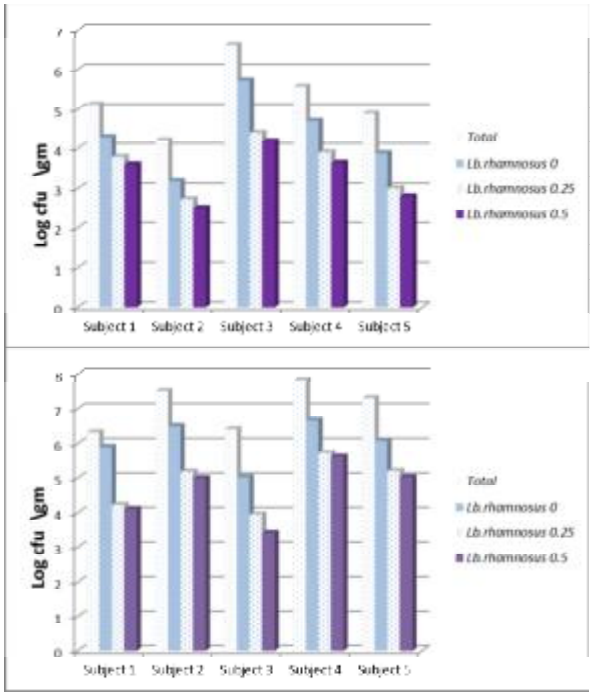

Figure 3: effect of the cell free extract of *Lb.rhamnosus*on aerobic & anaerobic bacterial plaque

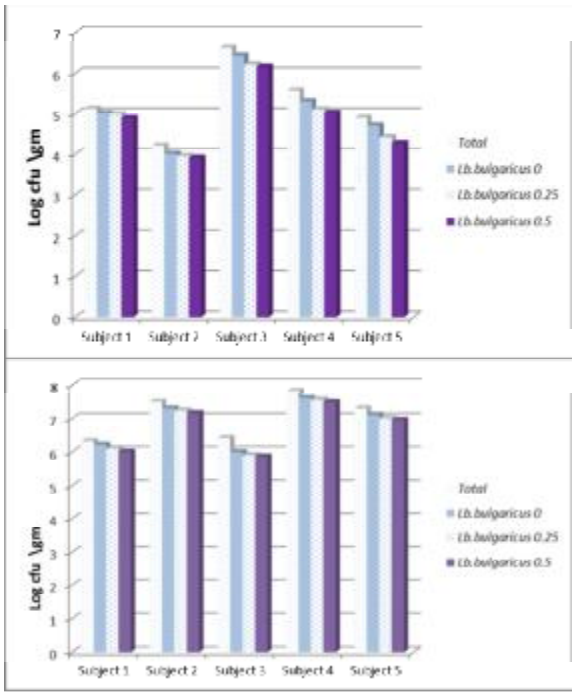

Figure 4: effect of the cell free extract of *Lb. bulgaricus.* on aerobic & anaerobic bacterial plaque

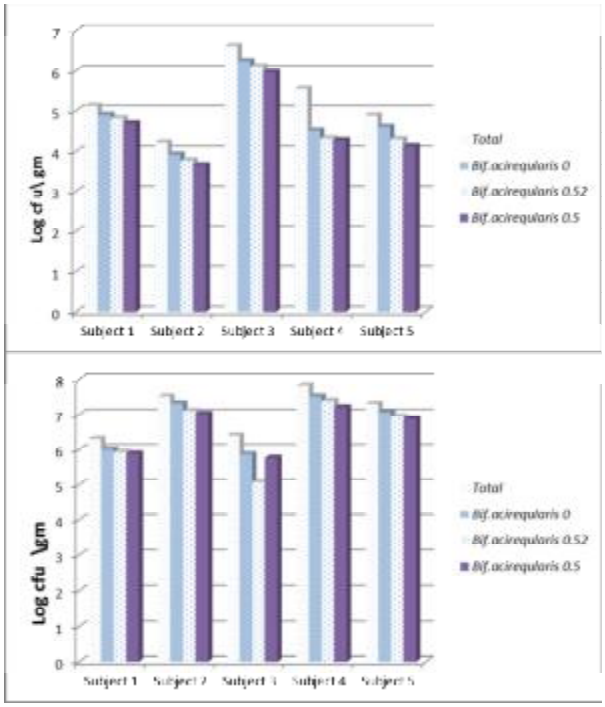

Figure 5: effect of the cell free extractof*Bif. acireqularis.* on aerobic & anaerobic bacterial plaque

Biofilms are increasingly cited as sources of infection and disease in humans. Dental plaque, respiratory infections, stomach ulcers, arteriosclerosis, kidney stones, ear infections, prostatitis, and numerous other microbial-caused ailments have been associated with biofilms [22]
The main pathogenic agents associated with periodontitis are P. gingivalis, Treponemadenticola, Tannerella forsythia and Aggregatibacteractinomycetemcomitans[23]
probiotic can play benefit role to reduce and modified plaque microorganisms [22].

As the result shown in figure 2-5 the total count of the aerobic for the 5 subjects were between 6.6 to 4.22 log cfu/gr while the total count for anaerobic for the same subjects were 6.4 to 7.82 log cfu/gr , this range between the subjects was normal according to the personal oral hygiene , type of food and drinks , flossing , smoking , pH [24].

Cell free extract of Lb. acidophilus probiotic skim milk dairy product was the most powerful in reducing both aerobic and anaerobic plaque bacteria among other probiotic bacteria fig 2 , it reduced 3.38 – 2.17 log cfu/gr aerobic bacteria while the same treatment reducing the anaerobic bacteria 2.5- 2.1 log cfu/gr, further reducing aerobic bacteria of (1.3-0.8) &(0.4-0.27) log cfu/gr after adding 0.25 & 0.5% yeast extract treatments respectively, also further reducing for

anaerobic bacteria between (1.51 – 1.22) & (0.41-0.22) for the same treatments respectively, the 0.25 % yeast extract treatment was significant $p < 0.05$, while the 0.5 yeast extract treatment was not significant, it could be anticipated that this might affect the pathogenic potential of the species based on antimicrobial activity, which in fact is another evaluation criterion for probiotics.

Antimicrobial activity of probiotics has been validated through various in-vitro and in-vivo studies. Sookhee and colleagues [25] isolated 3,790 strains of lactic acid bacteria from 130 individuals and found that the isolates identified as Lactobacillusacidophilus and Lactobacillus rhamnosus had a high capacity to antagonize important oral pathogens, including Streptococcus mutans and Porphyromonasgingivalis. Weissellacibaria, a gram positive facultative anaerobic lactic acid bacterium that has been isolated from humans, is present in fermented foods and is considered a potential probiotic agent [26].

Hydrogen peroxide as well as bacteriocin that acts against gram positive bacteria [26, 27].bacteriocins are ribosomally synthesized cationic peptides with a narrow spectrum of antimicrobial activity, whereas bacteriocin-like inhibitory substances have a broader spectrum Lb. acidophilus produced(acidophilin, acidolin, lactolin) bacteriocin-like [28] cell free extract fig(3) of Lb.rhamnosusskim milk dairy product reduced the aerobic plaque bacteria between (1.03- 0.82) and for anaerobic (1.38- 1.13) log cfu/gr further reducing for 0.25 & 0.5 % yeast cell free extracts extract treatments were observed for both aerobic bacteria (0.80- 0.39) & (0.32-0.18) log cfu/gr respectively, also anaerobic bacteria had further reducing for the same treatments (1.67 – 0.88) & (0.55- 0.19) log cfu/gr respectively.

The cell free extract fig (4 & 5) of Lb. bulgaricus&Bif. aciregularis probiotic dairy products overall maximum reducing for plaque aerobic bacteria were (0.70 , 1.30) respectively & for anaerobic bacteria (0.45 , 0.64) respectively.

As the result shown all the probiotic bacteria used were had the ability to reduced aerobic and anaerobic of plaque bacteria and there ability were not equal, Bagenda, & Yamazaki [29] observedthat Lb..acidophilus was to be the most sensitive indicator strain to the inhibitory effects of the SFC of the LAB isolates. However L. fermentum and L. plantarum were the least sensitive due to bacteriocin like substances or other factors such as pH and hydrogen peroxide were ruled out. Based on these results, most of the inhibitory effect observed for the LAB isolates was due to pH or hydrogen peroxide production. Therefore, the ability to produce bacteriocin , probiotic bacteria did not had the same antagonisms ability even in the same species [16].

Lb.acidophilusskim milk + 0.25 % yeast extract dairy product had been chosen to compare with chlorhexidine mouth wash because this treatment shown the most powerful antibacterial activity among other treatment and the flavor of yeast extract was not detected in vivo.

The mean baseline scores of PI & GI were similar for all the three groups. The mean PI value for all the three groups were 0 after scaling and polishing was done for all tooth surfaces ,the PI index score zero represent a tooth surface that was entirely free from clinically detectable plaque.

At day 14 when compares with the base line data was made ,there was a significant increase in the mean PI scores of the control group when compared with that of probiotic and cholorhexidine groups ($P<0.001$)and $P<0.001$) respectively.

Comparison of the mean plaque scores between the group is represented in table 1.

The degree of increment of mean plaque scores were more pronounced in the control group compared with that of the probiotic and cholorhexidine groups.

Probiotic and cholorhexidine groups had less plaque accumulation compared with the control group.

However there were no significant differences in the mean plaque accumulation between the probiotic and cholorhexidine group on examination on the 14 th day.

Test of subject for the three groups showed a plaque P value <0.001 thus the deference in mean GI for all the three groups were significant.

On the day 14 when comparison with the base line data was made ,there was significant decreases in the mean GI score of probiotic and cholorhexidine group when compared with that of the control group $P<0.001$and $P<0.001$, respectively .

The mean GI score between the group is represented in table 2.

But unlike the PI score, there was a significant difference in the GI between the probiotic and the cholorhexidine ($P=0.009$) probiotic group being better than the cholorhexidine group (mean=0.2300) (0.6805) respectively.

Table 1: The comparison of mean PI scores between the test groups

		Plaque index	
Base line	control	2.3	0.001
	probiotic	2.2	0.001
	chlorhexidine	2.5	0.001
14^{14} days	control	1.8	0.001
	probiotic	0.3	0.001
	chlorhexidine	0.5	0.001

Table 2: The comparison of mean GI scores between the test groups

		Gingival index	
Base line	control	2.1	0.001
	Probiotic	2.2	0.001
	chlorhexidine	2.1	0.001
14^{14} days	control	1.5	0.001
	probiotic	0.2	0.001
	Chlorhexidene	0.3	0.001

Chemical agents have been increasingly used as adjunct to mechanical plague control. They are intended to augment and not to replace mechanical plaque control.

Chlorhexidinegluconate is today thoroughly studied and the most effective antiplaque and ant periodontal disease agent. In oral use as a mouth rinse chlorhexudine has been reported to have a number of local side effects.

Antimicrobial mouth rinse act by nonspecifically reducing the levels of both friendly and harmful oral bacteria .in contrast,probiotic has been developed utilizing natural beneficial bacteria to promote a healthy balance of microorganisms in the mouth(30).

Probiotic technology represent a breakthrough approach to maintaining oral health by utilizing natural beneficial bacteria commonly found in healthy mouth to provide a natural defense against those bacteria thought to be harmful to teeth and gum[4]

The advantages of using a probiotic mouth rinse are that is contains friendly commensals, there isno issue of antibioticresistance, and there are no known or proven toxicities caused to their used.

However, inoral medicine this area of research is still in the cradle.

Probiotic in the form of mouth rinse has been tested among adult in one study. The result of this clinical trial showed that rinsing with probiotic result in a significant reduction of plaque accumulation and gingival inflammation.(31)

In this study it was observed that there was a significant difference in the mean PI and mean GI between the control chlorhexidine and probiotic mouth rinses groups after 14 days compared with the baseline (P<0.001).

However there were no significant differences in the mean plaque accumulations between the probiotic and chlorhexidine groups on the day 14 examination.

But unlike PI score, there was significant difference in the GI between the probiotic and the chlorhexidine groups (P=0.009), probiotic group (mean=0.2300 and 0.6805, respectively).The finding of this clinical study are in agreement with a previous study (31).

REFERENCES

1. Brown AC, Valiere A. Probiotics and medical nutrition therapy. Nutr Clin Care 2004; 7: 56-68.
2. Hinnebusch BF, Meng SF, Wu JT, Archer SY, Hodin RA. The effects of short-chain fatty acids on human colon cancer cell phenotype are associated with histone hyper acetylation. J Nutr 2000; 132: 1012-1017.
3. gustina R, Lukito W, Firmansyah A, Suhardjo HN, Murniati D, Bindels. The effect of early nutritional supplementation with a mixture of probiotic, fiber and micronutrients in infants with acute diarrhea in Indonesia. Asia Pac J Clin Nutr 2007; 16: 35-42.
4. aldeano CM, de LeBlanc A, de M, Vinderola G, Bonet MEB, Perdigón G. Proposed model: mechanisms of immunomodulation induced by probiotic bacteria. Clin Vaccine Immunol 2007; 14: 485-492
5. Lye HS, Rusul G, Liong MT. Removal of cholesterol by lactobacilli via incorporation of and conversion to coprostanol. J Dairy Sci 2010; 93: 1383-1392
6. cholz-Ahrens KE, Ade P, Marten B, Weber P, Timm W, Asil Y, Glüer CC, Schrezenmeir J. Prebiotics, probiotics and synbiotics affect mineral absorption, bone mineral content and bone structure. J Nutr 2007; 137: 838-846.
7. ian. An in vitro antimicrobial and safety study of *Lactobacillus reuteri* DPC16 for validation of probiotics concept. A master thesis. Massey University, Auckland, New Zealand, 2008.
8. ivek G, Bhavana G. Probiotics and periodontal disease: a current update. J Oral Health Comm Dent 2010; 4(Sp l): 35-37.
9. aglar E, Kargul B, Tanboga I. Bacteriotherapy and probiotics' role on oral health. Oral Dis 2005; 11:131-7.
10. Comelli EM, Guggenheim B, Stingele F, Neeser JR. Selection of dairy bacterial strains as probiotics for oral health. Eur J Oral Sci 2002;110:218-24.
11. Hojo K, Mizoguchi C, Takemoto N, Oshima T, Gomi K. Distribution of salivary lactobacillus and bifidobacterium species in periodontal health and disease. Biosci Biotechnol Biochem 2007;71:152-157

12. Iva Stamatova, Jukka H Meurman. Probiotics and periodontal disease. Periodontology 2000, 2009; 51:141-151
13. Antonio-Juan Flichy-Fernández 1, Teresa Alegre-Domingo 1, David Peñarrocha-Oltra 2, Miguel Peñarrocha- Diago , Probiotic treatment in the oral cavity: An update. Med Oral Patol Oral Cir Bucal 2010;15 (5):e677-80
14. Robinson RK. Dairy microbiology. The microbiology of milk products. Elsevier applied Sci. London & New York, 2007.
15. APHA (American Public Health Association), Standard Methods for the Examination of Dairy Products.14th Ed. Marth. E.H. (Ed). American Public Health Association. USA, Washington D.C. 2007.
16. Salam A. Syed, Walter J. Loesche. Efficiency of various growth media in recovering oral bacterial flora from human dental plaque. Applied Microbiology 1973; 26(4): 459-465.
17. Hamilton M. Testing antimicrobials against biofilm bacteria. Journal of AOAC international 2002; 85(2): 479-486 .
18. Silness J, Loe H. Periodontal disease in pregnancy. Acta Odontol Scand 1964; 22: 121.
19. Loe H,Silness J. Periodontal disease in pregnancy. Acta Odontol Scand 1963; 21:533-51.
20. Oliveira R, Oliveira de A, Glória MB. Screening of lactic acid bacteria from vacuum packaged beef for antimicrobial activity. Brazilian J Microbiol 2008; 39: 368-374
21. Ravula RR, Shah NP. Effect of acid casein hydrolysate and cysteine on the viability of yogurt and probiotic bacteria infermented frozen dairy desserts. Aust J Dairy Technol1992; 53:175–9.
22. Saminathan M Sieo CC, Kalavathy R, Abdullah N, Ho YW. Effect of prebiotic oligosaccharides on growth of *Lactobacillus* strains used as a probiotic for chickens. African Journal of Microbiology Research 2011; 5(1): 57-64.
23. Grudianov AI, Dmitrieva NA, Fomenko EV. Use of probiotics Bifidumbacterin and Acilact in tablets in therapy of periodontal inflammations. Stomatologiia Mosk 2002; 81: 39-43
24. Houle MA, Grenier D, Maladies parodontales: connaissance sactuelles. Current concepts in periodontal diseases. Medicine et Maladies Infecteuses 2003; 33(7):331-340
25. Kõll-Klais P, Mändar R, Leibur E, Marcotte H, HammarströmL, Mikelsaar M. Oral lactobacilli in chronic periodontitis and periodontal health: species composition and antimicrobial activity. Oral Microbiol Immunol 2005; 20: 354-61
26. Sookkhee S, Chulasiri M, Prachyabreud W. Lactic acid bacteria from healthy oral cavity of Thai volunteers: inhibition of oral pathogens. J Appl Microbiol 2001; 90(2):172-179.
27. Kang MS, Kim BG, Chung J, Lee HC. Inhibitory effect of Weissellacibaria isolates on the production of volatile sulphur compounds. J Clin Periodontal 2006; 33(3): 226-232.
28. Srionnual S, Yanagida F, Lin LH, Chen YS. Weisellicin. A newly developed bacteriocin from Weisella cibaria, isolated from plaasom, a fermented fish product from Thialand. Appl Environ Microbiol 2007; 73(7): 2247-2250.
29. Cintas LM, Casaus MP, Herranz C, Nes IF, Hernandez PE. Review: bacteriocins of lactic acid bacteria. Food Science and Technology International 2001; 7: 281–305.
30. Bagenda DK, Yamazaki K. Application of bacteriocins in food preservation and safety. Food 2007; 1: 137-148.
31. Meurman JH. Probiotic: dothey have a role in oral medicine and dentistry? Eur J Oral Sci 2005; 113: 188-96.
32. Noordin K, Kamin S. The effect of probiotic mouth rinse on plaque and gingival inflammation. Annal dent Univ Malaya 2007; 14: 19-25.

Correlation between biochemical analysis and periodontal health status and tooth loss in chronic renal failure patients

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ABSTRACT

Background: Periodontal disease is a chronic bacterial infection that affects the gingiva and bone supporting the teeth. The chronic renal failure is one of the serious systemic diseases. It causes general systemic changes which reflect themselves on the oral cavity components. The aims of this study is to determine and compare the periodontal clinical parameters and tooth loss between renal failure patients under hemodialysis and healthy individuals, to determine and compare the levels of C-reactive protein and albumin in saliva and serum between these two groups and to correlate the biochemical parameters with the clinical parameters for the renal failure patients.

Materials and Methods: Sample population consisted of (100) individuals. Males and females were included in this study aged from 45-55 years old. The human sample divided into two main groups; study and control groups. The study group subdivided into hepatitis positive and negative subgroups. Sample recruited for study were 73 patients attending the Artificial Kidney Centers in Baghdad city, all were under hemodialysis. The control group consisted of 27 healthy individuals no history of any systemic disease. All the clinical parameters were tested for both groups together with the levels of C-reactive protein and albumin in saliva and serum.

Results: The statistical analysis revealed highly significant differences between the study and control groups for all the clinical parameters and highly significant differences between the study and control groups when comparing the levels of albumin and C - reactive protein in saliva and serum. Non-significant differences were found when comparing males and females for each group for both clinical and biochemical parameters. But highly significant differences were illustrated when comparing between hepatitis +ve and –ve patients in the study group for all the parameters. Few strong correlations were revealed between the clinical and biochemical parameters; however, most of the correlations had confidence levels more than 75% which could have clinical significance.

Conclusions: The study group had worse periodontal health status than the control group. Also, there was a weak correlation between the clinical and biochemical parameters but with some interested numerical differences.

Keywords: Periodontal health status, C-reactive protein, renal failure, hemodialysis. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):100-105).

INTRODUCTION

Periodontal diseases are one of the most wide spread diseases of mankind, no nation and no region of the world being free from them [1].

The extent, severity, and course of periodontal diseases are affected by factors such as diet, genetics, personal oral hygiene, group (public) preventive services as well as personal dental preventive, diagnostic and therapeutic services [2].

Systemic diseases are among the factors that affect periodontal diseases. Chronic renal failure is a progressive disease that is characterized by the destruction of the kidney's functional units (nephrons) [3].

In order to prolong life, dialysis as an artificial means of removing nitrogenous and other toxic products of metabolism from the blood is the treatment of choice [4]. Improvements in dialysis and modalities for treating patients with renal failure are extending the life expectancy of the affected patient population and the likelihood that dentists will treat such patients is also increasing [5].

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The dental care of patients undergoing dialysis can be complex, given the prevalence of combined conditions such as diabetes, hypertension, renal osteodystrophy, immunosuppression, the presence of non-dental prosthetic devices, and the use of antihypertensives and anticoagulants or antiplatelets agents [6].

These patients appear to be predisposed to a variety of dental problems such as periodontal disease, narrowing of the pulp chamber, enamel abnormalities, premature tooth loss and xerostomia. Dental care, as well as primary preventive measures, seems to have been neglected in these patients [7].

MATERIALS AND METHODS

Instruments and supplies: many instruments and supplies were used in our study such as dental mouth mirror, periodontal probe (William's probe), pan for sterilized instruments, cotton wool, Pasture pipettes, special chemical kits for determination of albumin and C-reactive protein levels in serum and saliva, cooling centrifuge, cooling box and spectrophotometer for measuring levels of albumin in saliva and serum.

Human samples: Sample population consisted of (100) individuals. Males and females were included in this study aged from 45-55 years old. Sample recruited for study were patients attending the Artificial Kidney Centers in Baghdad city which included; Al-Kadhmiya Teaching Hospital, Al-Yarmook Teaching Hospital and Al-Karama Teaching Hospital, all were under hemodialysis.

The test group consisted of 73 patients who are divided into 2 subgroups; the first subgroup is hepatitis positive (+ve) (43 patients) and second subgroup is hepatitis negative (- ve) (30 patients).

The control group consisted of (27) adults with no history of systemic disease and with no history of drugs intake for the last month.

Clinical examination: The patients were clinically examined during the hemodialysis session which usually takes about 3-4 hours.

Control group was matched for the age range with the study group and also male and female were included. All the teeth were examined for each patient except the third molar was excluded.

Collected data included assessment of plaque index, gingival index, bleeding on probing, pocket depth, clinical attachment level, tooth mobility (mobility index) and number of missing teeth.

Biochemical analysis: The biochemical analysis include measuring the concentrations of albumin and C-reactive protein both in saliva and serum. Albumin in saliva and serum were measured by colorimetric method by the spectrophotometer using (Human) determination kits.

C-Reactive protein in serum and saliva was detected by using Latex Agglutination Slide Test (HumaTex CRP) for the qualitative and semi-quantitative determination of C-Reactive protein in non-diluted serum and saliva. HumaTex CRP is based on the immunological reaction between human C-reactive protein of a patient specimen and the corresponding anti-human CRP antibodies bound to Latex particles. The positive reaction is indicated by a distinctly visible agglutination of the Latex particles in the test cell of the slide. After that the positive samples were diluted by special fluid for semi-quantitative determination.

Figure 1: Chemical kits used for determination of albumin and C-reactive protein in saliva and serum.

Statistical analysis: Data were analyzed through the use of SPSS (Statistical Process for Social Science) version 11 application statistical analysis system and Excel.

The following statistical data analysis approaches were used in order to analyze and assess the results of the study:

I. Descriptive data analysis:

Tables (Frequencies and Percentages). arithmetic mean, standard deviation, standard error, two extreme values (min. and max.) of the calculated mean value and graphical presentation by using Bar chart.

II. Inferential data analysis:

These were used to accept or reject the statistical hypotheses, which included the Student t-test for equality of means of two independent groups.

Also, Pearson's Correlation Coefficient was used for testing the correlation between the two independent variables; the clinical and biochemical parameters.

RESULTS

Clinical parameters: For plaque index, the Statistical analysis comparing between the control and study groups, using t-test revealed a highly significant difference (p=0.000). For gingival index. The statistical analysis comparing between the control and study group using t-test revealed a highly significant difference (p=0.000).

For bleeding on probing, Statistical analysis comparing between the study and control groups revealed a highly significant difference between the two groups for score 0 (p=0.000) and for score 1 (p=0.002).

For pocket depth when comparing between the study and control groups, the statistical analysis showed a highly significant difference for all the 3 groups at (p=0.000). For clinical attachment level, the statistical analysis illustrated a highly significant difference between the study and control group for all the levels of attachment loss (p=0.000) except for (1-2) (p=0.001).

For mobility index, the statistical analysis comparing between the study and control group inform of a highly significant difference between them (p=0.000). For tooth loss.a highly significant difference was found between study and control group (p=0.000).

Biochemical parameters: For albumin level in saliva, the statistical analysis revealed a highly significant difference between the study and control groups (p=0.000).

For albumin level in serum, The statistical analysis showed a highly significant difference between the study and control groups (p=0.000).

While for C-reactive protein levels in saliva, a highly significant difference was found between the study and control group (p=0.000). And for c-reactive protein in serum, the statistical analysis announced a highly significant difference between the study and control groups (p=0.000).

Table 1: Description of significance between study and control groups for plaque index, gingival index, bleeding on probing, mobility index and missing teeth.

Parameters	t-test for Equality of Means		Sig.
	t-test	p-value	
PL	6.1	0.000	HS
GI	5.4	0.000	HS
BL on PR(0)	-10.0	0.000	HS
BL on PR(1)	7.4	0.000	HS
MI	6.1	0.000	HS
M T	5.4	0.000	HS

Table 2: Description of significance between study and control groups for pocket depth and clinical attachment level

Parameter	t-test for Equality of Means		Sig.
	t-test	p-value	
0-2 (PD)	-7.0	0.000	HS
3-5 (PD)	4.3	0.000	HS
≥ 6 (PD)	5.3	0.000	HS
0-2 (CAL)	3.6	0.001	HS
3-5 (CAL)	5.5	0.000	HS
≥ 6 (CAL)	-7.0	0.000	HS

HS : Highly Significant at P<0.01

Table 3: Description of means and significance between study and control groups for albumin levels in saliva and serum

Parameters	Groups	N	Mean	S.D.	S.E.	Min.	Max.	P-value	Sig.
ALB. Saliva	**Study**	73	266.75	45.44	5.32	200.00	382.40	0.000	HS
	Control	27	107.37	26.34	5.07	46.60	155.70		
ALB. Serum	**Study**	73	33.83	2.88	0.34	25.70	38.70	0.000	HS
	Control	27	45.54	2.66	0.51	40.20	53.40		

Table 4: Description of means and significance between study and control groups for C-reactive protein in saliva and serum.

Parameters	Groups	N	Mean	S.D.	S.E.	Min.	Max.	P-value	Sig.
CRP saliva	Study	29	22.1	6.6	1.2	12	30	0.000	HS
	Control	2	12.0	0.0	0.0	12	12		
CRP serum	Study	49	21.7	6.3	0.9	12	30	0.000	HS
	Control	3	18.0	0.0	0.0	18	18		

Correlation between clinical and biochemical parameters: For the study group, the statistical analysis revealed a significant difference comparing the GI with the CRP in saliva outcomes. Also a significant difference was found comparing the (3-5) level of attachment loss with the CRP in saliva outcomes. A significant difference was revealed comparing between MI and CRP in serum.

On the other hand, its important to mention some results that could have a clinical significance. For example for testing correlation between GI and CRP in serum . P-value for BOP (score 1) and serum CRP was 0.251. For correlation between BOP (score 1) and albumin in serum and others (Table 3-35).

Table 5: Pearson's Correlation Coefficients among CRP, ALB in (Saliva, Serum) and different of the studied parameters

Parameters	Classification / Correlations / P-value	CRP Saliva	CRP Serum	ALB. Saliva	ALB. Serum
PL	Pearson Corr.	-0.043	-0.096	0.060	0.037
	Sig. (1-tailed)	0.413	0.311	0.379	0.425
GI	Pearson Corr.	-0.334*	-0.113	0.001	0.056
	Sig. (1-tailed)	0.038	0.279	0.499	0.387
B O P(0)	Pearson Corr.	0.073	0.005	0.082	0.044
	Sig. (1-tailed)	0.353	0.489	0.337	0.410
B O P(1)	Pearson Corr.	-0.087	0.130	0.060	0.171
	Sig. (1-tailed)	0.327	0.251	0.378	0.188
MI	Pearson Corr.	0.114	-0.343*	-0.088	0.025
	Sig. (1-tailed)	0.278	0.034	0.325	0.448
M T	Pearson Corr.	0.031	-0.255	-0.193	-0.271
	Sig. (1-tailed)	0.437	0.091	0.158	0.077
1-2 (PD)	Pearson Corr.	-0.168	-0.032	-0.035	0.055
	Sig. (1-tailed)	0.193	0.435	0.429	0.388
3-5	Pearson Corr.	0.203	0.199	0.165	0.139
	Sig. (1-tailed)	0.145	0.150	0.197	0.236
6 ≥	Pearson Corr.	-0.006	-0.143	-0.049	0.073
	Sig. (1-tailed)	0.488	0.229	0.399	0.353
1-2 (CAL)	Pearson Corr.	0.225	0.047	-0.129	0.010
	Sig. (1-tailed)	0.121	0.405	0.253	0.480
3-5	Pearson Corr.	-0.318*	0.169	0.285	0.203
	Sig. (1-tailed)	0.046	0.191	0.067	0.145
6 ≥	Pearson Corr.	0.154	-0.237	-0.007	0.012
	Sig. (1-tailed)	0.213	0.108	0.486	0.476

(*) Sig. at P<0.05; (**) high Sig. at p<0.01

DISCUSSION

Clinical parameters :

The statistical analysis showed that the chronic renal failure patients have higher plaque index, higher gingival index, bleeding on probing, deeper periodontal pockets, more attachment loss, higher mobility index and more missing teeth than the healthy individuals. Also, the hepatitis +ve patients have higher values of clinical parameters than the hepatitis – ve. These results could be attributed to many reasons. These differences might be caused by the physiological and psychological condition of hemodialysis patients who seem to have a lower interest in their oral

hygiene condition and it might have accumulative effect as the disease progress with hepatitis after a period of time [8]. The chronic debilitating diseases such as syphilis, chronic nephritis and tuberculosis may predispose the patient to periodontal disease by impairing tissue resistance to local irritants creating a tendency toward gingivitis and alveolar bone loss [9]. Also, hepatitis patients always have higher risks for poor oral health. They are more likely to develop tooth decay, periodontal diseases, sensitive teeth, soreness of the mouth and the gums and bleeding [10]. Patients with chronic renal dysfunction will often have hematological problems, most commonly anaemia and bleeding. The presence of bleeding is widely accepted as an objective sign of inflammation in gingival and periodontal tissues. In fact many authors claim that bleeding observed with probing indicate pathological condition regardless of the probing depth [11]. Outcomes of our study suggest that the biochemical changes associated with chronic renal failure doubtly influencing the process of excessive destruction and deepening of periodontal pockets. Renal failure therapy can affect periodontal tissues including gingival enlargement in immune suppressed renal transplantation patients and increased levels of plaque, calculus and gingival inflammation and possible increased prevalence and severity of destructive periodontal diseases in ESRD patients on dialysis maintenance therapy [12]. On the other hand, the authors also postulate that periodontal disease is influenced by chronic renal failure because of insufficient bone metabolism. Earlier studies provide evidence that vitamin D polymorphisms may predispose to both chronic kidney disease and periodontitis. Hence it is possible that periodontal disease and chronic kidney disease might share common risk factors [13, 14].

Actually, Tooth mobility and drifting have been documented in CRF patients without appreciable pathological periodontal defects [15]. Tooth mobility is likely secondary, at least in part, to renal osteodystrophy, which result from secondary hyperparathyroidism. The classical signs of hyperparathyroidism of the mandible and maxilla are bone demineralization, loss of trabeculation, grand glass appearance and total loss of lamina dura mostly in the mandibular molars [7].

The high incidence of tooth loss in the hemodialysis patients might be caused by both accelerated alveolar bone loss resulting from renal osteodystrophy and poor oral hygiene. . Alveolar bone resorption plays an important risk of tooth loss. In addition, it has been suggesed that generalized bone loss contributes to the development of, or increases the severity of, alveolar bone loss in periodontal diseases [16].

Biochemical parameters:

Salivary albumin has been shown to be increased in medically compromised patients whose general condition has gotten worse. Immunosuppression, radiotherapy, kidney diseases and diabetes are examples of the states in which high concentrations of salivary albumin have been detected. It may be hypothesized that salivary albumin can be used to assess the integrity of the mucosal function in the mouth [17]. To a certain limit, It seems that the increase of salivary albumin in hepatitis patients may result from degeneration, destruction and lysis of periodontal cells [18].

On the other hand, Hypoalbuminemia, is highly prevalent in kidney failure and is associated with an increased mortality risk in this population. Decreased level of albumin in the blood in CRF patients are due to the protein loss to the urine which reflect the inability of the kidneys to maintain proteins, like albumin, in the blood. In theory, hypoalbuminemia may reflect either the inflammatory or nutritional status of patients with kidney failure [19].

C-Reactive Protein is an acute phase protein produced in the liver. The relationship between serum and salivary levels of CRP is not well understood. In a recent study, sick people admitted to a hospital had average salivary CRP levels 25 times higher than healthy people [20]. Salivary CRP may largely reflect local inflammation in the mouth, but some serum CRP can enter saliva through gingival tissues, especially if periodontal disease is present [21].

In serum, it was suggested that C-reactive protein (CRP) levels are elevated in patients with kidney failure and are independent predictors of cardiovascular mortality in this patient population [22].

Correlation between clinical and biochemical parameters: In testing the correlation between the clinical and biochemical parameters, most of the p-values should be studied carefully to understand the actual clinical significance not only the statistical significance. It was stated statistically that the study findings that are statistically not significant does not means the negative results are also clinically not significant, the opposite also holds true when a difference

between two study populations is found to be not statistically significant, even though it is biologically plausible. In this case, as in many other studies reported in the medical literature, the study probably had inadequate power, resulting from a low sample size. Many medical journals now require the use of confidence levels because they provide more information than the p-value in the setting of both positive and negative results. Conversely, if the findings are not significant, confidence levels can give an indication as to whether there is a potential for clinic significance if further studies are pursued, which usually happens when a larger sample size is needed [23,24].

REFERENCES

1. Buatongsri V, Songpaison Y, Hong prasong N, Phantumvanti P, Clark N**.** The distribution of severe periodontitis in urban (Bankok) and rural (payao province) high risk to stress group of this population. Cu Dent J 2002; 25:1-4.
2. Brown L, Jackson, Beverly A, Johns Thomas PW. The economics of periodontal diseases. Periodontol 2000-2002; 29: 223-34.
3. Ziccardi VB, Siani J, Demas PN, et al. Management of the oral and maxillofacial surgery patient with end stage renal disease. J Oral Maxillofac 1992: 90: 1207.
4. Davidovich E, Davidovitz M, Eielman E, Schwarts Z, Bimstein E. Pathology, therapy, and oral implications of renal failure in children and adolescents. Pediatr Dent 2005; 27: 98-106.
5. Grevel J, Reynolds KL, Rutzky LP, Kahan B**.** Influence of demographic factors on cyclosporine pharmacokinetics in adult uremic patients. J Clin Pharmacol 1989; 29:3: 261-6.
6. Carranza FA. Carranza's Clinical Periodontology.10th ed. Missouri: Saunders Company; 2009.
7. Klassen JT, Krasko BM. The dental health status of dialysis patients. J Can Dent Assoc 2002; 68: 1: 34-8.
8. Marakoglu I, Kahraman UG, Demirer S, Sezer H. Periodontal status of chronic renal failure patients receiving hemadialysis. Yonsei Medical J 2003; 44: 4: 648-52.
9. Nelson MA, Chaudhry AP. Effects of tocopherol (Vitamin E) deficient diet on some oral, para-oral and hematopoitic tissues of the rat. J Dent Res 1966; 45: 1072.
10. Robert MA. The relationship between hepatitis and oral health. Cosmetic Dentistry Guide 2011; 24: 66-71.
11. Barrington EP and Nevins M. Diagnosing periodontal disease. J Am Dent Assoc 1990; 121: 460-4.
12. Craig R. Interaction between chronic renal disease and periodontal disease. Oral Diseases 2008; Jan: 14:1-7.
13. Yoshihara A, Deguchi T, Hanada N, Miyazaki H. Renal function and periodontal disease in elderly Japanease. J Periodontol 2007; 78:1241-8.
14. Brito F, Almeida S, Figueredo CMS, Bregman R, Suassuna JHR, Fischer RG. Extent and severity of chronic periodontitis in chronic kidney disease patients. J Periodont 2011; 10: 11-6.
15. Carl W. Chronic renal disease and hyperparathyroidism; Dental manifestations and management. Compend Contin Educ Dent 1987; 8(9): 697-704.
16. Shoji K, Ohtusuka-Isoya M, Horiuchi H, et al. Bone mineral density of alveolar bone in rats during pregnancy and lactation. J Periodontol 2000; 1073-8.
17. Meurman JH, Pyrhönen S, Teerenhovi L, Lindqvist C. Oral sources of septicaemia in patients with malignancies. Oral Oncol 1997; 33: 389-97.
18. Cinquini I, Calisti L, Fierabracci V, Marrapese E, Egéa JC, Masiello P, Giuca MR . Enzymatic markers of salivary cell injury in saliva of type 1 diabetic children. Clin Oral Investig 2002; 6: 21-3.
19. Foley RN, Parfrey PS, Harnett JD, et al. Hypoalbuminemia, cardiac morbidity, and mortality in end-stage renal disease. J AmSoc Nephrol 1996; 7: 728–36.
20. Krasteva A, Perenovska P, Ivanova A, et al. Alteration in salivary components of children with allergic asthma. Biotechnology Biotehnological Equipment 2010; 24(2): 1866-9.
21. Higashi Y, Goto C, Jitsuiki D, et al. Periodontal infection is associated with endothelial dysfunction in healthy subjects and hypertensive patients. Hypertension 2008; 51(2): 446-53.
22. Arici M, Walls J. End-stage renal disease, atherosclerosis, and cardiovascular mortality: Is C-reactive protein the missing link? Kidney Int 2001; 59: 407–14.
23. Dowson B, Trapp EG. Basic and Clinical Biostatistics, 3rd ed. New York: Lange; 2001. pp 63-135.
24. Robert JN. Epidemiology and Biostatistics Secrets. 4th ed. Philadelphia: Mosby Elsevier Co.; 2006; pp 158-160.

Evaluation of inorganic ions and enzymes levels in saliva of patients with chronic periodontitis and healthy subjects

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ABSTRACT

Background: Chronic periodontitis is an inflammatory disease of tissues supporting the teeth. Inorganic ions and salivary enzymes have been most intensely studied as a potential marker for periodontal disease. This study aimed to detect sodium, potassium, magnesium and calcium ions level in saliva & also to assess the activity of creatin kinase (CK) and gamma glutamyle tranferase (GGT) enzymes in saliva of patients with chronic periodontitis and healthy subjects and correlate the mean salivary levels of these ions and enzymes with clinical periodontal parameters (PLI, GI, PPD and CAL).

Materials and Methods: The study sample consists of (23) patients with chronic periodontitis and (12) healthy subjects of both gender with age ranged (35-45) years .Plaque index (PLI). Gingival index (GI), probing pocket depth (PPD) and clinical attachment level (CAL) are the periodontal parameters used in this study, un stimulated saliva sample were collected from all subjects and the levels of sodium, calcium, Magnesium, potassium, CK and GGT enzymes in each specimen were analyzed for each group .A statistical analysis was done to estimate the levels of these ions and enzymes in saliva and correlate the mean salivary inorganic and enzyme levels with the clinical periodontal parameters.

Results: The present study showed that highly significant and significant differences in the levels of inorganic ions Na^{+} and Ca^{+2} respectively was found between chronic periodontitis and control group, while non significant differences in the level of Mg^{+2} and K^{+} ions were found between the study group and control group. Highly significant differences in the levels of salivary enzymes CK and GGT were found between the control group and chronic periodontitis group. This study showed a positive correlation between the activity CK enzyme and PLI, GI and CAL. Also there was a significant correlation found between Na^{+}, Ca^{+2} and CAL. Concerning PPD, there was no correlation between those ions and enzymes with PPD in chronic periodontitis patients.

Conclusion: estimation of those in organic ions and enzymes in saliva of chronic periodontitis may be used as potential diagnostic markers of disease status in periodontal tissues.

Keyword: Chronic periodontitis, inorganic ions, enzymes, saliva. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):106-111).

INTRODUCTION

Periodontal disease is a chronic disease of the oral cavity comprising a group of inflammatory conditions affecting the supporting structures of the dentition [1, 2]. Predominantly an aerobic gram negative bacteria present on the tooth surface as microbial biofilms and other microbial substance gain access to the gingival tissue and initit inflammatory reaction, which leads to the destruction of periodontal ligament and alveolar bone and finally tooth loss [3]. Clinical parameter such as probing pocket depth (PPD), gingival index GI, plaque index (PI), clinical attachment level (CAL), provide information on the severity of periodontitis but they do not measure disease activity, where as microbiological tests, analysis of host response, and genetic analysis have been proposed in an effort to monitor and identify patients at increased risk for periodontitis [4].

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Saliva has been found lately as an important biological material to the purpose to introduce new diagnostic tests which may contribute to making a diagnosis and explaining the pathogenesis of many systemic diseases.

A response of an organism to periodontal infection includes production of several enzymes families which are released by stromal, epithelial, inflammatory or bacterial cells. The analysis of these enzymes in salivary secretion, as well as in the gingival crevicular fluid can contribute to clarification of pathogenesis and to improvement of making a prompt diagnosis of periodontal disease [5].

Leading roles in this sense have the enzymes of tissue degradation, such as: elastase, collagenase, of protienase. The same intra cellular enzymes are increasingly released from the damaged cells of periodontal tissues into gingival crevicular fluid and saliva, as well as in the surrounding fluid. Those particularly relevant in this group of enzymes are: Creatine kinase (CK), gamma glutamyl transfease (GGT) and lactate dehydrogenase (LDH). These enzymes appear to

be useful to test the activity of periodontal disease or to measure the effectiveness of periodontal therapy [6, 7].

Salivary fluid is an exocrine secretion consisting of approximately 99% water and the other 1% is complex of organic and inorganic molecular [8]. Saliva contains the useful electrolytes of the body fluid (calcium, magnesium, sodium, potassium) [9].

Calcium is the most abounded mineral human body several studies indicate that salivary calcium concentration is an important factors in periodontal heath in industrialized countries. High level of salivary calcium is closely related to rapidly mineralized plaque, which turn is related to the poor oral hygiene. [10, 11]

Sodium is one of the major extra cellular ions that represent the principle electrolytes of human body fluid. The concentration of sodium in saliva increases with increase in salivary flow potassium play an important role in the cell function by regulating that osmotic pressure and neuromuscular transmission. It participates in the synthesis of protein and glycogen [12].

Magnesium's function includes acting as a component of bone nerve impulse transmission, protein synthesis and enzymes activity [13].

The aim of this study was the evaluation of the salivary minerals and enzymes in patients with chronic periodontitis and compared them to healthy subjects, also test the relationship between the enzymes and minerals with clinical periodontal parameters PLI, GI, PPD of CAL in chronic periodontitis patients.

MATERIALS AND METHODS

Subjects attending the periodontal department in the College of Dentistry / Baghdad University were enrolled in this study. We have 2 groups from both sexes; the first one is composed of 12 healthy persons and the second group is composed of 23 patients with chronic periodontitis, age 35-45 years, with not less than 5 periodontal sites with pocket depth of 4mm or greater.

All the subjects were in a good health, with no history of systemic disease, non smoker, and did not take vitamins or minerals supplements or medication of any type.

The periodontal examination includes:

1-Assessment of dental plaque by PLI of Silness and Loe[14].

2- Assessment of gingival condition by GI of Loe and Silness.[15]

3- Probing pocket depth (PPD) measurement according to Salvi[16]. the distance from the gingival margin to the bottom of the periodontal pocket was measured to the nearest millimeters by means of graduated periodontal probe. A scaled was design for ease of estimation and as follows:

Scale 1 = 4-5 mm

Scale 2 = 6 mm and greater

4- assessment of clinical attachment level (CAL). The distance from cementoenamel junction to the base of the pocket. A scale was designed for ease of assessment as follows:

Scale 1= 1-3 mm

Scale 2= 4-5 mm

Scale 3= 6-7 mm

Scale 4= 8-9 mm

Collection of saliva was performed under standard condition following instruction cited by Tenovuo [14].Essential elements of saliva were analyzed at the poisoning consultation center at the specialized surgeries hospital by flame atomic absorption spectrophotometer (buck Scientific, 210 VGP,USA) Following standardized procedure for Na^{+} , K^{+} , Mg^{+2} and Ca^{+2} accordance to Haswell [18].The salivary levels of the enzymes CK & GGT were measured using the commercially available kits. The kit used for CK analysis was manufactured by Biolabo SA, (France). The activity of this enzymes was determined by IFCC method on the UV (Ultra Violet)- Visible Spectrophotometer (Cecil, CE-7200), while the activity of GGT was determined by the kinetic colorimetric method according to persijin & van der silk [19, 20] – Standardized against recommended IFCC (International Federation of Clinical Chemistry) method.

The applied statistical analyses were the following: - mean value, standard deviation, correlation coefficient, student's t-test.

RESULTS

In this study the mean ±SD of plaque and gingival index in chronic periodontitis patients were (1.987±0.359, 1.757±0.13) respectively and in control group were (0.474±0.13, 0.66±0.155) respectively as shown in table (1). There was a highly significant difference in the mean of GI ($p<0.001$) Compared to Control group and a Significant difference in the mean of PLI ($p<0.05$) Compared to Control group, table (1), Figure (1).

Table (2) demonstrates the percentage distribution of sites according to different probing pocket depth grades and clinical attachment level grades .The results of this table showed that

78.3%of sites had scale 1 PPD, while 91.3 %of sites had scale1 CAL.

Descriptive statistics of four saliva mineral in patients with chronic periodontitis and control groups are shown in table (3); Figure (2). The results showed that the level of salivary Na^{+} in patients with chronic periodontitis was highly significant ($p<0.001$) than the control group, and the level of salivary Ca^{+2} in the patients group is significantly ($P<0.05$) Higher than Control group, while there is no significant difference in the levels of Mg^{+2} & K^{+} in patients and control group.

The obtained results have shown that the activity of examined Enzymes in patients with chronic periodontitis was significantly higher in relation to control group. The established difference showed the statistical significance of high level ($P<0.001$), table (4), Figure (2).

Correlation between the levels of the indicated salivary enzymes & Minerals and the values of clinical indexes showed a statistically significant correlation Coefficient between the values of gingival index (GI) and the level of K^{+} (-0.472) & CK activity (0.426) in patients Group, also there is a positive significant correlation coefficient between the values of PLI and the activity of CK (0.403) in patients Group. Table (5, 6, 7).There is significant correlation (-0.409) between Na^{+} and CAL (grade3) $p<0.05$, also there is significant correlation (0.497) between CK and CAL (grade3), $p<0.05$.Also significant correlation (0.407) between Ca^{+2} and CAL (grade 4) was observed.

DISCUSSION

In this study, a significant difference in PLI and highly significant difference in GI were found between chronic periodontitis patients and control group. This is obvious since the accumulation of plaque is the primary cause of periodontal disease and chronic periodontitis is a chronic inflammation of the gingiva and connective tissue [(2)].

Highly significant difference in salivary level of Na^{+2} was found between chronic periodontitis and control group. This result was an agreement with other studies [(21, 22)] and disagrees with AL Bahadili who found no significant differences between both groups [(23)].

This study shows a significant difference in Ca^{+2} ion levels between chronic periodontitis and the control group. This result in agreement with Acharya et al who found that periodontal diseases is associated with higher salivary calcium levels than that in healthy periodontium ,indicating that the calcium level of saliva could possibly be a risk factor for the development of periodontal disease [(21)]. These results disagree with Hisham Ali O who found that there was no significant difference in salivary levels of Ca^{+2} ions between chronic periodontitis and control group [(21)]. Calcium ions have been most intensely studied as a potential marker for periodontal disease in saliva. Sewon et al reported a higher concentration of Ca^{+2} detected in whole stimulated saliva from the periodontitis patients. The authors concluded that an elevated Ca^{+2} concentrations in saliva were characteristic of patient with periodontitis [(10)]. Nevertheless, the importance of the salivary of Ca^{+2} ions concentrations in relationship to the progression of periodontal disease is not defined.

In this study non significant differences in salivary levels of K^{+} and Mg^{+2} ions were found between chronic periodontitis and control group, these results agree with Husham Ali O [(21)], while disagree with Abid Aun W, who found significant difference in salivary levels of Mg^{+2} ions between chronic periodontitis and control group [(22)].

This controversy between the present study and other studies could be due different techniques applied, different sample size used and different age group.

Concerning CK and GGT enzymes activities in saliva, highly significant differences have been found between chronic periodontitis patients and control group. These results agree with another study done by Tatjiana et al [(5)]. Those enzymes are indicator of a higher level of cellular damage and their increased activity in saliva is a consequence of their increased released from the damaged cells of soft tissues of periodontium and a reflection of metabolic changes in the inflamed gingiva [(6, 7)].

The present study shows statistically significant correlation between PI and GI values, and the level of CK activities in chronic periodontities patients. These results agree with Tatjiana et al whom found high coefficient of correlation between the values of PI and GI and the activity of CK [(5)].

This study shows that there was no correlation in chronic periodontitis patients with PI and K^{+}, Na^{++} Mg^{+2} and Ca^{+2} ions level in saliva. This result agrees with Aid Aun W [(22)]. A significant correlation had been found between CK and CAL and this result agree with Mahmood M.SH who found strong correlation between CK enzyme and relative attachment level [(25)].

There was no significant correlation found between all salivary ions, enzymes and PPD.

These results agree with Abid Aun W, Ebru E and Ali E whom approved a non significant correlation between PPD and the mean salivary minerals. [22, 26]

As a conclusion, estimation of those inorganic ions and enzymes in saliva of chronic periodontitis may be used as potential diagnostic markers of active disease status in periodontal tissues.

REFERENCES

1. Giannobile WV, Beilker T, Kinney JS, Ramseier CA, Morelli T, Wong DT. Saliva as a diagnostic tool for periodontal disease: current state and future directions. Periodontol 2000 2009; 50: 52-64.
2. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4: 1-6.
3. Aituti FD, Granzian F, Tete S. Periodontitis from local infection to systemic diseases. Int. J. Immunopathol Pharmacol 2005; 18(3 Suppl): 1-12.
4. Sahingur SE, Cohen RE. Analysis of host responses and risk for disease progression. Periodontol 2004; 34: 57-83.
5. Tatyana Todorovic, Ivan Dozie, Mario Vincente, Besir Ljuskovic, Janko Pejovic Marjan Mound Milan K. Salivary enzymes and periodontal disease. Med Pral Patol oral Cir Bucal 2006; 11: E 115-9.
6. Numabe Y, Hisano A, Kamoi K, Yoshie H, Kurihara H. Analysis of saliva of or periodontal diagnosis and monitoring. Poriodontology 2004; 40: 115-9.
7. Kaufman E, Lamster IB. Analysis of saliva and periodontal diagnosis. J Clin Periodontal 2000; 27: 453-65.
8. Ahmadi MF, Davoodi P, Dalband M, Hendi SS. Saliva as a mirror of the body health. DJH 2010; 1(2): 1-15.
9. Sewon L, Lsin M, Karjalainen S, Poeoguins-Kaia A, Helenius H, Kiss E, Lethonen M. Effect of age on flow rate, protein of electrolytes compositions of stimulated whole saliva in healthy, non-smoking pregnant women. Open Dent J 2008; 29: 289-92.
10. Sewon L, Karjalainen S. Soderling H. Association between salivary calcium & oral health. J clinical periodontal 1998; 25: 915-919.
11. Sewon L, Karjalainen S, Sainio M. Calcium and other salivary factors in periodontitis affected subjects prior to treatment. J Clin periodontal 1995; 22: 267-70.
12. Sheng H. Sodium, Choride and Potassium. Biochemical and physiological aspects of human nutrition. 1st ed. Philadelphia: WB Saudnders Company; 2000: 686-700.
13. Groff JL. Gropper SS. Advanced nutrition human metabolism. Acta Odontol Scand 2000; 40: 87-95.
14. Silness J, Loe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odont Scand 1964; 22:121-35.
15. Loe H, Silness J. Periodontal disease in pregnancy I prevelance and severity. Acta Odont Scand 1964; 22:533.
16. Saliva GE, Linghe J, Lang NP. Examination of patients with periodontal disease. Clinical Periodontology and implant dentistry. 5th ed. Munksgaard: Copenhagen; 2008
17. Legerloff TJ. Saliva, in: Thylstup A, Fejerskovo (eds). Textbook of clinical cariology. 2nd ed. Manksgaard: Copenhagen; 1994. pp.17 -43.
18. Haswell SJ. Atomic Absorption Spectrometry theory, design and application. Tokyo: Elservier; 1991.
19. Persijn JP, Van der Silk WA. A new method for the determination of GT in serum. J Clin Chem Clin Biochem1976; 14: 421.
20. Tietz NW. Clinical guide to laboratory test. 3rd ed. Philadelphia: WB Saudnders Company; 1995. pp.180-187
21. Ali BG, Ali OH. Detection of salivary flow rate and minerals in smokers and non smokers with chronic periodontitis (clinical and Biochemical study). J Bagh College Dentistry 2012; 24(1):68-71.
22. Abid Aun WA. Inorganic level in saliva of patients with chronic periodontitis and healthy subject. J Bagh College Dentistry 2012; 24(3): 93-97.
23. Al-Bahadly MA. Quantitative determination of essential elements in saliva associated with different severities of periodontal disease and its correlation with histological picture. A master thesis, Department of Periodontics, College of Dentistry, University of Baghdad, 2007.
24. Acharya A, Kharadia MD, Dhavale R, Deshmukh VL, Sontakke AN. High Salivary calcium level associated with periodontal disease in Indian's subjects. A pilot study. Oral Health Dent 2011; 9(2): 195-200.
25. Mahmood M. Sh. Evaluation of the salivary biomarkers creatine kinase (CK) and blood urea nitrogen (BUN) activities before and after non surgical periodontal treatment in patients with chronic periodontitis. J Bagh College Dentistry 2011; 23(4): 107-11.
26. Ebru E, Ali E. The detection of salivary minerals in smokers and non smokers with chronic periodontitis by inductively coupled plasma atomic emission. J periodontal 2006; 77: 990-95.

Table 1: Mean and SD of PLI and GI in control group and patients with chronic periodontitis

Clinical parameter	Control Group		Patients Group		t-test	P-Value	Sig.
	mean	SD	mean	SD			
PLI	0.474	0.130	1.987	0.359	2.548	0.038*	S
GI	0.664	0.155	1.757	0.130	8.281	0.0**	HS

***P<0.05 Significant**
****P<0.001 High Significant**

Table 2: Percentage distribution of sites according to different probing pocket depth grades and clinical attachment level grades

	PPD		CAL			
	1(4-5mm)	2 (≥6mm)	1 (1-3mm)	2 (4-5mm)	3 (6-7mm)	4 (8-9mm)
No.	123	33	2326	142	64	15
%	78.8	21.2	91.3	5.6	2.5	0.58

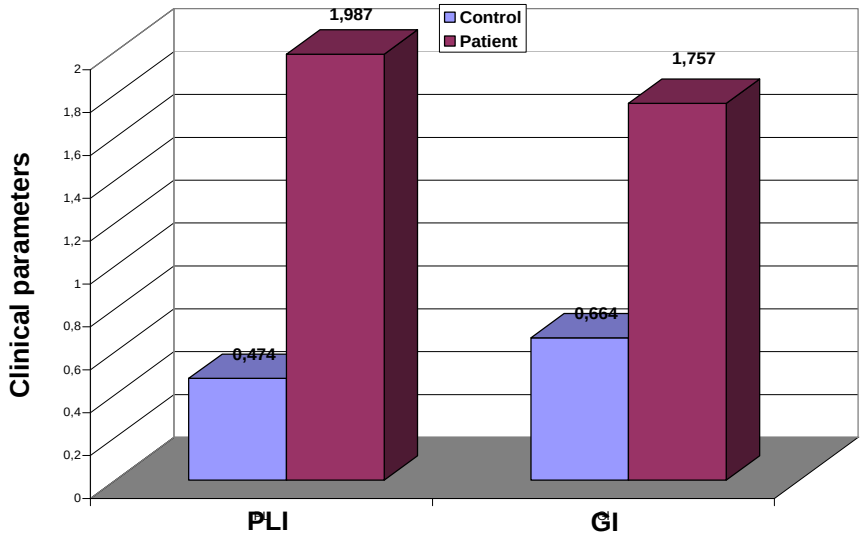

Figure 1: Plaque and Gingival index in patients with chronic periodontitis and control group.

Table 3: Mean and SD (mmole/L) of four salivary minerals in control and patients with chronic periodontitis.

Minerals	Control Group		Patients Group		t-test	P-Value	Sig.
	mean	SD	mean	SD			
Na^{+}	10.6	2.26	15.29	3.11	9.255	0.00**	HS
K^{+}	7.76	0.50	10.83	1.90	0.1168	0.4	NS
Mg^{+2}	0.34	0.04	0.364	0.10	0.9384	0.25	NS
Ca^{+2}	0.76	0.08	1.30	0.22	2.3120	0.025*	S

***P<0.05 Significant**
****P<0.001 High Significant**

Table 4: Mean and SD (IU/L) of two salivary enzymes in control and patients with chronic periodontitis

Enzymes	Control Group		Patients Group		t-test	P-value	Sig.
	mean	SD	mean	SD			
CK	4.02	0.78	37.04	5.34	8.06	P<0.01	HS
GGT	4.25	0.88	11.77	1.64	6.164	P<0.01	HS

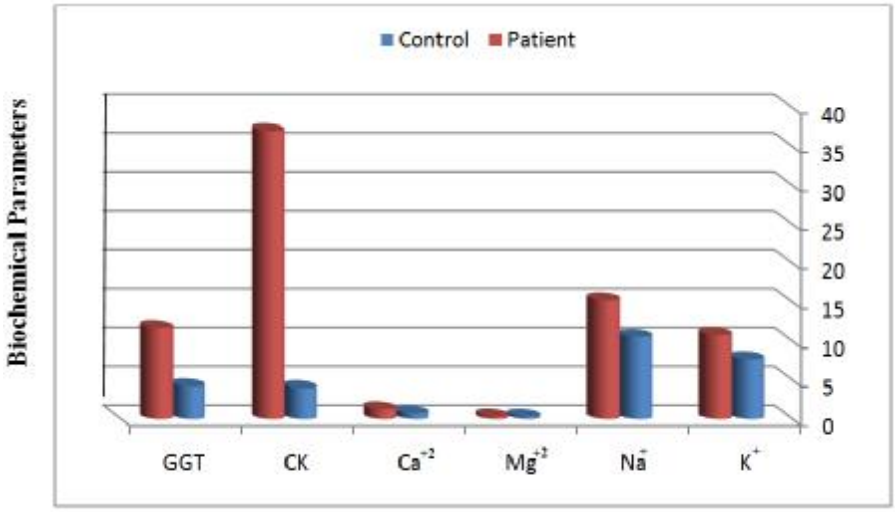

Figure 2: Salivary minerals and enzymes in patients with chronic periodontitis and control groups

Table 5: Correlation coefficient (r) among six saliva components and PLI & GI in control group and patients with chronic periodontitis

Clinical parameter		Correlation	K^+	Na^+	Mg^{+2}	Ca^{+2}	Ck	GGT
PLI	Patients	r	-0.163	-0.188	-0.260	-0.111	0.403	0.362
		p	0.457	0.389	0.231	0.613	0.049	0.089
	Control	r	-0.393	-0.094	0.350	0.056	0.310	0.353
		p	0.335	0.825	0.251	0.896	0.313	0.391
GI	Patients	r	-0.472	0.190	0.083	0.117	0.426	0.298
		p	0.023	0.386	0.705	0.596	0.042	0.167
	Control	r	-0.094	0173	0.311	0.034	0.346	0.157
		p	0.825	0.682	0.108	0.426	0.268	0.711

Table 6: Correlation coefficient (r) of salivary enzymes & minerals with PPD

PPD	Correlation	K^+	Na^+	Mg^{+2}	Ca^{+2}	CK	GGT
1 (4-5mm)	r	0.13	-0.244	0.35	0.312	0.124	0.210
	p	0.956	0.299	0.849	0.182	0.702	0.513
2 (≤6mm)	r	-0.242	-0.130	-0.188	-0.157	0.20	0.207
	p	0.304	0.585	0.427	0.508	0.520	0.520

Table 7: Correlation coefficient (r) of salivary enzymes & minerals with levels CAL

CAL	Correlation	K^+	Na^+	Mg^{+2}	Ca^{+2}	CK	GGT
1(1-3mm)	r	0.17	0.212	0.11	0.201	0.074	0.224
	p	0.943	0.370	0.964	0.395	0.818	0.483
2(4-5mm)	r	0.012	-0.409	0.061	0.242	0.170	0.247
	p	0.966	0.046	0.797	0.305	0.597	0.439
3(6-7mm)	r	-0.174	0.156	0.168	0.077	0.479	0.043
	p	0.462	0.512	0.478	0.748	0.015	0.409
4(8-9mm)	r	0.245	0.087	0.01	0.407	-0.135	-0.319
	p	0.298	0.714	0.98	0.048	0.676	0.312

The antioxidant effect of sulcular injection of green tea

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ABSTRACT

Background: Green tea is a popular neutraceutical as an antioxidant. The association between tea consumption, especially green tea, and human health has long been appreciated.
The aim of this study was to examine the antioxident effect of sulcular injection of green tea to evaluate its use in periodontal treatment.
Methods: Fifty –five male rabbit weighted 1-1.5 kg of the same species divided into three groups. The first group, group A, (test group) received 50µL/Kg of green tea dissolved in distilled water, the 2nd group, group B, received distilled water. The last group, group C, (control group) received no injection. Blood samples were taken at a time interval of (1, 3, 42, 72,168) hours for biochemical analysis of vitamin C and Malondialdehyde (MDA).
Results: Study showed there was a highly significant increase in mean concentration of serum vitamin C three hours after sulcular injection with 5%green tea extract ($P<0.01$),while a significant decrease in mean concentration of serum MDA after injection at the same time with the same extract($P<0.05$).
Conclusion: Green tea injected into sulcular had beneficial antioxidant effect, thus green tea can be used safely and successfully in periodontal treatment.
Key words: Green tea extract, oxidative stress, periodontal health. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):112-117).

INTRODUCTION

The tea plant (*Camellia sinesis* L.) is grown in about 30 countries worldwide [1]. Tea (*Camellia sinesis*) is the most widely consumed beverage worldwide for its desirable aroma, taste and putative positive physiological functions [2].

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals, and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage [3]. Catechin compounds are hypothesized to help protect against these diseases by contributing, along with antioxidant vitamins (i.e., vitamins C and E) and enzymes (i.e., superoxide dismutase and catalase), to the total antioxidant defense system [4]. In vivo studies showed that green tea catechins increase total plasma antioxidant activity [5]. Intake of green tea extracts also increases the activity of superoxide dismutase in serum and the expression of catalase in the aorta. These enzymes are implicated in cellular protection against reactive oxygen species [6]. This action is combined with direct action on oxygen species by a decrease in the nitric oxide plasma concentration [7].

Malondialdehyde (MDA), a marker of oxidative stress, also decreases after green tea intake [5]. These results suggest that catechins could have a direct (antioxidant) or indirect (increase of activity or expression) effect.

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Since catechins can act as antioxidants in vitro, they might prevent the oxidation of other antioxidants, such as vitamin E. However, ingestion of green tea catechins does not modify the plasma status of vitamins E and C in vivo [8]. This study is directed to evaluate the antioxidant effect of local injection of green tea into rabbit periodontium through estimation the levels of serum MDA and plasma vitamin C. The aim of this study was to assess the antioxidant effect of sulcular injection of green tea to evaluate its use in periodontal treatment.

ANIMALS AND METHOD

Study area and animal

This study was carried out at the Hawler Medical University, College of Dentistry, Department of Periodontology and department of basic science, Erbil city during the period from 1st Jan 2012 up to 30th May 2012. Fifty five (55) male rabbits of the same species and weight (1-1.5 kg) were left to acclimatization for seven days before starting the experiments, to maintain their standard diet and environmental condition were equal among all animals. Rabbits housed in an air –conditioned room (23-25˚C) with a 12-h light- dark cycle. They had free access to water and standard food during the experimental period, tags with different numbers were fixed on the rabbit's ear to mark them. The animals were divided into three intervals subgroups, the control (non- injected group), 5%green tea extract (test group) and the Distilled water (extraction solvent) group as shown in (Table 1). Sulcular injection technique was used through the labial gingival tissue of lower right central incisor. The depth of

penetration was measured by adjusting the sleeve to stop 5mm from the tip of the needle [9]. Test rabbit group was injected with 50 µL/Kg [10] of 5% green tea extracted in distilled water solution, while the base line control group was injected with distilled water alone.

Table 1: Classification of the studied groups used for biochemical assay

No. of rabbits	Time intervals of sample after injection (hr)	Groups		
		Control(non-injected group)	5%green tea extract injection group(test group)	Distilled water injected group
5	-	A	-	-
5	1	-	B1	C1
5	3	-	B2	C2
5	24	-	B3	C3
5	72	-	B4	C4
5	168	-	B5	C5
Total of no. of rabbits	-	5	25	25

Preparation of 5% green tea extract

Green tea was purchased from supermarket, dry Chinese green tea –Temple of Heaven Gun power, the green tea samples was expired at least one year later. Five (5) g of the selected green teas leaf, steeped for 1.5- 2 minutes in 100 ml of distilled water. The coolest brewing temperature was 160F/69˚C.The mixture was purified to obtained the 5% concentration solution of green tea [11].

Blood sample Collection

Cardiocentesis blood samples were collected after an overnight fast (12 – 14 hours). About 5 ml of blood was collected and dispensed into vacutainer® plain tubes. After centrifugation at 3000r.p.m. for five minutes, the serum was stored at – 80 °C.

Measurement of Oxidative Stress parameters

Serum Malondialdehyde (MDA)

Malondialdehyde (MDA) levels were determined by the MDA thiobarbituric acid (TBA) test using NWK-MDA01 assay kite/Northwest/USA. The test depends on the colori-metric reaction of MDA and TBA in acid solution. MDA, a secondary product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to generate a red-coloured product, which was detected spectrophotomet rically at 535 nm. The absorbance of the mixture was measured at 535 nm with a spectrophotometer and the results were expressed as µmol/l.

Serum vitamin C

Vitamin C was determined by the method of Stanly [12]. Ascorbic acid in plasma is oxidized by Cu (II) to form dehy-droascorbic acid, which reacts with acidic 2,4- dinitro-phenylhydrazine to form a red dihydrazone which is measured at 520 nm with a spectrophotometer

Statistical analysis

Data were evaluated statistically, analyzed and organized in tables and graphs. Computer program software SPSS (Statistical Package for Social Sciences); version 17 was used to analyzed the data .Quantitative variables were compared using the Student's t-test and expressed as (Mean ±S.E.M) .Results were considered significant, if the P value less than 0.05.

RESULTS

Lipid peroxidation (Serum malondialdehyde levels):

Table 2 and figure 1 show the mean and standard error (±SE) of control group (A) and groups after time intervals of intra sulcular injection with 5% green tea extract. The results showed that there is a significant decrease (P<0.05), only 3 hrs after green tea injection (groupB2), while the value was not changed significantly in the other groups those injected with green tea injected extract.

Table 2:The concentrations of serum Malondialdehyde (MDA) in groups after time intervals of sulcular injection with 5% green tea extract and non-injected rabbit (control) group.(P<0.05)

Rabbit Groups	No. of Rabbits	Time Intervals (hours)	Mean Conc. of MDA (µmol/L)	±SE	Sig.
A	5	Control	4.5	0.9	--
B1	5	1	4.7	1.48	N.S
B2	5	3	2.7	0.62	S
B3	5	24	3.3	1.39	N.S
B4	5	72	4.3	1.34	N.S
B5	5	168	4.5	1.06	N.S

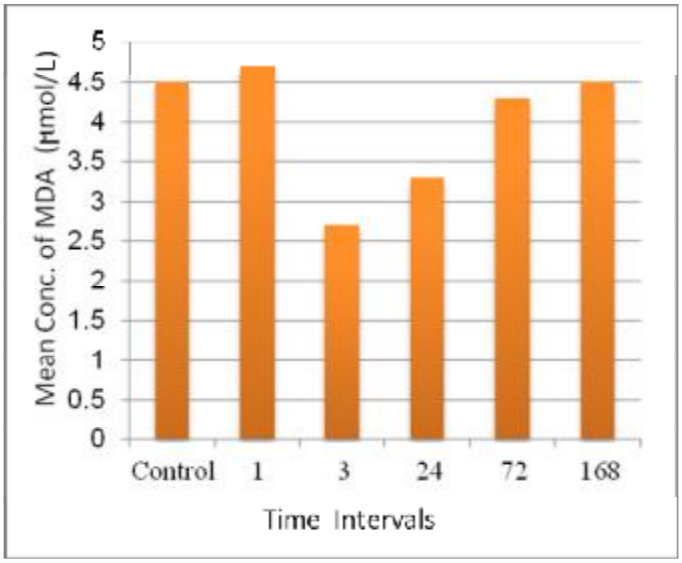

Figure 1: The concentrations of serum Malondialdehyde (MDA) in groups after time intervals of sulcular injection with 5% green tea extract and non-injected rabbit (control) group (P < 0.05).

The effect of distilled water (the extraction solvent) injection into sulcus on rabbit serum MDA is presented in Table (3). The results showed that there was no significant difference in mean concentration values between control group(A) and groups injected with distilled water during time interval(P>0.05).

Serum Ascorbic acid (vitamin C) in injected groups:

Table 4 and figure 2 show the mean and standard error (±SE) for serum ascorbic acid in rabbit groups injected with of 5% green tea extract into their sulcus. The results showed that vitamin C increased significantly in green tea injected groups comparing to control group, reaching the maximum concentration (0.218± 0.051mg/100g) after three hours of injection (group B2) (P<0.01), then the concentration dropped to control value, 24 hours after green tea extract injection (0.088±0.0164 mg/100g).

The effect of sucular injection of distilled water (the extraction solvent) on rabbit serum vitamin C is obtained in Table (5). The results showed that there was no significant difference in mean concentration values of serum ascorbic acid between groups injected with distilled water during time interval after injection and control group (P >0.05).

Table 3: The concentrations of serum MDA in rabbit groups after time intervals of sulcular injection with distilled and control group (A) (P>0.05) .

Rabbit Groups	No. of Rabbits	Time Intervals (hours)	Mean Conc. of MDA (µmol/L)	±SE	Significance
A	5	Control	4.5	0.9	--
C1	5	1	4.6	1.32	N.S
C2	5	3	4.4	0.63	N.S
C3	5	24	4.5	1.1	N.S
C4	5	72	4.1	0.76	N.S
C5	5	168	4.5	1.30	N.S

Table 4: The concentrations of serum vitamin C level in rabbit groups after time intervals of sulcular injection with 5% green tea extract and control group) (P<0.01)

Rabbit Groups	No. of Rabbits	Time Interval (hours)	Mean Conc.of Vit. C (mg/100 g)	± SE	Significance
A	5	Control	0.084	0.0212	--
B1	5	1	0.17	0.040	S
B2	5	3	0.218	0.051	H.S.
B3	5	24	0.088	0.0164	N.S
B4	5	72	0.086	0.0459	N.S
B5	5	168	0.090	0.0673	N.S

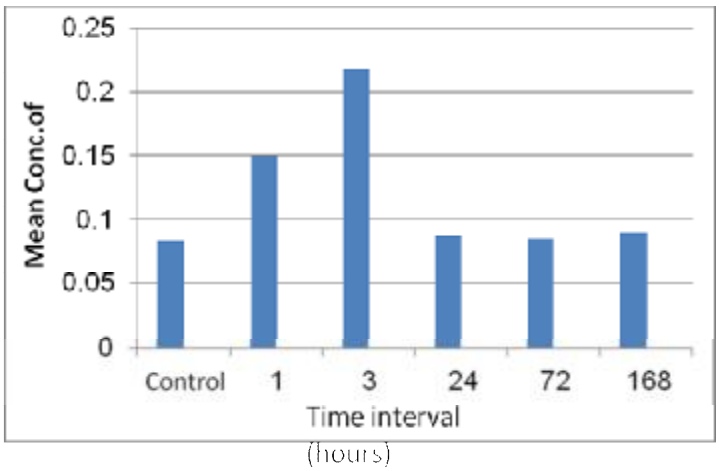

Figure 2: The concentrations of serum vitamin C level in rabbit groups after time intervals of sulcular injection with 5% green tea extract and control group (P>0.01).

Table 5: The concentrations of serum vitamin C level in rabbit groups after time intervals of sulcular injection with distilled water and control group

Rabbit Groups	No. of Rabbits	Time Intervals (hours)	Mean Conc. of vit C (mg/100gm)	±SE	Sig.
A	5	Control	0.084	0.0212	
C1	5	1	0.090	0.034	N.S
C2	5	3	0.107	0.077	N.S
C3	5	24	0.108	0.084	N.S
C4	5	72	0.103	0.043	N.S
C5	5	168	0.086	0.022	N.S

DISCUSSION

Lipid peroxidation in injected rabbit groups (Serum malondialdehyde levels):

The serum MDA level decreased only 3hrs after sucular injection with 5%green tea extracted with distilled water, while the sucular injection of distilled water alone had no effect on serum MDA level in the rabbit.

The above results demonstrated that, the sucular injection of 5% of green tea extract may have a significant role in in oxidative stress reactions. This may be due to catechins compounds that are presents in green tea which, can act a scavenger for free radicals that is produced from reactive oxygen species, thus decreasing or preventing cell damage that caused by free radicals [13].

Lipid peroxides, derived from poly saturated fatty acid, are unstable compounds that can decompose to form complex series of compounds. These compounds include reactive carbonyl compounds, of which the most abundant is MDA, a commonly used indicator for lipid peroxidation, oxidative stress, and subsequent cellular injury in the cell and tissue [14].

Silan *et al.* [15], showed that green tea extract blocked cellular inflammatory process as indicated from alleviation of perivascular edema and reduction in mononuclear leukocytes inflammatory cells infiltration. Green tea extract was able to normalized the elevated lipid peroxide (Thiobarbituric acid reactive substance) level and completely block lipid peroxidation.

The study of [16], showed a significant beneficial changes in lipid peroxidation after green tea drinking (decrease in MDA after green tea drinking).

Augustyniak *et al.*, [17] found that green tea decreased the oxidative stress; in addition to that, the administration of green tea increased the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH Px).

The results obtained in the present study indicated that a single dose of sucular injection of 5% green tea extract has a significant effect to reduce MDA level in sera rabbit(decrease lipid peroxidation , oxidative stress and cellular injury that caused by free radicals) .

Thus from the present study one can conclude that green extract injection into sulcus had beneficial effect, therefore green tea can be used safely and successfully in oral field.

Serum Ascorbic acid in injected rabbit groups:

In this study the serum vitamin C increased after sucular injection of 5% green tea extract into rabbit's sulculs. The increase in the mean concentration level started directly after 1 hour of local injection, then reached maximum level, 3 hours after injection, then the level declined and nearly returned to control value after 24 hours and remain till 168 hours after injection. The results indicated that the injection of distilled water (the extraction solvent) had no effect on vitamin C level in rabbit sera.

The oral injection of green tea into sulcus of rabbits, showed an improvement in the antioxidant status of plasma, considering that catechin supplementation has'nt only a direct action on antioxidant stress, but also it has a direct action , throughout increasing the levels of other antioxidant compounds (such as vit C) normally present in human serum [(18)].

Green tea rich in polyphenols in addition to ascorbic acid and minerals, these compounds could increase the green tea polyphenol antioxidant directly by chelate metal ions to prevent their participation and indirect by inhibition of prooxident enzyme. In addition, induction of antioxidant enzymes such as superoxide dismutase which had the ability to convert superoxide radicals into hydrogen peroxides which then metabolites by catalase (CAT) [(19)].

Hajimahmoodi *et al*., [(20)] reported that Chinas green tea had more potential antioxidant power comparable with Ahmed green tea. An antioxidant capacity was strongly correlated with the total phenolics content of the tea. It was published that green tea had reduced lipid peroxidation and caused an increase in the activity of antioxidant enzyme in diabetic rats [(21)].

The potential protective role played by green tea against injurious effect of reactive oxygen species, was studied in human micro vascular endothelial cells. The result showed that green tea polyphenol can acts as a biological antioxidant in a cell culture experimental model and prevent oxidative stress –induced cytotoxicity in endothelial cells [(22)].

From the results of the present study, one can conclude that the increase in serum level of vit C after single dose of green tea injection into intrasulcular may be due to the presence of this vitamine in green tea.

The present study suggests that there is an association between the intake of green tea and periodontal health condition. One can conclude that the increase in serum level of vit C after single dose of green tea injection into sulcus may be due to the presence of this vitamine in green tea. From the present study one can conclude that green tea injection into sulcus had beneficial effect, thus green tea can be used safely and successfully in oral field. Green tea sulcular injection could be used as a drug of choice in treatment of periodontal disease

REFERENCES

1- Graham HN. Green tea composition, consumption, and polyphenol chemistry. Prev Med 1992; **21**(3): 334–350.
2- Zhu QY, Hackman RM, Ensunsa JL, Holt RR, Keen CL. Antioxidative activities of oolong tea. J Agr Food Chem 2002; **50**(23): 6929-6934.
3- Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Oxford: Clarendon Press; 1985.
4- Abdel-Raheim MAM, Enas AH, Khaled AE. Effect of green tea extract and vitamin C on oxidant or antioxidant. Indian J Clin Biochem 2009; 24(3): 280-287.
5- Yokozawa T, Nakagawa T, Kitani K. Antioxidative activity of green tea polyphenol in cholesterol-fed rats. J Agric Food Chem 2002; 50: 3549-3552.
6- Negishi H, Xu JW, Ikeda K, Njelekela M, Nara Y, Yamori Y. Black and green tea polyphenols attenuate blood pressure increases in stroke-prone spontaneously hypertensive rats. J Nutr 2004; 134:38-42.
7- Yokozawa T, Nakagawa T, Lee KI, Cho EJ, Terasawa K, Takeuchi S. Effects of green tea tannin on cisplatin-induced nephropathy in LLC-PK1 cells and rats. J Pharm Pharmacol 1999; 51:1325-1331.
8- Alessio HM, Hagerman AE, Romanello M, Carando S, Threlkeld MS, Rogers J, Dimitrova Y, Muhammed S, Wiley RL. Consumption of green tea protects rats from exercise-induced oxidative stress in kidney and liver. Nutr Res 2003; 22:1177-1188.
9- Peterson JE, Motsson L, Nation W. Cementum and epithelial attachement response to the sulcular and periodontal ligament injection techniques. The American Academy of Periodontology1983; 5(4): 257-260.
10- Omer RM. Effect of local injection of α-L-Fucose on rabbit tongue muscle. Ph.D. thesis. Department of Biochemistry, Hawler Medical University, 2010.
11- Avwioro G, Lyiola S, Aghoghovwia B. Histological and biochemical markers of the liver of wistar rats on subchronic oral administration of green tea. North Am J Medical Sci 2010; 2(8):376-380.
12- Stanly TD, Howards S. Selected method for the determination of ascorbic acid in animal cells, tissues and fluid. Method in Enzymology. Vitamins and coenzymes part D 1979; 62: 3-11.
13- Liao S, Kao YH, Hiipakka RA. Green tea biochemical and biological basis for health benefits. Vitam Horm 2001; 62: 1-94.
14- Xu, Chi L, Row BW. Increased oxidative stress is associated with chronic intermittent hypoxia-mediated brain cortical neuronal cell apoptosis in a mouse model of sleep apnea. Neuroscienc 2004; 126: 313-323.

15- Silan C, Uzun O, Comunoğlu NU, Gokçen S, Bedirhan S. and Cengiz M. Gentamicin-induced nephrotoxicity in rats ameliorated and healing effects of resveratrol. Biol Pharm Bull 2007; 30: 79-83.
16- Coimbra S, Castro E, Rocha-Pereira P, et al.The effect of green tea in oxidative stress. Clinical Nutrition 2006; 25(5): 790-796.
17- Augustyniak A, Waszkiewicz E, Skrzydlewska E. Preventive action of green tea from changes in the liver antioxidant abilities of different aged rats intoxicated with ethanol. Nutrition 2005; 21(9):925-932.
18- Pietta P, Simonetti P. Dietary Flavonoids and interaction with physiologic antioxidant. In: Packer L, Hiramatsu M, Yoshikawa T (eds). Antioxidant food supplements in human health. San Diego7; Academic Press; 1999. p. 283–308.
19- Frei B, Higdon JV. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies J Nutr 2003; 133(10): 3275S–3284S.
20- Hajimahmoodi M, Hanifeh M, Oveisi MR, et al. Determination of total antioxidant capacity of green tea by the ferric reducing/antioxidant power assay. Iran J Environ Health Sci Eng 2008; 5(3): 167-172.
21- Badawoud MH, Al-Saggaf SM, Hagrasi MM. The effect of green tea on the oxidative stress and blood glucose level of diabetic rats. JKAU: Med Sci 2007; 14(3): 3-11.
22- Rah DK, Han DW, Beak HS, et al. Prevention of reactive oxygen species-induced oxidative stress in human microvascular endothelial cells by green tea polyphenol. Toxicol Lett 2005; 155(2): 269–275.

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Orthopantomogrphic pre-surgical assessment of mandibular third molar teeth form and structures using surgical findings as a gold standard

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ABSTRACT

Background: Mandibular third molars are the most frequently impacted teeth in human and their surgical extraction has become one of the commonest dentoalveolar surgeries. Accurate assessment of the position and morphology of the mandibular third molars is necessary to make a sound decision about the proposed surgical procedure. Today, orthopantomography is the imaging method of choice to provide information for adequate assessment of the impacted lower third molars, the related teeth, anatomical features, and the surrounding bone. The aim of this study is to determine the diagnostic accuracy of orthopantomographic view compared with postsurgical removal clinical finding in assessing the crown position, number and morphology of roots of the impacted lower third molar.

Materials and methods: Total sample of 50 patients (25 males and 25 females), age range from 19 to 35 years old with impacted lower third molars assessed radiographically by using Standardized orthopantomography for evaluation of crown position and roots number and morphology in comparison with surgical findings.

Results: According to the data obtained in this study, the comparison between the radiographic interpretation and the clinical findings revealed a complete agreement for crown position (100% K- value) while based on roots number the (K –value was 0.7, 10.66, 0.80) for teeth with one, two and complex roots respectively and according to roots morphology (K –value was 0.64, 0.67, 0.81) for normal, fused and dilacerated roots respectively with more frequent false negative findings

Conclusion: Although Orthopantomograph have a reasonable diagnostic value in the preoperative evaluation of the impacted lower third molars, but for more precise information modern radiographic modalities is advised to be used.

Keywords: Impacted lower third molar, Clinical morphology, Orthopantomography. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):118-122).

INTRODUCTION

Mandibular third molars, or wisdom teeth, are the most frequently congenitally impacted teeth [1,2]. They are the most often retained and impacted teeth, after the maxillary third molar, and are frequently the origin of considerable pain and complications [2,3] Although they normally erupt at ages ranging from 16 to 24 (mean"20") when they are in appropriate occlusion, about 40% of cases are partially or completely impacted [1,4].

The reported percentage of pathological changes such as infection, caries, root resorption, cysts and benign or malign tumors [5,6] is not high, but eruption failure usually causes irreversible damage even after mandibular third molars have been removed.[7] Therefore extraction of an impacted mandibular third molar is one of the most often performed procedures in oral surgery. [5]

The impacted lower third molars have been described in several methods of classification either based on its relationship to the long axis of the 2nd lower molar (vertical, horizontal, inverted, mesioangular, distoangular, buccoangular and linguoangular)[8] or based on roots curvature (straight, curved distally and curved mesially).[9] Killey and Kay classified the state of eruption into (erupted, partially erupted and unerupted) and the number of roots into three categories (tow, multiple, and fused roots). [10]

Clinical assessment should be carried out with the aim of assessing the status of the third molars and excluding other causes of the symptoms. [11] The knowledge of morphology of the third molar and prognosis on its eruption influences the clinical decision about its extraction and allows the prediction of complications of the procedure, [5] while the Radiological assessment is essential prior to surgery to provide the information necessary for adequate assessment of all third molar teeth. The radiographic examination of choice is a panoramic radiograph. [11]

Panoramic radiography is a radiological technique in which there is produced a single tomographic X-ray image of curved facial structures, including the maxillary and mandibular dental arches together with their supporting structures. On the basis of panoramic radiograms it is possible to evaluate developing third molars and their surrounding tissues. [12] The radiation dose of a panoramic radiograph is lower than

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from four periapical views and the diagnostic yield higher. [13, 14, 15] Doses from panoramic radiography can be further limited by using field size limitation to prevent exposing areas not required in the field of view. [16] Periapical or oblique lateral radiographs may be taken as an alternative. [11]

MATERIALS AND METHODS

A prospective study carried out on (50) patients with age range from 19 to 35 years old of equal sex distribution. All of them were requested for orthopantomographic images using (KODAK Dental Imaging Softwaer V6,12,10,0) with 10 mA, 73 KV) and underwent surgical extraction for their impacted lower third molars.

A complete clinical assessment was done which include: extraoral and intraoral examination with recording to the clinical statues of eruption (un-erupted or partially erupted) before extraction, followed by careful interpretation of the radiographs for the crown position (mesioangular, distoangular, vertical position), number and morphology of the roots and compare it to the clinical tooth anatomy after extraction.

Any patient with horizontally impacted lower third molar was excluded from this study because it may need surgical separation of the tooth crown from its roots and the roots from each other so there will be a practical difficulty on repositioning of the separated fragments postoperatively.

The statistical analysis of data was done by using Kappa measure of agreement (K- value). The comparison was done by true positive, false positive, true negative, and false negative.

RESULTS

Among the (50) studied patients (25 males & 25 females), aged from (19 to 35 years) with impacted lower third molar (ILTM), (44) patients were without any clinical complications, others were suffering from: recurrent pericoronitis, trismus and swelling respectively. According to eruption status (13) teeth were unerupted and (37) teeth were partially erupted as in table (1).

The radiographic interpretation of the crown position found that mesioangular is the most common position in this study (70%) Fig. (1), followed by distoangular and vertical position respectively.

According to this study sample the impacted lower third molar were mostly with two dilacerated roots(70%), table (2).

The comparison between the radiographic interpretation and the clinical findings was done to evaluate the sensitivity of orthopantomograph (OPG), the Kappa measure of agreement (K-value) for crown position was 100% as in table(3a), while based on roots number (K –value was 71%, 66%, 80%) for teeth with one, two and complex roots respectively as in table (3b) and according to roots morphology (K –value was 64%, 67%, 81%) for normal, dilacerated and fused roots respectively as in table (3c).

Table 1: Clinical findings of the impacted lower third molar

	Noncomplicated (%)	Swelling (%)	Trismus (%)	Pericoronitis (%)	Unerupted (%)	Partially Erupted (%)
ILTM in male subjects	23 (92)	2 (8)	1(4)	7 (28)	8 (32)	17 (68)
ILTM in female subjects	21 (84)	3(12)	6(24)	10 (40)	5 (20)	20 (80)
Total	44(88)	5(10)	7 (14)	17 (34)	13 (26)	37 (74)

Table 2: The diagnostic findings of the orthopantomograph.

	Crown position			No. of roots			Morphology of roots		
Radiographic findings	Mesio-angular (%)	Disto-angular (%)	Vertical (%)	One root (%)	Two roots (%)	Complex roots (%)	Normal (%)	Dilacerated (%)	Fused (%)
ILTM in male subjects	14 (56)	9 (36)	2(8)	1(4)	20 (80)	4 (16)	3 (12)	15 (60)	7 (28)
ILTM in female subjects	21 (84)	0 (0)	4 (16)	2(8)	21 (84)	2 (8)	2 (8)	20(80)	3 (12)
Total	35 (70)	9 (18)	6 (12)	3(6)	41 (82)	6 (12)	5 (10)	35(70)	10 (20)

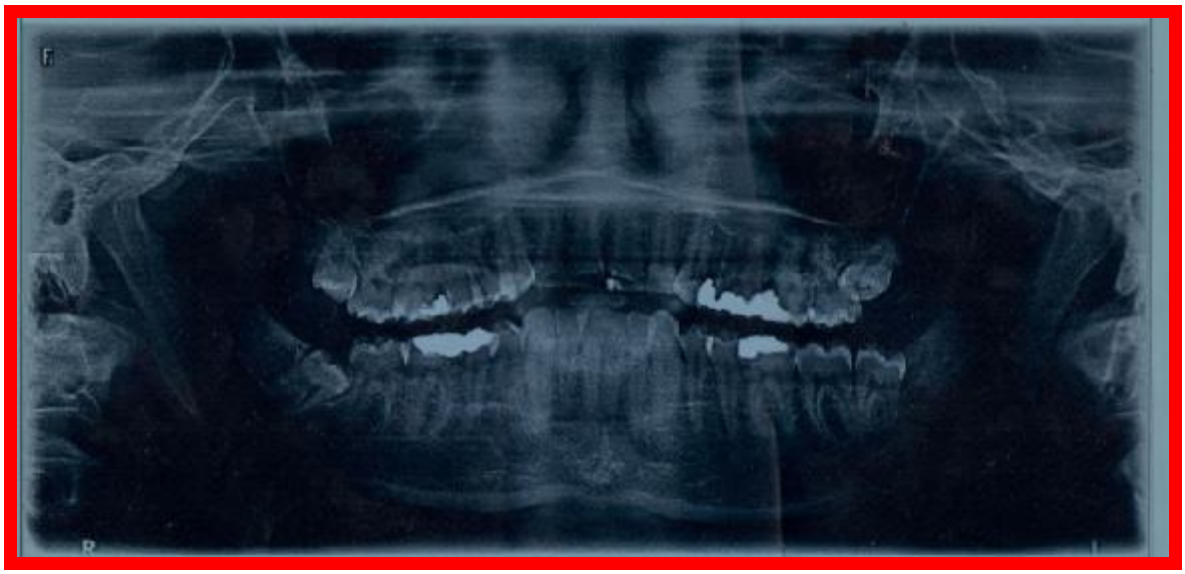

Fig. 1: Orthopantomographic image showing mesioangular ILTM.

Table 3 a, b, c: Comparison of orthopantomograph and clinical findings

Table 3-a

Crown position	Subjects	orthopantomograph	Clinical	TP (%)	FP	FN	K-value	Sig.
Mesioangular	Male	14	14	14(100%)	-	-	(100%)	non sig
	Female	21	21	21(100%)	-	-	(100%)	non sig
	Total	35	35	35(100%)	-	-	(100%)	non sig
Distoangular	Male	9	9	9(100%)	-	-	(100%)	non sig
	Female	0	0	0(100%)	-	-	(100%)	non sig
	Total	9	9	9(100%)	-	-	(100%)	non sig
Vertical	Male	2	2	2(100%)	-	-	(100%)	non sig
	Female	4	4	4(100%)	-	-	(100%)	non sig
	Total	6	6	6(100%)	-	-	(100%)	non sig

Table 3-b

Number of roots	Subject	Orthopantomograph	clinical	TP%	FP	FN	K-value	Sig.
One root	Male	1	2	1(50%)	-	1	(64%)	Non sign
	Female	2	3	2(66%)	-	1	(78%)	Non sign
	Total	3	5	3	-	2	(71%)	Non sign
Two roots	Male	20	16	16(100%)	4	-	(62%)	Non sign
	Female	21	19	19(100%)	2	-	(73%)	Non sign
	Total	41	35	35(100%)	6	-	(66%)	Non sign
Complex roots	Male	6	6	5	1	1	(80%)	Non sign
	Female	2	3	2(66%)	-	1	(78%)	Non sign
	Total	8	9	7(77%)	1	2	(80%)	Non sign

Table 3-c

Morphology of roots	Subject	Orthopantomograph	clinical	TP%	FP	FN	K-value	Sig.
Normal roots	Male	3	1	1(100%)	2	-	(48%)	Non sign
	Female	2	3	2(66%)	-	1	(78%)	Non sign
	Total	5	4	3(75%)	2	1	(64%)	Non sign
Dilacerated roots	Male	15	20	15(75%)	-	5	(58%)	sign
	female	20	18	18(100%)	2	-	(70%)	Non sign
	Total	35	38	33(86%)	2	5	(67%)	sign
Fused roots	Male	7	4	4(100%)	3	-	(68%)	Non sign
	Female	3	4	3(75%)	-	1	(84%)	Non sign
	Total	10	8	7(87%)	3	1	(81%)	Non sign

DISCUSSION

Prophylactic surgical extraction of asymptomatic impacted molars considerable controversy exists regarding some surgeons favor a conservative approach, while others prefer more interventional strategies. [17]

In this study the removal of asymptomatic or non complicated lower 3rd molar was the most common indication (88%), based on that the risk of surgical morbidity increases with increasing age which is coincide with Adeyemo et al.[18] This was similar with a study done by McArdle and Renton (2006) [19] which suggested that the early or prophylactic removal of a partially erupted mesioangular third molar could prevent many complications like distal cervical caries forming in the mandibular second molar. Recurrent pericoronitis was the 2nd frequent indication for removing impacted mandibular third molars in this study (34%), while other studies [20,21,22] reported that recurrent pericoronitis was the most frequent indication.

From a total 50 patients with impacted lower third molar (13) teeth were unerupted and (37) teeth were partially erupted this depend on the space available in the retromolar region, the teeth erupt if there is enough space and if their inclination is favorable. [23, 24]

The prevalence of third molars that remain unerupted and impacted varies in different areas of the world. It was found that the numbers of partially erupted and impacted lower third molars increased in populations of well developed countries, while they erupted early in underdeveloped regions of the world. [25, 26]

Panoramic view used in this study should be treated as a necessary aid in the diagnosis of mandibular third molar retention as they allow for evaluation of teeth morphology, position, inclination, they are also helpful in the prediction of eruption as well as prognosis on the difficulty of extraction of these teeth [27-29]

According to the radiographic interpretation of the crown position in this study, the misioangular position (70%) is higher than Stanley et al. study in which mesioangular position was (34%), [30] while the value of (82%) of the teeth in the present study with two roots is closed to the (88%) value of of Saraswati et al. [31]. The teeth with dilacerated roots in this study are (70%) which is near the findings of Saraswati et al. [31]

The variations could be attributed to the racial variation and differences in methodology of each study.

In the present study there is a complete agreement between the clinical and radiographic findings about the crown position (K- value 100%) as in table (3-a). The sensitivity of OPG was statistically significant for dilacerated roots with the *P*-value of <0.05 that mean the false negative findings were more frequent. The disagreement is due to the more inward placement of the roots which could result in a difference in the radiographic appearance and the path of passing X-ray beam could give different projections [30, 32]. a good assessment to the number and morphology of roots can reached by using more than one radiographic projection and techniques like Clark's and right angle technique which determines the three-dimensional orientation of impacted teeth, but the panoramic radiograph have been advocated the view of choice that demonstrate the whole impacted tooth ,with the investing bone , adjacent tooth, inferior dental canal and the anterior aspect of ascending ramus [33] in addition to its main advantage of low radiation exposure of the patient due to its ability to show the entire dental arch in one film. [34]

REFERENCES

1. Badawi F J, Levy JC, Yazbeck C, Cavezian R, Cabanis EA. Eruption of third molars: relationship to inclination of adjacent molars. Am J Orthod Dentofacial Orthop 2004; 125: 200-2.
2. Hattab FN, Alhaija ES. Radiographic evaluation of mandibular third molar eruption space. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999; 88:285-91.
3. Garcia RI, Chauncey HH. The eruption of third molars in adults: A 10-year longitudinal study. Oral Surg Oral Med Oral Pathol 1989; 68: 9–13.
4. Niedzielska IA, Drugacz J, Kus N, Kreska J. Panoramic radiographic predictors of mandibular third molar eruption. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 102:154-8; discussion 159.
5. Ganss C, Hochban W, Kielbassa AM, Umstadt HE. Prognosis of third molar eruption. Oral Surg Oral Med Oral Patho l 1993; 76:688- 93.
6. Tsai HH. Factors associated with mandibular third molar eruption and impaction. J Clin Pediatr Dent 2005; 30:109-13.
7. Punwutikorn J, Waikakul A, Ochareon P. Symptoms of unerupted mandibular third molars. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999; 87:305-10.
8. Winter GB. Impacted mandibular third molar. St Louis: AM medical book co; 1926. P. 40-65
9. Thoma KH. The Management of the malposed inferior third molar. J Dent Res 1932; 12:175.
10. Killey HC, Kay LW. Outline of oral surgery. Part I. London: E&S Livingstone Ltd.; 1987. p.52.
11. Management of unerupted and impacted third molar teeth section 4: Assessment and referral Scottish intercollegiate guidelines network. Elliot house, 8-10 Hillside Crescent, Edinburgh EH75EA web contact Duncan.service@nhs March 2000.
12. Hattab FN. Positional changes and eruption of impacted mandibular third molars in young adults. A radiographic 4-year follow-up study. Oral Surg Oral Med Oral Pathol Radiol Endod, 1997; 84: 604–608.

13. Gonzalo Nuno MM, Larena del Rosario ME. Radiographic study of the formation and calcification of the third molar. Dental Practice 1990; 11: 27-8.
14. White SC. Assessment of radiation risk from dental radiography. Dentomaxillofac Radiol 1992; 21:118-26
15. Faculty of General Dental Practitioners (UK). Selection criteria for dental radiography. London: Faculty of General Dental Practitioners; 1998.
16. Lecomber AR, Faulkner K. Dose reduction in panoramic radiography. Dentomaxillofac Radiol 1993; 22: 69-73.
17. Almendros-Marqués N, Alaejos-Algarra E, Quinteros-Borgarello M, Berini-Aytés L and Gay-Escoda C. Factors influencing the prophylactic removal of asymptomatic impacted lower third molars. Int J Oral Maxillofac Surg 2008; 37(1): 29-35.
18. Adeyemo WL, Ogunlewe MO, Ladeinde AL, Abib GT, Gbotolorun OM, Olojede OC, Hassan OO. Prevalence and surgical morbidity of impacted mandibular third molar removal in the aging population: a retrospective study at the Lagos University Teaching Hospital. Afr J Med Med Sci 2006; 35(4): 479-483.
19. McArdle LW and Renton TF. Distal cervical caries in the mandibular second molar: an indication for the prophylactic removal of the third molar? Br J Oral Maxillofac Surg 2006; 44(1): 42-45.
20. Almendros-Marqués N, Berini-Aytés L and Gay-Escoda C. Influence of lower third molar position on the incidence of preoperative complications. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 102(6): 725-732.
21. Gbotolorun OM, Olojede AC, Arotiba GT, Ladeinde AL, Akinwande JA and Bamgbose BO. Impacted mandibular third molars: presentation and postoperative complications at the Lagos University Teaching Hospital. Nig Q J Hosp Med 2007; 17(1): 26-29.
22. Bataineh AB, Albashaireh ZS and Hazza'a AM. The surgical removal of mandibular third molars: a study in decision making. Quintessence Int 2002; 33(8): 613-617
23. Ventä I, Turtola L, Ylipaavalniemi P. Change inclinical status of third molars in adults during 12 years of observation. J Oral Maxillofac Surg 1999; 57: 386–389.
24. Ventä I, Turtola L, Ylipaavalniemi P. Radiographicfollow-up of impacted third molars from age 20 to 32years. Int J Oral Maxillofac Surg 2001; 30: 54–57.
25. Odysanya S, Abayomi I. Third molar eruption among rural Nigerians. Oral Surg Oral Med Oral Pathol 1991; 71: 151–154.
26. Rajasuo A, Murtomaa H, Meurman J. Comparison of the clinical status of third molars in young men in 1949 and 1990. Oral Surg Oral Med Oral Pathol 1993; 76: 694–698.
27. Garcia RI, Chauncey HH. The eruption of third molars in adults: A 10-year longitudinal study. Oral Surg Oral Med Oral Pathol 1989; 68: 9–13.
28. Hattab FN, Alhaija ES. Radiographic evaluation of mandibular third molar eruption space. Oral Surg Oral Med Oral Pathol Radiol Endod 1999; 88: 285–291.
29. Ventä I, Turtola L, Ylipaavalniemi P. Radiographic follow-up of impacted third molars from age 20 to 32 years. Int J Oral Maxillofac Surg 2001; 30: 54–57.
30. Stanley HR, Alattar M, Collett WK, Stringfellow HR Jr, Spiegel EH. Pathological sequel of neglected impacted third molars. J Oral Pathol 1988; 17:113-7.
31. Saraswati FK, Balajirao B, Mamatha GP. Clinical and orthopantomographic evaluation of mandibular third molar. Contemp Clin Dent 2010; 1:27-30.
32. Littner MM, Kaffe I, Tamse A, Dicapua P. Relationship between the apices of lower molars and mandibular canal - a Radiographic study. Oral Surg Oral Med Oral Pathol 1986; 62:595-602.
33. Obiechina AE. Updatein the technique of third molar surgery. Annals of Ibadan Post grad Med 2003; 1: 40-45.
34. Bnediktstottir IS, Hintz H, Petersen JK, Wenzel A. Accuracy of digital and film panoramic radiographs for assessment of positions and morphology of mandibular third molars and prevalence of dental abnormalities and pathologies. Dentomaxillofac Radiol 2003; 32: 109-115.

Effects of diabetes mellitus types II on salivary flow rate and some salivary parameters (total protein, glucose, and amylase) in Erbil city

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ABSTRACT

Background: The concentration of some components of saliva may be associated with certain systemic illnesses, reflecting the hormonal, immunological, neurological, emotional, nutritional and metabolic states of the patient. The aim of this work was to assess salivary flow rate, and to evaluate saliva samples for levels of salivary total protein, glucose, and alpha amylase, in diabetics type II and healthy subject in both genders.

Subjects and methods: Unstimulated salivary flow rate, salivary total protein, glucose, and amylase were measured in 90 subjects, 60 with diabetes mellitus type II (30 controlled and 30 uncontrolled diabetic patients) and in 30 healthy subjects.

Results: Significant difference in salivary flow rate in diabetic patient when compared with healthy subject. The finding showed no significant differences between salivary total proteins in all groups. Significant difference in salivary glucose and amylase concentration was found between the healthy subjects and type 2 diabetic patients. According to the gender, there were only significant differences between male and female in salivary flow rate for healthy subjects

Conclusions: Patients with type 2 diabetes mellitus have higher concentration of salivary glucose and lower value of salivary flow rate and amylase. No significant difference was seen in protein value in all groups.

Key wards: Diabetes mellitus, healthy subject, salivary total protein, amylase, glucose. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):123-127).

INTRODUCTION

Diabetes mellitus is a complex multisystem disorder, it is a metabolic disease characterized by hyperglycemia due to defects in insulin production, insulin action, or both. Diabetes mellitus can impair the function of polymorphonuclear leukocytes which may predispose diabetic patients to greater risk of diseases including periodontal disease and oral candidal infections [1,2] and it has been consistently documented to be associated with altered salivary composition and function. This disrupts the homeostasis of the oral cavity, making it susceptible to various oral elements [3]. Oral fluid or whole saliva is a complex chemical milieu of teeth and oral soft tissues, consisting mainly of water, essential electrolytes, glycoproteins, antimicrobial enzymes and numerous other important constituents like glucose and amylase [4,5]. Although differences in the output and composition of saliva from diabetic and healthy subjects have been observed in a number of studies, some of these findings are contradictory [6-8].

The aims of this study were as follows: first, to estimate the constituents of salivary total protein, glucose and alpha amylase and salivary flow rate in order to aid in reaching firm conclusions about their alterations in diabetics as compared to healthy subjects; second, to compare and relate these parameters in controlled diabetics, uncontrolled diabetic and healthy subject variations between gender, in subjects aged (40-60) year in Erbil city.

SUBJECTS AND METHODS

This comparative study was conduct prospectively over a period of 4 month, from May 2011 to end of August. 90 subjects were selected in the study. Those 90 participants were attended to Layla kassim health center in Erbil city. Their age ranged between 40–60 years old were divided into three groups of 30 subjects for each group: The uncontrolled diabetic group (Group 1), 15 males, 15 female; the controlled diabetic group (Group 2), 15 male, 15 female, and the healthy subject group (Group 3), 15 male, 15 female. In diabetics groups fasting and post-meal blood glucose levels were evaluated and in healthy subjects, random blood glucose tests were performed in order to confirm them as non-diabetic. Blood glucose levels were taken as an indicator of metabolic control [9], the criteria were as shown in Table 1.

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Table 1: Criteria of blood glucose level as an indicator of metabolic control to categorize the patients as uncontrolled diabetics and controlled diabetics

Relation with food in (mg/dl)	For controlled diabetes	For uncontrolled diabetes
Fasting blood glucose level	<140	≥140
2 hour postprandial blood glucose level	<200	≥200

The hemoglobin A1c test is a simple lab test that shows the average amount of sugar in your blood over the last two to three months. For the identification between controlled and uncontrolled diabetic patient HbA1c kit were used (i-CHROMA^{TM} HbA1c, Republic of Korea). It's the best way to find out if your blood sugar is under control. Diabetes may be defined as having an HbA1c >6.5%, so, >6.5% = diabetes, <6.0% = not diabetic [10]. All the diabetic patient which included in the study were under medication (Metformine tablet, Glibenclamide tablet)

Exclusion criteria: Type 1 diabetic subjects, the patient which receiving insulin, patients with severe diabetic complications, presence of any other systemic illness or on medication other than those for diabetes, were all excluded.

Collection and pretreatment of whole saliva

The unstimulated salivary sample was collected, between 9 - 11 a.m., at least two hours after the subject's usual breakfast time. This was to ensure that the variability in salivary flow and compositions due to diurnal variation were minimized. If the participant was partial denture wearer, the denture was removed prior to saliva collection. The subjects were asked to rinse the mouth with water thoroughly to remove any food debris and after that subjects were comfortably seated and, after a few minutes of relaxation, they were trained to avoid swallowing saliva and asked to lean forward and spit all the saliva they produced for 5 minutes into a graduated test tube, through a glass funnel. The whole volume collected for 5 minutes was divided by (5) to determine the (USF) (ml/ min).

The subjects were instructed not to spit forcibly to avoid blood contamination. Resting saliva was collected for five minutes. Once the saliva was collected, the graduated sampling tube was placed in an ice carrier box and transferred to Hawler teaching hospital laboratory, for biochemical analysis. Total protein, glucose and alpha-amylase were determined immediately after collection in order to avoid daily variations caused by endogenous proteolytic activity.

Estimation of biochemical parameters in saliva

Saliva sample was centrifuged at 3000 rpm for 15 min to remove any particulate material. The supernatant was taken for analysis of, total proteins, glucose and amylase, using spectrophotometer (CECIL CI 2021)

Salivary total protein was determined using BIOLABO SA kit (02160 MAIZY- FRANCE). Briefly, 1ml of reagent solution was pipette into each of the two test tubes labeled Standard and Test. 1ml of standard was added to the test tube marked as Standard, followed by 0.02 ml of saliva. These were mixed well and all the tubes were kept in an incubator (Isotemp Fisher Scientific) at 37 C for 10 min before aspiration. Results were calculated and values were expressed as milligrams per deciliter (mg/dl)

Salivary glucose estimation was performed using BIOLABO SA kit (GLUCOSE GOD-POD Trinder reaction -FRANCE). Briefly, 1ml of reagent solution was pipette into test tube, add 0.01saliva, after that prepare another test tube add 1ml of standard add 0.01ml of saliva. These were mixed well and all the tubes were kept in an incubator at 37 C for 10 min before aspiration. Reagent blank was first aspirated in the analyzer, followed by standard solution, and finally, the test sample was aspirated and the reading was noted (spectrophotometer measuring at 505nm). Results were calculated and values were expressed as milligrams per deciliter (mg/dl)

Salivary Alpha amylase was determined using Alpha amylase kit BIOLABO (AMYLASE E-PNPG7 Method-FRANCE). Briefly, 1ml of reagent solution was pipette into test tube, add 0.025ml saliva, mix then start a timer. Record initial absorbance after 1min at 405nm, record the absorbance again every 3min. Results were calculated and values were expressed as units per deciliter (u/dl)

Statistical analysis: The data were analyzed using the SPSS statistical software. All value of biochemical parameters were expressed as mean ± SE. Intergroup comparisons of salivary parameters in the uncontrolled diabetic group, controlled diabetic group and healthy subject group were determined by using **t- test**. Levels of significance between group for all the parameters of the study with the gender were determined by employing Student's't' test.

RESULTS

Table 2 represent distribution of mean levels of salivary flow rate, total protein, glucose and alpha amylase in the controlled diabetic group, uncontrolled diabetic group and healthy subjects group. According to table 2 we had seen the higher value of salivary flow rate and salivary amylase, and lower value of total protein and glucose in healthy subjects.

Table 2: Distribution of mean levels of salivary parameters in the uncontrolled diabetic group, controlled diabetic group and healthy subject group

Parameters	Groups	Mean ±SE
USF (ml/ min)	**Controlled Diabetic group**	0 .45 (0.041)
	Uncontrolled Diabetic group	0.54 (0.050)
	Healthy Subject group	0.69 (0.049)
Salivary total protein (mg/dl)	**Controlled Diabetic group**	91.27 (3.36)
	Uncontrolled Diabetic group	90.17 (3.57)
	Healthy Subject group	80.21 (3.97)
Salivary glucose (mg/dl)	**Controlled Diabetic group**	17.98 (1.03)
	Uncontrolled Diabetic group	15.57 (1.03)
	Healthy Subject group	10.11 (1.08)
Salivary amylase (u/dl)	**Controlled Diabetic group**	54.33 (2.63)
	Uncontrolled Diabetic group	57.79 (3.10)
	Healthy Subject group	68.02 (3.39)

Table 3 represents intergroup comparisons of salivary parameters in the uncontrolled diabetic group, controlled diabetic group and healthy subject group. Statistical analysis showed significant differences between salivary flow rate, salivary glucose, and salivary amylase in healthy subject and diabetic patient (both controlled and uncontrolled diabetic patient). The finding showed no significant differences between salivary total proteins in all groups.

Table 3: Intergroups comparisons of salivary parameters in the uncontrolled diabetic group, controlled diabetic group and healthy subject group

Parameter	Intergroup comparisons	P value	Intergroup comparisons	P value
USF (ml/ min)	**Controlled group with uncontrolled group** **Controlled group with Healthy group**	0.198 0.00**	Uncontrolled group with Healthy group	0.013*
Salivary total protein (mg/dl)	**Controlled group with uncontrolled group** **Controlled group with Healthy group**	0.239 2.203	Uncontrolled group with Healthy group	1.978
Salivary glucose (mg/dl)	**Controlled group with uncontrolled group** **Controlled group with Healthy group**	0.120 0.00 **	Uncontrolled group with Healthy group	0.00**
Salivary amylase (u/dl)	**Controlled group with uncontrolled group** **Controlled group with Healthy group**	0.430 0.001 **	Uncontrolled group with Healthy group	0.05*
***Significant P value ≤ 0.05**			****Highly significant**	

Table 4 represents distribution of mean levels of salivary parameters between genders in the uncontrolled diabetic group, controlled diabetic group in compare to healthy subject group. According to the genders, there were only significant differences between male and female in salivary flow rate for healthy subjects group, uncontrolled Diabetic group and no significant differences between the genders for other parameters

Table 4: Distribution of mean levels of salivary parameters between the gender in the uncontrolled diabetic group, controlled diabetic group and healthy subject group

Parameter	Controlled Diabetic group		Uncontrolled Diabetic group		Healthy group	
	Males Females (n=15) (n=15) Mean ±SE Mean±SE	P value	Males Females (n=15) (n=15) Mean ±SE Mean±SE	P value	Males Females (n=15) (n=15) Mean±SE Mea±SE	P value
USF (ml/ min)	0.60 (0.07) 0.48(0.06)	0.064 NS	0.53(0.06) 0.36(0.04)	0.055* S	0.77 (0.07) 0.51(0.05)	0.056* S
Salivary total protein (mg/d)	91.3 (4.44) 91.1(5.19)	0.975 NS	90.1(5.35) 90.3(4.92)	0.971 NS	77 (6.96) 138(56.36)	0.285 NS
Salivary glucose (mg/dl)	17.4 (1.39) 18.5(1.56)	0.633 NS	14.4(1.40) 16.6(1.50)	0.180 NS	9.68(1.04) 10.5(1.93)	0.682 NS
Salivary amylase (u/dl)	55.9 (2.68) 52.7(4.59)	0.573 NS	52.4(3.95) 63.1(4.49)	0.136 NS	75.1(3.56) 61.0(5.30)	0.066 NS

*** Significant P value ≤ 0.05**

DISCUSSION

Diabetic mellitus can be defined as a metabolic syndrome characterized by hyperglycemia and disturbances in the metabolism of carbohydrates, protein and lipid [11] For salivary flow rates, no significant differences were seen between controlled and uncontrolled diabetics (p>0.05), This result comes in agreement to that reported by other previous study[6], while significant differences was seen in salivary flow rate between controlled, uncontrolled diabetic group and healthy non diabetic group, this result comes in agreement with some studies [12, 13, 14], this decreases in salivary flow rate or oral dryness occurring in diabetes can be multifactorial, either due to fatty infiltration of cells into the salivary glands or physical alteration of mucosal cells subsequent to dehydration due to polyuria or microvascular disease, local inflammation and irritation in the oral cavity, metabolic disturbances, and neuropathy affecting the salivary glands, and may be due to drug therapy for diabetes or concomitant drugs [15, 16] and the result of flow rate which was disagree with other studies [12, 17, 18] as they showed no significant differences in flow rate which may be due to sample selection.

Many studies have been done on the biochemical changes found in the saliva of diabetic patients, the results may differ from one study to another. These may be due to the diversity in selection criteria of the samples and type of design of each study, and variation in the environmental factors [14, 19].

With regard to salivary total protein, the present study results are consistent with previous studies [5, 12, 15], with no significant differences evident between diabetics and non-diabetics. However, recent studies have reported higher salivary total protein levels in diabetics [14, 15, 20], while Streckfus et al. [21] estimated significant lower protein concentrations in diabetics and emphasized protein utilization by other biochemical metabolic pathways an overall systemic response to glucose intolerance. Insulin is known to have the potential to alter protein metabolism.

Mean salivary glucose levels were clearly higher in the controlled and uncontrolled diabetic groups than in the healthy subject group and the differences were highly significant (Tables 3 and 4). Many authors found higher glucose salivary levels in diabetic patients than in non diabetics [3, 8,14]. While Marchetti et al. [22] reported no changes in salivary glucose levels in diabetics.

Higher salivary glucose levels favor the proliferation of microorganisms and enhance their colonization on teeth and oral mucous membranes [1,23]. The elevated salivary glucose level in diabetes confirms the effects of diabetic membranopathy, which leads to raised percolation of glucose from blood to saliva, thus altering the salivary composition in diabetes mellitus [24].

The highly significantly differences of salivary amylase levels in controlled diabetics when compared with healthy non- diabetics groups were seen in the present study and this supports the study by Yavuzyilmaz et al. [20], who linked them to hormonal and metabolic changes occurring in diabetic patients, and also the results showed a significant difference for uncontrolled diabetics and healthy non diabetic in this study. In contrast with studies [14,15] which they showed significant increases in salivary amylase levels in diabetics.

Table 4 represent the difference between the gender in salivary flow rate, total protein, glucose and alpha amylase in resting saliva in all groups. According to the gender, there was statistically

significant difference observed in the mean (USFR) between male and female in healthy group and uncontrolled diabetic males than in females, which was similar to the results of a study [25] and contradicted the results reported by Chavez et al [19], this may be due to smaller salivary gland size in females than males [26]. For salivary total protein levels and amylase, no significant differences were seen between genders in all groups and this result was disagree with Arati et al [6]. For salivary glucose levels, no significant differences were seen between gender in all groups, this result may be explained by the stages of the disease, metabolic control status of patients, the sex hormone status has no effect on the constituent of saliva in diabetic and healthy subject, and this was in agreement with Arati et al [6] although higher salivary glucose levels have been reported in males when compared with females [16].

REFERENCES

1- Manfredi M, McCullough MJ, Vescovi P, Al- Kaarawi ZM, Porter SR. Update on diabetes mellitus and related oral diseases. Oral Dis 2004; 10: 187-200.
2- Javed F, Näsström K, Benchimol D, Altamash M, Klinge B, Engström PE. Comparison of periodontal and socioeconomic status between subjects with type 2 diabetes mellitus and non-diabetic controls. J Periodontol 2007; 78:2112-9.
3- Holmes S, Alexander W. Diabetes and dentistry. Dent Pract 2004; 5: 16-9.
4- Little JW, Falace DA, Miller CS, Rhodus NL. Diabetes. In: Dental management of medically compromised patients. 6th ed. St Louis: Mosby; 2002. p. 248-70.
5- Meurman JH, Rantonen P, Pajukoski H, Sulkava R. Saliva albumin and other constituents and their relation to oral and general health in the elderly. Oral Surg Oral Med Oral Pathol Oral Radiol Endod2002; 94(4): 432-8.
6- Arati S. Panchbhai, Shirish S. Degwekar and Rahul R. Bhowte. Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India. J Oral Science 2010; 52(3): 359-68.
7- Carda C, Mosquera-Lloreda N, Salom L, Ferraris MEG, Peydró A. Structural and functional salivary disorders in type 2 diabetic patients Med Oral Pathol Oral Cir Bucal 2006; 11(4): 309-14.
8- Aydin S. A. comparison of ghrelin, glucose, alpha-amylase and protein levels in saliva from diabetics. J Biochem Mol Biol 2007; 40: 29-35.
9- American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2010; 33(Suppl. 1): S62–S69.
10- International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009; 32: 1327–34.
11- Vaziri PB, Vahedi M, Abdollahzadeh SH, Abdolsamadi HR, Hajilooi M and Kasraee SH. Evaluation of Salivary Albumin in Diabetic Patient. Iran J Public Health 2009; 38(3): 528-35.
12- Dodds MW, Dodds AP. Effects of glycemic control on saliva flow rates and protein composition in non-insulin-dependent diabetes mellitus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997; 83; 465-70.
13- Kimural I, Sasamoto H, Sasamura T, Sugiharay, Ohgaku S, Kabayashir M. Reduction of incretion-like saliva tin in saliva from patients with type 2 diabetes and parotid gland of streptozocin-diabetic BALB/c mice. Diabetes, Obesity and Metabolism 2001; 3(4): 254-8.
14- López ME, Colloca ME, Páez RG, Schallmach JN, Koss MA, Chervonagura A. Salivary characteristics of diabetic children. Braz Dent J 2003; 14: 26-31.
15- Pal P, Desai NT, Kannan N, Masur VN, Daniel MJ, Bhatt N. Estimation of salivary glucose, salivary amylase, salivary total protein and periodontal microflora in diabetes mellitus. J Indian Dent Assoc 2003; 74: 143-9.
16- Meurman JH, Collin HL, Niskanen L, Toyry J, Alakuijala P, Keinänen S, Uusitupa M. Saliva in non-insulin-dependent diabetic patients and control subjects: the role of autonomic nervous system. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 86, 69-76.
17- Collin HL, Niskanen L, Uusitupa M, Töyry J, Collin P, Koivisto AM, Viinamäki H, Meurman JH. Oral symptoms and signs in elderly patients with type 2 diabetes mellitus. A focus on diabetic neuropathy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 90: 299-305.
18- Collin HL, Uusitupa M, Niskanen L, Koivisto AM, Markkanen H, Meurman JH. Caries in patients with non-insulin-dependent diabetes mellitus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85: 680-5.
19- Chávez EM, Borell LN, Taylor GW, Ship JA. A longitudinal analysis of salivary flow in control subjects and old adults with type 2 diabetes. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001; 91: 166-73.
20- Yavuzyilmaz E, Yumak O, Akdo˘ ganli T, Yamalik N, Ozer N, Ersoy F, Yeniay I. The alterations of whole saliva constituents in patients with diabetes mellitus. Aust Dent J 1996; 41: 193-7.
21- Streckfus CF, Marcus S, Welsh S, Brown RS, Cherry-Peppers G, Brown RH. Parotid function and composition of parotid saliva among elderly edentulous African-American diabetics. J Oral Pathol Med 1994; 23: 277-9.
22- Marchetti P, Tognarelli M, Giannarelli R, Grossi C, Picaro L, di Carlo A, Benzi L, Ciccarone A, Navalesi R. Decreased salivary glucose secretory rate: usefulness for detection of diabetic patients with autonomic neuropathy. Diabetes Res Clin Pract 1989; 7: 181-6.
23- López Rubio AC, Garbero I, Testa MM, Cárdenas IL. Isolation of Candida species from different niches of oral cavity in periodontal patients, and its relationship with systemic conditions. Biocell 2006; 30(1):196.
24- Mealey B. Diabetes mellitus. In: Burket's oral medicine. Diagnosis and treatment, 10th ed. Greenberg MS, Gilck M (eds). Hamilton: BC Decker; 2003. p. 563-77.
25- Moore PA, Guggenheimer J, Etzel KR, Weyant RJ, Orchard T. Type 1 diabetes mellitus, xerostomia and salivary flow rates. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001; 92: 281-91.
26- Fenoll-Palomares C, Munoz-Montagud JV, Sanchiz V. Unstimulatcd salivary flow rate, and buffer capacity of saliva in healthy volunteers. Rev Esp Enferm Dig 2004; 96: 773-83.

Effect of Zamzam water on the microhardness of initial caries-like lesion of permanent teeth, compared to Casein Phosphopeptide-Amorphous Calcium Phosphate agents

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ABSTRACT

Background: chemically Zamzam water is suitable for drinking purposes contains calcium, magnesium, sodium, fluoride and other salts higher than other water that have an effective germicidal action. The aim of this study was to investigate the effect of Zamzam water on the microhardness of initial carious lesion compared to CPP-ACP agents.

Materials and methods: thirty two maxillary first premolars with enamel caries-like lesion randomly divided into one study group treated with Zamzam water and three control groups CPP-ACP, and CPP-ACP+NaF as a positive control and deionized water as a negative control (each group consists of 8 teeth). Teeth were subjected for microhardness assessment before and after pH cycling and treatment with the selected agents.

Results: Agents of study groups were statistically highly significant in elevation of the microhardness values, CPP-ACP+NaF caused highest change in the microhardness (158.58%) and less for CPP-ACP (81.48%) and lesser for Zamzam water (80.97%).

Conclusions: Zamzam water was effective in remineralization of the outer enamel caries-like lesions, which was reflected by increase in enamel microhardness values.

Key words: Zamzam water, pH-cycling, CPP-ACP, remineralization. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):128-132).

INTRODUCTION

Water is one of the main dietary components. Its quality plays an important role for the safety of food particularly for infants [(1)]. Millions of Muslims drink Zamzam water as sacred water, especially during pilgrimage and Umrah each year. Zamzam well is located in the holiest mosque of the Muslims in the city of Makkah, which is in the western province of the Kingdom of Saudi Arabia. The well is 4000 years old and the story of its formation is well known to Muslims. It is approximately 40 meters deep and surrounded by hills of igneous rocks [(2)]. The results of the water samples tested by the European laboratories showed that Zamzam water has a special physique that makes it advantageous water, that the main difference between Zamzam water and other water (city water) was in the quantity of calcium and magnesium salts, the content of these was slightly higher in Zamzam water. Additionally, the water contains fluorides that have an effective germicidal action. Moreover, the remarks of the European laboratories showed that the water was fit for drinking [(3)]. The preponderance of evidence indicates that fluoride can reduce the incidence of dental caries and that fluoridation of drinking water can provide such protection [(4,5)].

Multi-elemental and hydrochemical compositions of the holy Zamzam water have been studied. A total of 34 elements have been found with calcium (Ca), magnesium (Mg), sodium (Na) and chloride (Cl) in the highest concentrations [(6)]. The traditional understanding of the impact of diet on dental caries has focused on the importance of fermentable substrates in caries causation; however in the past 15 years there has been an increasing awareness of dietary components which can have hypo- or anti-cariogenic effects. These agents can be classified as either "active" or "passive" in terms of their caries preventive effects [(7)].

It is now clear that milk and milk products contain a variety of agents which can suppress caries progression and some which can exert "active" caries preventive effects. In the former group are protein buffers, calcium and phosphate ions, and whey proteins. Phosphopeptides have attracted considerable attention as caries preventive agents, and there is now a large body of evidence which indicates that phosphoproteins can modulate the mineralization of hydroxyapatite [(8,9)]. In recent years, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been demonstrated to have anticariogenic properties in both laboratory animal and human in situ experiments. Casein phosphopeptides (CPP) are peptides derived from the milk protein casein that are complexed with calcium and phosphate [(10,11)]. The CPP-ACP and fluoride have been

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shown additive effects in reducing caries [12]. This study was designed in order to test the effect of Zamzam water on the microhardness of the artificially initiated carious lesion of the outer enamel surface in comparison to casein phosphopeptide-amorphous calcium phosphate agents.

MATERIALS AND METHODS

Teeth samples in this study consisted of 32 maxillary first premolars extracted from 11-14 years old patients, referred for Orthodontic Department, College of Dentistry, University of Baghdad. Teeth were washed with de-ionized water, and then each tooth was wiped with acetone to remove any debris, then stored in 20 ml of de-ionized water to which 0.1% thymol was added to prevent microbial growth. Then teeth samples were kept in refrigerator at 4˚C until use [13]. Teeth were divided randomly into one study groups and three control groups and each group consisted of eight teeth. Enamel microhardness was measured initially for normal enamel and after induction of caries lesion by pH cycling procedure, and finally after treated by the selected agents (Zamzam water, CPP-ACP, and CPP-ACP+NaF). The microhardness measurement was done by Vickers microhardness device in the Department of Mechanical Engineering, University of Baghdad at a load of 500 gm for 30 seconds. A position of circular window of 6mm in diameter on the buccal surface of each tooth was standardized using orthodontic ruler, then an adhesive tape circle of 6mm diameter was cut and burnished on the buccal surface of the tooth using burnisher, after that an acid resistant nail varnish was used to paint the surfaces of the tooth, the adhesive tape was removed leaving a window on the buccal surface. Teeth were adapted in an acrylic model (the size of this model was 30 × 27 mm) using a red wax. The grit paper (grit 400) was placed in special manual device. Window of each tooth was ground and polished ten times in one direction. This procedure allowed a flat surface of each tooth for microhardness testing [14].

The Induction of Caries like Lesion on the enamel surface was conducted by preparation of demineralizing and remineralizing solutions and adjustment of pH. The demineralizing solution, which contained 0.075 M/L acetic acid, 1 mM/L calcium chloride, and 2 mM/L potassium phosphate had the pH adjusted to 4.3, while the remineralizing solution, which contained 150 mM/L potassium chloride, 1.5 mM/L calcium nitrate, and 0.9 mM/L potassium phosphate had a pH of 7. The pH cycling procedure was involved 6 hours of demineralization with 17 hours of remineralization, the procedure was repeated for a period of ten days, one time each day[15].

Statistical Analysis: Descriptive statistic including means and standard deviation. Estimation of the significance of differences among mean values using ANOVA and LSD tests. Confidence limit was accepted at 95%.

RESULTS

The mean values of the microhardness of the sound enamel surfaces, after demineralization and following treatment with Zamzam water, CPP-ACP and CPP-ACP+NaF are seen in Table (1). Statistically highly significant differences were recorded between different states of enamel for three agents. By using LSD test among variables (three steps) of microhardness, there is a highly significant reductions in enamel microhardness were observed after demineralization for all agents. A noticed increase in the microhardness values was seen after treatment with these three agents. These increases were statistically highly significant, Table (2). The mean values of the microhardness of enamel before and after pH cycling procedure and following the treatment with de-ionized water are shown in Table (3). ANOVA statistical test showed a highly significant difference between the three variables, although there was a highly significant reduction in values of enamel surface microhardness after pH cycling, the microhardness showed only a slight increase after treatment with deionized water which was statistically not significant Table (4).

However, none of the mentioned agents able to increase the elevation of the microhardness values from sound enamel, which is statistically significant, Figure (1). Figure (2) shows the changes in the microhardness values after treatment with selected agents estimated by special equation. Values from this figure reflect a very minor change in the microhardness for de-ionized water in comparison to other agents. CPP-ACP+NaF caused the highest change (158.58%), and less for CPP-ACP (81.48%) and lesser for Zamzam water (80.97%).

DISCUSSION

The chemical analysis of Zamzam water demonstrated highly significant readings in all inorganic elements including higher levels of fluoride, calcium and magnesium. Exposure to

fluoride in drinking water has been shown to be beneficial for oral and general health, especially in relation to dental caries [16]. Ionic calcium in water is the best form to use to insure its proper absorption by the bones and teeth [17].

An interesting result recorded in this study was the higher microhardness values for CPP-ACP+NaF and less for CPP-ACP and lesser for Zamzam water. This can be explained by The CPP-ACP and fluoride has been shown to have additive effects in reducing caries experience. The additive anticariogenic effect of the 1.0% CPP-ACP and 500ppm fluoride in the rat caries experiments led to the investigation of the potential interaction between the CPP-ACP and fluoride. The fluoride ion had incorporated into the ACP phase that was stabilized by the CPP to produce a novel amorphous calcium fluoride phosphate phase (ACFP) at the tooth surface. The identification of this novel amorphous calcium fluoride phosphate (ACFP) phase led to the proposition that the formation of this phase is responsible for the observed additive anticariogenic effect of CPP-ACP and fluoride [18,19].

In this study, Zamzam water recorded increase in the microhardness value of demineralized surface and this may be due to incorporation of Zamzam water elements (fluoride, magnesium, and calcium) in the appetite crystals increasing the resistance to acid dissolution. However presence of fluoride components in Zamzam water may be responsible for the chemical reaction between Zamzam water constituents and appetite crystals [20]. Changes in the microhardness of the demineralized surface following the treatment with Zamzam water were compared with CPP-ACP agents, although CPP-ACP+NaF gave the highest value than Zamzam water, but the difference between Zamzam water and CPP-ACP was not significant, the least changes was recorded for deionized water, one can expected from all these tests that Zamzam water is effective in remineralization of initial carious lesion and its effectiveness is not different from that of CPP-ACP. However, the cariostatic potential of Zamzam water need to be confirmed by further studies before giving any recommendation of using Zamzam water in the dental practice.

REFERENCES

1. Rudzka – kantoch Z and Weker H. Water in children's diet. Med Wieku Rozwoj 2000; 4:109 – 15.
2. Naeem A, Alsanussi M, Almohandis A. Multielemental and hydrochemical study of holy Zamzam Water. Journal New England Water Works Association 1983; 97: 158.
3. Analytical report of Zamzam water cited from the ministry of agriculture and water resources (personal communication) 1971.
4. Kaminsky LS, Mahoney MC, leach J, Melius J, Miller MJ. Fluoride: benefits and risks of exposure. Crit Rev Oral Biol Med 1990; 1(4): 261 – 81.
5. Ripa WL. A half century of community water fluoridation in the US .Review and commentary. J Public Health Dent 1993; 53: 17-44.
6. El-Zaiat Y. Inherent optical properties of zamzam water in the visible spectrum. Arab J science and engineering 2007; 32(24): 171-180.
7. Infeld T. Chewing gum-facts and fiction: a review of gum-chewing and oral health. Crit Rev Oral Biol Med 1999; 21(2): 86-90.
8. Reynolds EC. Remineralization of early enamel caries by anticariogenic casein phosphopeptide-amorphous calcium phosphate nanocomplexes. Dental Practice 2001.
9. Fahad A. Effect of Casein phosphopeptide-amorphous calcium phosphate on the microhardness and microscopic features of the sound enamel and initial caries-like lesion of permanent teeth compared to fluoridated agents**.** A master thesis. College of Dentistry, University of Baghdad, 2010.
10. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Acid resistance of enamel sub surface lesions remineralized by a sugar free chewing gum containing casein phosphopeptideamorphous calcium phosphate. Caries Res J 2004; 38: 551–6.
11. Morgan MV, Adams GG, Bailey DL, Tsao CE, Fischman SL, Reynolds EC. The anticariogenic effect of sugar-free gum containing CPP-ACP nanocomplexes on approximal caries determined using digital bitewing radiography. Caries Res J 2008; 66: 442-9.
12. Reynolds EC, Cain CJ, Webber FL, Black CL, Riley PF, Johnson IH, et al. Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptides in the rat. J Dent Res 1995; 74(6): 1272-9.
13. Barbakow F, Sener B, Lutz F. Dissolution of phosphorus from human enamel pretreated in vitro using SnF_2 stabilized with amine fluoride. Clin Prev Dent 1987; 9(5): 3-6.
14. Al-Sayyab M. The potential effect of combined CO_2 laser and fluoride on acid resistance of human dental enamel and root surface in vitro. PhD thesis, Preventive Dentistry, University of Baghdad, 2000.
15. Featherstone J, Oreilly M, Shariati M, Bruder S. Enhancement of remineralization in vitro and in vivo. In: Leach S.A. Ed. Factor relating to demineralization and remineralization of the teeth. Oxford IRL Press; 1986.
16. Brambilla E. Fluoride. Is it capable of fighting old and new dental disease? An overview of existing fluoride compounds and their clinical applications. Caries Res 2001; 35 (suppl 1): 6 – 9.
17. Al-Zuhair N, Khounganian R. A comparative study between the chemical composition of potable water and Zamzam water and its effect on tooth structure water in Saudi Arabia. Saudi Dental J 2006; 18: 843-55.

18. Reynolds EC, Cain CJ, Webber FL, Black CL, Riley PF, Johnson IH, et al. Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptides in the rat. J Dent Res 1995; 74(6):1272-9.
19. Walsh LJ. Preventive Dentistry for the general dental practitioner. Aust Dent J 2000; 45(2):76-82.
20. Al-Rawi N, Al-Alousi J, Al-Obaidy N. Effect of Zamzam water on the microhardness of initial carious lesion of permanent teeth enamel. M Dental Journal 2009; 6(2): 110-6.

Table 1: Microhardness (Mean* and Standard Deviation) of enamel surfaces treated by Zamzam water, CPP-ACP, and CPP-ACP+NaF after pH Cycling

Groups	Zamzam water		Casein phosphopeptides-amorphous calcium phosphate		Casein phosphopeptides-amorphous calcium phosphate+sodium fluoride	
Variables	**Mean**	**±S.D**	**Mean**	**±S.D**	**Mean**	**±S.D**
Sound enamel	263.03	12.59	295.98	16.18	247.81	14.87
Demineralization	54.93	11.49	57.68	9.02	49.9	6.97
Remineralization	99.39	8.03	104.66	8.46	129.03	8.51
ANOVA	F= 811.641		F= 921.701		F= 695.834	
	P= 0.000		P= 0.000		P= 0.000	
	df= 2		df= 2		df= 2	

*(VHN)

Table 2: LSD test among variables of Microhardness for Zamzam water, CPP-ACP, and CPP-ACP+NaF

Groups	Zamzam water		Casein phosphopeptides-amorphous calcium phosphate		Casein phosphopeptides-amorphous calcium phosphate+sodium fluoride	
Variables	**Mean difference**	**P-value**	**Mean difference**	**P-value**	**Mean difference**	**P-value**
Sound enamel× Demineralization	208.1	0.000*	238.3	0.000*	197.913	0.000*
Sound enamel× Remineralization	163.638	0.000*	191.313	0.000*	118.788	0.000*
Demineralization× Remineralization	-44.463	0.000*	-46.988	0.000*	-79.125	0.000*

*** Highly significant**

Table 3: Microhardness (Mean* Values and Standard Deviation) of enamel surfaces treated by de-ionized water

Variables	Mean	±S.D
Sound enamel	280.85	17.99
Demineralization	56.63	7.89
Remineralization	57.29	7.54
ANOVA	F= 905.545	
	P= 0.000	
	df= 2	

Table 4: LSD test among variables of microhardness of enamel surfaces treated by de-ionized water

Groups	Mean difference	P-value
Sound enamel × Demineralization	224.225	0.000*
Sound enamel × Remineralization	223.563	0.000*
Demineralization × Remineralization	-0.662	0.914

*** Highly significant**

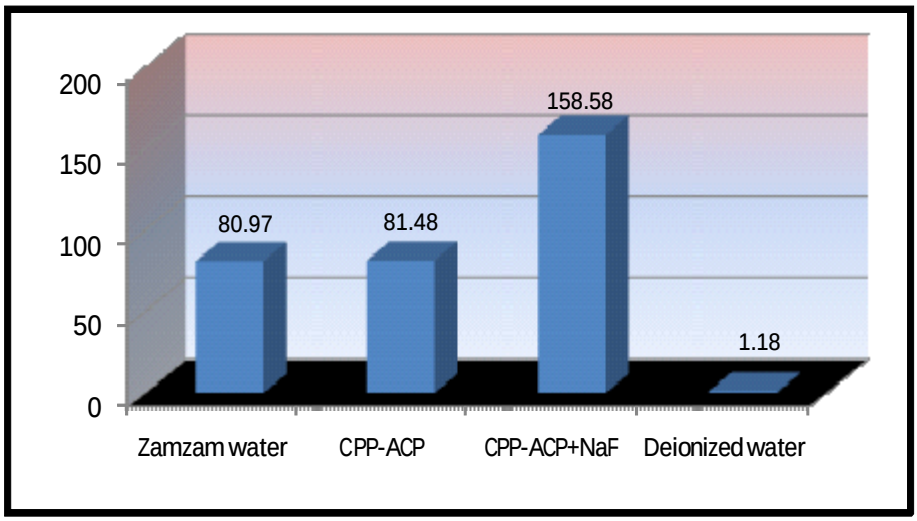

Figure 1: Mean values of the microhardness of the sound enamel surfaces, after demineralization and following treatment with the selected agents.

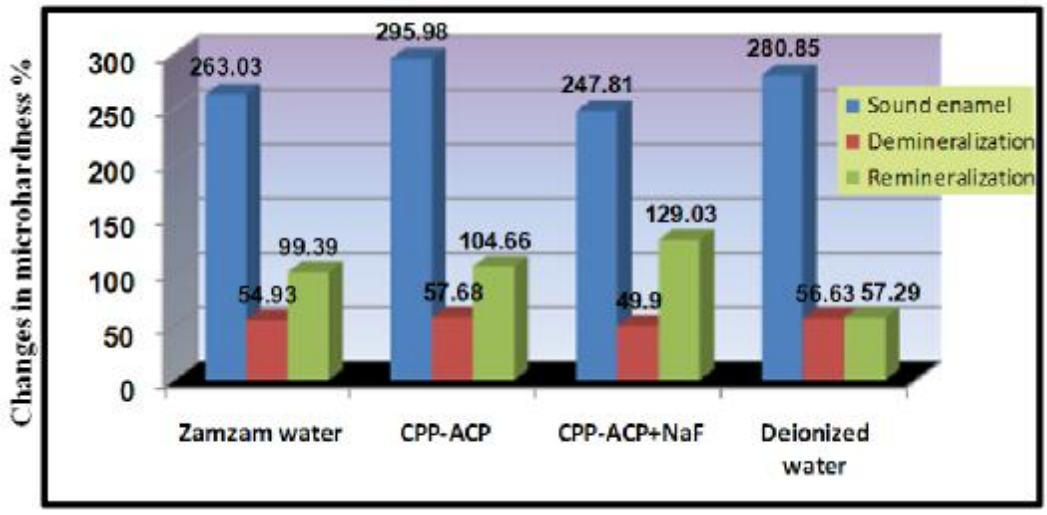

Figure 2: Changes in the microhardness values after treatment with the selected agents.

Biological evaluation of alveolar bone remodeling in methylprednisolone treated –rats during orthodontic tooth movement

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ABSTRACT

Background: Bone remodeling and metabolism associated with orthodontic tooth movement are regulated by a large number of local and systemic factors. The widespread useof therapeutic corticosteroids (GCs) today raise concerns with regard to their effects on mineralized tissue metabolism. This study aimed to investigate the effect of Methylprednisolone treatment on alveolar bone remodeling during orthodontic tooth movement.

Materials and Methods: A twenty-six 12-weeks old male Wistar albino rats were divided into 2 groups; control group (n = 13) without any drug administration during the study and steroidal group (n = 13) which received 5 mg/kg/day of methylprednisolone for 3 weeks. A split- mouth design was used performing orthodontic tooth movement on the upper right 1st molar by applying 20 g of mesial force using superelastic closed-coil spring attached to the incisors for 21 days while the upper left side served as the non-appliance side. Orthodontic tooth movement was evaluated on weekly basis using digital caliber. The rats were sacrificed after 3 weeks and alveolar bone remodeling process was evaluated by counting the number of osteoblast and osteoclast cells at the compression and tension sites at the coronal and apical levels of the mesiobuccal root of upper 1st molar in both appliance and non-appliance sides using digital microscope at 400× magnification. At day of sacrifice serum measurements for alkaline phosphatase (ALP) and acid phosphatase (ACP) activity were carried out.

Results: Showed that in the steroid group there was significantly greater amount of orthodontic tooth movement, greater reduction of bone formation and an increase in bone resorption with the presence of orthodontic appliance, increase in serum ACP activity and reduction of serum ALP activity as compared with the control group,($P \leq 0.05$).

Conclusion: The Methylprednisolone therapy in low-medium doses elicits a noticeable change in the bone turnover rate during orthodontic tooth movement.

Keywords: methylprednisolone, tooth movement, bone remodeling. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):133-142).

INTRODUCTION

Tooth movement during orthodontic treatment is achieved by the remodeling of the alveolar bone in response to mechanical loading as the forces of orthodontic appliances applied to the teeth are transmitted through the periodontal ligament (PDL) to the supporting alveolar bone, leading to deposition or resorption depending upon whether the tissues are exposed to a tensile or compressive mechanical strain.The transduction of mechanical forces to the cells triggers a biological response, which has been described as an aseptic inflammation because it is mediated by a variety of inflammatory cytokines. [1, 2]

Investigations of the actions of hormones on bone have revealed that glucocorticoids cause marked effects on bone metabolism and that continued exposure of skeletal tissue to excessive amounts of glucocorticoids results in osteoporosis. However, the exact mechanisms by which glucocorticoids act on bone are unknown. [3]

It has been shown that orthodontic tooth movement may be influenced by general and local administration of pharmaceutical agents.[4-9]As the prevalence of allergies and diseases that need corticosteroidtreatment is on the increase, it can be anticipated that an important number of orthodonticpatients can present variations from normal boneremodeling because of this steroid.[10] In most of thepublished animal experiments that studied glucocorticoidadministration and orthodontic tooth movement, the glucocorticosteroid dose has been high. These high doses made the animals osteoporotic. Daily injections (15 mg/kg) of glucocorticosteroid drug caused amarked state of osteoporosis in a short time period inthe rabbit [11, 12] and even higher doses (25 mg/kg) have been used in cats.[13]The dosages used in theabove-mentioned studies, however, are not compatiblewith the

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concentrations recommended for use inhumans, either for short or long durations. Yamane*et al.*[14]used a dosage of 10 mg/kg for only 7 days. Ong*et al.*[15] used a therapeutic dosage of 1 mg/kg in young rats for short-term, thus avoiding the risk ofsecondary hyperparathyroidism. Whereas a study performed by Kalia*et al.*[5] used a dosage of 8 mg/kg/day for shortand long-term administration, showed the mechanical load induced an enlargement of the alveolar wall that was less pronounced in both medicated groups, and in the short-term group the drug suppressed bone resorption and formation without mechanical stimulus. Force application resulted in significant increase in the relative extension of resorption and formation in both drug groups; it was particularly pronounced in the long-term group due to the secondary hyperparathyroidism state that the animals reached. The differences in the results of these studies probably reflect the combined effects of the dosages, the induction periods, and the amount of orthodontic force applied and the relative anti-inflammatory activity of the glucocorticoids tested.

In the present study, the effect of methylprednisolone (one of the most widely used corticosteroids) on bone metabolism in a rat model was tested with therapeutic dosages of 5 mg/kg/day to examine the effect of low dose prednisolone treatment on bone remodeling during orthodontic tooth movement.

The effect of treatment was evaluated by measuring the rate oforthodontic tooth movement, and analysis of bone remodeling patterns through thequantification of both the resorptive and formative components of the remodeling cycle (osteoclast and osteoblast cells counting), and by biochemical investigation of both alkaline phosphatase and acid phosphatase enzymes activity as the alkaline phosphatase enzyme is observed to be associated with osteoblastic activity whereas acid phosphatase enzyme is observed to be associated with osteoclastic activity. [16-18]

MATERIALS AND METHODS

Animals and Steroid treatment

Twenty-six 12-week-old adult male Wistar albino rats (average weight270.5 g) obtained from the animal department of (High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain university/Baghdad-IRAQ) were used in this study. Animals were acclimatized for 5 days in plastic cages (two per cage) with a standard 12-hour light/ dark cycle at a constant humidity and temperature of 25°C according to the National Research Council's guide for the care and use of laboratory animals and accessed to drinking water *ad libitum* with standard laboratory rat pellets. Body weights of all rats were measured daily.All rats received orthodontic treatment for 3 weeks and were divided randomly in two groups: control group (n=13) without corticosteroid treatment and steroid group (n = 13) administered daily doses of 5 mg/kg/day of methylprednisolone (Solu-medrol; Pharmacia NV/SA, Puurs - Belgium) intramuscularly for the prescribed number of days.

Orthodontic appliance treatment

Following acclimatization, an orthodontic appliance was inserted on the maxillary right first molar, and a mesially directed force of 20 g was applied. The orthodontic appliance consisted of a stretched superelastic (rematitan®)closed coil spring (9 mm in length, Dentaurum, Germany) ligated between the maxillary right first molar and 2 maxillary central incisors as described previously byMohammed-Salih[19]. The molar on the left side was used as the non-appliance side, (Fig. 1). The magnitude of tooth movement was determined by measuring the relative separation between the first andsecond maxillary molar using digital vernier calipers with sharpenedtips inserted into occlusal pits as the procedure modified by Ong*et al.*[15]. The distance betweenthe mesial occlusal pits on the first and second molars was measured intraorally before appliance insertion and at the end of the first, second and third week of the study (immediately after sacrifice). All appliances were checked weekly and at the time of sacrifice and all appliances were still in place and in good order.Measurements were performed by thesame operator and were repeated five times foreach side of the maxilla.Rats were sedated during appliance insertion using intramuscular injection of a mixture of ketamine (90 mg/kg body weight) and xylazine (10mg/kg body weight).

Histological Preparation

At 21 days post-appliance insertion, rats were sacrificed humanly under general anesthesia.Maxillae were immediatelyremoved, (Fig. 2) and dissected into halves, fixed in 10% neutral-buffered formalin solution for 24-48 hours and all the specimens from each group were decalcified by 10% formic acid for 3-4 weeks; it was checked every 4 days with changing of the acid, after that dehydration were done and paraffin cross- sections of 5 µm thick were prepared(parallel to the occlusal plane of molar teeth) with microtome. At the coronal and apical level two 5 µm thick horizontal sections, 150 µm apart, were cut. The coronal and apical levels

were defined using as a start the first section showing bone on the non-appliance side. Distance from the lower coronal section to the first apical section was 1150 µm.[20]The sections were stained with hematoxylin and eosin (H&E stain). Then sections were photographed by a photomicroscope (Olympus-Japan).

For evaluation of pathological changes consistent with the experiment. Tissues surrounding the mesiobuccal root were investigated on the appliance and contralateral non-appliance sides under digital light microscope atboth compression and tension sites and the following histomorphometric parameters were determined:

Evaluation of the Bone formation

Bone formation was evaluated at both compression/mesial andtension/distalsites at the coronal and apical levels on both appliance and contralateral non-appliance sides by estimating the number of osteoblasts cells were examined at ×400 magnification by the inbuilt image processing software of digital microscope (Micros Crocus II MCX100LCD Produktions und HandelsgmbH) that was fed directly to a TV monitor with a real time live camera.One area from each section was selected for the evaluation of bone neoformation.[21]

Evaluation of Bone Resorption

Bone resorption was evaluated at both compression/mesial and tension/distal sites at the coronal and apical levels on both appliance and contralateral non-appliance sides by estimating the number of osteoclasts cells were examinedinactive Howship's lacunae at ×400 magnification by the inbuilt image processing software of digital microscope (Micros Crocus II MCX100LCD Produktions und HandelsgmbH) that was fed directly to a TV monitor with a real time live camera. The histological criterion used to identify the osteoclast-like cells was the presence of multinuclear and eosinophilic cells on the bone surface or in bone resorptivelacunea. [6]

Serum Measurements

At sacrifice, blood was collected by cardiac puncture (2ml from each animal) after thoracotomy, into glass tubes and allowed to coagulate for30 minutes on ice. After centrifugation at ×3000 g for 20 minutes at 4C°, the serum was transferred to new tubes and frozen at − 20C°. Alkaline phosphatase (ALP) and acid phosphatase (ACP) activity were measured using method of determination as described previously by*Milne et al.* [22]

Statistical Methods

Data were expressed as the mean ± standard deviation of the mean (SD).The statistical analysis was carried out using SPSS version 15 computer program and the following tests were used:

-ANOVA test was used to determine if significant differences exist between the groups in the amount of tooth movement followed by least significant difference (LSD) test between each two groups.

-Mann-Whitney U test was used to compare between the two independent groups (control and steroid) for bone resorption and bone formation activity.

-T-test was used to compare between the means of the control and steroid groups for the serum level of ALP and ACP enzymes.

P value of ($P \leq 0.05$) was regarded as statistically significant.

RESULTS

Rate of tooth movement

On the basis of the weekly measurements, the pharmacological treatment resulted in a highly significant difference in the rate of orthodontic tooth movement which was faster in the steroid group than in the controlgroup by nearly two times after the 1st ,2nd and 3rd weeks post-appliance insertion ($p \leq 0.01$) ,(Table 1, Fig.3).

Histology

The alveolar bone remodeling process wasaffected dramatically inmedicated group than in control group with the presence of orthodontic appliance. Medicated rats differed from the controlson both the appliance and the non-appliancesides. Alveolar bone formation in the appliance sideat the compression site was significantlyreduced in the steroid group than in the control group at both levels (coronal and apical)($p \leq 0.05$),whereas non-significantly at the tension site ($p \geq 0.05$).At the non-appliance side although the results indicate there was a reduction in bone formation in the steroid group compared with the control group at both sites (mesial and distal sites)but non-significantly($p \geq 0.05$), (Table 2).

Alveolar bone resorption in the appliance side was significantly increased at both sites (compressionand tension sites) in the steroid group than in the control group at both levels (coronal and apical) ($p \leq 0.05$), except at the coronal level of the tension site was increased non-significantly($p \geq 0.05$).Also at the non-appliance side there was an increase in bone resorption in the steroid group than in the control group at both mesial anddistal sites but non-significantly($p \geq 0.05$), except there was a significant difference between them at the coronal levelof the mesial site($p \leq 0.05$), (Table 2).

Serum ALP and ACP levels

Serum ALP activity was found to have reducedsignificantly in the steroid group compared with the control group ($p \leq 0.01$), (Fig. 4). Whereas serum ACP activity showed a significantincrease in the steroid groupcompared with the control group($p \leq 0.01$), (Fig. 4).

DISCUSSION

Most in vivostudies of orthodontic tooth movement have concentrated on changes occurring within the PDL. However, the PDL can only provide a partial explanation for the mechanisms involved in dentoalveolar remodeling, and more attention has focused lately on the wider response of the alveolar bone. [23-26]Previous proposals have suggested that orthodontic loading may trigger bone remodeling by producing microdamage[27] or by stimulating the induction of a regional acceleratory phenomenon [23,25](a reaction to trauma in which the rate of bone remodeling exceeds normal tissue activity).

In the present study changes in the remodeling of alveolar boneupon 21 days of systemicglucocorticoidadministration were carried out in a rat model with and without orthodonticforces. The experimental model for mesial movement of rat molar has been repeatedly used in previous studies [5, 19, 25, 28-30]. The rat model is the standard method for the study of skeletal adaptation to mechanical stimuli[31]and to impaired metabolic conditions.[32-34]The total treatment duration of 3 weeks (pharmacological and orthodontic treatment) was chosen in order to interfere with bone metabolism for a minimumof one remodeling cycle (sigma), ranging according to various authors between 10 and 31 days [32]. According to Li *et al.*[35]the sigma of a rat changes as a function of age and at 6 months it is considered to beapproximately 21 days.

The effects of physiological and therapeutic doses of glucocorticosteroid administration (5 mg/kg/day) on alveolar bone as specified in this study with and without orthodontic movement have not been previously investigated which is comparable with low-oral dosesrecommended for more common diseases and to keep thedetrimental effects of bone loss minimal[36]. The short duration of corticosteroid administration in the present study makes the possibility of iatrogenic hypercortisonism and hyperparathyroidism remote.

The results showed ahigher rate of tooth movement was in steroid group than in control animals. This finding is consistent with a more rapid tooth movement found in animals in the acute phase of corticosteroid treatment [11, 19] and also with high bone turnover caused by secondary hyperparathyroidism during orthodontic tooth movement. [37]However, normal bone remodeling process is a fundamental to orthodontics; this increase could be explained by the effect of GCs on bone remodeling process. There is evidence that during the initial administration of corticosteroids, a period of very rapid bone loss occurs. This could be ascribed to the lack of balance between formation activities (inhibited or reduced by the drug) and the resorption activities (enhanced by drug administration) occurring in the initial phase of drug administration[19, 38, 39]. However, controversy exists as to the effects of corticosteroids on tooth movement. As noted previously, Ashcraft *et al.*[11] induced orthodontic molar tooth movement for 14 days in corticosteroid-induced osteoporotic rabbits, and showed a greater rate of tooth movement in steroid-treated rabbits. In contrast, Yamane *et al.*[14] reported that tooth movement in rats was inhibited by 10 mg/kg per day of hydrocortisone, while Davidovitch*et al.*[13] showed slower tooth movement in cats treated with cortisone acetate (12.5to 25 mg/day). These differences may be explained by variationswithin animal species studied, forces used to move teeth, duration of the experiment, dosage and time interval of administration, and potency of the steroid used. The present study used a standardized technique for inducing orthodontic tooth movement in rats as described previously by Brudvik and Rygh[40]. This technique mimics orthodontic tooth movement in humans.Experimental studies on tooth movement are often difficult to compare because of the use of different orthodontic appliances and different magnitudes, types, and duration of forces.

However, normally, a balance exists between the amount of bone resorbed by osteoclasts and the amount formed by osteoblasts to maintain a constant bone mass; in other words, bone resorption and formation are said to be coupled.

In the present study, the results showed that the steroid treatment disturbed the normal bone remodeling process in the presence of mechanical stimuli (at the appliance side) as the bone formation wasreduced at the compression (Fig.5) and tension sites (Fig.6).Alsoat the non-appliance side bone formation was reduced,but this is a reflection of steroid effect on bone, these findings consiestance with a decreased percentage of bone formation in the acute group carried by Kalia*et al.*[5] but in association with a decreased percentage of resorption activity. Such reduction of bone formation might be due to at least two different mechanisms, i.e., inhibition of osteoblast function

and inhibition of the proliferation or differentiation of precursor cells to osteoblasts. GCs have also been reported to promote the apoptosis of osteoblasts and osteocytes [41].GCs are known to have various effects on osteoblast gene expression, including down-regulation of type I collagen and osteocalcin. The expression of IGF-1, which is an important stimulator of osteoblast function, is also known to be decreased by GCs. [3]GCs at physiological concentrations are known to inducethe proliferation and differentiation of bone marrow stromalcells into cells that express a mature osteoblast phenotype,whereas GCs at higher concentrations or pharmacologicaldoses drastically reduce the proliferation ofosteoblast precursors [42]and inhibit the differentiationto mature osteoblasts.[43]

Bone resorption was increased at both appliance and non-appliance sides (Fig.7), when comparing scientific studies in the literature, itwas observed that glucocorticoids may produce antagonistic effects upon bone resorption during tooth movement. Hofbauer*et al.*[44]and Swanson *et al.*[45] affirm that corticosteroids stimulate *in vitro* bone resorption by osteoclast activity and/or formation increased, whileKalia*et al.*[5] used methylprednisolone 8 mg/kg/day under chronic and acute treatment and observed different results between the groups. In the acute, it was observed reduction on resorption percentage, while in the chronic, the tooth movement rate increased, due to secondary hyperparathyroidism. Ashcraft *et al.*[11] evaluated the effect of cortisone acetate on orthodontic movement in rabbits and observed a decrease in the mean incremental active tooth movement. Ong *et al.*[15] observed lower tartrate-resistant acid phosphatase-positive cells on the compression side after prednisolone administration. It is important to note that the glucocorticosteroid therapy is not only dose dependent but also time dependent. Many previous studies performed at 3, 14 and 21 days; there was a significant difference in the number of Howship'slacunae,therefore in the present study the use of steroid therapy for 21 days can be considered as a transition point from short to long-term of drug administration.

Studies testing the effect of glucocorticoids onbone resorption in vitro have not yielded uniformconclusions due to differences in the systems,culture conditions, and length of glucocorticoidtreatment used. Some researchers foundthat glucocorticoids inhibited PTH-stimulatedbone resorption in vitro.[46, 47]However; more recent studies havedemonstrated that glucocorticoids stimulatebone resorption in cultured calvaria.[48, 49]The effects ofglucocorticoids on osteoclast recruitment /differentiationand activity have been dissociated usingthe model system of bone chips implantedsubcutaneously into rats.[50]It was shown that glucocorticoids inhibited therecruitment and differentiation of bone resorbingcells, but stimulated the bone resorbing activity.This may be related to the hypothesized“coupling” of osteoblastic activity to bone resorption.[51]

An important interaction was noted betweenmechanical perturbation and the drug, leading to anincrease in the extension of mineralizing surfacesexceeding what was seen in the control animals.On the mesial aspect we might have generated a localized rapid acceleration phenomenon, where bone surface was subjected to a high local stress by the orthodontic appliance. This could lead to decreased resorption in some sites because of ischemia and increased in others reflecting a local repair process.

Biochemical markers of bone metabolism such as ALPand ACP levels in serum are frequently employed asadjuncts to bone mass measurements to detect systemicchanges of bone turnover in metabolic bone diseases. Eventhough serum ALP consists of several isoforms that originatefrom various tissues such as bone, liver, and kidney, it iscommonly used as a clinical marker for measuring osteoblastactivity and bone formation.[52]Thedecrease in serum ALP activity detected in the steroid group compared withthe controls was consistent with the reduction inbone formation capacity (no. of osteoblast cells) observed histologically in the present study. Since serum markers of bone metabolismreflect whole-body rates of bone formation and resorption,the loss of alveolar bone was clearly of rapid onset, resultinginsignificant osteopenia after just 2-4 days. Evidence frommicrogravity studies suggests that in addition to reducedosteoblast differentiation and function, [53, 54]osteoblast apoptosis[55] may have contributed to the osteopenia,although more recently, Bucaro*et al.*[56] reported thatthe effect of microgravity on osteoblasts was independentof the induction of apoptosis.

Theincrease in serum ACP activity suggests that bone resorption exceeds bone formation[57] may therefore be a reflection of the fact that bone formation and resorption, although both down-regulated by reduced mechanical loading,remained coupled, the outcome being a localized negativeskeletal balance of the tooth-supporting bone. Nevertheless, confirmation of this observation will require future assays of serum for the tartrate-resistant ACP5b isoform, a

unique bone resorption marker released fromresorbing osteoclast cells. [58]

Histological analyses in this study confirmed that the glucocorticoid drug (methylprednisolone) used under the conditions of this study elicits a noticeable change in the bone turnover rate. The effects on bone remodeling indicated a reduction of bone formation and increase in bone resorption and this effect was greater with the presence of theprocess of orthodontic tooth movement.

Clinically, it is fair to say that patients who are within the low-medium doses of this drug who are already undergoing orthodontic treatment should have their appointments scheduled with shorter intervals, as bone turnover will be enhanced and tooth movement would be faster to avoid and prevent any unwanted tooth movement.

REFERENCES

1. Garlet TP, Coelho U, Silva JS, Garlet GP. Cytokine expression pattern in compression and tension sides of the periodontal ligament during orthodontic tooth movement in humans. Eur J Oral Sci 2007; 115: 355-62.
2. Middleton J, Jones M, Wilson A. The role of the periodontal ligament in bone remodeling, the initial development of a time dependent finite element model. Am J Orthod Dentofacial Orthop 1996; 109: 155–62.
3. Delany AM., Dong Y, Canalis E. Mechanisms of Glucocorticoid Action in Bone Cells. J Cell Biochem 1994; 56: 295-302.
4. Ohkawa S. Effects of orthodontic forces and anti-inflammatory drugs on the mechanical strength of the periodontium in the rat mandibular first molar. Am J Orthod 1982; 81: 498-502.
5. Kalia S, Melsen B, Verna C. Tissue reaction to orthodontic tooth movement in acute and chronic corticosteroid treatment. Orthod Craniofac Res 2004; 7: 26-34.
6. Arias OR, Marquez-Orozco MC. Aspirin, acetaminophen, and ibuprofen: their effects on orthodontic tooth movement. Am J Orthod Dentofacial Orthop 2006; 130: 364-70.
7. de Carlos F, Cobo J, Díaz-Esnal B, Arguelles J, Vijande M, Costales M. Orthodontic tooth movement after inhibition of cyclooxygenase-2. Am J Orthod Dentofacial Orthop 2006; 129: 402-06.
8. Bartzela S, Türp JC, Motschall E, Maltha JC. Medication effects on the rate of orthodontic tooth movement: a systematic literature review. Am J Orthod Dentofacial Orthop 2009; 135: 16-26.
9. Gonzales C, Hotokezaka H, Matsuo K, Shibazaki T, Yozgatian J H, Darendeliler M., Yoshida N . Effects of steroidal and non-steroidal drugs on tooth movement and root resorption in the rat molar. Angle Orthod 2009; 79:715-26.
10. DIKE. Asthma, allergieandenoverfølsomhed Danmark- ogudviklingen1984–1987. Dansk Institute for Klinisk Epidemiologi, 1997.
11. Ashcraft MB, Southard KA, Tolley EA. The effect of corticosteroidinduced osteoporosis on orthodontic tooth movement. Am J Orthod Dentofacial Orthop 1992; 102: 310–9.
12. Thompson JS, Palmieri GMA, Eliel LP, Crawford RL. The effect of porcine calcitonin on osteoporosis induced by adrenal cortical steroids. Journal of Bone and Joint Surgery 1972; 54: 1490–500.
13. Davidovitch Z, Musich D, Doyle M. Hormonal effects on orthodontic tooth movement in cats – a pilot study. Am J Orthod. 1972; 62: 95–6.
14. Yamane A, Fukui T, Chiba M. In vitro measurement of orthodontic tooth movement in rats given beta-aminopropionitrile or hydrocortisone using a time-lapse videotape recorder. Eur J Orthod 1997; 19: 21–8.
15. Ong CK, Walsh LJ, Harbrow D, Taverne AA, Symons AL. Orthodontic tooth movement in the prednisolone-treated rat. Angle Orthod 2000; 70: 118–25.
16. Graber TM, Rakosi T, Alexandere GP. Dentofacial orthopedics with functional appliances. 1st ed. The C.V. Mosby Company. 1985; p: 48-53.
17. Bhaskar SN. Orban's oral histology and embryology. 11th ed. 1991; p. 203-59, 383, 452-60.
18. Basdra EK, Komposch G. Osteoblast-like properties of human periodontal ligament cells: an in vitro analysis. Eur J Orthod 1997; 19: 615-2l.
19. Mohammed-Salih HS. Orthodontic tooth movement in low-dose for different courses methylprednisolone-treated rats. International Journal of Advanced Biological Research 2012; 2: 545-51.
20. Verna C, Hartig LE, Kalia S, Melsen B. Influence of steroid drugs on orthodontically induced root resorption. Orthod Craniofacial Res 2006; 9: 57–62.
21. Knop L, Shintcovsk R , Retamoso L, Ribeiro J, Tanaka O. Non-steroidal and steroidal anti-inflammatory use in the context of orthodontic movement. Eur J Orthod 2012; 34(5): 531-5.
22. Milne TJ, Ichim I, Patel B, McNaughton A, Meikle MC. Induction of osteopenia during experimental tooth movement in the rat: alveolar bone remodelling and the mechanostat theory. Eur J Orthod 2009; 31: 221– 31.
23. Melsen B. Biological reaction of alveolar bone to orthodontic tooth movement. Angle Orthod 1999; 69:151–8.
24. Melsen B. Tissue reaction to orthodontic tooth movement-a new paradigm. Eur J Orthod 2001; 23: 671 –81.
25. Verna C, Zaffe D, Siciliani G. Histomorphometric study of bone reactions during orthodontic tooth movement in the rat. Bone 1999; 24: 371 –9.
26. Pavlin D, Zadro R, Gluhak-Heinrich J. Temporal pattern of osteoblast associated genes during mechanically-induced osteogenesis *in vivo:* early responses of osteocalcin and type I collagen. Connect Tissue Res 2001; 42: 135 – 48.
27. Verna C, Dalstra M, Lee TC, Cattaneo PM, Melsen B. Microcracks in the alveolar bone following orthodontic tooth movement: a morphological and morphometric study. Eur J Orthod 2004; 26: 459 – 67.
28. Rygh P, Bowling K, Hovlandsdal L, Williams S. Activation of the vascular system: a main mediator of periodontal fiber remodeling in orthodontic tooth movement. Am J Orthod 1986; 89: 453–68.
29. King GJ, Keeling SD, McCoy EA, Ward TH. Measuring dental drift and orthodontic tooth movement in response to various initial forces in adult rats. Am J Orthod Dentofacial Orthop 1991; 99: 456 – 65.
30. Ren Y, Maltha JC, Kuijpers-Jagtman AM. The rat as a model for orthodontic tooth movement- a critical

review and a proposed solution. Eur J Orthod 2004; 26: 483-90.
31. Jee WS, Li XJ. Ke HZ. The skeletal adaptation to mechanical usage in the rat. Cells and Materials supplement. 1991; 1:131-42.
32. Baron R, Tioss R, Vignery A. Evidence of sequential remodeling in rat radicular bone: morphology, dynamic histomorphometry, and changes during skeletal maturation. Anatomical Record 1984; 208: 137-145.
33. Frost H M, Lee W S. On the rat model of human osteopenias and osteoporoses. Bone and Mineral Research 1992; 18: 227-236.
34. Allain T I, Thomas M R, McGregor A M, Salisbury J R. A histomorphometric study of bone changes in thyroid dysfunction in rats. Bone 1995; l6: 505-9.
35. Li XI, Jee WS, Ke HZ, Mori S, Akamine T. Age-related changes of cancellous and cortical bone histomorphometry in female Sprague-Dawley rats. Cells and Materials supplement 1991; 7: 25-35.
36. Wang Y, Ohtsuka-Isoya M, Shao P, Sakamoto S, Shinoda H. Effects of Methylprednisolone on Bone Formation and Resorption in Rats. Jpn J Pharmacol 2002; 90: 236 – 46.
37. Verna C, Dalstra M, Melsen B. The rate and the type of orthodontic tooth movement is influenced by bone turnover in a rat model. Eur J Orthod 2000; 22:343–52.
38. Chavassieux P, Buffet A, Vergnaud P, Garnero P, Meunier PJ. Short-course effects of corticosteroids on trabecular bone remodeling in old ewes. Bone 1997; 20: 451–5.
39. Hirayama T, Sabokbar A, Athanasou NA. Effect of corticosteroids on human osteoclast formation and activity. J Endocrinol. 2002; 175: 155–63.
40. Brudvik P, Rygh P. The initial phase of orthodontic root resorption incident to local compression of the periodontal ligament. Eur J Orthod 1993; 15: 249–63.
41. Weinstein RS, Jilka RL, Parfitt AM, Manolagas SC. Inhibition of osteoblastgenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanism of their deleterious effects on bone. J Clin Invest 1998; 102: 274 –82.
42. Scutt A, Bertram P, Brautigam M. The role of glucocorticoids and prostaglandin E2 in the recruitment of bone marrow mesenchymal cells to the osteoblastic lineage: positive and negative effects. Calcif Tissue Int. 1996; 59: 154 – 62.
43. Boden SD, Hair G, Titus L, Racine M, McCuaig K, Wozney JM, Nanes MS. Glucocorticoid-induced differentiation of fetal rat calvarial osteoblasts is mediated by bone morphogenetic protein-6. Endocrinology 1997; 138: 2820 – 2828.
44. Hofbauer LC, Gori F, Riggs BL, Lacey DL, Dunstan CR, Spelsberg TC,Khosla S. Stimulation of osteoprotegerin ligand and inhibition of osteoprotegerin production by glucocorticoids in human osteoblastic lineage cells: potential paracrine mechanisms of glucocorticoid-induced osteoporosis. Endocrinology 1999; 140: 4382-9.
45. Swanson C, Lorentzon M, Conaway HH, Lerner UH. Glucocorticoid regulation of osteoclast differentiation and expression of receptor activator of nuclear factor-kappaB (NF-kappaB) ligand, osteoprotegerin, and receptor activator of NF-kappaB in mouse calvarial bones. Endocrinology 2006; 147: 3613-22.
46. Stern PH. Inhibition by steroids of parathyroid hormone- induces Ca45 release from embryonic rat bone in vitro. J Pharmacol Exp Ther 1969; 168: 211-7.
47. Raisz LG, Trummel CL, Wener JA, Simmons H. Effect of glucocorticoids on bone resorption in tissue culture. Endocrinology 1972; 90: 961-7.
48. Reid IR, Katz JM, Ibbertson HK, Gray DH. The effects of hydrocortisone, parathyroid hormone and the bisphosphonate, APD, on bone resorption in neonatal mouse calvaria. Calcif Tissue Int 1986; 38: 38-43.
49. Granowicz G, McCarthy MB, Raisz LG. Glucocorticoids stimulate resorption in fetal rat parietal bones in vitro. J Bone Min Res 1990; 5: 1223-30.
50. Defranco DJ, Lian JB, Glowacki J**.** Differential effects of glucocorticoid on recruitment and activity of osteoclasts induced by normal and osteocalcin-deficient bone implanted in rats. Endocrinology 1992; 131: 114-21.
51. Rodan GA, Martin TJ. Role of osteoblasts in hormonal control of bone resorption: A hypothesis. Calcif Tissue Int 1981; 33: 349-51.
52. Alvarez L, GuañabensN, PerisP, MonegalA, Bedim JL, Deulofeu R , de Osaba M. , Muñoz-Gomez J, Rivera-Fillat F, Ballesta AM. Discriminative value of biochemical markers of bone turnover in assessing the activity of Paget's disease. J Bone Mineral Res 1995; 10: 458 – 465.
53. Ontiveros C, McCabe L R. Simulated microgravity suppresses osteoblast phenotype, Runx2 levels and AP-1 transactivation. J Cell Biochem 2003; 88: 427 – 37.
54. Zayzafoon M, Gathings WE, McDonald JM. Modeled microgravity inhibits osteogenic differentiation of human mesenchymal stem cells and increases adipogenesis. Endocrinology 2004; 145: 2421-32.
55. Bucaro MA, Fertala J, Adams CS, Steinbeck M, Ayyaswamy P, Mukundakrishnan K, Shapiro IM, Risbud MV. Bone survival in microgravity: evidence that modeled microgravity increases osteoblast sensitivity to apoptogenic .Annals of the New York Academy of Sciences 2004; 1027: 64 – 73.
56. Bucaro MA, Zahm AM, Risbud MV, Avyaswamy PS, Mukundakrishnan K, Steinbeck MJ, Shapiro IM, Adams CS.The effect of simulated microgravity on osteoblasts is independent of the induction of apoptosis. J Cell Biochem 2007; 102: 483 – 95.
57. Raisz LG. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. J Clin Invest 2005; 115: 3318 – 25.
58. Janckila AJ , Takahashi K , Sun SZ , Yam LT. Tartrate-resistant acid phosphatase isoform 5b as serum marker for osteoclast activity. Clin Chem 2001; 47: 74 – 80.

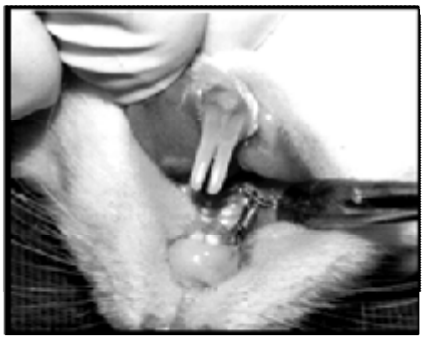

Fig.1: Experimental appliance inducing mesial traction of the rat molar (right) by a closed coil spring producing a force of 20g.

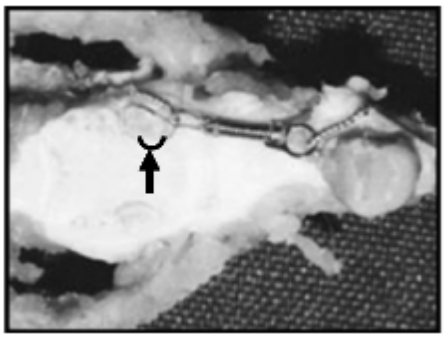

Fig.2: The steroid rat whole maxilla at sacrifice showing the distance formed at the appliance side between 1st and 2nd molar (arrow).

Table 1: The rate of orthodontic tooth movement (mm) after 1st, 2nd and 3rd weeks between the studied groups

		Control	Steroid	ANOVA	LSD
after 1 week	Range	(0.94 - 0.52)	(1.9 - 1.17)	P ≤0.01	1 week x 2 weeks P ≤0.01
	Mean	0.8	1.6		
	SD	0.1	0.3		
	SE	0.04	0.08		
	t-test	P ≤0.01			
after 2 weeks	Range	(1.24 - 0.82)	(2.62 - 1.54)		1 week x 3 weeks P ≤0.01
	Mean	1.1	2.2		
	SD	0.1	0.4		
	SE	0.04	0.11		
	t-test	P ≤0.01			
after 3 weeks	Range	(1.77 - 1.43)	(3.35 - 2.62)		2 week x 3 weeks P ≤0.01
	Mean	1.6	3.1		
	SD	0.1	0.3		
	SE	0.03	0.08		
	t-test	P ≤0.01			

Values are given as Range, mean, standard deviation (SD), and standard error (SE). P≤ 0.01: Highly Significant Difference.

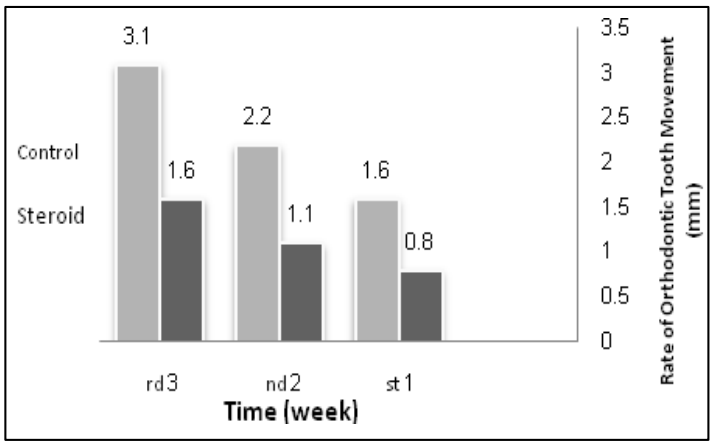

Fig. 3: The rate of orthodontic tooth movement (mm) after 1st, 2nd and 3rd weeks between the studied groups.

Table 2: Mann-Whitney U-test of groups for comparison of bone formation and resorption at different sides, sites and levels

				Control		Steroid		Mann-Whitney U- test	
				Mean	SD	Mean	SD	p	Sig.
Bone formation	Appliance	Compression	coronal	2.000	1.069	0.625	0.518	0.007	**
			apical	2.125	0.641	1.375	0.744	0.050	*
		Tension	coronal	4.750	1.909	3.375	1.302	0.130	NS
			apical	4.125	1.553	3.750	0.463	0.878	NS
	Non-Appliance	Compression	coronal	0.250	0.463	0.250	0.463	1.000	NS
			apical	0.625	0.518	0.250	0.463	0.234	NS
		Tension	coronal	1.125	0.835	0.750	1.165	0.382	NS
			apical	1.250	0.707	0.625	0.744	0.130	NS
Bone resorption	Appliance	Compression	coronal	3.375	1.302	6.250	1.669	0.001	**
			apical	2.375	1.061	5.250	1.282	0.001	**
		Tension	coronal	1.000	0.535	1.250	0.707	0.505	NS
			apical	0.250	0.463	1.000	0.535	0.028	*
	Non-Appliance	Compression	coronal	0.625	0.518	2.000	1.069	0.007	**
			apical	0.750	0.707	1.125	0.835	0.382	NS
		Tension	coronal	0.375	0.518	0.875	0.835	0.279	NS
			apical	0.250	0.463	0.625	0.744	0.382	NS

The values are given as mean and Standard Deviation (SD). (NS): Non-Significant ($p \geq 0.05$), (*): Significant Difference ($p \leq 0.05$), (**): Highly Significant Difference ($p \leq 0.01$).

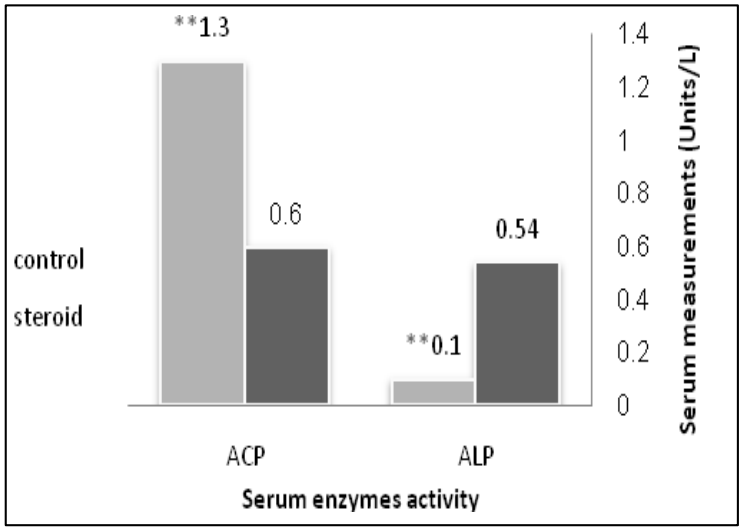

Fig. 4: Alkaline (ALP) and acid phosphatase (ACP) activity in serum (Units/L) between controls and steroid groups. **ALP significantly less in steroid than controls, P ≤ 0.01. While **ACP significantly higher in steroid than controls, P ≤ 0.01.

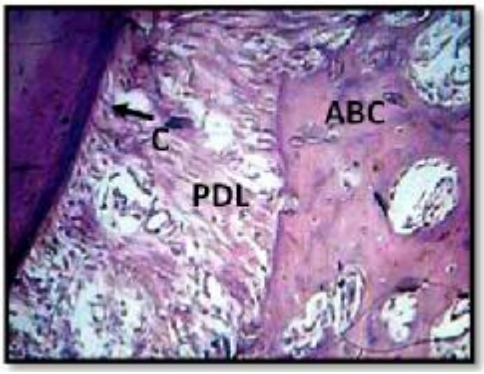

Fig. 5: Microphotograph view for the coronal portion of a steroidal rat tooth treated orthodontically at the compression site shows alveolar bone crest (ABC), cementum (C), and in between principle fibers of periodontal ligament (PDL). H&E, X200.

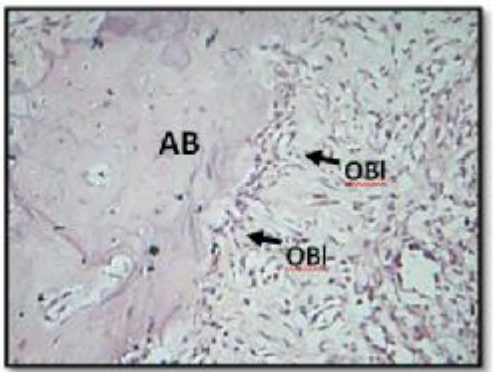

Fig. 6: Microphotograph view for the coronal portion of a steroidal rat tooth treated orthodontically at the tension site shows less no. of activated osteoblast cells (OBl) with minor apposition of alveolar bone (AB). H&E, X200.

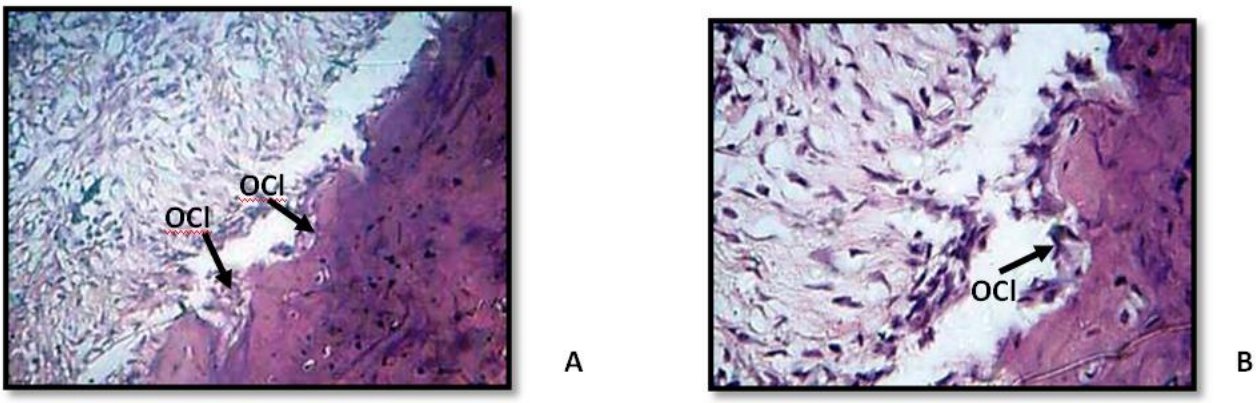

Fig. 7: Microphotograph view for the coronal portion of a steroidal rat tooth treated orthodontically at the compression site shows alveolar bone resorption. Note: proliferation of osteoclast cells (OCl) as multinucleated giant cells occupies Howship's lacunae. H&E, (A) X200, (B) X400.

The variation of facial soft tissue thickness in Iraqi adult subjects with different skeletal classes (A comparative cephalometric study)

Hiba M. Hussein Al-Chalabi, B.D.S., M.Sc. [1]

ABSTRACT

Background: The variation of facial soft tissue thickness is an important factor in facial reconstruction and superimposition methods in forensic dentistry as well as for orthodontist and plastic surgeons because they provide the basis for quantification and repeatability. The purpose of this study was to compare facial soft tissue thickness of Iraqi patients with different types of skeletal relations.

Materials and method: Lateral cephalometric study was conducted on 60 adult Iraqi patients with normal vertical dimensions (diagnosed clinically and radiographically as SN-Mandibular Plane angle 28°-36°), aged 18-30 years, classified according to skeletal sagittal relationship using ANB angle into three groups (each group consist of 10 male and 10 female subjects): Class I group (ANB2-4°), Class II group (ANB>4°) and Class III group (ANB<2°). Cephalometric analysis of soft tissue thickness was achieved by 10linearmeasurements using AutoCAD program 2007.

Results and Conclusions: This study showed that the facial soft tissue thickness measurements were significantly higher in male than in female in almost all measured midline landmarks, in comparing the three skeletal relation groups, Class III group show the highest readings when compared to Class I and Class II, Class II show the lowest results among the three groups (except for the labiomental fold area and pogonion area), while Class I group lies between the other two groups for all the measured values.

Key words: Facial soft tissue thickness, cephalometric study. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):143-149).

INTRODUCTION

Facial soft tissue thickness is not only of importance for plastic surgeons and orthodontists in order to plan the treatment procedure, but also for biologists to determine the facial appearance of ancient populations and forensic anthropologists for reliable identification of a victim [1, 2]. In the forensic field, facial reconstruction is a technique widely used in order to determine the facial appearance of a victim from skeletal remains [3].

Though the bony structure of the skull gives some information about facial appearance, this is not enough when used alone. Facial harmony and balance is determined by both the skeleton and the soft tissue [4]; however, most of the visual impact of the face is provided by the structure of the overlying soft tissues and their relative proportions [5]. Discriminative information is not provided about any single anatomic component of the face (fat or muscle) nor do these soft tissue depths give precise estimations of any individual's soft tissue thickness [6], despite this, soft tissue depth measurements play a significant role in both facial approximation and craniofacial superimposition methods because they provide a basis for quantification and thus, repeatability [4].

An evaluation of the soft tissue structures (nose, lips, and chin), besides the proportional relationship between the facial structures completes the hard tissue description [7].

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Knowledge of soft tissue depths pertaining to the growth and development period is important for dentistry and forensic anthropology [8]. It is also well established that in order to determine suitable tissue thicknesses, sex, age and ethnicity of the individual should be known; during facial reconstruction, plastic material should be placed on the skull depending on the facial soft tissue thickness at certain regions. Eye-sockets, forehead and the nasal septum, which are different for each individual, are precisely determined, and the face is finalized according to the age and the sex [9, 10].

Welcker [11] was the first to publish soft tissue depth tables for any application, and then in, Kollmann and Buchly [12] in 1898 were the first to conduct facial approximations using soft tissue depths, without knowing the facial appearance of the individual [4]. Later, Suzuki [13] compared Japanese adults with European adults and reported the racial differences with respect to sex. After that, various authors have studied facial tissue thickness in Caucasian adults [14], European [15], European-American [16], Japanese [17], and African-American [18]; in another study, Williamson et al. [10] emphasized the effects of aging on facial soft tissue thickness.

Dumont [5] studied soft tissue thickness in white children based only on types of dental occlusion, and Utsuno et al. [19], studied the facial soft tissue thickness differences among the occlusion classes in a relatively small sample of Japanese females. Facial soft tissue thickness has also been studied in the Turkish population [20,21].

For Iraqi population, many studies evaluate the facial soft tissue profile [22-24], Nevertheless, none of the studies to date has evaluated facial soft tissue thickness in Iraqi population according to the occlusion types as a direct measurement from bony to soft tissue landmarks. The main purpose of the present study is to determine the differences between facial soft tissue measurements among three skeletal relation types in Iraqi adults.

MATERIALS AND METHOD

The sample

The sample was selected from a group of Iraqi patients attending the Orthodontic Department at College of Dentistry, University of Baghdad and from students of the same college. Out of 207 clinically and radiographically examined patients, only 60 fit the selection criteria, all of the selected cases have normal vertical facial height (SN-Mandibular Plane Angle value 28◦-36◦ measured by AutoCAD program) then the sample was divided into three groups with regard to the value of ANB angle, each group consist of 10 male and 10 female subjects, Class I group for subjects with ANB 2◦-4◦, Class II group for subjects with ANB angle > 4◦, and Class III group for subjects with ANB angle <2◦.

The Selection criteria

1. The subjects are Arabic Iraqi in origin.
2. Adult patient (Age range 18-30 years).
3. No previous orthodontic treatment.
4. No severe craniofacial disorder, such as cleft lip and /or palate.
5. No apparent trauma of the jaws and facial soft tissue.
6. Full set of permanent dentition excluding the third molar.

The instruments

1. Diagnostic set (mirrors, probes).
2. Kidney dish.
3. Cotton.
4. Sterilizer (Memmert, Germany).
5. Disinfectant agent (Hibitane 5%).
6. Millimeter graded vernier (Dentaurum, Order-No. 042-751-00).

The Equipments

1. PM 2002 CC Proline Planmeca X-ray machine (Finland) available in the Collage of Dentistry at Baghdad University for lateral Cephalometric radiograph.
2. Personal computer (IBM Lenovo B570e Pentium IV).
3. Flash ram.
4. AutoCAD programs version 2007.

Method

1. History: including the name, age, medical history and dental history.
2. The intraoral examination includes: Open mouth examination to examine the maxillary and mandibular teeth and Closed mouth examination to measure the amount of anterior over bite by using intraoral vernier when the subject closing in centric occlusion.
3. Cephalometric analysis: Lateral cephalometric radiographs were taken for the subjects, then by specialized computer program (AutoCAD version 2007) used on Pentium IV computer, the problem of magnification of the lateral cephalogram is corrected by multiplying the readings by the magnification factor which is obtained as a ratio between the real distance measurement for a scale and the distance measurement for the same scale from radiographic image.

Skeleto-dental Cephalometric Landmarks:

The following landmarks were identified:

1. **Point S (Sella)**: the midpoint of the hypophysial fossa [25].
2. **Point N (Nasion):** the most anterior point on the nasofrontal suture in the median plane [26].
3. **Point G (Glabella):** the most prominent point of the bony forehead in the median plane [26].
4. **Point Me (Menton):** the lowest point on the symphysial shadow of the mandible seen on a lateral cephalograms [25].
5. **Point Pog(Pogonion):** most anterior point of the bony chin in the median plane [26].
6. **Point A (Subspinale):** the deepest midline point in the curved bony outline from the base to the alveolar process of the maxilla [26].
7. **Point B (Supramentale):** most anterior part of the mandibular base, it is the most posterior point in the outer contour of the mandibular alveolar process in the median plane [26].
8. **Point Pr (Prosthion):** alveolar rim of the maxilla; the lowest most anterior point on the alveolar portion of the premaxilla in the median plane between the upper central incisors [26].
9. **Point Id (Infradentale):** alveolar rim of the mandible; the highest most anterior point on the alveolar process in the median plane between the mandibular central incisors [26].
10. **Point U1:** the most anteriorprominent point on the crown of the most anterior maxillary central incisor [27].

Soft Tissue Landmarks:

1. Point g: soft tissue glabella [27].
2. Point n: skin nasion [26].
3. Point sn: subnasale[26].

4. Point ls: labralesuperius, border of upper lip [26].
5. Point sto: Stomion, central point of the interlabial gap [26].
6. Point li: labraleinferius, border of lower lip [26].
7. Point sm: submentale, labiomental fold [26].
8. Pointpog: skin pogonion [26].
9. Point me: soft tissue menton [27].

Cephalometric planes

1. **Sella-Nasion (SN) plane:** it is the anteroposterior extent of anterior cranial base [26].
2. **Mandibular plane (MP):** formed by a line joining Gonion and Menton [28].
3. **Nasion-Point A plane (**N-A plane) [26].
4. **Nasion-Point B plane (**N-B plane) [26].

Cephalometric Angular measurements

1. **ANB angle**: Differences between SNA and SNB which represent anteroposterior position of maxilla in relation to mandible; its normal range from (2◦ -4◦) [29,30].
2. **SN-Mandibular plane angle (SN-MP angle**): to assess the vertical problem, its normal range from (28◦ -36◦) [31].

Cephalometric Linear measurements according to Kurkcuoglu*et al*. [27]: (Figure 1)

1. G-g: Linear distance from the most prominent point on the frontal bone to the soft tissue prominence on the forehead
2. N-n: Distance from point Nasion to soft tissue nasion.
3. Rh: Perpendicular distance from the intersection of nasal bone and cartilage to soft tissue.
4. A –sn: Distance between subnasale and A point.
5. Pr-ls: Distance between the most prominent point of the upper lip and Prosthion.
6. St-U1: Distance between the most prominent point of the upper incisor and stomion.
7. Id-li: Distance between the most prominent point of the lower lip and infradentale.
8. B-lm: Distance from point B to labiomental sulcus.
9. Pog-pog: The distance between bony pogonion and soft tissue pogonion.
10. Me-me: The distance between bony Menton and soft tissue menton.

Statistical Analysis

The data were subjected to computerized Statistical analysis including Statistical Package for Social Sciences (SPSS) version 2006 computer program, the statistical analysis include:

A. **Descriptive Statistics**

1. Mean value.
2. Standard deviation (SD).

B. **Inferential Statistics**

1. Analysis of variance test (ANOVA) to get general comparison among the study groups.
2. LSD test for variables that show significant differences among the study groups in ANOVA test.
3. Independent Sample t-test for gender differences.

In the statistical evaluation, the following levels of significance are used:

Non-significant	NS	$P > 0.05$
Significant	*	$0.05 \geq P > 0.01$
Highly significant	**	$0.01 \geq P > 0.001$
Very highly significant	***	$P \leq 0.001$

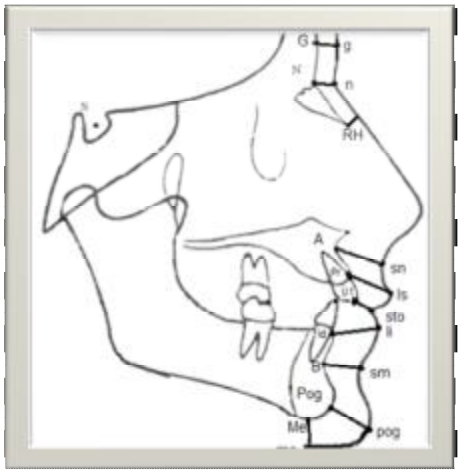

Figure 1: Cephalometric facial soft tissue thickness measurements

RESULTS

The descriptive statistics (including the mean and the standard deviation) and the gender differences of the fasial soft tissue thickness measurements for the three skeletal classes were shown in Table 1.

Table 2 show a comparism of fasial soft tissue thickness measurements among the three skeletal classes using ANOVA test, this comparism reveals a statistically insignificant difference of all the measured values among Class I ,Class II and Class III groups for the total sample and for male and female subjects except for the subnasal area in the total sample and female subjects and area of junction of upper and lower lips in the total sample and male subjects, also the male subjects show a significant difference in the area of upper lip border.

Table 3 show a comparism between each two skeletal classes for the meassurements that show a significant differences in ANOVA test

using LSD test which revealed a statistically significant difference for the whole sample between Class II and Class III groups in subnasal area and area of upper and lower lips junction, while Class I group show a non significant difference with the other two groups; for male subjects the results show a significant difference between Class I and Class III groups, Class II and Class III groups, while Class I and Class II groups show a non significant difference between them, the results also show a significant difference for male subjects in the area of upper and lower lips junction between each two compared groups; finally for female subjects, the results show a significant difference between Class II and Class III groups for the subnasal area show, while Class I group show a non signifact difference in comparim with Class II and Class III respectively.

DISCUSSION

Soft tissue depth measurements play a significant role both in facial approximation and craniofacial superimposition methods because they provide a basis for quantification and thus, repeatability [(4)].

In most of the studies, facial soft tissue depth values were reported as being greater in males than in females [(5,15,18,21,27)] , this agrees with the results of the present study as all the facial soft tissue measurements appear higher in males than in females of the same skeletal class for all the three groups, this increase is statistically significant for all measurements except for (out of ten measurements for each group) three in Class I (G-g, N-n, B-lm), five measurements in Class II (G-g, St-U1, B-lm, Pog-pog, Me-me) and two measurements in Class III (B-lm, Pog-pog).

The facial soft tissue thickness showed a different pattern when comparing the three study groups with each other, as Class III group show an increase in thickness over the other two groups, Class II group show a decrease, while Class I group lies between Class III and Class II groups, this is for the upper and middle facial midline measurements from the forehead till the border of the lower lip, at which the three groups were nearly equal in thickness, while the mandibular midline soft tissue thickness (B-lm, Pog- pog) show an increase in Class II group over Class I and Class III groups respectively, finally the mental area thickness (Me-me) show an increase in Class III group, Class I group and the lowest results in Class II group (Table 1).

The whole sample subjects showed a non-significant difference when compared the soft tissue thickness among the three skeletal classes (Table 2) except for two measurements which are the subnasal area that showed a significant incraese in Class III group over Class II group, with a non-significant increase over Class I group, the other measurement that showed a significant result is the area of junction between the upper and the lower lips which showed an increase in Class III group when compared to Class II group (Table 3) This difference could be due to retrusion of the mandible in Class II skeletal relation holding the lower lip with it and decreasing the between the upper and lower lip, in contrast to Class III relation which have a protruded mandible and an increase area of contact of upper and lower lips.

When comparing facial soft tisse thickness of the male subjects among the three skeletal classes (Table 2), the results show a non significant difference in: Glabella, Nasion, the area of junction between bone and nasal cartilage, the subnasal area, lower lip border area, labiomental fold area, pogonion and menton area; only two measurements show a statistically significant difference among the groups which are the area of upper lip border that show a significant increase in Class III group over Class I and Class II groups (Table 3), this increase in thickness in Class III group might be attributed to the relative retrusion of the maxillary bones while the soft tissue affected by the protrusion of the nose and the mandile leading to an increase in the distance between the soft tissue and bony landmarks. The other measurement that show a significant difference among the groups for male subjects is the area of junction between upper and lower lips, as Class III group show a significant increase when compared to the other two groups, Class II show a significant decrease when compared to the other two groups, while Class I group lies between Class III and Class II with a significant difference among them also (Table 3) this is also can be attributed to the position of the mandible.

For female subjects, when comparing the soft tissue thickness in the three study groups (Table 2), the results showed a non-significant difference for all the measurements except for the subnasal area which showed a significant increase in Class III group when compared to Class II group, while class I group showed a non-significant difference with the other two groups (Table 3) this is disagree with Kurkcuoglu ***et al.*** [(27)] as their results showed a significant increase in Class II and Class III groups overr Class I group in a Turkish sample.

In conclusions, the results of this study showed that the facial soft tissue thickness of Iraqi normo-divergent subjects is larger in males than in females of the same skeletal class, and class III

skeletal relation show the thickest facial soft tissue followed by class I, with the least thickness in class II skeletal relationship.

REFERENCES

1. George RM. The lateral craniographic method of facial reconstruction. J Forensic Sci 1987; 32: 1305-30.
2. Walker GF, Kowalski CJ. A two-dimensional coordinate model for the quantification, description, analysis, prediction and simulation of cranio-facial growth. Growth 1971; 35:191-211.
3. Yoshino M, Matsuda H, Kubota S, Imaizumi K, Miyasaka S, Seta S. International computer assisted skull identification system using video superimposition. Forensic Sci 1997; 90: 231–44.
4. Stephan CN, Simpson EK. Facial soft tissue depths in craniofacial identification (part I): an analytical review of the published adult data. J Forensic Sci 2008; 53: 1257-72.
5. Dumont RE. Mid-facial tissue depths of white children: an aid in facial feature reconstruction. J. Forensic Sci 1986; 31: 1463-9.
6. Simpson E, Henneberg M. Variation in soft-tissue thicknesses on the human face and their relation to craniometric dimensions. Am J Phys Anthropol 2002; 118: 121-33.
7. Koelmeyer TD.Videocamera superimposition and facial reconstruction as an aid to identification. Am J Forensic Med Pathol 1982;3: 45-8.
8. Rohrer-Ertl O. On a newly modified method for plastic reconstruction of the face using the skull (based on Kollmann data). AnthropAnz 1983; 41: 191-208.
9. Holdaway RA. Soft-tissue cephalometric analysis and its use in orthodontic treatment planning. Am J Orthod1983;84: 1-28.
10. Williamson MA, Nawroki SP, Rathbun TA. Variation in midfacial tissue thickness of African and American children. J Forensic Sci 2002; 47: 25-9.
11. Welcker H. Schiller's Schadel und Todtenmaske, nebstMittheilungenuberSchadel und Todtenmaske Kant's. Viehweg F and Son, Braunschweig 1883.
12. Kollmann J, Buchly W. Die Persistenz der Rassen und die Reconstruction der PhysiognomieprahistorischerSchadel. Archiv. Fur Anthropologie 1898; 25: 329–59.
13. Suzuki H. On the thickness of the soft parts of the Japanese face J Anthropol Soc Nippon 1948; 60: 7-11.
14. Rhine JS, Moore CE. Facial Reproduction: Tables of Facial Tissue Thicknesses of American Caucasoids in Forensic Anthropology. Maxwell Museum Technical Series no 1. Maxwell Museum, Albuquerque, 1982.
15. Ferrario VF, Sforza C, Poggio CE, Colombo A, Cova M. Effect of growth and development on cephalometric shapes in orthodontic patients: a Fourier analysis. Eur J Orthod 1997; 19: 669-80.
16. Alcalde RE, Jinno T, Orsini MG, Sasaki A, Sugiyama RM, Matsumura T. Soft tissue cephalometric norms in Japanese adults. Am J Orthod Dentofac Orthop 2000; 118: 84-9.
17. Manhein MH, Listi GA, Barsley RE, Musselman R, Barrow NE, Ubelaker DH. In vivo facial tissue depth measurements for children and adults. J Forensic Sci 2000; 45: 48-60.
18. Claes P, Vandermeulen D, De Greef S, Willems G, Suetens P. Craniofacial reconstruction using a combined statistical model of face shape and soft tissue depths: methodology and validation. Forensic SciInt 2006; 15: 159–61.
19. Utsuno H, Kageyama T, Deguchi T, Umemura Y, Yoshino M, Nakamura H, Miyazawa H. Facial soft tissue thickness in skeletal type I. Forensic SciInt 2007; 25: 137-43.
20. Baydas B, Erdem A, Yavuz I, Ceylan I. Heritability of facial proportions and soft-tissue profile characteristics in Turkish Anatolian siblings. Am J Orthod Dentofac Orthop 2007; 131: 504-9.
21. Gelgor IE, Karaman AI, Zekic E. The use of parental data to evaluate soft tissues in an Anatolian Turkish population according to Holdaway soft tissue norms. Am J Orthod Dentofac Orthop 2006; 129: 330-2.
22. Al-Ta'aniMA. Soft tissue facial profile analysis: acephalometric study of some Iraqi adults with normal occlusion; a master thesis submitted to the council of college of dentistry, Baghdad University, 1996.
23. Agha NF. Facial profile soft tissue analysis for Mosuli adults, class I normal occlusion; A master thesis submitted to the council of collage of dentistry, Mosul university, 1998.
24. Kadhum ZM. Soft tissue cephalometric norms for a sample of Iraqi adults with class I normal occlusion in natural head position; a master thesis submitted to the council of college of dentistry, Baghdad University, 2010.
25. Caufield PW. Tracing technique and identification of landmarks. In Jacobson A. (Ed). Radiographic Cephalometry from basics to video-imaging. Chicago: Quintessence publishing Co.; 1995. P: 60.
26. Rakosi T. An atlas and manual of cephalometric radiography. 2nd ed. London: Wolf Medical Publication; 1982.
27. Kurkcuoglu A, Pelin C, Ozenerb P, Zagyapan R, Sahinoglu Z, Yazıcı AC. Facial soft tissue thickness in individuals with different occlusion patterns in adult Turkish subjects. J Comparative Human Biology. Elsevier GmbH, 2011.
28. Salzmann JA. Limitations of roentgenographic cephalometrics. Am J Orthod 1964; 50(3), 169-88.
29. Riedel RA. The relation of maxillary structures to cranium in malocclusion and in normal occlusion. Angle Orthod 1952; 22(3): 142-5.
30. Steiner CC. Cephalometric for you and me. Am J Orthod 1953; 39(10):729-55.
31. Droel R, Isaacson RJ. Some relationships between the glenoid fossa position and various skeletal discrepancies. Am J Orthod 1972; 61: 64-78.

Table 1: Descriptive statistics and gender difference of facial soft tissue thickness measurements of the three skeletal classes groups

	Variables	Descriptive statistics						Gender differences	
		Total		Male		Female			
		Mean	S.D	Mean	S.D	Mean	S.D	t-test	p-value
Class I	G-g	6.09	1.08	6.30	1.17	5.89	1.00	0.84	0.413 (NS)
	N-n	6.27	1.23	6.55	1.37	5.98	1.06	1.04	0.313 (NS)
	Rh	2.67	0.54	2.94	0.42	2.40	0.52	2.54	0.021*
	A-sn	15.49	1.75	16.63	1.28	14.36	1.40	3.78	0.001***
	Pr-ls	13.20	2.15	14.47	1.76	11.92	1.74	3.26	0.004**
	St-U1	5.27	1.97	6.25	2.24	4.29	1.00	2.54	0.021*
	ld-li	14.46	1.05	15.13	0.89	13.80	0.76	3.60	0.002**
	B-lm	11.52	1.24	11.90	1.31	11.15	1.11	1.38	0.184 (NS)
	Pog-pog	12.47	2.18	13.70	1.95	11.24	1.70	3.00	0.008**
	Me-me	8.17	1.82	9.10	1.30	7.24	1.83	2.62	0.017*
Class II	G-g	5.93	0.80	5.77	0.79	6.08	0.81	-0.86	0.402 (NS)
	N-n	5.96	1.13	6.49	1.17	5.42	0.83	2.37	0.029*
	Rh	2.63	0.53	2.89	0.52	2.37	0.42	2.47	0.024*
	A-sn	14.99	2.20	16.49	1.91	13.50	1.25	4.14	0.001***
	Pr-ls	12.52	1.88	13.86	1.46	11.18	1.14	4.60	0.000**
	St-U1	4.26	0.96	4.32	1.20	4.20	0.69	0.27	0.793 (NS)
	ld-li	14.57	1.71	15.39	1.40	13.74	1.65	2.40	0.028*
	B-lm	11.67	1.56	12.02	1.91	11.32	1.12	0.99	0.333 (NS)
	Pog-pog	12.61	1.35	13.13	1.31	12.09	1.25	1.81	0.088 (NS)
	Me-me	7.54	1.63	8.09	1.64	6.99	1.51	1.55	0.138 (NS)
Class III	G-g	6.15	0.83	6.51	0.72	5.79	0.80	2.14	0.046*
	N-n	6.34	1.26	7.07	0.78	5.61	1.25	3.14	0.006**
	Rh	2.77	0.68	3.27	0.50	2.28	0.43	4.76	0.000**
	A-sn	16.81	2.63	18.02	2.69	15.60	2.02	2.27	0.036*
	Pr-ls	14.25	2.63	16.29	1.89	12.21	1.32	5.59	0.000***
	St-U1	6.37	2.85	8.53	1.84	4.21	1.83	5.28	0.000***
	ld-li	14.58	1.97	16.09	1.19	13.08	1.31	5.37	0.000***
	B-lm	11.38	0.93	11.71	0.85	11.06	0.93	1.64	0.119 (NS)
	Pog-pog	12.37	1.73	12.90	1.27	11.84	2.02	1.41	0.176 (NS)
	Me-me	8.10	0.96	8.71	0.78	7.48	0.72	3.67	0.002**

Table 2: A comparison of facial soft tissue thickness measurements for males, females and total sample among the three skeletal classes using ANOVA test

Variables	Total		Male		Female	
	F-test	p-value	F-test	p-value	F-test	p-value
G-g	0.33	0.72 (NS)	1.74	0.195 (N(NS)	0.29	0.751 (NS)
N-n	0.58	0.565 (NS)	0.79	0.464 (NS)	0.74	0.488 (NS)
Rh	0.32	0.725 (NS)	1.85	0.177 (NS)	0.19	0.826 (NS)
A-sn	3.57	0.034*	1.71	0.201 (NS)	4.43	0.022*
Pr-ls	3.03	0.056 (NS)	5.42	0.01**	1.4	0.263 (NS)
St-U1	5.16	0.009**	13.52	0.000***	0.01	0.987 (NS)
ld-li	0.03	0.969 (NS)	1.77	0.189 (NS)	0.97	0.393 (NS)
B-lm	0.25	0.78 (NS)	0.12	0.89 (NS)	0.16	0.853 (NS)
Pog-pog	0.09	0.915 (NS)	0.71	0.502 (NS)	0.67	0.522 (NS)
Me-me	1.04	0.361 (NS)	1.59	0.223 (NS)	0.29	0.749 (NS)

Table 3: A comparison of facial soft tissue thickness measurements for males, females and total sample between each two study groups using LSD test

Gender			Total		Male		Female
Skeletal Classes	Variables		A-sn	St-U1	Pr-ls	St-U1	A-sn
	I	II	0.478 (NS)	0.13 (NS)	0.434 (NS)	0.024*	0.234 (NS)
		III	0.066 (NS)	0.099 (NS)	0.025*	0.009**	0.093 (NS)
	II	I	0.478 (NS)	0.13 (NS)	0.434 (NS)	0.024*	0.234 (NS)
		III	0.012*	0.002**	0.004**	0.000 ***	0.006**
	II	I	0.066 (NS)	0.099 (NS)	0.025*	0.009**	0.093 (NS)
	I	II	0.002**	0.004**	0.000**	0.006**	0.012*

Effect of protein energy malnutrition (PEM) on oral health status of children aged 6 years old in Sammawa city

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ABSTRACT

Background: It has been realized that good nutrition is interdependent with good health, and the protein is the most important nutrient because it regulates the key process within the body, so if any deficiencies in protein occur this will lead to protein-energy malnutrition which is evaluated by using anthropometric measurements(height and weight). When PEM begins it affects adversely various aspects of growth and increase the severity of oral problems later. It has been reported that dental caries and enamel defect occur in malnourished children more than well nourished.
The aim of this study was to investigate the nutritional status of children by physical examination and its effect on dental caries and enamel anomalies in relation to gender.
Materials and methods: This study was conducted among urban primary school children aged 6 years in sammawa city which lies 300 Km south of Baghdad, were clinically evaluated to determine the prevalence of dental caries and enamel anomalies in relation to protein energy malnutrition. The sample size composed of 300 children distributed in primary schools which were randomly selected from different areas in sammawa city. The samples were examined physically by anthropometric measurements (weight and height) and orally for dental caries and enamel defects.
Results: The malnourished children with mild grade was the most prevalent grade in this study, males showed malnutrition more than females within the same age group, dmfs and DMFS according to nutritional status indicators were higher among malnourished children than well nourished group, the enamel opacities was higher in females than in males regarding gender differences and according to nutritional status indicators was higher among well nourished children than malnourished group in both primary and permanent dentition.
Conclusion: The prevalence of malnutrition was higher in boys than in girls, also the prevalence of dental caries was higher in malnourished children when compared with well nourished children, while enamel opacities was present only in well nourished children when compared with malnourished group, it was absent in malnourished children.
Key words: protein energy malnutrition; oral health; Sammawa city. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):150-155).

INTRODUCTION

Protein is derived from the Greek word meaning the first rank; it performs all the three functions of the nutrients which are growth maintenance, repair of body tissue and regulates the key process within the body. Protein next to the water is the most plentiful substances in the body. It can be obtained from meat, fish, eggs, milk, grain, vegetables and soybean [(1,2)].

Protein energy malnutrition occurs when there are deficiencies in protein or energy foods or both [(2,3)]. The main clinical symptoms associated with protein energy malnutrition are the failure of the body to grow in height and weight [(4)].

There are various studies of assessment of nutritional status of school children have been carried out at various parts of the world, however, most of these studies carried out in the middle east which were used anthropometric measurements(height and weight); in Iraq only few studies about the effect on protein energy malnutrition on oral health status[(5,6)].

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The mechanism whereby malnutrition during tooth development can make teeth more susceptible to dental caries may be due to its effect on the 1- morphology of the teeth: few animal studies have shown that the morphology of the tooth can be influenced by nutritional imbalance of protein fat and carbohydrate [(7)] 2- the quality of dental hard tissues [(8)].

Reports on the positive relationships between malnutrition and primary and permanent dentition caries in middle area in Iraq are available [(5,6, 9)].

The developing human tooth, like other organ in the body, is unalterable to sever nutritional deprivation if the later occurs during the critical period of dental growth [(10),] if diet includes little or non of essential amino acids during critical period of active growth, permanent damage can occurs [(11)].

Enamel hypoplasia is characterized by hypoplastic grooves and/or pits in the enamel, often horizontal or linear in appearance [(12).] A study conducted in Saudi Arabia found that enamel defect was higher among malnourished boys than well nourished groups [(13)].

On the other hand, in Iraq a study found that the prevalence of enamel defect was higher among well nourished children than underweight children in Baghdad city [(14)].

The present work was carried out to study the relationship between malnourishment and the

development of dental caries and enamel hypoplasia in children aged 6 years in Sammawa city comparing them with well nourished children.

MATERIALS AND METHODS

The sample

The study was conducted among urban primary school children aged 6 years old from 1-4-2010 to 15-5-2010. The sample size composed of 300 children include (155) males and (145) females aged 6 years old in Sammawa city in Iraq. The age was calculated according to the last birthday and any child with dmfs=0 and with systemic disease was excluded.

Materials

Plane dental mouth mirror no.4, dental probe No.00, tweezers, kidney dishes, cotton , gauze, disinfectants, portable lamp for artificial illumination, bathroom scale for weight records and height measuring board.

Anthropometric measurement

It represented by the measurements of height and weight. The measurement of weight was done by using bathroom scale for weight records nearest to 0.1 kg. The child was weighted with minimum clothes without touching anything with 500 gram was subtracted from the total weight to compensate for underneath clothes [6]. The scale was checked against a known weight of 10 kg and adjusted before starting the measurements and after weighting every 20 children [15,16].

The measurements of height was done by using the ordinary measuring tape fixed at the wall and the child standing up after removing the shoes with feet parallel to each other and pointed forward and the back is straight in upright position. The knee was straight and the head was in position that Frankfort Plane (the line between the lower border of the orbit and the upper margin of the external auditory meatus) was horizontal[6].

Malnutrition classification

The value of nutritional indicators was compared with the international reference values because the Iraqi standards were absent for comparison. The degree of malnutrition was counted according to Z-score as mild and moderate and severs as the following

Mild (Z=-1.0 SD to -1.99 SD)

Moderate (Z=-2.0 SD to -2.99 SD)

Sever (Z=-3.0 SD and below)

The Z score=individual value-median of reference population/standard deviation of reference population.

Calibrations

Inter-calibration and intra-calibration had been done to ensure the consistent utilization of diagnostic criteria for dental caries and enamel defect. Inter-calibration had been done with well experiential examiner. The examinations were done for 10 children who were examined twice with 14 days time laps. These two calibrations were assessing by using t-test. In both of them there were no significant differences as shown in tables 1 and 2.

Table 1: Intercalibration.

Data	No.	Mean -SD	Sig.
dmfs	10	16.17 -7.45 17.22-7.32	N.S.
DMFS	10	1.68-2.33 2.1-2.45	N.S.

Table 2: Intracalibration.

Data		Mean -SD	Sig.
dmfs	1st 2nd	18.55-7.79 17.22-6.80	N.S.
DMFS	1st 2nd	2.23-2.10 2.45-2.82	N.S.

Dental caries examination

Dental caries diagnosis was recorded according to WHO classification. The examination was started from the upper right first molar to adjacent tooth till reach upper left first molar and passing to the lower right first molar. Examinations were done with all surfaces of all teeth. A tooth was considered present in the mouth when any part of it was visible or could be touched with the tip of the tweezer without displacing soft tissue, if the same space was occupied by both primary and permanent tooth, the permanent tooth status was recorded. Alphabetic coding systems were used for recording the status of primary teeth and numerical coding system was used for permanent teeth. The index used for dental caries examination was dmfs/DMFS index [12].

Enamel anomalies examination

The examination was performed under normal natural day light. If there was any doubt about presence of any abnormality as tooth surface with single abnormality less than 1 mm was scored normal. If more than 2/3 of the tooth surface heavily restored or badly fractured was not examined. Ten teeth were examined on the buccal surfaces only, if any index teeth are missing, the area was excluded, these teeth are for permanent: upper left and right central and lateral incisors, canines, first premolars and the lower left and right first molar, while for primary teeth: upper left and right central and lateral incisors, canines, first molar and lower left and right second molar [12]. Enamel hypoplasia was differentiated from fluorosis, nonfluoride opacities are most

commonly creamy-yellow to brown in color, well demarcated, and on the smooth surfaces, also fluorosis is more symmetrical and more diffuse, with white striations or patches that do not have well-defined margins[15,16].

RESULTS

The result of mean height of boys 118.9cm while for girls was 118.5cm and the weight was 21.34kg for males and 20.81kg for females with no significant differences for both indicators (table3).

Table 3: Children height (cm) and weight (kg) by gender

Gender	No.	%	Height		Weight	
			Mean	SE	Mean	SE
male	145	48.3	118.9	0.422	21.347	0.285
female	155	51.7	118.5	0.724	20.81	0.685

The prevalence of malnutrition was high according to Z- score distribution. It showed that the well nourished boys (80.6%, 79.4%) for both height and weight indicators respectively was lower than well nourished girls(99.3%, 98.6%), therefore malnourishment was higher in boys than girls with mild degree of malnutrition was the most prevalent degree for both genders. The percentage of malnutrition was 19.4%, 20.6% in males and 0.7%, 1.4% in females for both height and weight indicators respectively as shown in table 4
.

Table 4: Distribution of children according to nutritional status indicators

Indicator	Gender	Normal above -1 SD		Mild (-1 SD to -1.99)		Moderate (-2 SD to -2.99)		Severe (-3 SD and below)	
		No.	%	No.	%	No.	%	No.	%
Weight	Male	123	79.4	26	16.8	6	3.8	0	0.0
	Female	143	98.6	2	1.4	0	0.0	0	0.0
Height	Male	125	80.6	15	9.7	14	9.1	1	0.6
	Female	144	99.3	1	0.7	0	0.0	0	0.0

Table 5 showed that the dmfs values according to nutritional status showed highly significant differences (P<0.01) between malnourished and well nourished children with higher dmfs in malnourished group.

Table 5: Caries experience (mean and S.E. of dmfs) according to nutritional status indicators

Indicator	Gender	Well nourished			Malnourished			t-test		
		No.	Mean	SE	No.	Mean	SE	d.f.	t-value	p-value
weight	Male	123	4.23	0.523	32	20.53	1.88	153	13.6	P<0.01
	Female	143	3.59	0.675	2	31.0	1.003	143	17.8	P<0.01
height	Male	125	3.69	0.361	30	23.86	1.629	153	18.6	P<0.01
	Female	144	3.77	0.685	1	32	0.0	143	22.8	P<0.01

**P<0.01 High significant

The mean of DMFS was higher in malnourished children than well nourished in both height and weight indicators, but the difference was significant (P<0.05) in males, while for females there was also higher DMFS values in malnourished than well nourished children with highly significant difference (P<0.01) in both height and weight indicators as shown in table 6.

Table 6: Caries experience (mean and S.E. of DMFS) according to nutritional status indicators

Indicator	Gender	Well nourished			Malnourished			t-test		
		No.	Mean	SE	No.	Mean	SE	d.f.	t-value	p-value
Weight	Male	123	0.541	0.091	32	2.37	0.243	153	4.62	0.03
	Female	143	0.507	0.084	2	4.0	0.0	143	12.6	P<0.01
Height	Male	125	0.451	0.073	30	2.866	0.192	153	3.99	0.02
	Female	144	0.531	0.086	1	4	0.0	143	14.6	P<0.01

***P<0.05 Significant **P<0.01 High significant**

The prevalence of enamel anomalies was higher in girls than boys; it was 15.2% for girls and 10.3% for boys for the same age group (table 7).

Table 7: Prevalence of enamel anomalies according to nutritional status indicators

Indicator	Gender	Well nourished		Mal nourished	
		No.	%	No.	%
Weight	Male	16	10.3	0	0
	Female	22	15.2	0	0
Height	Male	16	10.3	0	0
	Female	22	15.2	0	0

The values of enamel anomalies according to nutritional status indicators were higher among well nourished children than malnourished children (it was absent in malnourished children) as follows:-

- In primary teeth, the well nourished children had higher means of demarcated opacities than malnourished children with highly significant differences (P<0.01) for both genders and for both nutritional status indicators.
- In permanent teeth, it was higher enamel anomalies in well nourished than malnourished children for both genders and both nutritional status indicators with statistically highly significant differences (P<0.01) as shown in tables 8, 9.

Table 8: Mean number of primary teeth with demarcated opacities according to nutritional status indicators

Indicator	Gender	Well nourished			Malnourished			t-test		
		No.	Mean	SE	No.	Mean	SE	d.f.	t-value	p-value
Weight	Male	123	0.139	0.033	32	0	0	153	27.6	P<0.01
	Female	143	0.176	0.038	2	0	0	143	23.3	P<0.01
Height	Male	125	0.137	0.031	30	0	0	153	28.2	P<0.01
	Female	144	0.174	0.037	1	0	0	143	29.3	P<0.01

*P<0.01 High significant

Table 9: Mean number of permanent teeth with demarcated opacities according to nutritional status indicators

Indicator	Gender	Well nourished			Malnourished			t-test		
		No.	Mean	SE	No.	Mean	SE	d.f.	t-value	p-value
Weight	Male	123	0.327	0.082	32	0	0	153	25.6	P<0.01
	Female	143	0.366	0.081	2	0	0	143	25.9	P<0.01
Height	Male	125	0.322	0.076	30	0	0	153	24.3	P<0.01
	Female	144	0.363	0.079	1	0	0	143	26.3	P<0.01

*P<0.01 High significant

DISCUSSION

This study was designed to evaluate the nutritional status in relation to oral health among primary school children in Sammawa city in urban areas only due to few numbers of schools in rural areas and too far and difficult to be reached.

Malnutrition

In the present study, it was decided to use -1SD as cut off point in order to include mild malnutrition to have a significant effect on dental health[5,9], also WHO used -2 cut off point to calculate the prevalence of malnutrition in order to give help and support for the children with moderate and sever malnutrition. It would be helpful to consider indices to describe the nature of the problem; one of the indices is to use the mean Z-score, which summarize the entire population status. The Z-score standard deviation value system, which expresses the anthropometrical value as a number of standard deviation or Z-score below or above the reference mean, their major advantage for population based uses is that a group of Z-score can be subjected to summary statistics [17].

The prevalence of malnutrition measured by height for age, weight for age was found mostly in the present to be higher among males than females, this finding support the idea that boys are more active than girls and may be because of the work of the boys in order to earn income for their families, so they need more quantity and quality of food to carry higher energy requirement [18]. By

comparing the height and weight according to gender differences, males were found to be taller and heavier than females in all samples. This is in line with idea that the boys were heavier and taller than girls, this is in agreement with previous Iraqi studies in Al-Tammim governorate and the middle region of Iraq and in Sulaimania city [5,6].

Dental caries

The dmfs, DMFS index is more sensitive index for caries intensity since caries is measured in term of surface rather than teeth. The sensitivity of this index is at its height when radiographic examination accompanies the clinical examination [19]. However in this study, it was impossible to have radiographic examination accompanying the clinical examination because the examination of children was done in their schools, so it may expect underestimation of caries experience because inability to detect interproximal lesion clinically only. The mean of dmfs, DMFS in the study was higher in females than in males, this is could be due to earlier teeth eruption pattern in females than in males of the same age group [5], also ds, Ds represent the main component of dmfs, DMFS index may be due to failure of parents in realizing the importance of preserving teeth and seeking dental treatment [20]. It has been found in the present study that the mean dmfs,DMFS was higher among malnourished children than well nourished children which may be attributed to delay shedding of primary teeth and this lead to increase experience in primary teeth in comparison to their normal counter part children, and also could be attributed that the amount of nutrient intake to be lower among malnourished children than normal children and this lead to increase tooth susceptibility to dental caries through changing in tooth formation [21,22].

Enamel anomalies

The mean number of permanent teeth with demarcated opacities was found to be higher among females than males, it could be due to earlier eruption of teeth among girls than boys [23], also lower nutrient intake of calcium and phosphorus and iron among girls than boys which was found have a higher influence for its deficiency on enamel defect development [5]. The mean number of primary and permanent teeth with demarcated opacities was found to be in the present study to be lower among malnourished children by both nutritional status indicators(height and weight) than well nourished children described by the same indicators, this could be explained that plasma protein especially albumin are much reduced in protein energy malnutrition, the presence serum albumin inhibit enamel crystal growth and it has been found that protein fraction of enamel from patient with hypocalcified amelogensis imperfect contain albumin as one of the major constituent of the protein fraction [23,24].

REFERENCES

1. Nizel AE, Papas AS. Nutrition in clinical dentistry. 3rd ed. W.B.Saunders Company; 1989.
2. Daneshkazemi AR, Davari A. Assessment of DMFT and enamel hypoplasia among junior high school children in Iran. J Contemp Dent Pract 2005, 6:85-92.
3. Shils ME. Modern nutrition in health and disease. Williams and Wilkins. Philadelphia: Lippincot; 1999.
4. Latham MC. Protein energy malnutrition. In Brown ML. (ed). Present knowledge in nutrition. 6th ed. Washington D.C.: International life science institute; 1990.
5. Diab BS. Nutritional status in relation to oral health condition among 6-10 years primary school children in the middle region of Iraq. PhD thesis, Department of POP, College of Dentistry, University of Baghdad, 2003.
6. Droosh MK. Protein energy malnutrition in relation to oral health condition among 6 and 9 years old primary school children in Sulaimania city in Iraq. A master thesis, Department of POP, College of Dentistry, University of Baghdad, 2007.
7. Johansson I, Saellstrom AK, Rajan BP, Parames W. Salivary flow and dental caries in Indian children suffering from chronic malnutrition. Caries Res 1992; 26: 38-43.
8. Cecillia A, Tiffany MU. Nutrition and periodontal disease. 3rd ed. 2000; 1255-33.
9. Al-Obaidi W. Oral health status in relation to nutritional status among kindergarten children in Baghdad. A master thesis Department of POP, College of Dentistry, University of Baghdad, 1995.
10. Slayton RL, Warren JJ, Kanellis MJ, Levy SM, Islam M. Prevalence of enamel hypoplasia and isolated opacities in the primary dentition. Pediatr Dent 2001; 23: 32-36.
11. Johansson I, Saellstrom AK, Rajan BP, Parameswaran A. Salivary flow and dental caries in indian children suffering from chronic malnutrition. Caries Res 1992; 26(1):38-43.
12. World Health Organization. Oral Health Surveys. Basic Methods WHO; Geneva, Switzerland. 1997.
13. Rugg-Gunn AJ, Al-Mohammadi SM, Butler TJ. Effect of fluoride level in drinking water, nutritional status and economic status on the prevalence of developmental defects on dental enamel in permanent teeth in Saudi 14 year's old boys. Caries Res 1997; 31: 259-267.
14. Gatta E. Primary teeth emergence and enamel anomalies in relation to nutritional status among 4-48 month old children in Baghdad city/Iraq. A master thesis Department of POP, College of Dentistry, University of Baghdad, 2005.
15. Psoter WJ, Spielman AL, Gebrian B, St Jean R, Katz RV. Effect of childhood malnutrition on salivary flow and PH. Arch Oral Biol 2008; 53(3): 231-7.
16. Hong L, Levy SM, Warren JJ, Broffitt. Association between enamel hypoplasia and dental caries in primary second molars: a cohort study. Caries Res 2009; 43(5): 345-353.

17. Yip R, Sharp T. Acute malnutrition and high childhood mortality related to diarrhea. JADA 1993; 270: 587-590.
18. Dreze JG. Hunger poverty in Iraq. London school of economic and wider Rep. 1991, No. 32.
19. Psoter WJ, Reid BC, Katz RV. Malnutrition and Dental Caries. Caries Res 2005; 39: 441-447.
20. Al-Azawi L.A. Oral health status and treatment among Iraqi five years old kindergarten children and fifteen years old student (A national survey). PhD thesis, Department of POP, College of Dentistry, University of Baghdad, 2000.
21. Onis M, Montero, Akre J. Clugston. The world wide magnitude of protein emergence and physics of Velema children of south eastern Andhra Pradesh. Acta Odontol Pediatri 2001;7: 1-5.
22. Constans T, Alix E, Dardaine V. Protein –energy malnutrition. Diagnostic methods and epidemiology. Presse Med 2000; 29: 2171-6.
23. Takagi Y, Fujita H, Katano H, Shimokawa H, Kuroda T. Immunochemical and biochemical characteristics of enamel protein in hypo calcified amelogensis imperfect. Oral –Sug Oral-Med Oral-Pathol Oral-Radiol 1998; 85: 424-30.
24. Lin CA, Boslaugh S, Ciliberto HM. A prospective assessment of food and nutrient intake in a population of Malawian children at risk for Kwashiorkor. J Pediatr Gastroenterol Nutr 2007; 44(4): 487-93.

Evaluation of calcium and hydroxyl ions release from non-setting calcium hydroxide paste and mineral trioxide aggregate during apexification procedure

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ABSTRACT

Background: Several materials had been used as intracanal dressing to stimulate hard tissue formations during apexification procedure. Recently, a single appointment technique by using mineral trioxide aggregate (MTA) has been proposed as an alternative to the multiappointment calcium hydroxide apexification. The aim of this study was to evaluate the release of calcium and hydroxyl ions from calcium hydroxide paste and MTA through three different apical aperture sizes during pexification procedure.

Materials and Methods: The root canals of sixty extracted premolar teeth were instrumented to a master apical file No. 100, 120, and 140 and filled with either calcium hydroxide paste or MTA. Calcium ions concentrations and pH values of the surrounding media were measured at days 1, 3, 7, 14, 21, and 28 of the test period.

Results: Calcium ions concentrations and pH values of $Ca(OH)_2$ were more than that of MTA at days 1, 3, and 7, then the calcium ions concentrations of MTA increased with time and became more than that of $Ca(OH)_2$ which decreased with time. Ca^{+2} and OH^{-1} release from $Ca(OH)_2$ paste and MTA increased with larger apical aperture size at all time intervals.

Conclusions: MTA maintains a continuous calcium and hydroxyl ions release for longer time than that of $Ca(OH)_2$ paste.

Keywords: Apexification, calcium hydroxide, MTA. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):156-160).

INTRODUCTION

Apexification procedure had been historically used to establish apical closure and avoid surgery.This procedure requires the chemomechanical debridement of the canal followed by placement of an intracanal medicament to assist or stimulate apical healing and formation of an apical barrier [1].

The most common material used in exification is calcium hydroxide. Its mode of action is elieved to be dependent on its ability to release calcium ions and hydroxyl ions, which then diffuse into surrounding tissue [2, 3]. Their concentrations increase with larger apical aperture sizes [4].

Despite the clinical success of calcium hydroxide apexification technique, the length of treatment can be too difficult for the patient to maintain motivation in addition to the unpredictability of apical closure. An alternative for multiappointment calcium hydroxide procedure, a single-step technique using a new material, which is mineral trioxide aggregate (MTA), had been introduced [5].

The mechanism of action of MTA has some similarity with that of calcium hydroxide, although MTA does not have calcium hydroxide in its composition but it has calcium oxide (CaO) that could react with tissue fluid to form $Ca(OH)_2$ which dissociates into Ca^{+2} and OH^{-1} ions [6-8].

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MATERIALS AND METHODS

Sixty freshly extracted human premolars with single straight root canals and closed apices were used in this study. The crown portion of each tooth was removed at the cementoenamel junction (CEJ) of the buccal surface by using a diamond disk to permit ideal access to the root canal [9]. The working length was determined and standardized to 14 mm length.

The roots were divided into 3 groups, 20 roots for each, as follow:

Group A: 20 root canals were instrumented conventionally to the master apical file No. 100 until the tip of the master apical file extended 1mm beyond the apex to have 1mm aperture size.Group B: 20 root canals were instrumented conventionally to the master apical file No. 120 until the tip of the master apical file extended 1mm beyond the apex to have 1.2 mm aperture size.

Group C: 20 root canals were instrumented conventionally to the master apical file No. 140 until the tip of the master apical file extended 1mm beyond the apex to have 1.4 mm aperture size.

At the cervical portion of each root, a cavity of 2mm depth and 1mm floor around the circumference of the root canal was prepared to receive the cervical seal [10]. Two coats of clear nail polish were applied to the entire external root surface except the apical foramen, and allowed to dry at room temperature [11]. Each root was placed in a polyethylene vial containing 25 ml of

synthetic tissue fluid (STF) and incubated at 37°C for three days as a control period. After three days, the Ca^{+2} concentrations and pH values of the surrounding media were measured using the atomic absorption spectrophotometer (AAS) and digital pH-meter respectively [12]. The roots were taken out of the STF, the cervical seals were removed, and each group was subdivided into 2 subgroups (A1, A2), (B1, B2), and (C1, C2).

The canals of subgroups A1, B1, and C1 were packed with calcium hydroxide paste 2 mm shorter than the working length. A radiograph was taken immediately to assess the quality of the obturation and the extent of the filling material. The canals of subgroups A2, B2, and C2 were packed with 4mm apical plugs of MTA with the aid of an endodontic messing gun. Roots were radiographed to ensure that an adequate apical obturation had been performed. Gutta-percha and zinc oxide eugenol (ZOE) sealer filled the canals to the coronal ends of the apical plugs. The roots were radiographed to determine if the root canals were properly filled.

After replacing the cervical seals, the roots were kept in the same solutions in which they were immersed at the control period.

Calcium ions concentrations and pH values of the surrounding STF media were measured at days 1, 3, 7, 14, 21, and 28 of the test period.

RESULTS

$Ca(OH)_2$ subgroups (A1, B1, C1) revealed an increase in the release of calcium ions during the initial test period until they reached their maximum values followed by a decrease in Ca^{+2} release with time. Subgroups C1 showed the highest Ca^{+2} concentrations in the surrounding STF media, whereas subgroups B1 came next, and subgroups A1showed the lowest Ca^{+2} concentrations at all time intervals (Table 1).

MTA subgroups (A2, B2, C2) revealed a continuous increase in calcium ions concentrations throughout the test period until they reached their maximum values at day 28. Subgroups C2 showed the highest Ca^{+2} concentrations in the surrounding STF media, whereas subgroups B2 came next, and subgroups A2 showed the lowest Ca^{+2} concentrations at all time intervals (Table 2).

$Ca(OH)_2$ subgroups (A1,B1,C1) revealed an increase in the release of hydroxyl ions during the initial test period until they reached their maximum values at day 7 followed by a decrease in hydroxyl ions release with time. Subgroups C1 showed the highest pH values in the surrounding STF media, whereas subgroups B1 came next and subgroups A1 showed the lowest pH values at all time intervals (Table 3).

MTA subgroups (A2, B2,C2) revealed a continuous increase in pH values throughout the test period until they reached their maximum values at day 28. Subgroups C2 showed the highest pH values in the surrounding STF media, whereas subgroups B2 came next and subgroups A2 showed the lowest pH values at all time intervals (Table 4).

DISCUSSION

Aperture sizes of induced open apices canals were 1 mm, 1.2 mm, and 1.4 mm. This difference in the diameter of the apertures revealed significant increase in the surface area and circumference of the apical apertures [13].

STF was chosen to simulate the in vivo conditions in which Ca $(OH)_2$ paste and MTA were used [14].

The control period of three days concerned with the release of Ca^{+2} and OH^{-1} from roots structure after placing them in the STF solution. During this period, the maximum loss of Ca^{+2} and OH^{-1} from roots structure occurred [15].

The comparison of calcium ions release and hydroxyl ions release from $Ca(OH)_2$ paste and MTA through the canal of the same aperture size revealed that there was a delay of seven days in the release of calcium ions and hydroxyl ions from MTA as compared with $Ca(OH)_2$ paste. These results emphasize the fact that the $Ca(OH)_2$ paste dissociates directly into calcium and hydroxyl ions, whereas calcium oxide (CaO) which present within the composition of MTA reacts with tissue fluid and gives $Ca(OH)_2$ which then can dissociate into calcium and hydroxyl ions, this reaction between CaO and STF might delay the Ca^{+2} concentrations and pH values increase. After day 7, there was a continuous increase in the release of calcium ions and hydroxyl ions from MTA as compared with $Ca(OH)_2$ paste that had a decrease in its Ca^{+2} concentrations and pH values with time. This can be explained by the fact that $Ca(OH)_2$ paste undergoes disintegration over time, whereas the composition of MTA gives an idea that there are many sources of calcium ions other than CaO and a mixture of high concentrations of alkaline salts like tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium sulfate dihydrate that react with aqueous medium and then give calcium and hydroxyl ions. No previous study was done compared the calcium and hydroxyl ions release from Ca $(OH)_2$ paste and MTA through the canal of the same aperture size to compare this result with it.

Subgroups C showed the highest Ca^{+2} concentrations and pH values at all time interval followed by subgroups B, and then subgroups A. This indicates that more Ca^{+2} and OH^{-1} diffused from larger apical aperture size. This result is in agreement with Murray et al. [16] who reported that the dimension of the exposed sample surface area was an important physical constraint to Ca^{+2} and OH^{-1} release from non-setting products, whereas it was not an important physical constraint with setting products. This result is also in agreement with Robert et al. [4] who stated that Ca^{+2} and OH^{-1} diffusion depended on both the medicament and the size of the apical aperture in which their release increase with increase aperture size. This is because that the contact surface area with STF in the canal of large aperture size was greater than that in the canal of small aperture size that led to increase in Ca^{+2} and OH^{-1} release.

There were no significant differences in the pH values among $Ca(OH)_2$ subgroups or MTA subgroups during the test period. This may be attributed to the fact that the STF, in which the roots were immersed, is a buffer solution which has the ability to bind or release H^{+1} in solution, thus keeping the pH of the solution relatively constant despite the addition of considerable quantities of acid or base. Therefore, the alkalinity of $Ca(OH)_2$ paste and MTA is controlled by buffering action of tissue fluid to prevent it from being rise above the accepted biological level.

From the results of this study, it can be stated that "for apexification procedure, non-setting $Ca(OH)_2$ paste (Medical ™) is suitable for a considerable period of time regarding the aperture size and MTA (Pro Root MTA ™) is preferred to be an option to the multiple calcium hydroxide treatment because of a continuous calcium and hydroxyl ions release regardless the aperture size".

REFERENCES

1. Ghose LJ, Baghdady VS, Hikmat BYM. Apexification of immature apices of pulpless permanent anterior teeth with calcium hydroxide. J Endod 1987; 13(6):285-90.
2. Simon ST, Bhat KS, Francis R. Effect of four vehicles on the pH of calcium hydroxide and the release of calcium ions. Oral Surg. Oral Med Oral Pathol Oral Radiol Endod 1995; 80:459-64.
3. Pacios MG, Casa ML, Bulacio MA, Lopez ME. Calcium hydroxides association with different vehicles: in vitro action on some dental components. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 96(1):96-101.
4. Robert GH, Liewehr FR, Buxton TB, McPherson JC. Apical diffusion of calcium hydroxide in an in vitro model. J Endod. 2005; 31(1):57-60.
5. Duprez JP, Bouvier D, Bittar E. Infected immature teeth treated with surgical endodontic treatment and root-reinforcing technique with glass ionomen cement. Dent Traumatol 2004; 20(4):233-40.
6. Holland R, Souza V, Nery MJ, Otoboni Filho JA, Bernabe PFE, Dezan FJ. Reaction of rat connective tissue to implanted dentin tubes filled with MTA or calcium hydroxide. J Endod 1999; 25(3):161-6.
7. Duarte MAH, Demarchi ACC, Moraes IG. Determination of pH and calcium ions release provided by pure and calcium hydroxide-containing AHPlus. Int Endod J 2004; 37:42-5.
8. Takita T, Hayashi M, Takeichi O, Ogiso B, Suzuki N, Otsuka K, Ito K. Effect of MTA on perforation of cultured human dental pulp cells. Int Endod J 2006; 39:415-22.
9. Haenni S, Schmidlin PR, Muller B, Sener B, Zehnder M. Chemical and antimicrobial properties of calcium hydroxide mixed with irrigating solutions. Int Endod J 2003; 36:100-5.
10. Mohammed HY. Assessment of MTA As an endodontic material: a comparative study. Ph.D. thesis, Department of Conservative Dentistry, College of Dentistry, University of Baghdad, 2003.
11. Al-Atar AI. Evaluation of calcium and hydroxyl ions release from MTA mixed with four different vehicles: in vitro study. M.Sc. thesis, Department of Preventive and Pediatric Dentistry, College of Dentistry, University of Baghdad, 2003.
12. Calt S, Serper A, Ozealik B, Dalat MD. pH changes and calcium ions diffusion from calcium hydroxide dressing materials through root dentin. J Endod 1999; 25(5):329-31.
13. Cohen S, Burns RC. Pathway of the Pulp. 8th ed. St. Louis, Missouri. Mosby Year Book, Inc.; 2002: Ch.11, P: 415; Ch.16, P: 615-33; Ch.23, P: 825-44.
14. Sarkar NK, Caicedo R, Moiseyeva R, Kawashima I. Physiochemical basis of the biologic properties of MTA. J Endod 2005; 31(2): 97-100.
15. Gomes IC, Chevitarese O, Almeida NS, Salles MR, Gomes GC. Diffusion of calcium through dentin. J Endod1996; 22(11):590-5.
16. Murray PE, Lumley PJ, Smith AJ, Ross HF. The influence of sample dimensions on calcium and hydroxyl ions release from calcium hydroxide products. Endod Dent Traumatol 2000; 16(6):251-7.

Table 1: The differences in Ca^{+2} concentrations within and among the $Ca(OH)_2$ subgroups at all time intervals .

Sizes / Days	A1=1 mm n=10		B1=1.2 mm n=10		C1=1.4 mm n=10		F-value	P-value	Sig.
	Mean	±SD	Mean	±SD	Mean	±SD			
Control	7.56	0.041	7.59	0.032	7.54	0.025	3.630	0.083	NS
1day	7.65	0.033	7.83	0.029	8.32	0.032	11.934	0.004	HS**
3day	8.22	0.068	8.53	0.023	9.82	0.049	27.93	0.000	HS**
7day	8.84	0.044	9.52	0.027	9.66	0.035	14.56	0.000	HS**
14day	9.10	0.216	9.31	0.048	9.48	0.052	2.998	0.083	NS
21day	8.88	0.042	8.95	0.029	9.00	0.072	2.999	0.088	NS
28day	8.43	0.140	8.51	0.052	8.60	0.319	1.740	0.194	NS
P-value	0.001		0.001		0.001				
Sig.	HS**		HS**		HS**				

P>0.05 Non significant difference (NS)
****P<0.01 Highly significant difference (HS)**

Table 2: The differences in Ca^{+2} concentrations within and among MTA subgroups at all time intervals.

Sizes Days	A2=1 mm n=10		B2=1.2 mm n=10		C2=1.4mm n=10		F-value	P-value	Sig.
	Mean	±SD	Mean	±SD	Mean	±SD			
Control	7.52	0.018	7.58	0.038	7.53	0.018	1.442	0.172	NS
1day	7.60	0.035	7.63	0.022	7.64	0.029	3.000	0.083	NS
3day	8.13	0.026	8.15	0.087	8.18	0.034	1.990	0.156	NS
7day	8.36	0.034	8.38	0.026	8.41	0.035	3.002	0.083	NS
14day	9.72	0.034	9.75	0.044	9.78	0.093	2.250	0.125	NS
21day	11.80	0.279	11.84	0.042	11.89	0.052	0.740	0.487	NS
28day	14.37	0.090	14.38	0.046	14.40	0.249	0.090	0.911	NS
P-value	0.001		0.001		0.001				
Sig.	HS**		HS**		HS**				

P>0.05 Non significant difference (NS)
****P<0.01 Highly significant difference (HS)**

Table 3: The differences in pH values within and among the $Ca(OH)_2$ subgroups at all time intervals.

Sizes / Days	A1=1 mm n=10		B1=1.2 mm n=10		C1=1.4 mm n=10		F-value	P-value	Sig.
	Mean	±SD	Mean	±SD	Mean	±SD			
Control	7.35	0.005	7.36	0.005	7.36	0.005	1.170	0.324	NS
1day	7.40	0.011	7.43	0.014	7.44	0.028	3.721	0.078	NS
3day	7.48	0.007	7.58	0.020	7.70	0.015	3.800	0.099	NS
7day	7.68	0.008	7.78	0.013	7.90	0.020	3.912	0.090	NS
14day	7.65	0.043	7.72	0.020	7.81	0.029	3.879	0.090	NS
21day	7.60	0.014	7.69	0.010	7.78	0.020	3.853	0.090	NS
28day	7.59	0.004	7.65	0.017	7.73	0.024	3.834	0.090	NS
P-value	0.001		0.001		0.001				
Sig.	HS**		HS**		HS**				

P>0.05 Non significant difference (NS)
****P<0.01 Highly significant difference (HS)**

Table 4: The differences in pH values within and among the MTA subgroups at all time intervals.

Sizes / Days	A2=1 mm n=10		B2=1.2 mm n=10		C2=1.4 mm n=10		F-value	P-value	Sig.
	Mean	±SD	Mean	±SD	Mean	±SD			
Control	7.35	0.004	7.35	0.005	7.36	0.005	2.800	0.0780	NS
1day	7.40	0.008	7.41	0.018	7.42	0.073	0.250	0.7790	NS
3day	7.45	0.011	7.46	0.029	7.47	0.070	0.080	0.920 0	NS
7day	7.54	0.025	7.55	0.039	7.56	0.071	0.410	0.6650	NS
14day	7.78	0.014	7.79	0.022	7.84	0.029	3.915	0.0800	NS
21day	7.80	0.027	7.82	0.078	7.86	0.027	2.999	0.0788	NS
28day	7.93	0.033	7.96	0.020	7.98	0.010	3.990	0.0820	NS
P-value	0.001		0.001		0.001				
Sig.	HS**		HS**		HS**				

P>0.05 Non significant difference (NS)
****P<0.01 Highly significant difference (HS)**

Evaluation of serum anti-Cardiolipin antibody, hs-CRP and IL-6 levels in chronic periodontitis as possible risk factors for cardiovascular diseases

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ABSTRACT

Background: It has long been established that there is an association between cardiovascular disease and periodontitis. Evidence shows periodontally infected patients may be at a higher risk of thrombotic accidents via induced systemic inflammatory mediators' production and increase in serum levels of autoantibodies such as anti-cardiolipin antibody. The aim of the present study was to determine the presence of anti-cardiolipin antibody (ACLA)-IgG and -IgM, and to investigate the systemic levels of inflammatory markers of cardiovascular diseases like high sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6) in patients with chronic periodontitisin (CP) as well as to examine the relationships between these mediators and clinical periodontal parameters.

Subjects and Methods: Blood samples were collected from 45 patients with CP (20 with sever periodontitis and 25 with moderate periodontitis) and from 30 healthy age matched individuals served as controls. Clinical periodontal parameters used in this study were plaque index, gingival index, probing pocket depth, clinical attachment level and bleeding on probing. The levels of serum ACLA-IgG, ACLA-IgM, hs-CRP and IL-6 were determined using enzyme-linked immunosorbent assays.

Results: The current results revealed that serum levels of ACLA-IgG, hs-CRP and IL-6 were significantly higher in patients group as compared to healthy control group ($p<0.05$, $p<0.001$), whereas the serum level ACLA-IgM was not observed any significant differences between two groups ($p>0.05$). Concerning the comparison between two patient groups, sever CP group showed significant elevation in serum levels of ACLA-IgG, hs-CRP and IL-6 ($p<0.05$, $p<0.001$), while there is no differences in serum level of ACLA-IgM when compared to moderate CP patients group ($p>0.05$). Furthermore, in regards to the correlation between serum ACLA-IgG, ACLA-IgM, hs-CRP and IL-6, and clinical periodontal parameters, IL-6 level was showed significant positive correlation with clinical attachment level, whereas hs-CRP was showed significant positive correlation with each of probing pocket depth, clinical attachment level and bleeding on probing. Moreover; linear positive correlation was noticed between ACLA-IgG and clinical attachment level. Conversely, ACLA-IgM level did not show any correlation with clinical parameters of periodontitis ($p>0.05$).

Conclusion: Elevation in prothrombotic autoantibodies, ACLA-IgG and inflammatory mediators (hs-CRP and IL-6) factors may increase inflammatory activity in atherosclerotic lesions and potentially increasing the risk for cardiovascular events.

Key words: Chronic Periodontitis; Anticardiolipin; hs-CRP, IL-6. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):161-165).

INTRODUCTION

Periodontitis is a chronic infectious disease of the supporting tissues of the teeth and it has been consistently associated with cardiovascular diseases (CVD). CVD is the broad term used to categorize any abnormal condition characterized by dysfunction of the heart and blood vessels [(1, 2)]. According to the recent literature it is also possible that the apparent association between these two disease groups is related more to the existence of common risk factors and common underlying physiologies and pathophysiologies [(2, 3)]. Moreover; one explanation in this association is that periodontitis may also cause a prothrombotic state. The prothrombotic state is a propensity of blood to coagulate due to an abnormality in the coagulation and/or fibrinolysis system [(4, 5)]. The systemic dissemination of periodontal pathogens from periodontal lesions seems to be at least one cause for the systemic inflammation in periodontitis and elevation of CVD risk markers.

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The periodontal pathogens *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* have been shown in blood and biopsies from atherosclerotic plaques [(6, 7)]. Several parameters of systemic inflammation have been identified, including CRP, IL-1 and IL-6, however; some authors suggest that increase in these systemic markers of inflammation occurs together with increase in serum levels of auto-antibodies including ACLA [(8)].

Cardiolipin is a phospholipid (diphosphatidylglycerol) found in inner mitochondrial membrane primarily, but it is also a minor constituent of mammalian membranes in general. In diseases with mitochondrial damage cardiolipin can evoke an antibody response [(9, 10)]. Antiphospholipid antibodies are a class of auto-antibodies which have been found in 1-5% of systematically healthy population. These antibodies are also usually detected in patients with systemic lupus erythematosus and anti-phospholipids antibody syndrome, in addition are associated with adverse pregnancy outcomes [(8)]. The increased level of these antibodies has also

been observed in several situations including some infectious diseases, consequently infectious diseases may have a role in production of ACLA, it has also been suggested that patients with periodontitis might have a higher level of ACLA in comparison with periodontally healthy people. Thus, increased ACLA level might explain the association of such systemic disorders as prothrombotic accidents with periodontitis [11].

C-reactive protein is a plasma protein synthesized by the liver and adipocytes, being actually recognized as an important biomarker of a wide spectrum of conditions such as systemic inflammation, infections, neoplasias (lymphoma), and immune-mediated rheumatic disorders including rheumatoid arthritis and vasculites [12] . In addition, hs CRP measures cardiac and cerebrovascular risk being of special interest as risk factor [13]. Numerous cytokines have been identified at sites of chronic inflammation such as arthritis and periodontitis. One of these, IL-6 is an important pro-inflammatory cytokine involved in the regulation of host response to tissue injury and infection. It is produced by a variety of cells, such as monocytes, fibroblasts, osteoblasts and vascular endothelial cells in response to inflammatory challenges [14]. Moreover, it is widely accepted that IL-6 induces CRP production [15]. Since elevated plasma levels of IL-6 have been associated with unstable angina and CVD, IL-6 is actually related to other cardiovascular risk factors [13]. Due to the potential association between periodontitis and cardiovascular disease, the current work was carried out to assess ACLA, hs-CRP and IL-6 as possible risk factors for cardiovascular disease in patients with CP, and to determine the relationship of their elevated levels to the severity of periodontal disease.

SUBJECTS AND METHODS

The present study included 45 patients (19 females and 26 males), with an mean of age 42.1±8.071 years, and ranged between (30-55years), were from attendants seeking treatment in the department of periodontics, College of Dentistry, Baghdad University, during the period between December 2010 till May 2011. Diagnosis was made by specialized dentists (single examiner conducted the periodontal assessment in order to minimize the variation in the data), all the cases had received no treatment with no complain of other chronic or systemic diseases.Compared with 30 age and sex-matched apparently healthy individuals considered as controls. Clinical periodontal parameters used in this study were plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP). CP patients were divided in to two groups based on their clinical attachment loss (CAL), [25 moderate CP with CAL>2mm and 20 sever CP with CAL≥5mm].

Clinical attachment level is defined as the distance from the cement enamel junction (CEJ) to the location of the inserted probe tip. The measurements were made at four surfaces of each tooth.

Blood samples was collected from patients and controls to estimated serum concentrations of ACLA-IgG, ACLA-IgM, hs-CRP and IL-6 by using commercially available enzyme-linked immunosorbent assay (ELISA) kits and performed as recommended in leaflet with kits (ACLA-IgG & IgM-AESKU. Diagnostic. Germany; hs-CRP-Calbiotech, Inc. USA; IL-6-BioSource Europe S.A. Company, Belgium). Statistical analysis was assessed using P (Mann-Whitney-test), and (Kruskal-Wallis-test). Statistical analysis: It was assessed using P (Mann-Whitney-test), P (Bonferroni-test) and (Kruskal-Wallis-test). Correlation among different parameters was calculated by the spearman test and p values of $P<0.01$and $P<0.05$ were considered significant [16].

RESULTS

Forty five Iraqi patients with CP (19 females and 26 males) were recruited for the present study; their mean age was 42.1±8.071 years (range 30-55 years). Demographic results demonstrated that (58%) of patients were males and (42%) were females. The differences in clinical periodontal parameters between patients and healthy controls are summarized in table (1).

The current results revealed that mean serum levels of ACLA-IgG, hs-CRP and median serum level of IL-6 were significantly higher ($p<0.05$, $p<0.001$) in patients group (8.22±7.27 iu/ml, 1.86±1.51 mg/dl and 5.2 pg/ml respectively) as compared to healthy control group (6.11±3.83 iu/ml, 0.68±0.44 mg/dl and 2.15 pg/ml respectively), whereas the serum level ACLA-IgM was not observed any significant differences ($p>0.05$) between two groups as clearly shown in table (2). Concerning the comparison between two patient groups (sever and moderate CP), sever CP group showed significant elevation ($p<0.05$, $p<0.001$) in serum levels of ACLA-IgG, hs-CRP and IL-6 (12.13±8.48 iu/ml, 2.531±1.69 mg/dl and 6.5 pg/ml respectively), while there is no significant differences ($p>0.05$) in serum level of ACLA-IgM when compared to moderate CP patients group, table (3). Furthermore, in regard to

the correlation between serum ACLA-IgG, ACLA-IgM, hs-CRP and IL-6, and clinical periodontal parameters, linear positive correlation was noticed between ACLA-IgG and CAL (r=0.434, P=0.030). Conversely, ACLA-IgM level did not show any correlation with clinical parameters of periodontitis (p>0.05). Interestingly hs-CRP was showed significant positive correlation with each of PPD, CAL and BOP (r=0.389, P= 0.049; r=0.444, P=0.026; r=-0.579, P=0.002 respectively), whereas IL-6 level was revealed significant positive correlation with CAL (r=0.466; P=0.019) as observed in table (4).

DISCUSSION

Periodontitis is very common and is regarded as the second most common disease worldwide, after dental decay. Over the past two decades, there has been an increasing interest in the possible link between dental disease, specifically periodontal disease, and CVD [(1)]. Inflammation plays an important role in the pathogenesis of atherosclerosis, and markers of low grade inflammation have been consistently associated with a higher risk of CVD. It has been observed that people with periodontal disease are at a greater risk of systemic diseases such as CVD [(17)].

The present work is found increase in serum levels of ACLA-IgG in CP patients when compared to controls which is in accordance with the observations of the previous researchers [(18-20)]. Schenkein et al [(18)], evaluated serum ACLA level in patients with sever periodontitis, and suggested that increase in systemic markers of vascular endothelial inflammation occurs together with increase in level of serum ACLA. Consistency Faghihi and colleagues observed that the mean serum ACLA level of patient group was significantly higher than that of the control group although all cases had a normal range of ACLA [(19)], on the other hand, other study conducted by Sumanth et al. [(21)] denoted that serum ACLA-IgM and IgG levels were significantly higher in patients with acute myocardial infarction associated with CP than in patients with acute myocardial infarction. In addition, they showed significant alterations in concentrations of serum ACLA-IgM and IgG levels after phase I periodontal therapy. Interestingly, the present study failed to show any significant differences in serum ACLA-IgM levels between patients and control groups as well as between sever and moderate CP, this result was at variance with some other studies [(20, 21)], who found significant increase in serum ACLA-IgM level in sever CP patients when compared to healthy individuals. Correspondingly to our results Türkoğlu et al [(22)] found a positive correlation between ACLA-IgG levels and CAL, so they conclude that CP might be associated with an increased level of serum ACLA.

The possible explanation for the higher levels of serum ACLA-IgG may be due to the fact that since infectious diseases may induce the production of ACLA, it can be suggested that patients with periodontitis may show an increased level of serum ACLA. This increase might explain the presence of systemic disorders including prothrombic accidents (such as stroke) and fetal abortion in periodontitis patients [(23)]. Several inflammatory biomarkers have already been validated as cardiovascular risk factors, particularly CRP, an emerging and reliable biomarker of the acute phase response to infectious burdens and/or inflammation. In addition, IL-6 may also be listed among factors contributing to the association between chronic infections and CVD, displaying pro-inflammatory and pro-coagulant properties [(24)].

Other important findings in this study were the significant elevation of mean serum level of hs-CRP and median serum level of IL-6 in patients with periodontitis, particularly in the subgroup of patients with severe CP. These results are comparable to other previous results reported by Gani *et al.* [(25)], Fitzsimmons *et al.* [(26)], and Haba *et al.* [(27)]. More recent evidences, however, has indicated that patients with severe periodontitis have increased serum levels of CRP, hyperfibrinogenemia, moderate leukocytosis, as well as increased serum levels IL-6 when compared with unaffected control populations [(27)]. In contrast, Ide and co-workers reported that there were no statistically significant changes in the levels of any of the aforementioned systemic markers. They concluded that improvement in periodontal health also did not influence the levels of vascular markers [(28)]. However, different results in various studies may be due to the case selection, the volume of inflammatory tissues, or the methods used. Certainly, more studies taking into account other variables are required in this field.

Regarding the correlation between serum (hs-CRP and IL-6) and clinical periodontal parameters, hs-CRP was showed significant positive correlation with each of PPD, CAL and BOP, whereas IL-6 level was revealed significant positive correlation with CAL. The results obtained from the present study were similar to that reported by others investigators [(26, 27, 29)].

The elevated levels of CRP and IL-6 in periodontitis patients may occur when bacteria and bacterial products, such as

lipopolysaccharide, as well as locally produced pro-inflammatory cytokines enter the circulation. CRP and IL-6 may contribute, in part, to the observed associations between chronic infections and CVD. CRP may activate complement in damaged vessel walls whereas IL-6 has pro-inflammatory properties and a pro-coagulant effect. These properties may contribute to the pathogenesis of coronary syndromes. Furthermore, IL-6 stimulates the production of CRP by hepatocytes.

Finally, Azarpazhooh and Tenenbaum [30] reported that several studies showed a weak but statistically significant association between CVD and periodontal disease. Although the risk estimates might be considered modest, the high prevalence of both types of disease means that the absolute numbers of those affected is quite high. Hence, they reported that an individual with periodontitis is at greater risk of either having or developing CVD. In conclusion current results suggest that elevation in prothrombotic autoantibodies, ACLA-IgG and inflammatory mediators (hs-CRP and IL-6) factors may increase inflammatory activity in atherosclerotic lesions and potentially increasing the risk for cardiovascular events in sever CP patients.

REFERENCES

1. Roby VE, Shiba NK. Periodontitis: A possible risk factor for cardiovascular diseases. Archives Dental Sci 2011; 2(2): 5-9.
2. Humphrey LL, Fu R, Buckley DI, Freeman M, Helfand M. Periodontal disease and coronary heart disease incidence: a systematic review and meta-analysis. J Gen Intern Med 2008; 23: 2079-86.
3. Bizzarro S, van der Velden U, ten Heggeler JM, Leivadaros E, Hoek FJ, Gerdes VE, et al. Periodontitis is characterized by elevated PAI-1 activity. J Clin Periodontol 2007; 34:574-80.
4. Papapanagiotou D, Nicu EA, Bizzarro S, Gerdes VE, Meijers JC, Nieuwland R, et al. Periodontitis is associated with platelet activation. Atherosclerosis 2009; 202: 605-11.
5. Nicu EA, Van der Velden U, Nieuwland R, Everts V, Loos BG. Elevated platelet and leukocyte response to oral bacteria in periodontitis. J Thromb Haemost 2009; 7:162-70.
6. Bizzarro S, Nicu EA, Velden U, Laine ML, Loos BG. Association of serum immunoglobulin G (IgG) levels against two periodontal pathogens and prothrombotic state: a clinical pilot study. Thrombosis J 2010; 8:16.
7. Padilla C, Lobos O, Hubert E, Gonzalez C, Matus S, Pereira M, etal. Periodontal pathogens in atheromatous plaques isolated from patients with chronic periodontitis. J Periodontol Res 2006; 41:350-3.
8. Schenkein HA, Berry CR, Burmeister JA, Brooks CN, Barbour SE, Best AM, et al. Anticardiolipin antibodies in sera from patients with periodontitis. J Dent Res 2003; 82(11):919-22.
9. Schlame M, Rua D, Greenberg ML. The biosynthesis and functional role of cardiolipin. Prog Lipid Res 2000; 39(3):257-88.
10. Rozee KR, Acott P, Lee SH, Crocker JF, Gough D, MacDonald J, et al. Elevated anticardiolipin antibodies in acute liver failure. Biochim Biophys Acta 2003; 1637(3):183-6.
11. Blank M, Krause I, Fridkin M, Keller N, Kopolovic J, Goldberg I, et al. Bacterial induction of autoantibodies to beta2-glycoprotein-I accounts for the infectious etiology of antiphospholipid syndrome. J Clin Invest 2002; 109(6):797-804.
12. Ablij H, Meindes A. C- reactive protein; history and revival. Eur J Intern Med 2002; 13(7): 412-20.
13. Loos GB, Craandijk J, Hoek JF, Vandillen WM. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. J Periodontol. 2000; 71: 1528-34.
14. Song M, Kellum J. Interleukin-6. Crit Care Med 2005; 33(12): S463–5.
15. Castell JV, Gomez-Lechon MJ, David M, Andus T, Geiger T, Trullenque R. et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett 1989; 242: 237-9.
16. Sorlie DE. Medical biostatistics and epidemiology: Examination and Board review. 1st ed. Norwalk: Connecticut Appleton and Lange; 1995. pp. 47-88.
17. De Oliveira C, Watt R, Hamer M. Tooth brushing, inflammation, and risk of cardiovascular disease. Results from Scottish Health Survey BMJ 2010; 340: c2451.
18. Schenkein HA, Best AM, Brooks CN, Burmeister JA, Arrowood JA, Kontos MC, et al. Anticardiolipin and increased serum adhesion molecule levels in patients with aggressive periodontitis. J Periodontol 2007; 78(3):459-66.
19. Faghihi SH, Rokn AR, Ebrahimi R. Evaluation of Serum Anti-Cardiolipin Antibody Titer in Patients with Chronic Periodontitis. J Dentistry 2009; 6(2): 57-62.
20. Kumar YP. Quantitiative analysis of IgG, IgM anticardiolipin antibodies in healthy and chronic periodontitis patients. SRM University J Dental Sci 2011; 2(1):28-31.
21. Sumanth G, Chava V, Krishna BP. Effect of phase I periodontal therapy on anti-cardiolipin antibodies in patients with acute myocardial infarction associated with chronic periodontitis. J Periodontol 2011; 82(12): 1657-64.
22. Türkoğlu O, Bariş N, Kütükçüler N, SenarslanO, Güneri S, Atilla G. Evaluation of serum anticardiolipin and oxidized low-density lipoprotein levels in chronic periodontitis patients with essential hypertension. J Periodontol 2008; 79(2): 332-40.
23. Schenkein HA, Berry CR, Burmeister JA, Brooks CN, Barbour SE, Best AM, et al. Anticardiolipin antibodies in sera from patients with periodontitis. J Dent Res 2003; 82(11):919-22.
24. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. J Clin Periodontol 2008; 35(4): 277–90.
25. Gani DK, Lakshmi D, Krishnan R, Emmadi P. Evaluation of C-reactive protein and interleukin-6 in the peripheral blood of patients with chronic

periodontitis. J Indian Soc Periodontol 2009; 13(2): 69–74.
26. Fitzsimmons TR, Sanders AE, Bartold PM, Slade GD, Local and systemic biomarkers in gingival crevicular fluid increase odds of periodontitis, J Clin Periodontol, 2010; 37(1):30–6.
27. Haba D, Teslaru S, Ungureanu D, Hodrog D, et al. Evaluation of serum and gingival crevicular fluid C-reactive protein and IL-6 levels in patients with periodontitis and transient ischemic attacks. Rom J Morphol Embryol 2011; 52(4):1243–7.
28. Ide M, McPartlin D, Coward PY, Crook M, Lumb P, Wilson RF. Effect of treatment of chronic periodontitis on levels of serum markers of acute-phase inflammatory and vascular responses. J Clin Periodontol 2003; 30:334–40.
29. Mustapha I, Debrey S, Oladubu M, Ugarte R. Markers of systemic bacterial exposure in periodontal disease and cardiovascular disease risk: a systematic review and meta-analysis. J Periodontol 2007; 78:2289–302.
30. Azarpazhooh A, Tenenbaum HC. Separating fact from fiction: Use of high-level evidence from research syntheses to identify diseases and disorders associated with periodontal disease. Can Dent Assoc 2012; 78: 25-31.

Table 1: Demographic and Clinical l Parameters in Patients and healthy Control groups

	Patients (n=45)	Healthy Control (n=30)	P -Value
Demographic Parameters			
Age Range	30-55	25-50	0.14 NS
Age Mean ± SD	42.1±8.071	36.6±5.567	
Male	26	18	
Female	19	12	
Clinical Parameters			
PI	1.455	0.613	P<0.001**
GI	1.331	0.53	0.003*
PD	2.455	1.126	P<0.001**
AL	1.851	0	P<0.001**
BOP	28.62	4.766	P<0.001**

***P<0.05 Significant, **P<0.001 High significant**

Table 2: Patients and healthy control differences in serum concentration of ACLA-IgG, ACLA-IgM, hs-CRP and IL-6

	Range		Median		Mean		SD		P-Value
	patients N=45	Healthy N=30	Pat. N=45	Heal. N=30	Pat. N=45	Heal. N=30	Pat. N=45	Heal. N=30	
ACLA-IgG	23.8	17.1	6.2	6	8.22	6.11	7.27	3.83	0.002*
ACLA-IgM	51.9	18.9	6.2	4.75	9.36	5.68	9.90	5.03	0.747 NS
hs-CRP	6.2	2	1.2	0.6	1.86	0.68	1.51	0.44	P<0.001**
IL-6	10.6	8.6	5.2	2.15	7.3	3.46	3.83	2.55	0.003*

Table 3: Sever CP and moderate CP differences in serum concentration of ACLA-IgG, ACLA-IgM, hs-CRP and IL-6

	Range		Median		Mean		SD		P (Value)
	Sever N=20	Mode. N=25	Sever N=20	Mode. N=25	Sever N=20	Mode. N=25	Sever N=20	Mode. N=25	
ACLA-IgG	23.8	18	10.95	4.5	12.13	5.052	8.48	4.11	P<0.001**
ACLA-IgM	51.9	18.9	7	5.8	13.39	6.152	12.87	4.93	0.276 NS
hs-CRP	6.2	3.1	2.9	0.9	2.531	1.324	1.69	1.11	0.005*
IL-6	8.4	6.6	6.5	3.4	5.545	3.304	2.96	2.32	0.049*

Table 4: Correlation between serum s concentration of ACLA-IgG, ACLA-IgM, hs-CRP and IL-6 and clinical periodontal parameters in CP cases

Clinical Parameters	PI		GI		CAL		PPD		BOP	
	Correlation (Mann-Whitney)	P	r (Mann-Whitney)	P	r (Mann-Whitney)	P	r (Mann-Whitney)	P	r (Mann- Whitney)	P
ACLA-IgG	-0.054	0.797	-0.239	0.257	0.434*	0.030	-0.072	0.731	-0.306	0.137
ACLA-IgM	0.000	0.998	0.142	0.498	-0.149	0.479	-0.114	0.588	-0.126	0.548
hs-CRP	0.095	0.651	-0.105	0.616	0.389*	0.049	0.444*	0.026	-0.579*	0.002
IL-6	0.288	0.162	0.004	0.983	0.466*	0.019	-0.087	0.680	0.124	0.556

Antibacterial efficiency of chlorhexidine digluconate 0.2% against oral β- hemolytic streptococci and oral *Staphylococcus aureus* in immunocompromised patients

Firas H. Qanbar, B.D.S., M. Sc. [(1)]

ABSTRACT

Background: The use of antimicrobial agents was advocated for a number of years using different compounds that delivered through mouth rinses to control intra- and extra-oral disease in immunocompromised patients. The purpose of this research is to find out and to compare between the antibacterial properties of 0.2% chlorhexidine digluconate (CHX)on oral β- hemolytic streptococci and *Staphylococcus aureus* isolated from patient with renal failure.

Materials and methods: β- hemolytic streptococci and *Staphylococcus aureus* were isolated stimulated saliva samples collected from patients receiving steroids therapy. These bacteria were purified and diagnosed according to morphological characteristic, biochemical and antibiotic sucseptibility tests.

Results: Agar diffusion technique demonstrated that chlorexidine digluconate inhibited the growth of both types of isolates, but the antibacterial effect against *Staphyllococcus aureus* was less than that against β- hemolytic streptococci.

Conclusion: The use of CHX 0.2% as a mouth wash to remove those pathogens from the oral cavity to inhibit their infections in immunocompromised patients is highly indicated.

Key words: *Staphylococcus aureus*, β- hemolytic streptococci, chlorexidine, immunocompromised. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):166-169).

INTRODUCTION

The oral cavity is the most complex and the most accessible microbial ecosystem of the human body where the teeth, gingivae (gums), tongue, throat and buccal mucosa (cheeks) all provide different surfaces for microbial colonization [(1)].

The organisms present in the oral cavity are a mixture of commensals and pathogens, as many commensal bacteria can, under certain conditions be associated with human disease like in subjects whose immune systems are not working optimally, *i.e.* immunocompromised, are especially susceptible to infections by microbes that are commensal in healthy individuals. For these reasons, commensals are nowadays often referred to as opportunistic pathogens [(2)].

Staphylococci are found in the saliva of approximately 30% individuals, but they are considered transients rather than components of the resident oral microbiota and can play a significant role in oral and respiratory tract infections of a compromised host [(3)]. The staphylococci are gram-positive spherical cells, usually arranged in grapelike irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia [(4)].

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The pathogenic staphylococci often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins. Clumping factor, fibronectin- binding protein, and collagen-binding protein bind specifically to fibrinogen, fibronectin, and collagen, respectively, and are instrumental in adhesion to tissues and foreign bodies covered with the appropriate matrix protein. Protein A binds to the Fc portion of immunoglobulins (IgG). It is assumed that “false” binding of immunoglobulins by protein A prevents “correct” binding of opsonizing antibodies, thus hindering phagocytosis.. Staphylococci rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems [(5)].

Staphylococcus aureus (*S. aureus*) infection can result from direct contamination of a wound, eg, postoperative staphylococcal wound infection or infection following trauma like chronic osteomyelitis subsequent to an open fracture, meningitis following skull fracture. If *S. aureus* disseminates and bacteremia ensues, endocarditis, acute hematogenous osteomyelitis, meningitis, or pulmonary infection can result. The clinical presentations resemble those seen with other bloodstream infections. Secondary localization within an organ or system is accompanied by the symptoms and signs of organ dysfunction and intense focal suppuration [(6)]. Contact spread of staphylococcal infections has assumed added importance in hospitals, where a large proportion of the staff and patients carry antibiotic-resistant staphylococci in the nose or on the skin; in hospitals, the areas at highest risk for severe staphylococcal infections are the newborn

nursery, intensive care units, operating rooms, and cancer chemotherapy wards [7]. Massive introduction of "epidemic" pathogenic *S. aureus* into these areas may lead to serious clinical disease. Personnel with active *S. aureus* lesions and carriers may have to be excluded from these areas. In such individuals, the application of topical antiseptics may diminish shedding of dangerous organisms [8].

Streptococci are Gram-positive, non-motile, catalase-negative, facultatively anaerobic cocci that occur in chains or pairs. They are classified based on their hemolytic capacity (α- , β-, γ-hemolysis) and the antigenicity of a carbohydrate occurring in their cell walls (Lancefield antigen) [9]. β-hemolytic group A streptococci (*Streptococcus pyogenes*) cause infections of the upper respiratory tract and invasive infections of the skin and subcutaneous connective tissue. Depending on the status of the immune defenses and the genetic disposition, this may lead to scarlet fever and severe infections such as necrotizing fasciitis, sepsis, or septic shock. Sequelae such as acute rheumatic fever and glomerulonephritis have an autoimmune pathogenesis [10].

Streptococcal diseases can be classified as either acute, invasive infections or sequelae to them. Invasive infections occur as the pathogens enter through traumas or microtraumas in the skin or mucosa and cause invasive local or generalized infections. The rare cases of severe septic infection and necrotizing fasciitis occur in persons with a high-risk MHC II allotype [11].

Although humans can be asymptomatic nasopharyngeal or perineal carriers of *Streptococcus pyogenes*, the organism should be considered significant if it is detected by culture or other means. The ultimate source of group A streptococci is a person harboring these organisms. The individual may have a clinical or subclinical infection or may be a carrier distributing streptococci directly to other persons *via* droplets from the respiratory tract or skin. The nasal discharges of a person harboring *Streptococcus pyogenes* are the most dangerous source for spread of these organisms [12]. Resistance against streptococcal diseases is M type-specific. Thus, a host who has recovered from infection by one group A streptococcal M type is relatively immune to reinfection by the same type but fully susceptible to infection by another M type. Anti-M type-specific antibodies can be demonstrated in a test that exploits the fact that streptococci are rapidly killed after phagocytosis. M protein interferes with phagocytosis, but in the presence of type-specific antibody to M protein, streptococci are killed by human leukocytes [13]. Antibody to streptolysin O develops following infection; it blocks hemolysis by streptolysin O but does not indicate immunity. High titers (>250 units) indicate recent or repeated infections and are found more often in rheumatic individuals than in those with uncomplicated streptococcal infections [14].

Compromised hosts are people with one or more defects in their body's natural defenses against microbial invaders. Consequently immunocompromised people can become infected with any pathogen able to infect immunocompetent individuals they are much more liable to suffer from severe and life-threatening infections [15]. Modern medicine has effective methods for treating many types of cancers, is improving organ transplantation techniques and has developed technology that enables people with otherwise fatal diseases to lead prolonged and productive lives but a consequence of these achievements, however, is an increasing number of compromised people prone to infection [3].

Compromise can take a variety of forms, falling into two main groups:

- Defects, accidental or intentional, in the body's innate defense mechanisms
- Deficiencies in the adaptive immune response.

These disorders of the immune system can be further sub-classified as primary or secondary:

- Primary immunodeficiency is inherited or occurs by exposure in utero to environmental factors or by other unknown mechanisms. It is rare, and varies in severity depending upon the type of defect.
- Secondary or acquired immunodeficiency is due to an underlying disease state or occurs as a result of treatment for a disease [16].

Immunodeficiency results in:

- drastic effects on the structure of the lymphoid organs.
- gross reductions in the synthesis of complement components
- sluggish chemotactic responses of phagocytes
- lowered concentrations of secretory and mucosal IgA
- reduced affinity of JgG
- in particular, a serious deficit in T-cell number leading to inadequate cell-mediated responses.

The use of antimicrobial agents to control plaque and oral disease has been advocated for a number of years. Different compounds have been delivered through mouth rinses or tooth pastes or by topical application. Some chemical agents have proven to be helpful against plaque accumulation

and thereby to some extent also against caries [17]. Chlorhexidine (CHX) is a broad-spectrum antimicrobial agent whose effects are more potent on gram-positive microorganisms than on gram-negative microorganisms, and effective against aerobes, anaerobes and against organism associated with diseases of the oral cavity [18].

Chlorhexidine disrupts cell membrane and cell wall permeability of many Gram- positive and Gram-negative bacteria and interferes with the adherence of plaque-forming bacteria, thus reducing the rate of plaque accumulation; chlorhexidine can inhibit the adenosine triphosphatase (ATPase) which is an important enzyme that is linked to cytoplasmic membrane and thus can inhibit the process of returning potassium ions into cells in exchange for sodium and hydrogen ions, also inhibits metabolic enzymes such as phosphoenolpyruvate phosphotransferase [19].

MATERIALS AND METHODS

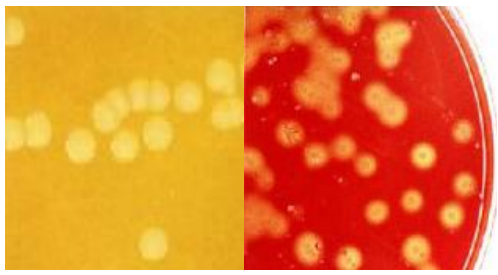
Stimulated saliva samples were collected under standard conditions to obtain 20 microbial samples from patients receiving steroid therapy aged 21-23 years were selected to participate in this study. Ten-fold serial dilutions were prepared using sterile normal saline. Two dilutions were selected and inoculated on blood Agar (B.A.) plates which are incubated anaerobically by using gas packs supplied in an anaerobic jar to isolate group A streptococci; and mannitol salt agar plates which are incubated aerobically for 24 hrs at 37°C for the isolation of *S. aureus*. Colonial morphology, cell morphology, biochemical activities and antibiotic sucseptibility tests were manipulated to diagnose the isolated bacterial species.

A single colony from each plate was transferred to 10 ml sterile BHI-B and then incubated for 24 hrs aerobically at 37°C to activate the inoculums. Agar diffusion technique was applied to study the antimicrobial effects of CHX against the isolates spreaded on Muller Hinton Agar (MHA); wells of equal sizes and depths were prepared in the agar using Kork porer. Each well was filled with 50µl of 0.2% CHX. Inhibition zones diameters were measured using a scientific ruler; resistance of the isolates to CHX was indicated when there were no zones of inhibition.

RESULTS

On mannitol salt agar plates, smooth circular golden yellow colonies appeared indicating (Fig. 1-A) from which colonies were subjected to catalase production test (+ ve) and tube coagulase production test (+ ve) .

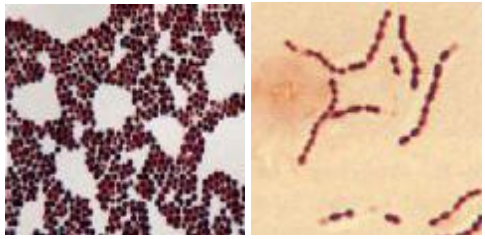
On blood agar plates, group A streptococci colonies appeared as small, circular colonies surrounded by clear zones of hemolysis (Fig. 1-B); bacitracin susceptibility test performed (bacitracin sensitive) to identify group A streptococci (*Streptococcus pyogenes*)



A **B**

Figure1: A: *S. aureus* colonies on mannitol salt agar. B: *Streptococcus pyogenes* colonies on blood agar

All the isolates were gram positive (Figure 2). The motility of all types of microbial cells was examined under microscope by direct smear and without staining; the isolates were non- motile.



A **B**

Figure 2: A: Gram stains showing *S. aureus*. B:Gram stain showing streptococci.

Diameters of inhibition zones for CHX were found to be indicator for the bacterial isolates sensitivity. Figure 3 illustrates the mean diameters of the inhibition zones in relation to CHX. Student's t-test showed highly significant differences among diameters of inhibition zones produced by CHX in the inoculated MHA plates.

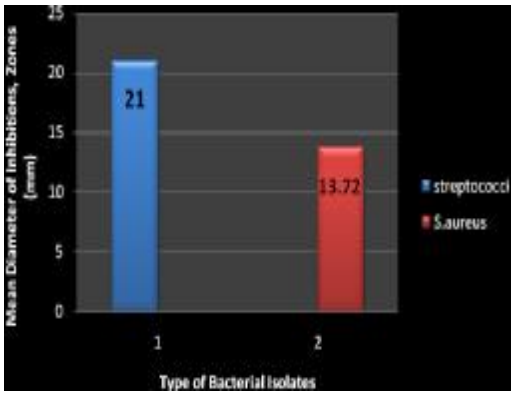


Figure 3: Comparison between the mean diameters of inhibition zones of CHX in relation to the isolates.

The comparison between the antibacterial effect of CHX in relation to *S. aureus* and *Streptococcus pyogenes* showed highly significant difference using T-test analysis (Table 1).

Table 1: Student's t-test for the comparison between the Effect of CHX on *S. aureus* and *Streptococcus pyogenes* (*in vitro*)

S.D.	t-test	P-value	Sig
0.891	40.867	P<0.01	HS

HS: highly significant difference at level P<0.05.

From the results shown above, it is quite obvious that CHX had exerted antimicrobial action against *S. aureus* and *Streptococcus pyogenes* but was less effective against *S. aureus* than *Streptococcus pyogenes* which could be due to the hereditary contents or attraction ability or the permeability of the cell wall of the microorganisms.

REFERENCES

1. Granato PA. Pathogenic and indigenous microorganisms of humans. In Murray PR *et al* (eds): Manual of clinical microbiology. 9th ed. ASM Press; 2007.
2. Lamont R J, Jenkinson HF. Oral Microbiology at a Glance. A John Wiley & Sons Ltd Publication; 2010. p 65.
3. Orr KE, Gould FK. Infection problems in patients receiving solid organ transplants. Rev Med Microbiol 1992; 3: 96-103
4. Bohach GA, Foster TJ. *Staphylococcus aureus* exotoxins. In Fischetti V A, Novick, R, Ferretti J, Portnoy D, Rood JI (eds). Gram- Positive Pathogens. ASM Press; 2000. p. 367–91.
5. Bronner S, *et al*. Regulation of virulence determinants in *Staphylococcus aureus*: Complexity and applications. FEMS Microbiol Rev 2004; 28:183.
6. Vandenesch F, Naimi T, Enright M, *et al*. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton–Valentine leukocidin genes: worldwide emergence. Emerging Infectious Diseases 2003; 9: 978–84.
7. Senna JP, Roth DM, Oliveira JS, *et al*. Protective immune response against methicillin resistant Staphylococcus aureus in a murine model using a DNA vaccine approach. Vaccine 2003; 21:2661–6.
8. Que YA, *et al*. *Staphylococcus aureus* (including staphylococcal toxic shock). In Mandell GL, Bennett JE, Dolin R (eds). Bennett's principles and practice of infectious diseases. 7th ed. Churchill Livingstone Elsevier; 2009.
9. Facklam R, Elliott JA. Identification, classification, and clinical relevance of catalase-negative, gram-positive cocci, excluding the streptococci and enterococci. Clin Microbiol Rev 1995; 8:479.
10. Bisno AL. Non-suppurative post-streptococcal sequelae: Rheumatic fever and glomerulonephritis. In Mandell GL, Bennett JE, Dolin R (eds). Bennett's principles and practice of infectious diseases. 7th ed. Churchill Livingstone Elsevier; 2009.
11. Bisno A L, Stevens D L. *Streptococcus pyogenes*. In Mandell GL, Bennett JE, Dolin R (eds). Bennett's principles and practice of infectious diseases. 7th ed. Churchill Livingstone Elsevier; 2010.
12. Stollerman G H, Dale J B. The importance of the group A streptococcus capsule in the pathogenesis of human infections: a historical perspective. Clin Infect Dis 2008; 46: 1038.
13. Kayser FH, Bienz KA, Eckert J, Zinkernagel RM. Medical microbiology. New York: Thieme; 2005.
14. Ruoff K L, Whiley R A, Beighton D. Streptococcus. In Murray PR, *et al*. (eds). Manual of clinical microbiology. 9th ed. ASM Press; 2007.
15. Brostoff J, Scadding GK, Male D, Roitt I M. Clinical immunology. London: Mosby International;1991.
16. Reese RE, Douglas RG. A practical Approach to Infectious Diseases. Baltimor / Toronto: Little Brown & Co; 1986.
17. Thylstrup A, Fejerskov O. Textbook of clinical cariology. 2nd ed. Copenhagen: Munksgaard; 1994.
18. Kolahi J, Soolari A. Rinsing with chlorhexidine gluconate solution after brushing and flossing teeth: a systemic review of effectiveness. Quintessence Int 2006; 37(8): 605-12.
19. Maltz-Turkienicz M I, Wyatt C, McBrid BC. Effect of chlorhexidine and iodine on *in vitro* plaque of Streptococcus mutans and Streptococcus sangius. Caries Sp Suppl Microbiol 1980; 3:895-66.