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RESEARCH ARTICLE

EVALUATION OF C-REACTIVE PROTEIN LEVEL IN PATIENTS WITH DIFFERENT GRADES OF CHRONIC PERIODONTITIS- A CLINICOPATHOLOGICAL STUDY

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ABSTRACT

Background: Periodontitis is a local inflammatory process mediating destruction of periodontal tissues triggered by bacterial insult. However, this disease is also characterized by systemic inflammatory host responses that may contribute, in part, to higher risk for cardiovascular diseases (CVD). The present study aims to examine C reactive protein (CRP) levels, as a marker of the inflammatory host response, in sera of subjects with varying grades of periodontitis and to correlate it to lipid profile thereby determining if CRP levels in chronic periodontitis can be used as an indicator of cardio vascular diseases**.**

Materials and method: Fasting blood samples from 60 patients comprising of 20 periodontally healthy patients, 20 patients with clinically moderate attachment loss and 20 patients with clinically severe attachment loss was collected and assessed for CRP, total cholesterol (Chol) & triglycerides (TG), as well as high & low density lipoproteins (HDL, LDL). The findings were then statistically analyzed.

Conclusion: A positive correlation between CRP levels and total cholesterol, triglycerides, & LDL and a negative correlation between CRP and HDL was observed with an increase in severity of periodontitis. Thus, CRP levels in chronic periodontitis could be an indicator of cardiovascular diseases.

INTRODUCTION

Chronic periodontitis degrades the attachment apparatus of the teeth, causing tooth loss. The more severe form of the disease is present in approximately 10–15% of adult population; whereas 35% exhibit moderate or mild signs of the disease. (Buhlin K(2013), Papapanou PN(1996) Direct and indirect host mediated effects of infectious agents may be responsible for the association between atherosclerosis and infections, in general, and periodontitis specifically. Both diseases also have several risk factors in common-e.g., smoking and diabetes. (Hujoel PP,2000) C Reactive Protein (CRP) is a sensitive and non- specific acute phase marker for inflammation. It is an acute phase reactant produced by the liver in response to diverse stimuli, including heat, trauma, infection, and hypoxia (Pepys and Baltz, 1983). (G.D. Slade,2000)

MATERIALS AND METHOD

Subjects included in the present study were randomly selected from the Out-Patient Department of Periodontics and the study was conducted in the Dept. of Oral and Maxillofacial Pathology.

A total of 60 subjects in the age range 35 to 60 years and with Body Mass Index ≤ 35 were divided into three study groups of 20 subjects each as follows:

Group A: Clinically healthy controls.
Group B: Patients diagnosed with moderate periodontitis. Clinical attachment loss (CAL) of 3-4 mm.
Group C: Patients diagnosed with severe periodontitis. CAL of 5 mm or more.

Exclusion criteria was Body Mass Index above 35**,** patients on Antibiotics, corticosteroids or NSAID'S within last month of sample collection, chronic smokers, patients with any other systemic disease like diabetes, patients with active viral, bacterial infection and trauma and pregnant females.

BMI was calculated to assess the obesity using the formula BMI = Weight/Height^2, measured in kilograms/meters.

Oral Hygiene Index - Simplified (OHI-S): was calculated to assess the oral hygiene status among all the three study groups applying the method formulated by Greene and Vermillion in which only one tooth surface in each sextant was assessed, equaling six surfaces.

PPD (percentage probing depth): was assessed for measuring severity of periodontal disease. Pockets were measured using Williams graduated probe, using the modifications of Third National Health and Nutrition Examination Survey (NHANES III) which included measurement in millimeters of periodontal pocket depth made at up to 4 sites per tooth.

5 ml venous blood sample was collected, out of which, 2 ml of the blood sample without EDTA was used for analysis of CRP and lipid profile and 2 ml of the blood sample mixed with EDTA for peripheral blood count.

Assessment of CRP levels

The blood samples were centrifuged at 3,000 r.p.m (revolutions per minute) for 5 minutes. Serum was separated for testing the CRP levels using the semi automatic analyzer (Erba Chem 5 Plus) with detectable level of 2mg/L in test specimen. CRP levels below 6mg/L were considered negative and levels above 6mg/L were considered positive.

Assessment of lipid metabolism

For lipid profile, blood was allowed to clot, centrifuged at 3000 rpm for 20 min., serum was separated and stored at -4 degree Celsius. Serum cholesterol (Choles), triglycerides (Tgl), high and low- density lipoproteins (HDL, LDL) were analyzed using ERBA Chem 5 Plus.

The differences between the three groups for different parameters (CRP and lipid profile) was analyzed for variability using one way analysis of variance (ANOVA) followed by post-hoc Tukey-Kramer test for pair wise comparisons.

Pearson's correlation was used to estimate the correlation between the CRP and lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides), whereas Spearman's rank correlation was used to correlate the periodontitis status (healthy, moderate periodontitis and severe periodontitis) with the CRP levels.

RESULTS

Mean CRP levels (mg/L) in different Groups. (Table 1)

Table 1 Mean CRP levels in different groups

				One-way ANOVA	
	N	*Mean*	*SD*	*F*	*'p'*
Group A	20	3.61	1.48	76.798	<0.0001
Group B	20	9.86	2.32		
Group C	20	24.69	9.17		
Pair-wise comparisons				**Tukey-Kramer test**	
Group A vs Group B					<0.05
Group A vs Group C					<0.05
Group B vs Group C					<0.05
Statistically significant p value					

In Group A mean CRP was 3.61 mg/L and the standard deviation(SD) was 1.48, in Group B mean was 9.86 and SD is 2.32, in Group C, mean was 24.69 and SD was 9.17. ANOVA test was applied and the differences were statistically significant. ($p < 0.0001$)

Pair wise comparisons between Group A, B & C using Tukey-Kramer test gave a significant p value ($p < 0.05$)

Mean Serum Total Cholesterol (mg/dl) in different Groups. (Table 2)

Table 2 Mean serum total cholesterol (mg/dl) in different groups

				One-way ANOVA	
	N	*Mean*	*SD*	*F*	*'p'*
Group A	20	191.97	17.50	81.899	<0.0001
Group B	20	221.81	14.33		
Group C	20	262.42	20.11		
Pair-wise comparisons				**Tukey-Kramer test**	
Group A vs Group B					<0.05
Group A vs Group C					<0.05
Group B vs Group C					<0.05
Statistically significant p value					

In Group A mean TC was 191.97 mg/dl and the SD was 17.50, in Group B mean TC was 221.81 mg/dl and the SD was 14.33, in Group C mean TC was 262.42 and SD was 20.11. ANOVA test was applied and the differences were statistically significant ($p < 0.0001$).

Pair wise comparisons between Group A, B & C using Tukey-Kramer test gave a significant p value. (p, 0.05)

Mean serum HDL cholesterol (mg/dl) in different Groups. (Table 3)

Table 3 Mean serum HDL cholesterol (mg/dl) in different groups

				One-way ANOVA	
	N	*Mean*	*SD*	*F*	*'p'*
Group A	20	50.80	6.43	81.416	<0.0001
Group B	20	42.59	5.00		
Group C	20	29.94	3.88		
Pair-wise comparisons				**Tukey-Kramer test**	
Group A vs Group B					<0.05
Group A vs Group C					<0.05
Group B vs Group C					<0.05
Statistically significant p value					

In Group A mean HDL was 50.80 mg/dl and the SD was 6.43, in Group B mean HDL was 42.59 mg/dl and the SD was 5.0, in Group C mean HDL was 29.94 and SD was 3.88. ANOVA test was applied and the differences were significant. ($p< 0.0001$)

Pair wise comparisons between Group A, B & C using Tukey-Kramer test gave a significant p value. ($p< 0.05$)

Mean serum LDL cholesterol (mg/dl) in different Groups. (Table 4)

Table 4 Mean serum LDL cholesterol (mg/dl) in different groups

				One-way ANOVA	
	N	*Mean*	*SD*	*F*	*'p'*
Group A	20	118.65	10.08	167.669	<0.0001
Group B	20	147.74	11.06		
Group C	20	181.13	11.22		
Pair-wise comparisons				**Tukey-Kramer test**	
Group A vs Group B					<0.05
Group A vs Group C					<0.05
Group B vs Group C					<0.05
Statistically significant p value					

In Group A mean LDL was 118.65 mg/dl and the SD was 10.08, in Group B mean LDL was 147.74 mg/dl and the SD was 11.06, in Group C mean LDL was 181.13 and SD was

11.22. ANOVA test was applied and the differences were significant. ($p < 0.0001$)

Pair wise comparisons between Group A, B & C using Tukey-Kramer test gave a significant p value. ($p< 0.05$)

Mean serum triglycerides (mg/dl) in different Groups. (Table 5)

Table 5 Mean serum triglycerides (mg/dl) in different groups

				One-way ANOVA	
	N	*Mean*	*SD*	*F*	*'p'*
Group A	20	145.94	7.39	141.150	<0.0001
Group B	20	180.35	14.26		
Group C	20	248.40	29.96		
Pair-wise comparisons				**Tukey-Kramer test**	
Group A vs Group B					<0.05
Group A vs Group C					<0.05
Group B vs Group C					<0.05
Statistically significant p value					

In Group A mean triglycerides was 145.94 mg/dl and the SD was 7.39, in Group B mean triglycerides was 180.35 mg/dl and the SD was 14.26, in Group C mean triglycerides was 248.40mg/dl and SD was 29.96. ANOVA test was applied and the differences were significant.($p < 0.0001$)

Pair wise comparisons between Group A, B & C using Tukey-Kramer test gave a significant p value. ($p< 0.05$)

Correlation of CRP levels and lipid profile: (Table 6)

Table 6 Correlation of CRP levels and lipid profile

CRP vs	'r'	95% C.I. for 'r'	'p'
Total Cholesterol	0.846	0.754 to 0.905	<0.0001
HDL- Cholesterol	-0.789	-0.868 to -0.668	<0.0001
LDL- Cholesterol	0.861	0.776 to 0.914	<0.0001
Triglycerides	0.930	0.885 to 0.958	<0.0001

Pearson's correlation was used to estimate the correlation between the CRP and lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides). The correlation was positive between CRP and total cholesterol (r =0.846), tyiglycerides (r= 0.930) and LDL (r = 0.861). It was negative between CRP and HDL (r = - 0.789)

Correlation of periodontitis with CRP: (Table 7)

Table 7 Spearman's rank correlation of periodontitis with CRP levels

CRP vs	'r'	95% C.I. for 'r'	'p'
Periodontitis status	0.931	0.887 to 0.959	<0.0001

Spearman's rank correlation was used to correlate the periodontitis status (healthy, moderate periodontitis and severe periodontitis) with the CRP levels. The correlation was positive (r= 0.931)

The results showed that the CRP levels increased with increased severity of periodontitis, from moderate to severe periodontitis. Also there was significant increase in the total cholesterol, LDL and triglyceride levels and decrease in HDL levels with increase in severity of periodontitis. Correlation between CRP and lipid profile was also statistically significant.

DISCUSSION

Periodontitis has been proposed to have an etiological modulating role in cardiovascular disease, (Ebersole JL1997, Loos BG,2000, Iacopino AM2001, Southerland JH2005, Mealey B2006) diabetes, respiratory disease(Saini R 2010, Scannapieco FA2003, Sharma 2011) and adverse pregnancy outcome(Verma S 2004). Inflammatory effects of periodontitis manifest systemically by dissemination of locally produced mediators such as CRP, Interleukin – 1Beta, Interleukin – 6 and Tumor necrosis factor Alpha (TNF – Alpha). (Loos BG 2000)

Ridker *et al* (1997) and Blake *et al* (2002) indicated that CRP levels in the upper quartiles of normality are predictors of future coronary events in healthy population. CRP measurements have been indicated as predictive value for development & prognosis of cardiovascular disease. (Nissen S2005) It has also been suggested that CRP may be a more powerful indicator for CVD than traditional risk factors such as low density lipoprotein (LDL), cholesterol. Indeed this has prompted the Centers for Disease Control (CDC) & the American Heart Association (AHA) to recently develop a summary on the inclusion of periodontal screening along with previously established criteria for risk assessment of CVD.

In this study, the control group (Group A) showed negative and Group B & C showed positive CRP levels. (Table 1; Graph1). This is in accordance with studies wherein they found a positive correlation between CRP and periodontitis patients[4,5,17].The results of this study showed that after adjusting for age and BMI, there was significant increase in CRP levels with increase in the severity of periodontitis, which is in agreement with the study by Noack *et al* (2001).[18]

Intergroup comparison for serum lipid levels revealed that the levels of total cholesterol, LDL, triglyceride were increased in Group B & Group C when compared with Group A, with the highest levels seen in Group C. (Table 2, 4, 5; Graph 2,4,5). Conversely the levels of HDL decreased in Group B and Group C when compared to Group A with lowest levels seen in Group C. (Table 3; Graph3) Thus with the increase in the severity of periodontitis, the levels of detrimental lipids, i.e. total cholesterol, LDL and triglycerides increased, whereas the levels of HDL which has a protective role, decreased. Spearman's rank correlation test gave a positive correlation between periodontitis status and CRP (r= 0.931). (Table 7)

There is substantial evidence that CRP may contribute directly to the pathogenesis of atherothrombosis. CRP is a ligand binding protein that binds to the plasma membrane of damaged cells. Aggregated but not soluble native CRP selectively binds Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) from whole plasma and could thereby precipitate in their atherogenic accumulation. Complexed CRP also activates complement and can be proinflammatory whereas CRP has recently been found to be a potent stimulator of tissue factor production by macrophages in vitro. Tissue factor is the main initiator of coagulation in vivo and its local concentration in the arterial wall is clearly related to coronary thrombotic events. However, the capacity of CRP to enhance tissue factor production suggests a possible causative link between increased CRP values and coronary events. CRP may activate the complement system and be involved in foam cell formation in atheromas.(Kanaparthy A2012)

Periodontal disease could result in repeated systemic exposure to bacterial endotoxin, lipopolysaccharide and other bacterial

products. The lipopolysaccharides of dental plaque diffuse into the systemic circulation and elicit a systemic lipopolysaccharide specific antibody response. This may lead to lipid metabolism disturbance and a hypercoagulable state through elevating circulating cytokines. (Wu T2000)

Also Monocyte derived cytokines, such as TNF-α, IL-1, 6 & 8 have powerful effects on hepatic protein synthesis (eg. in upregulating fibrinogen synthesis), tissue catabolism and lipid metabolism. Both TNF-α & IL-1 inhibit the production of lipoprotein lipase, causing lipid metabolism disturbance like increase in the level of serum cholesterol TGL and LDL. (Losche W2005)

Moreover, the infection with gram negative periodontal pathogens could prompt release of systemic IL-1 β and TNF-α, causing chronic hypertriglyceridemia and rise in level of which, leads to increase in triglyceride rich low density lipid. (Cutler CW 1999) The above observations and correlations between the different parameters in periodontitis indicate that these biochemical parameters may provide necessary information on the status of the periodontal disease and may also predict the future risk of cardiovascular diseases. Therefore these risk factors inspite being actual partakers can be used as biomarkers.

CONCLUSION

The possibility that periodontal disease and CVD share common risk factors or are manifestations of a similar underlying pathology remains unanswered. It will be necessary to conduct large-scale randomized intervention trials designed specifically to test these questions, as these would be of great interest not only from a scientific point of view but also from a public health perspective. Also microbial studies carried out in periodontal diseases will help in determining the pathogens which can increase the risk of CVD.

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