International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com

Research Article

HIGH SENSITIVITY C-REACTIVE PROTEIN AS AN INFLAMMATORY BIOMARKER IN MYOCARDIAL INFARCTION

Manjushree sugoor[1]*, Mirza Sharif Ahmed Baig[1], MS Dharapur[2] and Girish Desai[2]
[1]Department of Biochemistry, KBN Institute of Medical Sciences, Gulbarga, Karnataka, India
[2]Deparment of Biochemistry, M R Medical College, Gulbarga, Karnataka, India

Received for publication: December 8, 2013; **Revised**: January 21, 2014; **Accepted**: January 28, 2014

Abstract: Coronary artery disease is caused by the additive & interactive effects of inherited and environmental factors. Substantial evidence show that reactive oxygen species (ROS) plays a vital role in the etiopathogenesis of atherosclerosis. The study was conducted on 50 clinically diagnosed Myocardial Infarction patients along with equal number of age & sex matched controls. hs-CRP was measured as a marker of inflammation. It is measured by imuno-turbidimetric method. Statistical analysis was done by students paired 't' test and chi square test. They are expressed in terms of p value. The level of hs- CRP ($p< 0.001$) were found to be significantly elevated in MI. Our study revealed the significant role of hs-CRP in MI. hs-CRP is found to be the best predictor for the probability of severity of future coronary events.

Keywords: Myocardial infarction, hs-CRP, inflammation, atherogenesis.

INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of death among people of both sexes in industrialized countries of the world. The incidence of CVD is showing increasing trend even in developing countries like India due to industrialization and changing life style. Epidemiologists in India and international agencies such as World Health Organization (WHO) have been sounding an alarm on the rapidly rising burden of cardiovascular disease (CVD) for past 15 years. The reported prevalence of Coronary Heart Disease (CHD) in adult survey has raised four fold in 40 years and even in rural areas, the prevalence has doubled over the past 30 years. It is expected to be single most important cause of death in India by the year 2015[1].

Myocardial infarction (MI) is one of the important manifestations of coronary heart disease. Coronary atherosclerosis is a complex inflammatory process characterized by accumulation of lipids, macrophages and smooth muscle cells in intimal plaques in the large and medium size epicardial coronary arteries. The etiopathogenesis leading to atherogenesis is still unknown. But number of risk factors has been identified like modifiable and non-modifiable[2].

Current evidence supports that inflammation is a major driving force underlying the initiation of coronary plaques, their unstable progression, and eventual disruption; patients with a more pronounced vascular inflammatory response have a poorer outcome[3]. Pathological and clinical data suggests a prominent role of inflammation at every stage of atherogenesis. The vascular endothelium is a complex synthetic substance which is subjected to injury from potential insults like modified lipoproteins like oxidized LDL (ox-LDL), hemodynamic stress, oxidative stress etc. Endothelial cell injury causes upregulation of cellular adhesion molecules such as vascular adhesion molecule I (VCAM-I) and intercellular adhesion molecule-I(ICAM-I) that in turn causes chemokines mediated increased adhesion of leukocytes. On adherent to endothelium, leukocytes migrate into the intima of arterial wall. Recent research has shown that chemo attractant molecules like monocyte chemo attractant protein I (MCP-I) and T-cell chemo attractant family are responsible for direct transmigration of monocytes and lymphocytes respectively. Once resident in the arterial wall, these cells participate and perpetuate a local inflammatory response. Inflammatory mediators like macrophage colony stimulating factors (M-CSF) augment the expression of macrophage scavenger receptors leading to uptake of modified lipoprotein particles and formation of lipid laden macrophages (foam cells), that constitute a key element of atherosclerotic plaque. Mononuclear cells within the inflammatory infiltrate release cytokines, including interleukins (IL-I and IL-6), which reinforce cellular recruit and promote the oxidation and uptake of LDL as shown in figure 1.Inflammatory response stimulates migration and proliferation of smooth muscle cells and contributes to maturation of atherosclerotic lesion.

Thus, cycles of accumulation of mononuclear cells, migration and proliferation of smooth muscle

***Corresponding Author:**
Dr. Manjushree Sugoor,
Assistant Professor,
KBNIMS, Gulbarga, Karnataka, India.

cells and formation of fibrous tissue lead to further enlargement and restructuring of the lesions so that it becomes covered by a fibrous cap. Activated macrophages stimulate production of matrix metalloproteinase's (MMPs) such as collagenases, elastases and stromeolysins which degrades collagen in fibrous cap, rendering it thin and susceptible to rupture[4,5]. Plaque rupture results in release of highly procoagulant contents of atheroma core, which promote thrombus formation. In some cases, the overlying thrombus is non-occlusive and incorporates into maturing plaque when the forming thrombus leads to rapid compromise of arterial flow resulting in acute myocardial ischemia or infarction [4,5].

C-reactive protein is one of the predominant inflammatory biomarker. It is ubiquitous protein and acute phase reactant. Because the range of CRP reported in clinical studies of vascular risk was often far below thresholds detectable by standard CRP assays that have lower detection limits of 5-10 mg/L, several scientists worked to develop and validate high sensitivity methods for CRP measurement that over time became known as "hs-CRP".

According to the center for disease control and prevention and American heart Association (CDI/AA/A) guidelines 2002,CRP (as measured by high sensitivity assay (hs-CRP) is the inflammatory marker of choice, as it has more stability, assay precision, accuracy and availability[6].

The link between cardiovascular disease and hs-CRP come from following observations.

- hs- CRP found localized in atherosclerotic plaque[7].
- Acute /chronic infections that cause rise in circulating hs-CRP yield a higher risk for cardiovascular disease[8].
- hs- CRP is an independent cardiovascular risk factor even after correction for other risk factors[9].
- Baseline hs-CRP levels in apparently healthy persons with stable angina pectoris constitute an independent risk factor for cardiovascular events[7,10].
- The predictive value of hs-CRP was significantly higher than that associated with traditional biochemical CHD risk factors (TC, TG, and LDL) or other novel markers.
- After inflammation it reaches peak on 2nd day and returns to normal after 5th day
- hs-CRP plays a direct pathogenic role in arterial disease by
- T cell mediated endothelial destruction[11].
- Expression of adhesion molecules such as VCAM and E-Selectin[12].
- Stimulates macrophages to produce tissue factor[12].
- Activation of compliment[12].
- Attenuates nitric oxide production[12].
- Increase in the expression and activity of Plasminogen activator inhibitor-I in human endothelial cells[13].
- Inhibit angiogenesis[6].

hs-CRP is an independent predictor of future cardiovascular events. It has a potential role for the prediction of risk for developing CAD and may correlate with severity of CAD.

MATERIALS AND METHODS

The present study comprised of 50 normal healthy subjects in the age group above 30 years as controls (males 31, females 19) and 50 clinically diagnosed cases of Myocardial Infarction of the same age group (males 35, females 15) admitted at the Basaveshwar Teaching and General Hospital, attached to Mahadevappa Rampure Medical College, Gulbarga. An informed consent was obtained from the controls and cases before collecting the blood samples. The study was approved by Ethical and Research Committee of the Institution.

Collection of blood samples

Blood sample was withdrawn on 3rd day of MI as hs-CRP reaches peak after 2nd day and returns to baseline by 5th day. Total of 5ml venous blood samples (fasting) is collected from antecubital vein under all aseptic precautions in plain bulb. It is then allowed to clot and then centrifuged for serum separation. Serum hs-CRP was estimated by QUANTIA CRP-US Kit[9]using chem-5 semi auto analyser. Total cholesterol by CHOD-PAP method[14], Triglycerides by GPO-TRINDER method[15], HDL by Phosphotungstic acid method[16] and LDL, VLDL by friedwald formula[17].

The data analysis was done by unpaired student 't' test& chi square test. All results were expressed in mean± standard deviation. The difference in mean values of various parameters were analyzed for significance and the values expressed in terms of p value.

RESULTS

Table 1: Comparison of hs-CRP in controls and cases of Myocardial Infarction

	Controls (Mean±SD)	Cases (Mean±SD)	p value
hs-CRP (mg/L)	0.78 ± 0.13	3.62 ± 0.83	<0.001

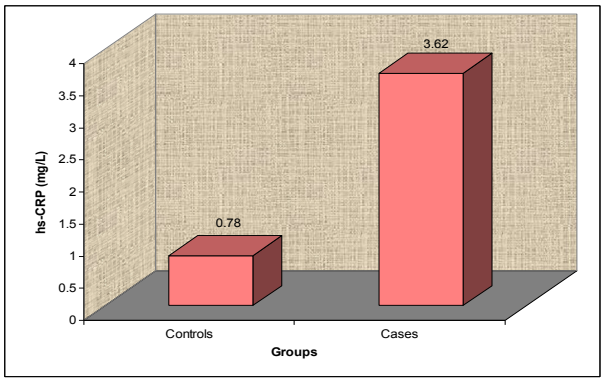

Fig. 1: Comparative analysis of hs-CRP in controls and cases

Comparative analysis of hs-CRP in controls and cases of Myocardial Infarction is shown in table 1 & figure 1. Statistical analysis by student 't' test shows that mean levels of hs-CRP were significantly increased in all cases compared to controls and are statistically highly significant (p<0.001).

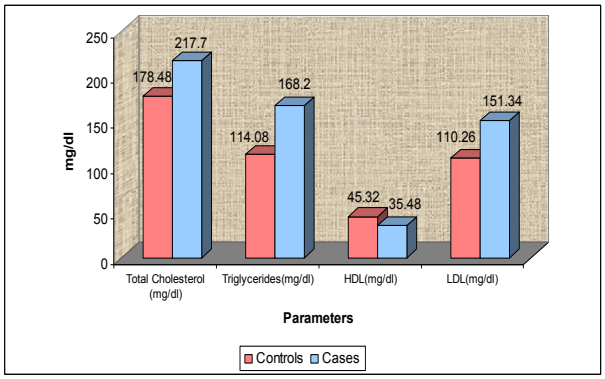

Fig 2: Comparative analysis of serum lipid profile in controls and cases of Myocardial Infarction.

Comparative analysis of various lipid profile parameters in controls and cases of Myocardial Infarction shows mean levels of TC,TG,LDL were significantly increased in all cases compared to controls and are statistically highly significant (p<0.001). Levels of HDL were lowered significantly in all cases compared to controls and are statistically highly significant.

DISCUSSION

Coronary Artery Disease, which frequently manifests as MI continues to exert an enormous toll in western society and also in developing countries like India. Despite progress in its measures in prevention, detection and treatment, it continues to be the leading cause of death.

Research over last decade proved the role of inflammation in pathophysiology of atherosclerosis. Inflammation is an important contributor to atherosclerosis, both accelerating the process and precipitating acute plaque rupture. Various traditional and newer biomarkers were suggested for diagnosis and prognosis of MI. In view of this, present study has been undertaken to assess clinical utility of some of the promising biochemical markers like hs-CRP and lipid profile which are inexpensive, identified by easy methods and validated that can be of some diagnostic and prognostic significance.

In our study, the levels of hs-CRP was found to be significantly elevated (p< 0.001) compared with controls. Our studies were in accordance with findings of Hon-Kan Yip[18], Benjamin Scirica[19] and Anderson[20]. The findings of our study are contradictory to the findings of Thompson[21] who demonstrated non-significance of hs-CRP measurement in MI. However in his study, a rather insensitive assay for CRP was used that was unable to differentiate levels with the normal range. There was a significant increase in Total cholesterol, Triglycerides and Low Density Lipoprotein levels in patients compared with controls where as High Density Lipoprotein levels were reduced in cases than controls. Hypercholesterolemia and hypertriglyceridemia are the major risk factors for the development of atherosclerosis, in turn causing MI.

CONCLUSION

In conclusion, our present study suggests that together with other biomarkers hs-CRP can improve the ability to detect absolute coronary risk, a critical issue because one half of all MI occur among individuals without overt hyperlipidemia. It plays a significant role in the risk assessment, prophylaxis and management of Myocardial Infarction.

BIBLIOGRAPHY

1. Goenka S, Prabhakaran D, VS Ajay and KS Reddy "Preventing cardiovascular disease in India-Translating evidence to Action" Current Science, Aug 2009; Vol 97,No 3.

2. Surekha R H, Srikanth B B M V, Jharna P, Ramachandra R V, Dayasagar R V, Jyothy A. "Oxidative stress and total antioxidant status in Myocardial Infarction". Singapore Med J, 2007:48(2); 137.

3. Inflammatory biomarkers in coronary artery disease Epaminondas Zakynthinos (MD), Nikolitsa Pappa (MD) b Journal of Cardiology (2009) 53, 317-333

4. David A et al. C - reactive protein, inflammation and coronary risk. Medical Clinics of North America, 2000; 84 (1): 149-161.

5. Russel Ross et al. Atherosclerosis, an inflammatory disease. New Engl J Med, 1999; 14(2): 115-126.

6. Shishehmbor M H et al. Using C-reactive protein to assess cardiovascular disease risk. Clev Clin J Med, 2003; 70(7): 634-640.

7. Michael B et al. C - reactive protein: A new risk assessment tool for cardiovascular diseases. JAOA, 2005; 105(9): 409-416.

8. Pai C Kao, Shu-Chu Shiesh and Ta Jen Wu; "Serum C-Reactive Protein as a marker for wellness assessment". Annals of Clinical and Laboratory, Science, 2006; vol 36: no 2.

9. Mendal M A et al.C-reactive protein and its relation to cardiovascular risk factors: A population based cross sectional study. BMJ, 1996; 312:1061-1065.

10. Suman B Sharma, Seema Gerg, Abhinav Veerwal & Sridhar Dwivedi. hs- CRP & oxidative stress in young CAD patients. A pilot study. Ind J Clin Biochem, 2008; 23(4): 334-336.

11. Johann Auer, Robert Berent, Elisabeth Lassnig &Bern d Eber. C-reactive protein & coronary artery disease. Jpn Heart J, Nov 2002; 43: 607-619.

12. James T, Willerson, Paul M Ridkar. Inflammation as a cardiovascular risk factor. Circulation, 2004; 109(2):II -2-II-10

13. Devaraj S et al. C-reactive protein increases Plasminogen Activator inhibitor–I expression & activity in human aortic endothelial cells: Implications for metabolic syndrome & atherothrombosis. Circulation, 2003; 107: 398-404.

14. Roeschlau P Bernt E and Gruber W A. Clin Biochem, 1974; 12(226).

15. McGowan MW. et al. Clin Chem, 1983; 29:538.

16. Burstein M. Scholnic HR, Morfin R.J. Lipid Res, 1970

17. Carl A Burtis, Edward Ashwood, Cardiac function. Teitz Textbook of Clinical Biochemistry and Molecular Diagnostics; 4th edn: Saunders publications. Page no 1619-1660

18. Hon–Kan Yip et al. Level of high sensitivity C-reactive protein is predictive of 30-day outcomes in patients with acute Myocardial Infarction undergoing primary coronary intervention. Chest, 2005; 127:803-808.

19. Benjamin M Scirica et al. Clinical application of C-reactive protein across the spectrum of acute coronary syndromes. Clinical Chemistry, 2007; 53: pg 1800-1807.

20. Jeffrey L Anderson et al. Evaluation of C-reactive protein, an inflammatory marker and infectious serology as risk factors for coronary artery disease and myocardial infarction. J Am Coll Cardiol, 1998; 32:35-41

21. Thompson SG et al. Hemostatic factors and the risk of MI or sudden death in patients with Angina Pectoris. N Engl J Med, 1995; 332:635-641.

Source of support: Department of biochemistry, MR Medical College
Conflict of interest: None Declared