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Serum Tumor Necrosis Factor Alpha and High Sensitive C-reactive Protein as Biomarkers in Periodontitis in Iraqi Patients with Osteoarthritis

A Thesis
Submitted to the council of college of dentistry / university of Baghdad in partial fulfillment of the requirement for the award of the degree of Master of Science in Periodontics

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Dedication

I dedicate my work to my lovely family,
My great father, wonderful mother for their help and support, my lovely angle sister (Rania) who always besides me give me love and support, my brother in law (Ahmed) thank you for everything.
To all my friend who make the world a better and joyful.

Enas

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Abstract

Background: Periodontitis is an infectious disease combined with a number of gram negative microorganisms, along with pathogenic bacteria, Periodontitis (PD) is as well a chronic inflammatory disease affecting the periodontal ligament and alveolar bone, Osteoarthritis (OA) is a chronic joint disease with compound reasons characterized by synovial inflammation, subchondral bone remodeling, also the formation of osteophytes, that cause cartilage degradation. Chronic periodontitis and osteoarthritis are considered widely prevalent diseases and related to tissue destruction due to chronic inflammation in general health and oral health, in recent times, the association between OA and metabolic diseases has been proposed, and the association between several systemic diseases such as rheumatoid arthritis, metabolic syndrome and periodontitis has been also concealed.

Aims of the study: determine the periodontal health status in patients having chronic periodontitis with osteoarthritis and compare them with those having chronic periodontitis without osteoarthritis and determine the serum levels of high sensitivity C-reactive protein (hs crp), tumor necrosis factor- alpha (TNF α) in both groups and compare the result with the control group (healthy periodontium and systemically healthy)and correlate these immunological markers with the clinical periodontal parameters including plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment level.

Materials and methods: This cross-sectional study done in a total of 80 subjects in both sexes aged (35-50) years,30 patients with osteoarthritis and moderate chronic periodontitis, 30 patient with moderate chronic periodontitis alone. And control group of 20 healthy subjects with healthy periodontitium. This study excluded the postmenopausal or pregnant woman and smokers

patient also. All patients were free of medication. All subjects were in good general health with no history of systemic disease. Participants with OA have documentation or radiographic imaging, consistent with degenerative arthritis in the absence of an inflammatory condition. Venous blood samples are drawn from each subject using pyrogen-free heparinized collection tubes. Tubes were centrifuge according to the manufacturer's guidelines, then stored at -15C° till analyse. Serum TNFα and hsCRP were measured using a enzyme-linked immunosorbent assay (ELISA).

Results: Patients with moderate chronic periodontitis and osteoarthritis had higher mean of dental plaque, gingival inflammation, and no significant difference in probing depth, bleeding on probing and attachment loss compared to those who had chronic periodontitis only.. A non significant differences between studied group regarding serum level of hs CRP and TNF-α, as well as, it revealed that the median of serum levels of hsCRP were little higher in chronic periodontitis with osteoarthritis group than chronic periodontitis group and control groups represent as (0.31,0.29,0.24) respectively .the median serum level of TNF-α was higher in chronic periodontitis with osteoarthritis than in other two groups (10.615,10.533,9.682) respectively. Regarding correlation, the present study revealed that there's a strong positive correlation between serum levels of hsCRP and TNF-α in the patients of all groups, with no correlation between clinical periodontal parameters and serum level of TNFα and CRP.

Conclusion: it was concluded that increasing the serum level of hsCRP and TNFα in patient with chronic periodontitis and osteoarthritis is non-significant relation that mean the correlation between osteoarthritis and periodontitis remain proposed and still revealed need further studies to prove this relationship between chronic periodontitis and their systemic effect on osteoarthritis.

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List of Abbreviation

Abbreviation	Meaning
A.a	Aggregatibacteractinomycetemcomitans
ANOVA	Analysis of Variance
B.O.P	Bleeding on probing
BMI	Body Mass Index
C	Control
CAL	Clinical Attachment level
CP	Chronic Periodontitis
CRP	C-reactive Protein
Df	Degree of freedom
ELISA	Enzyme linked immune
et al	et alia (and other)
GI	Gingival Index
HRP	Horse radish Peroxidase
HS	Highly Significant
IL	Interleukin
LPs	Lipopolysaccharides
Max.	Maximum
Mg	Milligram
ml	Milliliter
N	Number
NS	Non-Significant
PI	Plaque Index
PPD	Probing Pocket Depth
R	Coefficient of correlation
r.p.m	Revolutions per minute
SD	Standard deviation
Sig.	Significant
SPSS	Statistical Package for Social Science
TNF	Tumor Necrosis Factor
WHO	World Health Organization
OA	Osteoarthritis
ICAM	Intracellular Adhesion Molecule
PMN	Polymorph Nuclear Leukocyte
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs

CVD	Cardio Vascular Disease
LIF	Leukemic Inhibitor Factor
RA	Rheumatoid Arthritis
OD	Optimum Density
Ml	Microliter
CI	Confidence Intervals

INTRODUCTION

Introduction

Periodontitis (PD), is considered one of the common oral diseases, it is a destructive inflammatory disorder affecting the tissues that support teeth and it occur with many microorganisms (*Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia* together with *Aggregatibacter actinomycetem-comitans*,) (Saini, 2009). The diseases of periodontium range from simple type of (gingivitis) to more destructive type (periodontitis). These conditions can affect the general health and it has a primary effects on dentition. Periodontitis is characterized by both chronic inflammation of connective tissue and alveolar bone cause destruction and lead to periodontitis (DeStefano *et al.*, 1993). Sequentially, PD followed gingivitis, which involves inflammation of marginal periodontium. Although not every gingivitis develop into periodontitis. The progression of the periodontal destruction depend on the quantity, quality and virulence of the microorganisms and the immune response of the host (Saini, 2009).

Osteoarthritis (OA): the most important reason of disability in musculoskeletal worldwide (Ganley *et al*., 2012).And the major factor in physical limitation of old people. (Cheng *et al*., 2010). Also it is a chronic joint disease have compound etiologies characterized with synovial inflammation, remodeling of subchondral bone, and produce osteophytes, which in turn cause cartilage deterioration, Severity of osteoarthritis afflicted functional ability(El-Sherif *et al*., 2008). Hence, osteoarthritis in most of people leading to incapable or maintain proper oral hygiene, lead to accumulation of plaque and calculus, which increases hazard of dental caries and periodontal disease. (Pokrajac Zirojevic *et al*., 2002).

Cytokines act as a communication between immune and non-immune cells (Kinane and Lindhe, 1997). It has been projected that cytokines are

required to the pathogenesis of many diseases (Gemmell, 2001). C-reactive protein is a systemic marker released during the acute phase of an inflammatory response released. C-reactive protein is produced by the liver and is regulated by circulating cytokines, for example tumor necrosis factor and interleukin- 1, from local and or systemic inflammation like as periodontal inflammation, circulating C-reactive protein may pass on saliva via gingival crevicular fluid or the salivary glands. High levels of C-reactive protein have been correlated with chronic and aggressive periodontal diseases and with other inflammatory biomarkers (William *et al.*, 2000, D'Aiuto *et al.*, 2004). Also, it is a biomarkers of osteoarthritis (Conrozier *et al.*, 1998) and related to disease activity and bone quality. Elevated levels of CRP have been correlated with synovial fluid interleukin (IL-6) and degree of synovial fluid infiltration (Pearle, *et al.* 2007), as well as symptoms of pain and stiffness, radiographic gradings and disease progression.

Tumor necrosis factor-alpha (TNF-α) is a critical of pro-inflammatory mediator that result in destruction of periodontal tissues. TNF-α have many actions, mostly pro-inflammatory. Leukocyte recruitment and vascular permeability are facilitated by stimulating expression of selectins and adhesins by TNF-α, activating the osteoclasts similarly to way with IL-1 lead to resorption of bone and extracellular matrix (Moreland *et al*., 1997).

Monocytes and macrophages are the main cell types producing TNF-**α**. The local cellular effects of TNF-**α** facilitate the adhesion of polymorph nuclear leukocytes (PMNs) to endothelial cells, degranulation of PMNs, activation of phagocytosis and intercellular adhesion molecule (ICAM) -1 expression. The main role for TNF-α in alveolar bone resorption is in response to bacterial LPS, TNF-α triggers osteoclast activation, proliferation and differentiation resulting in bone resorption (Beutler *et al*., 1985).

These cytokine participitate in the pathogenesis of OA, they are responsible to a large extent for the loss of metabolic homeostasis of tissues

forming joints by promote catabolic and destructive processes. The key role they act in the pathogenesis of OA is a result of the effect of these compounds on the majority of cells that are in the joint and the contact via intracellular pathways of signal transduction on the production of cytokines as well as other inflammatory compounds and enzymes. The purpose of this study is to see if there is an association between osteoarthritis and chronic periodontitis.

Aims of the study

The present study aimed:

1. To determine the level of c-reactive protein and TNFα in patients with moderate chronic periodontitis and osteoarthtitis, periodontitis only and in a control group.
2. Find the correlation between these markers in each group.
3. Determine clinical periodontal parameters (PI,GI,BOP,PPD,CAL) and correlate with TNF alpha and hsCRP in each group

CHAPTER ONE
REVIEW OF LITERATURE

Literature Review

1.1 Periodontal Disease

1.1.1 Definition

Periodontal disease was defined as an infectious disease of the oral cavity initiated by Gram-negative bacteria, characterized by accumulation of inflammatory cells in the periodontal tissues (Page *et al*. 2000)it develop from the interaction between bacteria in the biofilm of plaque and the host immune response. Periodontitis cause destruction of the periodontium or the tooth supporting structures. Periodontitis can affect aesthetics and the masticatory function, lead to tooth loss, which not only decreases the quality of life but also causes acquired systemic disease (Novak, 2006).

Periodontitis is considered as a multifactorial disease modified by many risk factors. Which influence susceptibility for severity, extent, and course of the disease such as age, gender, socio-economic status and education level, diabetes, smoking, and also inherited factors (Genco, 1996).

Periodontitis (PD) develops through gingivitis, the inflammation of the marginal periodontium, but not every gingivitis will develop further into periodontitis it depend on the amount and the virulence of the microorganisms and the host resistance factors (risk factors and immune status). (Saini, 2009).

1.1.2 Etiology of periodontitis:

Periodontitis is the outcome of host inflammatory responses to the bacteria accumulation on tooth surfaces (Dental plaque) on supra and sub-gingival tissues. Periodontitis found in several different forms which differ in the etiology, rate and pattern of progression, history and the response to therapy. This variability can be due to differences in composition of the sub gingival

microbial flora, together with the factors that modify the host response to microbial attack. While the host response and environmental factors that effect on this response are important for disease manifestation, without the presence of bacteria, periodontitis cannot initiate. However, it must be noted that, although bacteria are necessary for disease initiation, they are not enough to cause disease progression unless there is an associated inflammatory response (Page *et al.*, 2000).

For periodontitis approximately 20 microbes are needed to be developed, which inhabit the sub gingival environment, are considered to be significantly pathogenic and associated with various forms of periodontitis. The most significant of these associated with periodontitis are *Aggregatibacteractinomycetemcomitans A.A*, *Porphyromonasgingivalis* and *tanerrella forsythia*. (Mercado *et al.*, 2003).

1.1.3 Clinical features of Periodontitis

The clinical features of periodontal tissue destruction are gingival bleeding on probing, increased pocket probing depth caused by apical migration of the junctional epithelium, periodontal bone loss and increased tooth mobility and finally tooth loss if disease activity continues (Mercado *et al.*, 2003).

1.1.4 Pathogenesis of periodontal diseases

One of the most common oral diseases is Chronic, plaque-associated inflammation of the periodontium and has a prevalence of 80% to 90% (Saini *et al.*, 2009) lead to destruction of soft and hard periodontal tissue and supsequently in tooth loss (Listgarten, 1996) Both the amount and virulence of the microorganisms and the resistance factors of the host (risk factors and immune status) are essential for the initiation and progression of the periodontal destruction. In addition to concept about microbiological, molecular, and

cellular mechanisms, which determine the strength and balance of the cellular and humoral host response in the tissues, it became clear that a complex and primarily endogenous periodontal microflora is responsible for disease initiation and progression. (Detert *et al.*, 2010).

1.1.5. Classification of chronic periodontitis:

The levels of chronic periodontitis can be classified as:

1. Slight or early periodontitis: There is progression of the gingival inflammation into the bone and early bone loss (less than 30%) is seen, resulting in slight clinical attachment loss of 1 to 2mm with pockets depth of 3 to 4mm.
2. Moderate periodontitis: A more advanced state of the previous condition, there is increased destruction of tooth supporting structures, attachment loss up to 4mm, moderate to deep pockets (5 to 7mm), moderate bone loss (30 to 50%), loose teeth and exposure of roots of teeth.
3. Severe or advanced periodontitis: There is further progression of periodontitis with severe destruction of the tooth supporting structures, attachment loss over 5mm, increased bone loss (more than 50%), increased pocket depth (usually 7mm or greater), increased tooth movement and severe exposure of teeth roots. (Moore WE and Moore LV,2000) figure (1-1)

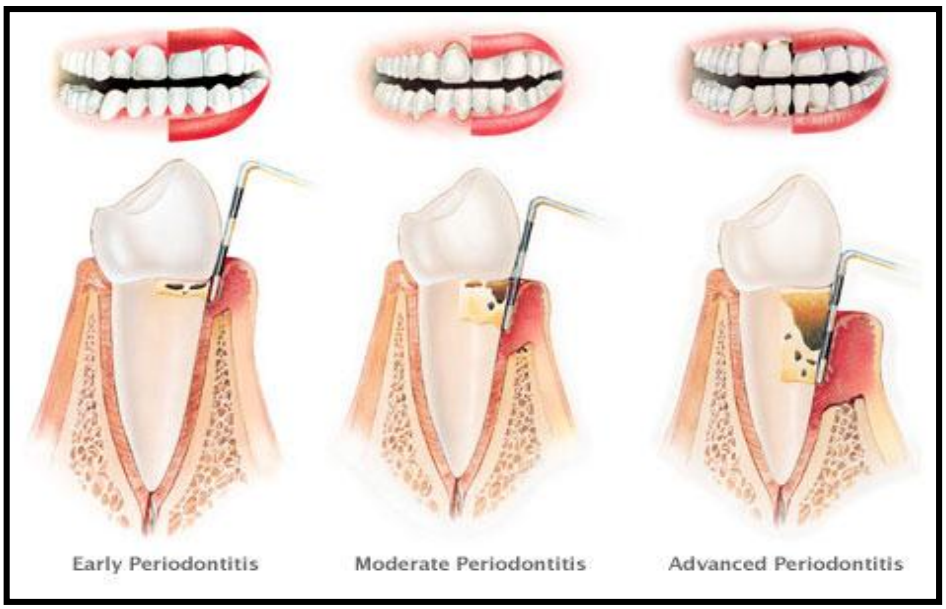


Figure 1.1: Classification of periodontitis

1.2. Osteoarthritis (OA)

Is a degenerative disease of synovial joints characterized by focal loss of articular cartilage with reactive changes in the subchondral and marginal bone, synovium, and para-articular structures (Scott, 2010). According to the National Institute for Health and Care Excellence (Conditions, 2014), OA refers to a clinical syndrome of joint pain accompanied by varying degrees of functional limitation and reduced quality of life. It is the most common form of degenerative joint disease, affecting 15% to 40% of people aged 40 and above (Corti & Rigon, 2003). The disease is a leading cause of disability and has a slow, progressive course that ends with joint failure and later disability (Roddy *et al*. 2005; Barron & Rubin 2007).The classification of osteoarthritis (OA) as a non inflammatory arthritis is an unlucky a consequence of early observations noting fewer leukocytes in OA synovial fluid compared with that of rheumatoid arthritis (RA), reactive arthritis, and even septic arthritis. In spite of this classification, investigators decades ago noticed a synovial inflammation in so-called ‘post-traumatic’ synovitis (Soren *et al*. 1976), and similar histopathology has been described among at least a subset of patients with what is now

dignosed primary OA. Given this greater approval for synovitis in patients with OA, inflammation has now been strongly concerned in the pathogenesis of OA (Scanzello and Goldring, 2012). This is not to mean that all OA pathogenesis is related to the synovium. Rather, synovitis is likely a secondary process induced by innate immune activation following cartilage damage that provides a important link in the chain of initiation and of OA. Osteoarthritis (OA) also the most common condition that affecting synovial joints, the most important reason of locomotor disability, and a major challenge for health - care providers, because OA increases significantly with age, it was long considered to be a degenerative disease that was an expected consequence of ageing and trauma. However, it is noticed now as a metabolically dynamic process characterized by an imbalance of joint breakdown in association with a mal adaptive and inadequate repair process. OA is result from abnormal biomechanical stresses (e.g. severe injury, repetitive excessive loading) superimposed on normal joint physiology. It may also result from normal stresses applied to an inherently compromised joint with abnormal physiology, for example weakened cartilage due to a genetic mutation in collagen II (figure 1-2). In fact, the genetic contribution to OA is equal or greater than the genetic contribution to the most common inflammatory arthritis in women — rheumatoid arthritis (RA). furthermore, the relative disability associated with these two conditions of arthritis is the same. Thus, number of interacting risk factors and processes, including genetic factors, gender, increasing age, excess weight, and injury, joint deformity and cupational exposures can be considered as the consequence or final common pathway of OA. Risk factors may differ in importance according to the site of involvement (Table 1.1), and risk factors for development of OA may differ from risk factors for progression. For example, high bone density is a risk factor for development of knee, hip and hand OA, but low bone density is a risk factor for more rapid radiographic progression of hip and knee OA.

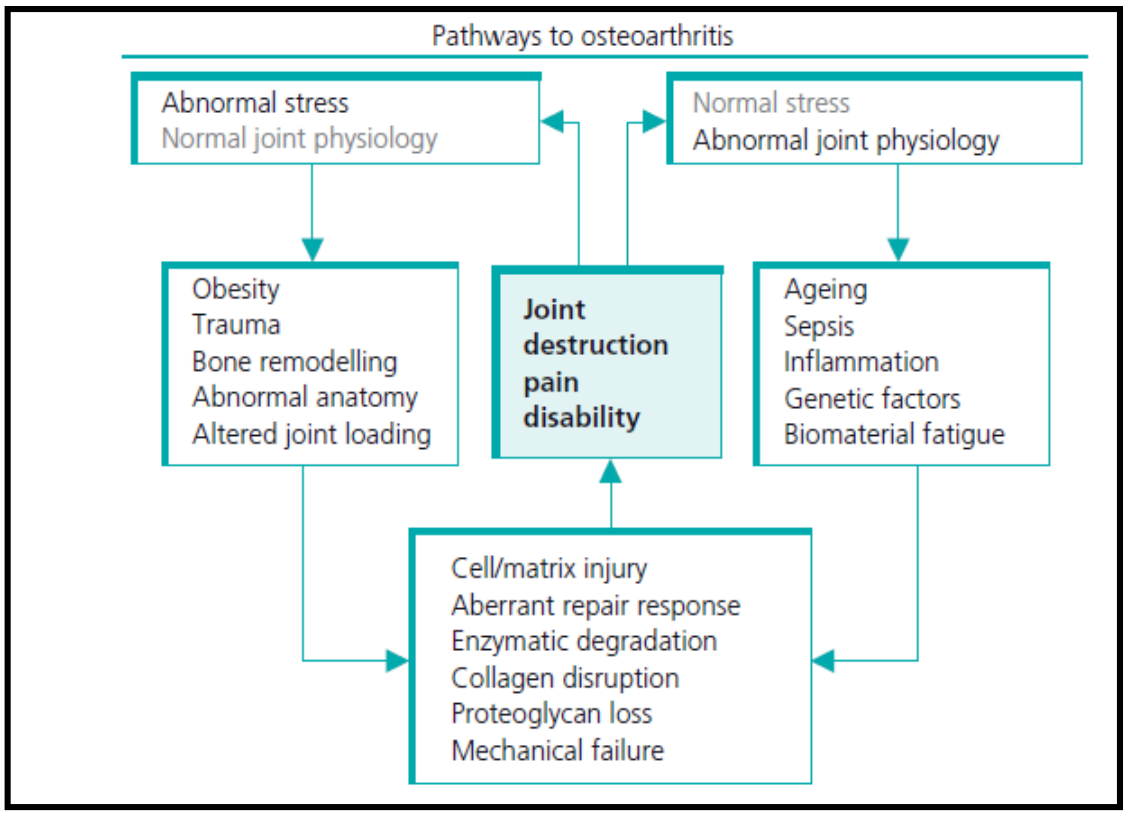


Figure 1-2: pathways to osteoarthritis. Taken from Poole *et al.*2007, withpermission of Dr.FarshidGuilak and the publisher, Lippincott Williams &Wilkins

Table 1.1: Important risk factors for osteoarthritis (Adebajo, 2011)

Risk factor	Notes
Genetics	Hand, knee and hip OA show strong heritability (40 – 60%); this probably results from combinations of multiple common polymorphisms rather than rare single genes with a large individual effect
Race	Knee OA is prevalent across the world, whereas hip OA is particularly prevalent in Caucasians
Age	Although not an inevitable consequence of ageing, OA is strongly age - related; this may reflect the cumulative effect of insults to the joint, aggravated by decline in neuromuscular function, or senescence of homoeostatic repair mechanisms
Sex	Women have a higher prevalence and radiographic severity of OA at all joint sites apart from the hip. Women are also more likely to have symptoms ifradiographic OA is present

Obesity	This is an important risk factor for knee OA, but a moremodest risk factor for hip and hand OA
Bone density	High density is a risk factor for development of knee, hip and hand OA; low density is a risk factor for more rapid progression of knee and hip OA
Abnormal joint shape and alignment	Acetabular dysplasia is a recognized cause of hip OA,and distal femoral dysplasia (often overlooked) maycontribute to knee OAvalgus malalignmentmay be a risk for development and more rapid progression of knee OA — this can have a majorinteraction with obesity
Joint trauma and Usage	Major joint injury is an important factor at the knee (especially if it causes subchondral fracture, meniscal injury or ligament rupture) and can cause OA at anysite; recognized occupational hazards include farming (hip OA), underground mining (knee OA), professionalsoccer (knee OA) and some heavy manual jobs (hand OA)

OA is conventionally separated into two main categories: primary and secondary. Primary OA typically joints in characteristic locations and is likely to result mainly from genetic predisposition, the case of abnormal joint physiology as described above. Multiple Heberden' s nodes (bony enlargement of distal inter phalangeal joints of the hand. appear in middle age and are a strong indicator for subsequent predisposition to knee OA and OA at other common target sites (" nodal generalized OA ") figure (1-3). However, OA can occur in any joint. When OA occurs in atypical joints, such as the ankle, the presentation alone should elicit consideration of secondary OA. Typical and etiologies of secondary OA include joint trauma, previous fracture and preceding inflammatory arthropathy such as gout, the case of abnormal despite the different etiologies. Pain, loss of joint motion and function, minimal (30 minutes) morning stiffness and short lived stiffness after joint stressors are the present manifestations of OA. Joint trauma is the most common of these, can lead to OA (15 – 20) years after the joint insult and can be a cause of young onset mono or pauci articular OA When abnormal joint stressors and abnormal

joint physiology happen together, the outcome is potentially even further severe. This is illustrated by the fact that severe meniscal damage to the knee is more likely to cause ultimate knee OA in patients with hand OA (evidence for a genetic predisposition to OA) compared with patients without hand OA (Englund *et al*., 2004).

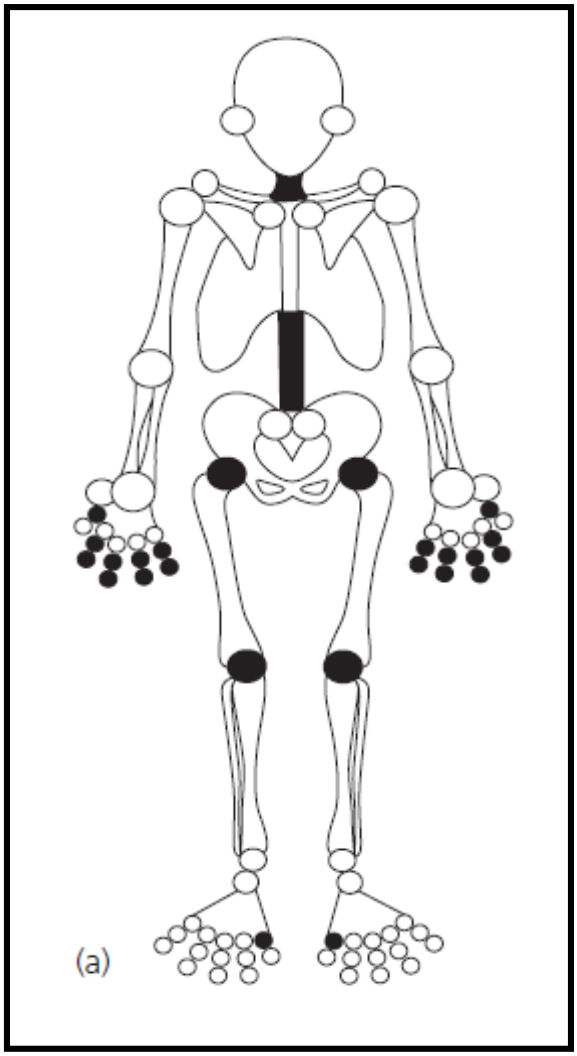


Figure 1-3: Pattern of joint distribution of primary OA (Adebajo, 2011)

1.2.1.Examination of osteoarthritis

The most important clinical features of OA are symptoms, functional impairment and signs. great discordance can exist between these three features. Pain may arise from several sites in and around an osteoarthritic joint Suggested mechanisms include increasing of intra - capsular and intra - osseous pressure,

subchondralmicro fracture and enthesopathy or bursitis secondary to muscle weakness and structural alteration table (1-2). Severity of pain and functional impairment are significantly influenced by depression, personality, anxiety, daily activity also reduced muscle strength and proprioception (muscle act an important proprioceptive role). Crepitus, deformity, bony enlargement, instability and restricted movement may take place together and predominantly reflect structural changes. Varying degrees of synovitis (warmth, effusion and synovial thickening) possibly superimposed, especially notice ablein knees, and muscle weakness or wasting is extremely widespread. Assessment aims to establish the source of symptoms in each patient. When diffuse and generalized tender points are identified at tendon insertion sites, then the co - occurrence of fibromyalgia should be considered and attention to improving sleep be included in the management considerations. Only a sufficient history and examination can determine how much structural and inflammatory change is exist and how much these contribute to a patient's problems (Mustanski *et al*., 2007).

Table 1.2: Types of joint pain in OA (Adebajo 2011)

Nature of pain	Probable aetiology of pain
Pain with use	Mechanical joint damage, enthesopathy from
Pain at rest	ligament or ligamentous attachments Inflammation with effusion and joint capsuledistension
Pain at night	Intra - osseous hypertension
Sudden flare of pain	Crystal synovitis, torn meniscus, exacerbation ofcartilage breakdown due to abnormal stressorwith secondary synovitis from pro – inflammatorycascade due to release of cartilage matrixfragments; consider sepsis as a rare possibility

1.2.2. Diagnosis of osteoarthritis

Typical OA can be diagnosed by history and examination alone. Recently the main investigation that can assist to confirm OA is the plain X - ray, with

demonstration of characteristic structural abnormalities, focal joint space narrowing (due to cartilage loss), marginal osteophyte or “ spur ” formation and subchondral sclerosis of bone (Figure 1-4).

It is gradually more recognized that biochemical abnormalities of the joint come first to radiographic abnormalities by as much as decades. therefore, much effort is currently being put into identifying more sensitive imaging modalities, such as magnetic resonance imaging, bone scintigraphy and ultrasound, beside of biochemical indicators in blood, urine or synovial fluid that might identify and quantify OA more precisely and earlier than by X - ray.

1.3. Management of osteoarthritis

The goals of medical management of OA are to:

(a) Give patient education and information access.

(b) Relieve pain.

(c) Optimize function.

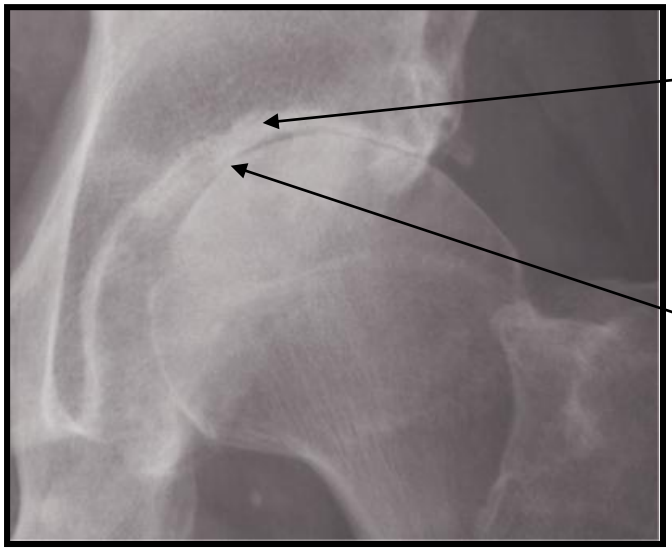
(d) Decrease disease progression.

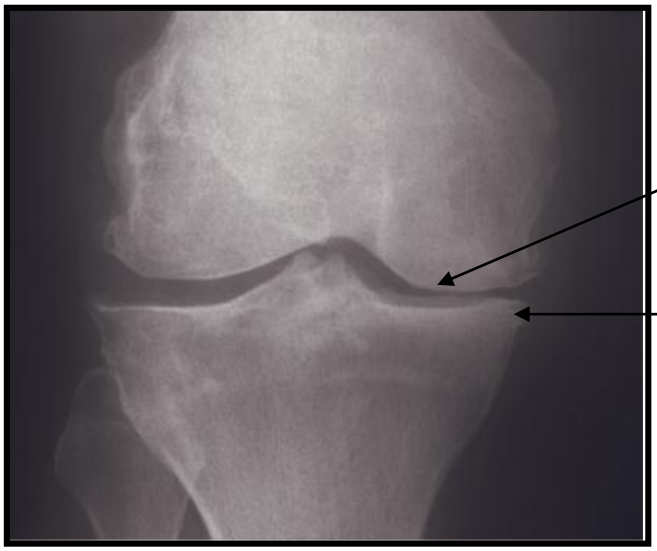
1.3.1 Patient education and information access

This is a professional responsibility, but education also improves outcome and is a treatment in its own right. The myth that OA is a progressive wearing - out of joints due to old age still persists; this invariably leads to inappropriate reductions in activity. A major contribution to managing OA has been the fi nding that a patient’s psychological status (anxiety, depression and social support) is an important determinant of symptomatic and functional outcome. Good evidence supports the use of educational programs to help patients understand OA and develop self management strategies.Symptoms of OA often are episodic, so it is advisable to provide armamentarium of treatment options to the patient to choose from during periods of relative quiescence and relative flare. table (1-3)

Table 1-3: European and US Guidelines for management of OA (Adebajo 2011)

European and US Guidelines for management of OA
• The management plan must be individualized, taking into account the site and severity of OA symptoms, any co - morbidity,concurrent medications and patient acceptability
• Non - pharmacological treatments are central — drug treatments are adjuncts
• A core and option approach is required — all patients should be offered education, an exercise programme, advice to reduce adverse mechanical factors and paracetamol as the fi rst oral analgesic to try; there is a wide range of other treatment options from which to select additional treatments, as required





A (Hip joint)

B (Tibiofemoral knee joint)

Figure 1-4: Radiographic OA: representative images of A (hip joint) and B (Tibiofemoral knee joint) with radiographic OA (Adebajo 2011)

1.3.2. Exercise

Strengthening exercise can reduce pain and disability and improve the physiological accompaniments of knee OA (muscle weakness, impaired proprioception and balance, tendency to fall). Aerobic activity also reduces pain and disability from OA, improves well - being and sleep quality, and is useful for common co- morbidities. Both forms of exercise need to be prescribed. Increased activity and exercise can be accomplished in a variety of ways (e.g. home exercise, group classes), tailored to the patient's wishes and lifestyle. Pool exercise, where in people weigh just one -eighth what they weigh on land, can mitigate negative effects of excessive joint loading due to obesity and allow freedom of joint movement and aerobic training for individuals with lower extremity OA. (Adebajo.2011)

1.3.3. Reduction of Adverse Biomechanical Factors

Spreading of physically hard jobs (e.g. housework, mowing the lawn) at intervals through the day, with breaks in between ("pacing"), can reduce persistent mechanical loading. Weight diminution can improve function and decrease pain in obese and overweight patients and may slow progression of knee and hip OA. Appropriate foot wear (thick soft sole, no raised heel, broad forefoot and deep soft uppers) can reduce impact loading in people with knee and hip OA, and wedged insoles can counteract knee virus deformity. Walking sticks and other walking aids reduce loading across OA joints. (Adebajo 2011)

1.3.4. Pharmacological Treatments

The high prevalence of OA, especially in the elderly, means that co - morbid conditions often exist, and management of OA must take into account these conditions and potential for drug interactions.pain is the main reason patients seek help. Long term, the non - pharmacological lifestyle measures

mentioned above can all reduce pain, but drugs are often helpful adjuncts to help quickly reduce pain during exacerbations of symptoms. Paracetamol should be the first oral analgesic to try, based on its excellent safety and reasonable efficacy. Topical non- steroidal anti- inflammatory drugs (NSAIDs) and topical capsaicin are also safe and are particularly useful for hand and knee OA.

Oral NSAIDs, including highly selective COX inhibitors and weak opioids (e.g. codeine, tramadol) may be considered for those patients who obtain insufficient relief from paracetamol and/or topical agents. The increased risk of gastrointestinal ulceration and bleeding from traditional NSAIDs can be decreased by concomitant prescription of a proton pump inhibitor or misoprostol. The highly selective COX inhibitors, although safer on the gut, may increase the risk of myocardial infarction and stroke, as indeed may many of the traditional NSAIDs. Traditional NSAIDs also cause adverse effects on renal function, especially in the elderly, and have multiple potential drug interactions. Oral NSAIDs and selective COX inhibitors therefore should be given at the lowest effective dose on an as - required, rather than regular, basis. Weak opioids, either alone or in combination with paracetamol, may provide good pain relief, but central nervous system side effects (e.g. constipation, headache, confusion) often limit their usefulness. Nutraceuticals provide an alternative in older, high – risk patients with co - morbidity because they have no associated renal or gastrointestinal side effects and are very popular with patients. Glucosamine is contraindicated in patients with shellfish allergy.

The initiation and use of either glucosamine or chondroitin sulphate requires monitoring of glucose in diabetics, as these agents are associated with mild insulin resistance animals. Intra articular corticosteroid injection is a helpful treatment that often gives fast effective relief of pain that may end just a few weeks to a few months. It is particularly useful to tide a patient over an important event (e.g. family wedding, holiday) and to improve pain during

initiation of other interventions such as an exercise program. A variety of hyaluronan preparations are also available, given as a single injection or a course of one per week for (3 – 5)weeks. Although a modest, relatively prolonged (several months) improvement in pain may result, the cost and logistics of this treatment are limiting.

1.3.5. Surgical

The success of prosthetic joint replacements has greatly advanced management of end - stage hip and knee OA. Surgery is also used increasingly now at the shoulder, elbow, and thumb base. Although issues of funding, waiting times, choice of prosthesis and revision have to be faced, there is no doubt that such surgery can transform a patient's life. Other surgical approaches (osteotomy, arthrodesis or joint fusion) may also be useful in specific circumstances. Arthroscopic debridement and lavage is indicated if a patient withOA describes mechanical locking.

Symptomatic improvement following joint lavage alone can last several months. The criteria for referral for consideration of joint replacement are not universally agreed upon, but include uncontrolled pain and severe impairment offunction despite conservative treatment.

Age it is not a contraindication. Is Autologous chondrocyte transplantation procedure that is currently typically reserved for young patients with ever chondral defects.

In summary, Mobility limitation resulting from osteoarthritis, particularly in the lower extremities, makes it difficult for those affected to make routine hygiene and treatment. A large national survey in Australia found that persons who reported that they had osteoarthritis were less preventive oral hygien than persons from the general population without arthritis. (Pokrajac- Zirojevic *et al*., 2002).

Osteoarthritis (OA) and periodontitis share physiopathological characteristics. OA patients also suffer from an important inflammatory component in the soft tissue and bone structural modifications. (Gurt, A. *et al*., 2014). Increased blood levels of proinflammatory mediators such as IL-6, TNF α, and hsCRP have been documented in adults with OA and PD. Both PD and OA are also associated with diabetes, CVD, obesity, and other proinflammatory conditions. However, the link between OA and PD remains unknown.

1.4. Cytokines

Cytokines is derived from a combined two greek words: “cyto” means cell and “kinos” means movement. They are small proteins that play an important role intransmitting signals between cells and aid in communication between cells and affect their behavior. Cytokines are a large family of molecules that includes the agents: chemokine’s, interferons, interleukins, lymphokines, tumer necrosis factor (Ibelgaufts, 2013) but in general it does not include hormones or growth factors.

Cytokines have a wide spectrum of sources from which they have been produced, including macrophages, B lymphocytes and T lymphocytes and mast cells, they are an immune cells, as well as endothelial cells, fibroblasts, and different stromal cells, different types of cells can produce these cytokines (Lackie, 2010; Ibelgaufts,2013).

They act through receptors, and are principally important in the immune system; cytokines modulate the balance between humeral and cell-based immune responses, and they regulate the maturation, growth, and responsiveness of particular cell populations. Some cytokines develop or inhibit the action of other cytokines in complex ways. They are unlike hormones, which are also important cell signaling molecules, in that hormones circulate in much lower concentrations and hormones tend to be made by specific kinds of

cells. Cytokines are important in health and disease, specifically in host responses to infection, immune responses." said by (Ibelgaufts, 2013).

1.5.Tumor necrosis Factor Alpha (TNF-α)

Tumor necrosis factor α (cachectic) is a member of a family of small biologically active proteins known as cytokines. TNFα plays a key role in the innate inflammatory response in vertebrates. When macrophages and T cells detect an invading pathogen, they produce TNFα, triggering local production of chemokine and additional cytokines, which then attract and activate leukocytes at the infection site (Heller and Krönke, 1994; Lentsch *et al*. 1999; Sternberg, 2006; Tanabe *et al.*,2010). TNFα also participates in the development of acquired immunity from the infection (Ugrinovic *et al.* 2003). TNFα plays a role in numerous other processes throughout the body, including remodeling of tissues and causing apoptosisof tumor cells (Goodsell, 2006). Dysregulation of TNFα production is involved in periodontal disease, heart disease, auto-immune conditions such as (Rheumatoidarthritis), and osteoarthritis (Torre-Amione*et al.*, 1996; Wu *et al.*, 1996; Fernandes Filho *et al*. 2002; Sharma *et al*. 2007; Cirelli *et al.* 2009). The primary role of TNFα is in the regulation of immune cells. TNFα, being an endogenous pyrogenic, is able to induce fever, apoptotic cell death, cachexia, inflammation and to inhibit tumor genesis and viral replication and respond to sepsis via IL-1 & IL-6 producing cells. Dysregulation of TNFα production has been implicated in a variety of human diseases including Alzheimer's disease (Dowlati *et al.*,2010), cancer (Locksley *et al.*, 2001), major depression (Dowlati *et al.*, 2010) and inflammatory bowel disease (IBD) (Brynskov*et al.*, 2002).

1.5.1.Tumor necrosis factor (TNF) and chronic periodontitis

Tumor necrosis factor (TNF) is one of pro-inflammatory cytokine that result in periodontal tissue destruction (Graves, 1999). Tumor necrosis factor α (TNFα) is part of the key cytokines in the beginning and maintenance of systemic inflammation which effect in progression and severity of Periodontitis (Nakajima *et al*., 2010).

TNFα has a a lot of of actions, mostly pro-inflammatory. Leukocyte recruitment and vascular permeability are facilitated by the stimulated expression of selectins and adhesins by TNF-α. Macrophage induced angiogenesis is also mediated by TNF-α and has a pivotal role in vascular proliferation in the periodontal granulation tissue formation (Leibovich SJ,1987). In response to bacterial LPS, TNF-α triggers osteoclast activation, proliferation and differentiation resulting in bone resorption. (Beutler, 1985).

1.5.2. Tumor necrosis factor alpha (TNFα) and Osteoarthritis:

Current evidence suggests that proinflammatory cytokine are responsible for the catabolic process occurring in the tissues. They appear to be first produced by the synovial membrane, and diffused into the cartilage through the synovial fluid, where they activate the chondrocytes to produce proinflammatory cytokines. In A synovial membrane, it is the synovial lining cells that play major role as inflammatory effectors of which interleukin (IL-1α), Tumor necrosis factor (TNFα), (IL-6), leukemic inhibitor factor (LIF) and IL-17 appear most relevant to the disease. (Martel-Pelletier, 1999)

Osteoarthritis data strongly support the concept that IL-1α, and perhaps TNF-α, are the major catabolic systems involved in the destruction of joint tissues, and may represent the source of articular tissue degradation. It is still uncertain whether IL-1α and TNFα act together or in concert to induce the pathogenesis of OA, or if functional chain of command exists between these proinflammatory cytokines. (Pelletier *et al*., 1997).

1.6. High sensitive C-Reactive Protein

It was first reported by Tillett and Francis in 1930 and was named so because it was discovered as a substance in the serum of patients with acute inflammation that reacted with the C- (capsular) polysaccharide of *Pneumococcus* (Suliman, 2015). It was initially thought that hsCRP might be a pathogenic secretion as it was elevated in people with a variety of illnesses including cancer however, discovery of hepatic synthesis demonstrated that it is a native protein (Gomaa, 2015). hsCRP plays a key role in the host's defense against infection. In the presence of calcium, hsCRP specifically binds to polysaccharides such as phosphocholine moieties present on the cell surface of many pathogenic microbes. Its binding activates the classical complement pathway and opsonises (prepares) ligands for phagocytosis. It also neutralizes the pro-inflammatory platelet-activating factor and down-regulates polymorphs (Ansar& Ghosh 2016), hsCRP is a pentameric plasma protein with homologs in vertebrates and many invertebrates that participate in the systemic response to inflammation. It is a pattern recognition molecule, that is extremely sensitive and non-specific acute-phase marker for inflammation, produced in response to many forms of injury other than binding to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens ((Singh *et al*., 2008), hsCRP are usually exist at relatively low level in the blood and raised within 72 hrs of tissue injury or with infection.

It is regulated by cytokines like interleukin-6 (IL- 6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) (Ebersole and Cappelli, 2000), (Bennett and Plum, 1996).These in turn cause systemic changes including hepatic release of a range of plasma proteins, activation of complement proteins and various metabolic changes (Kravitz and Shoenfeld, 2006). hsCRP levels have an association with smoking, obesity, triglycerides, diabetes and periodontal disease.It is proposed that changes in cellular and molecular

components of peripheral blood can be found in subjects with periodontitis because of inflammatory changes of the periodontal tissues. According to (Bansal et al., 2014), hsCRP is currently considered a key biomarker of systemic inflammation, and although it is mainly synthesized by hepatocytes in the liver in response to inflammation and tissue damage, it can also be produced locally by arterial tissue, hsCRP opsonizes bacteria for complement-binding and activates complement when complexes.

The acute-phase reaction represents an early and highly complex reaction of the organism to a variety of injuries such as bacterial, viral or parasitic infection, mechanical or thermal trauma, ischemic necrosis or malignant growth. This induces a complex series of non-specific, systemic, physiological and metabolic responses leading to increased synthesis and secretion of plasma proteins. This phenomenon is termed as 'acute phase response'. These changes are called 'acute' because most are observed within hours or days following the onset of infection or injury, although some acute phase changes also indicate chronic disease.

1.6.1 high sensitive C-Reactive Protein and Periodontal disease:

Periodontitis is defined as the inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both (Kinney *et al.*, 2014). This condition occurs in response to a predominantly gram-negative bacterial infection originating from dental plaque. However, the disease typically remains asymptomatic for decades and can be detected only by clinical examination with a periodontal probe or with intra-oral radiographs. It is only recently that researchers have begun to identify local and systemic inflammatory process that encourages a pathological response to an initial commensal microflora. Increased levels of acute-phase proteins have been noted

with gingival inflammation and periodontitis, reflecting the locally stressed environment (Bansal *et al*., 2014).

Since hsCRP is an acute-phase reactant produced by the liver in response to diverse inflammatory stimuli, recent studies have shown that their levels are elevated in periodontal disease. However not all studies have reported an association between destructive periodontal disease and hsCRP. It possibly reflect differences in destructive periodontal disease severity or disease progression in different study populations. hsCRP value <10mg/L were considered normal, while acute bacterial infections have been reported in 80% to 85% of patients with CRP values >100mg/L

Craig *et al*., (2003) Historically, CRP values >10 mg/l were regarded as diagnostic for a bacterial infection, while values <10 mg/l were neglected. This may be because CRP assays in the past were not very accurate and sensitive as in present and thus were less efficient in detecting the levels of CRP <10mg/l. So, these days' high-sensitivity assays for CRP have come into widespread use, allowing laboratories to determine hsCRP levels in serum as low as 0.15 mg/l.

According to (Hage and Szalai, 2007) hsCRP can bind *phosphoethanolamine* and phosphocholine of disrupted bacterial and host cell membranes as well as chromatin, small nuclear ribonucleo proteins, laminin and fibronectin in the presence of calcium. hsCRP, when bound to these ligands can activate the complement cascade. hsCRP receptors also exist on macrophages, monocytes and neutrophils and thus bound hsCRP can target bacterial and damaged host cells for phagocytosis and help direct and amplify the subsequent local inflammatory response to infection, trauma and necrosis. In acute inflammation serum CRP levels exceed 100 mg/L, and the level decreases in chronic inflammation.

Recent evidence has indicated that patients with severe periodontitis have increased serum levels of hs CRP, when compared with unaffected control population (Ebersole *et al*.,2016).But they fail little in indicating that

periodontitis was the cause for the observed serum hsCRP levels as CRP levels fluctuate with various confounding factors like aging, high blood pressure, alcohol use, smoking, low levels of physical activity, chronic fatigue, coffee consumption having elevated triglycerides, insulin-resistance diabetes, taking estrogen, eating a high-protein diet, suffering from sleep disturbances and depression (Au, 2014).

Various studies have proved a positive association between the presence of chronic periodontitis and high serum hsCRP levels (Gurav,2014). Because it is biologically plausible that inflammatory mediators (IL-1, IL-6 and TNF-α) are released under conditions of periodontitis and present the capacity to stimulate the hepatocytes to produce CRP. In the similar manner, it can be expected that, in the presence of chronic periodontitis, higher serum CRP levels would be found. To confirm that elevation in hsCRP is due to periodontal infection, it is essential to see whether periodontal treatment is effective in reducing CRP level. Following successful periodontal basic therapy, bacterial load is significantly reduced, while antibody titers to the specific pathogens are improved. As a result of these changes, local inflammation significantly decreases and there is a significant improvement of the clinical parameters.

1.6.2.High sensitive C- Reactive Protein and Osteoarthritis

High sensitive C-reactive protein (CRP) has been used to distinguish systemic inflammatory disorders such as rheumatoid arthritis (RA) from ‘‘noninflammatory’’ diseases such as osteoarthritis (OA). More recently, with the introduction of high-sensitivity CRP assays (hsCRP), which can detect CRP levels that are an order of magnitude lower than traditional assays, low-level elevations in CRP have been observed in diseases where there is a local, low-grade inflammatory component. (Ridker *et al*., 2005), (Hashimoto *et al*., 2001).

hsCRP production by hepatocytes, the main source of this acute-phase reactant, appears to be regulated primarily by the proinflammatory cytokines

interleukin (IL)-6 and IL-1. Furthermore, hsCRP itself may have important immune-modulating functions as it has been shown to have multiple roles in innate immune responses and tissue injury, and has the ability to activate the complement system, and enhance phagocytosis via opsonization (Du Clos and Mold, 2004).

Multiple studies have demonstrated that hsCRP is modestly elevated in the serum of patients with OA compared to age-matched controls (Wolfe F. The C-reactive protein but not erythrocyte sedimentation rate is associated with clinical severity in patients with osteoarthritis of the knee or hip. (Conrozier *et al*., 2000 (Sowers *et al*., 2002).

The acute phase protein C-reactive protein (CRP) is an important inflammatory regulator and measurement of circulating CRP is widely used as a diagnostic tool in acute inflammatory diseases. However, CRP has shown limited value in monitoring the low, but elevated level of inflammation in OA can be detected. (Dolzani *et al*., 2011) (Bejon *et al*., 2011)

Although the role of inflammation in osteoarthritis has been unclear for a long time and regard as noninflammatory arthritis, significant progress has been made in more recent years, and studies also show that there are ongoing inflammation and synovitis that result in permanent joint damage (Ehrlich, 2003)Given this greater appreciation for synovitis in patients with OA, inflammation has now been strongly implicated in the pathogenesis of OA (Scanzello and Goldring, 2012)

In many research studies have demonstrated that circulating levels of CRP, a marker of low-grade systemic inflammation, are modestly elevated in KneeOA and are associated with decreased cartilage volume and disease progression. (Sharif *et al*., 2000) Increased serum C reactive protein may reflect events that precede radiographic progression in osteoarthritis of the knee. (Stannus *et al*., 2010).

CHAPTER TWO
MATERIALS AND METHODS

Materials and Methods

2.1. Human sample

The sample in this study consisted of eighty males and females subjects with age range (35-50) years. moderate chronic periodontitis with osteoarthritis (CP+OA) group (30)patients were seeking the rheumatology clinic in Baghdad Teaching Hospital and the moderate chronic periodontitis (CP) groups (30)patients with control (C) group (20) subjects were recruited from the attendants to Iraqi National Blood Bank. The people enrolled voluntarily in the study in the period between December, 2016 – March, 2017 after taking an approval to perform this study (Appendix 2).

2.2. Design of the study:

After consents have been taken from all subjects include their agreement to submit this investigation. A self-reported questionnaire (Appendix 1) was taken include: Name, age, full medical history, dental history, clinical periodontal parameters {Plaque Index (PI), Gingival Index (GI),bleeding on Probing (BOP), Probing Pocket depth (PPD), Clinical Attachment Level (CAL).The blood was taken from suspected patient & control healthy individual and put it in plain tubes without anticoagulant, after centrifuging the serum was transferred immediately into other plain tubes and then preserved in freeze (-15C^{o}) until they have been assayed for CRP and TNFα.

2.2.1 Grouping of the studied sample

The sample was divided into three groups

1- Group (CP+OA) (chronic periodontitis /osteoarthritis):-

Thirty patients diagnosed to have moderate chronic periodontitis and osteoarthritis. The patients with osteoarthritis had documentation or

radiographic imaging consistent with degenerative arthritis in the absence of an inflammatory condition.

2- Group (CP) (chronic periodontitis / systematically healthy):-

Thirty patients diagnosed to have moderate chronic periodontitis and didn't have osteoarthritis. Moderate Chronic periodontitis in patients was defined as the presence of four sites with probing pocket depth ≥4-6mm with clinical attachment loss ≥2-4mm, this made according to the international classification system for periodontal disease (Lang *et at*, 1999).

3- Control Group (C) (clinically healthy periodontium / systemically healthy):-

Twenty patients with clinically healthy periodontium and healthy systemic status.

2.2.2. Exclusion criteria

- Pregnant and menopause ladies.
- Smokers
- Any patient had history of other chronic systemic diseases with known associations with CP as cardiovascular diseases and diabetes mellitus.
- Rheumatoid patient.
- Periodontitis patient who received periodontal treatment and /or antibiotics during the last 3months.

2.3. Materials

2.3.1 Clinical Instruments and materials:

1. Disposable dental mirrors.figure (2-1)
2. Periodontal probe (the University of Michigan O probe markings at (1,2,3,5,7,8,9,10 mm). figure (2-1)
3. Tweezers. Figure (2-1)

4. Mask and gloves.figure (2-1)
5. Kidney dish.figure (2-1)
6. Hot air oven (memmert).
7. Cotton. Figure (2-1)
8. Disposable towels.
9. Disposable cups.
10. Torniqa.
11. Disposable syringe (5mm).
12.plain gel tube. figure (2-2).
13.wound sticker
14.marker pen
15. Disposable vacuum tubes. Figure (2-2

2.3.2.Laboratory Instruments & Equipment's:

1. Disposable Plane tubes figure (2-2
2. Plane tube rack. figure (2-3)
3. Adjustable micro pipettes figure (2-4)
4. Multi-channel pipette capable of dispensing 50-100µl (SLAMED). figure (2-4)
5. Disposiple micropipette tip. figure (2-4)
6. Centrifuge machine (universal 16A, Germany) figure (2-5)
7. Disposable syringes.
8. Microplate ELISA Incubator. figure (2-6)
9. Microplate ELISA Reader device
10. Microplate ELISA Washer device figure (2-7).
11. Test kit, ELISA Kit (96-wells) for quantitative determination of serum human hs CRP kit. (sunlong biotech,.co.,ltd) figure (2-8)
12. Test kit, ELISA Kit (96-wells) for quantitative determination of serum humanTNF-α. (sunlong biotech,.co.,ltd.) figure (2-9)

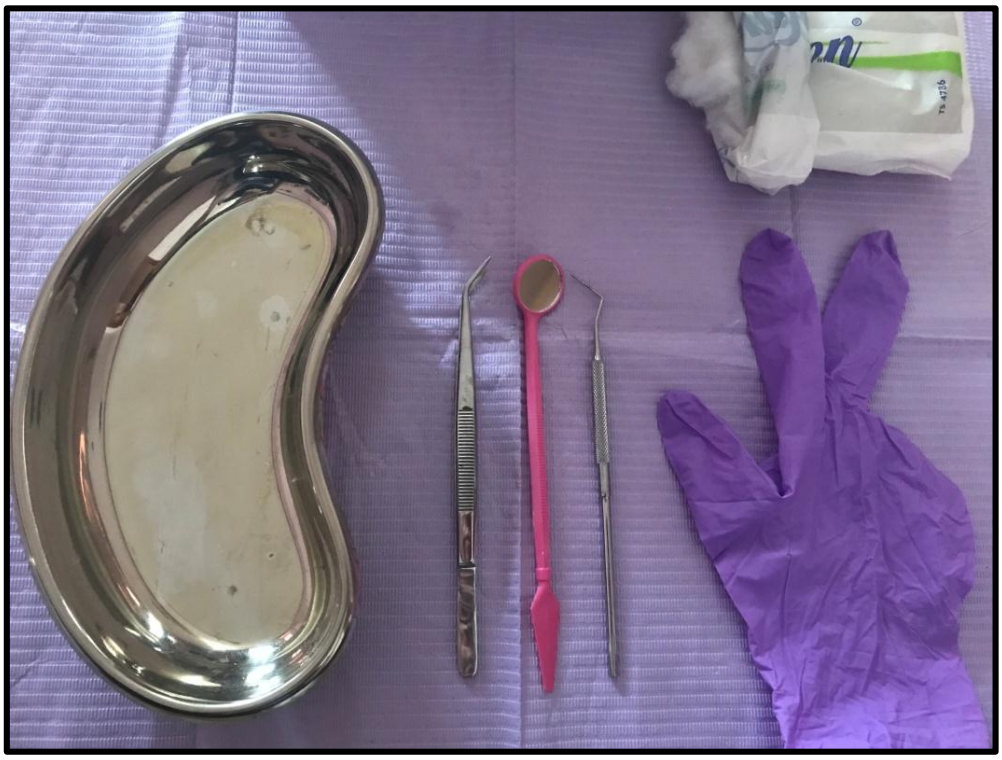


Figure 2-1: Kidney dish, disposable dental mirror, tweezer, periodontal probe, gloves and cotton.

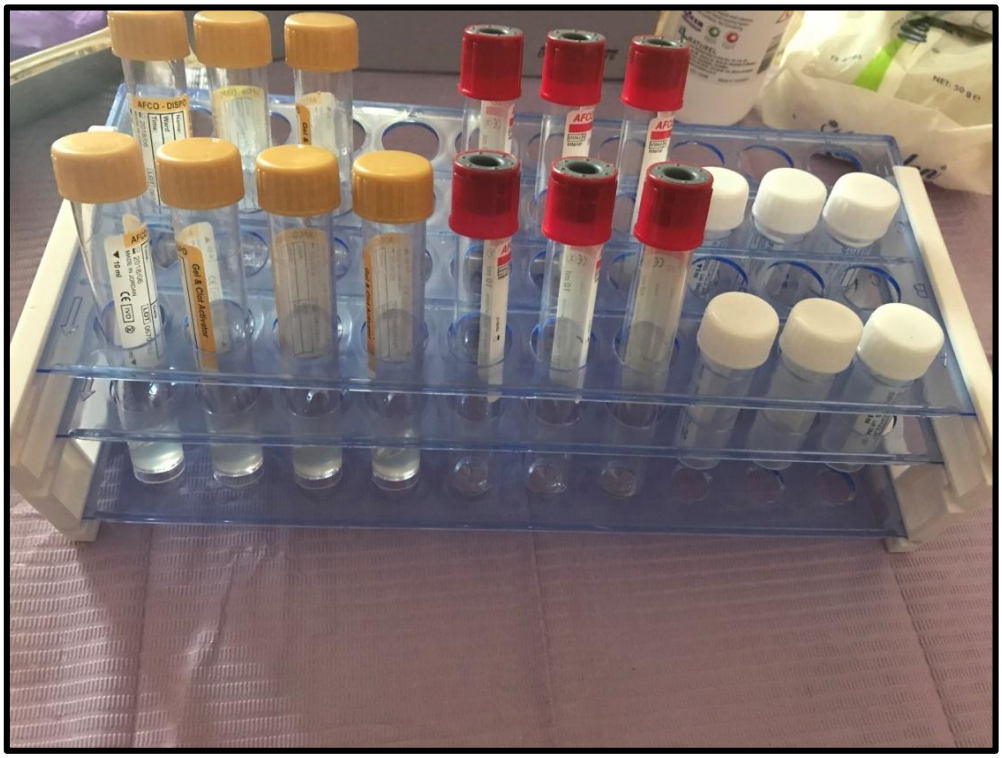


Figure 2-2: Disposable plain gel tube, vacuum tube, plain tube.

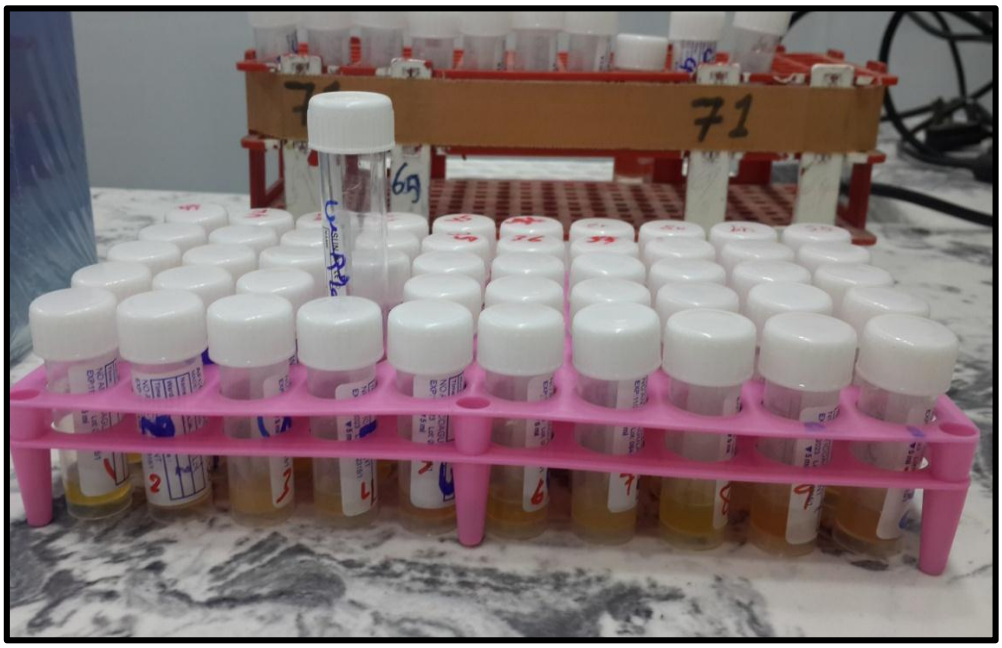


Figure 2-3: Plain tube rack

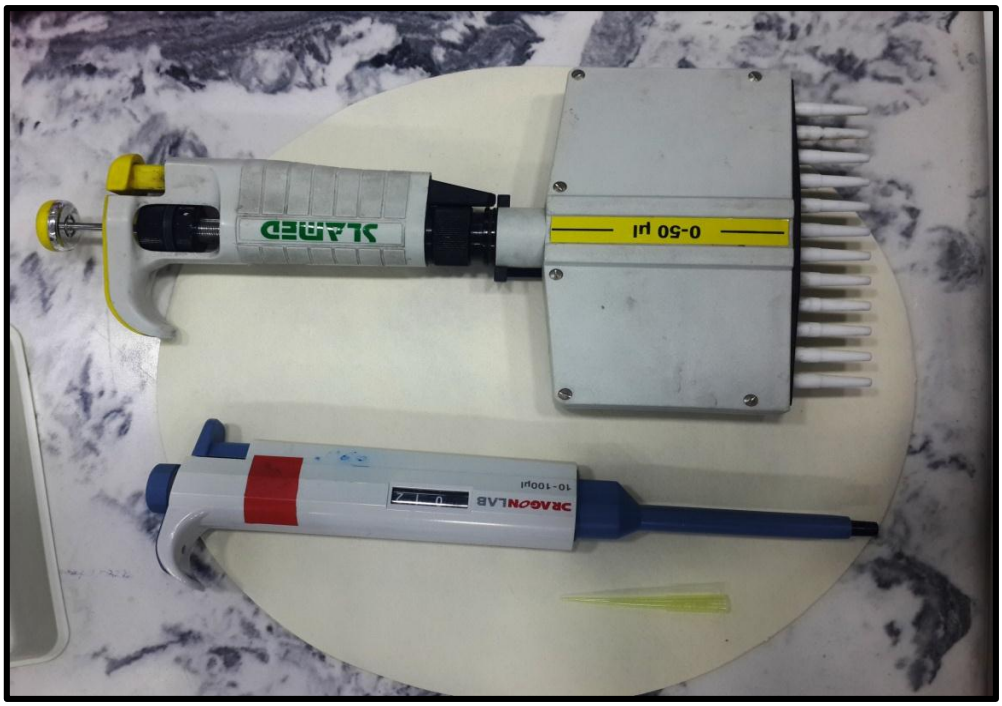


Figure 2-4: Adjutble micro pipette, multi-channel pipette & disposible tip.

Figure 2-5: Centrifuge machine (universal 16A)

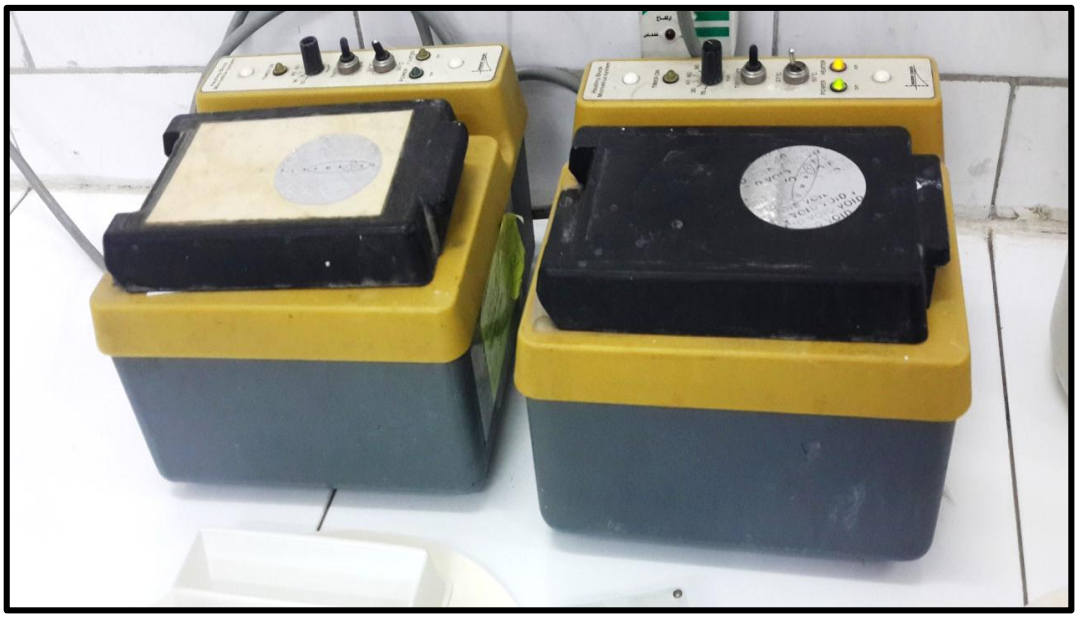


Figure 2-6: Microplate ELISA Incubator

Figure 2-7: microplate ELISA washer

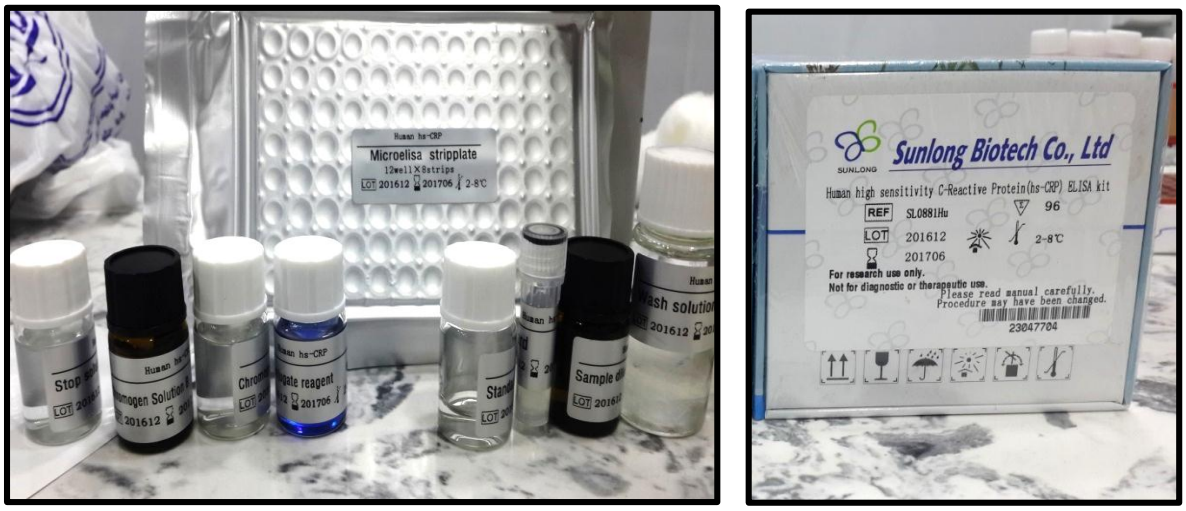


Figure 2-8: Human ELISA kit for hsCRP

Figure 2-9: Human elisa kit of TNFα

2.4. Methods

2.4.1. The clinical periodontal parameter

The four surfaces of each tooth except 3rd molar were examined and scored. Oral examination was performed according to the following criteria:

2.4.1.1 Plaque Index (PL):

According to Plaque Index by (Silness&Löe 1964).The score and criteria for this system as proposed by authors:

- Score criteria
- Score 0 no plaque in the gingival area.
- Score 1 a film of plaque adhering to the free gingival margin &adjacent area of the tooth surface, the plaque may be recognized only by running a probe across the tooth surface.
- Score 2 moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and or adjacent tooth surface which can be seen by naked eye.
- Score 3 abundance of soft matter within the gingival pocket and or on the gingival margin and adjacent tooth surface.

2.4.1.2 Gingival Index (GI):

The occurrence of gingival inflammation was assessed using the criteria of gingival index system (Löe 1967).

- Score 0: normal gingiva.
- Score 1: mild inflammation, slight change in color, slight edema, no bleeding on probing
- Score 2: moderate inflammation, redness and glazing, bleeding on probing.
- Score 3: sever inflammation, marked redness and ulceration, tendency tospontaneous bleeding.

2.4.1.3 Bleeding on probing (B.O.P):

Bleeding on probing defined as bleeding that occurred at any of the 4 sites of the tooth within 30 seconds of probing. BOP occurs by inserted the periodontal probe inside the pocket, if bleeding occurs within 30 seconds after probing, the site was given (score 1) where as a (score 0) was given for non-bleeding sites.

2.4.1.4 Probing Pocket Depth (PPD):

It is the distance from the gingival margin to the point at which periodontal probe stop in the gingival crevice. The sites for measurement were mid-buccalline, mid-palatal line, mesio-buccal and disto-buccal line angle. The PPD was estimated by using (Michigan O probe).

2.4.1.5 Clinical Attachment Level (CAL):

By definition CAL is the distance from the cemento-enamel junction (C.E.J) to the deepest point of the insertion of probe tip. The measurements were made at four surfaces (mesial, buccal, distal, palatal and lingual) of each tooth except 3rd molar. In case the gingiva covering the C.E.J. the istance was measured indirectly by subtracting the distance from the gingival margin to the (C.E.J) from probing pocket depth (Löe & Brown 1991). In some cases when

there was gingival recession, CAL was measured by adding the distance from the gingival margin to the (C.E.J) to the probing pocket depth (Linden and Mullally, 1994). The level of the (C.E.J) could be determined by feeling it with probe.

All the clinical measurements were done on what is look like a dental unit under light vision using mirror & periodontal probe required for measurements.

2.5. Collection of Blood Sample:

Five ml of venous blood sample were aspirated from anticubital vein of each individual, using disposable plastic syringes with 23 gauge stainless steel needle. The whole blood was collected in sterile disposable plain tubes. After collection of the whole blood, leaving it undisturbed at room temperature. then centrifuging at 2,000-3,000 rpm for 20 minutes. Aspirated and transferred immediately into another tube and frozen at (-15 C°) for subsequent analysis. Haemolyzed samples were discarded.

2.6. Principle of hsCRP:

The ELISA kit uses Sandwich-ELISA as the method. The Microelisastripplate provided in this kit has been pre-coated with an antibody specific to hs-CRP. Standards or samples were added to the appropriate Microelisastripplate wells and combined to the specific antibody.

Then a Horseradish Peroxidase (HRP)- conjugated antibody specific for hs-CRP was added to each Microelisastripplate well and incubated. Free components were washed away. The TMB substrate solution was added to each well. Only those wells that contain hs-CRP and HRP conjugated hs-CRP antibody would be appeared blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. Table (2-1)

The OD value is proportional to the concentration of hs-CRP. It can calculate the concentration of hs-CRP in the samples by comparing the OD of the samples to the standard curve. Figure (2 -10)

2.6.1 Procedure for hs CRP:

1. Dilution of Standards Ten wells were set for standards in a Micro Elisastrip plate. In Well 1 and Well 2, 100µl standard solution and 50µl Standard Dilution buffer were added and mixed well. In Well 3 and Well 4, 100µl solution from Well 1 and Well 2 were added respectively. Then 50µl Standard Dilution buffer were added and mixed well. 50µl solution was discarded from Well 3 and Well 4. In Well 5 and Well 6, 50µl solution from Well 3 and Well 4 are added respectively. Then 50µl Standard Dilution buffer were added and mixed well. In Well 7 and Well 8, 50µl solution from Well 5 and Well 6 were added respectively. Then 50µl Standard Dilution buffer were added and mixed well. In Well 9 and Well 10, 50µl solution from Well 7 and Well 8 were added respectively. Then 50µl Standard Dilution buffer were added and mixed well. 50µl solution was discarded from Well 9 and Well 10. After dilution, the total 5volume in all the wells were 50µl and the concentrations were 9 ng/ml, 6 ng/ml, 3 ng/ml, 1.5 ng/ml and 0.75 ng/ml, respectively.
2. In the Microelisastripplate,were leaved a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample were added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Incubation: incubated 30 min at 37℃after sealed with Closure plate membrane.
4. Dilution: the concentrated washing buffer was diluted with distilled water (30 times for 96T and20 times for 48T).

5. Washing: Closure plate membrane was peeled off and aspirated and refill with the wash solution. The wash solution was discarded after resting for 30 seconds. Repeated the washing procedure for 5 times.
6. 50 µl HRP-Conjugate reagents were added to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5
9. Coloring: 50 µl Chromogen Solution A and 50 µl Chromogen Solution B were added to each well, mix with gently shaking and incubate at 37 ℃for 15 minutes. Avoided light during coloring.
10. Termination: 50 µl stop solution was added to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Absorbance O.D. was Read at 450nm using a Microtiter Plate Reader. The OD value of the blank control well was set as zero. Assay should be carried out within 15 minutes after 9 ng/ml, 6ng/ml,3ng/ml 1.5ng/ml 0.75 ng/ml
12. Stop solution was added.

Table 2-1: The mean OD absorbance and concentrations level of CRP in all standards and ten samples from patients

Standards	mean OD absorbance	Concentration of hs CRP
S1	3.333	13.500
S2	3.119	9.000
S3	2.650	6.000
S4	1.714	3.000
S5	0.962	1.500
S6	0.504	0.750
S7	0.222	0.375
SAMPLE		
X1	0.162	0.291
X2	0.177	0.312
X3	0.195	0.338
X4	0.192	0.334
X5	0.209	0.357
X6	0.188	0.328
X7	0.211	0.360
X8	0.287	0.461
X9	0.130	0.242
X10	0.262	0.428

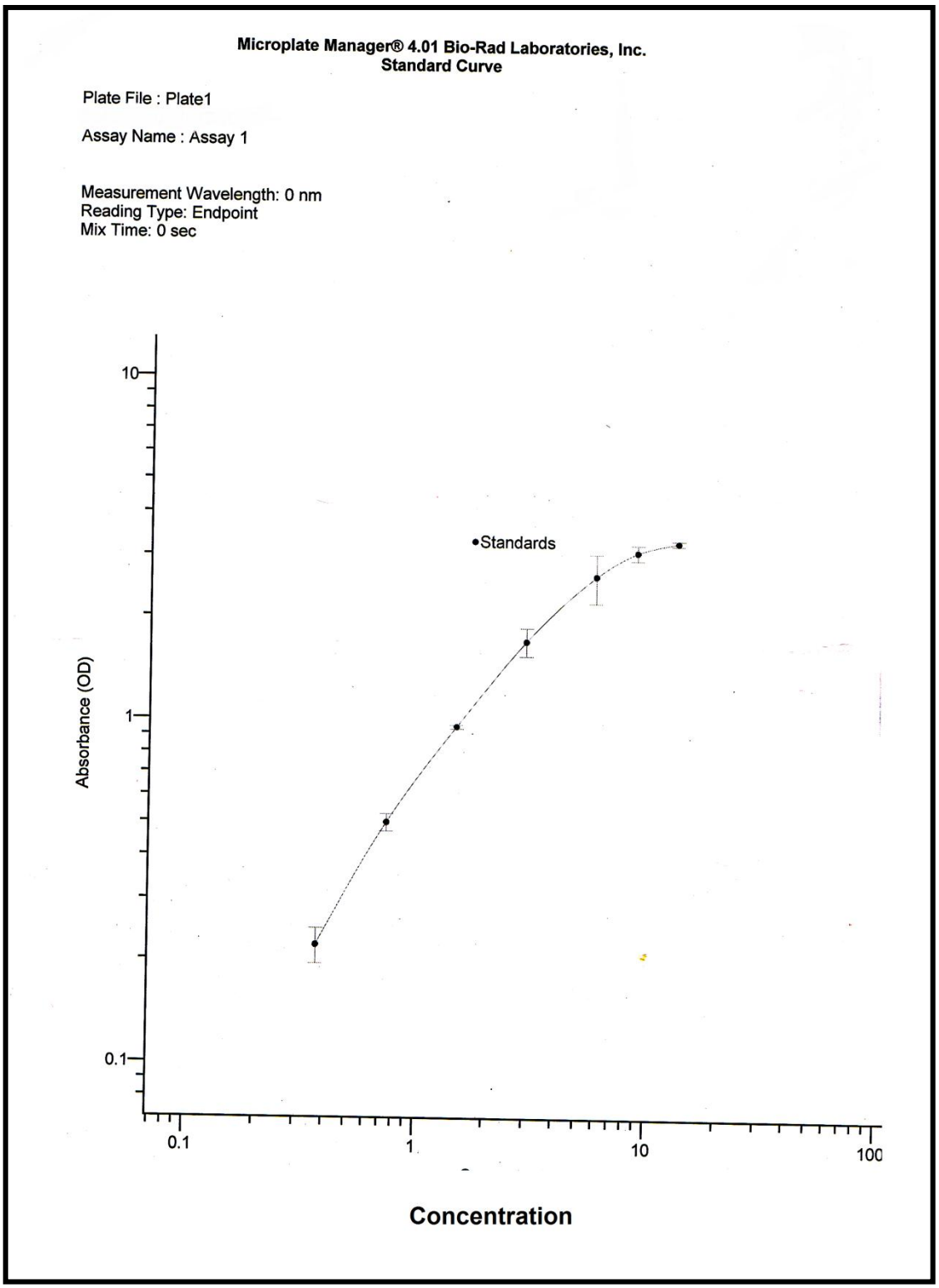


Figure 2-10: Standard curve fit for hsCRP

2.7. Principle of TNF-a test according to TNF-α (Human) ELISA Kit Protocol

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to TNF-α. Standards or samples were added to the appropriate Microelisa stripplate wells and combined to the specific antibody.

Then a Horseradish Peroxidase (HRP)- conjugated antibody specific for TNF-α was added to each Micro Elisastrip plate well and incubated. Free components were washed away. The TMB substrate solution added to each well. Only those wells that contain TNF-α and HRP conjugated TNF-α antibody appeared blue in color and then turn yellow after the addition of the stop solution figure (2-11). The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of TNF-α. It can be calculated the concentration of TNF-α in the samples by comparing the OD of the samples to the standard curve. Table (2-2) figure (2-12)

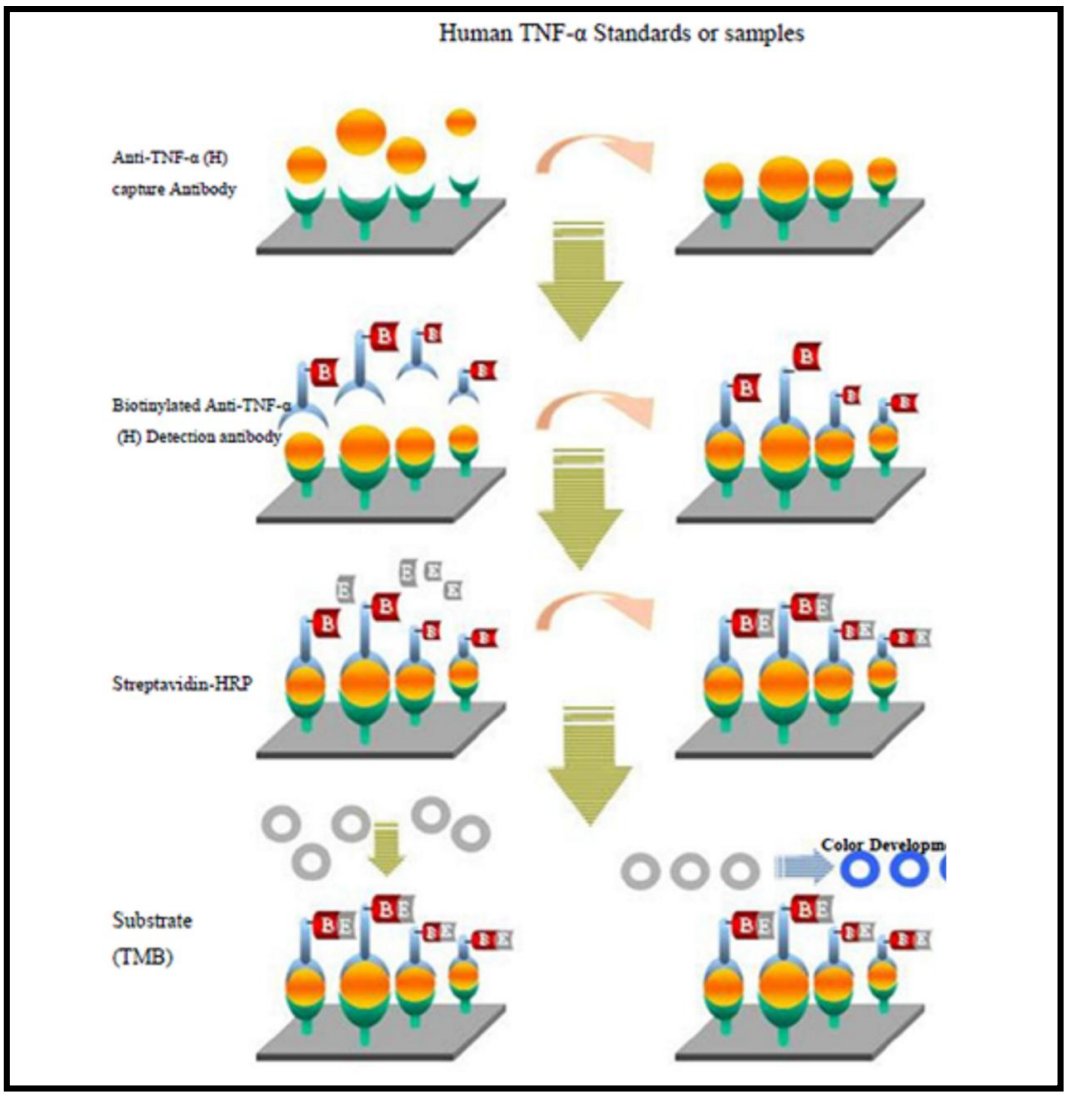


Figure 2-11: Assay principles for detection of anti TNFα antibody

2.7.1. Procedure

1. Dilution of Standards Ten wells was set for standards in a Micro Elisa strip plate. In Well 1 and Well 2, 100µl Standard solution and 50µl Standard Dilution buffer were added and mixed well. In Well 3 and Well 4, 100µl solution from Well 1 and Well 2 were added respectively. Then 50µl Standard Dilution buffer were added and mixed well. 50µl solution was discarded from well 3and Well 4. In Well 5 and Well 6, 50µl solution from Well 3 and Well 4 were added respectively. Then 50µl Standard Dilution buffer were added and mixed well. In Well 7 and Well 8, 50µl solution from Well 5 and Well 6 were added respectively. Then 50µl Standard Dilution

buffer were added and mixed well. In Well 9 and Well 10, 50µl solution from Well7 and Well 8 were added respectively. Then 50µl Standard Dilution buffer were added and mixed well. 50µl solution was discarded from Well 9 and Well 10. After dilution, the total 5volume in all the wells were 50µl and the concentrations are 300ng/L, 200 ng/L, 100 ng/L, 50 ng/L and 25 ng/L, respectively.

2. In the Micro Elisastrip plate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Incubation: incubate 30 min at 37°Cafter sealed with Closure plate membrane.
4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Add 50 µl HRP-Conjugate reagents to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Add 50 µlChromogen Solution A and 50 µlChromogen Solution B to eachwell, mix with gently shaking and incubate at 37 ℃for 15 minutes. Please avoid light during coloring.
10. Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microliter Plate Reader.

12. The OD value of the blank control well was set as zero. Assay carried out within 15 minutes after300 ng/L 450 ng/L 200 ng/L 100 ng/L 50 ng/L 25 ng/L 12.5ng/L
13. stop solutions was added

450 ng/L 200 ng/L 100 ng/L 50 ng/L 25 ng/L 12.5ng/L

Table 2-2: The mean OD absorbance and concentrations level of TNFα in all standards and ten samples from patients

STANDARD	MEAN OD ABSORBANCE	CONCENTRATION OF TNF
S1	3.399	450.00
S2	3.233	300.00
S3	2.854	200.00
S4	1.622	100.00
S5	0.865	50.00
S6	0.512	25.00
S7	0.249	12.500
SAMPLE		
X1	0.241	12.438
X2	0.172	9.320
X3	0.231	11.985
X4	0.258	13.210
X5	0.285	14.441
X6	0.112	6.586
X7	0.278	14.121
X8	0.339	16.932
X9	0.178	9.591
X10	0.240	12.121

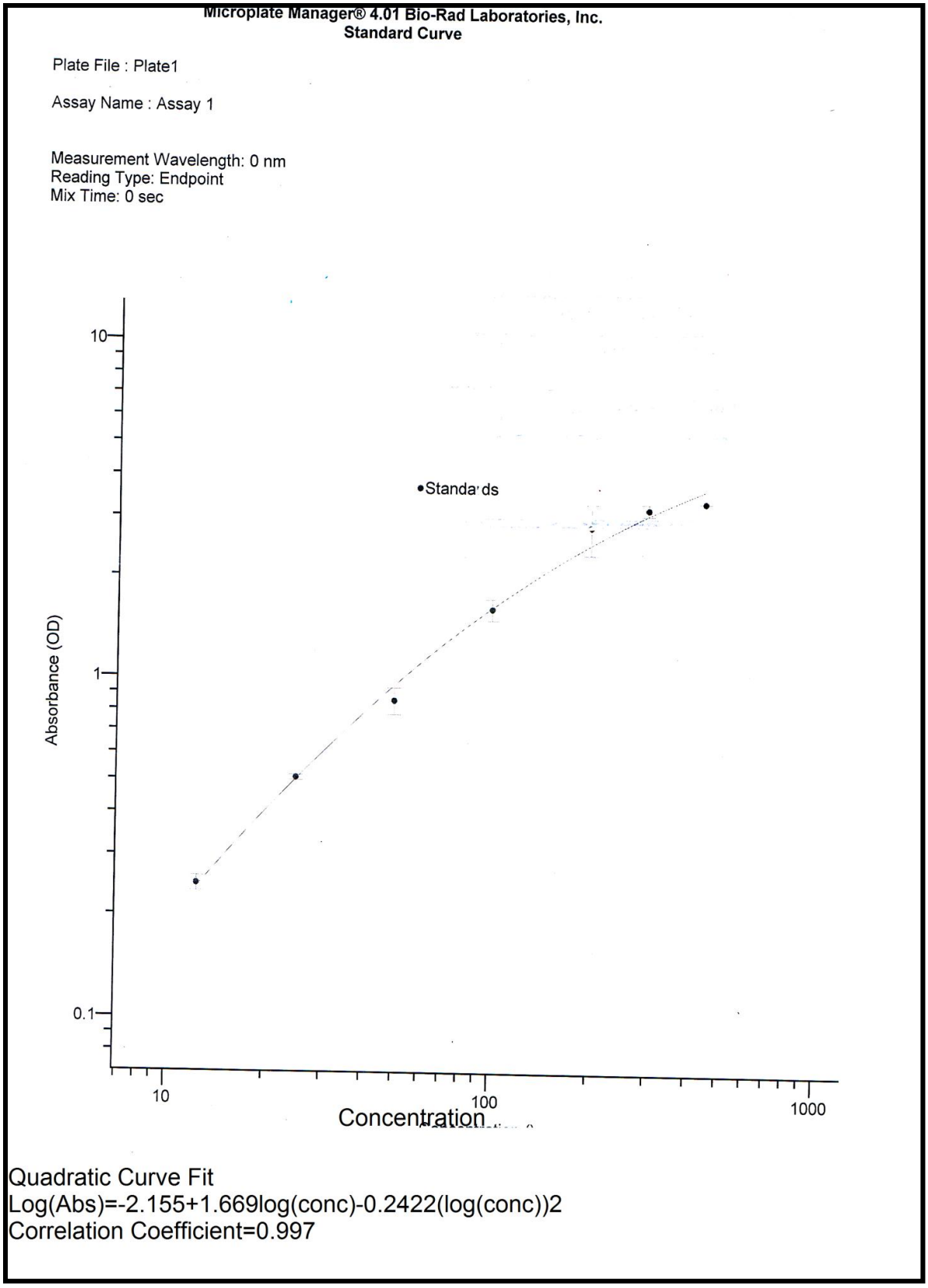


Figure 2-12: Standard curve fit for TNFα.

2.8 Calibration:

The accuracy and reproducibility of the examiner for the clinical periodontal parameters (PI, GI, BOP, PPD, CAL) were determined by:

1-Inter Examiner Calibration: The four sites of all teeth except the third molar were examined by the examiner and the supervisor for five subjects at the same times and the result was non-significant using paired t-test. Table (2-3)

Table 2-3: Descriptive and statistical test of Inter-calibration

Variables	Readings				Paired T test			Sig.
	Student		Supervisor					
	Mean	±SD	Mean	±SD	T#	df	P	
PLI	1.19	0.69	1.14	0.57	0.920	9	0.381	NS
GI	1.16	0.31	1.24	0.35	1.753		0.113	
PPD	1.90	1.45	2.10	1.29	0.802		0.443	
CAL	0.90	0.88	1.10	0.99	1.50		0.168	
BOP1	11.75	2.80	11.78	2.84	1		0.343	
BOP0	88.25	2.80	88.22	2.83	1		0.343	

NS=Not significant at P>0.05

2- Intra examiner calibration: The four sites of all teeth except the third molar were examined twice by the same examiner with an interval of one week between the two examination for (PPD,BOP and CAL).

All measurements of inter and intra calibration were computed and the result showed no significant difference (P-value >0.05) using paired t-test. Table (2-4)

Table 2-4: Descriptive and statistical test of Intra-calibration

Variables	Readings				Paired T test			Sig
	1st reading		2weeks					
	Mean	±SD	Mean	±SD	T#	df	p-value	
PPD	2	1.33	2.10	1.29	0.557	9	0.591	NS
CAL	0.80	0.79	1	0.94	1.50		0.168	
BOP1	11.58	2.61	11.59	2.62	1		0.343	
BOP0	88.42	2.61	88.41	2.61	1		0.343	

NS=Not significant at P>0.05

2.9 Statistical Analysis

Data description, analysis and presentation were performed using Statistical Package for social Science (SPSS version 21) Statistical analyses can be classified into two categories:

1-Descriptive Analysis:

A-Frequency, percentage for nominal variables, minimum, maximum, mean, standard deviation, median and mean rank for quantitative variables.

B- Simple and Cluster chart bars with and without error bars based on 95% confidence intervals (CI).

2- Inferential analysis:

A-Shapiro-wilk test: test the normality distribution of quantitative variables.

B-Fisher Exact Probability test: test used in the analysis of contingency tables when sample size is small.

C-Wilcoxon-sum rank test: non parametric test determine whether two independent samples were selected from populations having the same distribution.

D-Paired t- test: a parametric test analyzing two related quantitative dependent variables.

F-Kruskal wallis test: non parametric test to evaluate the difference between k independent variable with multiple Wilcoxon-sum rank post hoc test adjusted with Dunn-Bonferroni.

G-Spearman correlation: non parametric test to evaluate the monotonic relationship between two quantitative or ordinal variables.

CHAPTER THREE
RESULTS

Results

3.1. Descriptive statistical analysis of the demographic data

In this study 80 patients were included in 3 main groups. The first group consist of 30 patient with moderate chronic periodontitis+ osteoarthritis (CP+OA), second group include 30 patient with periodontitis only (CP), third group is control group consist of 20 healthy subjects (C). The age range for participants between (35-50) years.

The female number (32), represented (40.00 %) from the total samples and male (48), represented (60.00%) of the total samples. From the thirty patients in CP+OA group, (13) were males and the other were females that means females represent (56.67%) of the group. Similarly in CP group the number of females (9) and the males were (21) from this we observed that males were the dominant in this group and they consisted (70.00%) of the total samples. The control group, (6) females and (14) males.. Table (3.1), figure (3-1)

Table 3-1: Descriptive analysis of Gender distribution among groups.

Variables	Categories		Group		
			Control	CP. only	CP+OA
Gender	Male	NO.	14	21	13
		%	70.00	70.00	43.33
		% T	17.50	26.25	16.25
	Female	NO.	6	9	17
		%	30.00	30.00	56.67
		% T	7.50	11.25	21.25

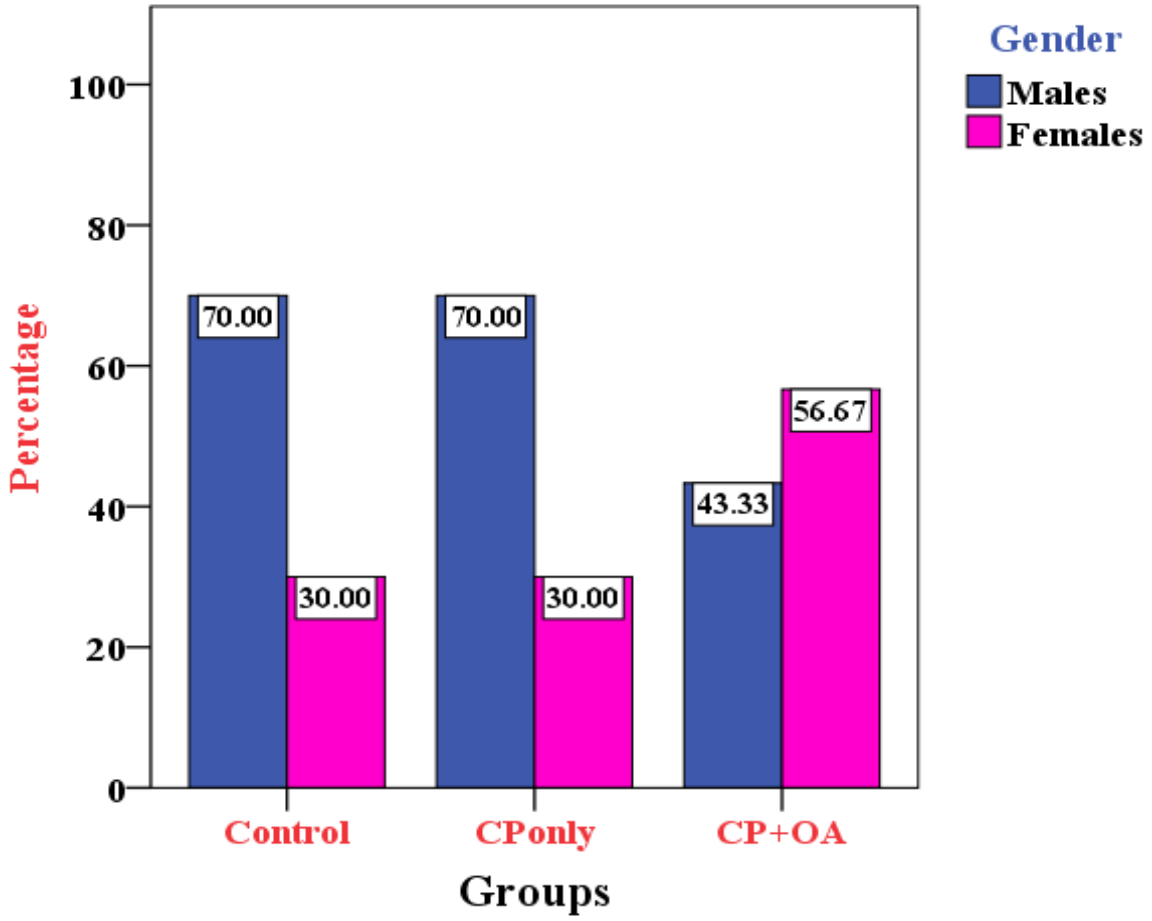


Figure 3-1: Bar chart for the percentage of male and female among the study groups.

3.2 Age parameter

Result in table (3-2) and figure (3-2) show that the age of the patient in this study was (35-50) years, in control group the patient age from (35-42) represent 75% of the total patient in this group and the age from (43-50)was only represent 25% in the group. The CP+OA group the percentage of age from (35-42) was 43% and (43-50) was 56%, in CP group the age of the patient from (35-42) was 53% from the total in this group and from (43-50) was 46%. In all these groups the age distribution had no significant difference.

Table 3-2: Descriptive and statistical test of Age distribution among groups

Variables	Categories		Group			Chi-square#	Sig.	Total
			Control	CP. only	CP+OA			
Age (Year)	35-42	NO.	15	16	13	4.916	0.086 NS	44
		%	75.00	53.33	43.33			55.00
		% T	18.75	20.00	16.25			55.00
	43-50	NO.	5	14	17			36
		%	25.00	46.67	56.67			45.00
		% T	6.25	17.50	21.25			45.00

NS: non significant at p>0.05

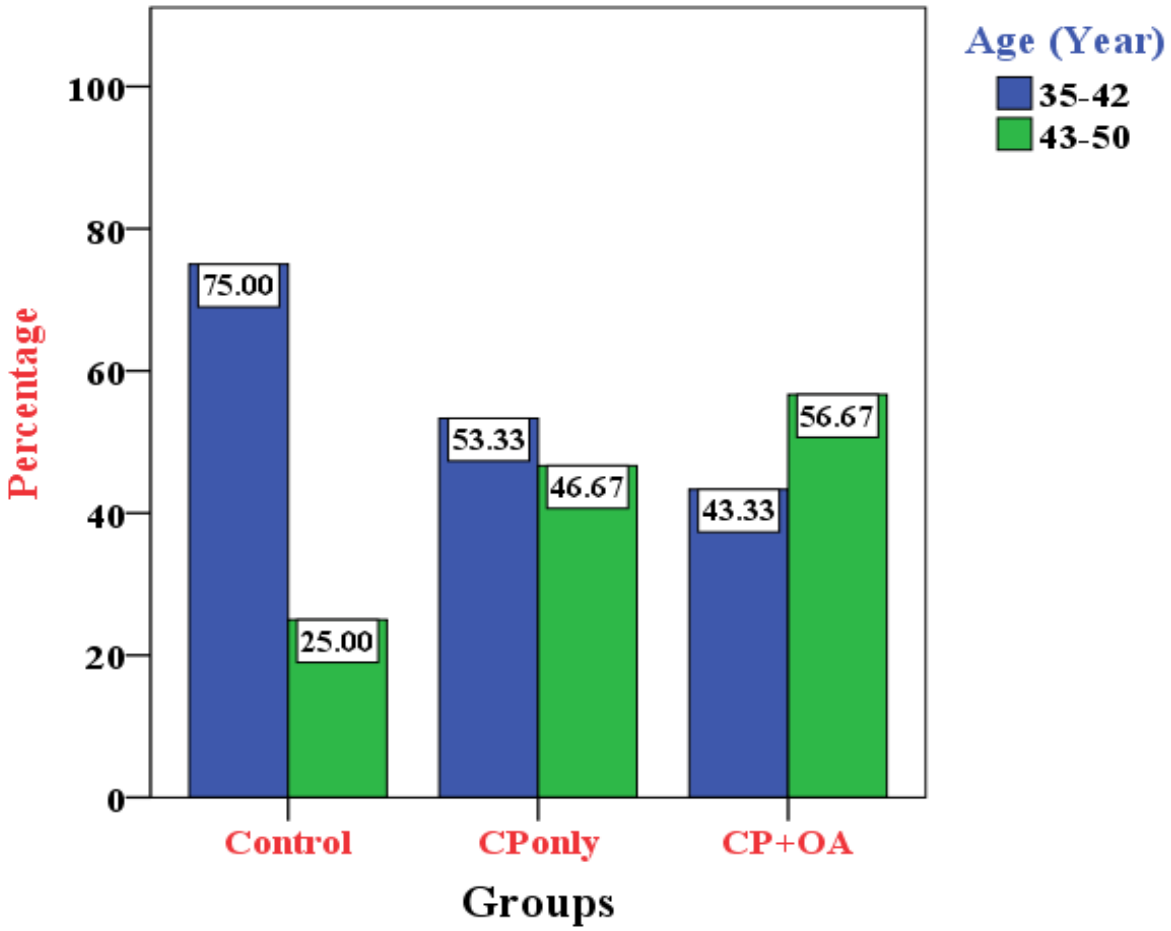


Figure 3-2: Bar chart for the percentage of age distribution among study groups

3.3 Clinical Periodontal parameters findings:

The data was not normally distributed and non-parametric, so in statistical analysis used Kruskal-Wallis test.

3.3.1. Plaque Index (PL):

Results in Table (3-3) and Figure (3-3) showed that both median and mean rank of PL were higher among CP+OA group (1.599, 52.82) than other the two groups as, CP group (1.40, 48.18) and the control group (0.271, 10.50) with highly significant difference between these groups P<0.01) and when compare each group with each other except between CP only and CP+OA, the result show no significant difference (P>0.05).

Table 3-3: Descriptive and statistical test of PL among groups

Group	Min.	Max.	Mean	±SD	Median	Mean Rank	Kruskal-Wallis# X^2	Kruskal-Wallis# Sig.	Multiple comparisons
Control	.030	.380	.251	.093	.271	10.50	45.15	0.000 HS	Control X CPonly=0.000
CPonly	.800	2.500	1.525	.498	1.400	48.18			Control X CP+OA=0.000
CP+OA	.700	2.500	1.625	.487	1.599	52.82			CPonly X CP+OA=1.00

Df=2,HS:highlysignificant at p<0.01

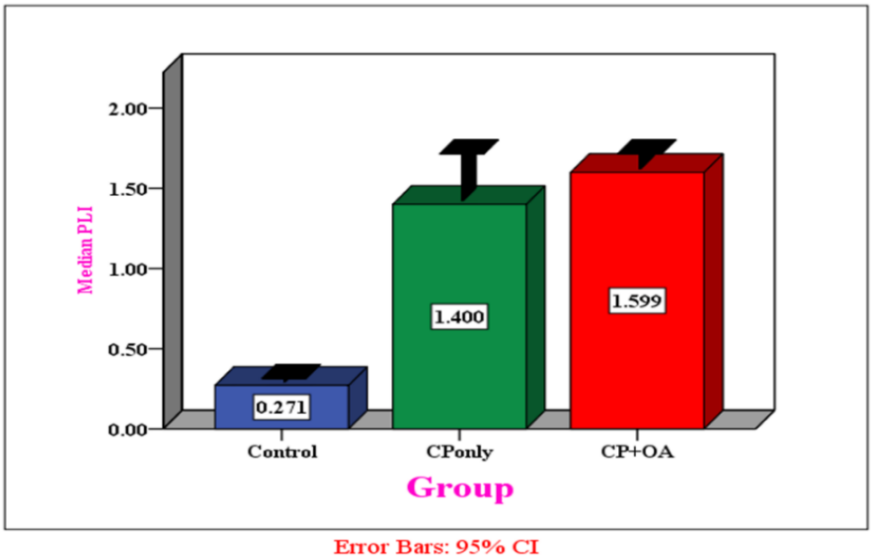


Figure 3-3: Bar chart for the median of PL parameter for all study groups

3.3.2.Gingival Index (GI)

Results in Table (3-4) and Figure (3-4) showed that both median and mean rank of GI were higher among CP+OA group (1.20, 55.32) than other two groups as CP group (0.90,45.68) and the control group (0.120, 10.50) with highly significant difference between these groups (P<0.01) and when compare each group with each other also it was highly significant difference. except between CP only and CP+OA, the result was statistically not significant difference (P>0.05).

Table 3-4: Descriptive and statistical test of GI among groups

Group	Min.	Max.	Mean	±SD	Median	Mean Rank	Kruskal-Wallis#		Multiple comparisons
							X^2	Sig.	
Control	.018	.295	.132	.083	.120	10.50	47.151	0.000 HS	Control X CPonly=0.000
CPonly	.600	2.000	1.068	.400	.900	45.68			Control X CP+OA=0.000
CP+OA	.500	2.200	1.303	.464	1.200	55.32			CPonly X CP+OA=0.324

HS: highly significant at p<0.01

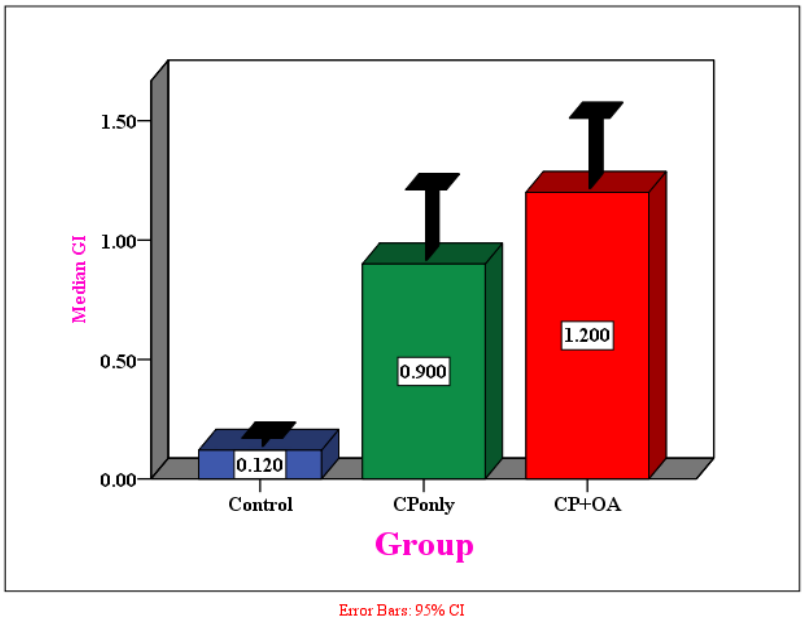


Figure 3-4: Bar chart for the median of GI index for all study groups

3.3.3. Bleeding on probing score (1)

Result in table (3-5) and figure (3-5) showed that both median and mean rank of BOP score1 were higher in chronic periodontitis CP with osteoarthritis group (10.350,32.57)than chronic periodontitis(CP)group (10.821,28.43) with non significant difference between these two groups P>0.05

Table 3-4: Descriptive and statistical tests of BOP score (1)between groups

Group	Min.	Max.	Mean	±SD	Median	Mean Rank	Mann-Whitney U test		Sig.
							Z	P-value	
CP. Only	7.400	18.500	11.612	2.892	10.320	32.57	0.917	0.359	NS
CP+OA	5.200	18.700	10.821	3.640	10.350	28.43			

NS:non significant at p>0.05

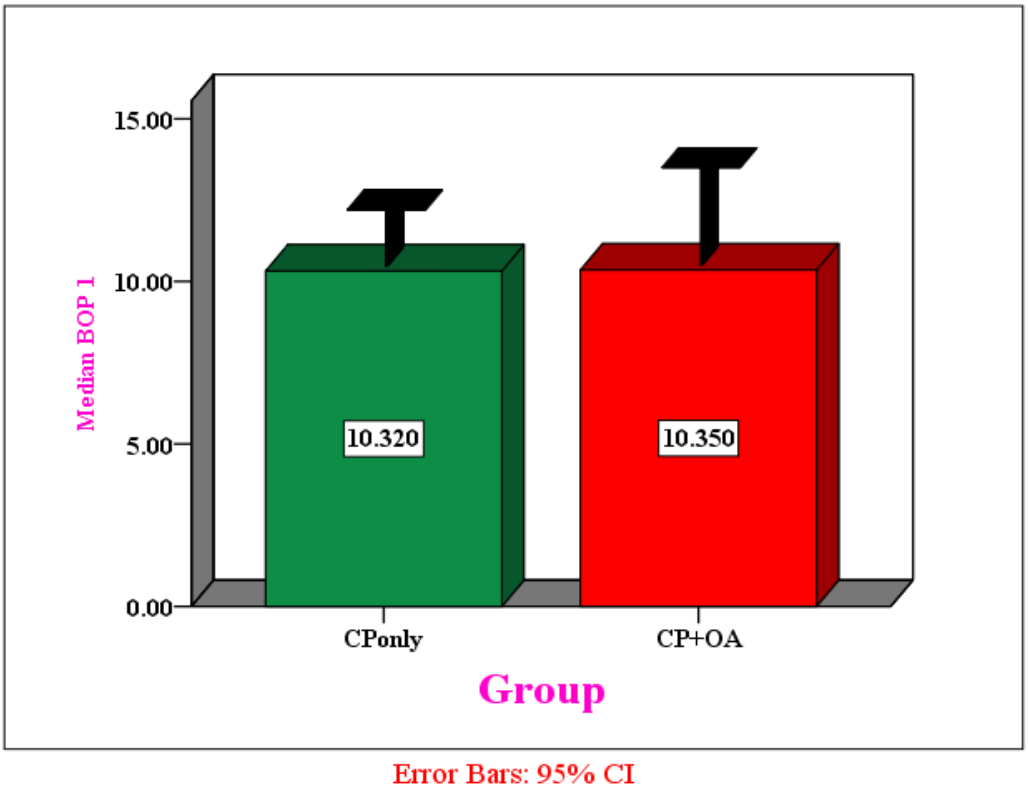


Figure 3-5: Bar chart for the median of BOP 1 score between study and control groups

3.3.4. Bleeding on probing score (0)

Result in table (3-6) and figure (3-6) showed that both mean and mean rank of BOP score0 was less in CP group (88.291, 32.55) than CP+OA group (89.090, 28.45) with non significant difference between these two groups P>0.05.

Table 3-6: Descriptive and statistical test of BOP score (0) between groups.

Group	Min.	Max.	Mean	±SD	Median	Mean Rank	Mann-Whitney U test		Sig.
							Z	p-value	
CP	81.400	92.500	88.291	2.894	89.580	28.45	0.910	0.363	NS
CP+OA	81.250	94.700	89.090	3.641	89.550	32.55			

NS:non significant at p value >0.05

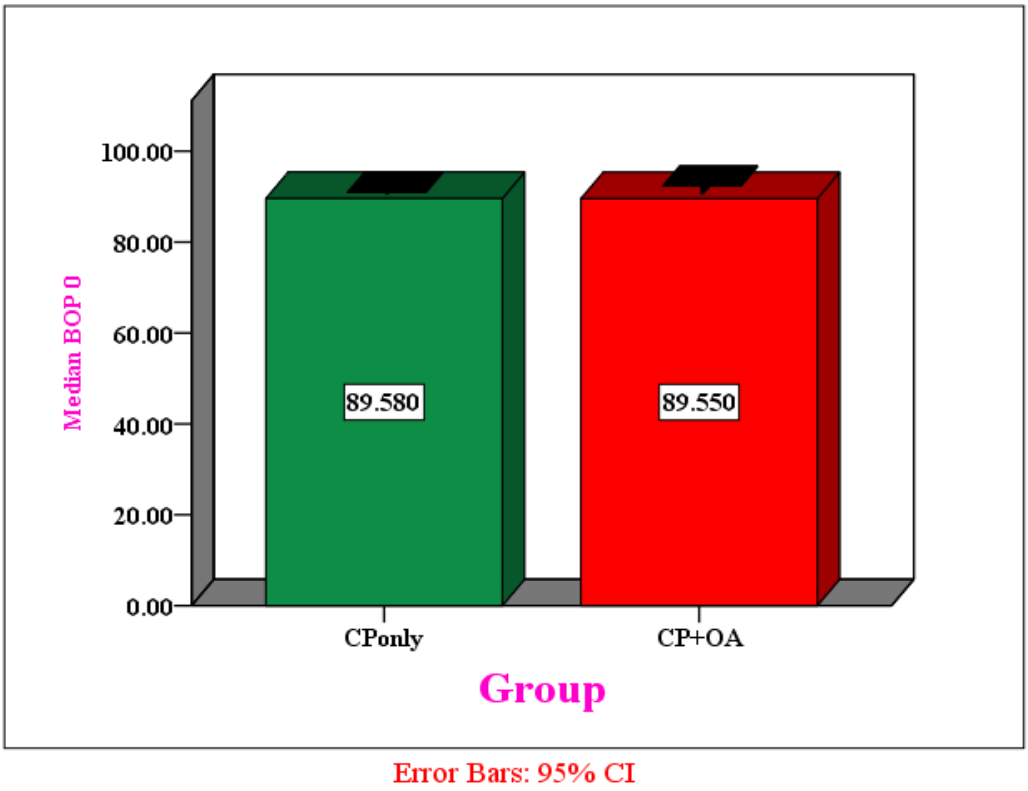


Figure 3-6: Bar chart of median BOP score (0) in the study and control group.

3.3.5. Probing pocket depth index:

The result as shown in table (3-7) and figure (3-7) the higher median and mean rank of PPD was in CP+OA group (1.250,31.38)while the median and mean rank of CP only was (1.200,29.62) and it was non significant difference between two groups P>0.05

Table 3-7: Descriptive and statistical test of PPD between groups

Group	Min.	Max.	Mean	±SD	Median	Mean Rank	Mann-Whitney U test		Sig.
							Z	p-vaue	
CP. Only	1.000	6.300	1.960	1.608	1.200	29.62	0.396	0.692	NS
CP+OA	1.000	6.400	2.791	2.065	1.250	31.38			

NS: non significant at p- value >0.05

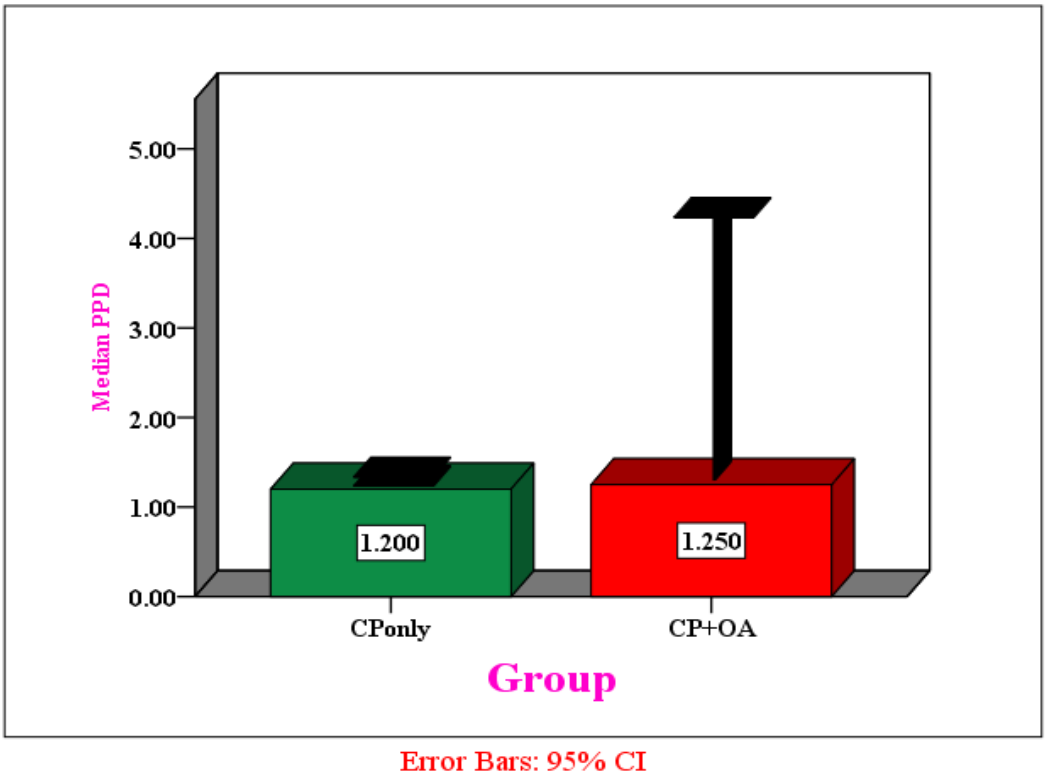


Figure 3-7: Bar chart of the median of PPD between groups

3.3.6 Clinical Attachment Loss (CAL):

The result in table (3-8) and figure (3-8) showed that the higher median and mean rank of clinical attachment loss (CAL) in CP group was (2.800, 31.40) while the median and mean rank in CP+OA group was (2.650, 29.60) and the result was statically not significant difference P>0.05.

Table 3-8: Descriptive and statistical test of CAL between groups

Group	Min.	Max.	Mean	±SD	Median	Mean Rank	Mann-Whitney U test		Sig.
							Z	p-value	
CP. Only	1.400	4.100	2.828	.686	2.800	31.40	0.400	0.689	NS
CP+OA	1.400	4.100	2.744	.615	2.650	29.60			

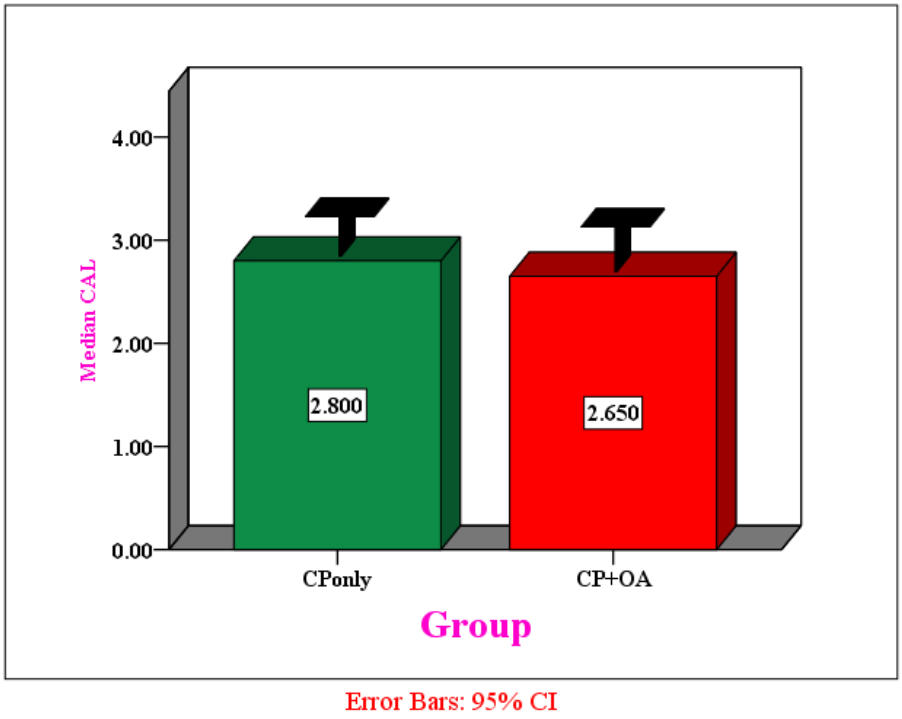


Figure 3-8: Bar chart of the median CAL between study groups and control group.

3.4. Immunological findings

3.4.1Tumor Necrosis Factor (TNFα):

Descriptive statistics of Serum cytokine level of (TNF-α) are clearly shown in table (3-9) and in figure (3-9). The current study pointed out that TNFα reported little increase in CP+OA group, the median is (10.615) as compared with Chronic Periodontitis group (10.533) and Control (C) group (9.682). Using Kruskal-Wallis test to show significant of statistical difference. It appeared that the difference was statically non significant.

Table 3-9: Descriptive and analytic test of TNFα among group

Group	Min.	Max.	Mean	±SD	Median	Mean Rank	Kruskal-Wallis	
							X^2	Sig.
Control	5.036	34.550	11.125	6.331	9.682	38.88	0.131	0.937 NS
CPonly	4.552	26.557	10.662	4.387	10.533	41.08		
CP+OA	4.552	16.375	10.190	2.948	10.615	41.00		

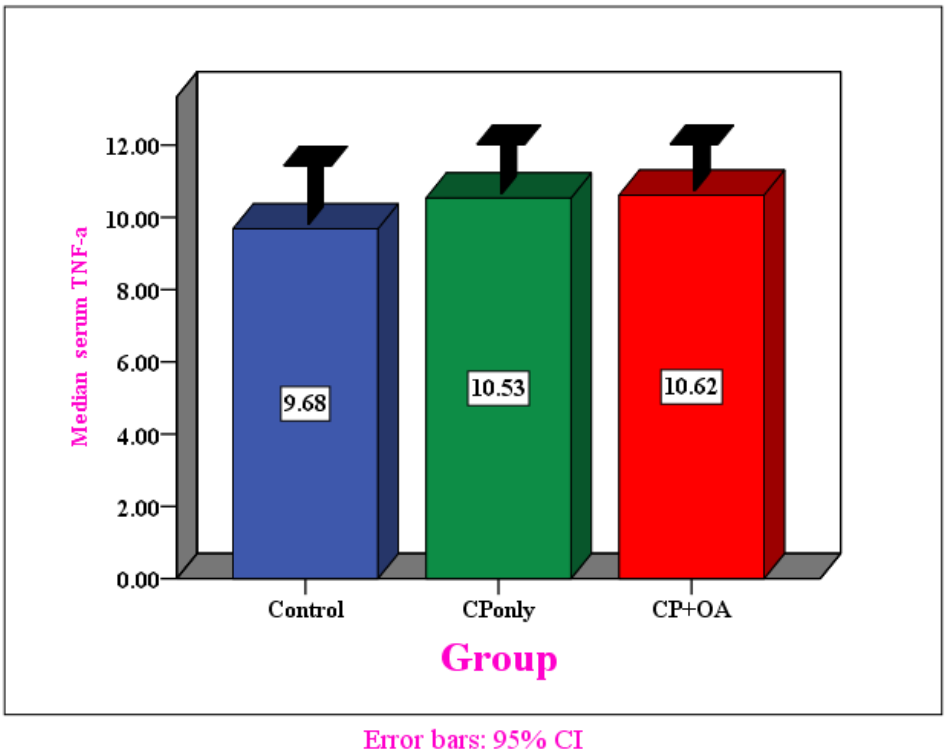


Figure 3-9: Bar chart of the median of serum TNFα among groups

3.4.2 High sensitivity C-reactive protein (hsCRP):

The result in table (3-10) and figure (3-10)show that the hsCRP found to be high in the chronic periodontitis with osteoarthritis the median is (0.314)followed by Chronic periodontitis (0.289),while the lowest median of CRP was in control group (0.242),and the result was statically nonsignificant difference p>0.05.

Table 3-10: Descriptive and statistical tests of CRP among groups

Group	Min.	Max.	Mean	±SD	Median	Mean Rank	Kruskal-Wallis		Sig.
							X^2	p-value	
Control	.101	1.681	.334	.335	.242	33.25	2.894	0.235	NS
CPonly	.117	1.052	.316	.164	.289	41.28			
CP+OA	.122	3.023	.389	.504	.314	44.55			

NS:non significant at p-value >0. 05

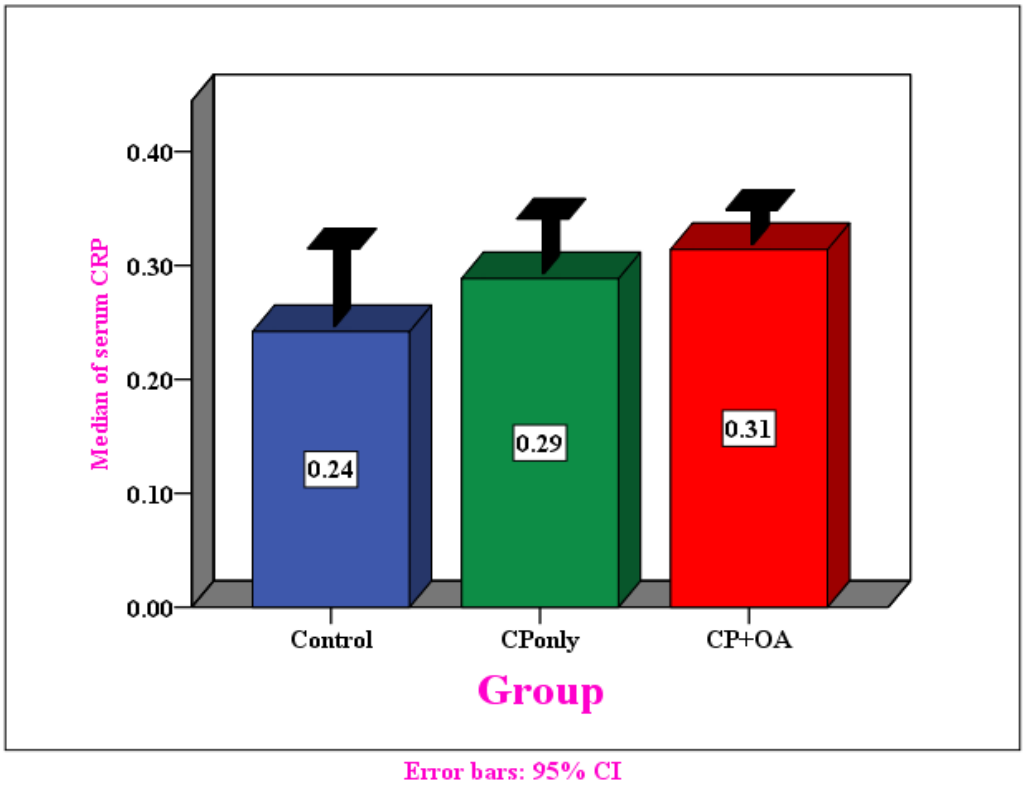


Figure 3-10: Bar chart of the median of serum CRP among groups

3.5. Spearman Correlation between TNFα, periodontal parameters and hs CRP among groups:

In table (3-11) the correlation between TNFα and periodontal clinical parameter in each group were weak non significant correlation, except with hsCRP in all groups were strong positive significant correlation, in control group (r=0.512, p=0.021). Figure (3-11). and in chronic periodontitis group (r=0.581, p=0.001), also TNFα in chronic periodontitis with osteoarthritis group was strong positive highly significant correlation with hsCRP (r=0.694, p=0.000).

3.6 The correlation between hsCRP, periodontal parameters and TNF-a among groups:

In table (3-12) The correlation between hs CRP and periodontal clinical parameters in each group were weak non significant but with TNFα the correlation in all groups were strong positive highly significant. Respectively in chronic periodontitis and osteoarthritis group was (r=0.694,p=0.000) (figure 3-12)also in chronic periodontitis group (r=0.581,p=0.oo1)and in control group hsCRP correlate strong positive highly significant with TNFα (r=0.521, p=0.021). figure (3-13)

Table 3-11: The correlation between TNFα, periodontal parameters and CRP among groups

Groups	Variables	R	P-value
Control	PLI	-.310	.184
	GI	-.303	.193
	CRP	**.512**	**.021**
CPonly	PLI	.074	.698
	GI	.227	.228
	CRP	**.581**	**.001**
	BOP1	.090	.635
	BOP0	-.090	.635
	PPD	-.018	.927
	CAL	.354	.055
CP+OA	PLI	.137	.470
	GI	.214	.256
	CRP	**.694**	**.000**
	BOP1	.203	.282
	BOP0	-.201	.286
	PPD	-.093	.625
	CAL	-.012	.949

Table 3-12: The correlation between CRP, periodontal parameters and TNFα among groups.

Groups	Variables	R	P-value
Control	PLI	-.112	.640
	GI	-.108	.649
	TNF-a	**.512**	**.021**
CPonly	PLI	.161	.397
	GI	.301	.106
	TNF-a	**.581**	**.001**
	BOP1	.282	.131
	BOP0	-.285	.127
	PPD	-.035	.856
	CAL	.104	.586
CP+OA	PLI	.159	.402
	GI	.136	.472
	TNF-a	**.694**	**.000**
	BOP1	.257	.170
	BOP0	-.254	.175
	PPD	.010	.959
	CAL	.059	.756

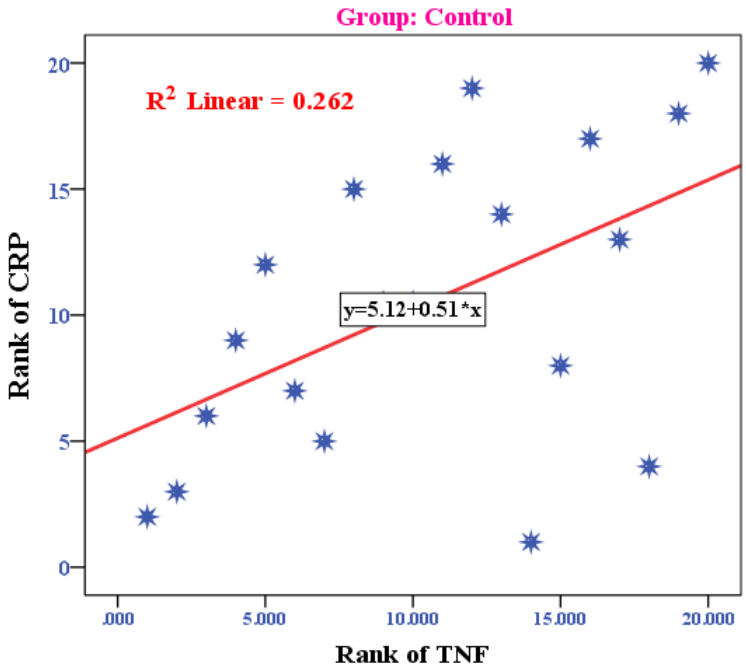


Figure 3-11: The correlation between hsCRP and TNFα in control group

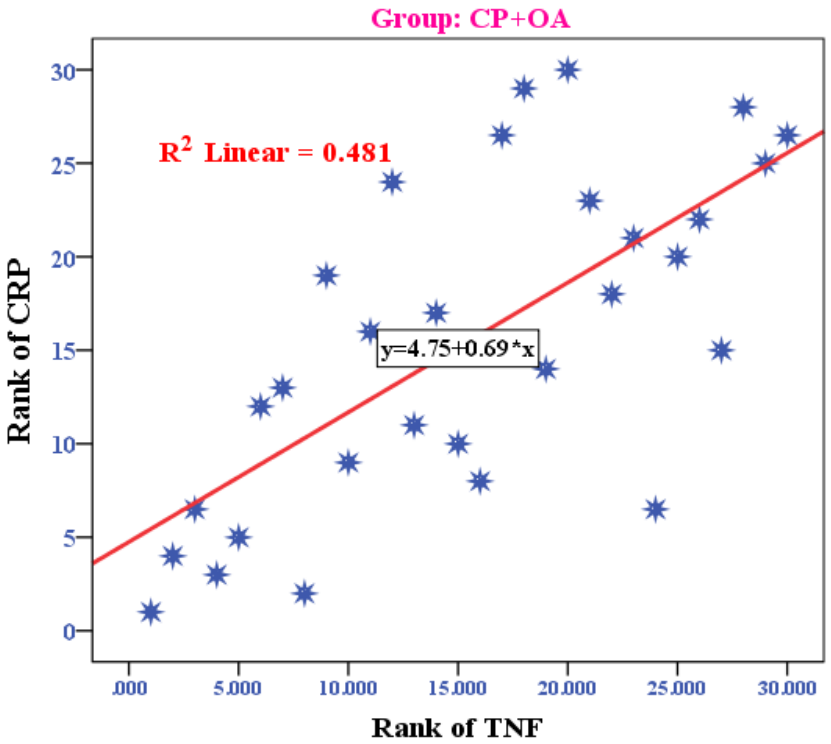


Figure 3-12: The correlation between hsCRP and TNFα in chronic periodontitis and osteartritis group

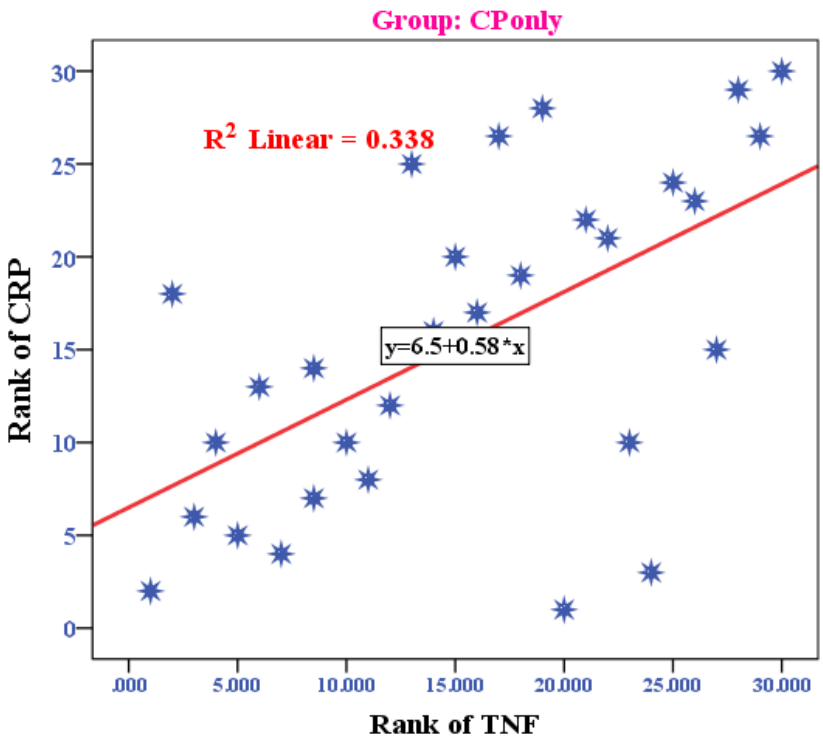


Figure 3-13: The correlation between hsCRP and TNFα in moderate chronic periodontitis group

CHAPTER FOUR
DISCUSSION

Discussion

Both musculoskeletal disorders and diseases of the oral cavity are widespread and potentially serious problems in the majority of people, until now little attention has been given to the links between them. Many musculoskeletal diseases, including osteoporosis, Paget's disease, and arthritic disorders, directly may involve the oral cavity and adjacent structures,a many of people with disabling osteoarthritis, rheumatoid arthritis, and other conditions have difficulty practicing good oral hygiene and traveling to dental offices for professional help. Various economical procedures can aid such individuals, including education of their caregivers and provision of antimicrobial mouthwashes and special tooth brush. Slight attention has been given to the relation between the two disease groups, but important linkages of inflammation exist. We discover the relations between some common musculoskeletal disorders (Osteoarthritis) and diseases of the oral cavity (chronic periodontitis) (Pokrajac-Zirojevic *et al*., 2002).

4.1 Demographic data

Osteoarthritis occurs in the population with a high incidence about in fourth of life. Supporting the above fact, the groups of OA patients in the current study consisted of more females in CP+OA group between (35-50) years , in CP group the female number 26, percentage (40%) and male number 34, percentage (60%) while the control group, (6) females and (14) males..In this study the age distribution among the study groups were that the percentage of age (35-42)in control group (C) was75%, in CP group was 53.33% and in CP+OA group was 43.33%. The age percentage of (43-50) in control group (C)was 25.00%, in CP group was 46.67% and in CP+OA group 56.67%.

4.2 Clinical Periodontal parameters

The present study reported that there was a higher in median values of gingival index and Plaque index among the study groups. This elevation of GI and PLI reflects a higher inflammation in the chronic periodontitis associated osteoarthritis than the chronic periodontitis (CP) group and could be related to limitation of patient movement which hinder oral hygiene practices lead to bacterial aggregation, formation of microbial bio--film (plaque)and bacterial invasion with its toxin which result in disruption of the epithelial barrier and more destruction to the supporting tissue this increase the exposed area for plaque accumulation on tooth surface (Carrenza, 2012), This result is agreed with te study of(Powers *et al*.,2013) which approved that patient with osteoarthritis had higher mean of GI. and disagree with the study (Torkzaban *et al.*, 2012)which found that no significant correlation was evident between OA and the mean percent of GI. reason for this finding is that the status of oral hygiene was poor (GI>50%) among all subjects with and without OA. Therefore, the difference between the two groups was not statistically significant.

Although The percentage of sites with BOP score 1 was higher in OA+CP group than CP group but the difference was non-significant which agree with the study (Chung *et al.*, 2017).

This may be attributed to the effect of plaque accumulation on the pathophysiololgic process and blood circulation in the inflamed tissue and the severity of bleeding correlated with the intensity of inflammation (carrenza2012).

Periodontal pockets vary in their location and depth; hence changes in the mean probing depths for the entire mouth provide reasonable information accordingly to this study PPD was higher in OA+CP group than CP group but statistically was no significant difference between the study groups. This agree

with the study (Chung *et al.*, 2017) who found CP+ OA and CP showed no significant difference in overall analysis according to clinical periodontal parameters. The increasing in pocket depth may be contributed to increase amount of plaque and bacterial invasion with its toxin which cause further destruction in sulcular and junctional epithelial, alveolar bone tissue and other supporting tissue.

Clinical attachment level refers to the distance from the cementoenamel junction (CEJ) to the location of the inserted probe tip. Thus, loss of fibers attachment expressed at the clinical level the cumulative effect of destructive pathological processes in periodontal disease, in our study show no significant difference between OA+CP group and CP group this was also agree with the study(Chung *et al.*, 2017) which found that there is no statically difference between the two groups, the osteoarthritis have no effect on the clinical periodontal parameters which found no significant difference except for plaque and gingival index which may be related to patient's poor ability to maintain proper oral hygiene may result in accumulation of plaque and calculus.

4.3. Serum hsCRP and TNFα levels

Periodontal inflammation begins as an acute inflammatory response after host-bacterial interaction and progresses into a chronic stage dominated by B lymphocytes and macrophages, following extreme T lymphocytes stage (Gemmell *et al*., 2001). Transition between these stages, accumulation and differentiation of immune cells in the inflammatory site are mediated by "cytokines". Cytokines are soluble mediators sharing many biologic processes such as wound healing, hematopoiesis, systemic and local inflammatory responses (Yücel, 2015). A lot of cell type other than immune cells like epithelial cells, gingival and periodontal ligament fibroblasts, and keratinocytes are able to produce cytokines when stimulated (Baek *et al*., 2013), (Liang *et al*., 2014).

Cytokines show pleiotropic effects on different target cells by regulate the cell activation, proliferation and function (Yücel, 2015).

Therefore the main effect of cytokines are direct the intensity and duration of immune response which may serve as a critical determinant of tissue destruction in many inflammatory chronic diseases like rheumatoid arthritis, inflammatory osteoarthritis and periodontitis (Graves *et al*., 2008) the current study was the first study in Iraq prompted to detect serum level of hs CRP and TNF-α in osteoarthritis patients with Chronic periodontitis.

Interestingly, the present study reported non significant difference of TNF α among the study groups, even though the median of TNFα was little increase in OA+CP group more than CP and controls group, TNFα play an important role in inflammatory arthritis and in degenerative joint disease (Westacott and Sharif, 1996), it appear from the fact that human articular chondrocytes from OA cartilage expressed a a lot of higher number of the p55 TNFα receptor which could make OA cartilage particularly susceptible to TNFα degradative stimuli (Fernandes *et al*., 2002).Also TNFα associated with the

level of OA severity. It is recognized that TNFα induces bone and extracellular matrix resorption by activating the osteoclasts. It is secreted by leukocyte, macrophage, lymphocyte in the local lesion of periodontitis (Zhou *et al*., 2013.) this agree with the study (Ieremie *et al*., 2014) observed that high serum levels of TNF-α detected in patients with chronic periodontitis.

So, it was clear that their levels increased in osteoarthritis and periodontal disease but in this study statically the difference was non significant which may be related to the newly dignosed disease, osteoarthritis is a non-inflammatory disease, and the proinflamatory TNFα level is still less in the serum of the patient and may be the severity of periodontitis was moderate effect on the destruction of the supporting tissue so affect little on the inflammatory markers in the seum.

In the current study the hsCRP was non-significant relation among the study groups but the median of it was higher in OA+CP group than CP, control group. hsCRP,is a protein mainly produced by the liver in response to an increase in interleukin-6 and TNFα,which is a part of the non-specific response to inflammation, infection and tissue damage (Martu *et al.*, 2017), there was strong evidence that individuals with chronic periodontitis have elevated hsCRP levels compared to the control groups as agree with the study (Martu *et al*., 2017). Also agree with the study done by (Salzberg *et al*., 2006)Which related to that primary host response to injuries, infections, ischemic necrosis or malignancy, initiated by the activation of local macrophages and other cells (including fibroblasts and endothelial cells), leading to the release of mediators such as TNF-a, IL-6 and IL-1β, sequentially cause systemic changes include hepatic release of proteins (including CRP), activation of complement proteins and various metabolic changes (Ide *et al*., 2003).the result of this study was disagree with the study (Beck *et al*., 2000) whom assessed the relationship between hsCRP and the periodontal status was not as clear. Also the study (Redman *et al*., 2016) reaveled that CRP levels were just above the level of

detection in nearly all subjects and not significantly higher in response to periodontitis. Also this biomarker is increased with the onset of osteoarthritis and increased in the serum in response to disease progression, the elevated hsCRP predict cartilage loss in osteoarthritis, this is agree with the study(Ishijima *et al.*, 2011). and disagree with the study done by (Lotz *et al.*, 2013) which find the level of hsCRP is not adequately discriminating to differentiate between patients with osteoarthritis and controls.

In spite of lack of previous study about the serum level of hsCRP and TNFα in patient with moderate chronic periodontitis and osteoarthritis, this study approved non significant difference of the markers in the study groups, it may be due to differences that can be influence hs CRP level such as different level of inflammation regarding osteoarthritis or amount of adipose tissue or the effect of body mass index (BMI), high blood pressure, alcohol use, chronic fatigue, diabetes, sleep disturbances, depression, lifestyle, diet may fluctuate hsCRP level. Other factors can be influence on proinflammatory cytokine construction and/or activity are the cytokines that have anti-inflammatory properties. Four such cytokines, namely transforming growth factor (TGFα), IL-4, IL-10 and IL-13, have been recognized as able to modulate various inflammatory processes.

4.4. Correlations between hsCRP, TNF-α and clinical periodontal parameters.

Local infection is considered to be as a contributer to the systemic inflammation burden.in the present study the serum level of hsCRP and TNFα show no correalation with clinical periodontal parameters in each group. This was agree with the study (Bretz *et al*., 2005)which found no correlation between circulating TNFα and IL-6 and any of the periodontal parameters), also agree with the study (Teles *et al*., 2012) And disagree with.the study (Zhou *et al*., 2013) Which revealed that there is positive correlation of TNFα, hsCRP, IL-6 and periodontal parameters. Also disagree with the study (Podzimek *et al*., 2015)which found the level of hsCRP is statically correlated to the clinical periodontal parameter especially (BOP, PD). These were studies also revealed positive correlation between clinical periodontal parameter and serum level of TNFα and CRP (Koppolu *et al*., 2013) (Ide *et al*., 2003) (Noack *et al*., 2001) (Montebugnoli *et al*., 2005)

Regarding current study it was approved that no correlation between the serums hsCRP, TNFα and periodontal parameters that may attributed to subject variables such as physical activity, food habits, socioeconomic conditions, obesity, age, stress, and lifestyle. These variables were difficult to control and may have influenced the results. Because of the small number of patients examined, data cannot be generalized for all patients.

In spite of that, this study found a strong positive correlation between TNFα and hsCRP in each group as agree with the study(Foss *et al*., 1993). Whom revealed the positive correlation between TNFα and hsCRP indicates that when patients have high TNF α levels, a relative increase in CRP concentrations also occurs, this correlation considered that when the acute-phase inflammatory response, changes in the concentrations of the level of many proteins are characteristically observed reflecting the reorganization of gene

expression of hepatocyte secretory proteins after inflammatory stimulus. A progressively increasing number of hepatocytes are recruited for hsCRP synthesis during the first days after the inflammatory reaction. Thus, steadily elevated hsCRP levels reveal active disease, and have been reported to occur in several infections, inflammatory reactions, tissue lesions and tumors. The extracellular signs that increase or decrease the expression of genes coding for acute-phase proteins in hepatocytes have revealed that several cytokines released from activated macrophages, such as tumor necrosis factor (TNF), interleukin-1 (IL-1) or IL-6 in combination with IL-1, are drawn in this process. These cytokines, by performing a regulatory function, act directly on the hepatocytes, inducing CRP synthesis, in other hand CRP modify the manners of effector cells, especially polymorphonuclear leukocytes, lymphocyte, monocyte and platelets during the inflammatory process. this study agree with (Kandati et al., 2014)which also concealed increased blood levels of pro inflammatory mediators such as IL-6, TNFα, and hs C-reactive protein have been documented in adults with OA and CP .However, the link between OA and CP remains unknown. osteoarthritis was not significantly associated with any CP category.

CHAPTER FIVE
CONCLUSIONS & SUGGESTIONS

Conclusions and Suggestions

5.1. Conclusions

1-Patients with moderate chronic periodontitis and ostearthritis although had a significant difference with patients of moderate chronic periodontitis and healthy patients in dental plaque,gingival health,but found no significant difference in bleeding on probing, probing depth, attachment loss with patient of moderate chronic periodontitis.

2-The serum level of hs CRP and TNF-α were higher in patients with moderate chronic periodontitis and osteoarthritis than patient with chronic periodontitis without arthritis but there was a no significant relation between the study groups.

3-There was no correlation between immunological parameters (hs CRP and TNF-α) and periodontal parameters: Plaque index (PL.I),gingival index (GI),bleeding on probing (BOP), clinical attachment level (CAL), probing pocket depth (PPD).

4- There was positive correlation between serum level of hs CRP and TNF-α in both studies groups with chronic periodontitis with osteoarthritis patient and those having only chronic periodontitis**.**

5- Findings from this cross-sectional study do not provide evidence for an association between CP and OA. Prospective research and studies using clinical criteria for diagnosis of symptomatic OA are needed to confirm these findings.

5.2 Suggestions for further research

1. Further studies are required for the measurement of the effect of the drugs on periodontal health status in osteoarthritis patients.
2. Further studies are required for measurement of other serum elements related to both diseases. (e.g: IL-6).
3. Further studies are needed to detection of periodontal pathogen in chronic periodontitis and osteoarthritis
4. Further studies including study anti-TNF-α drugs for the measurement of the effect on periodontal health status in osteoarthritis patients.
5. Further study for the correlation between two markers CRP and TNF-α and the effect of clinical treatment for both group chronic periodontitis with osteoarthritis and those with chronic periodontitis only on those parameters.

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APPENDICES

Appendix (1)

Case sheet

Participant's name: .....

Participant's age: .....

Participant's number: .....

Study group:

History of systemic disease:.....

Medication: .....

Notes:.....

Plaque Index

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Mean of PLI=

.....

.....

Gingival Index

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Mean of GI=

.....

.....

Bleeding on Probing

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

BOP%=

.....

.....

Probing Pocket Depth

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Mean of PPD=

.....

.....

Clinical Attachment Level

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Mean of CAL=

.....

TNF-α .....

hsCRP.....

Appendix (2)

موافقة للإشتراك في البحث العلمي

اسم الباحث:

عنوان البحث:

مكان إجراء البحث:

أنت مدعو(ة) للمشاركة ببحث علمي سريري سيجرى في ----------. الرجاء أن تأخذ(ي) الوقت الكافي لقراءة المعلومات التالية بتأن قبل أن تقرر(ي) إذا كنت تريد(ين) المشاركة أم لا. بإمكانك طلب إيضاحات أو معلومات إضافية عن أي شيء مذكور في هذه الاستمارة أو عن هذه الدراسة ككل من طبيبك.

في حال وافقت على المشاركة في هذه الدراسة، سيبقى اسمك طي الكتمان . لن يكون لأي شخص، ما لم ينص القانون على ذلك، حق الاطلاع على ملفك الطبي باستثناء الطبيب المسؤول عن الدراسة ومعاونيه.

موافقة المشترك:

لقد قرأت استمارة القبول هذه وفهمت مضمونها. تمت الإجابة على أسئلتي جميعها. وبناء عليه فأنني، حرا مختارا، أجيز إجراء هذا البحث و أوافق على الإشتراك فيه، وإني أعلم ان الباحث الدكتور _____ وزملاءه ومعاونيه او مساعديه سيكونون مستعدين للإجابة على أسئلتي، وأنه باستطاعتي الإتصال بهم على الهاتف _____ واذا شعرت لاحقا ان الأجوبة تحتاج الى مزيد من الإيضاح فسوف أتصل بأحد اعضاء لجنة الأخلاقيات. كما أعرف تمام المعرفة بانني حر في الإنسحاب من هذا البحث متى شئت حتى بعد التوقيع على الموافقة دون ان يؤثر ذلك على العناية الطبية المقدمة لي.

إسم المشترك:

توقيع المشترك:

التاريخ:

فرع امراض وجراحة ما حول الاسنان

كلية طب الأسنان/ جامعة بغداد

الخلاصه

الخلفيه:

التهاب انسجه ماحول الاسنان هو مرض التهابي مزمن يؤثر على اربطه وانسجه ماحول الاسنان والعظم السنخي،التهاب المفاصل هوايضا التهاب المفصل المزمن مع عده اسباب مركبه ومتداخله منها التهاب السائل الغضروفي واعاده تشكيل ماتحت العظام وتكوين النتوء الغضروفي والذي يؤدي الى انحلال وتحطم الغضروف.التهاب اللثة المزمن والتهاب المفاصل تعتبر من الأمراض منتشرة على نطاق واسع وتقوم بتدمير الأنسجة بسبب الالتهاب مزمن في الفم ومفاصل الجسم .

الهدف من الدراسه:

تهدف ادراسه الى تقييم الحاله الصحيه لانسجه ماحول الاسنان في مجموعتي الدراسه (المجموعه الاولى يضم مرضى التهاب انسجه ماحول الاسنان المزمن والمجموعه الثانيه مرضى التهاب المفاصل مع التهاب انسجه ماحول الاسنان المزمن)والمجموعه الضابطه و تحديد مستويات المصل من البروتين (c)التفاعلي ذو الحساسيه العاليه وعامل نخر الورم الفا في كلتا المجموعتين ومقارنتها مع المجموعه الضابطه .واختبارالعلاقه بين مرضى التهاب اللثه المزمن من نوع المتوسط والتهاب المفاصل من خلال تقييم مستويات المصل من البروتين (c)التفاعلي ذو الحساسيه العاليه وعامل نخر الورم الفا مع مؤشرات ماحول الاسنان السريريه في كلا المجاميع.

المواد وطرق العمل:

٨٠ مشارك من كلا الجنسين ادرجوا في هذه الدراسه ،تضم المجموعه الاولى ٣٠ مريضا تتراوح أعمارهم بين (٣٥ إلى ٥٠) سنةمع التهاب المفاصل والتهاب اللثة المزمن المتوسط،٣٠ مريض تتراوح أعمارهم بين (٣٥ إلى ٥٠) سنة من متوسط التهاب اللثة المزمن وحده. المجموعه الضابطه من ٢٠ مريضا اصحاء سريريا ولديهم انسجه سليمه ماحول الاسنان صحيه.استبعدنا في هذه الدراسه النساء مابعد سن اليأس والنساء الحوامل والمدخنين أيضا. المرضى المصابين بالتهاب المفاصل مزودين بوثائق أو التصوير الشعاعي، بما يتفق مع التشخيص السريري لالتهاب المفاصل مع عدم وجود حالة التهابية روماتيديه. يتم سحب عينات مصل الدم من كل مريض باستخدام أنابيب جمع خالية من عامل التخثر وتخضع لجهاز الطرد مركزي وفقا للمبادئ التوجيهية الشركة المصنعة، ثم تخزن في _C15 حتى تحليل المصل من عامل نخر الورم الفا و البروتين سي التفاعلي بواسطه الانزيم الرابط بالمناعه.

النتائج:

اظهرت النتائج وجود ارتفاع في المعدل الحسابي لمرضى التهاب اللثه المزمن المتوسط والتهاب المفاصل في مؤشرات التهاب اللثه والصفيحه الجرثوميه وعدم وجود اختلافات معنويه في مؤشرات عمق الجيوب والنزف عند التسبيروفي نسب درجات فقدان الانسجه الرابطه سريريا مع مجموعه المرضى المصابين بالتهاب اللثه المزمن المتوسط فقط.كما اظهرت ايضا وجود اختلافات غير معنويه بين مجاميع الدراسه لمستويات لمصل لكل من TNFα ,وhs CRP فقد ارتفع مستوى hsCRP قليلا في مرضى التهاب اللثه المرافق مع التهاب المفاصل من مرضى التهاب التهاب اللثه والضابطه (٠,٣١،٠,٢٩،٠,٢٤) ايضا مستوى TNFα كان اعلى بقليل في مجموعه التهاب اللثه المزمن المتوسط لمرافق لالتهابمفاصل من بقيه المجاميع (١٠,٦١٥،١٠,٥٣٣،٩,٦٨٢).

كما اظهرت الدراسه الحاليه ان هناك علاقه قويه ايجابيه بين مستويات المصل من TNFα, hs CRP في كلا المجاميع ولكن ضعيفه مع مؤشرات ماحول الاسنان السريريه في كلا المجاميع ايضا.

الاستنتاج:

اظهرت الدراسة الحالية أن الارتفاع البسيط في مستويات مصل الدم من البروتين c التفاعلي(hsCRP) و عامل نخر الورم(TNFα)في المرضى من مجاميع الدراسه ارتفاعا غير معنويا ولا يظهر تاثير او علاقه بين التهاب الانسجه ما حول الاسنان والتهاب المفاصل

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بغداد
كلية طب الاسنان

مستوى المصل لل TNFα وال hsCRP كمؤشرات حيويه لالتهاب انسجه ماحول الاسنان المزمن المرافق لمرضى التهاب المفاصل في العراق

رساله مقدمه الى
مجلس كليه طب الاسنان جامعة بغداد كجزء من متطلبات نيل شهادة الماجستير
في امراض وجراحة ماحول الاسنان

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١٤٣٩ هـ ٢٠١٧ م