Assessment of serum levels of MMP-8 and hs CRP in chronic periodontitis patients in relation to atherosclerotic cardiovascular disease

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ABSTRACT

Background: Periodontitis and Atherosclerosis Cardiovascular disease are chronic inflammatory diseases which are highly prevalent. During the last two decades, there has been an increasing interest in the impact of oral health on atherosclerosis and subsequent cardiovascular disease. The aims of the study were to evaluate the periodontal health status in study groups (Atherosclerotic cardiovascular disease patients with chronic periodontitis and patients having chronic periodontitis),to estimate the serum levels of Matrixmetalloproteinase-8(MMP-8) and high sensitive C-reactive protein(hs CRP) in study and control groups and compare between them. Also,test the correlation between the serum levels of MMP-8 and hs CRP with clinical periodontal parameters at each study group

Subjects, materials and methods: Eighty subjects, males and females were included in this study with age range (35-50) years old, they were divided into study groups [ATH+CP group: Atherosclerotic cardiovascular disease& chronic periodontitis group (*30 patients*), CP group: chronic periodontitis group (*30 patients*)] andcontrol group: (*20* systemically healthy subjects, have healthy periodontium). Periodontal health status was determined by measuring the following clinical periodontal parameters (Plaque index (PL.I), Gingival index (GI), Bleeding on probing (BOP), Probing pocket depth (PPD) and Clinical attachment level (CAL)).Serum levelsof (MMP-8and hs CRP) were determined by mean of ELISA.

Results: The results showed that the mean values of clinical periodontalparameters (PL.I, GI, PPD and CAL), were higher in the ATH+CP group than in the CP group with significant differences except in PL.I there was no significant difference. A higher percentage of score 1of BOP sites demonstrated by CP groupthan in ATH+CP group with highly significant difference.The levels of serum (MMP-8 and hs CRP) were higher in ATH+CP groupwhen compared with CP group and control group, with highly significant differences between all pairs of study and control groups.Regarding the Correlation between serum levels of (MMP-8 and hs CRP) and clinical periodontal parameters, were positive correlation in both study groups.

Conclusion: The present results my provide evidence that Chronic Periodontitis may contribute to the inflammation-associated to atherosclerotic process

Keywords: Atherosclerosis cardiovascular disease, chronic periodontitis, Matrix metalloproteinase-8, hs C-reactive protein. (J Bagh Coll Dentistry 2014; 26(4):141-146).

الخلاصة

الخلفية :يعد مرض النساغ ومرض التصلب العصيدي من الامراض الالتهابية المزمنة الواسعه الانتشار . خلال العقدين الماضيين كان هناك اهتمام متزايد في تاثير صحة الفم على التصلب العصيدي ومرض القلبي الوعائي

الاشخاص ,المواد وطرائق العمل :80 شخصا ذكورا واناثا ادرجوا في هذه الدراسة تتراوح اعمارهم بين (35-50)سنة. تم تقسيمهم الى مجموعتي الدراسة (ج1 مجموعه التصلب العصيدي لديهم النساغ المزمن (30مريضا) وج2 مجموعه النساغ المزمن (30مريضا) ج3 مجموعه الضابطه (20 شخصا اصحاء سريريا / يملكون انسجه ماحوا الاسنان صحية).قدرت الحالة للانسجة ماحول الاسنان عن طريق قياس موشرات ماحول الاسنان السريرية التالية (مؤشر الصفيحة الجرثومية ,مؤشر التهاب اللثه ,مؤشر النزف عند التسبير ,مؤشر عمق الجيوب بلاظافة الى فقدان الانسجة الرابطه سريريا). تم تحديد مستويات المصل لكل من المصفوفه الفلزية -8و البروتين الارتكاسي العالي الحساسيه باستخدام تقنية مقايسة الانزيم المرتبط الممتز المناعي

النتائج :اظهرت النتائج ان قيم المتوسط الحسابي لكل من ((مؤشر الصفيحة الجرثومية ,مؤشر التهاب اللثه , ,مؤشر عمق الجيوب بلاظافة الى فقدان الانسجة الرابطه سريريا) اعلى لدى ج1 من ج2 .هنالك نسبة عاليه لمناطق التسبير عندج2 من ج1 مع فروقات معنوية عالية . مستويات المصل لكل من من المصفوفه الفلزية -8و البروتين الارتكاسي العالي الحساسيه كانت اعلى عند ج1 عند مقارنتها مع ج2 وج3 مع وجود فروقات معنوية عالية بين كل ثنائي من مجموعات الدراسة والضابطه . فيما يخص العلاقه بين مستويات المصل لكل من من المصفوفه الفلزية -8و البروتين الارتكاسي العالي الحساسيه ومؤشرات ماحول الاسنان السريريه كان هناك ارتباط معنوي طردي

الاستنتاج :النتائج الحالية قد توفر دليل على ارتباط بين مرض النساغ المزمن ومرض التصلب العصيدي . وتشير الى ان مرض النساغ قد يلعب دورهاما في الالتهاب المصاحب لمرض التصلب العصيدي

INTRODUCTION

Periodontitis is described as a multifactorial, irreversible and cumulative condition initiated and propagated by both bacteria and host factors and is also associated with various systemic conditions (1). Periodontal disease (PD) and Atherosclerosis seemed to share a similar pathway and that certain individuals might be more likely than others to respond to higher levels of inflammatory stimuli. These factors lead to excessive production of cytokines and other inflammatory mediators, enhancing the development of periodontal and arterial cell wall lesions (2).

MMP-8 plays an important role in the pathogenesis of periodontal diseaseand it is catalytically the most competent proteinase to initiate type I collagen and extracellular matrix degradation associated with periodontal tissue destruction leading to tooth loss (3). MMP -8 has also been suggested to be implicated in atherosclerosishence, it degrades type I collagen, which is a major component of the fibrous cap, also MMP-8 possesses proteolytic activity on some non-matrix proteins such as angiotensin I (4).

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CRP, the classic acute-phase, is sensitive objective marker of inflammation, tissue damage and infection.The American Heart Association recommended hs CRP measurements as a part of the assessment of patients with a moderate risk of coronary heart disease [5]. Circulating oral bacteria and lipopolysaccharide (LPS) are also able to stimulate hepatocytes to secrete CRP [6].

The purpose of this study were to evaluate the periodontal health status in study groups (ATH+CPgroupand CP group),to estimate the serumlevels of MMP-8 and hs CRP in study and control groups and compare between them. Also, test the correlation between the serum levels of MMP-8 and hs CRP with clinical periodontal parameters at each study group.

SUBJECTS, MATERIALS AND METHODS

Eighty (80) subjects, males and females aged (35-50) years old were recruited in this study. They were from attendants seeking treatment in Iraqi center for heart diseases in Ghazy Alharery Hospital and patients were seeking periodontal treatment in department of periodontics at Teaching Hospital College of Dentistry, Baghdad University.All the individuals were informed about the purpose of this investigations andconsented to its protocol.

The subjects were divided into study groups and control group. Study groups include:

- ATH+CP group consists of thirty patients diagnosed to have chronic periodontitis and Atherosclerosis cardiovasculardisease (ASCVD)according to catherization and not more than one year and under anticoagulant drugs (Clopidogrel).
- CP group consistsof thirty patients diagnosed to have chronic periodontitis and didn't have history of any systemic diseases.Chronic periodontitis in patients was defined as the presence of at least four sites with PPD of ≥ (4) mm and clinical attachment loss of ≥ (1-2) mm, this made according to the international classification system for PD [7].
- Control group consistsof twentypatients with clinically healthy periodontiumthis was defined by GI scores<0.5 [8] with no pockets or clinical attachmentloss and no history of any systemic diseases.

Exclusion criteria included:

1. Any patient had history of other chronic, systemic diseases with known associations with PD as diabetes mellitus, Rheumatoid Arthritis, etc.
2. Smoker.
3. Medication (anti- inflammatory or antimicrobial therapy) within previous 3 months
4. Periodontal treatment within previous 3 months.
5. Pregnancy
6. Contraceptive pills.

Clinical periodontal parameters examination was performedby using Michigan O periodontal probe on four surfaces (mesial, buccal/ labial, distal and lingual/ palatal) of all teeth except third molar, all subjects must have at least 20 teeth. The collected data include:-

1. Assessment of Soft Deposits by the Plaque Index System (PL.I) according to Silness and Loe [9].
2. Assessment of Gingival Inflammation by the Gingival Index System (GI) according to Löe [8].
3. Assessment of Gingival Bleeding on Probing (BOP): Periodontal probe inserted to the bottom of the gingival crevice or pocket and is moved gently along the root surface. If bleeding occur within 30 seconds after probing, the site was given as score (1), and a score (0) for the non-bleeding site [10].
4. Assessment of Probing Pocket Depth (PPD): It's defined as the distance from gingival margin to the most apical penetration of the periodontal probe inserted into the gingival crevice or periodontalpocket [7].
5. Assessment of Clinical Attachment Level (CAL): It is the distance from the cemento-enamel junction to the location of the inserted probe tip (bottom of gingival crevice or periodontal pocket [7].

After the clinical periodontal parametersexamination, 5ml venous blood was collected from study and control groups. After centrifusion, serum samples were kept frozen at (-20) °C. Serum levels of (MMP-8 and hs CRP) were determined by mean ofEnzyme Linked Immuno- Sorbent Assay (ELISA).ELISA Kit (96-wells) for quantitative determination of serum MMP-8 of HCUSA BIO.ELISA Kit (96-wells) for quantitative determination of serum hs CRP of De Medi Tec.

Statistical analysis was assessed using t-test, Chi-square test, ANOVA test, LSD and Pearson's coefficient of correlation.

RESULTS

The current results revealed that mean values of (PL.I, GI, PPD and CAL) were higher in ATH+CP group than the CP group, with

significant differences between the two study groups except for the mean value of PL.I there was no statistical significant difference. The percentage of score 1 of BOP sites was higher in CP group than the ATH+CP group with highly significant difference.The statistical analysisof clinical periodontal parameters forATH+CP group and CP group are summarized in table (1).

Table (2) showed thatthe serum levels of MMP-8 and hs CRP were higher in ATH+CP group followed by CP group then the control group, the mean values with Std. Dev. for MMP-8 were(76±4.1, 67.7±3.1,27.1 ±4.8 respectively) and for hs CRP were (5.087±1.613,2.113 ±1.013, 0.558±0.138 respectively) with highly significant differences ($p<0.001$) among all the groups. Regarding both immunological parameters inter-groups' comparisons of mean values of serum MMP-8 as well as hs CRP between all pairs of the study and control groups demonstrated highly significant differences, as shown in table (3).

In ATH+CP group there was weak positive correlation between GI with serum MMP-8 levels at $p<0.05$. While there were highly significant, strong positive correlations between serum MMP-8 levels and each of (PL.I, BOP, PPD and CAL) at $p< 0.01$. For CP group, there were weak positive correlations between serum MMP-8 levels with PL.I ,GI and PPD, on the other hand,highly significant, strong positive correlations were observed between serum MMP-8 levels with BOP and CAL at $p<0.01$. It was demonstrated thatin ATH+CP group weak positive correlation between serum hs CRP levels with PL.I at $p<0.05$ as well as weak positive correlations with GI, BOP and PPD at $p>0.05$ in ATH+CP group,whilehighly significant strong positive correlation was observed between serum hs CRP levels with CAL at $p<0.01$.In CP group highly significant strong positive correlations were revealed between serum hs CRP levels with (GI, BOP, PPD and CAL) at $p<0.01$, while the correlation between serum hs CRP levels and PL.I, was weak positive at $p<0.05$, as shown in table (4).

Table 1: Statistical analysis of mean values of clinical periodontal parameters (PL.I, GI, BOP, PPD and CAL) for the ATH+CP group and CP group with comparison of significance

Groups	PL.I		GI		PPD		CAL		BOP			
									Score 1		Score 0	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	No	%	No	%
ATH+CP	1.83	±0.28	1.94	±0.39	5.04	±0.41	4.7	±0.82	1521	56.5	1155	43.5
CP	1.73	±0.29	1.73	±0.23	4.76	±0.62	4.2	±0.82	1722	60.5	1126	35.5
T-test	1.32		2.523		2.018		2.23		Chi	7.478		
Sig	NS		S		S		S		HS			

NS: Non-Significantat P>0.05 .S : Significant at P<0 .05. HS : Highly Significant at P<0.001

Table2: Statistical analysis of mean values of serum (MMP-8 and hs CRP) for ATH+CP group, CP group and control group with comparison of significance

Groups	MMP-8		hs CRP	
	Mean	S.D.	Mean	S.D.
ATH + CPgroup	76	±4.1	5.087	±1.613
CP group	67.7	±3.1	2.113	±1.013
Control group	27.1	±4.8	0.558	±0.138
F-test	965.094		98.928	
Sig.	HS		HS	

HS : Highly Significant at P<0.001

Table 3: Inter groups comparisons of the mean values of serum (MMP-8 nm/ml and hs CRP mg/l) between all pairs of the ATH+CP group, CP group and Control group

Groups	MMP-8			hs CRP		
	Mean difference	S.E.	Sig	Mean difference	S.E.	Sig
ATH+CPXCP	8.165	1.037	HS	2.974	0.30	HS
ATH+CPXControl	48.913	1.037	HS	4.528	0.33	HS
CPXControl	40.747	1.159	HS	1.554	0.33	HS

HS: Highly Significant at P<0.001

Table 4: Pearson's Correlation Coefficients among serum levels of (MMP-8and hs CRP) and clinical periodontal parameters in ATH+CP group and CP group

Immunological Parameters	Groups	Statistical analysis	PL.I	GI	BOP	PPD	CAL
MMP-8	ATH+CP	r	0.48	0.37	0.692	0.70	0.78
		P-value	0.007	0.03	0.000	0.000	0.000
	CP	r	0.13	0.252	0.69	0.323	0.709
		P-value	0.482	0.179	0.000	0.082	0.000
hs CRP	ATH+CP	r	0.365	0.245	0.036	0.284	0.507
		P-value	0.047	0.193	0.852	0.128	0.004
	CP	r	0.425	0.555	0.505	0.490	0.816
		P-value	0.019	0.001	0.004	0.006	0.000

DISCUSSION

The mean values of (PL.I , GI,PPD and CAL)were higher in ATH+CP group than CP group which could be explained in that plaque is the major etiological factor in periodontitis and it is expected to be accumulating more in chronic periodontitis because the presence of dental plaque is the main clinical finding for chronic periodontitis and it is coincide with the severity and the time beingof the disease, another possible explanation of such results as the hospitalized Atherosclerotic cardiovascular disease patients neglect the oral hygiene measures and didn't brush their teeth regularly, so that more gingival inflammation could be seen in ATH+CP group.Bacterial dental plaque elaborate various compounds(toxins,enzymes and H_2S), that elicit an inflammatory response that is protective but also is responsible for loss of periodontal tissue, pocket formation, loosening and loss of teeth[11].Also dyslipidaemia in patients of ATH patientshad a negative influence on all clinical measures of periodontal health status e.g. (greater clinical attachment loss, PPD and tooth loss) [12].

The percentage of score 1 of BOP sites demonstrated by CP group was higher when compared with ATH+CP group, this result may be due to the presence of more inactive sites during clinical periodontal examination of ATH+CP group ,also more number of examining sites in CP group than ATH+CP.Theresultsobtained from the present study were similar tothat reported by other investigators [11,13,14]. MMP-8 can initiate the digestion of type I collagen,whichis the major structural element and load-bearing molecule that provides tensile strength to the fibrous cap of an atherosclerotic lesion [15]. In addition to the inflammation caused by atherosclerosis, the sources of MMP-8 found in serum by the host response to the insult of periodontal pathogens involves local increases of MMP-8 level, which may most probably leak to circulation through inflamed periodontal tissues [16]. MMP-8 is the most competent proteinase associated with periodontal tissue destruction leading to tooth loss [3]. The results obtained from the present study regarding serum levels of MMP-8 were similar to that reported by other studies [17,18]. hs CRP is a predictive marker for cardiovascular disease .CRP arise in the atheromatous lesions and reflect the extent of inflammation that predisposes to plaque instability [19]. In patients with chronic periodontitis, periodontal pathogens and bacteremia occurs that initiates a systemic antibody response and the activation of the hepatic acute-phase response [20]. Numbers of studies were agreed with results of present study regarding serum levels of hs CRP [15,21-23].

Regarding positive correlation between serum levels of MMP-8 and clinical periodontal parameters, might be caused by the potential inflammatory effect through the dental plaque [18].

MMP-8 concentration correlated especially with the depth of the periodontal pocket in bleeding sites. This may in fact support the value of MMP-8 as an indicator of progressive periodontitis with advancing attachment,in addition to that neutrophils which are major cellular sources of MMP-8 are also present in an increased concentration in periodontal tissues in patients with periodontitis. Furthermore, bacterial proteinases present in microbial plaque can activate production of MMP-8 by neutrophils [24].

Numbers of studies showed there was positive correlation between serum levels of MMP-8 and clinical periodontal parameters [11,18,25]. The possible explanation of the positive correlations between serum levels of hs CRP and clinical periodontal parameters, as periodontal diseases are infections characterized by inflammation and destruction of the supporting tissues of the affected teeth. Gram negative anaerobes are present in large numbers in sub-gingival plaque in periodontal pockets,these periodontal pathogens create toxic LPS, which is released within the pockets and penetrates into the tissues, where the LPS interacts with macrophages to stimulate the release of inflammatory mediators. These inflammatory markers cause vasodilation and

destruction of the periodontal ligaments, activate fibroblasts and osteoclasts for tissue destruction and bone resorption [26]. Low levels of bacteraemia, endotoxins derived from gram negative microorganisms and other bacterial components may provide a stimulus for systemic inflammatory responses such as increased production of CRP due to activation of the cascade of inflammatory cytokine production by monocytes and other cells in the periodontal tissue [27].

Number of studies agreed with our results [14, 26, 28], while Ide et al. reported there were no correlations between serum levels of hs CRP and clinical periodontal parameters [29,30].

As conclusion; the present results my provide evidence of association between Chronic periodontitis and Atherosclerotic cardiovascular disease and suggest that periodontitis may play important role in activation and triggering immune response and patients with chronic periodontitis at risk of atherosclerosis and cardio-vascular disease.

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