Study of antioxidant enzymes, lipid peroxidation, lipid profile and immunologic factor in coronary artery disease in East Azarbijan

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ABSTRACT

Background: The oxidative stress and inflammation are cooperative events involved in atherosclerosis development. Aim: 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 diabetes were excluded from the study. Materials and Methods: Oxidative stress parameters for example malondialdehyde (MDA), antioxidant markers including: erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GPX), and 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). Conclusions: 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.

Key words: Antioxidant, lipid profile, lipid peroxidation, coronary artery disease

INTRODUCTION

Coronary Artery Disease (CAD) is the major cause of mortality and morbidity in the most countries.\cite{1} Among many traditional risk factors for CAD development including hypertension, hyperlipidemia, diabetes, age, sex, obesity, cigarette smoking and positive family history,\cite{2} oxidative stress and inflammation are now being considered as significant and novel risk factors.\cite{3-5} According to Kutuk \textit{et al.}, lipid peroxidation and inflammation are cooperative events involved in atherosclerosis development.\cite{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.\textsuperscript{[7,8]} It has been reported that endothelial dysfunction and increased oxidative stress may predict future events in patients with CAD.\textsuperscript{[9]} A significant risk factor of CAD is low density lipoprotein cholesterol (LDL). The lipoprotein is believed to have a central role in atherogenesis.\textsuperscript{[10-13]} High density lipoprotein (HDL) is one of the most important independent protective factors against atherosclerosis and CAD.\textsuperscript{[13,14]}

Malondialdehyde (MDA), a carbonyl group produced during lipid peroxidation, is used widely in determining oxidative stress.\textsuperscript{[5]} The activities of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) in erythrocytes and non-enzymatic antioxidants along with total antioxidant capacity (TAC) have been reported as predictive indices of CAD.\textsuperscript{[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.\textsuperscript{[15,16]}

The study was designed to evaluate the frequency of risk factors of CAD, inflammation markers, dyslipidemia and oxidative stress and the influence of antioxidant parameters on non-smoker and non-diabetic patients suffering from coronary artery disease.

**MATERIALS AND METHODS**

**Subjects**
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. Smokers and patients with chronic obstructive pulmonary disease, diabetes mellitus, renal disease, hepatitis and 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.

**Biochemical analysis**
All participation underwent blood sampling after 8-12 hour fasting. Blood samples were allowed to clot at room temperature for 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\textsuperscript{[17]} using commercially available Kit (Ransel: Randox laboratories Crumin U.K). The activity of SOD in blood samples were measured using available Kit (Ransel: Randox laboratories Crumin U.K). GPX and SOD results were reported as u/g Hb.\textsuperscript{[18]} ABTS (2, 2':-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and $H_2O_2$ 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.\textsuperscript{[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.\textsuperscript{[20]} Malonialdehyde (MDA) was assayed using Thiobarbituric acid- reactive substances assay using the molar absorption coefficient of 1.56x10$^5$ M$^{-1}$cm$^{-1}$ and spectrophotometry at 532nm.

**Data analysis**
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 ($P<0.05$) (Table1).
Table 1: The demographic and clinical data of the patient and control groups

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

* NS: None-Significant.
** A p-value < 0.05 considered significant.

The mean levels of MDA in the patient group were markedly higher than that of 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).

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients(n=60) mean±SD</th>
<th>Controls(n=60) mean±SD</th>
<th>* P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA(nmol/ml)</td>
<td>3.39±1.19</td>
<td>1.56±1.17</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>SOD(U/gHb)</td>
<td>972.49±216</td>
<td>1317.32±273</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>GPX(U/gHb)</td>
<td>40.61±10.09</td>
<td>48.23±8.85</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP(mg/dl)</td>
<td>3.81±2.57</td>
<td>1.64±1.43</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>TAC(mmol/L)</td>
<td>0.88±0.22</td>
<td>1.14±0.16</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

* A p-value < 0.05 considered significant.

Hs-CRP was also measured in the patient group and comparing with control group significant elevation was noticed (P<0.001) (Table 2, Figure 1). 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) (Table 3).

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient (n=60) mean ± SD</th>
<th>Control (n=60) mean ± SD</th>
<th>* P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>195.6±41</td>
<td>174.88±49</td>
<td>P=0.01</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>44.53±14.02</td>
<td>53.33±12.92</td>
<td>P=0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>130.67±41</td>
<td>112.43±35</td>
<td>P=0.01</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>163.21±65</td>
<td>133.06±61</td>
<td>P=0.01</td>
</tr>
</tbody>
</table>

* A P-value < 0.05 considered significant.
DISCUSSION

The role of oxidative stress in the development of coronary artery disease is well known. 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. Kostner et al. reported high MDA levels in coronary artery patients. 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. Our findings revealed a significant rise 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. SOD is an important antioxidant enzyme having an antitoxic effect against super oxide anion. The over-expression of SOD might be an adaptive response, and it results in increased dismutation of superoxide to hydrogen peroxide. Glutathione peroxidase (GPX), a selenium containing enzyme, is an important antioxidant enzyme of erythrocytes. GPX plays a significant role in the peroxyl scavenging mechanism, and in maintaining functional integration of the cell membranes. This study showed that SOD and GPX activities were significantly decreased in CAD patients. The results of this study are in agreement with those reported by Kayyum et al. SOD is an important enzymatic antioxidant which is positioned in the arterial wall where nitric oxide (NO) may be inactivated by $O_2^-$. Hence, pathological states like atherosclerosis which results in NO depletion may be associated with a fall 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. Fazendas et al. reported that TAC levels decreased in young survivors of acute Myocardial Infarction, Yegin et al. 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.

Inflammation has been shown to plays a significant role in the process of atherosclerosis and its attendant 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. 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. 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.
increase in CRP concentrations might be associated with the fact that CRP binds to the LDL particle in atherosclerotic plaques leading to activation of complement, thus, being proinflammatory and contributing to atherogenesis, and it may also increases ischemic tissue damage by complement dependent mechanism and tissue factor production by macrophages. [27]

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. [31] Hence, serum triglyceride may be a marker of LDL particle size.

CONCLUSION

It was concluded that inflammation causes atherogenesis via oxidative stress. The study lends credence to the simultaneous use of anti-inflammatory, lipid lowering, and anti-oxidative agents in the prevention of the onset and progression of atherosclerosis.

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PREVIOUS PUBLICATION

This study has been published in part as an abstract in the 12th Iranian Congress of Biochemistry and 4th International Congress of Biochemistry and Molecular Biology held in Mashhad, Iran, on 6th-9th September, 2011.


Conflict of Interest: None declared