Oxidative stress and Antioxidant status in Ghanaians with Type 2 Diabetes Mellitus


1&4 Senior Lecturers, Department of Chemical Pathology, U.G.M.S, Korle-Bu, Ghana
2Assistant Lecturer, Department of Chemical Pathology, U.G.M.S, Korle-Bu, Ghana
3MPhil. Student, Department of Chemical Pathology, U.G.M.S, Korle-Bu, Ghana
5Senior Lecturer, Department of Medical Laboratory Science, S.A.H.S, Korle-Bu, Ghana

ABSTRACT
An imbalance between reactive oxygen species production and antioxidant scavenging has been implicated in type 2 Diabetes Mellitus (DM). Reports indicate that several complications of Diabetes Mellitus results from increased activity of free radicals and accumulation of lipid peroxidation products leading to oxidative stress. The study aimed at investigating levels of Malondialdehyde, Glutathione Peroxidase and Superoxide Dismutase in type 2 Diabetic subjects. One hundred type 2 Diabetes Mellitus subjects and one hundred Non-Diabetic controls were included in this study. Fasting venous blood sample was obtained for the analysis of glucose, glycated hemoglobin, lipid profile, Malondialdehyde, Superoxide Dismutase and Glutathione Peroxidase. Diabetic subjects had significantly higher total cholesterol (6.8±1.2 Vs 4.3±0.7mmol/L), triglyceride (1.3±0.4 Vs 1.0±0.3mmol/L), LDL-cholesterol (4.0±1.5 Vs 2.7±0.6mmol/L), serum Malondialdehyde (4.40±1.96 Vs 2.75±1.05µM) compared to controls. However, HDL-cholesterol (1.4±0.5 Vs 1.6±0.1mmol/L), Superoxide Dismutase (3.81±1.64 Vs 10.4±2.55 U/Ml) and Glutathione Peroxidase (130.0±16.8 Vs 174.8±36.1 U/L) activities were significantly reduced in type 2 Diabetic subjects when compared with controls. Superoxide Dismutase and Glutathione Peroxidase were found to be decreased in type 2 Diabetes Mellitus patients due to increased oxidative stress.

KEYWORDS: Oxidative stress, Diabetes Mellitus, Antioxidants and Lipid peroxidation

INTRODUCTION
Oxidative stress is the result of imbalance in oxidant/antioxidant ratio in favor of the former, potentially leading to macromolecules and cell dysfunction [1, 2]. Enhanced oxidative stress leads to severe impairment of glucose-stimulated insulin secretion as a result of destruction of pancreatic β-cells and decreased β-cell numbers, resulting in the pathogenesis of Diabetes Mellitus [3]. In Diabetes Mellitus, impairment in the oxidant/antioxidant balance can damage cellular macromolecules, leading to protein modification and lipid peroxidation. Lipid peroxidation results in the generation of Malondialdehyde (MDA) [4-5]. Serum MDA has been used as a bio-marker of lipid peroxidation and has served as an indicator of free radical damage. Hyperlipidemia has also been reported as one of the causative factors for increased lipid peroxidation in diabetes mellitus [6-7].

Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) in blood have also been cited as markers for vascular injury in type 2 Diabetes Mellitus [8-9]. In patients with type 2 Diabetes Mellitus, increased oxidative stress and lower concentrations of antioxidants have been reported but results have been inconsistent [10-11]. MDA levels were found to be significantly lower in Diabetic subjects with complications [12-14] and without complications (15-16). Others have reported no change in oxidative stress [17-18]. Thus, the aim of this study was to investigate the association between lipid peroxidation and antioxidant status in type 2 Diabetics in Ghana.

MATERIALS AND METHODS
This was a hospital-based case-control study. The case subjects were made up of 100 diagnosed type 2 Diabetes Mellitus patients who had undergone routine clinical review at the out patients clinic of National Diabetes Management and Research Centre (NDMRC) of the Korle-Bu Teaching Hospital, Accra. The control group consisted of 100 Non-Diabetic age-matched subjects. Participants who gave their consent also answered a standard questionnaire. The questionnaire administered was intended to obtain information on the subject’s age, smoking habits, alcohol...
consumption, medications and duration of disease. The study was approved by the Ethical and Protocol Review Board of the University Ghana Medical School. Informed consent was obtained from each participant. Smokers and users of regular antioxidant supplements (vitamin C and folic acid) for at least one month before the start of the study were excluded. Anthropometric measurements such as weight and height were taken to obtain body mass index (BMI) of subjects. Blood pressure was measured using a mercury sphygmomanometer and stethoscope after subjects had rested for at least 15 minutes.

**Laboratory Procedure**

Blood sample were collected from subjects between 6 am and 8 am for all assays after 10-12 hours of overnight fast. Of the eight milliliters (8mls) of whole blood drawn, 2mls was dispensed into tubes containing fluoride oxalate and the plasma separated for the estimation of glucose; 2mls of blood was further dispensed into potassium dihydrogenphosphate oxalate and the plasma separated for the estimation of glycated hemoglobin (HbA1C); whiles the remaining 4mls were dispensed into serum separators for processing. The resulting sera were aliquoted into 1ml portions and stored at -20°C until required for analysis. Kits for the estimation of blood glucose, HbA1C, Total cholesterol, Triglycerides, High density lipoprotein cholesterol, were purchased from Vital Microlab 300M automated chemistry analyzer was used for biochemical analysis.

**Statistical Analysis**

Data was entered unto a spreadsheet and analyzed using Microsoft Office Excel 2007(Louisville, Kentucky). All data were expressed as mean plus/minus standard deviation (mean ± SD). The statistical package for social sciences (SPSS) version 20 was used for statistical evaluation. Significance of differences was determined using student t-test. Pearson’s correlation coefficient was determined within groups. Statistical significance was set at p-values < 0.05.

**RESULTS**

Hundred (100) subjects with type 2 Diabetes were matched for age with 100 Non-Diabetics. The clinical and biochemical parameters of the study population are shown in table 1.

### Table 1: Clinical and Biochemical Parameters of the Study Population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetics (N = 100)</th>
<th>Non-Diabetics (N = 100)</th>
<th>95% CI of mean difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>50.3 ± 5.4</td>
<td>48.8 ± 6.0</td>
<td>-1.990 – 7.810</td>
<td>0.1304</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>135.1 ± 19.1</td>
<td>102.9 ± 16.8</td>
<td>-10.860 – (-4.329)</td>
<td>0.0001**</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82.3 ± 12.4</td>
<td>70.9 ± 8.9</td>
<td>-3.394 – 0.1977</td>
<td>0.0001**</td>
</tr>
<tr>
<td>BMI (Kg/m^2)</td>
<td>29.7 ± 3.1</td>
<td>22.0 ± 2.5</td>
<td>-1.287 – (-0.438)</td>
<td>0.0001**</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>8.2 ± 2.6</td>
<td>4.8 ± 0.5</td>
<td>-5.917 – (-3.76)</td>
<td>0.0021*</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>8.9 ± 1.6</td>
<td>6.0 ± 0.9</td>
<td>-2.970 – (-1.813)</td>
<td>0.0018*</td>
</tr>
<tr>
<td>T. Chol (mmol/L)</td>
<td>6.8 ± 1.2</td>
<td>4.3 ± 0.7</td>
<td>-0.329 – (-0.036)</td>
<td>0.0014*</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.3 ± 0.4</td>
<td>1.0 ± 0.3</td>
<td>-0.733 – (-0.240)</td>
<td>0.0019*</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.4 ± 0.5</td>
<td>1.6 ± 0.1</td>
<td>-1.073 – 3.091</td>
<td>0.1760</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>4.0 ± 1.5</td>
<td>2.7 ± 0.6</td>
<td>-0.509 – 0.028</td>
<td>0.0052*</td>
</tr>
<tr>
<td>VLDL (mmol/L)</td>
<td>0.7 ± 0.4</td>
<td>0.5 ± 0.2</td>
<td>-0.514 – (-0.226)</td>
<td>0.0284*</td>
</tr>
<tr>
<td>MDA (µM)</td>
<td>4.40 ± 1.96</td>
<td>2.75 ± 1.05</td>
<td>-2.287 – (-0.944)</td>
<td>0.0017*</td>
</tr>
<tr>
<td>SOD (U/MI)</td>
<td>3.81 ± 1.64</td>
<td>10.4 ± 2.55</td>
<td>3.835 - 2.276</td>
<td>0.0023*</td>
</tr>
<tr>
<td>GPx (U/L)</td>
<td>130.0 ± 16.8</td>
<td>174.8 ± 36.1</td>
<td>49.274 - 63.331</td>
<td>0.0016*</td>
</tr>
<tr>
<td>Duration of DM (years)</td>
<td>7.70 ± 2.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1 shows the results for the clinical and biochemical parameters of the study population. Values are given as mean ± standard deviation. *mean difference is significant (p<0.05). **mean difference is highly significant (p<0.0001). HbA1C is glycated haemoglobin, MDA is Malondialdehyde, SOD is Superoxide Dismutase, GPx is Glutathione Peroxidase, Tchol is total cholesterol, TG is triglyceride, HDL is high density lipoprotein, LDL is low density lipoprotein, VLDL is very low density lipoproteins, SBP is systolic blood pressure, DBP is diastolic blood pressure, BMI is body mass index.
The mean ages of the case and control group were 50.3 and 48.8 years respectively. There was no significance difference in the mean age between type 2 Diabetics and controls (p= 0.13). The mean systolic and diastolic blood pressures were significantly higher in Diabetics compared with controls (p<0.001). Hypertension was prevalent (60%) among the type 2 Diabetic subjects. None of the healthy controls was hypertensive. The type 2 Diabetic subjects had significantly higher BMI than controls (p <0.001). The mean fasting blood glucose (FBG), serum total cholesterol, LDL, triglycerides and glycated hemoglobin levels were significantly higher in type 2 Diabetics compared to controls (p< 0.002). The mean Malondialdehyde (MDA) was significantly increased in the type 2 Diabetic subjects compared to controls (p < 0.002). There was significant reduction in the activities of both Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) in type 2 Diabetic subjects when compared to controls (p < 0.002).

Pearson’s correlation of biochemical variables and oxidative stress indices is shown in Table 2. There was significant negative correlation between plasma glucose, total cholesterol, triglycerides and low density lipoproteins levels with SOD and GPx. However, significant positive correlation was found between plasma glucose and total cholesterol levels with Malondialdehyde (MDA) levels in type 2 Diabetics.

### DISCUSSION

Oxidative stress and endothelial dysfunction have been implicated in patients with type 2 Diabetes Mellitus [19-20]. Alterations in lipid peroxidation, leading to increased free radical formation have also been associated with type 2 Diabetes Mellitus [21-23]. Peroxidation of polyunsaturated fatty acids in blood produces Malondialdehyde (MDA) that is frequently used to determine the oxidant/antioxidant balance in Diabetic patients [24-25]. In this study, serum MDA levels in type 2 diabetic subjects were significantly elevated compared to controls. Furthermore, there was a positive correlation between fasting blood (glucose, total cholesterol, triglycerides, LDL-cholesterol) levels and MDA levels.

This finding agrees with earlier studies [26-28]. Contrasting views have also been reported [29-30]. The increased levels of MDA could be as a result of increased glycation of proteins in Diabetes Mellitus [13, 31]. The glycated protein may themselves act as a source of free radical [27, 32]. Elevated levels of MDA could also be due to alteration in the function of erythrocyte membranes [13]. Blood glucose and glycated hemoglobin levels in this study were, significantly elevated in Diabetic subjects compared to controls and also showed positive correlations with total cholesterol, LDL-cholesterol and triglyceride levels. These findings supported prior studies [27, 31]. Hyperglycemia (poor glycemic control) leads to over production of reactive oxidants and subsequent destruction of various macromolecules in the body including lipids through the mechanism of oxidative stress [33].

Glycated haemoglobin levels above 7% may also trigger oxidative stress, which contribute to tissue damage through oxidation of low-density lipoprotein and exacerbation of endothelial dysfunction, leading to the development and progression of small vessel vaculopathies [34-35]. The significantly high level of serum lipids in type 2 Diabetic subjects found in this study may be as a result of increased mobilization of free fatty acids from peripheral depots, due to loss of the inhibitory action of hormone sensitive lipase in Diabetics [36]. The exact mechanism however, by which the elevated blood glucose leads to membrane lipid peroxidation

### Table 2: Pearson’s correlation table of biochemical variables and oxidative stress indices in the Diabetic subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>MDA(µ/M)</th>
<th>SOD(U/MI)</th>
<th>GPx(U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (yrs)</td>
<td>0.614</td>
<td>-0.511</td>
<td>-0.341</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>0.050</td>
<td>0.209</td>
<td>0.281</td>
</tr>
<tr>
<td>T. chol (mmol/L)</td>
<td>0.398</td>
<td>-0.349</td>
<td>-0.084</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.228</td>
<td>-0.327</td>
<td>-0.031</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>-0.312</td>
<td>-0.070</td>
<td>0.296</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>0.295</td>
<td>-0.282</td>
<td>-0.453</td>
</tr>
</tbody>
</table>

Table 2 shows the association of correlates (fasting blood sugar, total cholesterol, triglyceride, glycated haemoglobin, high density lipoprotein and low density lipoprotein) with Malondialdehyde, Superoxide Dismutase and Glutathione Peroxidase of Diabetic subjects. mean difference is significant at p<0.05.
is not fully understood. Some studies have shown that glucose can enolise and then reduce molecular oxygen to give α- keto aldehydes, hydrogen peroxide and reaction oxygen species (ROS). The ROS formed causes peroxidative breakdown of phospholipid fatty acids and accumulation of MDA [23, 31-32].

This study observed a significant decrease in Superoxide Dismutase (SOD) activity of type 2 Diabetic subjects compared to controls. Numerous reports indicate variations in the levels of antioxidants in Diabetic subjects [37-38]. Some authors report no change in SOD activity [15, 39] while others reported increased [40] and others decreased SOD activity [41-42]. Decreased SOD activity found in this study could be attributed increased lipid peroxidation processes, leading to increased production of superoxide free radical and the elevation of other reactive oxygen species such as hydroxyl radical and hydrogen peroxide (H$_2$O$_2$) [42].

Glutathione peroxidase (GPx) is one of the enzymes responsible for the removal of hydrogen peroxide (H$_2$O$_2$) produced as part of cellular metabolism. In this study, a deceased GPx activity was observed in type 2 Diabetic subjects when compared with controls. This was in disagreement with prior findings [43]. There is no consensus on the activity of GPx in the serum of diabetic subjects. Increased MDA levels and increased lipid peroxidation, taken together may also act to inhibit GPx activity [27]. A detailed history of exogenous antioxidant administration and other oxidative stress markers need to be evaluated and considered in future research for definite conclusions to be drawn.

CONCLUSION

Results from this study suggests that there is raised Malondialdehyde levels, decreased activities of Superoxide Dismutase and Glutathione Peroxidase along with increased body mass index and deranged lipid profile pattern in Ghanaians with type 2 Diabetes Mellitus.

ACKNOWLEDGEMENT

The authors thank the National Diabetes Management and Research Centre (NDMRC) of the Korle-Bu Teaching Hospital, Accra for technical, material and financial support. They also thank the Department of Chemical Pathology of the University of Ghana Medical School, for institutional support.

REFERENCES


34. Stefanovic J.K, Prakash M, Ibrahim M.S. Relationship between free iron and glycated hemoglobin in uncontrolled Type 2 Diabetic patients associated with complications. IJCB. 2010; 23 (1): 67-70


*Corresponding author: H. Asare-Anane
E-Mail: henryasare-anane.g@gmail.com