Interplay Between Inflammation and Hemostasis in Patients with Coronary Artery Disease

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Abstract Coronary artery disease (CAD) is a global epidemic currently. This study was planned to evaluate markers of inflammation and hemostasis and their possible association, if any, in patients with CAD. The study was carried out in 60 patients with acute myocardial infarction (AMI) and 60 age and gender matched controls. The following parameters were assayed in all study subjects— inflammatory-interleukin (IL)-10, high sensitivity C-reactive protein (hs-CRP), tumor necrosis factor (TNF)- α , fibrinogen; hemostatic-fibrinogen, fibrin D-dimer and a novel risk factor—homocysteine. Inflammatory markers (hs-CRP, TNF- α and IL-10), fibrinogen, fibrin D-dimer and homocysteine levels were significantly higher in the patients with AMI, as compared with controls. A positive correlation was observed between D-dimer and the inflammatory markers—hs-CRP and TNF- α . Upon multivariate analysis, TNF- α emerged as the best determinant of CAD in our study. Our results indicate that there is a possible interplay of inflammation and hemostasis in CAD,

underlining their synergistic role in the pathogenesis of CAD.

Keywords Acute myocardial infarction · Inflammation · Hemostasis · Interleukin (IL)-10 · High sensitivity C-reactive protein (hs-CRP) · Tumor necrosis factor (TNF)- α · Fibrinogen · Fibrin D-dimer · Homocysteine

Introduction

In recent times, coronary artery disease (CAD) has emerged as a major non-communicable disease, with considerable morbidity and mortality. It is characterized by the presence of a vulnerable atheromatous plaque, infiltration of inflammatory cells and subsequent intra-luminal thrombus formation. This is often followed by rupture of the plaque, with most coronary occlusive events occurring in the setting of fulminant intra-luminal thrombosis [1].

Besides local hyper-coagulability, recent studies have suggested a systemic imbalance of the hemostatic system in CAD, with a shift towards increased pro-coagulation and decreased fibrinolysis. Thrombosis and atherosclerosis are closely related phenomena as thrombosis of a disrupted atherosclerotic plaque triggers ischemic cardiovascular events. Some researchers have hypothesized that impaired fibrinolytic function may be a risk factor for ischemic events [1]. It has been hypothesized that endogenous markers of fibrinolytic function may identify certain otherwise healthy individuals, predisposed to thrombosis.

Recent consensus focuses on the less understood but more significant role of inflammation and hemostatic factors in CAD that underlines the importance of the association of circulating biomarkers of inflammation, endothelial dysfunction, coagulation and fibrinolysis with

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risk of major coronary events (MI or CAD death), after adjustment for traditional CAD risk factors [2].

We report a cross-sectional study to compare the association of the hemostatic variables and inflammatory markers with CAD risk in patients with acute myocardial infarction (AMI).

Methods

This study was undertaken in a tertiary health care facility in India (XXX). The study was approved by the Institute Ethics Committee. 60 age and gender matched healthy subjects and 60 AMI patients attending the hospital facility consecutively were recruited into the study, after obtaining written informed consent from all study subjects.

The parameters analyzed in our study included total homocysteine, Fibrinogen, fibrin D-dimer, IL-10, TNF- α and hs-CRP levels. TNF- α and IL-10 levels were measured by using commercial ELISA kits from Diaclone Research, Belgium. The measuring range of TNF- α was 25–800 pg/ml while that of IL-10 was 12.5–400 pg/ml respectively. Hs-CRP was measured in serum using immunoturbidimetric assay kit from Randox, UK with an assay range of –0.3–161 mg/l. Total homocysteine (Bio-Rad Lab, Oslo, Norway), plasma fibrinogen (GenWay Biotech Inc, California, USA) and D-dimer levels (American Diagnostica Inc, Stamford, USA) were measured by commercially available ELISA kits. The range for D dimer detection was 0–200 ng/ml while that for fibrinogen was 0.165–40 μ g/ml respectively. The assay range for total homocysteine level in blood was 2–50 μ mol/l.

Data Analysis

Data was expressed as mean $\pm$ S.D. Normal data was compared between the groups using student's *t* test and correlation analysis was done to assess the degree of association between the parameters and disease. Multiple logistic regression analysis was also done to analyse the nature of relationship between the variables. Statistical analysis was done on SPSS version 17.0. *p* < 0.05 was considered to be significant.

Results

The demographic profile of the study population is presented in Table 1. It is evident that the cases and controls were age and sex matched. There was no significant difference in the age between the control and the case groups (Table 1). All the parameters under evaluation have been

Table 1 Demographic features of the study population

	Cases (<i>n</i> = 60)		Controls (<i>n</i> = 60)		<i>p</i> value (Mann–whitney)
	Mean	Range	Mean	Range	
Age	52.5 $\pm$ 11.5	23–80	52.4 $\pm$ 11.5	23–78	0.9
Males (<i>n</i> = 50)	52.7 $\pm$ 11.9	24–76	54.0 $\pm$ 10.0	24–77	0.19
Females (<i>n</i> = 10)	52.1 $\pm$ 10.7	23–80	48.8 $\pm$ 13.8	23–78	0.06

Table 2 Comparison of the various biochemical parameters in the study population

Marker	Cases (<i>n</i> = 60)	Controls (<i>n</i> = 60)	<i>p</i> value
Total homocysteine (μ mol/l)	36.2 $\pm$ 9.11	10.6 $\pm$ 6.3	<0.001
Fibrinogen (mg/dl)	478.1 $\pm$ 69.9	310.2 $\pm$ 91.3	<0.001
Fibrin D-dimer (ng/ml)	611.5 $\pm$ 64.3	273.1 $\pm$ 23.7	<0.001
TNF- α (ng/ml)	65.6 $\pm$ 7.4	10.5 $\pm$ 3.2	<0.001
IL-10 (pg/ml)	16.2 $\pm$ 4.1	3.9 $\pm$ 1.6	<0.001
hs-CRP (mg/l)	7.6 $\pm$ 1.7	1.8 $\pm$ 0.7	<0.001

Table 3 Correlation analysis depicting the association between the different parameters under study

Correlation of	r value	<i>p</i> value
hs-CRP vs D-dimer	0.786	<0.001
TNF- α vs D-dimer	0.804	<0.001
hs-CRP vs Homocysteine	0.674	<0.001
TNF- α vs Homocysteine	0.912	<0.001
TNF- α vs disease incidence	0.883	<0.001
IL-10 vs disease incidence	0.834	<0.001
D-dimer vs disease incidence	0.899	<0.001
Homocysteine vs disease incidence	0.876	<0.001
CRP vs disease incidence	0.866	<0.001
Fibrinogen vs disease incidence	0.854	<0.001

compared in Table 2. Total homocysteine, fibrinogen, D-Dimer, IL-10, hs-CRP and TNF- α were elevated significantly in patients with AMI, when compared to control population.

On correlation analysis, a significant positive correlation was observed between CRP and D-dimer levels, as well as CRP and homocysteine levels. A similar correlation was observed for TNF- α also. TNF- α had a correlation coefficient (*r*) of 0.912 with Homocysteine signifying very strong association between these two parameters in subjects with CAD. Among all the parameters, D dimer showed the strongest association with disease incidence with a *r* value

Table 4 Multivariate regression analysis evaluating the role of the different parameters

Marker	β index	<i>p</i> value
TNF- α	4.7	<0.001
IL-10	3.4	<0.001
Total homocysteine	3.4	<0.001
CRP	2.0	0.03
Fibrin D-dimer	2.3	<0.001
Fibrinogen	2.4	<0.001

of 0.899 followed by homocysteine, fibrinogen and CRP respectively.

Inflammatory and hemostatic parameters also significantly correlated with disease incidence. However no significant correlation was seen between IL-10, homocysteine and D dimer levels (Table 3).

On multivariate analysis, TNF- α emerged as the best discriminator of CAD risk, with a beta index of 4.7 (Table 4). Beta index is a determinant of the strength of association. An index of 4.7 denotes that a unit increase in the level of TNF- α increases the chances of having the disease by an increment of 4.7. Homocysteine presented with beta index of 3.4, indicating its significant role in the pathogenesis of CAD.

Discussion

The existing approach for biomarkers in the diagnosis of CAD, usually places emphasis on one or a few potential candidates at a time. However the pathophysiologic changes in CAD are influenced by many factors, such as oxidative stress, inflammation, tissue necrosis and platelet activation [3]. So a radical approach that spans the interplay of all these factors, if not at least the predominant factors underlying the disease, would lead to a robust panel of biomarkers that would have ideal diagnostic properties for CAD such as early detection, high sensitivity and specificity, easy availability, and cost effectiveness. This would rather meet up with the diagnostic, prognostic and therapeutic needs of the golden hour. We focussed on the interplay between inflammation and hemostasis in patients with AMI.

IL-10 is an anti-inflammatory cytokine with pleiotropic effects in immune-regulation and inflammation. It down regulates the expression of T-helper 1 (Th1) cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production. IL-10 inhibits the synthesis of pro-inflammatory cytokines such as IFN- γ , IL-2, IL-3, TNF- α and GM-CSF secreted from the macrophages and regulatory T-cells. It also displays a potent ability to

suppress the antigen-presentation capacity of antigen presenting cells.

In a previous study by our group [4], we observed that IL-10 levels are elevated in patients with AMI, as compared with controls. Mälarstig et al. [5] reported that elevated IL-10 was an indicator of poor outcome and enhanced systemic inflammation in patients with acute coronary syndrome. In the present study, our results were concordant with their findings. Pérez Fernández et al. [6] showed that IL-10 expression within human atherosclerotic plaques has been demonstrated and animal experiments have shown that low levels of IL-10 lead to the development of extensive and unstable atherosclerotic lesions. Currently available evidence suggests a potential protective role for IL-10 in atherosclerosis [7].

C-reactive protein (CRP) is a proven marker of cardiovascular risk. Plasma CRP, an acute-phase reactant produced primarily by the liver in response to inflammatory cytokines such as IL-6, prospectively identifies asymptomatic individuals at risk for coronary events [8]. CRP induces the production of inflammatory cytokines and monocyte chemotaxis, increased cell adhesion molecules expression, down-regulation of endothelial nitric oxide synthase (NOS) expression and activity, increased LDL-C uptake by macrophages and tissue factor stimulation production by peripheral blood monocytes. Modestly elevated baseline concentrations of CRP, are associated with the long-term risk of coronary heart disease in general populations, whilst the major acute phase response of CRP following myocardial infarction is associated with death and cardiac complications [9]. It appears that for a given clinical scenario, the higher the CRP level, the greater the risk of future events. In a previous study by our group [4], we observed that CRP levels are elevated in patients with AMI, as compared with controls. In addition, Patel et al. [10] observed that for healthy persons at risk for coronary artery disease, CRP even within the normal range should not be disregarded. The above findings are in accordance to our present study.

TNF- α is a pro-inflammatory cytokine produced primarily by activated monocytes/macrophages. TNF- α has also been implicated in the pathogenesis as well as the progression of atherosclerotic plaques. The possible mechanisms postulated include the enhanced surface expression of ICAM-1, VCAM-1, E- and P-selectins on endothelial cells [11]. Increased chemokine and scavenger receptor expression is also attributed to TNF- α . Kosmala et al. [12] observed that in patients with stable CAD and preserved LV systolic performance, plasma levels of TNF- α and IL-6 are elevated. In a previous study by our group [4], we observed that TNF- α /IL-10 ratio is significantly increased in patients with AMI, as compared with controls. Hirsch et al. [13] concluded that the extent of

changes in serum TNF- α concentration is significantly related to estimates of infarct extent, obtained scintigraphically. The above findings are in accordance to our present study.

Fibrinogen, an acute phase protein and a clotting factor, appears to be an independent risk factor for cardiovascular disease, but the mechanism(s) are still uncertain. Fibrinogen controls plasma and red cell viscosity, so elevated levels of fibrinogen may decrease blood flow, particularly through stenotic vessels. Through conversion to fibrin, fibrinogen may promote thrombus formation [14]. A study in Framingham offspring population observed that fibrinogen was associated with traditional cardiovascular risk factors. Elevation of fibrinogen may provide a mechanism for risk factors to exert their effect. Also, fibrinogen levels were higher among subjects with cardiovascular disease compared with those without disease [15].

Recently, fibrin D-dimer, the degradation product of cross-linked fibrin, has garnered increasing interest for several reasons. First, it can be considered as a global marker of the turnover of cross-linked fibrin and activation of the hemostatic system. Second, in contrast to several other markers of haemostasis, elevated D-dimer levels have been found to predict the risk of future coronary events independent of conventional risk factors in initially healthy, middle-aged male and female subjects, in elderly men and women, as well as in patients with known peripheral arterial occlusive disease and after myocardial infarction [16]. Also modestly elevated circulating D-dimer values reflect minor increases in blood coagulation, thrombin formation, and turnover of cross-linked intravascular fibrin (which is partly intra-arterial in origin) and that these increases may be relevant to coronary heart disease according to Lowe et al. study [17]. We observed an elevated fibrinogen and fibrin D-dimer in the present study, in agreement with their findings.

It has also been demonstrated that plasma homocysteine level increases after tissue damage and promotes endothelial damage and adhesion of leukocytes to the endothelial surface. So, a high plasma homocysteine level is an indicator of continuing tissue damage and inflammatory vascular wall thickening, rather than an initiator of atherosclerosis [18]. In addition Humphrey et al. [19] observed that each increase of 5 $\mu\text{mol/l}$ in homocysteine level increases the risk of CAD events by approximately 20 %, independently of traditional CAD risk factors. In concordance with the above studies, we found a significant rise in homocysteine in CAD, as compared with controls.

We found a significant association between the inflammatory and hemostasis parameters and severity of the disease, thus indicating a significant interaction between inflammation and hemostasis mechanisms in the pathogenesis of AMI in concordance with a study conducted by

Woodward et al. [20]. In two recent studies [20, 21], Lowe et al. [21] and Yuan et al. [22] reported a similar positive correlation between CRP and D-dimer as observed by us in their study on patients with ischemic heart disease. The statistically significant correlation observed between the inflammatory markers namely CRP and TNF- α with the hemostasis marker-D dimer suggests a possible interplay between inflammation and thrombotic pathways in laying the foundation of the molecular perturbations which finally lead to the consequences inherent to atherosclerosis. ROS generation consequent to cytokine release leads to platelet activation and leukocyte chemotaxis along with alterations in the fibrinolytic pathway thereby promoting formation of thrombus in atherosclerotically narrowed arteries. Likewise, fibrin degradation end products such as D-dimer have multiple effects on the inflammatory cascade such as neutrophil activation, cytokine secretion and hepatic synthesis of acute phase reactants.

The strengths of this work are that it involves the CAD-prone North Indian population and it allows a detailed insight into the relatively unexplored area of interaction between hemostasis and inflammation in AMI. The limitation of our study includes the lack of follow-up data and the low sample size. Further, correlation of our findings with anatomical evidence of atherosclerosis as evidenced by angiography would have further validated our results.

Conclusion

Our study highlights the possible interaction between inflammation and hemostasis in patients with CAD. We found that on multivariate analysis, TNF- α emerged as the best discriminator of CAD risk. New insights into the interplay between hemostasis and inflammation in CAD may initiate the identification of novel diagnostic biomarkers to improve the outcomes of individuals with CAD.

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