

SERUM RATIO ANALYSIS OF CRP/IL-6 IN PATIENTS OF PERIODONTITIS AND CARDIOVASCULAR DISEASES

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Contribution

AS designed the study and did statistical analysis and manuscript writing. AMC did data collection. AS did review and final approval of manuscript. Both authors contributed equally to the submitted manuscript

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ABSTRACT

Objective: To explore the connection of periodontitis with cardiovascular diseases through ratio analysis of CRP/IL-6 in patients with both pathologies.

Methodology: It was an cross-sectional study and involved participants of 30-70 year of age. All the subjects were broadly classified into three classes, selected by convenience sampling technique in to healthy controls, periodontitis patients and cardiac patients having periodontitis. Their Serum levels for C-RP and IL-6 was assessed through sandwich ELISA technique. One Way ANOVA as statistical tool, was used to measure the significant difference amongst studied groups.

Results: About 95 participants were included with 56 males and 39 females. There were 21% healthy controls, 36% periodontitis and 41% cardiac patients. Females showed 1.7, 0.7 and 0.1 times raised serum ratio levels of CRP/IL-6 as compared to males, in cardiac patients with mild periodontitis, cardiac patients with severe periodontitis and mild periodontitis alone respectively. While in category of severe periodontitis, males exhibited 0.33 times more C-RP/IL-6 ratio concentrations in contrast to their counterpart females. Overall we found that with increase in periodontal and cardiac pathology, the ratio level of CRP/IL-6 is decreased in both genders separately and altogether, with significantly proved statistical difference ($p < 0.05$).

Conclusion: In both genders, with increase in pathology, serum ratio levels of C-RP/IL-6 are decreased, also females exhibited more C-RP/IL-6 values in comparison to males with only periodontitis and periodontitis in addition to cardiac pathology.

Key Words: Periodontitis, Cardiovascular Diseases, Ratio analysis, CRP, IL-6

INTRODUCTION

Chronic periodontitis is one of the prime oral pathology in adults across the world. It involves chronic inflammation of periodontal supporting structures namely, cementum of the tooth, periodontal ligaments, bony socket and soft gingiva.¹ Periodontitis is most of the times remained undiagnosed. Though it is observed that approximately 15% of adults world widely have severe state of periodontitis.² It is an infection caused by bacteria, however its progression mainly depends upon other factors, including immunity of the person, his/her genetic make-up and life style.³

Poor periodontal status obviously lead to compromised systemic health, as many studies advocated the link of periodontitis with other systemic disorders including primarily diabetes mellitus, then comes cardiovascular pathologies and last but not the least obesity.⁴ The causal relationship between periodontitis and systemic disorders is explained in a way that bacteria and their end products from infective periodontal tissues gain entry into systemic circulation and eventually terminating into systemic inflammation.⁵ CRP, IL-1 β , IL-6, and TNF- α , are regarded as the prime mediators in chronic periodontitis. CRP and IL-6 have also been called as potential risk factors for many cardiovascular disorders.²

IL-6 is a pro-inflammatory cytokine, which is released as an inflammatory mediator from macrophages and T-lymphocytes. The association of IL-6 with advanced stages of chronic periodontitis and cardiac diseases such as unstable angina, myocardial infarction and coronary heart pathologies, has been reported in many studies.^{1,6} It has also been stated that IL-6 causes inducement of C-RP (as an acute phase protein from hepatocytes) production.⁷ C-reactive protein is also a pronounced inflammatory mediator that acts by activating the complement system of immune reactions through its attachments on the surface of necrotic cells and even on the wall of bacteria, responsible for periodontal decay.⁸ Many studies confirmed it's raised concentration in the sera of cardiac patients. Also it is evident that C-RP is involved in association of periodontal infection and coronary heart diseases, as C-RP along with TNF- α initiates such cascade of reactions that ultimately lead to endothelial damage and intravascular cholesterol plaque formation.^{7,8}

The prime aim of the current study was to investigate the association of periodontitis and heart vascular diseases through serum ratio analysis of pro-inflammatory cytokines i.e. C-RP to IL-6.

METHODOLOGY

This cross-sectional study was conducted at a tertiary care hospital Lahore from 1st January to 31st July 2017. Patients were consented for sample collection and its use in the study. After taking consent, approximately 5 ml of blood was drawn through anti-cubital vein of each participant in sterile syringes. Then transferred this blood into glass vials for centrifugation at 3050 rpm for 4-6 minutes, in order to get sera, which were stored at -40°C.

Sandwich Enzyme linked Immunoassay technique was used for

biochemical assessment of C - reactive protein and Interleukin-6 on ELISA kits. The levels of each sample was read through interpolation on the calibration curve.

SPSS version 20 was used to analyze the ratio concentration of CRP to IL-6 in all the participants. Single tail ANOVA was used to observe the significant difference in all the categories in each group. Bar graphs were plotted to show intragroup comparison of each group.

RESULTS

A total of 95 participants were in the study, divided into 56 males and 39 females ranging in-between 30-70 year of age. Among 56 males, 10 were taken as control group i.e. healthy ones, 19 were with only chronic periodontitis (in which 12 were with severe and 7 were with mild state of periodontitis) and other 27 males were cardiac patients along with chronic periodontitis (again further divided into 12 with severe and 7 with mild periodontal issue). Females were also categorized in the same fashion i.e. from total 39 females, 10 were healthy, 15 were with chronic periodontitis alone (further subdivided into 10 with severe and 5 with mild state of periodontitis) and remaining 14 were cardiac patients along with chronic periodontitis (again sub-classified as 7 with severe and 7 with mild periodontal pathology).

IL-6 (pg/ml) levels in terms of number of participants, average mean values and standard error of mean involved in each of the five categories i.e. healthy, with mild chronic periodontitis, with severe chronic periodontitis, cardiac patients with mild chronic periodontitis and cardiac patients with severe state of chronic periodontitis; along with their broad division into three groups i.e. males, females and both genders respectively in Group 1, Group 2 and Group 3 (Table 1). The difference is found statistically significant in each studied Group with p-value < 0.05.

It is also observed that serum ratio of C-RP/IL-6 is 1.7 times increased in female cardiac patients with mild state of periodontitis as compared to their male counterparts. Similarly we found that female cardiac patients with severe periodontal pathology showed 0.7 times raised serum levels of C-RP/IL-6 in contrast to their counterpart males. While in patients having mild periodontitis alone, females showed 0.1 times raised levels as compared to males. However in patients having severe state of periodontitis alone, males showed 0.33 times raised ratio levels of C-RP/IL-6 as compared to females.

Table 1: Statistics of Serum Estimated Ratio of CRP/IL-6 (ng/pg per ml) among Studied Groups (n=95)

Categories of Subjects	CRP/IL-6 (ng/pg per ml)								
	Group 1 (Males)			Group 2 (Females)			Group 3 (All Males & Females)		
	n	Mean ± SEM	P value	n	Mean ± SEM	P value	n	Mean ± SEM	P value
Healthy	10	4142±612	0.0001*	10	3607±2497	0.025*	20	3874.5±1554.5	0.0021*
Mild Periodontitis	7	6793±1685		5	7421±2042		12	7054.7±1833.8	
Severe Periodontitis	12	4561±1528		10	3435±1539		22	4049.2±1533	
Cardiac Patients with Mild Periodontitis	6	289±60		7	778±252		13	552.3±163.4	
Cardiac Patients with Severe Periodontitis	21	353±74		7	612±104		28	417.8±81.5	

*Calculated through one-way ANOVA and confidence interval was set on 95%

The serum ratio of C-RP to IL-6 is detected to be significantly higher in patients with only mild state of chronic periodontitis compared to other four categories studied i.e. healthy, patients with severe state of chronic periodontitis, cardiac patients with mild periodontitis and cardiac patients with severe state of chronic periodontitis (Table-1). This pattern is consistently observed in all three studied groups i.e. males as Group 1, females as Group 2 and all studied subjects including both genders as Group 3 (Fig. 1, Fig. 2 and Fig. 3).

Similarly the lowest serum ratio levels are found in cardiac patients having severe chronic periodontitis, compared to all other studied categories and this pattern was observed in all males separately (Figure 1) and in all studied subjects including both genders (Figure 3). However in female group the lowest level

is detected in heart patients having mild state of chronic periodontitis. All results were found statistically significant in each studied group (Figure 2).

It is also perceived that with the advancement of pathology in terms of chronic periodontitis alone or along with cardiac disease, the serum ratio level of C-RP to IL-6 was declined in Females separately as Group 2 (Figure 2) and all studied subjects including both genders as Group 3 (Figure 3). While in males this trend is proved true for chronic periodontitis i.e. more serum ratio level of C-RP to IL-6 was observed in mild state as compared to severe state, but in cardiac patients with mild periodontitis, the ratio level is found more as compared to cardiac patient with severe periodontitis (Figure 1).

Figure 1: Comparison among different categories of males for serum C-RP/IL-6 ratio (p=0.0001).

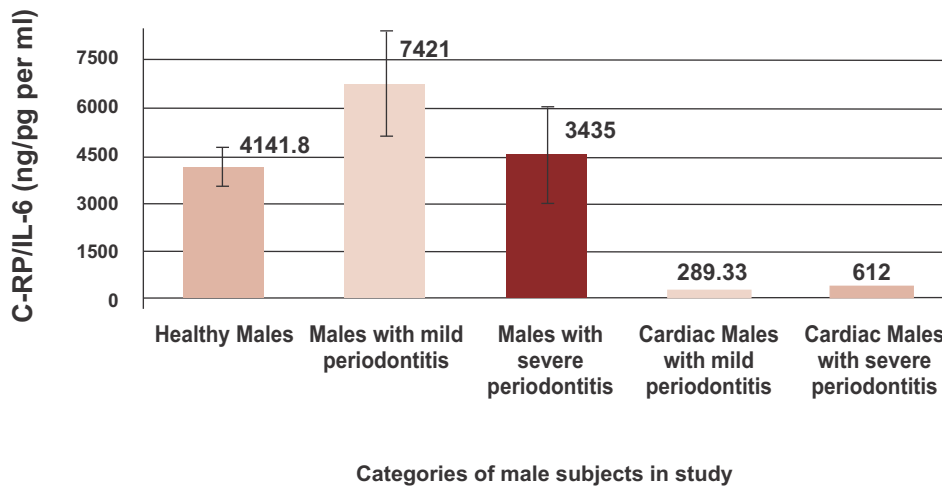


Figure 2: Comparison among different categories of females for serum C-RP/IL-6 ratio ($p=0.025$).

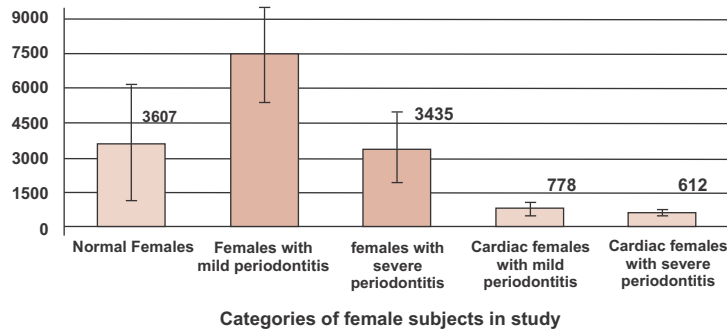
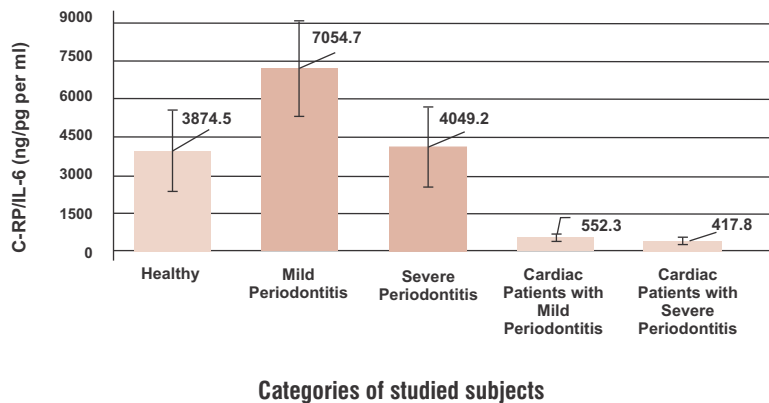


Figure 3: Comparison among studied categories for serum C-RP/IL-6 ratio ($p=0.0021$).



DISCUSSION

Inflammation is indeed a complicated defensive mechanism in which immunity is also exacerbated that further enhanced the release of inflammatory intermediaries as cytokines and chemokines that expedite the cellular process involved in local tissue decay.⁹ Talking about the risk factors for chronic periodontitis, smoking and obesity are the known ones in this connection, alongside genetic background and nutritional habits are also considered as a vital player in susceptibility to periodontal infection.¹⁰

Available epidemiological data has supported the notion that periodontitis is a threat for coronary heart diseases, as periodontitis acts as a constant source of bacterial infection to trigger immune inflammatory reactions; that finally turn into coronary atherogenesis.^{8,11} C-reactive protein possesses a special link in this regard. Other bio-inflammatory moderators involved in the pathogenesis of cardiovascular diseases originated primarily from periodontal decay are PGE2, IL-1, IL-6, and TNF- α . All these mediators are responsible for systemic acute inflammatory reactions, which enhance the development of atheromatous plaques that further contribute to more dangerous cardiovascular concerns.^{2,12}

At focal inflammatory sites IL-6 is secreted from monocytes, lymphocytes and even endothelial cells. Then it further excite neutrophils and thrombocytes in order to promote inflammatory

process. IL-6 also promotes the synthesis of proteins from hepatocytes such as C-RP, serum amyloid and fibrinogen.^{6,13} In patients with chronic periodontitis, periodontal pathogenic bacteria and their toxins gain systemic entry and an immune reaction is then initiated in which C-RP is observed as an important acute phase reactant.¹⁴ IL-6, TNF-alpha, IL-1 and C-RP all are pro-inflammatory cytokines, while IL-4 and IL-10 are labelled as anti-inflammatory mediators. In periodontitis the normal balance of pro and anti-inflammatory activity is lost, rather it is shifted towards more pro-inflammatory activity.¹⁵

Raised serum concentrations of C-RP and IL-6 have been observed in patients of chronic periodontitis and as well as cardiovascular pathologies.¹⁶ IL-6 is known as ubiquitous cytokine that exhibits its role in activation of acute phase reactant proteins. It is also considered a key moderator in promotion of atherothrombotic conditions.¹⁷ The claimed pathogenesis in relation to periodontitis leading to cardiovascular disturbances has explained in a way that local pathogenic virulent bacteria and their exotoxins along with other cytokines elevate the secretion of IL-6 at the inflammatory front in periodontitis.¹⁸ IL-6 also owns the capacity to enhance bone resorption activity by maximizing the osteoclast formation. Due to osseous destruction, clinical attachment loss is potentially elevated, causing more decay and severity in terms of periodontitis.¹⁹

The results of the present study displayed same consistent observations that C-RP and IL-6 both raised with the severity of

periodontitis and cardiovascular diseases, though we found a greater rise in serum concentration of IL-6 in comparison to C-RP with the increase in disease advancement, that's why the ratio analysis of C-RP/IL-6 showed lesser values with the advancement of pathology/pathologies (Periodontitis and cardiovascular diseases) in both males and females together and as well as separately, with statistical significance as $p < 0.05$ for each studied group.

LIMITATIONS

Nutritional habits, body mass index and smoking, all were ignored that could disturb the levels of pro-inflammatory cytokines and may play as confounders.

CONCLUSION

The average serum ratio levels of C-RP/IL-6 are observed to be found in an inverse relation with the degree and severity of disease, the more the disease the lesser would be the ratio serum levels of C-RP/IL-6, in the studied subjects. It is also noticeable that in categories of mild periodontitis, cardiac with mild periodontitis and cardiac with severe periodontitis, the female patients showed more concentration of C-RP/IL-6 as compared to males. Moreover the difference is found statistically significant among all studied categories i.e. mild periodontitis, severe periodontitis, mild periodontitis along with cardiac disorder, and severe state of periodontitis along with cardiac pathology, in both genders inclusively as well as separately.

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