

C-reactive Protein as Predictor of Increased Carotid Intima Media Thickness in Patients with Chronic Periodontitis

Roopali P Tapashetti¹, Sowjanya Guvva², Sudhir R Patil³, Sumit Sharma⁴, H M Pushpalatha⁵

Contributors:

¹Reader, Department of Periodontology, Al-Badar Rural Dental College and Hospital, Gulbarga, Karnataka, India; ²Reader, Department of Periodontology, SVS Institute of Dental Sciences, Mahabubnagar, Andhra Pradesh, India; ³Professor, Department of Periodontology, KLE Dental College, Bengaluru, Karnataka, India; ⁴Reader, Department of Periodontology, Jaipur Dental College and Hospital, Jaipur, Rajasthan, India; ⁵Senior Lecturer, Department of Pedodontics & Preventive Dentistry, Al-Badar Rural Dental College and Hospital, Gulbarga, Karnataka, India.

Correspondence:

Tapashetti RP. H-No. 2-907/15C, GouriSadan, Gubbi Colony, Near Gumbaz, Gulbarga - 585 105, Karnataka, India. Tel.: +91-9740944588. Email: roopali.perio@gmail.com

How to cite the article:

Tapashetti RP, Guvva S, Patil SR, Sharma S, Pushpalatha HM. C-reactive protein as predictor of increased carotid intima media thickness in patients with chronic periodontitis. J Int Oral Health 2014;6(4):47-52.

Abstract:

Background: C-reactive protein (CRP) - a prototypic marker of inflammation has been shown to be elevated in chronic periodontitis (CP) and also been shown to predict cardiovascular events. Increased carotid intima media thickness (CIMT) has been recently recognized as surrogate marker for atherosclerosis. In this context, we studied to correlate between CIMT and CRP in CP and to know whether CRP predicts the cardiovascular risk in CP.

Materials and Methods: The study consisted of 30 systemically healthy subjects aged over 40 years - 15 subjects with CP as cases and 15 subjects with no periodontitis as controls. All subjects were subjected to measurement of CRP levels and CIMT in addition to detailed periodontal evaluation. Quantitative determination of CRP was done by turbidimetric immunoassay. IMT of the common carotid arteries was estimated bilaterally using B-mode ultrasound at 6 sites. Positive CRP was defined as more than 10 mg/l.

Results: Mean CRP levels were significantly higher in subjects with CP (19.58 ± 17.03), then in non CP (NCP) (5.54 ± 1.63 , $P < 0.004$). Mean CIMT value was significantly higher in subjects with CP (1.09 ± 0.45) than in NCP (0.57 ± 0.06 , $P < 0.001$) and all periodontal indices correlated well with CIMT. Further, there was significant correlation between CRP and increased CIMT in subjects with CP ($r = 0.863$, $P < 0.001$).

Conclusions: The present study indicates CRP as a possible underlying pathway in the association between periodontal disease and the observed CIMT. CRP can be used as a risk predictor for atherosclerosis in patients with CP.

Key Words: Carotid intima media thickness, chronic periodontitis, C-reactive protein

Introduction

Periodontitis – a common, often underdiagnosed dental disease has been epidemiologically linked with cardiovascular diseases.¹⁻³ C-reactive protein (CRP), a prototypic marker of inflammation has also been shown to predict cardiovascular events.^{4,5} In this context, several studies have shown elevated CRP levels in periodontal patients being linked to cardiovascular diseases.⁶⁻⁸ However assessing coronary artery disease prospectively in clinical studies is often difficult and sometimes requires invasive procedures like coronary angiogram. Increased carotid intima media thickness (CIMT) measured non-invasively by B Mode ultrasound has been recently recognised as surrogate marker for atherosclerosis.⁹ The atherosclerosis risk in communities (ARIC) Study in the Western population has shown positive relationship of periodontal disease to increased carotid artery intima media thickness.¹⁰ Studies on this aspect in Indian population is limited and restricted to subjects with established atherosclerosis.¹¹ In this context, we undertook this study to know relationship between chronic periodontitis (CP) and increased CIMT and to know whether CRP can be used to predict this relationship.

Materials and Methods

Totally 30 adult systemically healthy subjects, all aged more than 40 years were included in study after obtaining informed written consent. They were divided into two groups – 15 subjects with CP as cases and 15 subjects with non-periodontitis (NP) as controls.

Exclusion criteria

- Any subject with recent febrile illness of <1 month duration
- Connective tissue disorders
- Recent use of non-steroidal anti-inflammatory drugs/antibiotics/steroids
- All diabetics and hypertensives.

These exclusion criteria were framed to remove confounding factors for elevated CRP and also to assess the risk for atherosclerosis in CP independent of conventional risk factors.

Chronic periodontitis was diagnosed based on standard clinical features of chronic inflammatory changes in the marginal gingival, presence of periodontal pockets, loss of clinical attachment and radiographically by evidence of bone loss.¹² Periodontal indices were recorded in all cases i.e., gingival

index, oral hygiene index – simplified, probing depth (PD) and clinical attachment loss (CAL). Fasting blood sample was collected for CRP (and lipid profile also) in all cases, and serum was isolated. Quantitative determination of CRP was done by turbidimetric immunoassay using kit from Erba Diagnostics Mannheim with detection limit of 5 mg/L. Positive CRP was defined as ≥ 10 mg/L.

IMT of the common carotid arteries was estimated bilaterally using B-mode ultrasound (Figures 1 and 2). Analyses were based on mean IMT of the far wall for 1 cm lengths of the carotid bifurcation and the internal carotid and common carotid, right and left.¹³ In this study, single sonologist did the scan for all the subjects.

The statistical software namely SPSS 11.0 and Systat 8.0 were used for the analysis of the data and Microsoft Word and Excel have been used to generate graphs, tables and so on. Chi-square test has been used to find the homogeneity of gender

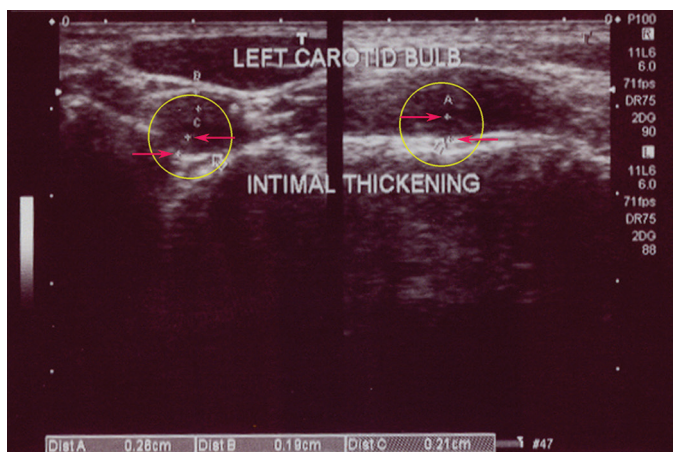


Figure 1: High resolution ultrasound images of measurement of carotid intima media thickness.

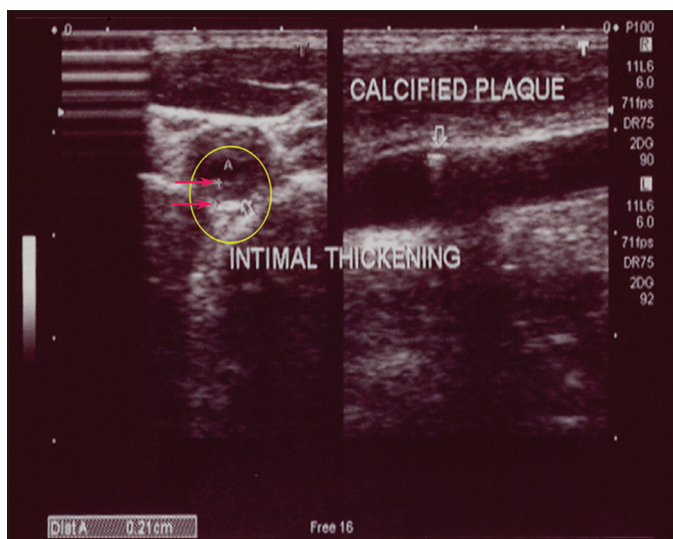


Figure 2: High resolution ultrasound images of measurement of carotid intima media thickness.

between the groups. Student’s *t*-test has been used to find the homogeneity of samples based on age in years. Student’s *t*-test has been used to find the significance of CIMT, CRP, and periodontitis parameters between the groups. Pearson correlation coefficient (*r*) has been used to find the significance of periodontitis parameters, CIMT and CRP levels in CP. Classification of correlation co-efficient (*r*) is as follows: Up to 0.1 is trivial correlation, 0.1-0.3 is small correlation, 0.3-0.5 is moderate correlation, 0.5-0.7 is large correlation, 0.7-0.9 is very large correlation, 0.9-1.0 is nearly perfect correlation and 1 is perfect correlation.

The receiving operating characteristics (ROC) curve was plotted to assess the accuracy of CRP as diagnostic marker. In a ROC curve, the true positive rate (sensitivity) is plotted in function of the false positive rate (100-specificity) for different cut-off points. Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions) has a ROC curve that passes through the upper left corner (100% sensitivity, 100% specificity). Therefore, the closer the ROC curve is to the upper left corner, the higher the overall accuracy of the test.¹⁴

Results

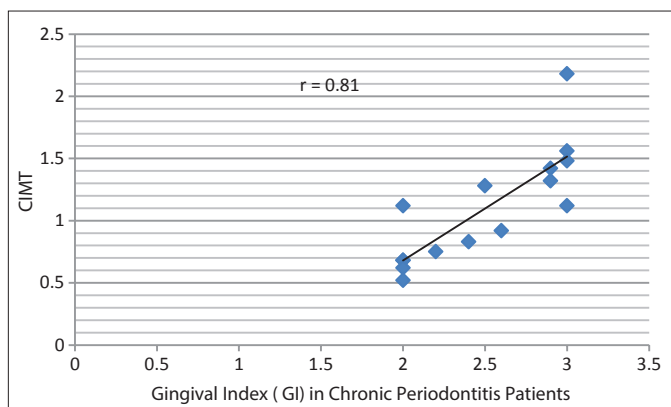
The baseline characteristics in the study groups were well matched (Table 1). The CRP levels were significantly higher in CP (19.58 ± 17.03) when compared to CRP levels in NP (5.54 ± 1.63) (Table 2). The mean CIMT value was also significantly higher in CP (1.09 ± 0.45) when compared o CIMT values in NP (0.57 ± 0.061) (Table 2). CIMT has a large and significant correlation with CRP ($r=0.86$). CIMT also had significant Pearson correlation with all periodontal indices. All correlations depicted in Graphs 1-5.

Discussion

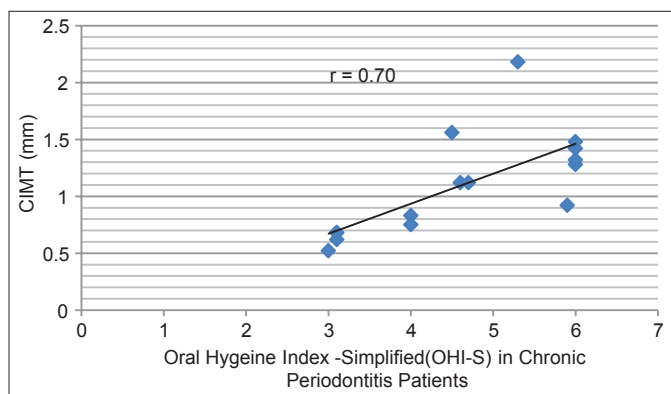
Majority of studies related to periodontitis – cardiovascular relationship have looked at clinical cardiovascular events or at prevalent atherosclerosis population. We investigated this relationship at subclinical level by measuring CIMT in Indian subpopulation. It’s well-known that CRP levels are elevated

Table 1: Study characteristics.			
Study characteristics	Subjects with CP	Subjects with NCP	P value
Age in years	47.67±3.37	46.53±2.80	0.325
Sex (%)			1.000
Male	9 (60.0)	9 (60.0)	
Female	6 (40.0)	6 (40.0)	
Smoking (%)			0.361
No	11 (7.3)	13 (86.7)	
Yes	04 (26.7)	02 (13.3)	
LDL	120.07±11.18	118.47±14.76	0.743
HDL	44.13±3.35	45.46±4.46	0.388

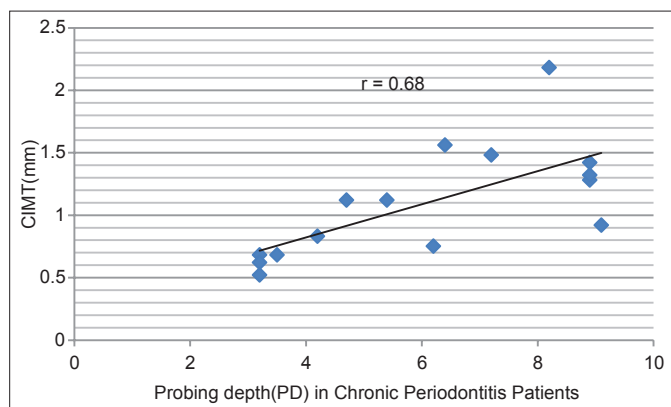
LDL: Low-density lipoprotein, HDL: High-density lipoprotein, CP: Chronic periodontitis, NCP: Non-chronic periodontitis.



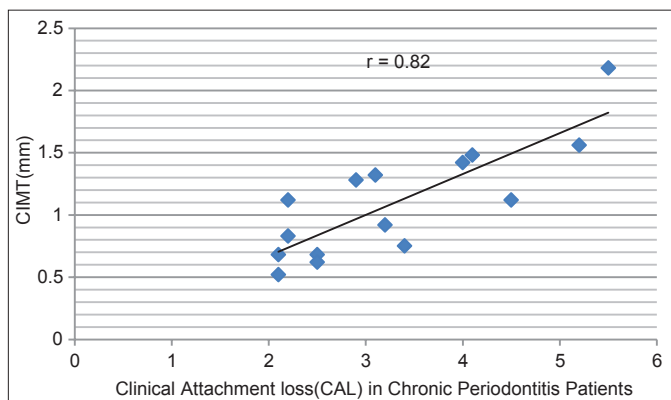
Graph 1: Pearson correlation between CIMT and GI.



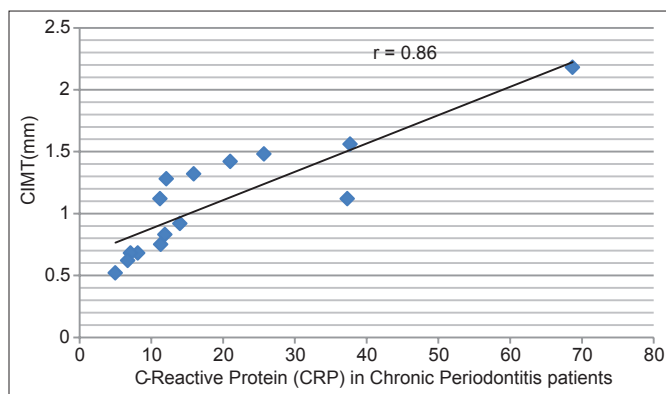
Graph 2: Pearson correlation between CIMT and OHI-S.



Graph 3: Pearson correlation between CIMT and PD.



Graph 4: Pearson correlation between CIMT and CAL.



Graph 5: Pearson correlation between CIMT and CRP.

Table 2: Comparison of mean CIMT values, frequency of positive CIMT, mean CRP levels and frequency of positive CRP.

Parameter	Subjects with CP	Subjects with NCP	P value
Mean CIMT (mm)	1.09±0.45	0.57±0.06	<0.001
Positive CIMT (>1 mm) (%)	8/15 (53)	0/15 (0)	<0.002
Mean CRP level (mg/L)	19.58±17.03	5.54±1.63	<0.004
Positive CRP (>10 mg/L) (%)	11/15 (73)	1/15 (7)	<0.001

CP: Chronic periodontitis, NCP: Non-chronic periodontitis, CRP: C-reactive protein, CIMT: Carotid intima media thickness.

in CP and linked to cardiovascular disease.⁶⁻⁸ Here, we looked at CRP-CIMT relationship and whether CRP can predict the risk of atherosclerosis in CP. Among the indexed articles in PubMed, only few periodontal studies have looked at CRP-CIMT relationship at subclinical level.¹⁵⁻¹⁷ To the best of our search, this is probably the only Indian study in this context.

The baseline characteristics in the study groups were well matched (Table 1). Like many other studies,¹⁸⁻²³ in the present study too, subjects with CP had significantly higher levels of CRP when compared to NP (19.58 ± 17.03 in CP vs. 5.54 ± 1.63 in NP, Table 2). Effect of CP on CRP was large (1.13) and when we looked at prevalence of positive CRP, it is 38 times more likely to occur in CP than NP.

In the present study, subjects with CP had significantly higher mean CIMT value of 1.09 ± 0.45 when compared with mean CIMT value of 0.57 ± 0.06 in NP. Further, when we looked at prevalence of positive CIMT (value > 1 mm); it was seen to occur in 53% of subjects with CP compared with none in NP (Table 2). Beck *et al.* were the first to demonstrate a higher mean CIMT value in severe and moderate periodontitis than in NP (0.82, 0.77, 0.74 respectively).¹⁰ In the same ARIC study, the prevalence of positive CIMT (defined as >1 mm) was totally 26.4% (15.5% in severe and 10.9% in moderate periodontitis, odds ratio 2.0 and 1.4 respectively) compared to 7.8% in controls. In another recent study, López *et al.* have shown significantly higher CIMT value in subjects with periodontitis (0.77 ± 0.26) than those without it (0.68 ± 0.13).¹⁵ Even in younger population, periodontitis has

been shown to be associated with subclinical atherosclerosis.¹⁶ The present study observed a very high mean CIMT value and higher prevalence of positive CIMT in this region of India. This could probably be related to multiple factors such as ethnicity, late diagnosis, lower socioeconomic status, ignorance and lack of access for treatment in the regional population.

However the limitation of mean CIMT value in small sample size is that it can get skewed due to single large value. So to overcome this, we analyzed how well CIMT value correlates well with periodontal indices. CIMT had a large and significant correlation with all periodontal indices (Table 3, Graphs 1-5) with strongest correlation seen with CAL ($r = 0.822$, $P < 0.001$). This corroborated that periodontitis can predict atherosclerosis at subclinical level. López *et al.* had shown periodontitis to be independent predictor of CIMT.¹⁵

Cairo *et al.* had similarly demonstrated in a cross sectional study, wherein the mean PD and full mouth bleeding score may predict sub-clinical atherosclerosis and systemic inflammation in young adults with severe periodontitis.¹⁷

However, when it comes to day to day dental practice, assessment of cardiovascular risk by measuring CIMT is often cumbersome, time consuming and relatively expensive. On the other hand, measurement of CRP is quick, inexpensive and does not require skilled expertise of a radiologist. It is well-known that CRP is itself an important general marker of increased risk for cardiovascular events and has also been shown to add to risk prediction based on conventional risk factors.²⁴ So after having already shown higher CRP levels and higher CIMT values in patients with CP, our next aim was to assess predictive utility of CRP by testing Pearson

correlation and diagnostic characteristics of CRP (sensitivity and specificity).

The Pearson correlation between CRP and CIMT showed a very large and significant correlation ($r = 0.863$; Table 3 and Graph 5) thereby supporting role of CRP as predictor for atherosclerosis in CP. In terms of diagnostic characteristics of CRP for a positive CIMT in subjects with CP, the sensitivity and specificity of CRP was 100% and 57%, respectively with accuracy of 79% (Table 4). Lower specificity is biologically plausible because first of all acute phase response is essentially, and inescapably a non-specific response.²⁵ Then secondly, a positive CRP antedates the development of atherosclerosis by several years and it is the persistent elevation of CRP which would contribute to the pathogenesis of atherosclerosis. Rifai *et al.* in their debate on specificity on CRP for vascular disease have shown that the relative risk for future cardiovascular events increased significantly with each increasing quartile of baseline CRP level.²⁶ Hence, those subjects with positive CRP and negative CIMT would be probably those cases whom atherosclerosis may occur later.

Regardless of the specificity and underlying mechanisms, the empirical measurement of CRP has high negative predictive value i.e., a negative CRP would rule out the risk for atherosclerosis in such individuals. Conversely, a positive CRP would motivate both the clinician and the patients for taking preemptive measures not only for CP but also for all other existing modifiable risk factors. The sensitivity and specificity of CRP for CP in the total study population was 73% and 93% respectively with accuracy of 83% (Table 5). To add statistical power to this, we derived the ROC curve area. The area under curve was more than 0.9 with curve being closer to the upper left corner (Graph 6). Here, the higher specificity of CRP is obvious because of chronic inflammation happening in CP.

To summarize, the effect of CP on CRP is large (1.13) and a positive CRP is 38.5 times more likely to occur in CP than controls. Positive CRP strongly correlates with positive CIMT. Positive CRP is highly sensitive albeit non-specific indicator of

Table 3: Pearson correlation of CIMT with CRP and CIMT with each periodontal index in subjects with CP (Graphs 1-5).

Subjects	CIMT	GI	OHI-S	PD	CAL	CRP
1	0.83	2.4	4	4.2	2.2	11.9
2	0.75	2.2	4	6.2	3.4	11.3
3	2.18	3	5.3	8.2	5.5	68.7
4	1.28	2.5	6	8.9	2.9	12.1
5	1.12	3	4.6	5.4	4.5	37.3
6	0.92	2.6	5.9	9.1	3.2	14
7	0.68	2	3.1	3.5	2.1	8.1
8	1.12	2	4.7	4.7	2.2	11.2
9	1.32	2.9	6	8.9	3.1	15.9
10	1.42	2.9	6	8.9	4	21
11	1.56	3	4.5	6.4	5.2	37.7
12	0.68	2	3.1	3.2	2.5	7.1
13	1.48	3	6	7.2	4.1	25.7
14	0.62	2	3.1	3.2	2.5	6.7
15	0.52	2	3	3.2	2.1	5.0
Pearson (r)		$r=0.810$ Graph 1	$r=0.700$ Graph 2	$r=0.686$ Graph 3	$r=0.822$ Graph 4	$r=0.863$ Graph 5

CRP: C-reactive protein, CIMT: Carotid intima media thickness, CP: Chronic periodontitis, GI: Gingival index, OHI-S: Oral hygiene index-simplified, PD: Probing depth, CAL: Clinical attachment loss.

Table 4: Sensitivity and specificity of CRP in predicting positive CIMT in CP.

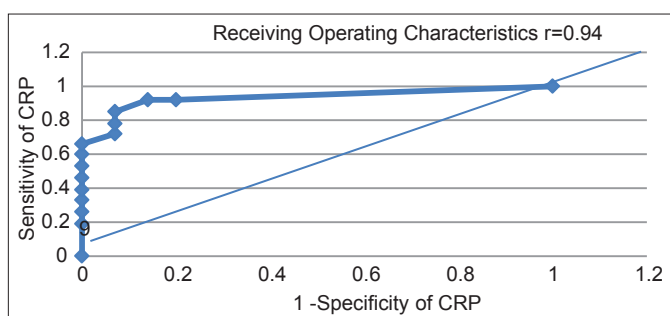
Test result	CIMT positive	CIMT negative	Diagnostic characteristics
CRP positive	8	3	Sensitivity – 100%, Specificity – 57% Accuracy – 79%
CRP negative	0	4	

CRP: C-reactive protein, CIMT: Carotid intima media thickness, CP: Chronic periodontitis.

Table 5: Sensitivity and specificity of CRP in total study population.

Test result	CP	NP	Diagnostic characteristics
CRP positive	11	1	Sensitivity - 73%, Specificity - 93% Accuracy - 83%
CRP negative	4	14	

CP: Chronic periodontitis, CRP: C-reactive protein, NP: Non-periodontitis.



Graph 6: Receiving operating characteristic curve of CRP in the study population.

an ongoing disease process in the body that deserves serious and careful medical attention.

Conclusion

Increased CIMT is observed in subjects with CP, which is independent of conventional risk factors. CRP is a useful and sensitive marker to predict cardiovascular disease in such subjects.

References

- Mattila KJ, Valle MS, Nieminen MS, Valtonen VV, Hietaniemi KL. Dental infections and coronary atherosclerosis. *Atherosclerosis* 1993;103(2):205-11.
- Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. *J Periodontol* 1996;67(10 Suppl):1123-37.
- Genco R, Offenbacher S, Beck J. Periodontal disease and cardiovascular disease: Epidemiology and possible mechanisms. *J Am Dent Assoc* 2002; 133 Suppl: 14S-22S.
- Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. C reactive protein and its relation to cardiovascular risk factors: A population based cross sectional study. *BMJ* 1996;312(7038):1061-5.
- Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* 2003;107(3):363-9.
- Wu T, Trevisan M, Genco RJ, Falkner KL, Dorn JP, Sempos CT. Examination of the relation between periodontal health status and cardiovascular risk factors: Serum total and high density lipoprotein cholesterol, C-reactive protein, and plasma fibrinogen. *Am J Epidemiol* 2000;151(3):273-82.
- Slade GD, Ghezzi EM, Heiss G, Beck JD, Riche E, Offenbacher S. Relationship between periodontal disease and C-reactive protein among adults in the atherosclerosis risk in communities study. *Arch Intern Med* 2003;163(10):1172-9.
- Joshi KJ, Wand HC, Merchant AT, Rimm EB. Periodontal disease and biomarkers related to cardiovascular disease. *J Dent Res* 2004;83(2):151-5.
- Grobbee DE, Bots ML. Carotid artery intima-media thickness as an indicator of generalized atherosclerosis. *J Intern Med* 1994;236(5):567-73.
- Beck JD, Elter JR, Heiss G, Couper D, Mauriello SM, Offenbacher S. Relationship of periodontal disease to carotid artery intima-media wall thickness: The atherosclerosis risk in communities (ARIC) study. *Arterioscler Thromb Vasc Biol* 2001;21(11):1816-22.
- Thakare KS, Deo V, Bhongade ML. Evaluation of the C-reactive protein serum levels in periodontitis patients with or without atherosclerosis. *Indian J Dent Res* 2010;21(3):326-9.
- Nova MJ, Novak KF. Chronic periodontitis. Newman MG, Takei H, Klokkevold PR, Carranza FA, (editors). *Textbook of Clinical Periodontology*, Ch. 31. Philadelphia: WB Saunders; 2011. p. 494-5.
- Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, et al. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: The atherosclerosis risk in communities (ARIC) study, 1987-1993. *Am J Epidemiol* 1997;146(6):483-94.
- Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: A fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;39(4):561-77.
- López NJ, Chamorro A, Llancaqueo M. Association between atherosclerosis and periodontitis. *Rev Med Chil* 2011;139(6):717-24.
- Cairo F, Castellani S, Gori AM, Nieri M, Baldelli G, Abbate R, et al. Severe periodontitis in young adults is associated with sub-clinical atherosclerosis. *J Clin Periodontol* 2008;35(6):465-72.
- Cairo F, Nieri M, Gori AM, Rotundo R, Castellani S, Abbate R, et al. Periodontal variables may predict sub-clinical atherosclerosis and systemic inflammation in young adults. A cross-sectional study. *Eur J Oral Implantol* 2009;2(2):125-33.
- Ebersole JL, Machen RL, Steffen MJ, Willmann DE. Systemic acute-phase reactants, C-reactive protein and haptoglobin, in adult periodontitis. *Clin Exp Immunol* 1997;107(2):347-52.
- Slade GD, Offenbacher S, Beck JD, Heiss G, Pankow JS. Acute-phase inflammatory response to periodontal disease in the US population. *J Dent Res* 2000;79(1):49-57.
- Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, De Nardin E. Periodontal infections contribute to elevated systemic C-reactive protein level. *J Periodontol* 2001;72(9):1221-7.
- D' Aiuto F, Ready D, Tonetti MS. Periodontal disease and C-reactive protein-associated cardiovascular risk. *J Periodontol Res* 2004;39(4):236-41.
- Loos BG. Systemic markers of inflammation in periodontitis. *J Periodontol* 2005;76 11 Suppl:2106-15.
- Salzberg TN, Overstreet BT, Rogers JD, Califano JV, Best AM, Schenkein HA. C-reactive protein levels in patients with aggressive periodontitis. *J Periodontol* 2006;77(6):933-9.

24. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation* 1998;97(20):2007-11.
25. Koenig W, Pepys MB. C-reactive protein risk prediction: Low specificity, high sensitivity. *Ann Intern Med* 2002;136(7):550-2.
26. Rifai N, Buring JE, Lee IM, Manson JE, Ridker PM. Is C-reactive protein specific for vascular disease in women? *Ann Intern Med* 2002;136(7):529-33.