Clinical Significance of the Biomarker C-Reactive Protein in Acute Myocardial Infarction: A Preliminary Laboratory Evaluation

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Abstract: Acute Myocardial Infarction (AMI) represents a severe manifestation of the spectrum of Acute Coronary Syndrome (ACS). The incidence of AMI has attained alarming proportions in both the developed and developing countries. Our preliminary short-term study focuses on the clinical application of the biomarker C-Reactive Protein (CRP) in the laboratory diagnosis potential of AMI. Besides the conventional markers, CRP and other biochemical markers and their role in cardiovascular diseases, has become a subject of interest in recent times. In the present study we evaluated the CRP values using "Chromatest" kit latex-agglutination technique on the venous blood samples of a total of 40 patients of whom 20 were admitted with AMI and 20 healthy volunteers (control). Other disease conditions that could raise CRP values were considered and excluded in the controls. The results were tabulated and statistical analysis indicated that CRP has significant potential as a biochemical marker that could be concomitantly used along with other established markers. We report our evaluation of CRP levels on the day of AMI occurrence and after a period of 10 days. The results (p<0.005) indicate that mean CRP value of AMI for the day of admission (D1) was 6.092 mg L^{-1}. The mean values for males and females exclusively with AMI on D1 were 6.406 and 5.779 mg L^{-1}, respectively. The mean value of CRP 10 days post infarct (D2) was 2.793 mg L^{-1}. The mean values of CRP exclusively for males and females with AMI on D2 were 2.495 and 2.393 mg L^{-1}, respectively. The mean value of CRP in control group on D1 was 0.834 mg L^{-1}. The mean values of CRP on D1 for males and females exclusively were 0.839 and 0.824 mg L^{-1}. The mean values of CRP on D2 exclusively for males and females were 0.84 and 0.828 mg L^{-1}, respectively. Our study clearly shows the higher values of CRP in patients with AMI in comparison to normal subjects. The high values clearly indicate the detrimental effects of inflammation and myocardial necrosis. Males have slightly higher CRP values than females in both the control and patients with AMI. The study warrants the need for further research in the potential of clinical application of CRP and accentuates the possibility of concomitant use of CRP with other biomarkers due to its reasonable efficacy and cost.

Key words: Acute myocardial infarction, biochemical marker, C-reactive protein, laboratory assay, clinical biochemistry

INTRODUCTION

Ischemic heart disease is the leading causing of death in developed countries and the third major cause of mortality in developing countries where only HIV/AIDS and lower respiratory infections outnumber coronary disease (WHO, 2002). Alarmingly, ischemic heart disease is projected to be the number one killer in 2030 as compared to 2002 rankings of major killers (Mathers and Loncar, 2006). Acute myocardial infarction belongs to the spectrum of acute coronary diseases. As a rule, the diagnosis of acute myocardial infarction is usually made on the clinical presentation, electrocardiographic findings and confirmed by the changes in plasma enzyme activities. With a better understanding of the pathophysiolgy of cardiovascular diseases and the advent of an era of genomics and proteomics, has lead to a proliferation in the number of biomarkers available to clinicians (Arrell et al., 2001). In addition to the recent conventional plasma enzyme estimation, research is being undertaken on various other markers in AMI . The plasma enzyme estimations of greatest value in AMI are creatine

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kinase, lactate dehydrogenase (or hydroxybutyrate dehydrogenase) and aspartate transaminase (Mayne, 1996). The levels of Creatine Kinase (CK) and especially of CK-MB (the myocardial-specific isoform) have served as essential components of decision making in emergency rooms for over 25 years (Hamm,1994). In a broad sense we can classify the current trends in biomarker use as established, potentially outdated, emerging and developing markers (Allan *et al.*, 2006). Cardiac troponin is considered as an established marker that provides robust prognostic information (Antman *et al.*, 1996). Creatine kinase-MB and m yoglobin are now known to be potentially outdated (Adams *et al.*, 1993). C-reactive protein and B-type natriuretic peptide are included as emerging markers while sCD40 ligand, myeloperoxidase, ischemia-modified albumin (cobalt albumin), pregnancy-associated plasma protein-A, choline, cystatin C, fatty acid binding protein were included as developing markers (Allan *et al.*, 2006).

C-Reactive Protein (CRP) is an acute-phase reactant protein that is synthesized in the liver. CRP is a 115-kDa pentamer synthesized under the control of interleukin-6 in the setting of the innate, non specific immune response to a number of pathophysiological conditions including infection, inflammation, cell damage and neoplasm (Pepys and Hirschfield, 2003). There is controversy regarding the variability of CRP levels (Rifai and Warnick, 2004). Values >3 mg L^{-1} are associated with higher risk and values <1 mg L^{-1} are associated with low risk and those between 1 and 3 mg L^{-1} are considered intermediate (Pearson *et al.*, 2003). Multiple assays exist for CRP and one of them is high sensitive or hsCRP. The approaches that have been used to measure hsCRP, include the labeling of anti-CRP antibodies with either enzyme (ELISA), or fluorescent compound and attaching the antibody either monoclonal or polyclonal to polystyrene beads (Gunduz *et al.*, 2005). The values of CRP differ in women using oral contraceptives, men and women not using oral contraceptives in the order in a decreasing order respectively (Reise *et al.*, 2002). CRP may help predict short- and long-term cardiovascular outcomes and the additional CRP screening to traditional lipid testing has the potential to identify individuals at high risk for future cardiovascular events who may benefit from target preventive interventions (Koenig, 2005). CRP is easily and inexpensively measured and standardized high sensitivity (hsCRP) assays are commercially available that provide similar results in stored, fresh or frozen plasma (Rifai *et al.*, 1999). C-reactive protein is also linked to hypertension as both the cause and reverse causation (Schillaci and Pirro, 2006).

MATERIALS AND METHODS

Patient selection: The study population included 40 patients of which 20 patients (10 males and 10 females) were admitted to the intensive unit of the internal medicine and cardiology department of the Bihor county hospital, Oradea for suspected acute myocardial infarction. All patients met the following criteria for diagnosis: prolonged central chest pain, elevated Creatine Kinase MB (CK-MB) and ST elevation on the electrocardiogram. With the aid of a thorough clinical examination and history taking if it was possible we made it certain that none of the patients had any clinical signs of concomitant disease or interfering non cardiac diseases like malignancy, infection, recent surgical intervention, trauma or other inflammatory disorders. Patients admitted up to 12 h after symptom onset received streptokinase, unless contraindicated. All patients received anti platelet therapy (aspirin or clopidogrel), unfractioned heparin, GP 2b/3a inhibitors, beta blockers and, if indicated, oral ACE inhibitors. No-inflammatory drugs, except aspirin, were administered. The other 20 patients (10 males and 10 females) served as the control group. These included healthy blood donors who were clinically examined for any ailments (non smokers, non diabetics and non hypertensive) and any known history that could change the CRP values.

Procedure and equipment: Venous blood samples (5 mL) were obtained on admission and after the diagnosis of AMI was ascertained. The day of admission was taken as Day1 (D1). Serum samples for determining CRP were stored at -20°C and assayed at the end of the study. The procedure applied was "Chromatest" kit and reagent was applied through the latex-agglutination method. Here, the latex suspension when mixed with serum containing elevated CRP levels on a slide; clear agglutination is seen within 2 min. The entire procedure was carried out after 10 days post-AMI denoted as (D2). Similarly for healthy volunteers (controls) blood samples were taken after 10 days in tandem to patients with AMI. The results (CRP values mg L^{-1}) were noted and statistically tabulated using student *t* test. The emphasis of the result was made on variations in CRP values according to sex in both patients with AMI and controls; the most frequent CRP values were also taken into consideration. The results were compared in both controls and patients with AMI on D1 and D2.

RESULTS AND DISCUSSION

Sex and CRP: The mean age of 20 patients was 59 years the range being 47-73. In Fig. 1, it is observed that male patients with AMI have a higher value in comparison
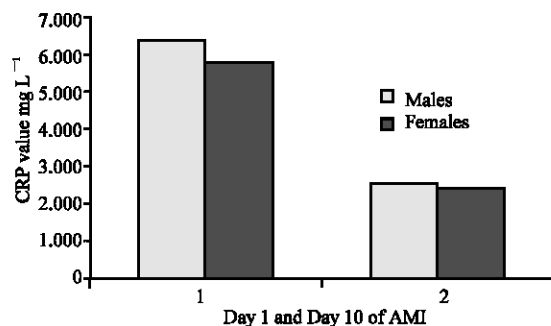
Fig. 1: Mean CRP values in males and females on Day 1 and Day 10 (p<0.005)
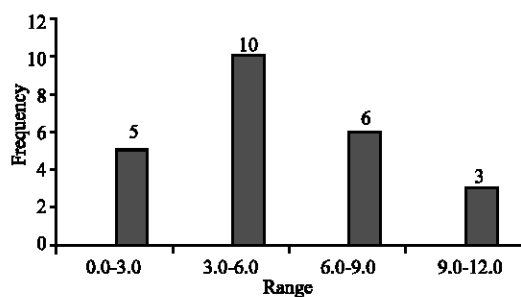

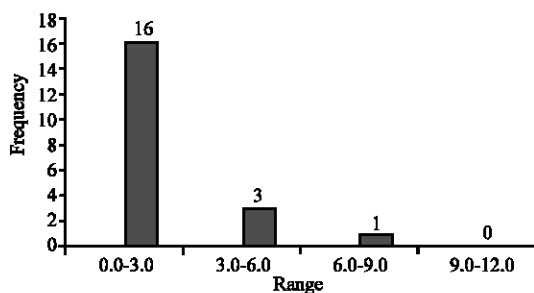Fig. 2: The frequency of CRP values on Day 1 of AMI

Fig. 3: The frequency of CRP values on day 10

to their female counterparts. The mean values for males and females exclusively with AMI on D1 were 6.406 and 5.779 mg L^{-1}, respectively.

Frequency of CRP values: In Fig. 2, we observe that the majority of patients on the day of admission or D1 have values within the range of 3.0-6.0 mg L^{-1}. On the other hand, there were 3 patients who had values below 3.0 mg L^{-1}; almost half the patients had a CRP value above 6.0 mg L^{-1}. In Fig. 3, as a marked contrast we observe that on D2, almost three quarters of the patients have a CRP value below 3.0 mg L^{-1} while only a quarter have a CRP concentration between 3.0 and 9.0 mg L^{-1} with remote existence between 6.0 and 9.0 mg L^{-1}.

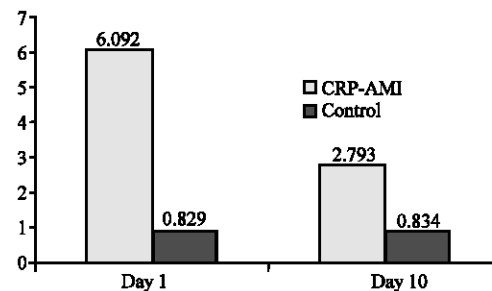**AMI patients versus control:** In Fig. 4, we clearly observe the higher values of CRP in patients with

Fig. 4: Mean CRP values of patients with AMI and Control group on Day 1 and 10 days after AMI (p<0.005)

AMI in comparison to the controls. The mean value of CRP in patients with AMI (both males and females) on D1 is 6.092 mg L^{-1} while the mean value of CRP on D2 is 2.793 mg L^{-1}. Furthermore, the mean CRP value on D1 for control is 0.829 mg L^{-1} while D2 is 0.834 mg L^{-1}.

The circulating concentration of human CRP, the classical acute phase protein, is always increased after acute myocardial infarction, starting within 4-6 h of onset of symptoms and reaching a peak after 50 h (Kushner, 1998). C-reactive protein is able to bind to phospholipids of damaged cells, with subsequent activation of complement system and enhanced uptake of these cells by macrophages. The myocardial necrosis post infarction is a potent stimulus. Increase in CRP is generally regarded as a sign of inflammation, although it is associated with non inflammatory conditions associated with cellular distress and injury (Schillachi and Pirro, 2006). On D1 we observe the dramatic rise in CRP levels, mean value from our study being 6.092 mg L^{-1}. This value is several folds higher than the normal limits found in the control. On D2 the value decreased but remained higher in comparison to control. These increased values of CRP make it an ideal marker. But the case may be still controversial. According to Kushner *et al.* (2006) CRP values in about one third American population are in the range of 3-10 mg L^{-1}. CRP could thus be elevated due to gene polymorphism, diet, demography, socio-economical groups and non inflammatory conditions. Hence, the attribution to atherosclerosis or acute myocardial infarction makes it confusing and controversial. Precise sequential measurements of CRP may thus serve as a sensitive test for myocardial necrosis in the context of ischemic heart disease. Increased CRP without an increased CK-MB suggests non infarctive or non cardiac lesion (De Beer *et al.*, 1982). Gender differences in CRP values can be supported by the study (Riese *et al.*, 2002) where it is mentioned that oral contraceptive using women have higher values than men who in turn have higher values

than non oral contraceptive using women. In our study, both on D1 and D2, for control and AMI patients, the values of males were higher than females. There is a decrease in CRP values after 10 days according to our study and we may consider the implication of treatment and the gradual resolution of necrotic tissue. In the present study we tried to correlate the number of patients to a specific value of CRP. According to the study, on D1 more than half the patients had a CRP concentration between 3.0-6.0 mg L^{-1} whiles more than a quarter between 6.0 and 9.0 mg L^{-1}. Ten days later, however, the values below 3.0 mg L^{-1} are found in majority of the patients. Our study is in accordance with that of Kushner *et al.* (1998). According to Kitsis *et al.* (2006), myocardial damage can be limited by the inhibition of CRP and in the mouse model of myocardial infarction with the occlusion of a coronary artery, the Bis(PC)-H bound to CRP considerably decreased the infracted area.

The present study has certain limitations due to the lesser number of patients and controls used, it is imperative that the proposed model be carried out on a higher number of subjects. CRP is ideal due to its lower cost and can be measured at any time of the day. Similar results are provided for frozen and fresh plasma and it is stable at room temperature (Gunduz *et al.*, 2005).

CONCLUSION

The rationale of our study was to evaluate the possibility of using C-reactive protein as a marker for an acute myocardial infarction rather than a risk factor for one. We conclude that the distinguished high value of CRP in AMI compared to healthy population makes it a suitable and cost effective marker that can be used along side CK-MB and other markers. Our study warrants the need for further research on CRP and AMI in various health centers through out the world.

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