Evaluation of plasma glutathione peroxidase (GPX) enzyme in type 1 and type 2 chronic diabetes mellitus patients in Yenegoa, Bayelsa State of Nigeria

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Diabetes mellitus is a metabolic disease which is characterised by absolute or relative deficiency in insulin secretion, insulin action or both. Glutathione peroxidase is one of the antioxidant enzymes that protect, prevent or reduce the level of oxidative damage to cells in the body. Decreased activity of this antioxidant enzyme may increase the susceptibility of diabetic patients to oxidative injury. Adequacy of the enzyme helps in preventing some clinical complications associated with diabetes. The study compared the level of this enzyme in type 1 and type 2 of chronic diabetic patients and determined its significance difference from diabetic subjects that have not suffered the disease for up to ten years. 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 ten years. Enzyme linked immunosorbent assay (ELISA) method was used in the quantisation of the enzyme level. The result showed a mean ± S.D of 2.29 ± 0.38, 3.37 ± 0.61 and 4.51 ± 0.53 pg/ml for chronic Diabetic type 1, type 2 and control subjects respectively. A mean ± S.D of 3.73 ± 0.40 and 3.94 ± 0.33 pg/ml for Diabetic type 1 and type 2 respectively for subjects that have suffered the disease for less than ten years. Statistically, there was a significant difference (P<0.05) between the levels of the enzyme in type 1 and type 2 diabetic patients with respect to the control at 95% confidence level. The plasma levels of the enzyme did not show any Statistical difference at this confidence level (P>0.05) between the chronic and non chronic sufferers of the disease. Correlation analysis (r = -0.05) did not indicate any association between the results of type 1, type 2 diabetes and the non-diabetic healthy subjects.

Key words: Glutathione peroxidase, diabetes mellitus, evaluation, antioxidant enzymes, hyperglycaemia, glycated haemoglobin, oxidative stress, carbohydrate metabolism, chronic.

INTRODUCTION

Insufficient levels of antioxidant or inhibition of the antioxidant enzymes cause oxidative stress and may damage or kill cells (Ceriello and Motz, 2004). Oxidative stress cause damage to cell structure and cell function by overly reactive oxygen containing molecules and chronic excessive inflammation. This play a significant role in many human diseases including cancers (Lenaz, 2001). Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions (West, 2000).

\[ 2O_2 + H^+ \rightarrow H_2O_2 \]

\[ O_2 + H_2O_2 \rightarrow O_2 + OH + H \]

Glutathione peroxidase (GPX) is an enzyme with peroxidase activity and performs a biological role that protects the organism from oxidative damage. This is done by reducing the lipid hydro peroxides to their corresponding alcohols and to reduce free hydrogen.

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peroxide to water (Brownlee, 2001). Glutathione peroxidase is one of the endogenous proteins that work in combination to protect cells from reactive oxygen species (ROS) damage. Increased level of the products of oxidative damage to lipids and proteins has been detected in the serum of diabetic patients and this can lead to complications (Brownlee, 2001; Hisalkar et al., 2012).

All forms of diabetes are characterized by hyperglycemia – a relative or absolute lack of insulin action (Sayday et al., 2001). oxidative stress plays a pivotal role in the development of diabetes complications, both Microvascular and cardiovascular. The metabolic abnormalities of diabetes cause mitochondrial superoxide over production in endothelial cells of both large and small vessels, as well as in the myocardium. The increased superoxide production causes the activation of 5 major pathways involved in the pathogenesis of complications (Giacco and Brownlee, 2010).

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) (Likidilid et al., 2010) (Ezeiruaku and Michael, 2015). Depletion of blood glutathione (GSH) has been documented in many clinical situations such as malnutrition, severe burn injury, human immunodeficiency virus (HIV), infection and diabetes, both type 1 and type 2. Glutathione depletion may have adverse consequences in diabetic patients, independent of glycemic control and it may weaken the defence against oxidative stress. This could cause damage to protein, DNA or membrane lipids and thus potentially lead to cell dysfunction in various tissues. In diabetes, increased oxidative stress is known to play a decisive role in the pathogenesis of vascular complications (Dominique et al., 2005).

Over all, diabetic microvascular complications are caused by prolonged exposure to high glucose levels, in addition to genetic determinants of individual susceptibility and as with atherosclerosis from hypertension and dyslipidemia (Giacco and Brownlee, 2010). Because every cell in the body of people with diabetes is exposed to abnormally high glucose concentrations, there is an increased ROS peroxidation, there is a defensive system consisting of antioxidant enzymes that play an important role in scavenging ROS. The organisms’ susceptibility to free radical stress and peroxidative damage is therefore related to the balance between the free radical load and the adequacy of antioxidant defences (Hisalkar et al., 2012).

Several studies (Lodovici et al., 2009) (Bigagli et al., 2012) have reported lower concentration of non-enzymatic antioxidants as well as enzymatic oxidants in type 2 diabetes. This study investigated /estimated the level of the glutathione peroxidase in the plasma of chronic diabetes subjects of type 2 in Yenegoa, Bayelsa state, Nigeria and compared it to the level in diabetic subjects that depends solely on insulin (insulin dependent type 1 diabetes) and have had hyperglycemia for ten years and above period. This is with a view to determine if there is a reduction in the level of plasma glutathione peroxidase in type 1 and type 2 chronic diabetes patients compared to those that have not suffered the disease for up to ten years when compared to non diabetic apparently health 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 15 km 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 7.0 mMoL/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. The 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 heparinized tubes for the various biochemical measurements that included fasting blood glucose, glycysylated haemoglobin (HBAIC) and glutathione peroxidase 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; glutathione. It applies to in-vitro quantitative determination of glutathione peroxidase concentration 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 AND DISCUSSION

The results obtained and inference from the study is as presented in the Tables 1 and 2. In diabetes, exposure to steady hyperglycemia, whether of type 1 (insulin resistance) or type 2 (non insulin dependent) has been implicated in altered oxidative metabolism. This has been reported as a cause of increased production of oxygen free radical through glucose auto oxidation and non enzymatic glycation (Ford et al., 2003).

The complications in the long run leads to the morbidity and mortality associated with the disease (Patel et al., 2008). Free radical is the main causes of oxidative stress, which may react with various bio molecules and macromolecules of connective tissue (Robertson and Harmon, 2006).

The oxygen and other free radicals are difficult to measure directly because of their unstable nature. This necessitated the use of glutathione peroxidase (GPX), one of the antioxidant enzymes as an indicator of free radical activity. The study showed significant decrease in the level of glutathione peroxidase enzyme (GPX) activity in diabetes type 1 and type 2 subjects who have suffered this disease for over ten years as compared to healthy individuals which is an indication of marked oxidative stress. This means that there is a decreased scavenging capacity of glutathione-dependent antioxidant defensive system against elevated lipid peroxidation process in these subjects.

From the study, the plasma glutathione peroxidase level in type 1 diabetes subjects is 2.29 ± 0.38. When compared to the level in type 2 diabetic subjects (3.37 ± 0.61), there was no statistical significance difference at P>0.05, 95% confidence level. The type 1 and type 2 results when compared to the level for healthy, non diabetic subjects showed a statistical difference in the level of this antioxidant enzyme.

The correlation analysis result of r = -0.05 did not indicate any association between the results of type 1 and type 2 diabetes and the non diabetic healthy individuals.

Several difference mechanisms have been proposed to explain why oxidative stress is increased in diabetic mellitus. Increased production of reactive oxygen species (ROS) and decreased antioxidant defence (West, 2000) as seen in several pathological conditions. Glutathione peroxidase is one of the enzymes responsible for the removal of H2O2 produced as part of cellular metabolism and there is significance occurrence in the presence of lipid peroxidation with reduced levels of GPX in the diabetic group with the resultant destruction of lipid hydroperoxide (Turk et al., 2002).

With regards to the duration of the disease in the studied subjects, there was no significance difference (p<0.05) between the patients that have suffered diabetes mellitus for over ten years (chronic) and those that are below ten years. The study also showed a significance difference between the non chronic (less than ten years) subjects to non-diabetic, healthy subjects. These findings are in accordance with the observations made earlier by Chugh, et al in 1999, that chronic exposure to hyperglycemia and insulin resistance has been implicated in altered oxidative metabolism. This means that excessive plasma and tissue glucose can exert pathological effects through non enzymatic glycosylation which leads to the production of superoxide and hydrogen peroxide (Mercuri et al., 2000)

Conclusion

Plasma GPX 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, with its associated complications and particularly when it’s not controlled. The findings from this study therefore suggest the estimation of plasma antioxidant level, with other routine investigations 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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Table 1. The plasma levels of Glutathione peroxidase (GPX) in type 1 and type 2 chronic diabetes mellitus patients.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Number of subjects</th>
<th>HBAIC (percentage)</th>
<th>GPX (µg/ml) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic type 1</td>
<td>90</td>
<td>8.9 ± 1.94</td>
<td>2.29 ± 0.38*</td>
</tr>
<tr>
<td>Diabetic type 2</td>
<td>110</td>
<td>8.2 ± 1.13</td>
<td>3.37 ± 0.61*</td>
</tr>
<tr>
<td>Control (non diabetes)</td>
<td>110</td>
<td>5.1 ± 0.72</td>
<td>4.51 ± 0.53^</td>
</tr>
</tbody>
</table>

* Showed no statistical difference at P>0.05; ^ Showed a statistical difference at P<0.05.

Table 2. The plasma level of Glutathione peroxidase (GPX) in type 1 and type 2 diabetes subject that have suffered the disease for less than ten years.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Number of subjects</th>
<th>HBAIC (percentage)</th>
<th>GPX(Ug/ml) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic type 1</td>
<td>68</td>
<td>8.3 ± 1.74</td>
<td>3.73 ± 0.40*</td>
</tr>
<tr>
<td>Diabetic type 2</td>
<td>90</td>
<td>7.8 ± 1.19</td>
<td>3.94 ± 0.33*</td>
</tr>
<tr>
<td>Control (non diabetes)</td>
<td>110</td>
<td>5.1 ± 0.72</td>
<td>4.51 ± 0.53^</td>
</tr>
</tbody>
</table>

* Showed no statistical difference at p>0.05; ^ Showed a statistical difference at P<0.05.

REFERENCES


