ABSTRACT

Background: Diabetes Mellitus (DM) is the most common disorder characterized by metabolic abnormalities and long term complications. Patients with type 2 diabetes mellitus are more prone to Coronary Artery Disease (CAD). Although oxygen free radicals are known to contribute to the development of CAD and diabetes. Oxidative stress occurs as a result of increased level of lipid peroxides and free radical intermediates, as well as the decreased in total antioxidant capacity.

Aim: In the present study, our aim was to investigate the lipid peroxidation, lipid profile and antioxidant enzymes in diabetic patients with and without CAD.

Materials and Methods: The study was carried out in 62 patients suffering from diabetics, 59 patients suffering from diabetes with CAD and 78 healthy controls were randomly selected. Various parameters like serum lipid profile, Malondialdehyde used as an index of oxidative stress, antioxidant enzymes like Glutathione peroxidase (GPx), Superoxide dismutase (SOD), and Paraoxonase (PON) were measured and compared.

Results: Increased levels of MDA concentration, total cholesterol, triglycerides, LDL-cholesterol, while decreased levels of HDL-cholesterol, GPx, SOD and PON were significantly low (p<0.001) in diabetes with CAD patients compared to diabetes without CAD patients.

Conclusion: The hypothesis of the current study indicates that increased concentration of lipids and lipid peroxide levels may be contribute to decreased levels of antioxidant enzymes that are associated with increased consequences of diabetes leading to Coronary artery disease.

Keywords: Lipid peroxidation, Lipid profile, Glutathione peroxidase, Superoxide Dismutase, Paraoxonase.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder resulting from a number of factors in which an absolute or relative deficiency of insulin or its function occurs [1]. World health organization has reported that global prevalence of diabetes will increase more than double from 135 million to 300 million by 2025. India stands as the first in whole world to have the largest number of diabetes mainly Non Insulin Dependent Diabetes mellitus (NIDDM) [2].

Coronary Artery Disease (CAD) is a rising problem in developing countries like India. Epidemiological surveys indicate that there could be 15-20 million cases of CAD in India. The incidence is higher in urban than rural population [3].

Diabetes mellitus threatens to become a global health crisis; treating diabetes and its complications is going to dominate future health care expenditures. NIDDM accounts for about 90% of the total diabetic population, and coronary artery disease (CAD) is the most common cause of morbidity and mortality. Cardiovascular deaths are increased up to fourfold in diabetics compared with their nondiabetic counterparts. The
development of atherosclerosis is closely associated with risk factors such as hypertension (HTN), obesity; smoking, dyslipidemia and mainly diabetes have been identified [4].

Oxidative stress is defined as the interruption of balance between oxidants and reductants within the body due to the excess production of peroxides and free radicals. This imbalance will cause damage to cellular components and tissues in the body leading to oxidative stress and as well as the decrease in total antioxidant capacity [5].

Hyperglycemia, a hallmark of diabetic depletes natural antioxidant and facilitates the production of reactive oxygen species (ROS) which has the ability to react with all biological molecules like lipids, proteins, carbohydrates, DNA etc and exert cytotoxic effects on cellular components. Thus as increased ROS and impaired antioxidant defense contribute for the initiation and progression of micro and macro vascular complications in diabetes [6].

DM is characterized by hyperglycemia together with biochemical alternations of glucose and lipid peroxidation. Lipid peroxidation is a free radical related process, which is potentially harmful because its uncontrolled, self-enhancing process causes disruption of membranes, lipids and other cell components. A lot of oxygenated compounds, particularly aldehydes such as Malondialdehyde (MDA) are produced during the attack of free radicals to membranes, lipoprotein and polyunsaturated fatty acids [7]. Thus lipid peroxidation in the blood provides useful information for the prognosis of diabetes in which secondary disorders are often fatal.

Serum Paraoxonase (PON) synthesized in the liver and it is High density lipoprotein cholesterol (HDL-C) associated enzyme that prevents oxidative modification of Low density lipoprotein cholesterol (LDL-C). Serum PON is responsible for the antioxidant activity of HDL-C [8].

Sufficient levels of antioxidants are important to live with healthy condition for human beings and most important for the prevention of chronic diseases such as cancer, diabetes and CAD. Low plasma levels of antioxidant as well as low intake of dietary antioxidants have been associated with an increased risk of atherosclerotic heart disease [9]. Enzymatic Glutathione peroxidase (GPx), Superoxide Dismutase (SOD) and PON play an important role in alleviating tissue damage due to formation of free radicals. Moreover, the body defense mechanism would play an important role in the form of antioxidants and try to minimize the damage adapting itself to the above stressful situation.

Hence a systemic approach has been made in the present study to focus on the Diabetes and Coronary artery disease. Oxidative stress was measured by the serum levels of MDA, which is widely used as an index of the extent of oxidative damage, stress related enzymes such as GPx, SOD and PON were measured and compared in type 2 diabetic subjects with and without Coronary Artery Disease along with normal healthy subjects.

**MATERIALS AND METHODS**

The study was case controlled in design. We have selected the patients as they are presented. The study was only male oriented. Patients included in the present study were all admitted to the medicine unit or attending the Out Patient Department (OPD) of medicine and some patients were admitted to the intensive coronary care unit (ICCU) of Al- Quwayiyah Government General Hospitals, Shaqra University, Kingdom of Saudi Arabia, during the period from October 2011 to August 2012 were included in the study.

Consecutive 121 patients with type 2 diabetes mellitus, admitted to hospital were selected for the study and they were between 40-65 years. They were further classified into two groups. 62 patients were diabetes mellitus without CAD and another group of 59 patients were diabetes mellitus with CAD. The criteria for the diagnosis of type 2 diabetes was fasting blood glucose >126.0 mg/dl [10] and glycated hemoglobin (HbA1c) 6.2% [11].
The criteria for the diagnosis of CAD was made on the basis of clinical history, history of myocardial infarction, 12 leads electrocardiogram (ECG) and coronary angiography findings. Smoking was defined as regular smoking of cigarettes / Beedies (a local type of tobacco). Those patients whose body mass index (BMI) >25 were considered as obese. Controls had 78 healthy age matched, non diabetes, non myocardial infarction, non smoking and non alcoholic healthy individuals.

All diabetic patients selected for this study were on irregular treatment for diabetics and none of the study subjects was an antioxidant supplementation or lipid lowering drugs. Subjects suffering from renal, hepatic disease and any chronic or acute inflammatory illness were excluded from the study.

All participants gave written informed consent and this protocol was approved by the ethical and human research committee of College of Applied Medical Sciences, Shaqra University, Al-Quwayiyah, Kingdom of Saudi Arabia.

Fasting venous blood samples were collected from all the study subjects after an overnight fast. Fasting glucose levels were estimated by enzymatic methods [12]. Glycemic control was assessed by measuring glycated hemoglobin by the resin-ion exchange method [13]. The lipid profile was done by fully auto analyzer (ERBA-XL-300). The concentration of serum Cholesterol was estimated by CHOD- PAP method [14], Triglycerides level was estimated by GPO (trinder) method [15], while HDL-C estimation was done by Phosphotungestic method [16] and LDL-C levels were estimated by enzymatic methods [17]. Serum levels of MDA, a marker of lipid peroxidation were measured by thiobarbutric acid (TBA) method [18]. The haemolysate prepared from the red cells was used for the estimation of antioxidant enzyme activities. GPx was measured by the method of Paglia and Valentine [19]. SOD estimation was based on the reaction between superoxide radicals and 2-4-

iodophenyl 3-4- nitrophenol- 5-phenyl tetrabuzium chloride to form a red formazon dye [20]. PON activity was estimated by using 5.5 Mm p-nitro phenyl acetate (sigma chemicals Co.,) as a substrate. The change in the absorbance at 412 nm due to the formation of p-nitro phenol was measured by using ELICO spectrometer [21].

**STATISTICAL ANALYSIS**

All values are expressed as mean ±SD. Student t-test was used to estimate the significant difference between the groups. Pearson’s correlation analysis was used to test the correlation between various parameters and considered significant when p<0.05. SPSS for windows 13.0 was used for statistical analysis.

**RESULTS**

The clinical characteristic of the two groups of type 2 diabetes mellitus patients and control subjects are presented in Table I. In present study number of smokers and hypertensive were more in diabetes mellitus with CAD compared to diabetes mellitus without CAD and controls.

Serum levels of fasting glucose, HbA1c, total cholesterol, triglycerides, LDL-C were significantly higher in diabetes with CAD compare to controls (p<0.001), whereas decreased HDL-C (p<0.001) levels in diabetics with and without CAD patients as compared to controls Table II. Antioxidant enzymes such as GPx, SOD and PON were significantly decreased (p<0.001) in diabetics without CAD and further decreased in diabetes with CAD as compared to controls (p<0.001). Whereas MDA levels were significantly increased (p<0.001) in diabetes without CAD and further decreased in diabetes with CAD as compared to controls (p<0.001). Whereas MDA levels were significantly increased (p<0.001) in diabetes with CAD compared to diabetes without CAD and controls as shown in Table III.

In order to ascertain any relationship between HbA1c levels with various antioxidant enzymes and stress factors, linear correlation analysis was carried out. Results revealed that GPx, SOD and PON activities were negatively correlated with HbA1c level. Whereas MDA concentration was
positively correlated with HbA1c levels in diabetics without CAD and diabetics with CAD as shown in Table IV.

**DISCUSSION**

Type 2 (Non-Insulin-Dependent) Diabetes is associated with a marked increase in the risk of coronary heart disease. It has been debated whether patients with diabetes who have not had myocardial infarctions should be treated as aggressively for cardiovascular risk factors as patients who have had myocardial infarctions [22]. Although it has been apparent for some time that coronary heart disease (CHD) is the major cause of morbidity and mortality in patients with type 2 diabetes. Framingham study demonstrated a direct association between diabetes and heart failure [23]. CAD occurs due to a number of factors in diabetics; both insulin resistance and elevated lipid levels, common in diabetics primarily triggers atherogenic injury. It is also suggested that endothelium in diabetic arteries is more prone to atherogenic injury due to decreased production of endothelial nitric oxide, known to be antiatherogenic, and increased production of plasminogen activator inhibitor [24].

Tobacco Smoking is one of the most powerful modifiable risk factor for the development of CAD and diabetes patients [25]. Our data showed that prevalence of smoking was significantly higher in diabetes with CAD patients as compared to controls. Like other studies [26, 27] in our study also, hypertension and obesity was found to be significantly high in diabetic patients and further increased in diabetic with CAD patients. Atherosclerosis is a process for which there is substantial evidence of a role for oxidative stress. Hypercholesterolemia and triglyceridemia are independent risk factor that alone or together can accelerate the development of CAD and progression of atherosclerotic lesions. HDL may be protective by reversing cholesterol transport, inhibiting the oxidation of LDL and by neutralizing the atherogenic effects of oxidized LDL [28].

MDA is a natural product of lipid peroxidation and reflects the oxidant status of the biological systems. It has been demonstrated that high MDA levels are associated with high oxidative stress in diabetes mellitus and CAD [29]. Several authors have reported increased levels of lipid peroxidation in diabetic patients [30, 31] while a few could not find any significant increase in lipid peroxidation in diabetes [32, 33]. Few authors have reported increased levels of MDA in CAD patients [6, 34]. In our study also, increased levels of MDA in diabetic patients and further increased in diabetic with CAD patients compared to controls, because at a time two diseases have a still greater degree of oxidative stress.

Earlier studies on the relationship between the lipid peroxides and glycemic controls have yielded conflicting results. Kesavulu et al [35] and Losada et al [36] have shown a positive correlation between MDA and the measure of blood glucose control such as HbA1c, while several authors could not [37]. Similarly, positive correlation between the MDA concentrations versus HbA1c levels was observed in the present study. The estimation of lipid peroxidation in the diabetes and CAD patients is very useful as it may serve as a useful monitor to judge the prognosis of the patients.

Free radicals scavenging enzymes such as GPx and SOD are the first line of cellular defense against oxidative injury, which are involved in the disposal of superoxide anions and hydrogen peroxide [28]. One of the most important antioxidant enzymes found in the humans is GPx; it protects the cell damage by catalyzing the reduction of lipid hydroperoxides and also protects the heart from damage by oxidative stress due to oxygen free radicals through its antioxidant effect [38]. A few authors have reported increased GPx activity in the RBC of type 2 diabetes [39]. In contrast, some reports have described a decreased activity of erythrocyte GPx in diabetic patients...
[35] and CAD patients [40]. Similarly in the present study also, it was found significantly decreased activity of GPx in diabetic patients due to decreased activity of glucose-6-phosphate dehydrogenase in erythrocytes [28]. However hypercholesterolemia is also one of the reasons for the decreased GPx activity in CAD patients. So we have observed further decreases in diabetic with CAD patients. Another one of the important antioxidant enzymes present in human body is SOD. Some studies reported reduced levels of SOD activity in diabetes [41] and Suresh Chari et al [42] says decrease in the activity of SOD in CAD patients. On the other hand jain et al [43] reported on increased levels of SOD in diabetes and MM Kesavulu et al [44] revealed that there is no change in the activity SOD in CAD patients, but Sree hari babu et al [45] says decreased levels of SOD activity in diabetic patients. Whereas in the present study decreased activity of erythrocyte SOD was observed in diabetic without CAD patients and further decreased in diabetic with CAD patients because of superoxide is the main reactive oxygen species which react with nitric oxide radical and forms peroxynitrite thereby causing oxidative stress, cellular damage and also increased levels of lipid peroxidation and conjugated dienes [46]. This would seem unexpected because in a disease with elevated oxidative compounds, a compensatory increase in antioxidant enzymes would be desirable.

It was tested whether there was a correlation between HbA1c levels and SOD, GPx activity and our results showed a significant negative correlation between HbA1c versus GPx and SOD levels, which suggest that the enzymes could be glycated. Similarly Faure et al [47] and Nath et al [48] revealed that there was a significant negative correlation between HbA1c versus SOD and GPx level.

In recent studies, reduced serum PON activity has been reported to be associated with increased risk of insulin resistance [49]. Mackness et al [21] and Leivev et al [50] reported the PON activity was significantly lowered in patients with diabetes mellitus and CAD. Lower serum PON activity has been associated with increased susceptibility to atherosclerosis, neuropathy, retinopathy and other complications in diabetic population compared with healthy controls. In the present study also, a decreased PON activity was observed in diabetes and this further decreased in diabetes with CAD patients. Thus there was a significant negative correlation between HbA1c and serum PON activity.

There were some limitations in the present study, sample size was small and it was a hospital based study, so can’t represent whole population. There is need to perform such studies on larger and community based population.

CONCLUSION

It is very clear from this study that there are abnormalities in lipid profile, lipid peroxide levels and antioxidant enzymes in diabetic patients with and without CAD. We hypothesized that reduced antioxidant enzyme activities and increased MDA levels may contribute to the increased susceptibility for the development of insulin resistance and CAD in patients with NIDDM. The present study illustrates that reduced consumption of alcohol, smoking, animal saturated fat and increased consumption of n-3 fatty acids, intake of fruits and vegetables, tree nuts, natural of antioxidants, supplementation of trace elements, physical activity and maintenance of healthy body weight and secondary measures like control of hyperglycemia and HTN are the measures to mitigate the devastating consequences of diabetes which further may lead to cardiovascular diseases. Thus, further investigations of therapeutic strategies to prevent or delay the progression of diabetic cardiovascular complications are needed.

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Table 1. Clinical details of the study subjects

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Controls (n=78) Mean ±SD</th>
<th>Diabetes without CAD (n= 62) Mean ±SD</th>
<th>Diabetes with CAD (n= 59) Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>47.6 ± 7.9</td>
<td>50.2 ± 12.3</td>
<td>53.8 ± 11.4 *</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.7 ± 8.1</td>
<td>25.2 ± 3.5</td>
<td>28.0 ±4.2 *</td>
</tr>
<tr>
<td>HTN %</td>
<td>7 %</td>
<td>59 %</td>
<td>70 % *</td>
</tr>
<tr>
<td>Smokers %</td>
<td>9 %</td>
<td>62 %</td>
<td>72 % *</td>
</tr>
</tbody>
</table>

* P<0.001, Highly significantly compared to controls. BMI= Body mass Index, HTN= Hypertension, CAD= Coronary Artery Disease.
Table 2: Biochemical details of study subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n=78) Mean ±SD</th>
<th>Diabetes without CAD (n= 62) Mean ±SD</th>
<th>Diabetes with CAD (n= 59) Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Blood Glucose (mg/dl)</td>
<td>90.3 ± 13.5</td>
<td>189.0 ± 17.9*</td>
<td>223.0 ± 21.2*</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>5.1 ± 0.6</td>
<td>7.9 ± 0.8*</td>
<td>8.2 ± 0.7*</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>146.0 ± 17.5</td>
<td>181.0 ± 18.4*</td>
<td>287.0 ± 28.1*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>111.2 ±15.8</td>
<td>122.0 ±16.3*</td>
<td>230.0 ±22.2*</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>50.1 ±5.0</td>
<td>46.0 ±5.3*</td>
<td>32.2 ±5.9*</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>72.0 ±21.8</td>
<td>90.0 ±24.2*</td>
<td>192.0 ±30.2*</td>
</tr>
</tbody>
</table>

* P<0.001, Highly statistically significantly vs controls. CAD= Coronary Artery Disease, HbA1c= Glycated Haemoglobin, HDL-C= High density lipoprotein cholesterol, LDL-C= Low density lipoprotein cholesterol.

Table 3: Activities of erythrocyte Antioxidant enzymes and MDA Concentration in study subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n=78) Mean ±SD</th>
<th>Diabetic without CAD (n= 62) Mean ±SD</th>
<th>Diabetic with CAD (n= 59) Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (U/gHb)</td>
<td>36.2 ± 13.1</td>
<td>25.2 ± 3.7*</td>
<td>19.5 ± 2.8*</td>
</tr>
<tr>
<td>SOD (U/gHb)</td>
<td>8.9 ± 1.9</td>
<td>7.0 ± 0.86*</td>
<td>5.6 ± 0.46*</td>
</tr>
<tr>
<td>Serum PON (U/ml)</td>
<td>196.1 ± 17.8</td>
<td>160.1 ± 16.2*</td>
<td>130.2 ± 10.2*</td>
</tr>
<tr>
<td>MDA (nmoles/ml)</td>
<td>3.6 ± 0.5</td>
<td>6.8 ± 0.40*</td>
<td>8.7 ± 1.3*</td>
</tr>
</tbody>
</table>

*P<0.001, Highly statistically significantly compared to controls. GPx=Glutathione peroxidase, SOD= Superoxide dismutase, PON= Paraoxonase, MDA= Malondialdehyde.

Table 4: Correlation Analysis of HbA1c levels between Diabetics with and without CAD patients

<table>
<thead>
<tr>
<th></th>
<th>Diabetes without CAD ‘r’</th>
<th>Diabetes with CAD ‘r’</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c / GPx</td>
<td>- 0.62*</td>
<td>- 0.69*</td>
</tr>
<tr>
<td>HbA1c / SOD</td>
<td>- 0.73*</td>
<td>- 0.79*</td>
</tr>
<tr>
<td>HbA1c / PON</td>
<td>- 0.88*</td>
<td>- 0.92*</td>
</tr>
<tr>
<td>HbA1c / MDA</td>
<td>0.80*</td>
<td>0.89*</td>
</tr>
</tbody>
</table>

*significant at p<0.05, r= correlation coefficient. GPx=Glutathione peroxidase, SOD= Superoxide dismutase, PON= Paraoxonase, MDA= Malondialdehyde, HbA1c=Glycated Haemoglobin.