



EVALUATION OF PERIODONTAL STATUS IN ACUTE MYOCARDIAL INFARCTION PATIENTS AND ITS ASSOCIATION WITH SERUM HIGH SENSITIVE C – REACTIVE PROTEIN AND FIBRINOGEN LEVELS - A CROSS-SECTIONAL STUDY

Dental Science

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ABSTRACT

BACKGROUND: In recent years, periodontal disease has been implicated in the onset and development of cardiovascular disease. Aim of this study was to assess the periodontal status, in acute myocardial infarction (AMI) patients and its association with the levels of hs-CRP and fibrinogen.

METHODS: 200 patients, diagnosed with AMI by cardiologist were included in the study. Periodontal status was recorded using Ramjford's Periodontal Disease Index. hs-CRP levels were assessed using immunoturbidometric assay and fibrinogen levels by coagulation assay.

RESULTS: Elevated levels of hs-CRP were found in 22.5% of patients with gingivitis and 96.8% of patients with periodontitis, which was statistically significant. Elevated levels of fibrinogen were found in 12.5% of patients with healthy gingiva 15% of patients with gingivitis and 75% of periodontitis patients which was statistically significant.

CONCLUSION: Elevated levels of hs-CRP and fibrinogen in AMI patients with periodontitis suggested significant association of periodontitis with hs-CRP and fibrinogen levels.

KEYWORDS

Acute Myocardial Infarction, C reactive protein, Fibrinogen, Periodontal disease

INTRODUCTION

Periodontal disease is the most common multi factorial disease, afflicting a very large proportion of the adult population. Destructive periodontal disease is predominantly associated with an anaerobic gram negative micro flora species such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia* and *Treponema denticola*. (Slots J, Ting M. 1999, Socransky SS, Haffajee AD. 2005, Holt SC, Ebersole JL. 2005) Epidemiologic studies on the prevalence of periodontal disease in countries around the world have shown that many adults have periodontal disease, which is a major cause of tooth loss¹ and approximately 10% of the adult population and approximately 30% of individuals more than 50 years of age have severe periodontitis. (Aida J A et al., 2006, Ribecro J et al. 2005, Grossi SG, Genco RJ, 1998)

In recent years, periodontal disease has been implicated in the onset and development of cardiovascular disease, including atherosclerosis, cerebrovascular disease and coronary artery disease, (Wu T et al. 2000^[1], Demmer RT & Desvarieux M, 2006) out of which cardiovascular disease is the leading cause of death in western industrialized countries (World Health Organization, 1995) Bacteremia resulting from periodontal disease with bacteria and inflammatory cytokines are believed to play a large role in the systemic sequelae of periodontal disease (Ishikawa I et al, 1997).

Of the various inflammatory cytokines, interleukin-6 acts on the liver to produce C- reactive protein (Gabay C & Kushner I, 1999, Libby P & Ridker PM. 1999), and these pro-inflammatory cytokines are released into the blood stream, causing a systemic inflammatory response, measurable as an increase in C-reactive protein levels (Buhlin K et al, 2003)

Recent studies provide evidence that periodontal disease is associated with cardiovascular risk factors, including acute-phase proteins, CRP and plasma fibrinogen. Both CRP and fibrinogen contribute to atheroma formation via several possible mechanisms (Wu T et al. 2000^[2], Slade GD et al. 2000) Aim of this study was to assess the periodontal status of acute myocardial infarction (AMI) patients, analyse and correlate serum levels of high sensitive C-reactive protein (hs-CRP) and fibrinogen with their periodontal status.

MATERIALS AND METHODS

The study population consisted of 200 acute myocardial infarction patients aged between 30-60 years, diagnosed at Jayadeva Institute of Cardiology, Bangalore, who had not undergone any type of periodontal treatment during the past one year and patients who had not taken antibiotics during past 6 months were included in the study. Cardiac status was assessed using Electrocardiogram (ECG), Low density lipoprotein (LDL), High density lipoprotein (HDL) and Creatinine phosphokinase MB (CPK-MB) levels. The subject hs-CRP levels were assessed using immunoturbidometric assay and fibrinogen levels by coagulation assay. All participants answered a questionnaire regarding their health, habits and family history. Their body mass index (BMI) was calculated. Their levels of serum total cholesterol, LDL, HDL and triglyceride concentration was also recorded. Ramjford's Periodontal Disease Index (PDI) was used to record the periodontal status of the subjects (Ramjford S P, 1967).

HS-CRP ANALYSIS

Two ml of venous blood was collected from antecubital vein. The sample was centrifuged at 2000 rpm for 15 minutes and serum was separated. **Test procedure:** Four, hundred micro liter of activation buffer (R1) and 100 micro liter of quantia – CRP latex reagent (R2) were pipetted into a measuring cuvette. Reagents were mixed and incubated for five minutes at 37°C. To this 5 micro liter of test specimen was added and mixed gently and the cuvette was placed in the analyzer and was started. The turbidity of the specimen was measured at wavelength between 505-578 nm by spectrophotometer (Figure 1 A & B). The digital output was recorded for that specimen.

FIBRINOGEN ANALYSIS

Two ml of venous blood was collected from antecubital vein and transferred into a vial containing 3.2% of sodium citrate (anticoagulant). Nine parts of blood was mixed with one part of anticoagulant. The sample was centrifuged at 1500 rpm for 15 minutes and plasma was separated. Plasma was removed from the tube within 60 minutes using a plastic pipette. **Test procedure:** Ninety micro liters of test imidazole buffered saline (IBS) was added to 10 micro liter of plasma and mixed well. This was incubated at 37°C for 120 seconds. 0.2 ml of dilution was mixed with 0.1 ml of bovine thrombin to this 50 micro liter of



Figure 1: A: Hs-crp Reagents, B: Hs-crp Analyzer By Immunoturbidimetric Method, C: Fibrinogen Reagents, D: Fibrinogen Analyzer By Coagulation Method

fibrinogen reagent was added and the sample was run. The clotting time was read from the digital output. The fibrinogen levels were obtained from the pre calibrated standard curve from the obtained clotting time (Figure 1 C & D).

RESULTS

The present study involved 200 patients with AMI with the mean age of 53.99±13.15 years (range 30 to 97 years) comprising of 78% of men and 22% of women. Of the total 200 acute myocardial infarction patients, 8% were periodontally healthy, 60% presented with gingivitis and 32.5% of patients had periodontitis. The mean body mass index (BMI) was 24.50±4.37 Kg/m² (range 15.2 to 40.14 Kg/m²). All the patients showed ST elevation and raised CPKMB levels. Elevated levels of hs-CRP was found in 44.5% of the patients who had PDI of 3.34±0.74 while those with normal levels had PDI of 1.96±0.48 which was statistically significant (p=0.001). Further, elevated levels of hs-CRP were found in 22.5% of patients with gingivitis and 96.8% of patients who presented with periodontitis, (p= 0.001) (Table 1, Graph 1).

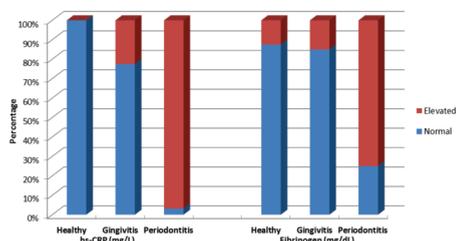
The mean fibrinogen level was 353.84 ± 98.55 mg/dl. Elevated levels of fibrinogen were found in 34 % of the patients who had PDI of 3.22±0.88, while those with normal levels of fibrinogen had PDI of 2.25±0.75 which was statistically significant (p=0.001). Elevated levels of fibrinogen were found in 12.5% of patients with healthy gingiva 15% of patients with gingivitis and 75% of periodontitis patients (p= 0.001) (Table 1, Graph 1).

Table 1: Association Of Hs-crp And Fibrinogen With Periodontal Index

Study Variables	Periodontal Disease Index			P*
	Healthy (n=16)	Gingivitis (n=120)	Periodontitis (n=64)	
hs-CRP (mg/L)				
Normal	16(100.0%)	93(77.5%)	2(3.1%)	<0.001**
Elevated	0	27(22.5%)	62(96.8%)	
Fibrinogen (mg/dl)				
Normal	14(87.5%)	102(85.0%)	16(25.0%)	<0.001**
Elevated	2(12.5%)	18(15.0%)	48(75.0%)	

*P values are obtained 2x3 Fisher Exact test; **P <0.001 is considered to be statistically significant. n - number of subjects; hs-CRP - high sensitive C reactive protein; mg – milligram; dl - decilitre

Graph 1: Association of hs-CRP and Fibrinogen with Periodontal Index



DISCUSSION

Cross-sectional and prospective studies have established that elevated peripheral blood levels of several systemic inflammatory markers

including CRP and fibrinogen are associated with the risk of cardiovascular diseases and severity of atherosclerosis (LiuZZ G et al., 1994, Toss H et al, 1997, Danesh J^[1] et al., 1998). It has been proposed that these markers could be elevated due to undiagnosed chronic infectious processes; subsequently their pro-inflammatory properties may increase the existing inflammatory activity in plaque associated lesions in coronary arteries and thus predispose for cardiac events (Danesh J^[2] et al., 1998). This study was aimed to evaluate the periodontal status of patients with acute myocardial infarction and association between the two. This study also aimed at analyzing the serum levels of hs-CRP and fibrinogen in these patients and correlating their levels with periodontal status. Of the total 200 AMI patients, 8% were periodontally healthy, 60% of patients presented with gingivitis and 32.5% of patients had periodontitis. Radafshar G et al. (2006) reported periodontitis in 70.5% of patients with AMI, Geerts SO et al. (2004) reported periodontitis in 91% of the patients with angina pectoris or AMI, which showed slightly higher incidence when compared to the present study. This discrepancy may be due to the number of study samples taken which were 50 and 108 patients with AMI patients respectively. While Deliargyris EN et al. (2004) reported periodontitis in 48% of patients with AMI which was similar with the current study.

In the present study, elevated levels of hs-CRP were found in 44.5% of the patients who had PDI of 3.34±0.74 while those patients with normal levels had PDI of 1.96±0.48 which was statistically significant (p=0.001). Elevated levels of hs-CRP were found in 0% of the patients with healthy gingiva, 22.5% of gingivitis patients and 96.8% of patients with periodontitis, which was statistically significant (p= 0.001). This was consistent with previous reports showing increased rates of AMI in cases with elevated CRP levels. Radafshar et al. (2006) reported mean levels of hs-CRP of 21.88mg/L and 9.26mg/L in patients with and without periodontal disease respectively, who had acute myocardial infarction. Observed elevation in CRP levels could be explained by the cascade of events caused by the invading oral flora during periodontal disease. Also Ebersole et al. (1997) reported significant higher level of CRP among individuals with adult periodontitis especially those having more active sites.

In the present study, the mean fibrinogen level was 353.84±98.55 mg/dl. Elevated levels of fibrinogen was found in 34% of the patients who had PDI of 3.22±0.88, while 66% of those with normal levels had PDI of 2.25±0.75 which was statistically significant (p=0.001). Elevated levels of fibrinogen were found in 12.5% of periodontally healthy patients, 15% of gingivitis patients and 75% of periodontitis patients which was also statistically significant (p= 0.001). Taylor et al. (2006) reported mean fibrinogen levels of 3.8g/L (2.9-4.9g/L) in his study on patients with periodontitis with cardiovascular risk factors, which was a similar finding to the current study.

Chronic infections may lead to atherogenesis by two different pathways:

- 1) a direct invasion of the arterial wall by periodontal pathogens, and
- 2) the release of systemic inflammatory mediators with atherogenic effects in response to infection (Mehta JL 1998).

Periodontitis has been proposed as having an etiological or modulating role in cardiovascular disease (Matilla KJ et al. 1999, Beck JD et al. 1996) Several mechanisms have been proposed to explain or support this hypothesis. One of these is based around the potential for the inflammatory phenomenon of periodontitis to have effects by the systemic dissemination of locally produced mediators such as C-reactive protein, interleukin -1β, interleukin – 6 and tumor necrosis factor α (Gemmell E, 1997, Kornman KS 1997) Endotoxins can induce changes in the metabolism of lipids and secondarily cause an increase of CRP and fibrinogen in the plasma (Genco RS 1998).

CONCLUSION

From the present study, it can be concluded that: in patients with AMI, the incidence of gingivitis was more compared to periodontitis. Study showed elevated levels of hs-CRP and fibrinogen in AMI patients with periodontitis when compared with AMI patients without periodontitis. The elevated levels of hs-CRP may also be due to the presence of gingivitis which subsequently may lead to periodontitis if oral hygiene is not maintained adequately. With the present study we have observed a significant association of periodontitis with hs-CRP and fibrinogen levels. Therefore, we propose that periodontitis may be one such

undiagnosed chronic infectious process which might predispose for cardiovascular disease.

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