

# Serum Tumor Necrosis Factor Alpha and High Sensitive C-Reactiveprotein as Biomarkersin Periodontitis in Iraqi Patients with Osteoarthritis

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## ABSTRACT

**Background:** Periodontitis (PD) is well-known chronic 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. The aim of this study is todetermine the association of chronic periodontitis and osteoarthritis in patients by analysing tumor necrosis factor alpha TNF $\alpha$  and high sensitive c-reactive protein (hsCRP) in the serum.

**Materials and Method:** A total of 80 patients of both sexes aged 35-50 years ,30 patients with osteoarthritis and moderate chronic periodontitis (CP+OA), 30 patients of both sexes aged 35-50 years with moderate chronic periodontitis alone (CP). And control group (C) of 20 healthy patients with healthy periodontium participated in this cross sectional study. we excluded the postmenopausal and pregnant woman from female patient and smoker patient also. All patients are free of medication and have 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 were drawn from each subject using pyrogen-free heparinized collection tubes. Tubes were centrifuged according to the manufacturer's guidelines, then stored at -15°C till analyse. serum TNF $\alpha$  and hs CRP were measured using an enzyme-linked immunosorbent assay (ELISA).

**Results:** A non-significant differences between studied group regarding serum level of hsCRP and TNF- $\alpha$ , as well as, the result 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, and 0.24. Similarly, the medium serum level of TNF $\alpha$  was higher in chronic periodontitis with OAth than in other two groups (10.615, 10.533, and 9.682 respectively). The age and gender showed a non-significant difference between the groups

**Conclusion:** The present study revealed that there's a strong positive correlation between serum levels of hsCRP and TNF $\alpha$  in patients of all groups but there is no correlation between Osteoarthritis and chronic periodontitis.

**Keywords:** chronic periodontitis, osteoarthritis, TNF $\alpha$ , hsCRP. (J Bagh Coll Dentistry 2018; 30(3): 7-12)

## INTRODUCTION

PD, is considered one of the common oral diseases, it is a destructive inflammatory disorder affecting the tissues that support teeth and it is mostly associated with range of pathogenic bacteria (*Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia* together with *Aggregatibacteractinomycetem-comitans*)<sup>(1)</sup>, PD is characterized by both chronic inflammation of connective tissue and alveolar bone destruction which lead to loss of tooth-supporting apparatus<sup>(2)</sup>. Sequentially, PD followed gingivitis, which involves inflammation of marginal periodontium. However, not every gingivitis develops into PD. The progression of the periodontal destruction depends on the quantity, quality, virulence of the microorganisms and the immune response of the host<sup>(1)</sup>.

OA is the most important reason of disability in musculoskeletal worldwide<sup>(3)</sup>, and the major factor in physical limitation of old people.<sup>(4)</sup>

Also, it is a chronic joint disease that has compound etiologies characterized with synovial inflammation, remodeling of subchondral bone, and produce osteophytes, which in turn cause cartilage deterioration, Severity of OA afflicted functional ability.<sup>(5)</sup> Hence, OA in most of people leads to maintain proper oral hygiene, lead to accumulation of plaque and calculus, which increases hazard of dental caries and periodontal disease<sup>(6)</sup>. Cytokines act as a communication between immune and non-immune cells<sup>(7)</sup>. It has been proposed that cytokines are necessary to the pathogenesis of many diseases<sup>(8)</sup>. C-reactive protein is a systemic marker released during the acute phase of an inflammatory response. C-reactive protein is produced by the liver and is regulated by circulating cytokines, such as TNF $\alpha$  and interleukin- 1, from local and/or systemic inflammation such as periodontal inflammation<sup>(9)</sup>. TNF $\alpha$  is a critical pro-inflammatory mediator that results in destruction of periodontal tissues. TNF $\alpha$  has many

actions mostly pro-inflammatory. Leukocyte recruitment and vascular permeability are facilitated by stimulating expression of selectins and adhesins by TNF- $\alpha$ , activating the osteoclasts

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similarly to way with IL-1 lead to resorption of bone and extracellular matrix. Monocytes and macrophages are the most important cell types producing TNF- $\alpha$ <sup>(10)</sup>. These cytokines are normally found in the blood and increase with inflammation so in this study we determine the level of TNF $\alpha$  and hsCRP in the serum of the patient to determine the degree of inflammation in chronic periodontitis and osteoarthritis.

## MATERIALS AND METHODS

Sample population consisted of eighty male and female subjects with age range 35-50 years. Moderate chronic periodontitis with OA (CP+OA) group 30 subject seeking treatment in the rheumatology clinic in Baghdad Teaching Hospital and the CP groups 30 subject, 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 and March 2017. They were subjected to a questionnaire including question about their name, age, full medical history, dental history, medications and they were diagnosed for detecting a periodontal disease. Followed by blood collection for the assessment of serum level of TNF $\alpha$  and hsCRP.

**Exclusion criteria:** Pregnant and menopause ladies, smokers, any patient with history of other chronic systemic diseases which are known to be associated with CP such as cardiovascular diseases and diabetes mellitus. Also, Rheumatoid patient and PD patient who received periodontal treatment and /or antibiotics during the last 3months.

**Collection of blood Samples:** After the subjects have been selected, 5ml of venous blood sample were aspirated from antecubital 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, centrifuging at 2,000-3,000rpm for 20 minutes. Then aspirated and transferred immediately into another tube and frozen at (-15°C) for subsequent analysis. Haemolyzed samples were discarded. Then, using ELISA Kit (96-wells) for quantitative determination of serum human TNF- $\alpha$ , hs CRP (Sunlong Biotech Co. Ltd, China) The Microelisa strip plate provided in this kit has been pre-coated with an antibody specific to TNF $\alpha$  and hsCRP. Standards or samples were added to the appropriate Microelisa strip plate wells and combined to the specific antibody.

**Statistical Analysis:** Each patient assigned a serial identification number. The data were reviewed, cleaned with double check entry into

the computer using Statistical Package for Social Sciences (SPSS) version 21. The overall comparison of mean values among the different study groups was done by Kruskal–Wallis. Comparison between any two study groups was done by Mann–Whitney U-test. The correlation between TNF- $\alpha$ , hs CRP and the clinical variables in each group was done by Spearman's correlation test.

## RESULTS

In this study, 80 patients were included in 3 main groups. The first group consist of 30 patients with moderate CP+OA, second group include 30 patients with moderate CP, third group is the C group consisting of 20 subjects. The age range for participants between 35-50 years. The 32 females represent 40% from the total samples and 48 males represent 60% of the total samples. From the 30 patients in CP+OA group, 13 are males (43.33%) and the others are females representing 56.67% of the group, but in CP group the number of females (9) and the males are 21 from this we observed that males are the dominant in this group and they represent 70% of the total samples. The C group, 6 females and 14 males. There were no statistical association between males and females (Table 1).

The age of the patient was 35-50 years, in C 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 5% 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% and from 43-50 was 46%. In all these groups the age distribution had no significant difference (Table 2).

The current study indicated that TNF $\alpha$  exhibited little increase in CP+OA group, the median was 10.615 as compared with CP group (10.533) and C group (9.682). Analysis of results using Kruskal-Wallis test showed that the difference was statistically non-significant (Table 3).

In table 4, data showed that the hsCRP was found to be high in the CP with OA the median is 0.314 followed by CP (0.289), while the lowest median of CRP was in C group (0.242), and the result was statistically not significant ( $P > 0.05$ ). There was strong positive correlation between TNF $\alpha$  and hsCRP in each study group as shown in table 5.

**Table 1: Gender distribution among study groups.**

		Group			Chi-square <sup>#</sup>	p-value	Total	Sig		
		Control	CP. only	CP+OA						
Male	NO.	14	21	13	5.556	0.062	48	NS		
	%	70.00	70.00	43.33			60.00			
	% Total	17.50	26.25	16.25			60.00			
Female	NO.	6	9	13			5.556	0.062	32	NS
	%	30.00	30.00	56.67					40.00	
	% Total	7.50	11.25	21.25					40.00	

**Table 2: Age distribution among study groups.**

		Group			Chi-square <sup>#</sup>	Sig.	Total		
		Control	CP. Only	CP+OA					
35-42 Years	NO.	15	16	13	4.916	0.086 NS	44		
	%	75.00	53.33	43.33			55.00		
	% Total	18.75	20.00	16.25			55.00		
43-50 Years	NO.	5	14	17			4.916	0.086 NS	36
	%	25.00	46.67	56.67					45.00
	% Total	6.25	17.50	21.25					45.00

**Table 3: Illustrates the analytic test of TNF $\alpha$ .**

Group	Min.	Max.	Mean	$\pm$ SD	Median	Mean Rank	Kruskal-Wallis	
							X <sup>2</sup>	Sig.
Control	5.036	34.550	11.125	6.331	9.682	38.88	0.131	0.937 NS
CP only	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		

**Table 4: Illustrates the analytic test for hsCRP.**

Group	Min.	Max.	Mean	$\pm$ SD	Median	Mean Rank	Kruskal-Wallis		Sig
							X <sup>2</sup>	p-value	
Control	0.101	1.681	0.334	0.335	0.242	33.25	2.894	0.235	NS
CP only	0.117	1.052	0.316	0.164	0.289	41.28			
CP+OA	0.122	3.023	0.389	0.504	0.314	44.55			

**Table 5: Illustrates the correlation of TNF $\alpha$  and hsCRP among study groups.**

Groups	R	P-value	Sig
Control	0.512	0.021	S
CP	0.581	0.001	HS
CP+OA	0.694	0.000	HS

**DISCUSSION**

OA occurs in the population with a high incidence about in fourth decade of life. Supporting the above fact, the groups of OA patients in the current study consisted of more females between 35-50 years, in CP+OA group, CP group the number of female was 26 (40%), and number of male 34 (60%), while the C group consisted of 6 females and 14 males. Statistical analysis showed that there were non-significant differences regarding gender and these

demographic data is coinciding with a study done in Europe by Lagonja *et al.* (11) they mentioned that no significant difference in terms of gender and age between CP+OA group and CP group and also agree with study of Torkzaban *et al.* (12) which found no correlation between gender and any of periodontal indices. this study disagrees with the study done by Pokrajac-Zirojevic *et al.* (13) which found the association between OA and gender was proven to be highly significant with female more commonly affected with OA than male (P=0.001).

Interestingly, the present study reported non-significant correlation of TNF $\alpha$  among the groups, although TNF $\alpha$  was increased in OA+CP group more than CP and C group, TNF $\alpha$  has an important role in inflammatory arthritis and in degenerative joint disease (14) it may be from the fact that human articular

chondrocytes from OA cartilage expressed a significantly higher number of the p55 TNF $\alpha$  receptor which could make OA cartilage particularly susceptible to TNF $\alpha$  degradative stimuli<sup>(15)</sup>. also associated with the level of OA severity. It is known that TNF $\alpha$  induces bone and extracellular matrix resorption by activating the osteoclasts, also it is secreted by leukocyte, macrophage, lymphocyte in the local lesion of PD.<sup>(16)</sup> This agree with Fernandes et al.<sup>(17)</sup> who observed that high serum levels of TNF $\alpha$  detected in patients with CP.

so it was clear that their levels increased in OA and periodontal disease but in this study the difference was statistically non-significant which may be related to the newly diagnosed disease, OA is a non-inflammatory disease, and the proinflammatory TNF $\alpha$  level is still less in the serum of the patient and may be the severity of PD had moderate effect on the destruction of the supporting tissue which is reflected on the level of inflammatory markers in the serum.

Also, in this study the hsCRP was non-significant related among the study groups but it was higher in OA+CP group than CP, C group. CRP, is a protein mainly produced by the liver in response to an increase in interleukin-6 and TNF $\alpha$ , which is part of the non-specific response to inflammation, infection and tissue damage<sup>(18)</sup>. There is a strong evidence that individuals with CP have elevated CRP levels as compared to the C groups which agrees with Previous studies conducted by Martu et al. and Salzberget al.<sup>(19)</sup>. CPR is related to the initial host response to injuries, infections, ischemic necrosis or malignancy. It is initiated by the activation of local macrophages and other cells (including fibroblasts and endothelial cells), leading to the release of mediators such as TNF $\alpha$ , IL-6 and IL-13. These in turn cause systemic changes including hepatic release of a range of plasma proteins (including CRP), activation of complement proteins and various metabolic changes.<sup>(20)</sup> This result disagrees with the study of Beck et al.<sup>(21)</sup> whom assessed the relationship between CRP and the periodontal status was not as clear. In spite of lack of previous study about the serum level of hsCRP and TNF $\alpha$  in patient with moderate CP and OA, this study indicated anon-significant correlation between the markers in the study groups, it may be due to differences that can

be influence CRP level such as different level of inflammation regarding OA 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, in addition diet may fluctuate CRP level. Other factors can have influence on proinflammatory cytokine production and/or activity are the cytokines having anti-inflammatory properties. For such cytokines, namely transforming growth factor (TGF) $\alpha$ , IL-4, IL-10 and IL-13, have been identified as modulator for various inflammatory processes.

This study found a strong positive correlation between TNF $\alpha$  and CRP in each group which agree with Foss et al.<sup>(22)</sup>whom revealed a positive correlation between the level of TNF $\alpha$  and hsCRP that indicate when patients have high TNF $\alpha$  levels, a proportional increase in CRP concentrations also occurs, this correlation considered that when the onset of acute-phase inflammatory response changes the concentrations of many plasma proteins, reflecting reorganization of the gene expression of hepatocyte secretory proteins after the inflammatory stimulus hsCRP is the acute phase protein occurring in humans, Normally, these proteins are present in small amounts in plasma, and an exacerbated increase in their rate of synthesis occurs after stimulation. A progressively increasing number of hepatocytes are recruited for CRP synthesis during the first days after the inflammatory reaction. Thus, persistently elevated CRP levels reflect active disease, and have been reported to occur in several infections, inflammatory reactions, tumors, and lesions. As conclusion of this study, CP and OA showed no significant association in overall analysis. Findings from this cross-sectional study do not provide evidence for a correlation between CP and OA. Prospective research and studies using clinical criteria for diagnosis of symptomatic OA are needed to confirm these findings.

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## الخلاصة

**الخلفية:** التهاب اللثة هو مرض التهابي مزمن يؤثر على اربطة وانسجة ما حول الاسنان والعظم السنخي، التهاب المفاصل هو ايضا التهاب المفصل المزمن مع عدة اسباب مركبة ومتداخلة منها التهاب السائل الغضري وفي اعادة تشكيل ما تحت العظام وتكوين النتوء الغضري وفي والذي يؤدي الى انحلال وتحطم الغضروف. التهاب اللثة المزمن والتهاب المفاصل تعتبر أمراض منتشرة على نطاق واسع وتقوم بتدمير الأنسجة بسبب الالتهاب مزمن في الصحة الجسم العامة وصحة الفم.

**الهدف من الدراسة:** لتقييم الحالة الصحية لانسجة ما حول الاسنان في مرضى التهاب المفاصل واختبار العلاقة بين مرضى التهاب اللثة المزمن والتهاب المفاصل من خلال تحديد مستويات المصل من البروتين (c) التفاعلي ذو الحساسية العالية وعامل نخر الورم الفا. **المواد وطرق العمل:** مجموعة 30 مريضا من كلا الجنسين الذين تتراوح أعمارهم بين 35 إلى 50 سنة مع التهاب المفاصل والتهاب اللثة المزمن المتوسط، 30 مريض في كلا الجنسين الذين تتراوح أعمارهم بين 35 إلى 50 سنة من متوسط التهاب اللثة المزمن وحدة. المجموعة الضابطة من 20 مريضا اصحاء سريريا ولديهم انسجة ما حول الاسنان صحية. استبعدنا في هذه الدراسة النساء ما بعد سن اليأس والنساء الحوامل والمريض المدخن أيضا. المرضى المصابين بالتهاب المفاصل مزودين بوثائق أو التصوير الشعاعي، بما يتفق مع التشخيص السريري لالتهاب المفاصل مع عدم وجود حالة التهابية روماتيضية. 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 والبروتين سي التفاعلي.

**النتائج:** وجود فروق غير معنوية بين المجموعة المدروسة فيما يتعلق بمستوى مصل الدم من البروتين سي التفاعلي وعامل نخر الورم الفا وكذلك كشفت أن متوسط مستويات مصل الدم من البروتين سي التفاعلي كانت أعلى قليلا في التهاب ما حول الاسنان المزمن مع

مجموعة التهاب المفاصل من مجموعة التهاب ماحول الاسنان المزمن ومجموعات السيطرة تمثل (0.31،0.29،0.24). وبالمثل كان مستوى مصّل الدم من عامل نخر الورم الفا أعلى في التهاب ما حول الاسنان المزمن مع التهاب المفاصل مما كانت عليه في المجموعتين الأخرين (10.615،10.533،9.682) على التوالي. وكان العمر ونوع الجنس فرقا غير معنويا بين المجموعات. كشفت الدراسة الحالية أن هناك علاقة إيجابية قوية بين مستويات مصّل الدم من البروتين سي التفاعلي ذو الحساسية العاليه وعامل نخر الورم الفا في المرضى في جميع المجموعات.

**الاستنتاج:** اظهرت الدراسة الحالية أن هناك علاقة إيجابية قوية بين مستويات مصّل الدم من البروتين سي التفاعلي (hsCRP) وعامل نخر الورم (TNF $\alpha$ ) في المرضى من جميع المجموعات ولكن لا يوجد تأثير او علاقة بين التهاب الانسجة ما حول الاسنان والتهاب المفاصل.