Original Article

Association of Serum C-Reactive Protein and LDL:HDL with Myocardial Infarction

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Abstract

Objective: To identify frequency of the following markers of atherosclerosis risk: high C-Reactive Protein (CRP) and ratio between serum levels of low density lipoprotein (LDL) and high density lipoprotein (HDL) and to determine the association of high serum CRP levels and LDL:HDL in the patients presenting at NICVD with first myocardial infarction.

Methods: This case control study was conducted at National Institute for Cardiovascular Disease (NICVD), in Karachi, comprising of 50 male patients of acute myocardial infarction (without any other co-morbidity) and 50 matching controls.

Results: In this study, CRP levels were significantly high ($p = 0.003$) and serum HDL levels significantly low ($p = 0.006$) in patients as compared to controls. Serum LDL levels and the ratio of LDL and HDL were not significantly different among the two groups.

Conclusion: High serum CRP levels rather than high LDL:HDL are associated with myocardial infarction in the patients presenting at NICVD with first myocardial infarction (JPMA 56:318;2006).

Introduction

Atherosclerosis is the dormant pattern of arteriosclerosis characterized by the formation of intimal fibro-fatty plaques that have a central grumous core rich in lipids.[1] It is one of the leading causes of mortality and morbidity around the world but etiology is unknown. A number of risk factors are associated with atherosclerosis. Dyslipidemia is acknowledged as a major risk factor for atherosclerosis, especially hypercholesterolemia and hypertriacylglyceremia. Ishaq et al.[2] reported that the age-adjusted incidence of acute myocardial infarction in males is 192.8/100,000 as compared to 19.6/100,000 in females in Pakistan. Samad et al.[3] showed that the local population of Karachi presented with coronary artery disease at an earlier age than the Western population, with majority being males (65.5%). It was observed that there is a much higher risk of coronary artery diseases in South Asians as compared to European or Chinese ethnic groups in Canada.[4]

Many large scale studies have shown a high correlation between total plasma cholesterol and LDL levels and the severity of atherosclerosis as judged by the mortality rate from ischemic heart disease.[5] This is why, it was considered a consequence of hyperlipidemia, especially, hypercholesterolemia, but recent evidence shows that it is an inflammatory disease.[6] Current views regard atherosclerosis as a dynamic and progressive disease arising from the combination of endothelial dysfunction and inflammation. In fact, the lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can best be described, in aggregate, as an inflammatory disease.[7]

The inflammatory response in atherosclerosis includes phagocytic recruitment, chemoattractant generation, cytokine formation and production of some proteins called acute phase reactants, which help to potentiate the host defense system.[1] The most characteristic acute phase reactant is C-reactive protein (CRP)[8], which is mainly synthesized and secreted by hepatocytes 6 hours after an acute stimulus and shows rapid turnover (half life: 19 hours).[9]

CRP is implicated in vascular dysfunction and in the progression of atherosclerosis[10] and was found to be elevated in many cases of myocardial infarction.[11]

Inflammation, systemic or local, stimulates production of IL-6, which induces expression of hepatic genes encoding acute-phase reactants found in blood, including CRP and serum amyloid-A (SAA).[12]

Evidence suggested that measuring blood concentration of CRP could add useful information.[5] Large scale prospective studies have shown that plasma levels of CRP are a strong independent predictor of risk for future MI, stroke, peripheral arterial disease and vascular death among individuals without known cardiovascular disease. In addition, among the patients with acute coronary ischemia, stable angina pectoris, and history of myocardial infarction, levels of the CRP have been associated with increased vascular event rate.[12]

There are more chances of having increased levels of CRP in South Asians where there are more infectious disorders present in the community which might prove as the risk factor in causing atherosclerosis.

The present study was conducted to study the association of serum CRP levels and LDL:HDL ratio in the patients presenting at National Institute of Cardiovascular Diseases (NICVD) with first myocardial infarction.

Methods

This was a case control study, carried out on 100 male subjects (age range: 30-75 years) of which 50 were with acute myocardial infarction (MI) patients and 50 were normal subjects taken as controls. The patients were selected consecutively and enrolled from National Institute of Cardiovascular Diseases (NICVD), Karachi, and Emergency Department, Ziauddin Medical University Hospital, North Nazimabad, Karachi. The patients included were diagnosed as acute myocardial infarction by a consultant cardiologist on the basis of history, clinical examination, ECG, and laboratory investigations. The controls were age, sex and socio-economically matched persons not having any heart disease. The participants were excluded if they had a past history of any heart disease, diabetes mellitus, any infection or inflammatory disease. Screening with complete blood count, ESR, fasting blood glucose was performed, and if abnormal, were excluded.

The study included the quantitative estimation of serum C-Reactive Protein (CRP), Low-density Lipoproteins (LDL), High-density Lipoproteins (HDL), Fasting Blood Sugar (FBS) as well as Complete Blood Count (CBC) and Erythrocyte Sedimentation Rate (ESR). C-reactive protein was assayed with the help of a standard kit (Roche Tinaquant® 1299859) based on the principle of immunological agglutination.[13]

The homogenous enzymatic colorimetric test was used for the determination of both serum HDL and LDL-cholesterol.[14] CBC (by automated analyzer as well slide read) and ESR (by Westergren method.) were done to screen for any infection or inflammation. Fasting blood glucose was determined by glucose oxidase method.[15]

This study was conducted according to the recommendations of the Ethical Committee of our institution. Informed written consent was taken from every participant at the time of enrolment.

The data was entered in "Microsoft Excel" and analysis was done on SPSS (Statistical Package for Social Sciences) version 8.0. The results are given in the text and tables as mean $\pm$ standard deviation (S.D.) for quantitative variables like age, weight, height, BMI, CRP, serum HDL and LDL, while for qualitative variables like smoking the values are given as number and percent. Statistical comparison was made between the controls and patients by student's t-test. Geometrical means were also calculated for serum CRP levels since some of the subjects had very high values. In all statistical analyses, p-value < 0.05 was considered significant

Results

In this study, 50 patients with acute myocardial infarction were compared with 50 age-matched healthy controls.

Table 1 shows the baseline characteristics of the two groups. BMI, temperature and respiratory rate in patients were significantly higher than in controls. Pulse rate and systolic and diastolic blood pressure did not differ significantly in the two groups. Preprandial glucose, haemoglobin, haematocrit, MCV, MCH, MCHC and percentage of basophils also did not differ significantly in the patients and controls. TLC and neutrophil percentage were significantly higher in patients as compared to controls. RBC count and percentage of lymphocytes, eosinophil and monocyte as well as platelet counts were significantly lower in patients than in controls.

In Table 2, the inflammatory markers are compared among the patients and controls included in the study. ESR did not differ significantly among the two groups, while CRP was significantly higher among the patients. While comparing geometric means of the serum CRP levels, three control subjects were not included since their serum CRP was undetectable and log [CRP] of these values would become infinity. Serum HDL and LDL-cholesterol lipid levels were also compared. HDL-cholesterol was found to be significantly lower in patients as compared to controls. All other serum lipid levels were not significantly different.

ESR, serum CRP and serum HDL and LDL-cholesterol levels were analyzed among the subjects in relation to their LDL:HDL. Table 3

Among the subjects who had LDL:HDL ratio > 3 (Table 3), the patients had significantly higher CRP levels, while significantly lower levels of HDL-cholesterol as compared to the controls. Geometric mean of the CRP levels were calculated in various groups.

Discussion

Elevated CRP levels have been regarded as a risk factor for CAD.[16] Similar findings have also been observed by large prospective studies.[17] In the present study we compared males suffering from first MI with matching

Table 1. Baseline characteristics of patients and controls.

Parameter	Patients n=50	Controls n=50	P-value
Age (Years)	50.3 ± 11.02	49.2 ± 11.06	0.667
BMI (wt.kg/height.m2)	30.5 ± 6.9	27 ± 4.48	0.003*
Pulse (per min)	79.2 ± 10.5	75.6 ± 9.95	0.08
BP systolic (mmHg)	117.7 ± 18.6	122.5 ± 8.47	0.1
BP diastolic (mmHg)	75.6 ± 14.1	79.5 ± 6.59	0.07
Resp Rate (per min)	20.5 ± 2.1	17.2 ± 1.73	<0.001*
Temperature (^{O}F)	98.1 ± 0.2	97.9 ± 0.52	0.003*
Pre-prandial S. Glucose (mg%)	89.6 ± 10.04	89.5 ± 9.3	0.95
RBC count (×10^6/uL)	4.9 ± 0.7	5.2 ± 0.56	0.01
Hemoglobin (G%)	14.1 ± 1.9	14.4 ± 1.46	0.32
Hematocrit (%)	42 ± 5.7	43.3 ± 4.12	0.21
MCV (fl)	86.4 ± 6.9	83.3 ± 8.27	0.07
MCH (pg)	30.3 ± 9.5	27.9 ± 3.18	0.1
MCHC (g/dl)	33.5 ± 1.0	33.4 ± 1.33	0.6
TLC (×10^3/uL)	10.4 ± 2.8	7.5 ± 1.69	<0.001*
Neutrophil %	74.7 ± 13.6	61.6 ± 8.11	<0.001*
Lymphocyte %	19.9 ± 8.9	32.3 ± 7.32	<0.001*
Eosinophil %	1.9 ± 0.8	2.9 ± 2.68	0.009*
Monocyte %	1.8 ± 1.2	2.7 ± 2.16	0.007*
Basophil %	0.1 ± 0.3	0.1 ± 0.34	0.76
Platelets (×10^3/uL)	232.7 ± 71.5	265.2 ± 67.39	0.02:

All the values are given as mean ± standard deviation.
* significant p-value.

Table 2. Comparison of inflammatory markers and lipid levels between MI patients and controls.

Parameter (unit)	Patients (n=50)	Controls (n=50)	P-value
Serum HDL Cholesterol (mg/dl)	32 ± 7.9	36 ± 7.92	0.006*
Serum LDL Cholesterol (mg/dl)	109 ± 29.5	119 ± 35.55	0.13
LDL:HDL	4 ± 1.27	4 ± 1.11	0.37
ESR (mm 1st hr)	7 ± 3.4	8 ± 5	0.34
Serum CRP (mg/L)	16 ± 27.51	4.1 ± 2.99	0.003*
Geometric means of serum CRP levels			
All subjects	2 ± 1.13	1.3 ± 0.73	< 0.001*
Subjects with age 45 years and younger.+	1.8 ± 1.03 (n =20)	1.2 ± 0.86 (n=17)	0.04*
Subjects with age 46-60 years.+	2.2 ± 1.1 (n=23)	1.2 ± 0.67 (n=22)	0.001*
Subjects with age more than 60 years.	2 ± 1.5 (n=7)	1.4 ± 0.67 (n=8)	0.34

All the values are given as mean ± standard deviation.
* significant p-value.
+ Subjects with undetectable serum levels of CRP, were not included in the analysis.

Table 3. Comparison of inflammatory markers and serum lipid levels between various groups.

Parameter (unit)	Patients (n=50)	Controls (n=50)	P-value
Comparison of Inflammatory Markers and Serum Lipid Levels between various groups with LDL:HDL ratio ≤ 3			
ESR (mm 1st hr)	7 ± 4	9 ± 5	0.26
Serum CRP (mg/L)	20.3 ± 38.7	4 ± 3.3	0.07
Geometric means of serum CRP levels	2 ± 1.4	1.1 ± 0.87	0.04*
Serum HDL Cholesterol (mg/dl)	36.6 ± 8.56	40 ± 8.16	0.2
Serum LDL Cholesterol (mg/dl)	87.1 ± 20	97 ± 21.7	0.15
LDL:HDL	2.4 ± 20	2.5 ± 0.7	0.75
Comparison of Inflammatory Markers and Serum Lipid Levels between various groups with LDL:HDL ratio > 3			
ESR (mm 1st hr)	7 ± 4	1.3 ± 0.73	0.88
Serum CRP (mg/L)	14.2 ± 21.46	4.1 + 2.84	0.01*
Geometric means of serum CRP levels	2 ± 1.09	1.1 ± 0.87	0.002*
Serum HDL Cholesterol (mg/dl)	29.5 ± 6.63	33.2 ± 6.44	0.02*
Serum LDL Cholesterol (mg/dl)	118.8 ± 27.94	133.4 ± 33.95	0.07
LDL:HDL	4.17 ± 1.15	4 ± 0.86	0.67

All the values are given as mean ± standard deviation.
* significant p-value.

controls. Serum CRP levels were significantly higher among patients. This elevation of CRP was less likely to be secondary to myocardial injury, since the blood samples were drawn within 1- 1½ hours after the onset of MI.

The results of this study are in agreement with the findings of Ford et al.[18], who have observed a tendency of higher CRP levels with increasing age. Table 2 shows that among the healthy subjects mean CRP levels were highest in age groups > 60 years. Geometric mean of CRP levels among patients and controls showed that patients had significantly higher values. Although the mean CRP levels in patients of age group > 60 years was higher as compared to controls for the same group, the statistical significance was not observed due to relatively higher CRP levels as compared to controls in other age groups.

Koenig et al.[11] have reported that CRP has important prognostic relevance to the coronary heart disease in middle aged men. This study also supported the same finding. In addition, it was observed that it is similarly important in age group ≤ 45.

Low HDL is shown to be associated with higher prevalence and incidence of CAD.[19] This study also had similar observations with majority of patients having LDL:HDL ≥ 3. Low HDL in individuals in age group 46-60 years, was also associated with greater prevalence of AMI. The younger age group showed a similar trend but statistical significance was not observed.

This association can be explained on the basis of various studies describing the action of HDL-C as it reverses the cholesterol transport and delivers the excess cholesterol from the peripheral tissues to liver for disposal [19] due to the presence of LCAT in HDL.[20] HDL also reverses the endothelial cell dysfunction, stimulates prostacyclin production, inhibits the endothelial cell apoptosis, decreases platelet aggregability and inhibits LDL oxidation.[18,19] Increasing the levels of HDL-C may also reduce atheromatous plaque volume.[20]

Some of the recent reports from India and Pakistan did not show an association of HDL with AMI.[21,22] In contrast to this study, one of these studies[21] had limitations such as recruitment of hospital-based, socio-economically unmatched controls, inclusion of patients suffering from hypertension and diabetes mellitus, inclusion of a substantial number (≥ 30) of all participants being vegetarians. However, the study regarded obesity as a risk factor. Similarly, Iqbal et al.[22] also reported no association of HDL with AMI in their study, which had differences with this study such as ratio between controls and cases being 1:1.7, rather than being at least 1:1, inclusion of both sexes rather than males only. Thus, absence of association with HDL by these studies couldn't be validated.

LDL-C has been regarded as a risk factor for atherosclerosis.[6] The reason for this is that LDL particles are responsible for delivering cholesterol from liver to the peripheral tissues. Oxidation of LDL particles is a key process in the development of atherosclerosis. Within vascular endothelium, oxidized LDL particles induce inflammation, proliferation and apoptosis of endothelial and smooth muscle cells.[6,23]

In this study, we did not find any association between AMI and serum LDL-C levels. However, it was found that LDL levels were higher among controls rather than patients of AMI. LDL:HDL was also not found significantly different among study subjects in various groups and subgroups. Zwaka et al.[24] have demonstrated experimentally that CRP binds to oxidized LDL, which is then taken up by macrophages which in turn become foam cells. These foam cells are integral part of atheromatous plaques. They showed that CRP also binds to native LDL particles and opsonizes them, which are then phagocytosed by macrophages. Loidl et al.[23] have reported that minimally modified LDL may lead to the apoptosis of smooth muscle cells, which is a hallmark in the development of atherosclerosis. Moreover, LDL is positively correlated with the incidence of CAD.

In contrast, other studies[25] have shown that individuals who develop cardiovascular events had almost equal LDL-C levels as compared to those who did not develop such events.

In this study, higher CRP levels in patients were associated with lower HDL, as compared to controls. Higher CRP levels were also found associated in patients with high BMI and age group 46-60 years.

This study did not show significantly higher levels of LDL among patients within various groups. CRP appeared to be more strongly associated with CAD as compared to serum HDL, LDL-cholesterol or their ratio in all groups.

This study has observed that high serum C-reactive protein is significantly associated with myocardial infarction rather than high LDL:HDL, especially in age group 46-60 years. Low serum levels of high density lipoproteins were also associated with acute myocardial infarction.

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