Anti-phosphoryl Choline and hs - CRP in Serum of Atherosclerosis Cardiovascular Patient with Chronic Periodontitis

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Abstract

Background: Periodontitis and Atherosclerosis Cardiovascular disease are chronic inflammatory diseases which are highly prevalent. Over the last two decades, the amount of evidence corroborating an association between dental plaque bacteria and coronary diseases that develop as a result of atherosclerosis has increased.

The aim of study: was to evaluate the periodontal health status in study groups

(Atherosclerotic cardiovascular disease patients with chronic periodontitis (ATH+CP) and patients having chronic periodontitis (CP)**)**, to investigate the serum level of antiphosphorylcholine antibody-IgG(Anti-PC IgG) , and the systemic level of inflammatory marker of cardiovascular diseases like high sensitivity C-reactive protein (hs-CRP) in chronic periodontitis with and without atherosclerosis cardiovascular disease as well as to examine the relationships between these mediators and clinical periodontal parameters.

Material and Methods: Blood samples were collected from sixty patients (30 CP patients and 30 CP+ATH patients) and from 25 apparently healthy volunteers were enrolled in this study. Periodontal parameters used in this study were plaque index (P.I), gingival index (G.I), bleeding on probing(BOP), probing pocket depth(PPD) and clinical attachment level (CAL) and Serum levels of Anti-PC IgG was estimate by enzymelinkedimmunosorbent assays (ELISA) and hs-CRP was estimate by immunoturbidimetric.

Results: The current data revealed a significant elevation ($p<0.01$) in median level of hs-CRP in CP group and CP+ ATH group in comparison to that in healthy control, moreover, the comparison between two groups of patients showed significant differences between two groups ($p<0.05$) .Whereas the serum level of antiphosphorylcholine-IgG was not observed significant differences between study groups ($p>0.05$) except for CP group when compared to control group ($p\leq0.05$).Moreover, in regards to the correlation between serum antiphosphorylcholine –IgG and hs-CRP, and clinical periodontal parameters, did not show any correlation with clinical parameters of periodontitis ($p>0.05$).

Conclusion: inflammatory mediator (hs-CRP) may increase inflammatory activity in atherosclerotic lesions and potentially increasing the risk for cardiovascular events in CP+ATH, furthermore, about the effect of Anti-PC IgG in ATH +CP group, further studies are needed to obtain more understanding result.

Key words*: Atherosclerosis cardiovascular disease, chronic periodontitis, Antiphosphorylcholine, hs C-reactive protein.*

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)**

Atherosclerosis and its cardiovascular ischaemic complications are the most common causes of death and disability worldwide **(2)**. It's a chronic lipid-driven inflammatory disease of the arterial wall characterized by the involvement of the innate and adaptive immune systems **(3).** Inflammation plays a critical role in the atherosclerotic process, starting from endothelial dysfunction through all stages of plaque build-up until its detrimental clinical ischemic complications **(4).**

Associations between periodontitis and atherosclerosis would be predicted based on inflammatory mechanisms initiated by bacteria associated with periodontal lesions, locally or systemically, that then influence the initiation or propagation of the atherosclerotic lesion. Such lesions may be initiated by inflammatory stimuli including systemic and locally produced inflammatory cytokines and chemotactic agents that cause changes in the endothelium such as up-regulation of adhesion molecules. These changes promote interactions with leucocytes, such as monocytes, that promote leucocyte migration into the intimal layer of the artery [(5)].

C-reactive protein is an acute-phase reactant that is mainly produced in the liver in response to a variety of inflammatory cytokines such as IL-6. It therefore serves as a marker for systemic inflammation in a variety of conditions [(6)].Abd et al. reported that elevated cell- and cytokine mediated markers of inflammation, including C-reactive protein, fibrinogen, and various cytokines, are associated with periodontal disease. The same elevated proinflammatory factors in PD have also been linked with atherothrombogenesis [(7)].

Phosphorylcholine (PC) is an immunogenic epitope in the capsular polysaccharide of Streptococcus pneumoniae and it has been suggested that the presence of serum anti-PC is partly a result of exposure to this organism[(8)].However, periodontitis patients exhibit elevated levels of anti-PC and this antibody reacts with 30–40% of bacteria in dental plaque samples, including periodontitis-associated bacteria and is demonstrated by higher serum levels of antibodies directed toward phosphorylcholine (anti-phosphorylcholine IgG) in patients with attachment loss, in comparison with those with healthy gingiva[(9)].

Shaw et al., in 2003 suggests that antibodies produced against certain periodontal bacteria would also react to phosphorylcholine bearing oxLDL[(10)] and, therefore, magnify the uptake of this lipid by foam cells, promoting further progress of atherosclerosis.

Subjects and Methods

A total of 60 patients consist of ((30 CP patients and 30 CP+ATH patients) and 25 apparently healthy volunteers) considered as control, were their ages range from (35-64) years with a mean age of (45.33 and 52.53, and 43.68 years respectively).

They were from attendants seeking treatment in the cardiology center in Al Sadr Teaching Hospital, patients were seeking periodontal treatment in department of periodontics at College of dentistry, University of Basra and people from the Iraqi national blood bank during the period between November 2018 till April 2019. Periodontal parameters used in this study were P.I, G.I, BOP, PPD, and CAL. Blood samples were collected from all patients and controls, After centrifusion, serum samples were kept frozen at (-20) °C, to estimate the serum level of antiphosphorylcholine-IgG by enzyme-linked immunosorbent assay, while hs-CRP was estimate directly by immunoturbidimetric.

Statistical analysis: It was assessed using P (ANOVA test), P (Chi-square). P (Kruskal Wallis test) and P (Mann-Whitney). Correlation among different parameters was calculated by the Spearman correlation coefficient test, P value less than the 0.05 was considered statistically significant.

Results

The demographic results of patients groups and controls group included in this study are presented in table**1**, there was male's predominance among study groups when compared to females. Furthermore, the differences in clinical periodontal parameters in patients and healthy controls are summarized in table**2**.

The current results revealed a significant elevation in median serum level of hs-CRP in CP group (0.210 mg/dl) and in CP+ ATH patients (0.575 mg/dl) as compared to healthy control (0.180 mg/dl), ($p<0.01$). Moreover, the comparison between two groups of patients showed that the median level of hs-CRP was increase in CP group but statistically not significant ($p>0.05$).Whereas the serum level of antiphosphorylcholine-IgG was not observed significant differences between study groups ($p>0.05$) except for CP group when compared to control group ($p\leq0.05$) as revealed in table 3. Furthermore, in regard to the correlation between serum hs-CRP and Anti-PC IgG, and clinical periodontal parameters did not show any correlation with clinical parameters of periodontitis ($p>0.05$) as shown in table4.

Table 1: Demographic characteristics of study group

Gender and age			CP cases n=30	CP+ATH cases n=30	Controls n=25
Male	NO.		19	27	12
	%		63%	90%	48%
Female	NO.		11	3	13
	%		37%	10%	52%
age	**Range**	35-44	14	4	12
		45-54	9	13	9
		55-64	7	13	4

Table 2: Clinical Periodontal Parameters in Study Groups

Clinical periodontal Parameters	Study group			P-value
	CP	CP + ATH	+ control	
P.I	1.69(±0.36)	2.04(±0.29)	0.50(±0.20)	0.0001**
G.I	1.43(±0.27)	1.37(±0.23)	0.47(±0.17)	0.0001**
BOP	32.91%	40.54%	0.0	0.0001**
PPD	4.91(±0.590)	4.61(±0.601)	0.0	0.05*
CAL	4.51(±0.741)	5.09(±1.115)	0.0	0.022*

**** = Highly significant difference (p=0.0001),*=significant difference**

Table3: The differences in median serum levels of hs-CRP (mg/dl) and Antiphosphorylcholine-IgG (u/ml) among study groups.

Immunological Parameters		CP	CP+ATH	control	P-value (Kruskal Wallis test)
hs-CRP	Mean rank	40.65	53.45	33.28	0.008**
	Median	0.210	0.575	0.180	
Anti-PC IgG	Mean rank	46.08	46.83	34.79	0.134NS
	Median	1.525	1.700	1.250	

Cont... **Table3: The differences in median serum levels of hs-CRP (mg/dl) and Antiphosphorylcholine-IgG (u/ml) among study groups.**

groups	P(Mann-Whitney)	
	Hs-CRP	Anti-PC IgG
Control X CP	0.220NS	0.05*
Control X CP +ATH	0.004**	0.106NS
CP X CP +ATH	0.034*	0.773NS

***P<0.05 Significant, **P<0.001 High significant, NS: non-significant**

Table 4: Spearman Correlation Coefficients among serum levels of (hs CRP and Anti-PC IgG) and clinical periodontal parameters in ATH+CP group and CP group

Immunological Parameters	Groups	Statistical analysis	P.I	GI	BOP	PPD	CAL
hs-CRP	CP	r	.330	.152	.256	.108	.327
		p-value	.075NS	.424NS	.171NS	.570NS	.078NS
	CP+ATH	r	.084	.068	.108	.141	.176
		p-value	.659NS	.720NS	.571NS	.458NS	.352NS
Anti-PC IgG	CP	r	.088	-.102	-.128	-.113	.251
		p-value	.644NS	.593NS	.502NS	.551NS	.181NS
	CP+ATH	r	-.186	.099	.090	-.020	-.073
		p-value	.325NS	.605NS	.636NS	.916NS	.702NS

Discussion

The possible association between periodontitis (PD) and atherosclerotic cardiovascular disease (ASCVD) has received much attention over the past two decades a comprehensive review was performed by an American Heart Association (AHA) working group [(11)], which concluded that "periodontal disease is associated with atherosclerotic vascular disease independent of known confounders"

In the present study a predominance of CP group among males than a female as male less attitude toward good oral hygiene[(12)] as well as in ATH+CP as the Estrogen is protect female as it has multiple effects including effects on lipids, nitric oxide, vascular tone and antioxidant properties[(13)].

The current result was found that the mean value of (P.I ,GI, and CAL) were higher in ATH+CP group as compared to CP group which is in accordance with the observations of the Kumar et al.[14], as they found that there was significant increase in mean of periodontal parameter (PI,GI) in ATH with CP patient compared to healthy control, Similarly Mohamad et al.[15] found that the mean value of each P.I, G.I were significantly higher in periodontitis patients when compared to healthy controls, while Thakare et al.[16] Deepa et al.[17] found that CAL was more in ATH with CP as compared to CP group. The possible explanation the presence of dental plaque is the main clinical finding for CP and it is coincide with the severity of the disease and the time being with the disease and it is expected to be accumulating more in CP, another possible explanation of such results as the hospitalized ASCVD patients neglect the oral hygiene measures and didn't brush their teeth regularly, In addition, CAL indicates the amount of the root surface denuded of periodontal attachment, thus it was used as an indicator of disease severity. The percentage of score 1 of BOP sites and CAL demonstrated by CP group were higher when compared with ATH+CP group, and these result was consistent with Androsz-Kowalsa et al.[18] and Raheem at el.[19] they found mean bleeding index in chronic periodontitis was significantly higher than in CAD with chronic periodontitis and control. The possible explanation for that percentage of BOP sites demonstrated by CP group was higher when compared with ATH+CP group, may be due to the presence of more inactive sites during clinical periodontal examination of ATH+CP group, also in CP the examining sites more than in ATH+CP group so ASCVD might be lead to tooth loss.

The present work is found increase in serum levels of hs-CRP in CP+ATH patients when compared to CP and control groups which is in accordance with the observations of the previous researchers[16,20], The possible explanation for positive association existed between the presence of chronic periodontitis and high serum CRP levels because it is biologically plausible that inflammatory mediators, especially IL-1 and -6 and TNF-a are released under conditions of periodontitis and present the capacity to stimulate hepatocytes to produce CRP[21], In addition direct actions of CRP which contribute to the induction of a prothrombotic state may be the enhancement of the procoagulant activity[22]. on the other hand, regarding IgG antiphosphoylcholine revealed significant difference between CP group when compared to control group and these result in agreement with[9,23] who found that patients with periodontal attachment loss have higher concentrations of anti-PC IgG than do individuals who demonstrate no attachment loss. The possible explanation for the higher level of serum IgG anti-phosphoryl-choline antibodies among CP patients in our study that inflamed periodontal tissues permit ingress of antigens from oral bacteria which leads to increased systemic production of anti-PC[23].

Regarding the correlation between serum (hs-CRP and anti-PC IgG) and clinical periodontal parameters, hs-CRP did not show significant correlation. And these was agreed with Ide and co-workers reported that there were no correlation between serum hs CRP and clinical periodontal parameters and they concluded that improvement in periodontal health status by non-surgical periodontal treatment did not influence the serum levels of hs CRP[24], in contract were inconsistent to that reported by others investigators [16,25] they found that there was significant positive correlation between serum CRP levels and clinical periodontal parameters in ATH with CP and CP group. Finally, antiphosphoylcholine IgG did not show significant correlation with clinical periodontal parameters and our result disagreed with Schenkein et al.[11] as they reported higher serum levels of antibodies (antiphosphorylcholine IgG) directed toward phosphorylcholine in patients with attachment loss, in comparison with those with healthy gingiva. The discrepancies observed between various studies could be caused, in part, to the differences in the sample size of each study, differences in types of samples used for each study and differences in sampling methods.

In conclusion current results suggest that elevation in inflammatory mediators (hs-CRP) may increase inflammatory activity in atherosclerotic lesions and potentially increasing the risk for cardiovascular events in CP patients. While for Anti-PC IgG the data show that many commonly occurring bacterial species found in dental plaque contain PC antigen and as a consequence of inflammation and periodontal attachment loss may influence systemic anti-PC antibody concentration, and on his role in atherosclerosis cardiovascular disease, further studies are needed to obtain more understanding result.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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