DENTISTRY

Artificial Neural Network for Prediction of Unerupted Premolars and Canines

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ABSTRACT

Objective: **The purpose of this study was to establish Neural Network for prediction the size of unerupted premolars and canines in Iraqi population.**

Design: **Prospective cohort study**

Subjects and Methods: **for this study, ninety four adult patients (41 males and 53 females) seeking for orthodontic treatment with the age range of 15-20 years were recruited. Data were obtained from intra oral photographs. ANN was developed as new and accurate method for prediction of unerupted teeth using MATLAP program.**

Result: **High degree of correlation was obtained for this Network between the summation of mesiodistal width of premolars, canines of the target and the actual output (r = 90794).**

Conclusions: **This study suggests that artificial intelligence systems with neural network machine learning would be useful as an accurate method in orthodontics for prediction of unerupted teeth and its performance was achieved by components such as proper selection of the input data, preferable generalization and appropriate organization.**

KEY WORDS

digital technology; Artificial Neural Network; MATLAP program.

INTRODUCTION

The nature of malocclusion in orthodontic results from dental, skeletal problems, or a combination of these problems[1)]. A large number of cases of malocclusion develop during the mixed dentition stage as showed by many orthodontic literatures especially during the interval from the 6th to the 12th year of life[2)].

One of the main reasons for patients who seek orthodontic treatment is crowding of teeth since, it is an unaesthetic problems, and furthermore maintenance of oral hygiene also becomes difficult. Intervention can be done to treat or to reduce its severity if it can be diagnosed early during mixed dentition stage[3,4)].

So, the purpose of the analysis during mixed dentition period is to predict as accurately as possible the space required for the alignment of the canines and the premolars[5)].

An important factor in managing the developing occlusion of a growing child is predicting the size of un erupted teeth during the mixed dentition period[6)]. Great importance is given to predict the sizes of un erupted posterior teeth in the mixed dentition especially if a good treatment plan is to be established[7)]. Answering the traditional question of whether the available space in the posterior segments is sufficient depend on accurate prediction to allow the permanent teeth to erupt freely with good alignment in their respective arches[8,9)].

Many reports have indicated attempts to predict the width of un erupted permanent canine and premolars since 1940's[10)]. These methods, namely prediction tables of Moyers (1963) and Tanaka and Johnston equations (1974) are the most largely used because of their simplicity[11,12)]. These methods could be classified into three main categories based on the predictor (independent variable): 1) the evaluation based on the erupted teeth 2) measuring un-erupted teeth on radiographs 3) the combination of the first and second methods is used as a predictor[13)].

An important criteria for a predictive method are accuracy, safety, and simplicity to become a part of the comprehensive case analysis in contemporary orthodontic practice[14)].

However, the accuracy of these methods on other races is doubtful since, they were developed on Caucasian populations only[15)]. Still other methods use regression equations based on the high linear correlation between relevant groups of teeth. The common factor in this category is the possibility of predicting the sizes of unerupted teeth by using the widths of other fully erupted permanent teeth[16-20)].

Globally, digital technology is becoming constantly one of the most important procedures in the clinical activities and, thus, orthodontic digital revolution has been added more and more by orthodontists in their clinical practice[21)].

Currently, many multiple-factor analysis methods are available for medical use and among these Artififcial Neural Network (ANN) model analysis is very commonly used. Recently, there have been many studies about artificial intelligence and bioinformatics[21,22)]. One approach is machine learning using a Neural Network system[23)].

In true sense, ANNs are the simple clustering of the primitive artificial neurons and this clustering occurs by creating layers, which then are connected to one another. As showed in Figure 1, the input layer consists of neurons that receive input from the external environment. The output layer consists of neurons that communicate the output of the sys-

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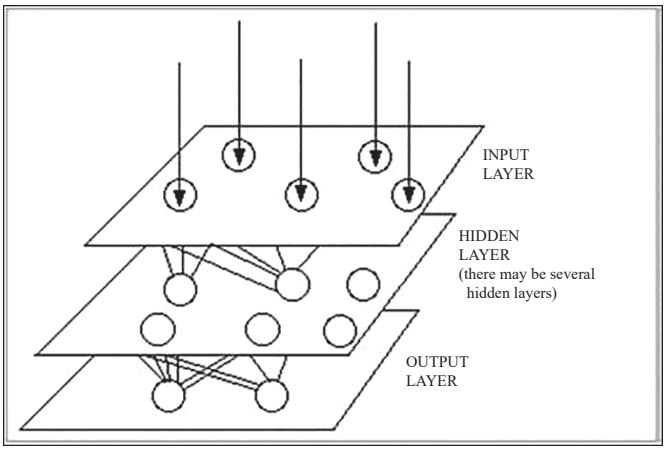

Figure 1: The structure of an Artificial Neural Network.[25)]

tem to the user or external environment. There are usually a number of hidden layers between these input and output layers; however, Figure 1 is just a simple structure with only one hidden layer. When the input layer receives the input, its neurons produce output and this becomes input to the other layers of the system. The process continues until a certain condition is satisfied or until the output layer is invoked and fires their output to the external environment[24)].

Previously, in orthodontics, the use of ANN was restricted for the decision of extraction; the prediction of change in lip curvature; and for the prediction of arch form[25-27)]. The mentioned studies found that, ANN model analyses were more accurate as compared to the conventional ones.

To our knowledge, no studies have employed the ANN for the prediction of un erupted premolars and canines during mixed dentition analysis using intra-oral photographs. Thus this study aimed to make a new artificial intelligence decision-making model for prediction of un erupted teeth through only photographs using neural network machine learning.

MATERIALS AND METHODS

A total of ninety four patients were recruited for this prospective study, with age range of 15-20 years, (41 males and 53 females) seeking for orthodontic treatment. This study was conducted in the Al-Shaab specialist dental center in Baghdad. Inclusion criteria were patients with age range (15-20) years, no previous orthodontic or surgical treatment, all permanent teeth erupted up to second molar included, no craniofacial trauma, and no congenital anomalies Exclusion criteria were patients who were not fit for orthodontic treatment (poor oral hygiene, multiple caries), patients with systemic diseases or pregnant patients, patients were not within the age range. Ninety four dental impressions were taken for both upper and lower arches of patients by using polysiloxane impression material. Maxillary and mandibular occlusal photographs were taken for all the patients using digital camera (Nikon D7500 DSLR Camera, Tokyo, Japan) and Nikon AF-S VR Micro-NIKKOR 105 mm f/2.8 G IF-ED lens. In this technique, a modified intra oral combination mirror was used[28)]. A 35 mm trimmed scale was bonded on the front surface of an occlusal cheek retractor which is used for calibration purposes.

These images were subsequently uploaded into Autodesk Auto-Cad software (21.0). The contact points of lower incisors, upper and lower premolars and canines were marked indirectly.

After that manual space analysis were performed by conventional method using digital Vernier gauge caliper to determine mesiodistal width of lower incisors, mesiodistal width of upper and lower canines and premolars. These dental measurements were validated against the digital dental measurements from intraoral photographs that uploaded into AutoCad software.

Inter and intra examiner calibrations were performed out on a sample of 27 subjects (15 males and 12 females) for assessment of dental factors from intraoral digital photograph and physical plaster dental casts.

In ANN programming, all the data of dental measurements had uploaded into MATLAP program (R2020a v 9.8.0/2020) from the Microsoft Excel. The data was randomly divided into 70% of data for learning (PTrian = 0.7) and 30% for testing. Feedforward backpropogation Network was used. The learning function was Bayesian Regularization Neural Network.

To prevent overfitting, iterative learning was stopped at the minimum error point of the training set. Next, through evaluation of the test set, the adequacy and accuracy were evaluated, and the best-fit model was chosen. The Network was trained by entering the mesiodistal width of the lower incisors in mm values as inputs values for the Network while the outputs values were the mesiodistal width of upper and lower premolars and canines of 94 patients.

After several attempts the best architecture of the network was 1 variable as input values which was the mesiodistal width of lower incisors, 1 hidden layer composed from 5 neurons and 2 variables as output values which was the mesiodistal width of upper and lower premolars and canine.

Statistical Analysis

Data was subjected to statistical analysis using the Statistical Package for the Social Sciences, version 16.0 (SPSS Inc, Chicago, Ill). Descriptive statistics were performed for mesiodistal width of lower incisors and upper and lower premolars and canines. Sexual dimorphism was evaluated by independent sample t-test. Paired t test was used for comparison of side difference and phot-cast difference for the dental measurements. Interclass correlation coefficients (ICCs) were estimated from repeated dental measurements to evaluate the repeatability and reproducibility of the method. Mesiodistal width of lower incisors was compared with mesiodital width of upper and lower premolars and canines to assess Pearson correlation coefficients. Linear regression analyses were made after designing the Networks for real targets of mesiodistal width of premolars and canines (dependent variables) and actual output of mesiodistal width of premolar and canines (independent variables). Levels of $P < 0.05$ were considered statistically significant.

RESULTS

Shapiro-Wilk test was done to check the normality of distribution of data, the findings showed non-significant difference (P-value > 0.05) that means the data were normally distributed.

The intraclass correlation coefficient (ICC) was used to evaluate the test-retest reliabilities of measuring the mesiodistal width of the teeth on casts and on intra oral photos; the values were scored as follows: ICC less than 0.4, poor reliability; ICC between 0.4 and 0.75, moderate reliability; and ICC greater than 0.75, excellent reliability[29)]. The ICC values in this study ranged from 0.97 to 0.99, demonstrating excellent reliability.

Independent sample t-test was used for comparison of gender difference for the dental measurements which showed significant difference only for the mesiodistal width of canines (Tables 1 and 2).

Paired samples T test was done for dental measurements of the intraoral photos and study models. All the results showed non-significance difference between the intra oral photos and study models dental measurements and no significant difference between the mesiodistal width of right and left premolars and canines with strong correlation between these measurements (Table 3).

Since, there were no significant difference and good correlation between intra-oral photo and study model dental measurements. Pearson correlation coefficient was estimated between the summation of mesiodistal width of lower incisors and upper and lower right premolars and canines from the intra oral photo dental measurements. Significant correlations were found ($P \leq .001$) between these measurements (Table 4).

Linear regression analysis was estimated for 70% of the collected data after designing the Neural Network. It showed high coefficients of correlations between the summation of mesiodistal width of right premolars and canines of the targets and the actual output after designing the Neural Network during the training process ($R = 0.831$ during training part, $R = 0.898$ during testing part of training process and $R = 0.840$ as a whole) (Figure 2) with best training performance which was 0.44681 at epoch 6 (Figure 3).

Following testing process linear regression analysis was estimated for the other 30% of the collected data after designing the Neural

Table 1: Gender difference for the Intra-oral photo dental measurements

Measurements of intra-oral photos	Male subjects n = (41)				Female subjects n = (53)				T test	P value	Significance
	Min	Max	Mean	SD	Min	Max	Mean	SD			
MD of lower right central incisors	4.72	6.40	5.50	0.40	4.82	6.66	5.56	0.42	-0.62	0.53	NS
MD of lower right central incisors	5.51	7.03	6.07	0.39	5.32	6.86	5.99	0.41	0.92	0.36	NS
MD of lower right canines	6.27	8.01	7.03	0.40	5.60	7.37	6.73	0.37	3.64	0.00	S
MD of lower right 1st premolars	5.70	7.80	7.07	0.47	6.00	8.34	7.02	0.53	0.40	0.69	NS
MD of lower right 2nd premolars	6.25	8.22	7.13	0.51	5.97	8.57	6.88	0.47	2.34	0.12	NS
MD of lower left central incisors	4.88	6.71	5.58	0.39	4.89	6.60	5.59	0.42	-0.08	0.94	NS
MD of lower left lateral incisors	5.44	7.33	6.17	0.43	5.16	6.76	6.05	0.39	1.33	0.19	NS
MD of lower left canines	6.43	8.00	7.08	0.40	5.76	7.33	6.67	0.38	5.00	0.00	S
MD of lower left 1st premolars	5.82	7.75	7.12	0.50	6.14	8.21	6.98	0.48	1.29	0.20	NS
MD of lower left 2nd premolars	6.29	8.67	7.21	0.49	5.91	8.44	6.99	0.53	2.02	0.15	NS
MD of upper right canines	6.98	8.90	7.64	0.36	6.64	8.10	7.27	0.29	5.31	0.00	S
MD of upper right 1st premolars	6.10	8.50	6.89	0.46	6.10	7.54	6.78	0.37	1.25	0.21	NS
MD of upper right 2nd premolars	6.00	8.40	6.95	0.51	6.20	7.77	6.81	0.37	1.48	0.14	NS
MD of upper left canines	6.88	8.60	7.60	0.32	6.71	7.98	7.30	0.29	4.53	0.00	S
MD of upper left 1st premolars	6.13	8.21	6.89	0.44	6.00	7.54	6.80	0.35	1.07	0.29	NS
MD of upper left 2nd premolars	6.12	8.33	6.94	0.48	6.27	7.72	6.82	0.36	1.26	0.21	NS

*MD indicates mesiodistal width of teeth; min: minimum; max: maximum; SD: standard deviation.

Table 2: Gender difference for the dental casts measurements.

Measurements of dental casts	Male subjects n = (41)				Female subjects n = (53)				T test	P value	Significance
	Min	Max	Mean	SD	Min	Max	Mean	SD			
MD of lower right central incisors	4.75	6.60	5.51	0.40	4.85	6.62	5.55	0.41	-0.40	0.69	NS
MD of lower right central incisors	5.50	7.04	6.07	0.39	5.30	6.85	5.98	0.40	1.13	0.26	NS
MD of lower right canines	5.85	7.96	6.98	0.48	5.63	7.37	6.72	0.36	2.97	0.00	S
MD of lower right 1st premolars	5.75	7.91	7.08	0.46	6.03	8.20	7.02	0.51	0.57	0.57	NS
MD of lower right 2nd premolars	6.25	8.22	7.12	0.50	5.95	8.27	6.91	0.46	2.13	0.14	NS
MD of lower left central incisors	4.88	6.63	5.58	0.37	4.90	6.61	5.56	0.41	0.26	0.79	NS
MD of lower left lateral incisors	5.46	7.30	6.17	0.41	5.16	6.73	6.03	0.38	1.60	0.11	NS
MD of lower left canines	6.40	8.00	7.08	0.40	5.76	7.78	6.69	0.40	4.61	0.00	S
MD of lower left 1st premolars	5.80	7.75	7.11	0.50	6.20	8.20	6.97	0.47	1.37	0.17	NS
MD of lower left 2nd premolars	6.30	8.65	7.21	0.49	5.88	8.40	6.96	0.54	0.66	0.13	NS
MD of upper right canines	6.99	8.93	7.63	0.36	6.67	7.99	7.26	0.30	5.34	0.00	S
MD of upper right 1st premolars	6.10	8.55	6.92	0.47	6.11	7.45	6.79	0.35	1.53	0.13	NS
MD of upper right 2nd premolars	6.10	8.41	6.95	0.51	6.23	7.70	6.83	0.37	1.34	0.18	NS
MD of upper left canines	6.87	8.55	7.60	0.32	6.72	7.88	7.29	0.29	4.67	0.00	S
MD of upper left 1st premolars	6.14	8.23	6.91	0.43	6.10	7.43	6.83	0.34	0.92	0.36	NS
MD of upper left 2nd premolars	6.14	8.32	6.94	0.50	6.21	7.70	6.84	0.36	1.15	0.25	NS

*MD indicates mesiodistal width of teeth; min: minimum; max: maximum; SD: standard deviation.

Network. It showed high coefficients of correlations between the summation of mesiodistal width of right premolars and canines of the targets and the actual output R = 0.90794 (Figure 4).

DISCUSSION

One of the most important aspects of odontometric studies is reliability of measurement, which is the ability to obtain the same measurement consistently over sequential measures[30]. In order to improve the reliability of the measurements studied herein, the following steps were employed: using high-quality dental stone for casts fabrication, using of digital calipers to greatly reduce the possibility of reading error and eye fatigue, and assessing intra-examiner variability using inter class correlation coefficient[31].

Therefore, any differences in the mesiodistal tooth widths, if observed, would result from the tooth size variability in the present sample and the prediction methods studied.

In this study, there were no statistically significant differences between the left and right sides. These findings indicate that the right or the left side measurements could be used to represent the mesiodistal tooth widths for this sample. This finding agreed with the usual practice of using teeth on one side of the jaw, or the average of the two, for analyzing the mesiodistal widths of teeth[32,33].

The results showed that there were statistically significant differences in the tooth widths between the male and female subjects. However, the difference was only statistically significant ($p < 0.05$) for the canines. This finding agree with the results of many studies[34-36], who found that only the canines in both the jaws exhibited a significant sexual difference while the other teeth did not. In a continuation of the same studies, they also determined that there was no statistically significant

Table 3: Comparison of photo-cast and side difference for the dental measurements.

Groups		Pair t test	P value	significance	Correlation	Significance
LR centrals p	LR centrals M	-0.04	0.97	NS	0.99	0.00
LR laterals P	LR laterals M	0.86	0.39	NS	1.00	0.00
LR cainines P	LR canines M	1.29	0.20	NS	0.93	0.00
LR 1st premolars P	LR 1st premolars M	-0.19	0.85	NS	1.00	0.00
LR 2nd premolars P	LR 2nd premolars M	-1.06	0.29	NS	0.98	0.00
LL centrals P	LL centrals M	2.12	0.06	NS	0.99	0.00
LL laterals P	LL laterals M	1.16	0.25	NS	0.98	0.00
LL canines P	LL canines M	-0.85	0.40	NS	0.96	0.00
LL 1 st premolars P	LL 1st premolars M	1.96	0.07	NS	0.99	0.00
LL 2nd premolars P	LL 2nd premolars M	1.14	0.26	NS	0.97	0.00
UR canines P	UR canines M	1.58	0.12	NS	0.98	0.00
UR 1st premolars P	UR 1st premolars	-1.70	0.09	NS	0.96	0.00
UR 2nd premolars P	UR 2nd premolars M	-1.87	0.06	NS	0.99	0.00
UL canines P	UL canines M	0.88	0.38	NS	0.99	0.00
UL 1st premolars P	UL 1st premolars M	-2.43	0.06	NS	0.95	0.00
UL 2nd premolars P	UL 2nd premolars M	-1.77	0.08	NS	0.99	0.00
LR canines	LL canines	-0.62	0.53	NS	0.72	0.00
LR 1st premolars	LL 1st premolars	0.45	0.66	NS	0.79	0.00
LR 2nd premolars	LR 2nd premolars	-1.66	0.10	NS	0.73	0.00
UR canines	UL canines	-0.51	0.61	NS	0.93	0.00
UR 1st premolars	UL 1st premolars	-0.92	0.36	NS	0.88	0.00
UR 2nd premolars	UL 2nd premolars	0.15	0.88	NS	0.97	0.00

*LR indicates lower right; LL: lower left; UR: upper right; UL: upper left; P: intraoral photo; M: study model.

Table 4: Correlation coefficients between the summation of mesodistal width of right premolars, canines and mesiodistal width of lower anterior teeth.

Measurements		All subject (n = 94)		Male subjects (n = 41)		female subjects (n = 53)	
		Correlation	Significance	Correlation	Significance	Correlation	Significance
MD of lower incisors	MD of LR premolars and canines	0.84	0.00	0.86	0.00	0.80	0.00
MD of lower incisors	MD of UR premolars and canines	0.83	0.00	0.85	0.00	0.86	0.00

*MD indicates mesiodistal width of teeth; LR: lower right; UR: upper right.

difference between the left and right sides suggesting that measurements of teeth on one side could be truly representative when the corresponding measurements on other side was unobtainable.

In contrast to other studies which showed that there were statistically significant differences in the tooth widths between the male and female subjects for all teeth in both mandibular and maxillary dental arches[37,39]. Moreover, the measurements obtained from the photographs demonstrated a statistically significant high degree of correlation with dental cast measurements with no significant difference between them. So, in this study the measurements were obtained from the intra-oral photos. Although Vernier caliper measurements were regarded as the "gold standard," against which other measurement techniques are compared. Manual tooth-size analysis can be time-consuming in a busy practice, as well as prone to recording and calculation errors[40].

Difficulty in creating a standardized position for the mirror in this area or the angle formed between the lens and mirror when obtaining the occlusal photograph might be the result of slight difference in measurements between dental cast and intra oral photograph[41]. However, this method shows excellent reliability and only minor errors. These data emphasize using this method as a reliable way of obtaining tooth size and dental arch dimensions. The present study is in accordance with many studies[41,24].

Equations developed by data collected from one ethnic group to predict the size of unerupted permanent teeth might not be applicable to another due to racial and ethnic groups present variations in the mesiodistal widths of their permanent teeth[43,44]. Thus, In contrast, the Artificial Neural Network in this study adjusts its structure based on the training samples presented to the system. Therefore, it can be used to predict the size of unerupted teeth in different ethnic groups, provided that an appropriate training data set is presented to the system which have been documented by Moghimi *et al* in 2011[45].

To minimize overfitting and to verify the fitness of the model, the samples were randomly divided into 70% of data for learning (PTrian = 0.7) and 30% for testing from the beginning in our study. In addition, the learning set was divided into the training set and the testing set and all set to make a generalized model. As a result of this, the success rate of the model was better generalized; this has been descripted by jung and kim[46].

An ideal prediction method should result in no difference between the predicted and actual widths of the permanent canines and premolars[45]. In this study figure 6 showed high degree of correlation between the real target and the output (r = 0.90794) with best training performance which was 0.44681 at epoch 6 which make this method very accurate for prediction of un erupted teeth as compared with other conventional methods.

This study has several limitations due to the regional nature, the proposed prediction method was only tested in one ethnic group. More generalized studies in different ethnic groups are needed to validate the feasibility of the proposed method.

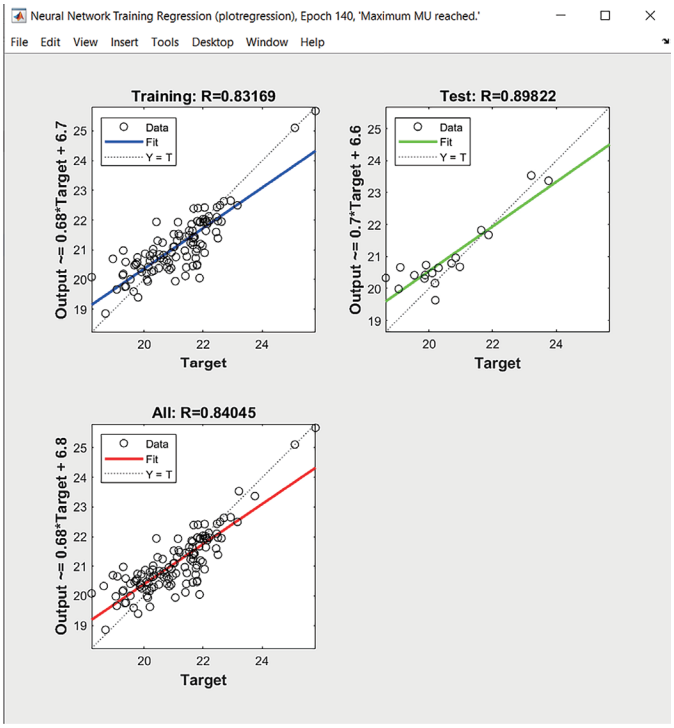

Figure 2: Scatterplots illustrating linear regression results between the summation of mesiodistal width of right premolars and canines of the targets and the actual output after designing the Neural Network during training process.

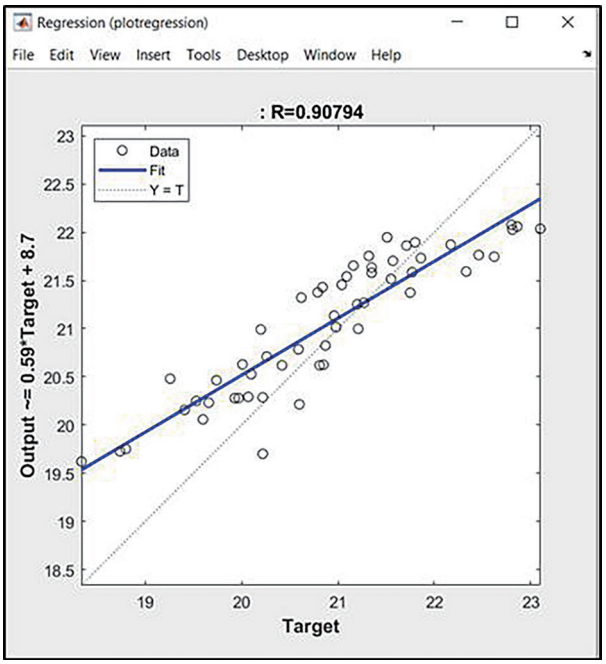

Figure 4: Scatterplot illustrating linear regression result between the summation of mesiodistal width of premolars and canines of the targets and the actual output after designing the Neural Network during testing process.

CONCLUSIONS

The proposed method is a promising accurate tool for forecasting the sizes of un erupted premolars and canines, particularly the architecture of this Network can be adjusted based on the data collected from different ethnic groups.

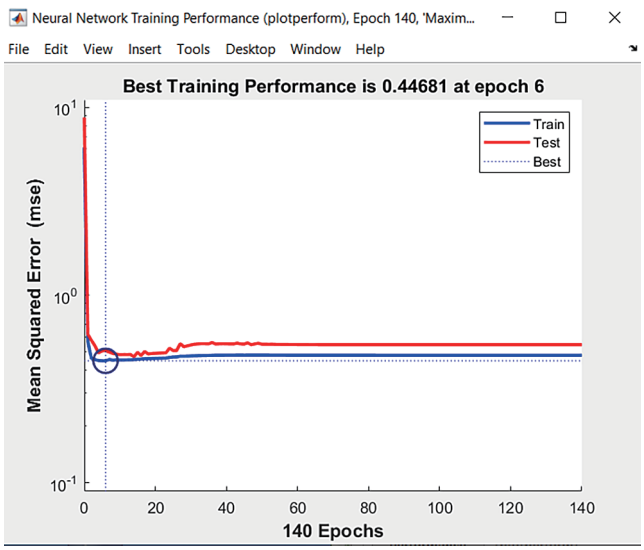

Figure 3: Best training performance for the Network between the summation of mesiodistal width of right premolars and canines of the targets and the actual output after designing the Neural Network during training process.

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Development and validation of the Arabic version of the Pain Sensitivity Questionnaire (PSQ)

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Abstract

Pain sensitivity level is correlated with the treatment outcomes for individuals. Although pain perception, patient's personality and attitude have a great role, fear of pain may halt individuals from seeking treatment. This study aimed to translate pain sensitivity questionnaires (PSQ) into the Arabic language, perform a cross-cultural adaption of the PSQ, and validate the Arabic version of PSQ in healthy patients. Methodology: The PSQ was translated forward and backward following cross-cultural adaptation guidelines. The Arabic version of the PSQ was tested on 50 healthy patients. Test-retest reliability and intraclass correlation coefficient was used to check the reliability, additionally; Cronbach's alpha and factor analysis was used to estimate the internal consistency. Results: The mean PSQ-total, PSQ-moderate, and PSQ-minor were 4.86 ± 1.54, 6.12 ± 1.62, and 3.56 ± 1.79 respectively. A very good internal consistency was shown for the PSQ- total, PSQ-moderate, and PSQ-minor of the Arabic version as determined by Cronbach's Alpha (0.918, 0.881, and 0.867, respectively). For convergent validity, the PSQ scores of the Arabic version showed significant correlations with pain catastrophizing scale (PCS) ($r = 0.506$, $P < 0.001$; $r = 0.466$, $P = 0.001$; $r = 0.407$, $P = 0.003$ for PSQ-total, PSQ-moderate, and PSQ-minor of the Arabic version, respectively). For test–retest reliability which was evaluated in an interval of 4 weeks, the intraclass correlation coefficients were 0.928, 0.948 and 0.842 for PSQ-total, PSQ-moderate, and PSQ-minor respectively. Conclusions: the validated current Arabic version of PSQ is a cross-culturally equivalent and reliable tool for pain sensitivity assessment.

Keywords: Pain, pain sensitivity questionnaire, cross-cultural, validity, reliability, Arabic, Arabic translation, pain sensitivity, assessment of pain

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CLINICAL PHARMACY

Effect of Accelerated Canine Retraction by Vitamin D3 Local Administration on Apical Root Resorption, Alveolar Bone Integrity, and Chair-side Time: A Prospective Clinical Study

Noor R. Al-Hasani[1)], Ali I. Ibrahim[2)]

ABSTRACT

Objectives: **To evaluate the effect of vitamin D3 local injections on apical root resorption, alveolar bone integrity, and chair-side time following three and six months of canine retraction.**

Subjects and Methods: **Seventeen adult patients (18-35 years old) of class I and II malocclusions were recruited, who required bilateral maxillary 1st premolars extraction before starting maxillary canines retraction. The experimental side received 25 pg dose of vitamin D3 injected locally into the distal periodontal sulcus of the canine (before force application) every three weeks, while the control side received retraction force only. Periapical radiographic evaluation was conducted after 3 and 6 months of the start of canines' retraction.**

Results: **At both time points (3 and 6 months), radiographic findings revealed uniform alveolar bone contour with non-significant differences ($p > 0.05$) between the experimental and control sides regarding canine root resorption scores. The experimental side exhibited better alveolar bone turnover as indicated by well-defined periodontal ligament widening. However, vitamin D3 injection entailed four minutes extra chair-side time.**

Conclusion: **Enhancement of canine retraction by local injection of vitamin D3 induced better periodontal response, does not impose detrimental apical root resorption nor adversely affect the alveolar bone integrity, and the procedure does not entail significant extra chair-side time.**

KEY WORDS

vitamin D_3 injection, accelerated orthodontics, root resorption, alveolar bone integrity

INTRODUCTION

Orthodontic treatment (OT) can induce significant impact on the psychological and social well-being of the patients via correcting teeth disorders, function and facial aesthetics[1)]. However, OT is characterized as a lengthy procedure, in addition to other drawbacks such as caries, root resorption, gingival, alveolar bone and periodontal adverse effects[2,3)]. Accordingly, enormous effort has been harnessed to shorten the OT duration using either surgical or non-surgical approaches, with emphasis to reduce the adverse effects, cost and chair-side time[4)]. The surgical approach is known for its invasiveness and, hence, not preferred by most orthodontic patients. On the other hand, most non-surgical methods are premised on the use of biological materials as a locally administered medication, with no or minimal local and/or systemic side effects[5)].

Although literature is replete with studies investigating the rate of orthodontic tooth movement following local administration of corticosteroids, thyroxine and prostaglandins, most of these studies were conducted on animals[4,6,7)]. In contrast, trials of using vitamin D to accelerate tooth movement involved both animal and fewer human studies, albeit the effect was merely investigated on short-term basis[8-10)]. These studies reported various rates of accelerated tooth movement post local administration of vitamin D. The first clinical study on humans was conducted in 2011, in which three doses of vitamin D3 were used: 15, 25, and 40 pg/0.2 mL. The local injection of 25 pg/0.2 mL recorded a faster tooth movement (51%) in the experimental side in comparison to the control side than the other tested doses[6)].

Orthodontic tooth movement using conventional techniques can induce trauma to the teeth and surrounding tissues such as apical root resorption, loss of alveolar bone integrity and damage of the periodontium[3,11-13)]. In addition, it has been assumed that techniques concerned with accelerating tooth movement can lead to root resorption and periodontal trauma in a higher rate[14,15)]. Although the use of 25 pg/0.2 mL local injection of calcitriol (vitamin D_3) was reported successful in accelerating tooth movement[8)], the results were based on 4 weeks follow-up duration without evaluating the extra chair-side time and the possible damaging effects on the root and alveolar bone. Therefore, the aim of this study was to evaluate the long-term effect of vitamin D_3 injection on the apical root resorption and integrity of the alveolar bone at two time points: after 3 and 6 months of the start of canines' retraction stage. In addition to calculating the average extra chair-side time required to perform the local administration procedure. The null hypothesis stated that acceleration of canine retraction by local injection of vitamin D_3 does not exacerbate apical root resorption nor adversely affect the alveolar bone integrity, and the procedure does not entail sig-

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nificant extra chair-side time.

SUBJECTS AND METHODS

The study involved 30 adult patients (18-35 years old) who sought accelerated orthodontic treatment to be conducted at Baghdad Teaching Hospital (College of Dentistry) and a private clinic in Baghdad, Iraq for the duration from December 2019 to July 2021. However, only 17 patients were managed and followed up according to the study design due to the frequent lock-down periods imposed by the COVID-19 pandemic, which started in Iraq in March 2020.

Based on previous study findings, which involved blood samples collection after vitamin D3 (calcitriol) local injections[8], all patients were informed about the safety and non-significant systemic influence of locally administered calcitriol, and signed consent forms were obtained with an ethical approval (Reference number: 094419) by the College of Dentistry, University of Baghdad. Patients of class I and II malocclusions were recruited, with a treatment plan that requires bilateral maxillary 1st premolars removal followed by maxillary canines retraction to close the extraction space. The selected patients had no history of systemic disease or craniofacial deformities, no previous orthodontic treatment, and no history of chronic use of medications. Panoramic radiographs were taken before treatment to confirm healthy periodontium, alveolar bone and no root resorption.

Pre-adjusted orthodontic brackets (stainless steel MBT 0.022" system, Pinnacle, Ortho Technology, USA) were used for all patients. After leveling and alignment phase, maxillary canine retraction commenced in the second phase using rectangular (0.017 x 0.025") stainless steel archwire with a distalizing force (applied using transparent elastomeric chain, Ortho Technology, USA) of 200 g measured by a pressure gauge.

Vitamin D_3 (1,25-dihydroxyvitamine D_3, the active form of vitamin D_3) ampoules (Calcitriol, Mibe, Germany) were used to prepare a diluted dose (25 pg) for local injections. Dimethylsulfoxide (0.2 ml) (DMSO, Bisolve B.V., Netherlands) was used for dilution and worked as a vehicle[8].The maxillary arch of each patient was divided into experimental (right side) and control (left side). The 25 pg dose of calcitriol was injected locally into the distal periodontal sulcus of the maxillary right canine before retraction force application, while the maxillary left canine received orthodontic retraction force only as a control. The local injections were administered on each orthodontic visit (every three weeks) until extraction space closure (an average duration of six months). A stopwatch was used to calculate the average time required for preparing and injecting the calcitriol on each visit.

Radiographic Evaluation

A high quality portable X-ray camera (Remex K100, Korea) was used at (70 kV, 2 mA) to evaluate the long-term effect of calcitriol injection on apical root resorption and integrity of the alveolar bone at two time points: after 3 months of the start of canines' retraction stage and at the end of extraction space closure (an average of six months). Evaluation of apical root resorption was conducted using the scoring system of Levander and Malemgren[16] (figure 1):

0: no root resorption.
1: mild resorption, where the root is of normal length and has an irregular contour.
2: moderate resorption, with small areas of root loss and the apex having almost a straight contour.
3: severe resorption, with a loss of almost one third of the root length.
4: extreme resorption, with a loss of more than one third of the root length.

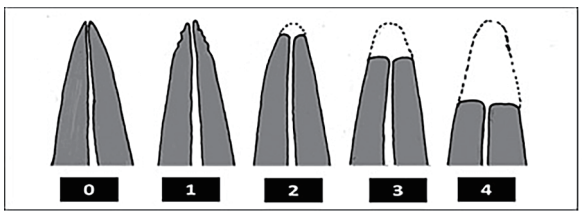

Figure 1: Root resorption index for quantitative assessment of root resorption.

Statistical Analysis

Data were statistically evaluated using the Statistical Package for Social Sciences (version 27, SPSS Inc., IBM, USA) at a level of significance $p < 0.05$. Mann-Whitney test was applied for testing the difference between the control and experimental sides regarding the radiographic root resorption scores.

RESULTS

Radiographs of five patients were selected randomly following three months of canine retraction in order to examine the root and alveolar bone areas for any deleterious effects post frequent local administration of vitamin D3, as shown in figure 2. On the other hand, figure 3 demonstrates the radiographic findings of five patients after six months of canine retraction.

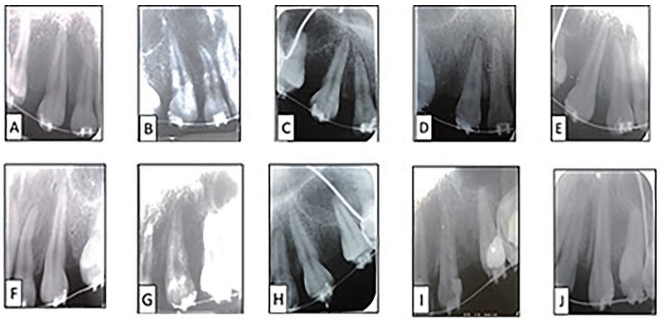

Figure 2: Radiographic evaluation of root resorption and alveolar bone integrity after 3 months of canine retraction. Top row (A-E) represents the experimental (right) side, while bottom row (F-J) represents the control (contralateral left) side of the same patient.

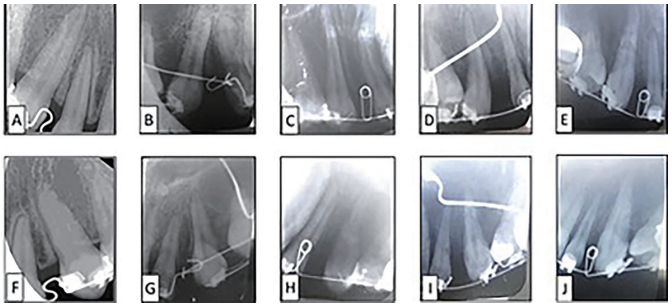

Figure 3: Radiographic evaluation of root resorption and alveolar bone integrity after 6 months of canine retraction. Top row (A-E) represents the experimental (right) side, while bottom row (F-J) represents the control (contralateral left) side of the same patient.

At both time points (3 and 6 months), three main scores were encountered (according to the scoring system of root resorption): 0, 1, and 2; with the highest frequency of score 0 indicating absence of root resorption on both control and experimental sides (Table 1).

After 3 months, the experimental (vitamin D injection) side of 6 patients exhibited score 1 (examples are shown in figure 2: A and D), while 7 patients showed score 1 on the control side (as demonstrated in figure 2: G, H, I). All other radiographs depicted score 0 (examples are shown in figure 2: B, C, E, F, J).

Following 6 months, local vitamin D administration into the experimental side of 8 patients resulted in score 1 (examples are shown in figure 3: A, B, C), and 8 patients showed score 1 on the control side (as depicted in figure 3: G and H). Score 2 was recorded by two patients on the experimental side, and by three patients on the control side (an example is shown in figure 3: F). All other radiographs yielded score 0 (examples are shown in figure 3: D, E, I, J). The statistical comparisons (Mann Whitney test) between the experimental and control sides at both time points revealed non-significant differences ($p > 0.05$) concerning

the distribution and severity of root resorption scores, i.e. the influence on canine root resorption.

Table 1: Canine root resorption scores after 3 and 6 months of retraction, with inferential statistics between the control and experimental sides.

Sequence of Patients	After 3 months			After 6 months		
	Control	Experimental	Mann Whitney Test	Control	Experimental	Mann Whitney Test
1	0	0		1	1	
2	1	0		1	0	
3	0	0		0	1	
4	1	1		1	1	
5	0	0		0	0	
6	0	0		1	0	
7	0	0		2	2	
8	1	1		2	1	
9	0	1	**U = 97.00**	1	1	**U = 92.00**
10	1	1	**p = .533**	1	2	**p = .387**
11	0	0		0	0	
12	1	0		1	0	
13	0	0		0	0	
14	0	1		0	1	
15	0	0		0	0	
16	1	1		2	1	
17	1	0		1	1	

Regarding the integrity of alveolar bone surrounding the distalized canines, the radiographs showed more well-defined periodontal ligament widening around the canines on the experimental side than the control side at both time points (figures 2 and 3). The alveolar bone contour surrounding the canines exhibited comparable uniformity on the experimental and control sides at both 3 and 6 months follow-up periods. However, the level of alveolar bone height showed radiographic variations between the right and left sides at both time points.

The calculated average time for preparing (including addition of vehicle to the active ingredient) and administering an injection of vitamin D was 4 minutes, representing the time required before placing the power chain elastic for canine retraction; versus zero minute on the control side as power chain placement was carried out immediately without any preceding injection.

DISCUSSION

This study aimed at investigating any potential damaging effect of local vitamin D3 local injections used to accelerate orthodontic canine distalization over a relatively long-term follow-up period that covers the average time required for canine movement to close an extraction space. The rationale was premised on the significant role of vitamin D3 in enhancing remodeling of bone, which can be harnessed in orthodontics to accelerate bone resorption and formation, an essential process for the success of orthodontic treatment. In 2011, the first clinical study investigated the role of vitamin D3 in accelerating canine retraction depending on a short-term (one month) follow-up[8]. Although tooth movement was shown to be accelerated, this short period of time is not enough to reveal the detrimental effects on the root and alveolar bone, if any, radiographically. Therefore, longer follow-up durations (3 and 6 months) were sought in the current study as these periods represent the average time required for active orthodontic canine movement, depending on the amount of space to be closed[17].

This study revealed no significant adverse effects on root resorption of distalized canines following frequent vitamin D local injections, as indicated by the frequency of certain root resorption scores. Non-significant differences were found between the control and experimental sides in terms of the distribution and severity of root resorption scores. Although the root resorption scoring system adopted in this study was composed of 5 categories[16], only three categories (scores 0 through 2) were recorded by both the control and experimental sides, with the majority of score 0 (no root resorption) and absence of severe root resorption categories (scores 3 and 4). The description of these scores (0, 1, and 2) has been commonly reported in previous studies and described as a normal reversible phenomenon accompanying orthodontic tooth movement[16,18-20]. This finding came in agreement with Ciur *et al* (2016) who studied the effect of vitamin D_3 (0.2 mL of 42 pg/mL) on the rate of tooth movement during orthodontic treatment and reported minimal root resorption. However, their study did not include a standardized method for root resorption assessment, and involved a very small sample size (6 patients only)[21]. In another study conducted on rats by Seifi et al, they used prostaglandin E2 alone versus a combination of thyroxine and prostaglandin E2 to boost orthodontic tooth movement. They reported acceleration in tooth movement with both formulations; however, prostaglandin E2 alone yielded more destructive effect on the root in comparison with the combination of thyroxine and prostaglandin E2, which showed minimal root resorption[22]. It has been previously reported that thyroxine can induce a protective effect on the root surface[23]. Therefore, tooth movement can be accelerated without deleterious effects on the root, depending on the active ingredient used to enhance the movement.

In comparison with the control side, the radiographs of the current study showed more well-defined periodontal ligament widening around the distalized canines on the experimental side at both 3 and 6 months follow-up periods, with almost identical uniformity of the alveolar bone contour. The level of alveolar bone height showed radiographic variations between the right and left sides at both time points, which reflects the normal individual variation as all the teeth were checked clinically and radiographically (by routine panoramic radiographs) for any alveolar bone destruction and tooth mobility before starting the treatment. These findings can be explained by the significant role of vitamin D_3 in the process of bone turnover. It has been reported that vitamin D_3 supports the bone remodeling effect by providing a good balance between the osteoclast and osteoblast activation in the bone[24,25] and alveolar bone[9,10,26,27]. In addition, previous studies showed that intraligamentous and submucosal injections of an active metabolite of vitamin D_3 caused an increment in the osteoclasts number leading to a greater tooth movement during canine retraction with light forces in cats and rats, respectively[9,26]. On the other hand, it has been found that 1,25-dihydroxyvitamin D_3 can enhance orthodontic movement retention by augmenting the osteoblasts role in bone deposition at the tension side[28]; hence, it has a dual effect as it contributes to both bone resorption and deposition.

To study the dual effect of vitamin D_3, Kale *et al* (2004) compared the effect of administrating prostaglandin and the active form of vitamin D_3 on tooth movement in rats, which was markedly increased in comparison to the controls. However, they found that the effect of vitamin D_3 on the differentiation and number of osteoclast on the tension side surpassed the effect of the prostaglandin[10]. Moreover, another study tested the effect of the active form of vitamin D_3 on bone remodeling in rats during orthodontic tooth movement, and recorded an increment in the mineral appositional rate on the alveolar bone surface, suggesting that locally administered vitamin D could strengthen and reinstate the supporting tissue, specifically the alveolar bone[27]. These findings support the outcomes of the current clinical study, which confirmed that the active metabolite of vitamin D_3 could boost a healthy tooth movement by balancing the bone resorption and deposition processes during orthodontic treatment. Radiographic evaluation of changes in the alveolar bone has been reported as a reliable method for clinical assessment of bone remodeling around the teeth[29,30].

Local injections of vitamin D into the periodontal ligament is a simple procedure that does not require special training; however, pain and the need for slow injection of the medicine were reported as the main drawbacks[8]. In addition, the current study estimated the average chairside time for preparing (including addition of vehicle to the active ingredient) and administering an injection of vitamin D, which was around 4 minutes. This extra time is non-significant when considering the benefit of acceleration in tooth movement, reported to be 51% faster canine retraction in the experimental side in contrast to the control side[8]. For proper release and distribution of the active ingredient (1,25-dihydroxyvitamin D_3), a vehicle solution (DMSO) was mandatory to be added before injection, a step that necessitated extra time. Therefore, future work to prepare a formulation of a prolonged shelf-life and stability that contains vitamin D3 mixed with the vehicle is recommended to

reduce the time required for injection preparation.

CONCLUSION

Based on radiographic findings, it was concluded that acceleration of canine retraction by local injection of vitamin D3 enhanced the periodontal ligament response, does not exacerbate apical root resorption nor adversely affect the alveolar bone integrity, and the procedure does not entail significant extra chair-side time.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interests.

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Knowledge, Attitude, and Practice of Infection Control by Dental Students at Pedodontic Clinic, College of Dentistry, University of Baghdad, Iraq

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Knowledge, Attitude, and Practice of Infection Control by Dental Students at Pedodontic Clinic, College of Dentistry, University of Baghdad, Iraq

Mohammed Kahtan Salih

ABSTRACT

Objectives: **This study explored knowledge, attitude, and practice of infection control by dental students at College of Dentistry/ University of Baghdad, Iraq.**

Material and Methods: **Three hundred dental students participated in this study. A self administrated questionnaire with 21 close ended questions related to use of personal protective equipments, infection control awareness, vaccination status, percutaneous exposures, and attitude towards treatment of patients with hepatitis B (HBV)/ or human immunodeficiency virus (HIV) was distributed to dental students. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 21. Fisher exact and Chi-square test were used with significance level set to 0.05.**

Results: **The response rate was high (92%). The majority of students received hepatitis B vaccine (undergraduates 84.04% and postgraduates 91.11%). Needle stick injuries was the most common type for percutanuous injuries with larger number of injuries occurred among postgraduates (85.56%) in comparison to (55.85%) in undergraduates ($p < 0.05$). Majority of students used personal protective equipments, but only a small proportion of them washed their hands before donating gloves. There was a significant difference ($P = 0.021$) between undergraduates (15.96%) and postgraduates (27.78%) attitude towards treatment of patient with history of HBV or HIV.**

Conclusion: **More efforts must be spent to improve students' attitude and compliance with infection control policy. Also, a continuous and rigorous assessment of students' knowledge and practice of infection control measure must be done to ensure that the best is delivered by our dental students while treating their patients.**

KEY WORDS

infection control, dental students, knowledge and attitude, survey

INTRODUCTION

At dental surgery both patients and dentists are at risk of exposure to highly infective pathogens. This may include a variety of viruses like herpes virus, hepatitis B and C virus, cytomegalovirus and human immunodeficiency virus as well as a wide range of pathogenic microorganisms that habitat the oral cavity and respiratory tract like mycobacterium tuberculosis, staphylococcus and streptococcus species[1]. Pathogenic microbes can be transmitted in the dental setting via one or more routes. This can occur either in a direct way by contact with blood, saliva or other body fluids or indirectly by contacting contaminated surface and instruments as well as sharp injuries or mucous membrane splashes. Also airborne infection can occurs as a result of inhalation of droplets and aerosol that are held in air of dental surgery which develop from either dental procedures that produce aerosol (for example scaling) or as a result of patient sneezing and coughing[2,3].

The United States centre for disease control has published guidelines to aid in control of infection in dental setting and a standard precaution measures were applied as a rule for the general patient care. The use of barriers, gowns and other personal protective appliances (PPA) was advised and regarded as an essential element to control infection in dental surgery[4]. Hand hygiene is also considered as a core element in preventing cross contamination. That is why dental health care givers must wash their hands with antimicrobial soap or rub them with detergents when they become soiled; before putting gloves and after removing them in order to prevent cross contamination[5].

It has been shown that gloves are useful barrier that protect cross contamination between patients and dentist. There were reported cases of dentists who captured hepatitis B virus as a result of treating patients without using gloves as well as cases where a carrier dentist infected his patients. Blood products were found under finger nails of dentists who did not use gloves while treating their patients after five days[6]. Since dental treatment to some extent may involve contact and breach of patient mucous membrane and gingival tissues which imply a medium to high risk chance of cross contamination thus there is a need to sterilize instruments and the health care workers must adhere to strict cross infection prevention protocols particularly the use of gloves and autoclave[7]. The world health organization has published an information leaflet on gloves use with description of proper practice and safe placement and disposal of gloves while caring for patient. The gloves should be used once for each patient and disposed as soon as possible whenever the care for patient is ended. Also, they described the use of double gloves as an appropriate practice especially in countries where high prevalence of hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) where a prolonged surgical pro-

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Table 1: Students distribution according to gender and academic level.

Academic level	Gender		
	Male	Female	Total
Undergraduates	40 (21.3%)	148(78.7%)	188(67.6 %)
Postgraduates	32 (35.6%)	58 (64.4%)	90(32.4 %)
Total	72 (25.9%)	206 (74.1%)	278(100 %)

Table 2: Vaccination status for both undergraduates and postgraduates students.

	Undergraduate vaccination	Postgraduate vaccination	p-value vaccinated	Total male vaccinated	Total female	p-value
yes	158 (84.04%)	82 (91.11%)	0.108	55 (76.39%)	185 (89.81%)	0.004
no	30 (15.96%)	8 (8.89%)		17 (23.61%)	21 (10.19%)	

Table 3: Student responses to questions related to needle stick injuries (Undergraduates = UG; Postgraduates = PG).

Questions related to needle stick injuries	UG	PG	*P* - value	Gender		*P* - value
				male	female	
Have you got needle stick injury before?						
Yes	55.85%	85.56%	0.000	65.28%	65.53%	0.000
No	44.15%	14.44%		34.72%	34.47%	
Frequency of needle stick injury:						
once or twice a year	82.86%	76.62%	0.297	82.98%	79.26%	0.581
more than 3 times a year	17.14%	23.38%		17.02%	20/74%	
Reason for needle stick injuries:						
not paying attention while recapping needle	69.52%	77.92%	0.207	74.47%	72.59%	0.803
became nervous because of uncooperative child	30.48%	22.08%		25.53%	27.41%	

Use of personal protective equipments (PPE)

Table 4: Students response to questions related to use of personal protective equipments (undergraduate = UG, postgraduate = PG). *Fisher exact.

Questions related to use of PPE	UG	PG	*P*-value	Gender		*P*-value
				Male	female	
Wearing gloves						
yes	97.34%	100%	0.118	98.61%	98.06%	1.00*
no	2.66%	-		1.39%	1.94%	
Wearing double gloves						
yes	15.96%	35.56%	0.000	41.67%	15.53%	0.000
no	84.04%	64.44%		58.33%	84.45%	
Changing gloves between patients						
yes	95.21%	91.11%	0.182	94.44%	93.69%	1.00*
no	4.79%	8.89%		5.56%	6.31%	
Wearing mask						
yes	95.74%	82.22%	0.000	87.50%	92.72%	0.175
no	4.26%	17.78%		12.50%	7.28%	
Wearing scrub or lab. Coat						
yes	96.81%	90%	0.025*	91.67%	95.63%	0.227*
no	3.19%	10%		8.33%	4.37%	
Providing patients with safety goggles						
yes	9.57%	15.56%	0.144	8.33%	12.62%	0.326
no	90.43%	84.44%		91.97%	87.38%	

cedure or contact with large amount of body fluids or blood is anticipated[8]. The Occupational Safety and Health Administration (OSHA) sanitation standard with respect to eating and drinking in workplace areas has prohibited drinking and eating in premises where exposure to infectious or toxic materials is expected[9]. Since dental health care workers are at high risk of HBV then vaccination is very important not only to prevent infection and possibility of chronic liver disease but also to limit the chance of providing a carrier pool that may transmit the disease to susceptible subjects[10].

There is a need to train dental health care staff about proper management of medical wastes. Many regulated waste can be generated at dental surgery and they may pose a danger for environment as well as to personnel who come in contact with, if not dealt with properly. The center for disease control has issued a guideline for Environmental Infection Control in Health-Care Facilities where segregation and labeling of medical waste is mandatory and detailed and safe disposal methods are explained[11].

At teaching dental hospital where a large number of patients are being treated with less experienced undergraduate students there is a need to focus on infection control policies and to strictly adhere to infection control guidelines. More efforts and time must be spent on student training in infection control as there is increased risk of cross contamination compared to more experienced dentist and hence they may harm themselves and / or their patients[12].

The aim of this study was to investigate knowledge, attitude, and practice of infection control by dental students at Pedodontic Clinic, College of Dentistry/ University of Baghdad, Iraq.

SUBJECTS AND METHODS

Ethical approval was obtained from College of Dentistry / University of Baghdad. The study was conducted at Department of Pedodontics and Preventive dentistry / College of Dentistry/ University of Baghdad in October 2017. Students were fully informed and participated voluntarily. The sample comprised the 5th year undergraduate as well as postgraduate students whom were treating patients in the pedodontics clinic. The sample size was 300 (n = 200 undergraduates, and n = 100 postgraduates). The questionnaire was distributed to students in the classroom and after ten minutes was collected.

A self administrated questionnaire that included twenty one close ended questions related to use of barriers and other personal protective equipments, infection control practice and awareness, safe disposal of dental wastes, HBV vaccination status, percutaneous exposure, and attitude towards treating patients with HBV or HIV. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 21. Fisher exact and Chi-square test were used with significance level set to 0.05.

RESULTS

The total number of respondent was 278 (188 undergraduate and 90 postgraduate students) with response rate of 92%. The gender distribution and academic level is shown in Table 1.

Table 5: Student practices' and awareness towards spread of infection as well as attitude towards treatment of patients with infectious diseases.

Variables	UG	PG	*P* - value	Gender		*P* - value
				male	female	
Hand washing before placing gloves						
Yes	34.57%	57.78%	0.000	48.61%	39.81%	0.193
No	65.43%	42.22%		51.39%	60.19%	
Hand washing after gloves removal						
Yes	81.91%	76.67%	0.304	77.78%	81.07%	0.546
No	18.09%	23.33%		22.22%	18.93%	
Dental bracket table disinfection before towel placement						
Yes	63.83%	77.78%	0.019	68.06%	68.45%	0.951
No	36.17%	22.22%		31.94%	31.55%	
high speed and low speed handpiece disinfection						
Yes	83.51%	83.33%	0.970	77.78%	85.44%	0.132
No	16.49%	16.67%		22.22%	14.56%	
Use of mobile phone while gloves on during patient care						
Yes	51.06%	38.89%	0.057	45.83%	47.57%	0.799
No	48,94%	61.11%		54.17%	52.43%	
Eating and drinking in clinical premises						
Yes	14.89%	30%	0.003	22.22%	18.93%	0.546
No	85.11%	70%		77.78%	81.07%	
Would you treat patient with history of HIV or Hepatitis B.						
Yes	15.96%	27.78%	0.021	26.39%	17.48%	0.102
No	84.04%	72.22%		73.61%	82.52%	

Vaccination status and percutaneous injuries

The majority of students received HBV vaccine. 84.04% of undergraduates in comparison to 91.11% of postgraduates (Table 2). There was a significant difference between genders with respect to HBV vaccine (p - value = 0.004).

The main reason reported for percutaneous injuries was needle stick injury. The percent in undergraduates was 55.85% while in postgraduates it was 85.56% with a significant difference between them ($p < 0.05$). 71.22% of both undergraduates and post graduates students reported to recap the local anesthetic needles using both hands rather than single hand pen grasp technique. Table 3 shows student responses to questions related to needle stick injuries.

Use of personal protective equipments (PPE)

All postgraduate students (100%) reported to use gloves compared to 97.34% of undergraduates with no significant difference between them. The use of double gloves was done by small percent of undergraduates (15.96%) compared to (35.56%) of postgraduates with significant difference between them ($P < 0.05$). A small percent (6.12%) of undergraduates and postgraduates failed to comply with infection control policy and they reported not to change gloves between patients. The use of face mask and scrubs while treating patients was slightly higher in undergraduates compared to postgraduates students with significant difference between them. Unfortunately, the majority of students (88.49%) failed to provide their patients with safety goggles. The students' replies to use of (PPE) are shown in Table 4.

Practices, attitude and awareness towards spread of infection

A small number of students reported to wash their hands before placement of gloves. The difference was significant ($p = 0.000$) between undergraduates and post graduates. At end of treatment more students reported to wash their hands after gloves removal. The students reported to wipe the dental bracket table with 70% isopropyl alcohol disinfectant before placement of towels and this was done by 63.83% of undergraduates compared to 77.78% of postgraduates with significant difference between them ($p = 0.19$). The high speed and low speed handpiece were only disinfected by wiping them with 70% isopropyl alcohol and this was reported by 83.45% of both undergraduates and postgraduates students. With respect to mobile phone, 51.06% of the undergraduates reported to touch or answer their mobile phone while their gloves on during patient care compared to 38.89% of postgraduates. Despite that eating and drinking is prohibited in the clinical premises but some students did not abide with the regulation. 14.89% and 30% of undergraduates and postgraduates respectively reported to eat in dental surgery. The majority of postgraduate and undergraduate students reported that they would not treat any patient with history of HBV or HIV ($P = 0.021$). Table 5 shows student practices' and awareness towards spread of infection.

Table 6: Handling of dental wastes by undergraduates (UG) and postgraduates(PG).

Questions related to handling of medical and dental wastes.	UG	PG	*P*- value	Gender		*P*- value
				Male	female	
Amalgam waste separation						
Yes	15.96%	21.11%	0.291	19.44%	16.99%	0.638
No	84.04%	78.89%		80.65%	83.01%	
Sharp dental waste separation and disposal in sharps container						
Yes	32.98%	53.33%	0.001	48.61%	36.41%	0.068
No	67.02%	46.67%		51.39%	63.59%	

Handling of hazardous wastes

The student replies to questions related to handling of dental wastes are shown in Table 6. The amalgam remnants and empty amalgam capsules were separated from the rest of medical waste by very small percent (17.63%) of both undergraduates and postgraduates. The sharp instruments were disposed safely in sharp box by 32.98% of undergraduates compared to 53.33% of postgraduates ($p = 0.001$).

DISCUSSION

This was the first study to be conducted at College of Dentistry/ University of Baghdad. The aim was to evaluate students' knowledge and attitudes towards infection control and to check whether the gold standards are abided by dental students. Infection control is being taught for undergraduates as part of their curriculum in medical microbiology in their third year while it is given as an individual topic in the curriculum of postgraduates. The undergraduate students start their clinical training and treat patients when they reach to the fourth and fifth academic year. At this level they start to apply theory to practice as they are being led by their tutors at dental institute.

Hepatitis B vaccination is not mandatory for students and staff who treated patients at dental school. The total number of undergraduate and postgraduate students whom reported to receive Hepatitis B vaccine comprised 86.33% and this finding was comparable to some of studies that are held at dental schools in Middle East countries. HBV vaccination is variable worldwide. Some studies reported a high percent of vaccination like in United Arab Emirates (93.6%)[13], Saudi Arabia (80%)[14], Brazil (90.8%)[15] and Canada (100%)[16] while others reported a moderate percentage like in Yemen (71.7%)[17] and even much less like in India (38.8%)[18]. It was reported that non sterile occupational injuries are more likely to occur in the dental setting than other medical profession probably due to small operative field, patient movement and variety of sharp instruments[15]. Also dental students are at increased risk of these injuries probably because they have less experience[16].

The non sterile occupational injuries in this study for both undergraduates and postgraduates students were 65.47%. This finding was similar to Al-Essa and Al-Mutairi study in 2017 (65%) for dental students at King Saud University[19] and lower than what was reported in the Canadian study by McCarthy *et al*. in 2000 (82%)[16] and comparable to findings by Halboub *et al*. at Sana' a University (62%)[17], Alshiddi (57%)

at prosthodontic department in King Saud University[20] and Rahman *et al*. (53.8%) at University of Sharja[13] but higher than what was reported by De Souza *et al*. (31%) in Brazil[15]. Needle Stick injuries were the main category of the non sterile occupational injuries reported by our students in this study and it was higher than what was reported in the study of Rahman *et al*. (31%)[13] and Al-Essa (21.1%)[19]. Postgraduate students in this study sustained more sharp injuries than undergraduate students (PG 85.56%, UG 55.85%). This probably because they tend to treat younger (3-5 years old) and probably uncooperative children in their clinical sessions which will add more stress on them especially when treating those children under local anesthesia without sedation. A large number of our student (71.22%) reported to use both hands to recap dental syringe which was not the correct practice. 80.22% sustained at least one or two needle stick injuries per year while 19.78% had more than three. The overall percent of students to suffer sharp injuries were 182 (65.47%) which was comparable to the study of Khader *et al*. in Jordan (66.5%)[21]. 73.08% (n = 133) of student attributed these needle injuries because of not being careful while recapping while 26.92% (n = 49) attributed this to being stressed while treating uncooperative child. This indicates that more meticulous training is needed for students to reduce these injuries and not to use both hands to recap local anesthesia and/or probably to change the armamentarium by using ultra safety dental syringe to eliminate the need for recapping.

The student compliance with use of gloves was high (98.20%) and this was similar to previous findings in studies conducted in Yemen (96.6%)[17], Saudi Arabia (99.3%)[19], (100%)[20], United Arab Emirates (100%)[13], Brazil (99.5%)[15], and Canada (100%)[16]. The use of double gloves was reported by small proportion of students (22.30%) as a precaution in case their first one was torn. Other protective barriers usage like face mask (91.37%), laboratory coat and/or scrubs (94.60%) were also high and this was in agreement with previous studies[14,16,19,20]. 93.88% of the students reported to change their gloves between patients and this was slightly lower than what was reported in the previous studies from Saudia Arabia[19] and Yemen[17]. The Occupational Safety and Health Administration (OSHA) standard for eye and face protection necessitates the provision of protective equipments whenever the eye or face is subjected to hazards from flying particles, molten metal, liquid chemicals, acids or caustic liquids, chemical gases or vapors, or potentially injurious light radiation[22]. Unfortunately, a small percent (11.31%) of both undergraduates and post graduates students provided their patients with safety goggles. This means that the health and safety rules are in need to be reinforced in the dental setting and students' need to be aware of patients' eyes safety.

Hand hygiene is considered as one of the most effective measures used to reduce spread of infection by health care workers[23]. It has been shown that hands of health care providers could be a reservoir for many pathogens[24] including those that are drug resistant[25]. Although there was a significant difference (p = 0.000) in hand hygiene practice between postgraduates (57.78%) and undergraduate students (34.57%), but the compliance was still poor. This was much less than what was reported by Singh *et al*. in India (95.5%)[18] and McCarthy and Britton (90.9%) in Canada[16] and comparable to what was reported in a study from Germany by Mutters *et al*.[23]. Hand hygiene practice by undergraduates and post graduates students tend to increase after removal of gloves (80.22%). This finding is comparable to the results from the study of McCarthy and Britton (84.8%) in Canada[16] and is higher than what was reported in the study from Saudia Arabia (67%)[19], UAE (47.9%)[13], Yemen (43%)[17] and Nigeria (46.7%)[26]. It has been shown that the operatory surfaces and nearby bench top or bracket table can be contaminated by aerosol produced during dental procedures and once germs are settled they can survive for an extended period of time in which case these surfaces can become a potential source for cross infection unless they are being disinfected or sterilized[27]. 68.35% of students (PG = 77.78%; UG = 63.83%; *P value* = 0.019) reported to disinfect the dental bracket before placement of towel, however this was less than what was reported (98.3%-100%) by Ogden *et al*.[28] in their study in Dundee, UK. 83.45% of students reported to disinfect dental handpiece by use of 70% isopropyl alcohol. This practice is still considered unsafe as research has shown incomplete eradication of microbes even those whom were susceptible to alcohol[29]. Wiping the external surface of dental handpiece and omitting the complexity of its internal structure which might become contaminated with infectious materials that if not properly cleaned and sterilized may pose a serious danger and cause cross infection if used for the next patient[30].

Studies showed that potentially hazardous microbes can colonize on electronic devices like pagers, personal digital assistant and mobile phones that are used by health care providers. These devices can become a possible source for cross contamination if not cleaned and disinfected properly[31-33]. About half of the students (47.12%) reported to touch their mobile phone while they were providing dental care. They used the mobile to check for time, or to make or receive a call and this was done with gloves on. This percent was much higher than what was reported by Singh *et al*. (18%). In their study they reported a microbial growth and contamination rate of staff and trainee mobile phones which varied from 95.65-100% respectively. The bacterial load (mean colony forming unit) was much higher in the trainee mobile[34].

80.22% of students refrained from eating and drinking in the clinical premises and this was similar to the findings by Gershon *et al*.[35]. The students attitude to treat patients with HBV and HIV was low (19.78%) in comparison to other studies from Yemen (54.1%)[17], KSA (66.7%)[19], UAE (44-66%)[13], and Brazil (87.8)[15].

Unfortunately, only (39.57%) reported to separate sharps from other medical wastes and this was much less than what was reported by studies from KSA (90%)[14], Maryland, USA (87.9%)[35] and Canada (90.9%)[17]. Also a small percent (17.63%) of students reported to separate amalgam wastes and empty amalgam capsules in special containers while the rest of them mixed these hazardous wastes with other clinical wastes.

CONCLUSION

The infection control represents an ever changing evidence based topic that requires continuous monitoring and follow up to ensure that the standards of health care are met. An infection control policy especially tailored for the teaching dental clinics that is routinely revised and updated must be formulated and it should implemented by all staff, students and teaching faculty. The appointment of infection control officer to ascertain that infection control rules are met may ensure a better compliance than the tutor instruction to students while supervising them during their clinical sessions. Also the teaching curriculum may need to be changed to become more student centered with 'hands-on' application together with limitation of student class size while teaching infection control. This will enhance student interest and ensure a deep learning approach.

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DENTISTRY

Surface Analysis of the PEKK Coating on the CP Ti Implant Using Laser Technique

Aseel Mohammed Al-Khafaji[1)], Thekra Ismael Hamad[2)]

ABSTRACT

Objective: **Evaluation of the poly ether keton keton polymer (PEKK) coating material on the commercial pure titanium disks (CP Ti) with or without laser surface structuring.**

Design: **In vitro experimental study of PEKK polymer coated material on the CP Ti disks with or without laser surface structuring.**

Materials and methods: **coating the surface of the commercial pure titanium (CP Ti) disks with PEKK polymer was performed via using frictional mode CO_2 laser, then the samples disks analyzed by using FESEM.**

Results: **the FESEM reveal good adherence and distribution of the PEKK coated material over the CP Ti substrate by using the frictional mode CO_2 laser at 2 watt and 6 ms pulse duration.**

Conclusion: **the frictional mode CO_2 laser considered an effective and suitable method for PEKK coating on the CP Ti substrate.**

KEY WORDS

commercial pure titanium, PEKK polymer, laser, laser surface structuring

INTRODUCTION

The titanium and its alloy can be considered as "gold standard" material for endosseous dental implants between all the available dental implant materials, they position distinguished due to its many desirable properties beside their long-term clinical survival rates for several decades. The titanium and its alloys able to interact closely with the tissue bone beside its highly biocompatible (spontaneous build-up of an inert and stable oxide surface layer)[1)].

The usage of Ti and its alloys as dental implants may be correlated with some disadvantages despite the well evidenced of its usage like, the elastic moduli difference between the titanium implant and the surrounding bone, which led to stress in the bone-implant interface and periimplant bone loss[2)], its dark grayish color[3)] and hypersensitivity to titanium[4)]. Those limitation of the titanium and its alloy coupled with the patients demanding for dental implants metal-free led to using the dental implants made from ceramic and polymer[1,3,5)]; but unfortunately, due to high young`s modulus of the ceramic led to preferring using of polymer[6)].

The dental implants surface modification, specifically the topographical, considered as an effective method for improving bioactivity of dental implants[7)]. Implant rough surface could be provided by utilizing laser that improve the osseointegration. Several studies showed that the implant surface modification by the laser technique can reduce the dental implants contamination with implants torque removal increasing after their implantation in rabbit tibia and femur[8-10)]. The laser surface modification techniques could offer better osseointegration due to formation of surface microstructures with significantly hardness enhancement, corrosion resistance, standard roughness, a high degree of purity and increasing of the oxide layer[11)]. **Bereznai *et al***. stated that the oxide layer increases extra than doubles after implant surface laser treatment[12)].

The high-performance polymers had better properties than commodity plastics. The group of high-performance polymers who had utilized in the dental field was called poly aryl ether ketones (PAEK). The PEAKs had pulled so much attention because of the feasibility of utilizing them as a substituted to metal in a wider range of applications such as removable dentures and implanted prostheses beside it's used as provisional implant abutment[13,14)]. The PEKK chemical structure was the best mechanical properties of all PAEK family. The compression strength of the PEKK was up to 80% greater than PEEK[15)].

PEKK polymer is a biocompatible material from the PAEK family like PEEK. It's used in medical implants field due to its biocompatibility beside its high mechanical strength; PEKK have high strength, high rigidity, high resistance to hydrolysis, and suitable for extremely demanding conditions. When thermoplastics are processed the chemical properties not change and just its form that is altered. The PEKK also does not shown any porosity or monomers[16,17)].

MATERIALS AND METHODS

Samples Preparation

Circular disks (7 mm diameter and 2 mm thickness) of CP Ti grade (II) (Orotig Srl EU Company, Italy) were cut with wire cut machine (Knuth Smart DEM-Germany), then those disks were bringing to a mirror smooth uniform appearance via rotation machine with Sic Papers proceeded from 500 to 2400 grit. For removing the contamination and

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Table 1: The water contact angle of PEKK coating with different duration time of CO_2 laser

Laser pulse duration time	1	2	3	4	5	6	7	8	9	10
Degree of water contact angle (o)	38.73	34.28	30.72	24. 15	22	20.04	25.89	34.55	43.63	48

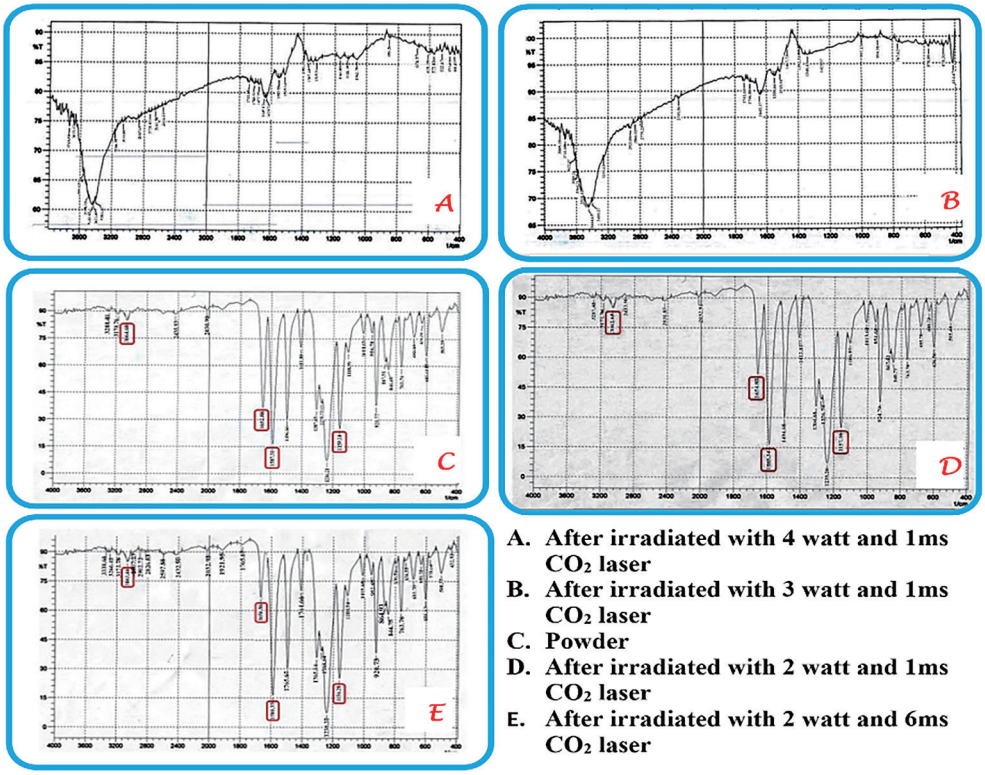

Figure 1: FTIR Spectrum of PEKK

the debris the samples were putted in the ultrasonic cleaning device for (15 minutes with ethanol) then for (10 minutes with distilled water) respectively. Finally, the samples were dried at room temperature for 15 minutes[18].

Surface Structuring by Laser

The laser system performed the desired profile on the titanium disk surface. The surfaces of the CP Ti were structuring under normal atmosphere by using pulse mode CNC fiber laser machine (Jinan JinQiang 20W laser– China) with laser power 20-Watt, wavelength 1064 nm, and scanning speed up to 7000 mm/sec. The design that used for structuring the samples was the dot design over the whole surface of the sample with 0.01 mm space between each adjacent dot in all directions. Corel Draw software (version XII) was used for drawing the dot design shapes. The samples-laser source disk distance was 20 cm. When the system triggered on, the sample was starts shooting by the laser with a continuous series of laser pulses in an ablation process to form the dot design[18].

Laser Coating Technique

Approximately 1 g of PEKK polymer powder (GAPEKKTM, 3200P, Gharda chemicals. India) was dispersed in 100 ml of distilled water that contain 3g of para chlorophenol (HiMEDIA, India) that used to gain a homogenous slurry (The para chlorophenol was suitable solvent solution for solvent the PEKK), which was preplaced as a powder bed on the surface of the CP Ti; the substrate was spray-coated with the slurry[9,18], thin layer of PEKK powder was preplaced on the CP Ti substrate when the solvent was evaporated.

A frictional mode CO_2 laser (I2itek, Fractional, HQ Dublin, Ireland) was used for coating the PEKK on the CP Ti samples at 10.6 µm wavelength, 0.1 mm spot diameter and 0.1 mm distance between each adjacent exposed spot (So laser was completely exposed to all regions of the PEEK); the disk to lens distance was 5 cm. Numerous trials were carried out for achievement appropriate laser coating parameters for best coat layer.

During coating procedure all the laser parameter was fixed while the CO_2 laser power was only the parameter that change, three different laser powers was examined for the PEKK coatings, which are 2, 3 and 4 Watt. the PEKK coated layer were examined visually and then by FTIR (Biotech, FTIR-600, UK) to be sure that the CO_2 laser does not cause any damage effect or decomposition to the PEKK.

The visual results of using CO_2 laser PEKK coated shown that, the PEKK coated layer become brown in color when exposed to (3 and 4) watt power this means that damage was happened to the PEKK polymer, the damage effect was confirmed by the FTIR spectrum Figure 1(A and B) that revealed the main bands of the PEKK polymer was not found when compared with the spectrum of the PEKK powder figure 1 (C), {The main bands groups of PEKK molecule are (C = H) benzene ring which have 3064 Cm^{-1} absorption bands, ketone(C = O) which have 1652 Cm^{-1} absorption bands, (C = C) aromatic ring which have 1587 Cm^{-1} absorption bands, and (C-O) ether bond which have 1159 Cm^{-1} absorption bands}. While the results of CO_2 laser PEKK coated at 2 watt shown that the PEKK coated layer remain white in color, the FTIR spectrum of the PEKK coated layer that exposed to 2 watt figure 1(D) shown the present of the main bands of the PEKK; so this means that the 2 watt power with 1 ms pulse duration of CO_2 laser not caused any decomposition or any structural chemical changes to the PEKK polymer.

Ten duration time from (1 to 10) ms were used for selection the best duration time of PEKK coating by using 2 watt CO_2 laser. Since the wetting properties is very important in implantable material and considered as indicator for future good osseointegration, so surface wettability test (water contact angle test) was used to measure the amount of the PEKK coated layer wettability for the ten tested duration times (1-10) ms. The disk with low contact angle measurement (high wetting surface) was chosen for PEKK coating of the final CP Ti samples. Table 1 shown the degree of water disk contact angle for each laser duration time (1-10) ms at 2 watt CO_2 laser.

The results of the water contact angle test shown that the PEKK coating by the CO_2 laser at 2 watt with 6 ms duration time gives the best

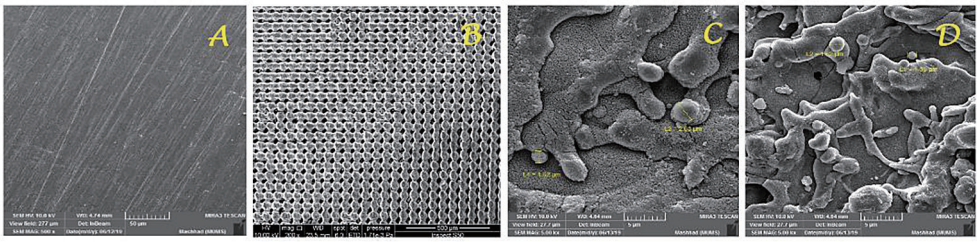

Figure 2: FESEM analysis of A) control, B) LS group, C) P group, D) LS-P group

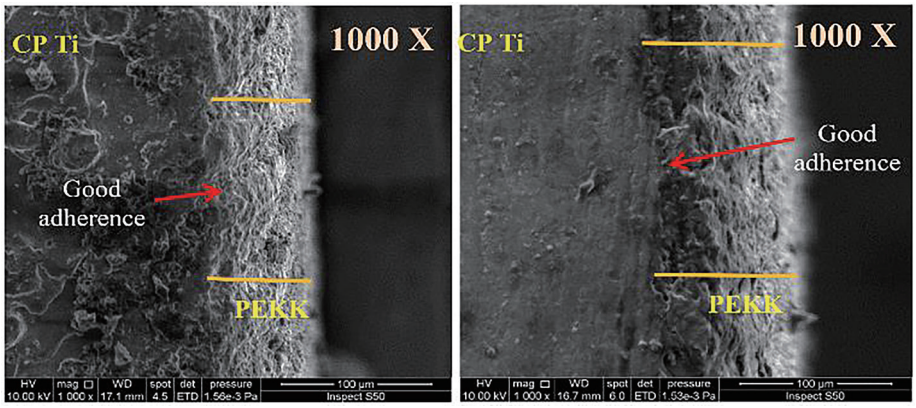

Figure 3: The cross-section images of the FESEM, group P left images, group LS-P the right images

hydrophilicity of the PEKK polymer (the low value of water contact angle).

The FTIR spectrum was performed at 6 ms duration time to be sure it was safe to use for PEKK coating. The main groups of the PEKK polymer were found in the FTIR Figure 1(E). So, no damaging effect to the PEKK was occurred. Therefore, the laser coating technique was performed by using CO_2 laser (fractional mode) at 2 watt power, 6 ms pulse duration, 10.6 µm wavelength for the final CP Ti disks coating with PEKK polymer. Lastly the top surface of the samples of all groups was analyzed by FESEM.

Samples Grouping

The CP Ti disks were divided into four groups which are:

1. **The control group (C):** CP Ti disk without any laser surface structuring and coating.
2. **Laser surface structuring group (LS):** CP Ti disk with laser structuring.
3. **PEKK coating group (P):** CP Ti disk with PEKK coating
4. **Laser surface structuring with PEKK coating group (LS-P):** CP Ti disk with laser structuring and with PEKK coating.

Field Emission Scanning Electron Microscopy (FESEM)

The field emission scanning electron microscopy (MIRA3 TESCAN, Czech Republic) was used to examine surface morphological topography of the C, LS, P and LS-P groups, PEKK particle size, the PEKK coating thickness and the PEKK adherence to the CP Ti substrate.

RESULTS

Surface Microstructure

The surface morphological analyses of CP Ti samples were seen in Figure 2 (A), the group LS shown in figure 2 (B), while the samples that coated by PEKK polymer groups (P and LS-P) were seen in Figure 2 (C, D) respectively.

Particles Size Analysis

The coated PEKK particles size was investigated by using FESEM, the particles size was ranged from (1-3) µm for the groups P and LS-P as seen in Figure 2 (C and D).

PEKK Coating Thickness

From the cross-section images of the FESEM the thickness of the PEKK coating could be obtained figure 3. The thickness of the PEKK coating material for group P was about 31.95 µm as, while the thickness of the PEKK coating material for group LS-P was about 38.6 µm.

PEKK Coat Adherence to the CP Ti Substrate

The cross-section images of the FESEM of the PEKK coated material figure 3 shown nearly uniform distrubuted on the CP Ti substrat with very good adherence in all the coated areas without present of any gaps between them.

DISCUSSION

Surface Microstructure

The FESEM of the group C had shown nearly smooth surface with linear lines that happen due to manufacturing and processing; while for the groups LS, P and LS-P had shown surfaces with roughness and porous like the structure of the bone, which had a beneficial effect to cell adhesion, this result in agreement with **Meng *et al.* in 2016 and Safi in 2019**[19,20].

The images of LS group shown very fine clear and uniform dots structure all over the examined samples with no defect or any inconsistent structures on them, while the images of the PEKK coated layer of the groups P and LS-P displayed homogenous continuous surface, which means that PEKK evenly and uniformly scattered on the CP Ti with, this uniform distribution layer of PEKK was possibly due to the technique that used during coating by utilizing homogenous slurry of the PEKK with para chlorophenol during the coated layer on the surface of the substrate, in addition to the using of CO_2 laser with proper parameters for PEKK coating that helped in proper distribution of the coated layer, this comes in agreement with **Malek *et al.*; Goodarzi, and Safi**[20-22].

PEKK Coating Thickness

The average PEKK coating thickness for group LS-P was greater than the group P; this may be due to the effect of the surface structuring that increase in roughness which may lead to inflowing of the PEKK to the pits that gives this thickness.

PEKK Coat Adherence to the CP Ti Substrate

The cross-section images of the FESEM shown good adhesion of the PEKK coat layer on the CP Ti substrate with and without laser surface structuring and absent of any gaps between them. this result was similar to the results of **Gary *et al*. in 2005; Shivamurthy *et al*. in 2008, Safi in 2019 and Al-Khafaji in 2020**[16,20,23,24]; whose used the cross section image of FESEM to seen the adherence of the coated material with the substrate.

SUMMARY

Coating of the CP Ti substrate with PEKK by using CO_2 laser method can be produced coating with uniformed distribution and excellent bonding to the CP Ti substrate (with and without laser surface structuring).

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DENTISTRY

The Effect of Adding Poloxamer Surfactant on Cleaning Efficiency of NaOCl and NaOH (SEM Study)

Thaer A. Mukhlif[1], Raghad A. Al-Hashimi[2]

ABSTRACT

Objectives: **The purpose of this in vitro study was to compare the effect of adding a poloxamer surfactant to the irrigant solutions on its cleaning efficiency.**

Design: **In this study the roots of extracted permanent premolar teeth were used and evaluated by using Scanning Electronic Microscopy (SEM).**

Materials and Method: **72 human single tooth of permanent premolar (8 for each group) were used in this in vitro study. Roots after sectioning at cervical area to get 15 mm were embedded in a plastic container filled with impression silicon, then instrumented with ProTaper rotary instruments till size F4. Each group (8 root) were irrigated with one of the nine solutions used in study: three concentrations of NaOH [5% (A1), 2.5%(A2), 0.5%(A3)], three concentrations of NaOH with Poloxamer surfactant [5% (B1), 2.5% (B2), 0.5% (B3)], NaOCl 5.25% (C1), NaOCl 5.25% with Poloxamer surfactant (C2), and Normal Saline (D). After instrumentation, each tooth was finally washed with normal saline. Then by using diamond disc, all roots were sectioned longitudinally in buccolingual direction to obtain two halves of each root, the more intact root was selected to be prepared for scanning electron microscopy test. Finally, the data was analyzed by using the SPSS 25 program with a non-parametric Kruskal-Wallis test. Additionally, scores were analyzed by using the chi-square test. The significance level for all tests was set at $p \leq 0.05$.**

Results: **the study showed that Sodium hypochlorite was higher in smear layer removal than that of Sodium hydroxide, and there was also a significant increase in the cleaning efficiency of sodium hydroxide with increasing concentration of solutions.**

Conclusion: **The adding of a poloxamer surfactant didn't significantly increased the cleaning efficiency of both solutions used. No significant difference between NaOCl and NaOH.**

KEY WORDS

poloxamer, cleaning efficiency, SEM, NaOCl, and NaOH

INTRODUCTION

The removal of pulpal tissue and debris, and the reducing of bacterial load from the root canal spaces are considered the main goal of root canal treatment. This goal is usually restricted by many challenges such as the morphological complexity of root canal system. Inadequate root canal disinfection, and bacterial debridement was recorded by previous studies regardless of the instrumentation techniques. Therefore, the new research of irrigating solutions has focused on its tissue-dissolving ability, and powerful antibacterial activity. Sodium hypochlorite (NaOCl) solution has a high surface tension, which could limit his penetration into dentinal tubules, fins and isthmuses. It also does not prevent the recolonization of reminded microorganisms because absence of residual antimicrobial activity[1].

Smear layer is shown to consist of not only dentin but also necrotic and viable tissue, including remnants of odontoblastic processes, pulp tissue, and microorganisms and their by-products (Pashley *et al*., 1998). It has been shown to hinder intra-canal disinfectants and sealers penetration into dentinal tubules and has the potential of compromising root filling seal. Pashley *et al*., 1978. An intact smear layer may obstacle the penetration of bacteria into dentinal tubules, and may cause leakage and reinfection when degraded after treatment[2].

The most common root canal irrigant is sodium hypochlorite (NaOCl) which have a broad antimicrobial activity against microorganisms, as well as having a potential removal of organic components. Dentin wettability may be increased by NaOCl as mentioned by previous studies, but due to its high surface tension, microorganisms in the depth of tubules may not be reached[3]. The inability of NaOCl and Ethylenediaminetetraacetic acid (EDTA) to remove smear layer in the apical third of root canals may be attributed to their high dynamic viscosity[4].

NaOCl is composed of hypochlorous acid and hypochlorite ions in different proportions. Together, they produce the free available chlorine (FAC), which improves antimicrobial activity and organic dissolution, which are affected by pH of NaOCl. At alkaline pH, organic dissolution is greater, while at acidic pH, antimicrobial activity is greater. Surfactants could be having an effects on the physicochemical properties of NaOCl, such as contact angle, surface tension, free active chlorine, pH, and its penetration into dentinal tubules[5].

In numerous applications, surfactants are used due to their surface-active properties, that when the molecules of hydrophilic head and hydrophobic tail, gathering at a phase boundary (for example an air/water interface) leading to surface tension reduction and system stabilization. The critical micelle concentration (CMC) is the concentration at which an increases in surfactant concentration, lead to no further reduc-

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Table 1: descriptive statistic for Mean Rank of smear layer removal for all solutions used at Apical, Middle and Coronal sections.

Groups	N	Mean Rank Coronal	Mean Rank Middle	Mean Rank Apical
NaOH 5%(A1)	8	26.63	25.56	22.50
NaOH 2.5%(A2)	8	32.38	31.75	33.25
NaOH 0.5%(A3)	8	59.63	58.94	58.00
NaOH 5% + Polox(B1)	8	22.75	22.75	27.00
NaOH 2.5% + Polox(B2)	8	23.75	29.69	27.75
NaOH 0.5% + Polox(B3)	8	56.00	53.13	52.50
NaOCl 5.25%(C1)	8	23.75	22.75	24.25
NaOCl 5.25% + Polox(C2)	8	19.88	19.94	19.75
Normal Saline (D)	8	63.75	64.00	63.50

Table 2: descriptive statistics for Mean of smear layer removal of all root sections.

	N	Mean	Std.Dev.	Minimum	Maximum	Percentiles		
						25th	50th	75th
Coronal	72	2.986	1,1926	1.00	5.00	2.00	3.00	4.00
Middle	72	3.305	1.1462	2.00	5.00	2.00	3.00	4.00
Apical	72	3.7222	.99608	2.00	5.00	3.00	4.00	4.75

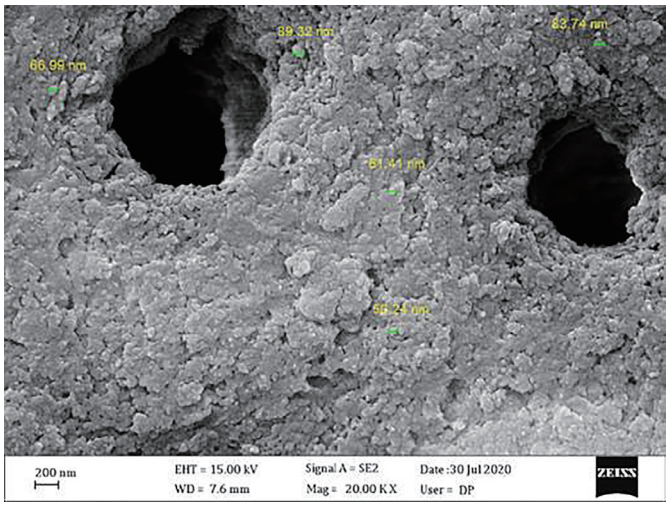

Figure 1: opened dentinal tubules under 20000X magnification.

tion in surface tension. Above the CMC, micellization process formed due to aggregation of surfactant molecules in the bulk phase. Solubilization and antibacterial properties of surfactants are related to the CMC point. This point provides a clear reference for concentration of surfactant, enabling direct comparison of performance by different surfactants through use of the concentration relative to the CMC[6].

Poloxamer 407 has 12,600 Dalton molecular weight (70% POE). It is an amphiphilic synthetic copolymer which consist of a hydrophobic central part of poly (oxypropylene) block between two peripheral hydrophilic poly (oxyethylene) blocks. Molecules of poloxamer can readily self-assembled to form micelles because of their amphiphilic nature, this feature depending on the temperature and concentration. Poloxamer is characterized by its non-toxicity, stability, biodegradability, and the ability to carry drugs. Therefore, it is suitable to be used as a controlled-release agent[7]. This study was used to evaluate the effect of Poloxamer surfactant on the cleaning efficiency of NaOH and NaOCl, and the effect of concentration of NaOH on its cleaning efficiency.

MATERIALS AND METHODS

A sample of 72 single-rooted mandibular premolar teeth extracted for periodontal and orthodontic reasons were used in the study. To verify root canal anatomy with only one central root canal, two digital radiographs were taken in a bucco-lingual and mesio-distal direction. Inclusion criteria were permanent teeth, intact apices, no previous root canal treatment, or extensive coronal restoration., whlie exclusion criteria was root caries, cracks, oval canals, and fracture lines[8]. To standardize the root length to 15mm, the teeth were decoronated using flexible diamond disc and the samples were divided randomly into nine experimental groups (8 roots for each group)[9].

Following access cavity preparation, patency was checked using a size 10 file. Simultaneously, root canal working length was visually determined by subtracting 0.5 mm from the measurement, when the file passes the major foramen. Instrumentation and irrigation protocol of root canal was used, and prior to canal preparation, all roots were embedded in small plastic container filled with silicon impression material to guarantee a closed system. Root canal preparation was performed by only one experienced operator with ProTaper gold rotary systems to size F4. Eight roots for each solution group (Nine) were used as the followings: NaOH at three concentrations [5% (A1), 2.5% (A2), 0.5% (A3)], NaOH with a Poloxamer surfactant at three concentrations also [5% (B1), 2.5% (B2), 0.5% (B3)], NaOCl 5.25% (C1) as Positive control, NaOCl 5.25% with a poloxamer surfactant (C2), and Normal saline (D) as a negative control. 2.5 ml of each solution with a 30-g open-ended needle (sinalident, China) was used to irrigate each root canal by inserting needles inside root canal not deeper than one mm short of the working length without binding with canal wall. Preparation cycle of each instrument during instrumentation consisted of three pecks (up and down) with an amplitude of not more than 3 mm[8].

SEM preparation

By using a diamond disc at a slow speed, a longitudinal groove was made on the lingual, and buccal surfaces of each root without penetrating the canal. The roots were gently split into two halves using a chisel and were stored in deionized water at 37 until SEM analysis. The specimens were dehydrated by using 100% ethyl alcohol and were placed in a furnace at 600 for 24 hours. On a metallic stubs the samples were mounted, and gold sputtered by using an ion sputter. Then, under scanning electron microscope (LEO 440i, Carl Zeiss, Tokyo, Japan) was examined for the presence or absence of the smear layer. The surface morphology photomicrographs were taken by 1000 X magnification (Fig. 1) of the root canal walls at coronal (11-14 mm from apex), middle

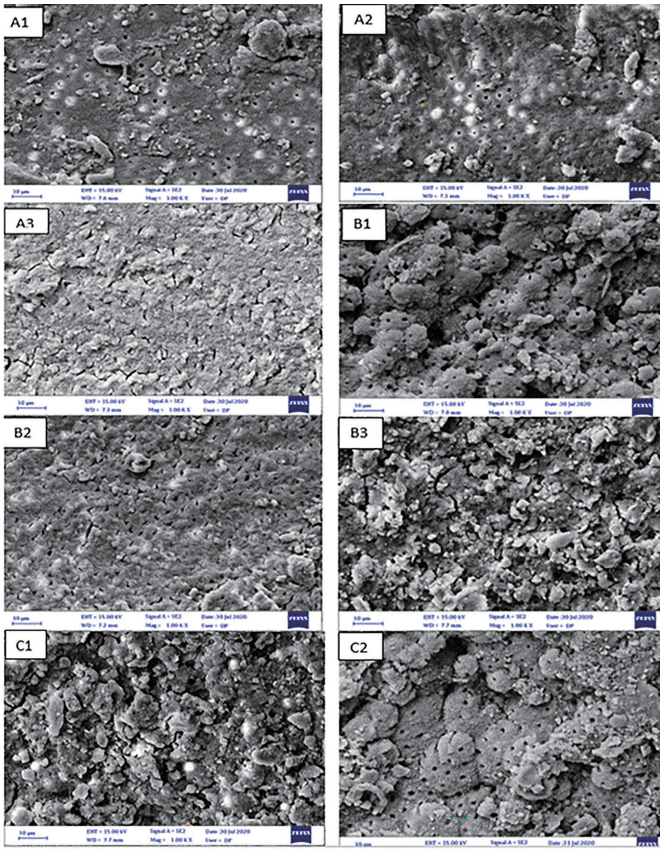

Figure 2: Smear layer at coronal section for different solutions under scanning electron microscope at 1000X magnifications: NaOH [5% (A1), 2.5% (A2), 0.5%(A3)], NaOH with Poloxamer [5% (B1), 2.5% (B2), 0.5% (B3)], NaOCl 5.25% (C1), NaOCl 5.25% with Poloxamer (C2).

Table 3: Chi-Square ruskal wallis test of Coronal, Middle, and Apical sections.

	Coronal	Middle	Apical
Chi-Square	50.790	47.029	44.735
df	8	8	8
Asymp. Sig.	.000	.000	.000

(6-9 mm from apex), and apical (1-3 mm from apex) thirds of each specimen[9]. All photomicrographs were examined by Photpshop C software and the amount of opened dentinal tubules was calculated to detect the score.

The scores were as the following (remnants of vital or necrotic pulp tissue, dentine particles, bacterial components, and retained irrigant):

Score 1: orifice of dentinal tubules patent, and no smear layer.

Score 2: Some opened dentinal tubules, and a small amount of smear layer.

Score 3: Very little opened dentinal tubules, and homogenous smear layer along almost the entire canal wall.

Score 4: No opened dentinal tubules, and the entire root canal wall covered with a homogenous smear layer.

Score 5: The entire root canal wall covered with a thick, homogenous smear.

The data was analyzed by using the SPSS 25 program with a non-parametric Kruskal-Wallis test. Additionally, scores were analyzed by using the chi-square test. The significance level was set at $p \leq 0.05$[8].

RESULTS

Because the data was nonparametric, the scoring was used in this test. The data was recorded and analyzed by using Kruskal Wallis and Wilcoxon rank Sum (Mann Whitney U) Test. The descriptive statistic for the present study was shown in Tables 1 and 2 which revealed that the more opened dentinal tubules (Figure 1 and 2), and the best smear layer removal (19.88) was recorded by group C2 (NaOCl 5.25% with Poloxamer) at a coronal section, while the worst smear layer removal (63.50) was recorded with Group D (Normal Saline) at the apical section.

From chi-square test (Table 3) it was clear there was no significant difference in cleaning efficiency among solutions used in present study in all sections (apical, middle, and coronal). Mann Whitney U Test (Table 4) was used for multiple comparison among groups. It showed that adding surfactant didn't increase the cleaning efficiency of both solutions used (NaOCl and NaOH), also there was a significant effect of concentration between the higher concentration (5%) and the lower concentration (0.5%) on cleaning efficiency of NaOH and NaOH with Poloxamer surfactant (Figure 2).

DISCUSSION

Root canal morphology is complex with irregularities areas that contain pulpal tissues and microorganisms that cannot be approached by instruments. Therefore, proteolytic and antiseptic fluid is necessary to clean and disinfect these areas. The primary goal of root canal treatment is the spreading of irrigant onto the canal to reache the non-instrumented areas[10].

Sodium hydroxide (NaOH) was used in this study because it has been shown to be effective in removing proteins and nucleic acids, and also effective for inhabiting most bacteria and its endotoxins, yeasts, and viruses. In industrial manufacturing the common practice is to save time by salt adding, such as sodium chloride, to NaOH solution to combine sanitization with cleaning. Sodium hydroxide as a cleaning agent, dissolves proteins and saponifies fats. In general, it can solubilize precipitated proteins, and in the presence of chlorine its hydrolyzing power is enhanced. Sodium hydroxide is very effective in inactivating a number of yeasts and bacteria and that this inactivation is dependent upon concentration, contact time, and temperature[11].

The type of a surfactant selected for this study was Nonionic surfactant than other types of surfactant because Non-ionic surfactants could provide a better wetting ability than ionic surfactants because they having the ability of hydrogen bonding on its polar hydrophilic portion, which can form a strong connection with molecules of water[12].

Non-ionic surfactants were used for about 45% of industrial production of all surfactants, it is used in this study because it is don't ionizing in aqueous solution because of their nondissociable hydrophilic group type[13].

The use of Poloxamer other than other types of nonionic surfactant was due to it is previous use with drugs as solubilizer, emulsifier and stabilizer, and due to its being non-toxic and non-irritant and also used as wetting agents in ointments[14]. Also these molecules pose the ability to form a non-covalent bonds with mucus, thus favoring an intact interaction with various biological tissues, giving the substantively by prolonging the residence time at the application site[15].

The use of Scanning Electron Microscope (SEM) was due to its precise and has been the most widely used methods to evaluate the removal of the smear layer[16]. Many parameters could be evaluated by SEM such as: smear layer, pulpal debris, inorganic debris, surface profile. All techniques of root canal instrumentation are unable to completely remove pulpal debris from irregularities such as dentin grooves and depressions[17]. The conclusion by many previous studies is that the use of NaOCl during or after instrumentation produces canal walls superficially clean, but the smear layer still present[18].

From the result of this study there was no significant difference between NaOCl and NaOH, that both solution didn't have the ability to remove smear layer effectively, especially the inorganic portion of smear layer[16]. This non-significant differences may be related to the similarity in dissolving mechanism for both solutions because both solutions depend in its tissue dissolving ability on hydroxyl ions for neutralization and saponification action, NaOCl acts as solvent to degrade organic and fat, and transform them into a soap (fatty acid salts) and glycerol (alcohol), to reduce the surface tension of the remaining solu-

Table 4: Mann Whitney U Test among different solutions used at apical, middle, and coronal section.

Solutions	Groups	N	Coronal		Middle		Apical	
			P-Value	Sig.	P-Value	Sig.	P-Value	Sig.
NaOH 5% (A1)	A2	8	.442	NS	.442	NS	.195	NS
	A3	8	.000	Hs	.000	Hs	.001	Hs
	B1	8	.645	Ns	.721	Ns	.645	Ns
	B2	8	.721	Ns	.645	Ns	.442	Ns
	B3	8	.001	Hs	.003	Hs	.005	Hs
	C1	8	.721	Ns	.721	Ns	.798	Ns
	C2	8	.382	Ns	.442	Ns	.798	Ns
	D	8	.000	Hs	.000	Hs	.000	Hs
NaOH 2.5% (A2)	A3	8	.000	Hs	.002	Hs	.003	Hs
	B1	8	.279	Ns	.328	Ns	.505	Ns
	B2	8	.234	Ns	.798	Ns	.442	Ns
	B3	8	.003	Hs	.028	Hs	.021	Hs
	C1	8	.234	Ns	.328	Ns	.279	Ns
	C2	8	.130	Ns	.195	Ns	.083	Ns
	D	8	.000	Hs	.000	Hs	.000	Hs
NaOH 0.5% (A3)	B1	8	.000	Hs	.000	Hs	.002	Hs
	B2	8	.000	Hs	.001	Hs	.001	Hs
	B3	8	.645	Ns	.505	Ns	.442	Ns
	C1	8	.000	Hs	.000	Hs	.001	Hs
	C2	8	.000	Hs	.000	Hs	.001	Hs
	D	8	.234	Ns	.234	Ns	.442	Ns
NaOH 5% + Polox (B1)	B2	8	.878	Ns	.442	Ns	.959	Ns
	B3	8	.001	Hs	.002	Hs	.010	Hs
	C1	8	.878	Ns	1.00	Ns	.798	Ns
	C2	8	.798	Ns	.721	Ns	.442	Ns
	D	8	.000	Hs	.000	Hs	.000	Hs
NaOH 2.5% + Polox (B2)	B3	8	.000	Hs	.015	Hs	.005	Hs
	C1	8	1.000	Ns	.442	Ns	.645	Ns
	C2	8	.574	Ns	.279	Ns	.234	Ns
	D	8	.000	Hs	.000	Hs	.000	Hs
NaOH 0.5% + Polox (B3)	C1	8	.001	Hs	.002	Hs	.005	Hs
	C2	8	.001	Hs	.001	Hs	.002	Hs
	D	8	.161	Ns	.105	Ns	.105	Ns
NaOCl 5.25% (C1)	C2	8	.574	Ns	.721	Ns	.574	Ns
	D	8	.000	Hs	.000	Hs	.000	Hs
NaOCl 5.25% + Polox (C2)	D	8	.000	HS	.000	HS	.000	HS

tion. Sodium hypochlorite neutralizes amino acids forming water and salt[19]. The difference in mechanism between both solutions may be related to chlorination reaction between chlorine and the amino group (NH) to form chloramines that interfere in cell metabolism. This reaction was related to NaOCl, but not to NaOH.

The finding of the present study revealed there was no significant difference in smear layer removal among different concentration used (5% ,2.5%, and 0.5%) of NaOH or NaOH plus surfactant, just between 5% and 0.5%. This was in coincidence with **Torabinejad *et al*. in 2003**[20] who investigated by scanning electron microscopy there was no significant differences among the abilities of 1.3%, 2.6%, and 5.25% NaOCl when used with MTAD as a final irrigant to remove smear layer. **Beltz *et al*. in 2003**[21] agreement with our study showed that the use of 1.3% NaOCl during instrumentation, instead of a higher concentration of NaOCl was effective in smear layer removal when combined with EDTA.

Marion *et al*. in 2012[22] concluded that both 2.5% NaOCl and 5.25% NaOCl have similar properties, but the first one is less cytotoxic, therefore being more indicated for root canal endodontic treatment. As presented by previous studies the NaOCl ability to dissolve organic tissue is directly related to its concentration. This statement confirms Baumgartner and Cuenin study, which observed that the higher the concentration, the more rapid the tissue dissolution[22].

In contrast with present study **Shahriari *et al*. in 2017**[23] found that a significant difference in smear layer removal among three concentrations of NaOCl (1%, 2.5% and 5%) when activated with Laser. This differences in results may be not related to the concentration of solution used but it may be related to the use of laser in their study which didn't used in present study.

The finding of this study showed there was no significant difference in smear layer removal when the surfactant was added to solutions, this was in agreement with **Guerreiro *et al*. in 2020**[24] who found that the addition of surfactants to NaOCl did not impact on the removal of the accumulated hard tissue debris (AHTD) from mesial canals of extracted mandibular molars. None of the irrigation solutions were able to render root canals free from AHTD. In accordance to that, **Turker *et al*. in**

2012[25] showed that the adding a surfactant failed to improve the performance of EDTA in terms of smear layer removal. This finding is in agreement with a study done by Lui *et al*. in 2007 which demonstrated that addition of surfactants to EDTA did not result in better smear layer removal compared to EDTA alone. According to **Zehnder *et al*. in 2005**[26] calcium chelating ability of an endodontic chelator did not improved by surfactant addition to reduce the surface tension. In contrast to finding of this study, **Dua *et al*. in 2015**[27] concluded the addition of surfactants to EDTA in Smear Clear would have resulted in better smear layer removal compared with EDTA alone. This contrast with present study may be attributed to the differences in type of solution used, that they used EDTA, instead of NaOCl and NaOH which were used in present study.

CONCLUSIONS

From this findings, we concluded that the addition of poloxamer surfactant didn't significantly improve the smear layer removal. There was no significant difference in smear layer removal between NaOCl and NaOH. The increased concentration of NaOH to certain level (from 0.5 % to 2.5 %) didn't significantly increase the removal of smear layer, but when increased from 0.5% to 5%, the difference was significant.

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The Role of Soluble TLR-2 in the Immunopathogenesis of Gingivitis

Batool Hassan Al-Ghurabi

ABSTRACT

Objective: **TLRs has a vital role in stimulating the immune system and identifying the pathogen, as TLR2 signaling has significant part in the innate immunity and the conservation of gingival validity. Therefore, this study was designed for evaluate role of sTLR2 in immune-pathogenesis of gingivitis.**

Study Design: **A case-control study.**

Materials and Methods: **The study was conducted on 20 patients with generalized gingivitis and 20 healthy controls. Saliva was collected from all subjects and ELISA was done for estimation the level of sTLR2 in patients and controls. The primary outcome of the study was that inflammation leads to high expression of TLRs in periodontal disease.**

Results: **The findings noted significant higher level of salivary sTLR-2 was among gingivitis than that to control.**

Conclusion: **The salivary level of sTLR-2 might be a good biological marker in periodontal disease.**

KEY WORDS

gingivitis, toll like receptors, innate immune response

INTRODUCTION

Gingivitis is a non-destructive type of periodontal disease, it is a reversible inflammatory status of the soft tissue around the teeth (gingiva) with no involvement of attachment structure, but untreated gingivitis can progress to periodontitis. Periodontitis involve the deeper structure of periodontal tissues leading to loss of attachment with the devastation of gingiva, cementum, periodontal ligament and alveolar bone[1]. Gingiva, which considered the primary defense location against bacteria in oral cavity, is continually at risk to expose to about 500 different types species of pathogenic bacteria which is either Gram-positive or Gram-negative in nature[2]. Toll-like receptor (TLR) including TLR-2 is consider one main type of gingival epithelial cell receptors that identify pathogen associated molecular patterns (PAMPs)[3]. TLR-2 considers a receptor which is located on the surface of specific cells to distinguish foreign materials and transmits the suitable signals to the immune system cells[4]. Soluble form of TLR-2 has been identified in body fluids. It has been proposed that the soluble TLR-2 (sTLR-2) able to sequester pathogens[5,6]. It acts as a key role in the immune system and plays an essential role in pathogen recognition and innate immunity activation. They distinguish PAMPs that are shown on the infectious molecule and activate the production of cytokine which is play vital role in effective immunity development, cytokines participating in this include TNF-α and various interleukins[7]. The purpose of the current case-control research was designed for evaluate role of sTLR2 in immune-pathogenesis of gingivitis, our null hypothesis stated that the TLR2 has no role in immunopathogenesis of gingivitis

MATERIALS AND METHODS

Study design and subject sampling

To calculate sample size, TLR-2 biomarker was used as primary outcome of the study, which was used to calculate sample size using online tool EPITOOLS (*https://epitools.ausvet.com.au/casecontrolss*) at 95% confidence interval, 5% error margin. The acceptable sample size will consist of 20 patients. We were adding a control group consisting of 20 subjects systemically healthy with healthy periodontuim. Therefore, the total sample size in this study will be 40 subjects divided into two groups: 20 patients and 20 controls were enrolled in this case control study to estimate salivary level of sTLR2 (MyBiosource, USA) by sandwich ELISA. Patients (10 males and 10 females) were selected from attending the department of periodontics in the teaching hospital of College of Dentistry /University of Baghdad. The diagnosis of gingivitis was done according to the criteria proposed in 2017 by the Workshop for the Classification of Periodontal and implant Diseases and Conditions[8]. While subject in healthy control (10 males and 10 females) were free of any systemic disease with healthy intact periodontuim selected from attenders of other department in the college of dentistry

Inclusion and exclusion criteria

The inclusion criteria were including; the presence of at least 20 or more natural teeth, good general health with no history of any systemic diseases, subjects in the gingivitis group had generalized gingivitis on intact periodontuim with bleeding on probing more than 30% and gingival sulcus depth ≤ 3 mm no sites with clinical attachment loss (CAL). While healthy control subject with healthy intact periodontuim characterized by the following criteria: bleeding on probing less than 10% with probing pocket depth ≤ 3 mm no sites with clinical attachment loss. The age and gender of healthy control group were matched with

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Table 1: Demographic and Clinical characteristic of patients and controls.

Characteristics	Gingivitis group	Control group	P-value
Age			0.88NS
Mean ± SE	27.2 ± 1.50	25.6 ± 1.25	
Gender			1.00NS
Male	10	10	
Female	10	10	
PLI			
Mean ± SE	1.35 ± 0.07	0.62 ± 0.06	0.000**
GI			
Mean ± SE	1.51 ± 0.09	0.24 ± 0.03	0.000**
BOP	50% ± 3.9	6.7% ± 0.07	0.000**

Table 2: Salivary mean levels of sTLR2 (ng/ml) in patients and controls.

Salivary sTLR2	Patients group	Controls group	p-value
Minimum	2.2	0.6	
Maximum	12.7	4.59	
Mean	6.96	2.61	0.000**
SE	0.70	0.28	

Table 3: Pearson correlation of salivary sTLR2 with clinical periodontal parameters.

Salivary sTLR2	Periodontal Parameters		
	PLI	**GI**	**BOP**
Correlation (r)	0.46	0.41	0.57
p-value	0.003**	0.003**	0.001**

patients group. The exclusion criteria were including; previous periodontal therapy for the last 6 months, systemic diseases, history of smoking or alcohol drinking and the use of antibiotics and/or anti-inflammatory medication in the last 3 months.

Collection of salivary samples

Saliva sample was collected between 9-12 am, after the patients and controls washes their mouth many times by sterilized water and then waiting for 1-2 min for water clearance, 2 ml of whole unstimulated saliva was collected into polyethylene tubes and centrifuged at (3000) rpm for 10 min and the resulting supernatant layer was stored at -40°C in eppendorf tubes until assayed.

Clinical periodontal examination

Plaque Index (PLI) was measured according to criteria of Silness and Löe[9]. Gingival Index (GI) was measured based on criteria of Löe and Silness[10], bleeding on probing index were measured for the presence or abscence of bleeding for all teeth except 3rd molar, A periodontal probe inserted to the base of the periodontal pocket/sulcus for four surfaces of each tooth and is moved lightly along the tooth (root) surface.After probing, the site was given score (1) if bleeding occurred within 30 seconds and score (0) for the non-bleeding site[11].

Inter Examiner's calibration: prior for enrollment in the current study, calibration sessions between the main examiner and expert periodontist will be conducted until agreement level more than 75% is reached for all clinical parameters.

Intra Examiner's calibration: Reproducibility of the examiner will be assessed by carrying out clinical periodontal data collection on five patients. Each subject will be assessed twice in one visit, over a 1-h interval. The second set of measurements will be carried out to mask the first assessment. Reproducibility of the data collection will be determined for each site by calculation of the percentage of the sites examined where the scores are identical or within 1 mm. Assessment of the mean difference in the scores (with 85% accuracy) between visits indicate that there is no systematic bias in the measurements between visits.

Statistical analysis: Comparison of salivary sTLR2 level between two groups was calculated by student T-test, p-value of $p < 0.05$ was deemed important.

RESULTS

The findings presented in the current study were based on testing 20 gingivitis patients compared with 20 healthy periodontium as controls. The Patient's age of patients ranged between (18-45) years with an average of (27.2 ± 1.50) year. There were no considerable change ($p > 0.05$) related age and gender among patients and controls groups. The demographic and clinical variables of the 40 subjects enrolled in this study were illustrated in Table 1. The current results found significant elevation in salivary sTLR2 concentrations among gingivitis subjects, with mean (6.96 ± 0.70 ng/ml) when compared to the healthy controls (2.61 ± 0.28 ng/ml), ($P < 0.05$) as clearly shown in Table 2. Moreover, Pearson's correlation between salivary sTLR2 levels and clinical parameters (PLI, GI and BOP) in patients shows that there is significant positive association as observed in Table 3.

DSCUSSION

Because the gingiva is always susceptible to bacteria found in plaque biofilm and as sTLR-2 is responsible for recognizing the peptidoglycan of Gram positive bacteria, sTLR-2 signaling play a crucial part in the innate immunity as well preservation of periodontal health[12]. Along with immune cells, periodontal tissues as well expressed TLRs[13], and as the gingival disease developed by superficial bacterial colonization of gingiva resulting in an inflammation of gingival tissue[14]. Subsequently, tissue destruction can cause by increase of cytokine release because of chronic energizing of TLRs[13]. The findings of the present study indicate that salivary level of sTLR-2 is increase in gingivitis group as compared to control group. In agreement with our study[15,16] reported that sTLR-2 was elevated in patients and may be correlated with disease activity. Similarly, Beklen *et al*.,[17] revealed that the expression of TLR-2 was increase in gingivitis tissues as compared to healthy control tissues. The possible explanation for the high level of TLR2 in gingivitis could be attributed to inflammation which rise expression of TLRs leading to an increased identification of TLRs in saliva, which may be helpful as a diagnostic assay for periodontal diseases. Generally, change in expression of TLR-2 and TLR-4 has been demonstrated formerly in periodontal disease. In addition, TLR-2 and TLR-4 are stimulated by periodontal pathogens, and then these receptors mediated signals and regulate release of cytokine in oral epithelial cells[18]. Moreover, sTLR-2 level showed a significant positive correlation with gingival parameter (PLI and GI) in study group, this could reflect its association with initiation of periodontal disease. The result is in accordance with Sarah *et al*.[19] who reported that sTLR-2 levels significantly correlated to gingival parameter and reported that TLR-2 increased as the periodontal disease develops. In addition, other studies[13,14] concluded that higher level of sTLR-2 in gingivitis subjects was significantly correlated GI and BOP. In contrast other study[18] reported that sTLR-2 levels showed a negative association with clinical determinants of gingivitis group. A limitation of the current study is to study only one type of TLR (TLR2), as TLR4 is also implicated in periodontal disease and needs to be investigated with TLR2 for an association with gingivitis. In conclusion the current findings suggest that salivary level of sTLR-2 might be a good biological marker in periodontal disease.

CONFLICTS OF INTEREST

The authors have disclosed no potential conflicts of interest.

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