Original Article

Evaluation of hs-CRP, Antioxidant Markers and MDA in Patients of Coronary Artery Disease (CAD) Containing Non-Smokers and Non-Diabetics

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Abstract

Background: *The oxidative stress and inflammation are cooperative events involved in atherosclerosis development. In the present study we assessed the association of MDA, antioxidant markers, high sensitive C - reactive protein (hs-CRP) and lipid status parameters in the patients with coronary artery disease (CAD). Significant risk factors such as cigarette and diabet were excluded from the study.*
Methods: *Oxidative stress parameters for example Malondialdehyde (MDA), antioxidant markers including: erythrocyte superoxide dismutase(SOD), Glutathion peroxidase (GPX), Total antioxidant capacity(TAC), The inflammation marker and serum lipid status parameters were measured in 120 subjects including 60 CAD patients with angiographically diagnosed CAD and 60 CAD-free subjects as a control group, also diabetics, smoking patients, patients with malignancy, renal and liver disease, and other disease were excluded from the study.*
Results: *The serum MDA and hs-CRP levels were increased significantly as compared to controls. However, erythrocyte SOD, GPX activities and TAC level were reduced significantly in patients ($P<0.05$ in all cases). The levels of total cholesterol, Triglyceride, LDL-C were significantly higher and that of HDL-C was meaningfully lower than those of control ($P<0.05$ in all cases).*
Conclusion: *The association between oxidative stress parameters, antioxidant markers, the inflammation index and lipid status parameters suggest their involvement in atherosclerosis development that may lead to CAD progression.*
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Introduction

Coronary Artery Disease (CAD) is the major cause of mortality and morbidity in most countries.[1] Among many traditional risk factors for CAD development including: hypertension, hyperlipidemia, diabetes, age, sex, obesity, cigarette smoking and positive family history;[2] oxidative stress and inflammation are now being considered as significant and novel risk factors.[3-5] According to Kutuk et al., lipid peroxidation and inflammation are cooperative events involved in atherosclerosis development.[6] Endothelial dysfunction occurs in conjunction with CAD. The risk factors of CAD have been almost universally associated with a degree of endothelial dysfunction in humans.[7, 8] It has been reported that endothelial dysfunction and increased oxidative stress may predict future events in patients with CAD.[9] Low density lipoprotein cholesterol (LDL) is considered as the most important risk factor of CAD. The lipoprotein is believed to have a central role in atherogenesis.[10-12] High density lipoprotein (HDL) is one of the most important independent protective factors against atherosclerosis and CAD.[13, 14] Malondialdehyde (MDA), a carbonile group produced during lipid peroxidation, is used widely in determining oxidative stress.[5] The activities of antioxidant enzymes, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX) in erythrocytes and non-enzymatic antioxidants along with total antioxidant capacity (TAC) have been reported as predictive indices of CAD.[1,3] The lipoprotein (a), hs-CRP, fibrinogen and homocystein are new risk factors and inflammation markers which are used in prediction of atherosclerosis and CAD.[15,16] The study designed to evaluate the frequency of risk factors of CAD, inflammation markers, dyslipidemia and oxidative stress and influence of antioxidant parameters in decrease this disease in non-smoker and non-diabetic patients suffering from coronary artery disease.

Methods

We studied 60 CAD patients and 60 controls. The CAD group included 30 females and, 30 males with a mean age of 58 years, ranging from 40-78 years. They had various degrees of stenosis in one or more of the main branch of coronary artery documented by coronary angiography. Patients with diabetes mellitus,

renal disease, chronic obstructive pulmonary disease, hepatitis and smoker were excluded from the study group. The controls included 30 females, 30 males with a mean age of 57 years, ranging from 40-76 years. The subjects proved to be healthy by health screening and had no obstructions in the coronary artery by angiography. Blood samples were collected in the morning by venipuncture after an overnight fast and were allowed to clot at room temperature for about 1 hour. Sera were separated from cells by centrifugation at 1500xg for 10 min and kept at -80°C and blood samples were stored at -20°C until analysis.

The lipid and lipoprotein parameters were measured by standard methods. GPX method is based on that of Paglia and Valentine.[17] Glutathione Peroxidase (GPX) catalyses the oxidation of glutathione (GSH) by Cumene Hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH the oxidized Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm is measured. The activity of GPX in blood samples were measured using commercially available Kit (Ransel: Randox laboratories Crumin U.K). The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical ($O_2\circ$), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. The The activity of SOD in blood samples were measured using commertially available Kit (Ransel: Randox laboratories Crumin U.K). GPX and SOD results were reported as u/g Hb.[18] ABTS (2, 2́-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H2O2 to produce the radical cation ABTS+. This has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree which is proportional to their concentration.[19] Plasma total antioxidant capacity was determined using Randox total antioxidant status Kit (Randox) (NX2332). Hs-CRP was measured by Commercial Kit (PARS AZMON.IRAN) by Immuno Torbidometry.[20] Malonedialdehyde (MDA) was measured using Thiobarbituric acid- reactive substances assay employing the molar absorption coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ and spectrophotometry at 532nm. Data were analyzed with t-test and expressed as mean ± SD. Data were compared in the groups by using SPSS

software version 16. P<0.05 was chosen as the level of significance.

Results

Table 1 indicates the general characteristics of the observed study population. No differences were noticed between the mean values of age, sex and family history of CAD in patient and control groups. The percent of hypertensive subject in the patient groups was significantly higher than that of control group (P‹0.05) (Table 1). The mean levels of MDA in the patient group were markedly higher than that of the control group (P<0.05) (Table 2). The activities of SOD and GPX and mean value of total antioxidant capacity in the patients group were meaningfully lower than those of the control group (P<0.001) (Table 2). Hs-CRP was also measured in the patient group and comparing with control group significant elevation was noticed (P<0.001) (Table2, Figure). The mean levels of total cholesterol, Triglyceride and LDL-C in the patient group were significantly higher, but that of HDL-C was lower than those of control group (P<0.001 in all cases) (Table3).

Table 1- The demographic and clinical data of the patient and control groups (mean ± SD)

Characteristics	Patients (n=60) mean ± SD	Controls (n=60) mean ± SD	P Value
Female/male, n	30/30	30/30	NS*
Age, years	58 ± 9.92	57 ± 11.02	NS
Hypertension, n (%)	33 (55%)	22(36.7%)	P=0.04
Family history, n (%)	25 (41.7%)	19 (31.7%)	NS

* NS: None-Significant.

Table 2- Status of oxidants/antioxidants and hs-CRP levels in CAD patients and healthy controls

Parameter	Patients(n=60) mean±SD	Controls(n=60) mean±SD	P value
MDA(nmol/ml)	3.39±1.19	1.56±1.17	p‹0.001
SOD(U/gHb)	972.49±216.96	1317.32±273.78	p‹0.001
GPX(U/gHb)	40.61±10.09	48.23±8.85	p‹0.001
hs-CRP(mg/dl)	3.81±2.57	1.64±1.43	p‹0.001
TAC(mmol/L)	0.88±0.22	1.14±0.16	p‹0.001

Table 3- Serum lipid profiles in CAD patients and healthy controls

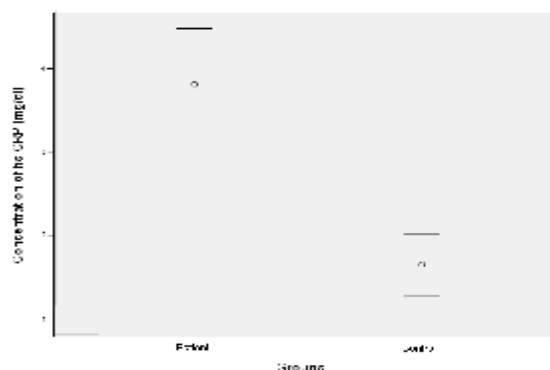| Parameter	Patient (n=60) mean ± SD	Control (n=60) mean ± SD	P value
TC (mg/dl)	195.6±41.71	174.88±49.26	P=0.01
HDL-C (mg/dl)	44.53±14.02	53.33±12.92	P=0.001
LDL-C (mg/dl)	130.67±41.67	112.43±35.07	P=0.01
TG (mg/dl)	163.21±65.21	133.06±61.37	P=0.01

Fig- The Difference in hs-CRP levels in the patient and control groups.

Discussion

The role of oxidative stress in the development of coronary artery disease is well known.[5] Reactive oxidant species can damage all types of biomolecules including; lipids, proteins and DNA. Antioxidative defence comprising of enzymatic and non enzymatic defence inactivate reactive species.[15, 16] Kostner et al. reported high MDA levels in coronary artery patients.[21] In a study of cavalca et al. in which serum free and total MDA were measured synchronously, significantly higher MDA levels were noticed in coronary artery patients in comparison to the control group.[22] Our study on CAD patients shows a significant increase in serum MDA levels. This finding is in accordance with the finding of Kostner et al. This increase in MDA levels might be resulted from increase in lipid peroxidation which increased lipid peroxidation itself is resulted from an increase in oxidative stress levels. NO is a potent stimulus for the expression of SOD.[23] SOD is an important antioxidant enzyme having an antitoxic effect against super oxide anion. The overexpression of SOD might be an adaptive response, and it results in increased dismutation of superoxide to hydrogen peroxide.[24] Glutathion peroxidase (GPX), a selenium containing enzyme, is an important antioxidant enzyme of erythrocytes.[15, 16] GPX plays a significant role in the peroxyl scavenging mechanism, and in maintaining functional integration of the cell membranes.[1] Our study indicated a significant decrease in SOD and GPX activities in CAD patients. The results of this study are in agreement with those reported by Kayyum et al.[3] SOD is a key antioxidant enzyme located strategically between the endothelium and

vascular smooth muscle cells in the compartment of the arterial wall where nitric oxide (NO) is capable of being inactivated by $O2^{\circ-}$ .Therefore, conditions resulting in NO depletion, such as atherosclerosis, could be associated with a concomitant decrease in extracellular SOD. In their study TAC levels were also significantly lower in CAD patients and association was observed between the measured parameters and the severity of the disease.[3] Fazendas et al,[25] reported that TAC levels decreased in young survivors of acute MI, Yegin et al,[26] also showed reduction in the level of TAC. Our findings are in consistent with them, and a decreased TAC level might be associated with an enhanced protective mechanism to oxidative stress in CAD. It is the conclusion of the authors that because of low levels of antioxidant parameters in our subjects, we advocate diet supplementation with antioxidants (vitamins E and C) for CAD patients. This will reduce the progression of cardiovascular diseases through endothelial dysfunction and lipoprotein oxidation. There is increasing evidence that inflammation plays an important role in pathogenesis of atherosclerosis and its complications. It has been suggested that hs-CRP may not only be a marker of generalized inflammation but also directly and actively participate in atherogenesis.[27] Increased hs-CRP concentration is associated with metabolic disorders such as dyslipidemia. Thus, increased CRP levels and dyslipidemia have significant risk prediction value compared with those based on lipids alone in CAD patients. Many studies have shown an increase in serum hs-CRP levels in CAD patients of more than 50 years of age in males and females.[27, 28] Present studies on CAD patients revealed a significant increase in the level of hs-CRP in these patients. The results are in agreement with those reported by Espliguero et al. This increase in CRP concentrations might be associated by the fact that CRP binds to the LDL particle in atherosclerotic plaques leading to activation

of complement, thus, being proinflammatory and contributing to atherogenesis. CRP may also increases ischemic tissue damage by complement dependent mechanism and tissue factor production by macrophages. Dyslipidemia has been shown to be an important risk factor for CAD.[29] The high concentration of triglycerides and LDL-C and low levels of HDL-C have been reported in most studies.[30] Our study showed significant increase in serum LDL, TG and TC levels in CAD patients and also significant decrease in serum HDL in the patients. This might be associated by 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 may be an indirect marker of LDL particle size.

Conclusion

It was concluded that oxidative stress is a consequence of low level inflammation one of the mechanisms by which inflammation causes atherogenesis. The study supports the simultaneous use of antioxidative, anti-inflammatory agents and also lipid lowering regimen against the onset and progression of atherosclerosis.

Acknowledgment

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