Extracellular Superoxide Dismutase (EC.SOD) Evaluation in Type 1 and 2 Diabetes Mellitus Subjects in Yenagoa, Bayelsa State, Nigeria

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Abstract
Background: Extracellular superoxide dismutase (EC-SOD) is a secretory glycoprotein located in blood vessel walls at high levels and may be important in the antioxidant capability of vascular walls. Diabetes mellitus, a group of metabolic disorder of carbohydrate metabolism in which glucose is underutilized, producing hyperglycaemia is characterized by absolute or relative deficiency in insulin secretion, action or both. Oxidative stress which occurs due to over increase in production of free radicals or impaired compensatory response to antioxidant defence system has been assumed to be involved in the pathogenesis of some diabetic complications. The study compared the level of this enzyme in type 1 and 2 of chronic diabetes patients and determined its significance difference from diabetic subjects that have not suffered the disease for up to ten years.

Method: A total of 468 subjects were used for this study. This comprised of 90 diabetic subjects of type 1, 110 of type 2 and 110 apparently healthy subjects that never had hyperglycaemia and with HBAic value of less than 6.0%. The study also included 68 type 1 and 90 type 2 diabetic patients that have suffered the disease for less than 10 years. Enzyme linked immunosorbent assay (ELISA) method was used for this study.

Result: The result showed a mean ±S.D of EC-SOD level of 24.95 ± 9.71ng/ml and 31.05 ± 12.21ng/ml for chronic diabetic type 1 and type 2 respectively. A mean ±S.D of EC-SOD level of 39.44 ± 10.50ng/ml, 44.10 ± 12.38ng/ml for diabetic type 1 and type 2 subjects respectively that have suffered the disease for less than 10 years. An EC-SOD level of 63.78 ± 28.21 was gotten for non-diabetic subjects. From the result, there was no statistical difference (P>0.005) between the levels of the enzyme in type 1 and type 2 diabetic patients, but significantly differ (P<0.005) from the values obtained for non diabetic subjects at 95% confidence level. Statistical difference also exists at this confidence level between the chronic and non chronic sufferers of this disease.

Keywords: Diabetic mellitus, Hyperglycaemia, Superoxide dismutase, Chronic, Oxidative stress, Carbohydrate metabolism.

INTRODUCTION
Superoxide dismutases are a group of low molecular weight metalloproteins present in all aerobic cells of plants, animals, and micro-organisms. They provide protection against damaging reaction with the superoxide radical anion (O$_2^-$) by catalyzing its disproportionation into oxygen and hydrogen peroxide (Garcia-Gonzalez et al., 1999).

There is compelling evidence that superoxide excess induced by diabetic hyperglycaemia plays a central role in diabetic vascular cell damage (Brownlee, 2005). High glucose flux increase the production of superoxide anion (O$_2^-$) by mitochondrial electron transport chain, and the over produced superoxide enhances the major pathways of hyperglycaemic vascular cell damage, including protein kinase C, advanced glycation end products (AGE) products and hexosamine pathways (Brownlee, 2001).

In addition, superoxide is produced by multiple pathogenic pathways of diabetes. These include increased nicotinamide adenine dinucleotide phosphate [(NAD (P) H] oxidadase activity, uncoupled endothelial nitric oxide synthase (eNos), and enhanced signalling of AGE’s, angiotensin 11 and oxidized-LDL receptors (Li and Shah, 2004). Excessive production of superoxide anion results in the formation of secondary reactive oxygen species (ROS) including peroxynitrite and hydroxyl radicals, leading to the damage of DNA, proteins and lipids and causes vascular cell injury(Evans et al., 2002).

This superoxide overproduction is considered as a pathogenic pathway in diabetic vascular complications. A net accumulation of superoxide anion is determined by a balance between superoxide production and antioxidant capacity. In this context, antioxidant defence system could play a critical role in diabetic vascular damage (Fridovich, 1995) (Kamel et al., 2015). Superoxide dismutase is the major antioxidant enzyme for superoxide removal, which converts superoxide into hydrogen peroxide (H$_2$O$_2$) and molecular oxygen (Fridovich, 1995) (Hisalkar et al., 2012).

\[
20^2 + 2H\rightarrow_{SOD} H_2O_2 \\
O_2 + H_2O_2 \rightarrow O_3 + OH + OH^-
\]

The hydrogen peroxide is further detoxified to water (H$_2$O) by catalase or glutathione peroxidise.
In mammals, three SOD isoforms exist. Cytoplasmic CuZnSOD (SOD$_1$), mitochondrial MnSOD (SOD$_2$) and extracellular CuZn SOD (SOD$_3$) or (EC.SOD) (Faraci and Didioa, 2004). Each SOD isoform is derived from distinct genes but catalyzes the same reaction, producing H$_2$O$_2$ and O$_2^-$. There is substantial evidence that SOD activity in peripheral blood cells is reduced in the diabetic patients with diabetic nephropathy as compared with those without diabetic complications (Soedamah-muthu et al., 2006) (Seshosai et al., 2011) (Giacco and Brownlee, 2010).

Superoxide dismutase, an enzyme found in all living cells, is taken orally for removing wrinkles, rebuilding tissue and extending the length of life. However, there is no evidence that SOD products that are taken by mouth are absorbed by the body (Segui et al., 2004). Different studies have provided evidences of increased oxidative stress with depleted antioxidant enzymes and vitamins in both type 1 and type 2 diabetes (Lapolla et al., 2007) (Lodovici et al., 2008) (Likidlilid et al., 2010) (Al-Rawi, 2011), and have also reported lower concentration of non enzymatic antioxidants as well as enzymatic antioxidants in type 2 diabetes (Bigagli et al., 2012). This study evaluated the status of the superoxide dismutase (EC.SOD) in type 1 and type 2 diabetic patients that have suffered the disease for over ten years and compared the levels with patients that have suffered the disease for less than ten years. This is with a view to determine the effect of having uncontrolled hyperglycaemia and of course diabetic complications on this enzyme in chronic diabetic subjects.

**STUDY AREA**

Samples for this study were collected from Yenegoa, Bayelsa State and its environs, specifically from diabetic patients attending Federal Medical Centre and Niger Delta University Teaching Hospital (NDUTH) Okolobiri, about 15km from Yenegoa, Bayelsa State of Nigeria.

**STUDY SUBJECTS**

A total of 468 subjects were used for this study. This comprised of 90 patients suffering from diabetes type 1 and 110 patients of type 2 that have suffered the disease for over ten years. Their status were confirmed after a fasting blood sugar test with values above 7.0mMol/l and glycated haemoglobin (HbAic) values of above 7.0%. The study subjects also included 68 type 1 and 90 type 2 patients that have not suffered the disease for up to ten years. The chronic diabetes patients were confirmed, known subjects that have been suffering from this disease for over ten years by the physician in these hospitals. The basic information of age, sex, family history, duration of disease, habits of smoking, and alcohol consumption, including complications like hypertension, eye and renal disease was obtained from the subjects. 110 non diabetic subjects were carefully selected from the population in the same locality after determining their fasting blood glucose level (normally <6.0mMol/L) and glycated haemoglobin level (<6.0%). The study age bracket was between 30 and above years for both diabetic and non diabetic subjects. Informed consent was gotten from all the participants in this study and the management of the hospitals. This study was carried out between May, 2010 to January, 2015.

**SAMPLE COLLECTION**

The study subjects (diabetic and non diabetic) were properly instructed to fast over night for 12-14hr before coming for sample collection. About 10ml of venous blood was collected from the anterior cubital vein and discharged into fluoride; EDTA and heparized tubes for the various biochemical measurements that included fasting blood glucose, glycosylated haemoglobin (HBAIC) and enzyme superoxide dismutase estimations.

**METHODS**

The enzyme linked immunosorbent assay (ELISA) method was used for this study. The Elabscience Biotechnology co ltd (ELISA) kit was specifically used for the study. The components of the ELISA kit used were specifically designed to analyse the antioxidant enzyme superoxide dismutase”. It applies to in-vitro quantitative determination of superoxide dismutase concentrations in plasma (Uotila et al., 1981) (Peter et al., 2001)

**STATISTICAL ANALYSIS**

The data are expressed as mean ± standard deviation. The paired t-test (test of significance) was done using the student’s t-test to compare the groups. Differences were considered significant at P<0.05 (95% confidence level). Correlation between the groups studied was tested using the regression analysis.

**RESULTS**

The results obtained and inference from the study is as presented in the table 1 and table 2 below.
Table 1: The plasma levels of Extracellular superoxide dismutase (EC.SOD) in type 1 and type 2 poorly controlled, chronic diabetic mellitus patients

<table>
<thead>
<tr>
<th>STUDY GROUPS</th>
<th>NUMBER OF SUBJECTS</th>
<th>HBAIC (%)</th>
<th>EC.SOD (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic type 1</td>
<td>90</td>
<td>8.9 ± 1.94</td>
<td>24.95 ± 9.71*</td>
</tr>
<tr>
<td>Diabetic type 2</td>
<td>110</td>
<td>8.2 ± 1.13</td>
<td>31.05 ± 12.21*</td>
</tr>
<tr>
<td>Non Diabetic (control)</td>
<td>110</td>
<td>5.1 ± 0.72</td>
<td>63.78 ± 28.21*</td>
</tr>
</tbody>
</table>

*Showed a statistical difference at P<0.05

Table 2: The plasma levels of Extracellular superoxide dismutase (EC.SOD) in type 1 and type 2 diabetic subjects that have suffered the disease for less than ten years.

<table>
<thead>
<tr>
<th>STUDY GROUPS</th>
<th>NUMBER OF SUBJECTS</th>
<th>HBAIC (%)</th>
<th>EC.SOD (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic type 1</td>
<td>68</td>
<td>8.3 ± 1.94</td>
<td>39.44 ± 10.5*</td>
</tr>
<tr>
<td>Diabetic type 2</td>
<td>90</td>
<td>7.8 ± 1.13</td>
<td>44.10 ± 12.38*</td>
</tr>
<tr>
<td>Non Diabetic (control)</td>
<td>110</td>
<td>5.1 ± 0.72</td>
<td>63.78 ± 28.2*</td>
</tr>
</tbody>
</table>

*Showed a statistical difference at P<0.05

DISCUSSION
In healthy subjects, antioxidant compounds counter the effect of free radicals. Oxidative stress results due to over increase in production of free radicals or decreased level of antioxidants and several mechanisms increase the intracellular and extracellular concentration of glucose resulting in oxidative stress. In humans, three forms of SOD exist, but the presence of E.C.SOD on the endothelial cell surface at high level might have an important protective effect against superoxide in the vascular wall, supporting the antioxidant role of nitric oxide and its role in prevention of LDL oxidation (Takatsu et al., 2001) and this stimulated the interest of the extracellular superoxide dismutase (EC.SOD) in this study.

In this study, chronic exposure to hyperglycaemia and of course insulin resistance really depleted the antioxidant enzyme, superoxide dismutase of extracellular origin. The result showed that because of the altered oxidative metabolism, the EC.SOD was reduced in poorly controlled, chronic diabetes when compared to the diabetic subjects that have suffered the disease for less than ten years. Statistically, at 95% confidence level (P<0.05) there was a significant difference in these two groups of diabetic subjects. The values obtained in both groups were statistically different (P<0.05) from the values for non diabetic (control) subjects. The correlation studies showed that, there is an association (r=0.34) between continued hyperglycaemia and enzymatic antioxidant agent like extracellular superoxide dismutase. The reduction in the level of the antioxidant enzyme is an indication in the increased level of oxidative stress. Several mechanisms have been proposed to explain why oxidation is increased in diabetes mellitus. Increased production of ROS and decreased antioxidant defences. Hyperglycaemia in diabetes may increase ROS production via changes in the redox potential of glutathione and decreased antioxidant defences due to production in total antioxidant capacity in plasma. (West, 2000) (Hodgkinson et al., 2003) (Colak et al., 2005) (Hiroki et al., 2009). The study showed that excessive plasma and tissue glucose can exert pathological effects through non enzymatic glycosylation which leads to the production of superoxide and hydrogen peroxide.

CONCLUSION
Plasma EC.SOD in both type 1 and type 2 diabetes are decreased as a result of antioxidant mechanism seen in pathological conditions of oxidative stress. This is further depleted as the age of the disease increases, particularly when it’s poorly controlled. The findings from this study therefore suggest the estimation of plasma antioxidant levels with other routine investigation in diabetic patients. This may be useful in the prevention of the diabetic complications which can be prevented by supplementing the antioxidant rich components of the diet- hence avoiding further diabetic events.

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