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Full Length Research Paper

Plasma Catalase Evaluation in Type 1 and Type 2 Chronic Diabetic Patients in Yenegoa, Bayelsa State, Nigeria.

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The complications of Diabetes Mellitus, a group of metabolic disorder of carbohydrate metabolism in which glucose is underutilized, producing hyperglycemia are far less common and less severe in people who have well controlled blood levels. In chronic or uncontrolled situations, insufficient levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress which occur due to over increase in production of free radicals or impaired compensatory response to antioxidant defense system. Catalase is an enzyme present in most of the aerobic cells; it protects the cells from oxidative stress by catalysing the rapid decomposition of hydrogen peroxide (H_2O_2) in two types of reactions depending on its peroxidative and catalytic activities. This study compared the level of the plasma catalase enzyme in type 1 and type 2 chronic diabetic patients to diabetic patients that have suffered the disease for less than 10 years with respect to control subjects. A total of 468 subjects were used for this study. This comprised of 90 diabetic subjects of type 1, 110 of type 2 that have suffered the disease for over ten years and 110 apparently healthy subjects that never had hyperglycemia and with HBAIC value of < 6.0%. The study also included 68 type 1 and 90 type 2 diabetic patients that have suffered the diseases for less than 10 years. Enzyme linked immunosorbent assay (ELISA) method was used for this study. The result showed a mean ± S.D plasma catalase level of 22.46 ± 14.94 pg/ml, 26.60 ± 10.65 pg/ml and 43.10 ± 23.28 pg/ml for diabetic type 1, type 2 and control subjects respectively. A plasma catalase level of 25.50 ± 13.15 pg/ml and 28.48 ± 12.72 pg/ml for diabetic type 1 and type 2 respectively for diabetic subjects that have suffered the disease for less than 10years. Analysis of the result, showed that there was no statistical difference (P > 0.05) between the levels of the enzyme in type 1 and type 2 of both chronic and non chronic diabetic patients, but significantly differs from the values obtained for non diabetic, apparently healthy subjects at this 95% confidence level. This showed that Plasma catalase enzyme level is decreased in diabetic patients (chronic and non chronic) in Yenegoa, Bayelsa State of Nigeria with respect to non diabetic (control) Subjects.

Keywords: Catalase, Antioxidants, Chronic, Diabetes mellitus, Hyperglycaemia, Oxidative stress, immunoassay, Glycation.

INTRODUCTION:

Catalase is an enzyme commonly found in all living organism that require oxygen for existence (Bacteria, plant and animals) (Abdul-salam et al., 2000). It is a very important enzyme in protecting the cell from oxidative damage by Reactive Oxygen Species (ROS) (Ho et al., 2004). It is involved in any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression etc) as a result of a reactive oxygen species stimulus. ROS include singlet oxygen superoxide and

oxygen free radicals. The enzyme catalase, catalyzes the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese co-factor (Chilikani et al., 2004). The reaction of catalase in the decomposition of hydrogen peroxide in living tissue is represented as $2H_20_2 \dashrightarrow 2H_20+0_2$. This reaction according to Boon et al., 2007 occur in two stages:

H_20_2+Fe (iii)---E $\dashrightarrow$ H_20+O-Fe (iv)-E(+)

H_2O_2+O=Fe (iv)-E (+)$\dashrightarrow$$H_20$+Fe = (iii)-E+$O_2$

It is widely known that oxidative stress may play a relevant role in the pathogenesis of vascular complications (Fancesco et al., 2004). Increased production of reactive oxygen metabolites and species is a direct consequence of high glucose concentrations (Bayens and Thorpes, 1996) (Gingliand et al., 1996). Hyperglycemia is able to increase the levels of oxygen radical scavenging enzymes in cultured endothelial cells (Ceriello et al., 1996) and in the kidney of rats with diabetes induced by streptozotocin (Sechi et al., 1997). Finally, hyperglycemia can induce formation of free radical and activation of oxidative stress through non enzymatic glycation of proteins (Mullarkey *et al* ., 1990), auto oxidation glycation (Bigagli et al., 2012) activation of protein Kinase C. (Anderson et al., 2007) and increased polyol pathway (Javed *et al*.,2015). In normal individuals, exposure to high glucose concentrations induced an antioxidant defensive mechanism in skin fibroblasts in adult with type 1diabetes with macro albuminuria and in overt nephropathy, this defensive mechanism is absent (Ceriello et al., 2000).

The presence of catalase in microbial or tissue sample can be tested by adding a volume of hydrogen peroxide and observing the reaction. The formation of bubbles of oxygen indicates a positive result.

Catalyse can also catalyse the oxidation by hydrogen peroxide of various metabolites and toxins, including formaldehyde, formic acid, phenols, acetaldehyde and alcohols (Chelikani et al., 2004). Hydrogen peroxide is a harmful by-product of many normal metabolic processes and to prevent damage to cells and tissues, it must be quickly converted into other less dangerous substances. To this end, catalase is frequently used up by cells to rapidly catalyse the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani et al., 1996).

Catalase is used in the food industry for removing hydrogen peroxide from milk prior to cheese production. It is used in the food wrappers where it prevents food from oxidizing and also in the textile industry, for removing hydrogen peroxide from fabrics (Hengge, 1999).

Reactive oxygen species (ROS) are substances released during oxidative metabolism. The reactions of ROS with macromolecules can lead to DNA mutations, changes in the structure and function of proteins and peroxidative damage of cell membrane lipid (Abdul-Salam et al., 2000). Since free radicals have been implicated in the pathogenesis of a variety of human disease, this has prompted interest in the evaluation of cellular levels of antioxidant agents. Catalase is an antioxidant enzyme with high specific activity present in all aerobic cells. In this study, the levels in plasma of chronic diabetic subjects is measured to compare with non-chronic and non-diabetic subjects to determine the effect of long exposure of cells to hyperglycemia and the associated complications of uncontrolled, chronic diabetes mellitus and its oxidative stress found in altered oxidative metabolism.

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 %`(WHO, 2011)

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 physicians 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 thirty years and above for both diabetic and non diabetic subjects. Informed consent was gotten from all the participants in this study and this was done between May 2010 and January, 2015.

Sample Collection

The study subjects (diabetic and non diabetic) were properly instructed to fast over night for 12-14 hours 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 Catalase estimations.

METHODS

Fasting plasma glucose (FPG) was estimated quantitatively using the Glucose Oxidase method as modified by Randox Laboratories Limited (United Kingdom). HbAic levels were estimated quantitatively using immunoassay method as described by Chek diagnostics (USA). The enzyme linked immunosorbent assay (ELISA) method was used for the Catalase enzyme estimation. The Elabscience Biotechnology Company Ltd (ELISA) kit was specifically used for the study. The components of the ELISA kit used were specifically designed to analyse the antioxidant enzyme; it applies to in-vitro quantitative determination of Catalase concentrations in plasma (Uotila et al., 1981) (Peter et al., 2001)

Statistical Analysis

The data generated 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 and the analysis of variance (ANOVA). The results were considered statistically significant at 95% confidence interval (P<0.05).

RESULT

The results obtained and inference from the study is as presented in the tables and figures below:

Table 1: The plasma levels of catalase enzyme in type 1 and type 2 poorly controlled chronic (>10years) Diabetic mellitus patients in Yenegoa, Bayelsa State, Nigeria

STUDY GROUP	NUMBE OF SUBJECTS	HBAIC (%)	ENZYME LEVEL (pg/ml)
Diabetic type 1	90	8.9 + 1.94	22.46 ± 14.94 #
Diabetic type 2	110	8.2 ± 1.13	26.60 ± 10.65 #
Non Diabetic (control)	110	5.1 ± 0.72	43.10+ 23.28 *

showed no statistical difference at P >0.05 * showed a statistical difference at P < 0.05

Table 2: The plasma levels of catalase enzyme in type 1 and type 2 diabetic subjects that have suffered the disease for less than ten years in Yenegoa ,Bayelsa State, Nigeria

STUDY GROUPS	NUMBER OF SUBJECTS	HBAIC (%)	ENZYME LEVEL (pg/ml)
Diabetic type 1	68	8.3 ± 2.19	25.50 ± 13.15 #
Diabetic type 2	90	7.8 ± 1.54	28.48 ± 12.72 #
Non-diabetic (control)	110	5.1 ± 0.72	43.10 ± 23.28 *

showed no statistical difference at P > 0.05 * showed a statistical difference at P < 0.05

. **Table 3:** ANOVA Comparing controls, Type1 DM <10 yrs and Type1 DM > 10 yrs

	Catalase (Pg/ml)
Control n=110	43.10 ± 23.28
Type1 DM < 10 yrs n=68	25.50 ± 13.15
Type 1 DM > 10 yrs n=90	22.46 ± 14.94
P value	P > 0.05

- Values are expressed as mean ± SEM.
- n= number of subjects.
- not significant between type 1 < 10years and type 1> 10years, but significantly differ from the controls

Table 4: ANOVA Comparing controls, Type2 DM <10 yrs and Type2 DM > 10 yrs

	Catalase (Pg/ml)
Control n=110	43.10 ± 23.28
Type2 DM < 10 yrs n = 90	28.48 ± 12.72
Type 2 DM > 10 yrs n = 110	26.60 ± 10.65
P value	P > 0.05

- Values are expressed as mean ± SEM.
- n= number of subjects.
- not significant between type 2 < 10years and type 2 > 10years, but statistically differ from the controls.

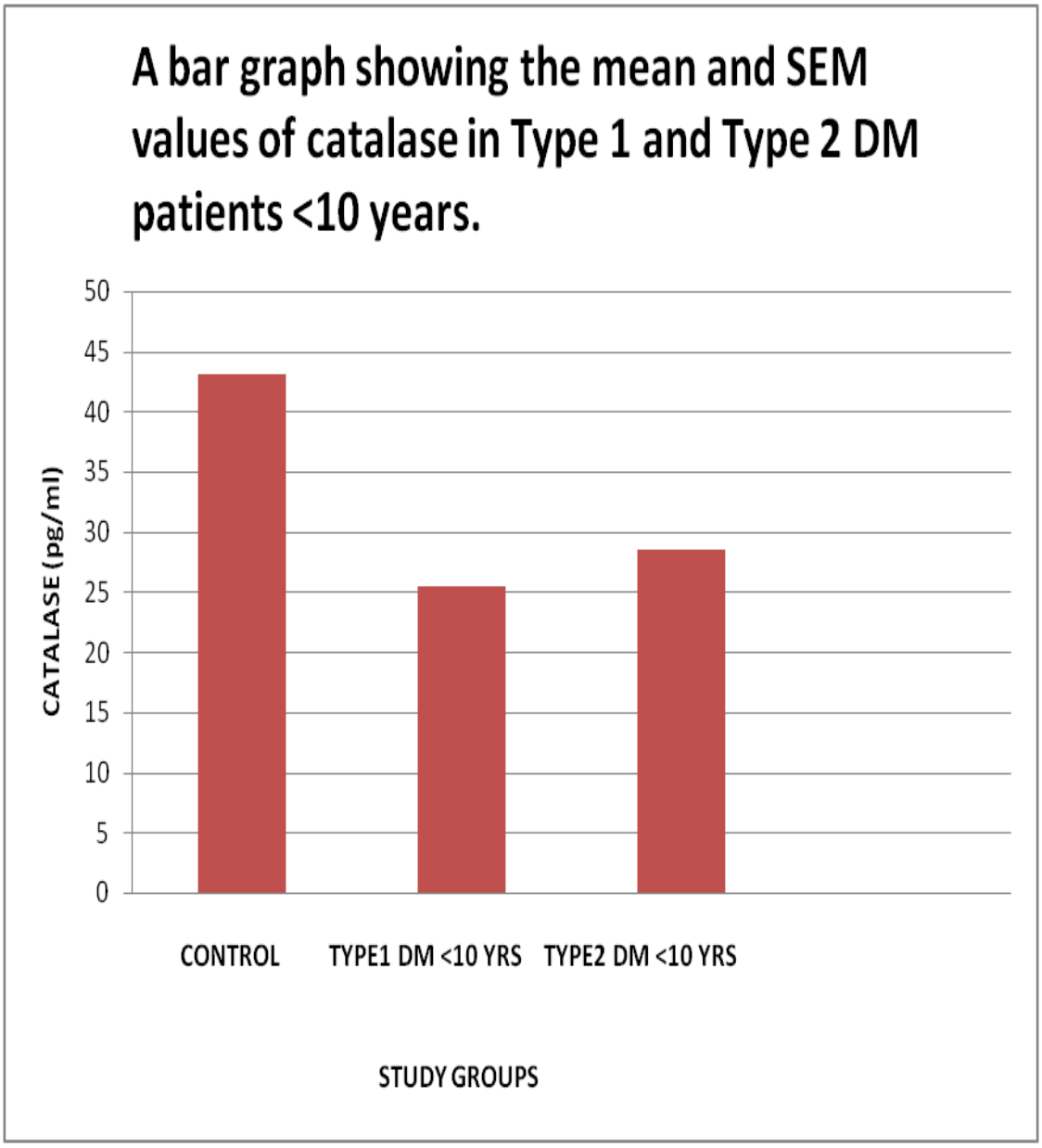

Figure 1: A bar graph showing the plasma Catalase enzyme level in non chronic sufferers of DM type 1 and type 2. Statistically there was no difference between the two diabetic types, but significantly different from the non diabetics

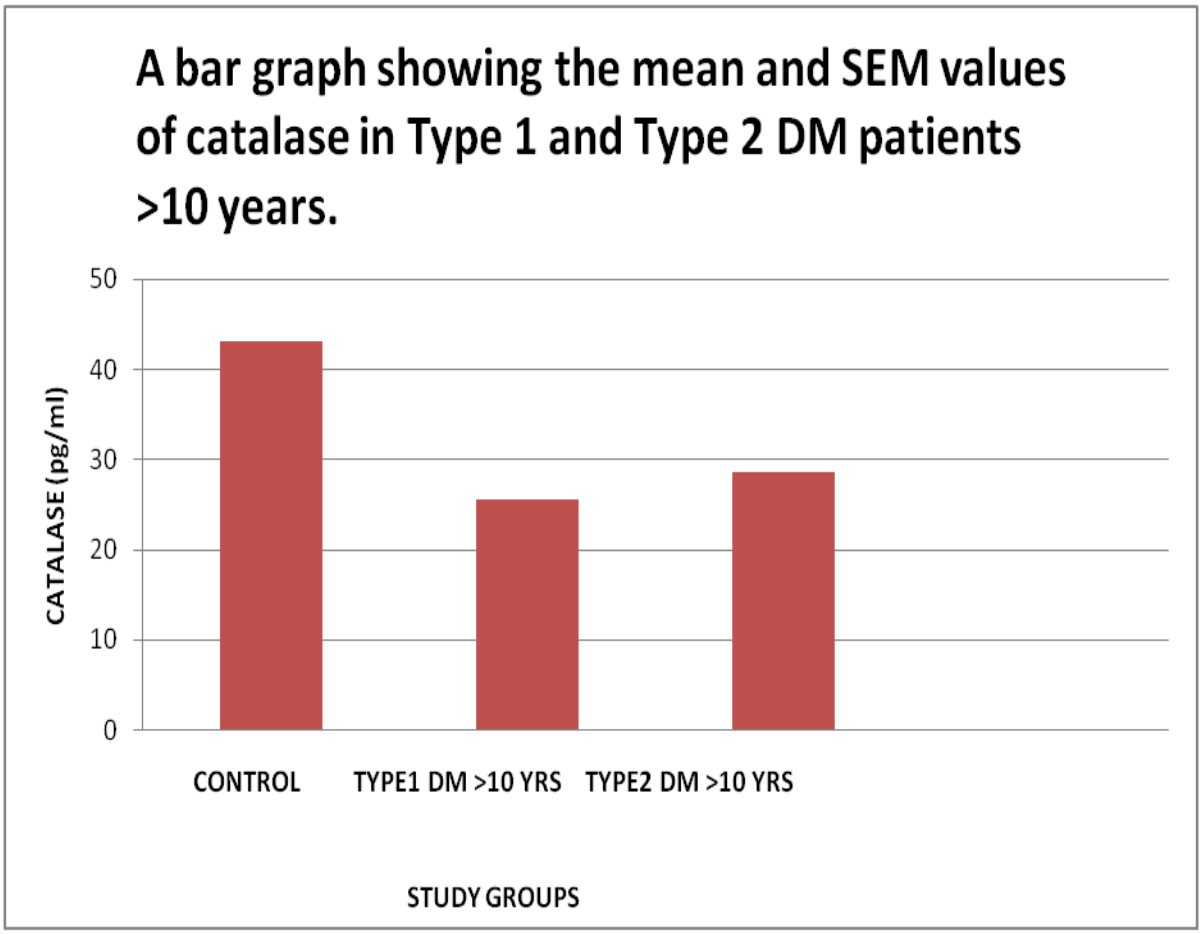

Figure 2: A bar graph showing the plasma Catalase enzyme level in chronic sufferers of DM type 1 and type 2. Statistically there was no difference between the two diabetic types, but significantly different from the non diabetics.

DISCUSSION

In diabetics, 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 radicals through glucose autoxidation and non-enzymatic glycation (Ford et al., 2003).

In healthy subjects, antioxidant compound 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. The oxidative stress may play a relevant role in pathogenesis of diabetic vascular complications (Bayens and Thorps, 1996).

Catalase is an enzyme exerting a dual function; it catalyzes the decomposition of hydrogen peroxide to produce water and oxygen, which is a catalatic function and oxidation of H donors, which is a peroxidative function.

In this study, the assayed plasma levels of this enzyme in chronic diabetic subjects showed no significant decrease from diabetic subjects that have suffered the disease for less than 10years (Table 1 and 2). The value of 22.46 $\pm$ 14.94 pg/ml for chronic diabetic type 1 and 26.60 $\pm$ 10.65 pg/ml for chronic diabetic type 2 as compared to the value of 25.50 $\pm$ 13.15 pg/ml for type 1 and 28.48 $\pm$ 12.72 pg/ml for type 2 diabetic subjects that have suffered the disease for less than 10 years. Both cases (chronic and non chronic)have values that differ significantly ($P<0.05$) from the non diabetic , control subject, with a plasma catalase value of 43.10 $\pm$ 23.28 pg/ ml in Yanegoa, Bayelsa State, South of Nigeria. Test of variance (ANOVA) did not indicate any significant difference between the type 1 and type 2 and between the chronic and non chronic diabetic subjects (Table 3 and 4). Statistical difference ($P<0.05$) existed between the diabetic types and non diabetics (control).

The correlation analysis result of r= -0.08 did not indicate any association between the result of type 1, type 2 diabetics and the non- diabetic healthy individuals.

Several different mechanisms have been proposed to explain why oxidative stress is increased in diabetic mellitus. Increased production of Reactive oxygen species (ROS) and decreased antioxidant defenses (West, 2000) as seen in several pathological conditions. Catalase enzyme is one of the enzymes responsible for the decomposition of the hydrogen peroxide to produce water and oxygen which is part of the cellular metabolism and there is significant occurrence in the presence of lipid peroxidation with reduced levels of antioxidant enzymes (Turk et al., 2002).

CONCLUSION:

Plasma catalase enzymes are decreased in both type 1 and type 2 diabetes when compared to non-diabetic, apparently healthy subjects. This is as a result of antioxidant mechanism seen in pathological conditions of oxidative stress like diabetics mellitus. The findings from the study therefore suggest the estimation of plasma antioxidant levels with other routine investigations in diabetic patients. This may be useful in the prevention of diabetic complications which can be prevented by supplementing the antioxidant rich components of the diet- hence avoiding further diabetic events.

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