Indian Journal of Clinical Biochemistry, 2008 / 23 (4) 334-336

hs-CRP AND OXIDATIVE STRESS IN YOUNG CAD PATIENTS: A PILOT STUDY

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ABSTRACT

Premature coronary artery disease (CAD) is common in India. We, therefore, studied oxidative stress, dyslipidemia, and high sensitivity-C reactive protein (hs-CRP) levels in young CAD patients. Present study consisted of male CAD patients below 40 years and age and sex matched healthy controls (n=30 each). Fasting blood samples were analyzed for serum lipid profile, malondialdehyde, antioxidant enzymes and hs-CRP levels. Dyslipidemia was observed in 90% of the young CAD patients, of which 72.2% showed increased serum triglycerides and decreased HDL-cholesterol. LDL-cholesterol levels were high in 77.8%. Serum malondialdehyde and hs-CRP levels were increased significantly ($p<0.0001$) as compared to controls. hs-CRP levels were in high risk range in all the young patients. However, glutathione peroxidase activity was reduced significantly ($p<0.05$). Our data suggests that elevated hs-CRP levels along with dyslipidemia and oxidative stress adds to the predictive value of premature CAD in young Indians.

KEY WORDS

Dyslipidemia, high sensitivity-C reactive protein, Premature CAD, Malondialdehyde.

INTRODUCTION

Coronary artery disease (CAD) is one of the commonest causes of mortality and morbidity all over the world. Indians are prone to CAD at a younger age(1). In INTERHEART study, Yusuf et al reported that mean age of presentation with new MI was 52 years in South Indians, 9.7% of these cases were younger than 40 years of age. Premature CAD is defined as CAD occurring before the age of 54 in men and 65 years in women. In its severe form it is defined as CAD occurring below the age of 40 years. Risk factors for CAD include conventional factors like hypertension, diabetes mellitus, smoking, hyperlipidemia and central obesity at a younger age. New risk factors include Lipoprotein(a), hs-CRP, fibrinogen, hyperhomocysteinemia etc. CAD in Indian population is appearing in younger age group and is associated with increased incidence of hospitalization, morbidity and mortality.

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Oxidative stress parameters have been evaluated in CAD frequently(2,3). However, there has not been much published work related to oxidative stress in young CAD patients in India. Similarly, not much literature is available on hs-CRP levels and its association with lipids and oxidative stress in CAD patients in younger age group in India. We, therefore evaluated these parameters in young CAD patients.

MATERIALS AND METHODS

Our study comprised of 30 male patients below the age of 40 years, who were clinically diagnosed of CAD and 30 healthy matched controls. Selection of the patients was done after confirmation of CAD by ECG, specific enzymes like CK-MB and SGOT. Patients with smoking habits and those suffering from other diseases like diabetes mellitus, renal disease, chronic obstructive pulmonary disease and hepatitis were excluded from the study. Blood samples were drawn after overnight fasting (within 24 hours of MI in patient group) and analysed for oxidative stress parameters, lipid profile, hs-CRP using standard methods.

Blood was collected in EDTA vial and was used for analysis of reduced glutathione($GSH^{'}$) by the method described by Beutler et al(4). Hemolysate was prepared from the whole

blood by the method described by Paglia and Valentine (5) for estimation of glutathione peroxidase(GPx) activity. Serum malondialdehyde (MDA) levels were estimated by the method of Satoh (6). Serum total cholesterol, HDL-cholesterol (HDL-C), serum triglyceride and LDL-cholesterol (LDL-C) were estimated by standard methods. The results for lipid profile were analysed further for prevalence of dyslipidemia. Dyslipidemia was defined when any of the lipid fractions was deranged i.e. serum cholesterol >200mg%; HDL-C <40 mg%; LDL-C >100 mg%; serum triglycerides >150mg% (7). Inflammatory marker hs-CRP was estimated by using solid phase ELISA technique (Calbiotech Inc, USA). The study was approved by the ethical committee on human research and all the participants gave their written consent.

Statistical analysis was performed using unpaired 't' test. The value of $p<0.05$ was taken as significant.

RESULTS

The present study was conducted on 30 male premature CAD patients and the results were compared with healthy controls. Mean age in the study group was 36.8 ±4.4 years and that in control group was 35.9±4.8 years.

Serum MDA levels in young CAD patients were significantly ($p< 0.0001$) higher as compared to controls. GSH levels were low in patients as compared to controls though the difference was not significant. However, GPx enzyme activity decreased significantly ($p>0.05$) in young CAD patients (Table 1).

Table 1 : Status of oxidants/antioxidants and hs-CRP levels in healthy controls and young CAD patients (mean ± SD)

Parameter	Control (n=30)	Patients (n=30)	P Value
GPx (U/gm Hb)	35.62±17.91	23.28± 15.10	0.02
GSH (mg/gm Hb)	2.10± 0.69	1.74± 0.79	0.12
MDA (µmol/L)	1.05± 0.36	2.89± 1.40	<0.0001
hs-CRP (µg/ml)	1.97± 1.12	8.98± 2.03	<0.0001

Serum triglycerides levels were significantly high ($p=0.003$) and HDL-C levels were low ($p= 0.02$) in CAD patients. Serum cholesterol and LDL-C levels though were high in the patients, the difference was not significant (Table 2). 90% patients showed dyslipidemia. Amongst them, 29% had high serum cholesterol levels (>200 mg%), 72.2% showed decreased HDL-C fraction(<40 mg%), 72.2% patients had high levels of serum triglycerides(>150 mg%), 77.8% had high levels of LDL-C(>100 mg%).

Table 2. Serum lipid profile in healthy controls and young CAD patients (mean ± SD)

Parameter	Control (n=30)	Patient (n=30)	P Value
TC (mg%)	163.9± 20.91	181.20±24.68	0.056
HDL-C (mg%)	42.58±8.86	34.95±10.12	0.02
LDL-C (mg%)	99.85±23.96	107.15±21.68	0.313
TG (mg%)	109.0±31.96	188.65±100.8	0.003

hs-CRP levels were raised significantly ($p< 0.001$) in young CAD patients (Table 1). The levels of hs-CRP were above 3µg/ml in all the patients, which is considered abnormally high (8). However, only 5% cases in control group had hs-CRP level >3µg/ml.

DISCUSSION

Reactive oxidant species can damage all types of biomolecules. Oxidative damage to lipids, proteins and DNA can be deleterious. Antioxidative defence comprising of enzymatic and non enzymatic defence brings about inactivation and/or removal of reactive species. Glutathione peroxidase (GPx) is one such antioxidant enzyme. GPx, a selenium containing enzyme, is an important antioxidant enzyme of erythrocytes. Serum MDA which is a marker of lipid peroxidation and GPx have been studied extensively to investigate oxidative stress in patients of CAD (2, 3). These studies indicated an increase in plasma MDA levels and decrease in GPx activity in older age group patients. Our study on young CAD patients showed a significant increase ($p<0.0001$) in serum MDA levels and a significant decrease ($p=0.02$) in GPx activity. An increase in lipid peroxidation product MDA and antioxidant GPx indicates an increase in oxidative stress.

Dyslipidemia has been shown to be an important risk factor for CAD (7). Indians world wide demonstrate a triad of high triglycerides, high LDL-C and low HDL-C levels. Our study also demonstrated presence of dyslipidemia in young patients as well. Since triglycerides bring change in LDL particle size, density, distribution and composition producing small dense LDL which is more atherogenic. Thus estimation of serum triglyceride levels is an indirect measurement of LDL particle size. In Indian patients with CAD, prevalence of high triglyceride levels are found more than high cholesterol levels (1).

There is increasing evidence that inflammation plays an important role in pathogenesis of atherosclerosis and its

complications. CRP is a member of Pentraxine family of proteins and is an acute phase reactant. A growing number of studies suggest that CRP is an independent risk factor for atherosclerotic vascular disease. It has been suggested that hs-CRP may not only be a marker of generalized inflammation but directly and actively participate in atherogenesis (9). Many studies have shown an increase in serum hs-CRP levels in CAD patients of more than 50 years of age in both males and females (9,10). Therefore, it was relevant to study changes in hs-CRP in young patients. Present study on young CAD patients revealed that hs-CRP is significantly increased in these patients. Moreover, all the patients exhibited hs-CRP levels greater than 3µg/ml. Increased hs-CRP concentration has been shown to have significant correlation with age and is associated with metabolic disorders such as dyslipidemia (9, 10). CRP binds to the LDL particle in atherosclerotic plaques leading to activation of complement thus being proinflammatory and contributing to atherogenesis. CRP may also increase ischemic tissue damage by complement dependent mechanism and tissue factor production by macrophages (1, 9).

In contrast to the study conducted by Espliguero et al (9) who have shown that increased hs-CRP levels correlate with age, our study shows an increased level of hs-CRP in young patients of CAD as well. It is evident that hs-CRP in young patients with dyslipidemia brings about atherogenic changes and ischemic tissue damage leading to premature CAD.

Thus, increased CRP levels and dyslipidemia have significant risk prediction value compared with those based on lipids alone in young CAD patients.

The present study was conducted under the short term summer training program funded by Indian Council for Medical Research. Therefore, number of cases studied was less due to time limitation. However, further studies may be carried out in a larger population.

ACKNOWLEDGMENT

Authors are thankful to Indian Council of Medical Research (ICMR), New Delhi for the award of short term research studentship for the year 2006.

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