

Original Article

Association between inflammatory factor, lipid peroxidation and total-antioxidant in non-diabetic patients of coronary artery disease

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Abstract

Introduction: The oxidative stress and inflammation are cooperative events involved in atherosclerosis development. In the present study, we assessed the association of malondialdehyde (MDA), antioxidant markers, high sensitive C-reactive protein (HS-CRP) and lipid status parameters in non-diabetic patients with coronary artery disease (CAD) or vessel heart disease (VHD). Significant risk factors such as diabetes were excluded from the study.

Methods: Oxidative stress parameters for example MDA, antioxidant markers including: erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GPX), total antioxidant capacity (TAC), inflammation marker and serum lipid status parameters were measured in 120 subjects including 60 CAD patients (non-diabetic) with angiographically diagnosed CAD and 60 CAD-free subjects as a control group, also diabetic 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 the controls. However, erythrocyte SOD, GPX activities and TAC level were reduced significantly in patients (non-diabetic) ($P < 0.05$ in all cases). The levels of total cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-c) were significantly higher and that of high-density lipoprotein cholesterol (HDL-c) was significantly lower than those of controls ($P < 0.05$ 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 many countries.¹ Among many traditional risk factors for CAD development, there are hypertension, hyperlipidemia, diabetes, age, sex, obesity, cigarette smoking and positive family history;² oxidative stress and inflammation are now considered as significant and novel risk factors.³⁻⁵ According to Kutuk and Basaga, lipid peroxidation and inflammation are comorbid 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-c) 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

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lipoprotein (HDL) is one of the most important independent protective factors against atherosclerosis and CAD.^{13,14}

Malondialdehyde (MDA), a carbonyl 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), high sensitive C-reactive protein (HS-CRP), fibrinogen and homocysteine 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 decreasing this disease in non-diabetic patients suffering from CAD.

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 and hepatitis were excluded from the experimental 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 fasting and were allowed to clot at room temperature for about 1 hour. Sera were separated from cells by centrifugation at 1500 x g 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 GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and Nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized glutathione (GSSG) was immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance at 340 nm was measured. The activity of GPX in blood samples were measured using commercially available Kit (Ransel: Randox laboratories Crumlin, U.K.). The role of SOD is to accelerate the dismutation of the toxic superoxide radical (O₂[•]), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. The activity of SOD in blood samples were measured using commercially available Kit (Ransel: Randox laboratories Crumlin U.K.). GPX and SOD results were reported asu/g Hb.¹⁸ ABTS (2, 2'-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 color, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree which is proportional to their concentration.¹⁹ Plasma TAC was determined using Randox total antioxidant status Kit (Randox) (NX2332). HS-CRP was measured by Commercial Kit (PARS AZMON. IRAN) by Immuno Turbidimetry.²⁰ MDA was measured using thiobarbituric acid-reactive substances assay employing the molar absorption coefficient of 1.56 × 10⁵ M⁻¹cm⁻¹ and spectrophotometry at 532nm.

Data were analyzed with t-test and expressed as mean ± Standard deviation. Data were compared in the groups using SPSS for Windows (version 16; SPSS Inc., Chicago, IL, USA). P < 0.05 was considered as a statistically significance level.

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 non-diabetic patient groups was significantly higher than that of control group ($P < 0.050$). The mean levels of MDA in the patient group were markedly higher than that of control group ($P < 0.050$) (Table 2).

The activities of SOD and GPX and mean value of TAC 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 ($P < 0.001$) (Table 2). 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 the control group ($P < 0.001$ in all cases) (Table 3).

Discussion

The role of oxidative stress in the development of CAD is well known.⁵ Reactive oxidant

species can damage all types of biomolecules including lipids, proteins and DNA. Antioxidative defense comprising of enzymatic and non-enzymatic defense 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.

Nitric oxide (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

Table 1. The demographic and clinical data of the patients (non-diabetic) and control groups

Characteristic	Patient (non-diabetic) (n = 60)	Control (n = 60)	P
Female/male, No.	30/30	30/30	NS
Age (years)	56 ± 6.81	54 ± 8.95	NS
Hypertension, No. (%)	28 (46.7%)	17 (28.3%)	** < 0.05
Family history, No. (%)	21 (35.0%)	16 (26.7%)	NS

Values are mean ± SD; *NS: Non-significant; ** A P-value < 0.05 considered statistically significant

Table 2. Status of oxidants/antioxidants and HS-CRP (High sensitive C-reactive protein) levels in patients (non-diabetic) and control groups

Parameter	Patients (non-diabetic) (n = 60)	Controls (n = 60)	P
MDA (nmol/ml)	4.59 ± 2.09	2.16 ± 1.08	< 0.01
SOD (U/gHb)	790.34 ± 114.36	1135.21 ± 165.69	< 0.01
GPX (U/gHb)	37.52 ± 09.06	46.12 ± 7.54	< 0.01
HS-CRP(mg/dl)	2.17 ± 1.32	0.82 ± 0.03	< 0.01
TAC (mmol/L)	0.65 ± 0.13	1.02 ± 0.08	< 0.01

Values are mean ± SD; * A P-value < 0.05 considered significant; MDA: Malondialdehyde; SOD: Superoxide dismutase; GPX: Glutathione peroxidase; HS-CRP: High sensitive C-reactive protein; TAC: Total antioxidant capacity

Table 3. Serum lipid profiles in patients (non-diabetic) and control groups

Parameter	Patient (non diabetic) (n = 60)	Control (n = 60)	P
TC (mg/dl)	199.00 ± 52.03	179.88 ± 50.12	< 0.05
HDL-c (mg/dl)	41.04 ± 11.08	51.12 ± 11.73	< 0.05
LDL-c (mg/dl)	134.54 ± 32.54	117.24 ± 26.12	< 0.05
TG (mg/dl)	180.13 ± 44.13	148.02 ± 52.25	< 0.05

Values are mean ± SD; * A P-value < 0.05 considered significant; TC: Total cholesterol; HDL-C: High-density lipoprotein; LDL-c: Low-density lipoprotein; TG: Triglyceride

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 Shaikh and Suryakar³ SOD is a key antioxidant enzyme located strategically between the endothelium and vascular smooth muscle cells in the compartment of the arterial wall where NO is capable of being inactivated by $O_2^{\cdot-}$. 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 myocardial infarction (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 advocated 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 Arroyo-Espliguero et al.²⁷ 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 with 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.

Conflict of Interests

Authors have no conflict of interest.

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