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Original article

Study of inflammatory markers and coronary artery disease

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ABSTRACT

Background: CAD is leading cause of Morbidity and Mortality in men and women. Various risk factors involved include hardening of coronary arteries, reduced blood flow, hypertension, smoking, deranged lipid profile, obesity, diabetes and stress. However, current research reveals that atherosclerosis is an inflammatory disease and not a simply due to accumulation of lipids in coronary arteries. Therefore, recent research in the developed countries is focused on new biochemical and inflammatory markers.

Material and Methods: This is a case control study. The study was carried out at Department of Biochemistry, in collaboration with Dept. of Medicine Lady Hardinge Medical College & Dept. of Cardiology at G.B Pant Hospital. The study population consisted of 100 consecutive patients above 35 years of age with documented stable coronary artery disease. Control group consisted of 100 subjects age and sex matched with no clinical or ECG evidence of Coronary Artery Disease. Plasma levels of hsCRP interleukin-2, interleukin-6 were assayed by ELISA. Serum glucose, cholesterol, triacylglycerol, urea and creatinine were measured by using standard kits on fully automated auto analyzer. Multivariate analysis was performed to evaluate the associations between blood pressure and circulating levels of IL-2 and IL-6.

Results: We compared the values of serum hsCRP, IL-6, IL-2, cholesterol and triacylglycerol of group B with control group-A and significance difference was found with p value < 0.001.

Conclusion: Raised levels of hs C-reactive protein interleukin-2 and interleukin-6 may have a role in the development of coronary artery disease and can be makers for CAD.

Key Words: Coronary artery disease (CAD), myocardial infarction (MI), high sensitivity C-reactive protein

INTRODUCTION

The etiology of CAD is multi-factorial. It is result of interplay between lifestyle and environmental factor. Epidemiological and experimental studies have revealed an association between biochemical markers of systemic inflammation and cardiovascular disease such as atherosclerosis, heart failure and hypertension. CHD results due to atherosclerosis which is a progressive inflammatory disorder of arterial wall, characterized by focal lipid rich deposits in the intimal layer, principally in large medium sized-elastic and muscular arteries which can lead to ischemia of the heart.[1,2] In spite of treatment and preventive measures against the established risk factors, adopted strategies have failed to control this killer disease. Extensive research is being going on for finding new markers to diagnose and identify the individuals at high risk of the disease. CRP has a long half-life, its levels remain stable over time without exhibiting circadian variability, and fasting blood samples are not required. Currently, CRP levels <1 mg/L, 1 to 3 mg/L, and >3 mg/L are used to denote low, intermediate, and high-risk groups.(C).

Therefore, hs-CRP is considered to be an appropriate marker to study endothelial dysfunction and atherosclerosis. hs-CRP has been found to activate a number of processes involved in inflammatory reactions.[3-8]

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Cytokines are involved in a variety of immunological, inflammatory and infectious diseases. Interleukin-6 (IL-6) is pro-inflammatory cytokine derived from activated T-lymphocytes that induces the growth and differentiation of B cells to produce antibodies and induction of hepatocyte secretion of acute phase inflammatory proteins. Epidemiological data evaluating the role of IL-6 in atherogenesis is sparse. In one of the prospective study in apparently healthy men, it was found that elevated levels of IL-6 were associated with increased risk of future myocardial infarction, thus it supports a role of cytokine mediated inflammation in the early stages of atherogenesis.[9] Another study reported that in fatty streaks and in the atheromatous 'cap' and 'shoulder' regions, macrophage foam cells and smooth muscle cells (SMC) express IL-6, which suggests its role in the progression of atherosclerosis. IL-6 is also associated with elevated levels of C reactive protein and fibrinogen in patients with acute coronary syndrome.[10] IL-2 is normally produced by the body during an immune response.[10] IL-2 is necessary for the development of T cell immunologic memory. Hs-CRP is risk factor for CAD. It stimulates the endothelial cells to express adhesion molecules and potent attractant. HsCRP may play a direct role in the initiation and the progression of atherosclerotic lesion. It was the first acute phase protein to be described and is highly sensitive systemic marker of inflammation and tissue damage. High CRP levels after acute myocardial infarction (AMI) indicates an unfavorable outcome even after correction of other risk factors. Thus, CRP constitutes an independent cardiovascular risk factor.[11-14] In the present perspective this study has been conducted to evaluate these inflammatory markers in our population.

MATERIAL AND METHODS

The study has been conducted in the Department of Biochemistry with collaboration of Department of Medicine Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital and Department of Cardiology, GB Pant Hospital with the approval of the institutional review committee and ethical committee. Informed consent was obtained from all subjects.

STUDY DESIGN:

This was a case-control study conducted on two hundred individuals who were selected from the outpatient department of the cardiac clinic of GB Pant Hospital and from department of Medicine at Lady Harding Medical College and Smt. Sucheta Kriplani Hospital.

The patient group comprised of 150 male patients, age ranging from 35-59 years, with Angiographically confirmed stable coronary artery disease. They were all newly diagnosed patients and hence were not on any medication before withdrawing blood samples.100 apparently healthy subjects attending health check up clinic at G B Pant Hospital, served as a control group. None had a history or physical or laboratory findings of any cardiovascular, renal, liver, or metabolic disease before the study, none had history of diabetes, hypertension or dyslipidemia and none were taking any medication.

Analytical Measurement

After overnight fast 10 ml venous blood sample was collected. Two ml blood was transferred to test tube containing potassium fluoride for serum glucose assay after centrifugation. Rest of the blood was allowed to coagulate and centrifuged. Serum was stored at -40°C.

Serum hsCRP were assayed using solid phase enzyme immunoassay based on Sandwich Principle. The kit used for hs-CRP estimation was procured from Diaclone.

Serum IL-2 and IL-6 were assayed using solid phase enzyme immunoassay. The kits used for IL-2 and IL-6 were procured from Diaclone.

Serum glucose, triacylglycerol, cholesterol, urea and creatinine were measured on Fully Automated auto analyzer Beckman CX-9 using standard kits.

Statistical Analysis

All values are given as mean±standard error mean .Normality of the sample distribution of each continuous variable was tested with the Kolmogorov–Smirnov test. The Student's t or Mann–Whitney U test, depending on the shape of the distribution curves, was used for evaluation of differences in continuous variables. Spearman's rank correlation was applied to test for association between continuous variables. A two-tailed p value<0.05 was considered statistically significant

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and those < 0.1 were considered marginally significant. Statistical analysis was carried out using SPSS for windows 14.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

This evaluation was done by performing various biochemical tests (fasting glucose, urea and Creatinine) in both groups. The levels of inflammatory markersinterleukin-2, interleukin-6, and hs C-reactive protein were measured in both groups. Routine blood investigations as cholesterol, triacylglycerol, fasting plasma glucose, urea & creatinine were also assayed. Demographic data as age, sex, body mass index, smoking, family history, blood pressure and pulse rate were also recorded and noted in both groups.

Group-A (Patients) and Group – B (Control)

Group A have significant higher values of BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), in comparison to control group. No significant difference was found in the mean values of age and pulse rate of group-A and group-B. as shown in Table 1.

Table1: Comparison of demographic data between Group A and Group B. The values are expressed in mean ± SEM. The number of observations is given in parenthesis.

Parameters	Group A (n-150)	Group B (n-100)	P-value
Age (males)	50.7±1.6	51.2±1.5	>0.05
Smokers	116	33	<0.001
Family history	71	30	<0.001
Body Mass Index(kg/m^2)	* 27±4.3	23±2.1	<0.001
Systolic(mmHg)	*145.4±58.	125.1±6.5	<0.001
Diastolic(mmHg)	*92.02±7.5	74.2±4.9	<0.001
Pulse Rate/min	68.3±6.8	68.1±8.60	>0.05

Group A have significant higher values of fasting blood glucose (FBG), serum creatinine, total cholesterol, triglyceride (TG), low-density lipoprotein cholesterol (LDL-c) but they have lower value of high-density lipoprotein cholesterol (HDL-c) in comparison to Group B. no significant difference were found in urea level between Group A and Group B. as shown in Table -2

Table 2: Serum values of Total cholesterol, Triacylglycerol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol fasting glucose, creatinine and urea in group-A and group-B. The values are expressed as mean ± SEM. The number of observations is given in parenthesis.

Parameters	Group-A (n)=100	Group-B (n)=100	P-value
Total cholesterol (mg/dl)	*299.3±5.32	176.50±9.57	<0.001
Triacylglycerol (mg/dl)	*348.32±8.16	113.20±7.06	<0.001
LDL-c (mg/dl)	*145.4±30.2	75.7±5.4	<0.001
HDL-c(mg/dl)	*38.61±3.81	48.4±4.22	<0.001
Fasting glucose (mg/dl)	*88.98±1.29	85.53±1.51	<0.001
Creatinine (mg/dl)	0.93±0.02	0.89±0.02	>0.05
Urea (mg/dl)	28.80±1.13	29.50±1.22	>0.05

*P < 0.001. The values in group-A (Case) are significantly different as compared to group-B (Control).

Plasma values of interleukin-2 of the individuals in group-A and group-B were 594.7±15.5 pg/ml and 131.6±2.3 pg/ml. Plasma values of interleukin-6 of the individuals in group-A and group-B were 60.8±0.67 pg/ml and 2.2±0.9

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pg/ml respectively. Plasma values of Hs C-reactive protein of the individuals in group-A and group-B were 11.0±3.6 mg/L and 0.6±0.4 mg/L respectively (Table 3).

Table 3: Comparison of Plasma levels of interleukin-2, interleukin-6, hs C-reactive protein in Group-A and Group-B. The values are expressed in mean ± SEM. The number of observations is given in parenthesis.

Parameters	Group-A (n)=100	Group-B (n)=100	P-value
IL-2 pg/ml	*594±15.5	131.6±2.3	<0.001
IL-6pg/ml	*60.8±0.67	2.2±0.9	<0.001
Hs-CRP mg/L	*11.0±3.6	0.6±0.4	<0.001

*P < 0.001. The values in group-A (Case) are significantly different as compared to group-B (Control).

Table 4: Correlation of free high sensitivity C reactive protein with other data in group A.

	Pearson Correlation	Significance (P value)
Age	-0.423	0.001
BMI	-0.554	0.0001
SBP	-0.601	0.0001
DBP	-0.477	0.001
FBG	-0.556	0.0001
Total cholesterol	-0.653	0.0001
TG	-0.545	0.0001
LDL-c	0.116	0.041
HDL-c	0.036	0.758
Serum Creatinine	0.071	0.314
IL-2	-0.687	0.0001
IL-6	-0.756	0.0001

DISCUSSION

Cardiovascular diseases are the most common cause of Morbidity and Mortality all over the world. Researchers are going on for new up-coming markers which will help in early diagnosis and treatment. In the present study we have studied hsCRP, IL-2 and IL-6 levels in Indian population in angiographically proven cases. Study population was divided in two groups with 100 subjects in each group. Group A comprised of patients and Group B was taken as control group. To our knowledge, this is the first reported study of the association between the hs-CRP and Proinflammatory cytokines IL-2, IL-6 and coronary artery disease. HsCRP is a marker of inflammation and a risk marker for cardiovascular disease and is now included in the Framingham 10-year risk score (FRS).[1] The American Heart Association and Centers for Disease Control and Prevention has recommended that in patients with an FRS of 10% to 20%, a CRP between 3 and 10 mg/l is a risk factor of cardiovascular disease.[2] In the present study, levels of hsCRP were found to be significantly increased in study group compared to control group (11.0±3.6 Vs 0.6± 0.4 mg/ml; p<0.001). Many studies are available which have shown that high level of hs-CRP is considered as marker of inflammation and atherosclerosis[3-5] whereas one of the study did not find raised hs-CRP levels in atherosclerosis.[6] In another study, it was found that CRP binds to the phosphocholine layer of oxidized low density lipoprotein (OX-LDL)[7] thus, increases uptake of low density lipoprotein into

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macrophages.[8] CRP also up-regulates the expression of adhesion molecules in endothelial cells and increases plasminogen activator inhibitor-1 expression and activity.[9]

CRP is mainly synthesized in hepatocyte. Induction of CRP in hepatocyte is regulated at the transcriptional level by interleukin-6 (IL-6). This effect is enhanced by interleukin-1 β(IL-1β).[10] Both IL-6 and IL-1β control expression of CRP genes through activation of the transcription factors STAT3, C/EBP family members, and Rel proteins (NF-κB).[11] Interactions among these factors results in enhanced stable DNA binding of C/EBP family members and result in maximum induction of the gene.[12]

In the present study, levels of IL-6 were found to be higher in study group compared to control group (60.8±0.67 Vs 2.2±0.9pg/ml; $p<0.001$). The results are in concordance with the results obtained in other studies.[13-15] In an experimental study on mice, direct involvement of IL-6 in atherosclerosis was suggested.[16] In another large multicenter study, IL-6 gene polymorphism was found to be correlating with the severity of coronary artery disease and also with the risk of myocardial infarction.[17-20] IL-6 also induces production of smooth muscle cells and enhances expression of ICAM-1 and thus, helps in foam cell formation.[21-23] In the present study, a positive correlation was found between hs-CRP and IL-6, which substantiates the finding that elevated levels of IL-6 and its hepatic byproduct C-reactive protein are associated with increased risks of coronary atherosclerosis. Similar findings were also reported in another study conducted by Verma et al, Vander Meer IM et al. and Rajappa et al.[24-26]

In the present study, Serum IL-2 levels in study group were found to be significantly higher than in the control group (594 $\pm$ 15.5 Vs 131.6 $\pm$ 2.3pg/ml; $p<0.001$). A study conducted by Mazzone et al[27] and Mizia-Stec et al[28] also reported the similar findings. IL-2 acts as a proatherogenic agent by inducing formation of more T helper cell subtype Th1 phenotype. T cells are present in atherosclerotic lesions, and Th1 cells actively promote atherogenesis. IL-2 promotes the expansion and activation of T cell subset, and, thus, consequently, plaque development.[29,30] However, one of the study reported the anti-atherogenic role of IL-2.[31] In the present study, we have also found a positive correlation between IL-2 and IL-6 in the study group. Furthermore it is also reported that elevated IL-2 and IL-6 levels are strongly associated with future cardiac events and mortality in a population with stable CAD during a long term follow up. Each increase of 1 pg/ml in IL-6 was associated with increased chances of subsequent MI or sudden death.[32] In our study the levels of IL-2 and IL-6 have been found significantly raised with p value < 0.05 and support the idea that IL-2 and IL-6 can be used as prognostic marker in clinical setup.

CONCLUSION

From the present study we conclude that, raised levels of interleukin-2, interleukin-6 and hs C-reactive protein may have a role in the development of coronary heart disease. CRP may be both ‘marker’ and ‘maker’ of atherothrombosis. Cytokines are being increasingly recognized as a potential therapeutic target in a wide variety of diseases. Inhibition of signaling by IL-2, IL-6 may be beneficial in the treatment of atherosclerosis. However, It is still unclear whether these inflammatory markers merely are markers or they actively contribute to the development and progression of atherosclerotic disease at their own. Further researches are required which will provide new opportunities for Diagnosis, prediction of disease and will lead to new treatments for atherosclerosis.

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