The Antioxidant Enzymes (Superoxide Dismutase; Glutathione Peroxidase; Catalase) Status in Chronic and Non Chronic Diabetic Mellitus Type 1 and Type 2 Subjects in Yenegoa, Bayelsa State, Nigeria.

[1]Ezeiruaku, F.C., [2] Wankasi, M.M. and [3] George, G.S.
[1,2,3]Department of Medical Laboratory Science,
Faculty of Basic Medical Sciences,
College of Health Sciences,
Niger Delta University,
Bayelsa State, Nigeria.
Correspondence Authors e-mail: feddyezeh@yahoo.com.

ABSTRACT:

Background*: Diabetes mellitus is a metabolic disease which is characterised by absolute or relative deficiency in insulin secretion, insulin action or both. This leads to glucose underutilization with the concomitant diabetic complication and oxidative stress. Oxidative stress results due to increase in production of free radicals or decreased level of antioxidants. Increased activity of the antioxidant enzymes in mopping up free radicals during oxidative stress, results in its depletion.* ***Aim of study****: To investigate the level of these antioxidant enzymes (Superoxide dismutase, Glutathione peroxidase and Catalase) both in chronic and non chronic sufferers of the disease with respect to apparently healthy, non diabetic subjects in Yenegoa, Bayelsa state of Nigeria. Also to determine the effect of disease duration, on the levels of the antioxidant enzymes.* ***Method****: A total of 468 subjects were used for this study. This comprised of 158 diabetic subjects of type 1, 90 of them have suffered the disease for more than ten years (Chronic) and 68 of them for less than ten years (Non chronic). A total of 200 type 2 diabetic subjects, of which 110 of them were chronic sufferers and 90 of them non chronic with 110 apparently healthy subjects that never had hyperglycaemia and with HbAIC value of less that 6.0%. A questionnaire was used in the collection of demographic and lifestyle data from the subjects used for the study. Enzyme linked immunosorbent assay (ELISA) method was used for the determination of the enzymes level.* ***Result****: From the study, the results showed that the mean plasma value for the antioxidant enzymes – SOD, GPX and CAT were significantly decreased in diabetic type 1 and type 2 compared to non diabetic group(control) at $P<0.05$ confidence level. The study indicated that there is a significant decrease in plasma SOD enzyme level in chronic diabetics type1 and type 2 when compared to those diabetic patients(non chronic) that have suffered the disease for less than ten years. This is quite different from the plasma GPX and CAT that did not show any statistically significant difference between the chronic and non chronic diabetic subjects. The plasma antioxidant enzymes decreased with an increase in haemoglobin glycosylation and increase in the duration of illness. This means that as the disease condition progresses, the antioxidant enzymes studied are further depleted.* ***Conclusion****: The levels of the antioxidant enzymes where reduced in both type 1 and type 2 diabetics as compared to non diabetics, also the levels of the antioxidant enzymes decreased with increasing duration of illness.*

Key Words*: Diabetic mellitus; Antioxidants; Superoxide dismutase; Glutathione peroxidise; Catalase; Oxidative stress; Haemoglobin glycation; Chronic; Hyperglycaemia.*

INTRODUCTION:

Aerobic organisms including humans possess antioxidant defence systems that deal with reactive oxygen species (ROS) produced as a consequence of aerobic respiration (Erika *et al*., 1999). Reactive oxygen is related to both the arrest of growth and the start of cell differentiation. Low concentration of reactive oxygen intermediates may be beneficial or even indispensable in processes such as intracellular messaging and defence against micro-organisms but higher amounts of active oxygen may be harmful to cells and organisms. A wide array of non enzymatic and enzymatic antioxidant defences exists, including superoxide dismutase (SOD), Glutathione peroxidase (GPX) and catalase (CAT) (Mate's *et al.*, 1999).

Antioxidant enzymes are endogenous proteins that work in combination to protect cells from reactive oxygen species damage. Increased levels of the products of oxidative damage to lipids and protein have been detected in the serum of diabetic patients and their presence correlates with the development of complications (Brownlee, 2001). Intracellular antioxidant defence is primarily provided by antioxidant enzymes, which catalyse decomposition of reactive oxygen species. The three major antioxidant enzymes, superoxide dismutase, glutathione peroxidase and catalase, differ from each other in structure, tissue distribution and cofactor requirement.

Superoxide dismutases are a group of low molecular weight metalloproteinase present in all aerobic cells of plants, animal, and micro organisms. They provide protection against damaging reaction with the superoxide radical anion **(0^{2-})** by catalysing its disproportionation into oxygen and hydrogen peroxide (Garcia-gonzalez *et al*., 1999). In mammals, three SOD isoforms exist. Cytoplasmic CuZnSOD (SOD_1), Mitochondrial Mn SOD(SOD_2) and extracellular CuZnSOD (SOD_3) or (EC.SOD) (Faraci and Didioa, 2004). Each SOD isoform is derived from distinct genes but catalyzes the same reaction, producing **H_20_2** and **0_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) (Ezeiruaku and Micheal, 2015).

Glutathione peroxidase (GPX) is an enzyme with peroxidase activity, a selenium dependent enzyme and its extracellular form is a glycoprotein, while the intracellular and mitochondrial forms also possess different antigenic structures. It 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 peroxide to water (Brownlee, 2001) (Hisalkar *et al*., 2012).

Catalase is an enzyme commonly found in all living organism that requires oxygen for existence (Abdul salam *et al*., 2000). The enzyme catalyses the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese co-factor (Cheli kani *et al*, 2004).

Different studies have provided evidence 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) (Hisalkar *et al* .,2012). Hyperglycemia, a hallmark of diabetic condition, depletes natural antioxidant and facilitates the production of ROS, which has the ability to react with all biological molecules like lipids, protein, carbohydrates, DNA and exert cytotoxic effects on cellular components (Dincer, 2002). Thus, increased ROS and impaired antioxidant defence contribute for initiation and

progression of micro and macro vascular complications in diabetes (Ceriello et al., 1998) (Ceriollo and Motz, 2004).

Antioxidant reverses many of the effect of hyperglycemias on endothelia dependent relaxation and delayed cell replication (Manisha, 1999). To control lipid peroxidation, there is a defensive system consisting of antioxidant enzymes that play an important role in scavenging ROS. The organism susceptibility to free radial stress and peroxidative damage is related to the balance between the full radical load and the adequacy of antioxidant defences. Abnormally, high level of lipid peroxidation and the simultaneous decline of antioxidation mechanism can lead to damage of cellular organelles and oxidative stress (Manjulata *et al*., 2009). The quantity and quality of the reactive species is determined by metabolic pathways within the organism, influenced by exogenous factors such as radiation, food, stress etc. The adverse effects of free radicals are recognised in several disorders, but care should be taken when assessing their causative role (Erika *et al*., 1999). As elucidated by Patel *et al.*, 2008, long term complications are the main cause of morbidity and mortality. Because free radicals are difficult to measure directly as a result of their highly unstable nature, this study was therefore designed to evaluate the plasma levels of these three antioxidant enzymes EC, SOD, GPX and CAT.

Several studies have reported lower concentration of non enzymatic antioxidants as well as enzymatic antioxidant in type 2 diabetes (Djordjevi *et al*. , 2011), but there is no data regarding the actual status of antioxidant enzymes in both type 1 and type 2 diabetes patients with respect to the duration of disease. This study was therefore planned to investigate the level of these antioxidant enzymes both in chronic and non-chronic sufferers of the diseases with respect to apparently healthy, non diabetic subjects in Yenegoa, Bayelsa state of Nigeria.

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 158 patients suffering from diabetes type 1, of which 90 of them have suffered the disease for more than ten years (chronic sufferers) and 68 of them (non chronic) have suffered the disease for less than ten years. The studied subjects also included 200 of type 2 diabetic patients that were made up of 110 chronic sufferers and 90 non chronic sufferers. Their status was confirmed after a fasting blood sugar test with values above 7.0mMol/l and glycated haemoglobin (HbAic) values of above 7.0 %`(WHO, 2011). 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%) (ADA, 2015). 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. Also 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 with an administered questionnaire. 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 results were also made available to them. This study was carried out between May 2010 - January, 2015.

SAMPLE COLLECTION

The study subjects (diabetic and non diabetic) were properly instructed to fast over night for 10-14hours 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 enzymes : glutathione peroxidase, superoxide dismutase and catalase estimation.

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 enzymes estimation. 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 enzymes; glutathione peroxidase, superoxide dismutase and Catalase. It applies to in-vitro quantitative determination of the enzyme concentrations in plasma (Uotila *etal*., 1981) (Peter *et al*., 2001)

STATISTICAL ANALYSIS

The data are expressed as mean $\pm$ 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 analysis of variance (ANOVA). The results were considered statistically significant at 95% confidence interval ($P<0.05$).

Results

The result of the study, the values for the various parameters and the analysis is as presented in the following table below.

Table 1: Plasma level of the antioxidant enzymes, the mean $\pm$ S.D of HbAic in type 1 and type 2 poorly controlled chronic diabetic mellitus patients.

Study groups	Number of subjects	HbAic (%)	SOD (ng/ml)	GPX (ug/ml)	CAT(pg/ml)
Diabetic type 1	90	8.9±1.94	24.95±9.71	2.29± 0.38	22.46 ±14.94
DIABETIC TYPE 2	110	8.2±1.13	31.05±12.21	3.37±0.61	26.60±10.65
NON DIABETIC (CONTROL)	110	5.1±0.72	63.78±28.21	4.51± ±0.53	43.10±23.28

P Value = P<0.05	

Statistically at 95% confidence level (P>0.05), there was no difference between the mean values for type 1 and type 2 chronic diabetic patient in the antioxidant enzymes assayed.

Statistical difference exist (P<0.05) between the chronic diabetics and the non diabetic (control) subjects.

Table 2: Plasma level of the antioxidant enzymes, the mean ±S.D of the HbAic in type 1 and type 2 diabetes mellitus patients that have suffered the disease for less than ten years.

Study groups	Number of subjects	HbAic (%)	SOD (ng/ml)	GPX (ug/ml)	CAT (pg/ml)
Diabetic type 1	68	8.3±1.74	39.44±10.50	3.73±0.40	25.50±13.15
DIABETIC TYPE 2	90	7.8±1.19	44.10±12.38	3.94±0.33	28.48±12.72
Non diabetic (control)	110	5.1±0.72	63.78±28.21	4.51±0.53	43.10±23.28

P Value =P<0.05

Statistically at 95% confidence level (P>0.05) there was no difference between the mean values for type 1 and type 2 diabetic patients that have suffered the disease for less than ten years in the antioxidant enzymes studied. The mean values differ significantly (P<0.05) with the value obtained for non diabetic (healthy) control subjects. From the table 1 and 2, there is a statistical difference at this confidence level between the values obtained for EC. SOD for chronic and non- chronic DM.

Table 3: The glycated haemoglobin level and the antioxidant level of the enzymes with the age of disease in type1 DM.

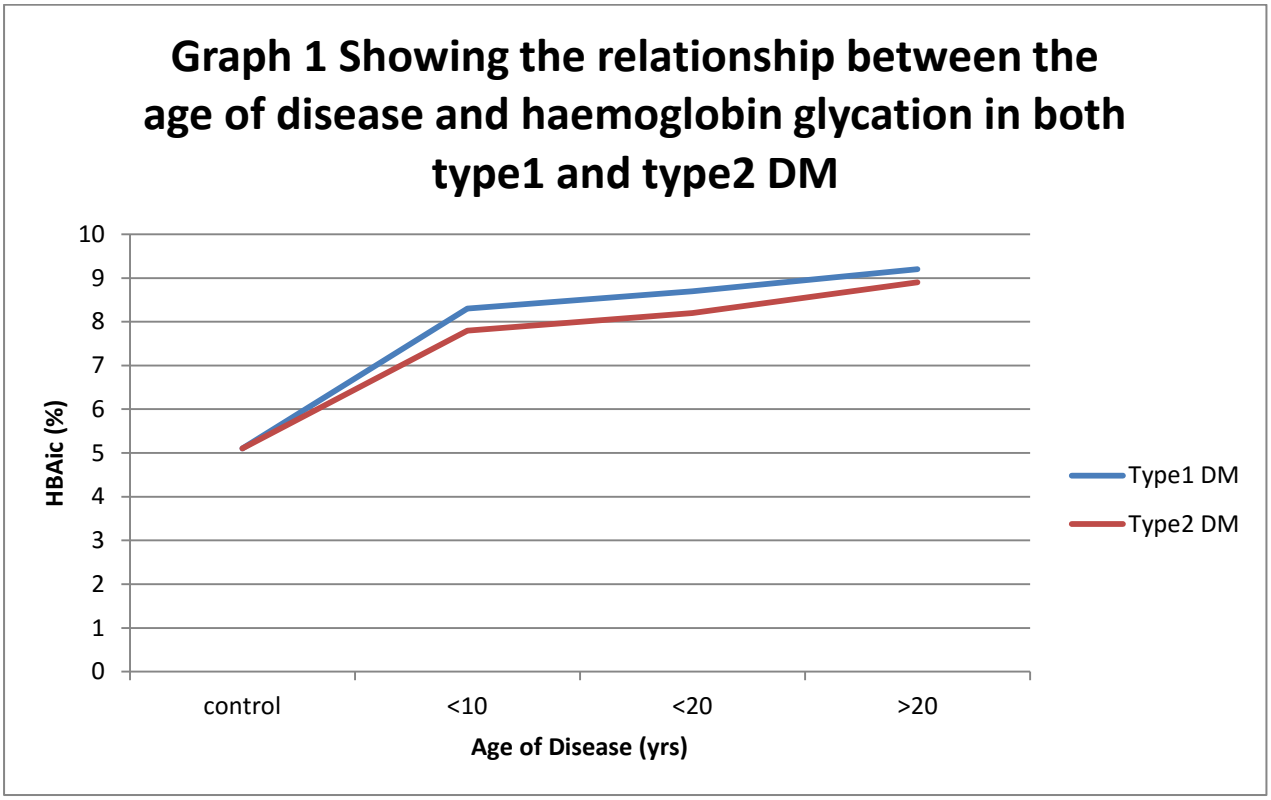
Study groups	Age Of Disease(yrs)	Number of subjects	HbAic (%)	SOD (ng/ml)	GPX (ug/ml)	CAT (pg/ml)
Dm type1	< 10	68	8.3± 1.74	39.44 ± 10.50	3.73 ±0.40	25.50 ± 13.15
Dm type1	11-20	52	8.7 ± 1.91	26.49 ±9.15	2.66 ±0.52	23.93 ± 12.56
Dm type1	21 and above	38	9.2 ± 0.98	21.74 ±10.79	2.03 ±0.32	21.40 ± 12.98
Control		110	5.1 ± 0.72	63.78± 28.21	4.51± 0.53	43.10 ± 23.28
P Value =	P<0.05					

Table 4: The glycated haemoglobin level and the antioxidant level of the enzymes with the age of disease in type2 DM.

Study groups	Age Of Disease(yrs)	Number of subjects	HbAic (%)	SOD (ng/ml)	GPX (ug/ml)	CAT (pg/ml)
Dm type2	<10	90	7.8 ± 1.19	44.10 ±12.38	3.94 ±0.33	28.48 ± 12.72
Dm type2	11-20	65	8.2 ± 1.85	36.20± 13.16	3.54± 0.41	25.83 ± 9.93
Dm type2	21 and above	45	8.9 ± 1.38	28.22 ±11.58	3.14 ±0.29	23.99 ± 13.10
Control		110	5.1 ± 0.72	63.78± 28.21	4.51± 0.53	43.10 ± 23.28
P Value =	P<0.05					

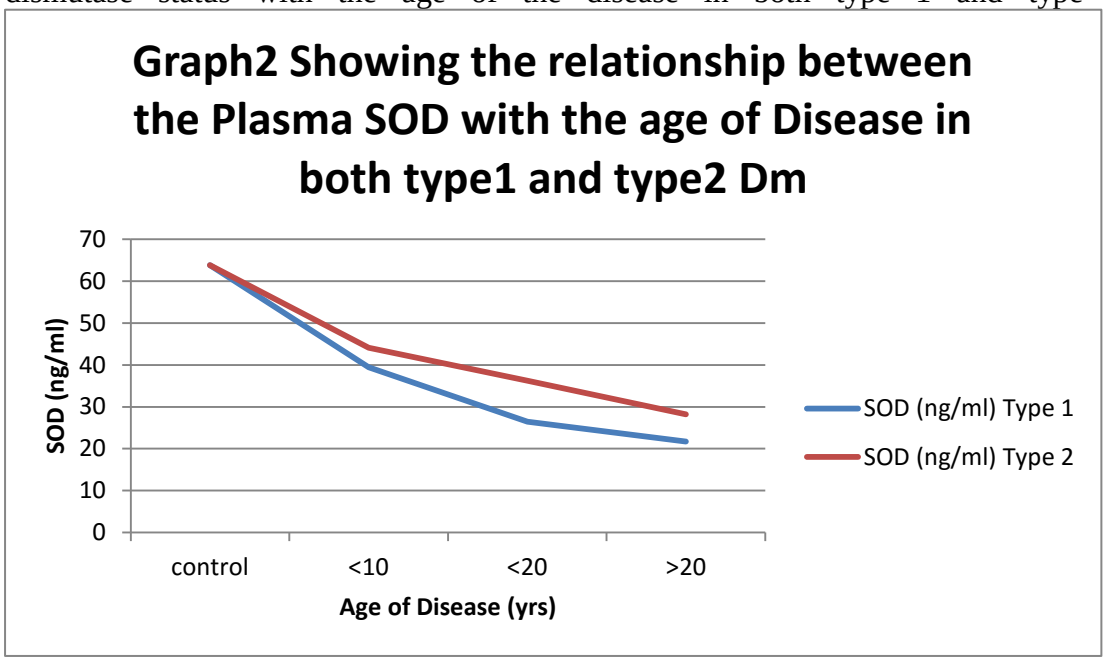
Table 3 and 4 showed that, with increase in the duration of illness, the enzymes are depleted and with concomitant increase in haemoglobin glycosylation

GRAPH 1: A line graph showing the relationship between the age of the disease and haemoglobin glycation in both type 1 and type 2 DM.



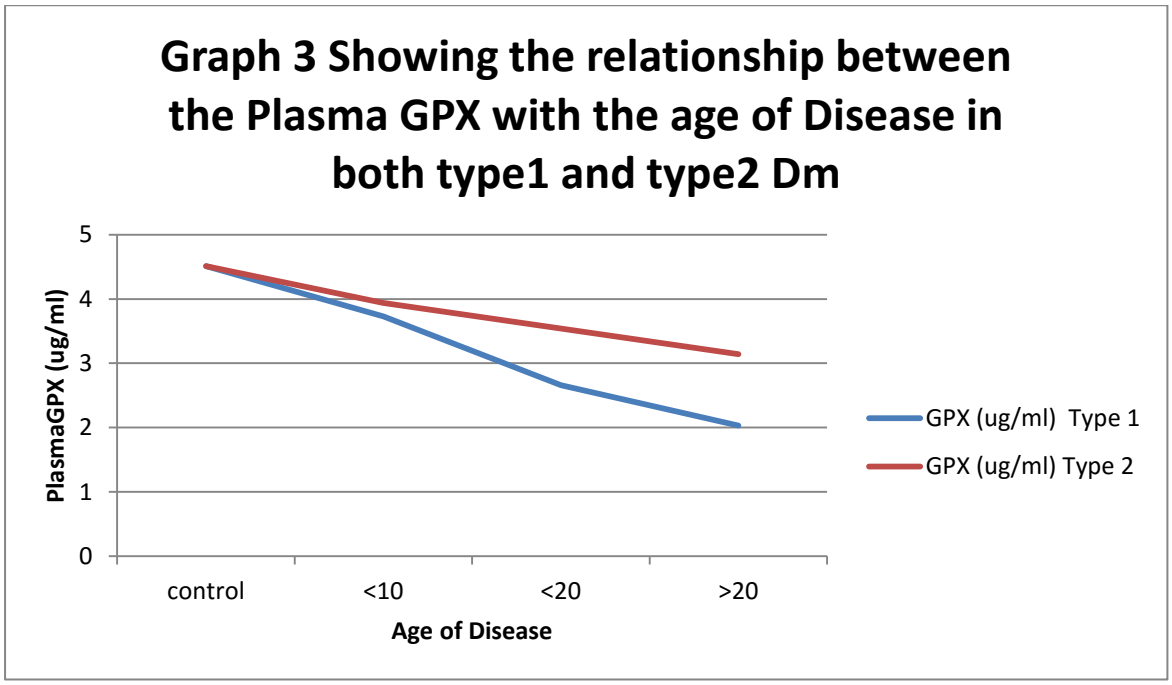
The line graph showed that haemoglobin glycosylation increased significantly with increase in the duration of the disease.

Graph 2: A line graph showing the relationship between the antioxidant enzyme, superoxide dismutase status with the age of the disease in both type 1 and type 2 DM.



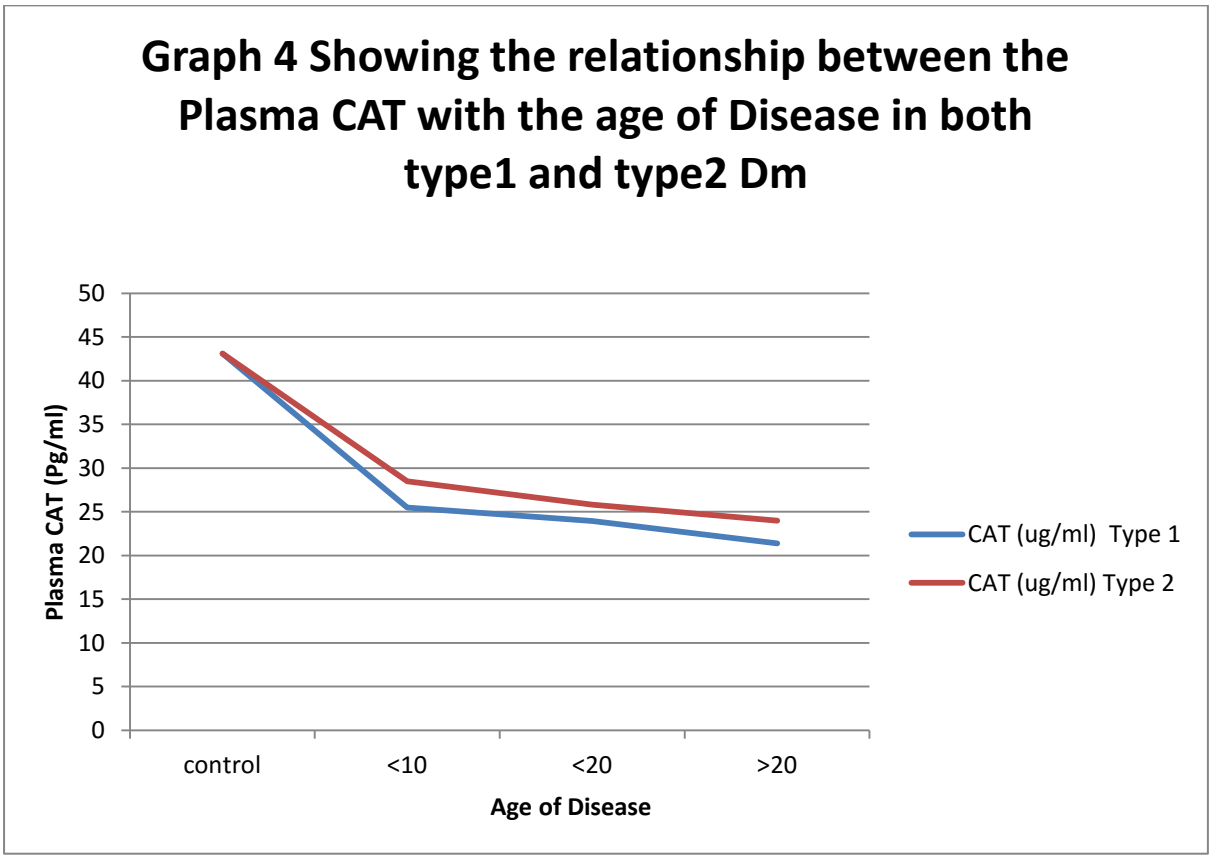
The line graph showed that the enzyme, Superoxide dismutase significantly decreases as the age of the disease increases.

Graph 3: A line graph showing the relationship between the antioxidant enzyme, Glutathione peroxidase status and the age of the disease in both type 1 and type 2 DM.



The line graph showed that the antioxidant enzyme, Glutathione peroxidase significantly decreases with increase in the age of the disease.

Graph 4: A line graph showing the relationship between the antioxidant enzyme, Catalase status and the age of the disease in both type 1 and type 2 DM.



The line graph showed that the Catalase enzyme, significantly decreases as the age of the disease increases.

DISCUSSION:

In diabetic mellitus patients, the pertinacity of hyperglycemia has been reported as a cause of increased production of oxygen-free radicals through glucose auto-oxidation and non enzymatic glycation. This means that the antioxidant enzymes activity in diabetic patients is always increased with concomitant depletion of the enzymes superoxide dismutase, glutathione peroxidase and catalase responsible for the reverse of the effects of hyperglycaemia at the different levels of the cell and its functions. Regulation of the antioxidant system must provide sufficient, properly located, anti-oxidant compounds and enzyme

From the study, we observed that the mean value of antioxidant enzymes-SOD, GPX and CAT was significantly decreased in diabetic type 1 and type 2 compared to non-diabetic group (CONTROL) ($p<0.05$) (Table 1). They are indicators of decrease in the protective antioxidant mechanism. This study in Yenegoa, Bayelsa State, Nigeria is in agreement with several studies (Bohr *et al*., 1998, Poljsak and Milisav, 2013). This study reveals a significant fall in SOD levels, which could be due to excessive oxidative stress. The values decreased significantly in the chronic type 1 and type 2 ($P<0.05$) with respect to the subjects that have suffered the disease for less than ten years (Table 3 and 4)

Similarly, the GPX and CAT were reduced in type 1 and type 2 DM, but not significantly ($P>0.05$) different from those that have suffered it for less than ten years. The decrease is as a

result of scavenging capacity of glutathione-dependent antioxidant defensive system against elevated lipid peroxidation processes in these diabetic patients. Hence, the resultant diabetic complications and insulin resistance as a consequence of increased production of free radicals and changes in activity levels of antioxidant enzymes in order to scavenge free radicals. 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 level of antioxidant enzymes (Turk *et al*., 2002).

With regard to the duration of the diseases, the enzymes were reduced significantly in both type 1 and type 2 DM (Graph 2-4). As the patient age with the disease, there is an implication that there will be an insulin resistance that impacts on the cell management of hyperglycaemia with increased free radical production. This is different from the subjects that have suffered the disease for less than ten years and non diabetic controls.

These findings are in accordance with the observations made by chugh *et el*., 1999, Prechl *et al*., 1997, that chronic exposure to hyperglycaemia and insulin resistance has been implicated in altered oxidative metabolism. Excessive plasma and tissue glucose can exert pathological effect through non-enzymatic glycosylation which leads to the production of superoxide and hydrogen peroxide (Mercuri *et al*., 2000). In this study, there is an increased haemoglobin glycation with the age of the disease.

CONCLUSION

The plasma SOD, GPX and CAT are decreased in both type 1 and type 2 diabetes mellitus when compared to non diabetic, apparently healthy subjects. The EC. SOD levels are significantly reduced in chronic type 1and type 2 with respect to the subjects that have suffered the disease for less than ten years. The depletion of the antioxidant enzymes reduce with the duration of the disease and with an increase in the haemoglobin glycosylation. This means that autoxidation of glucose due to persistent hyperglycaemia in diabetes mellitus is the major cause for generating oxidative stress.The findings from the study therefore suggest the estimation of plasma antioxidant enzymes with other routine investigations in diabetes 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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REFERENCES

Abdul Salan, A.A., Faizeh, A.A. and Ghad, N.A (2000). Catalase evaluation in different human diseases associated with oxidative stress. *Saudi Medical Journal* **21**(a):826-830.

American Diabetes Association (2015). Standards of medical care in diabetes. *Diabetic Care* **38**(1): 01 – 93.

Al-Rawi, N.H. (2011). Oxidative stress, antioxidant status and lipid profile in the saliva of type 2 diabetes. *Diabetes and Vascular Diseaes Reseach* **8**:22-28

Bigadli, E., Raimondi, L., Mannucci, E., Colombi, C., Badini, G., Rotella , M *et al* (2012). Lipid and protein oxidation products, antioxidant status and vascular complications in

poorly controlled type 2 diabetes. *The British Journal of diabetes and vascular disease* **12**:33-39.

Bohr, V., Anson, S., Mazur, R. And Dianov, G.(1998). Oxidative DNA damage processing and changes with aging. *Toxicology Letters **102-103**: 47 - 52*

Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature* **414**: 813- 820.

Ceriello, A. and Motz, E. (2004). Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes and cardiovascular disease? The common soil hypothesis revisited. Artherioselerosis, *Thrombosis and vascular Biology* **24**:816-823

Ceriello, A., Bortolo Hi, N., Crescentini, A., Motz, E., Lizzio, S., Russo A, et al., (1998). Antioxidant defences are reduced during the oral glucose tolerance test in normal and NDDM subjects. *European Journal of clinical investigation* **28**:329-333

Chelikani, P., Fita, I. and Loewen, P.C (2004). Diversity of Structures and properties among catalases *Cellular and Molecular life Sciences* **61** (2): 192-208.

Chugh, S.N., Kakkan R., Kaira, S. and Sharma A. (1999). An evaluation of oxidative stress in Dm during uncontrolled and controlled state and after Vitamin E supplementation. *The journal of the Association of physicians of India **47**(4): 380-383.*

Dincer, Y., Akcay, T., Alademir, Z and Llkova, H. (2002). Effect of oxidative stress on glutathione Pathway in RBC's from patients with IDDM. *Metabolism*. **51**:1360-1362

Djordjevic, G.M., Djuric, S.S., Djordjevic, V.B., Apostolski, S. and Zivkovic, M. (2011). The role of oxidative stress in pathogenesis of diabetic neuropathy. In C. Croniger (ed), *Role of the Adipocyte in Developmentof type 2 Diabetes*. Rijeka, Croatia InTech. PP153 - 172

Ezeiruaku, F.C and Micheal, K.C. (2015). Extra cellular superoxide dismutase (EC. SOD) evaluation in type 1 and type 2 DM Subjects in yanegoa, Bayelsa State, Nigeria. *Journal of Natural Sciences Research* **5**(22):20-24.

Erika, S., Jozsef, P., Janos, F. And Aniko, S. (1999). Alterations in enzymatic antioxidant defence in diabetes mellitus –a rational approach. *Postgraduate Medical Journal* **75**: 13 - 17

Faraci , F.M and Didioa, S.P (2004). Vascular protection superoxide dismutase isofrms in the vessel wall. *Arterioscles Thrombocytic Vascular Biology* **24**: 1367-1373.

Garcia – Gonzalez, A., Harrera – Abarca, L. and Ochoa, J. L. (1999). Effect of superoxide dismutase from bovine erythrocytes on different activity parameters in Adjuvant induced arthritis. *Archives of Medical Research* **30** (2): 132-134.

Giacco, F. and Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circular Research* **07**(a) 1058-1070.

Hisalkar, P.J., Fawade, M.M and Karmik, A.C (2012) Evaluation of plasma superoxide dismutase and glutathione peroxide in type 2 diabetes patients. *Biology and Medicine* **4**(2): 65-72.

Lapolla, A., Piaralli, F. and Sartore, G. (2007). Advanced glycation end products and antioxidant status in type 2 diabetic patients with and without peripheral arteres disease. *Diabetes care* **30**:670-676.

Likididlilid, A., Patchanans, N. and Peerapatdit, T. (2010). Lipid peroxidation and antioxidant enzyme activities in erythrocytes of type2 diabetic patients. *Journal of the Medical Association of Thailand* **93**:682-693

Lodovici, M., Giovannelli, L., Pitozzi, V., Bigagli, E., Bardini, G. and Rotella, C.M (2008).Oxidative DNA damage and plasma antioxidant capacity in type 2 diabetic patients with good and poor glycaemic control. *Mutation Research* **638**:98-102

Lodovici, M., Bardini, G, and Rottela, C.M (2009). Lipoperoxidation and antioxidant

capacity in patients with poorly controlled type 2 diabetes. *Toxicology and industrial Health* **52**:337-341.

Manjulata, K., Manju, B.P., Veena, S.G. and Neelima, S. (2009). Status of antioxidant enzymes and lipid peroxidation in type 2 DM with micro vascular complications. *Open Endocrinology Journal* **3**: 12 – 15.

Manisha, T. (1999). Antioxidant and their role in M, Heart disease and cancer. *Journal of the diabetic Association of india*. **39**(3):69-73

Mercuri, F., Quagliaro, L., and ceriello, A. (2000). Oxidative Stress in Diabetes. *Diabetes technology and Therapeutics* **2**(4):589-600.

Mates, J.M. and Sanchez, J.F. (1999). Anti oxidant enzymes and their implications in pathophysiology process. *Front Biosience Journal **4**: 339-345.*

Patel, A., Macmahon, S. and Chalmers J. (2008). Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *The New England Journal of Medicine* **358**: 25660-2572.

Peter, H., Sott, E. W and Steven, A.T (2001). Enzyme linked Immuno Sobert assays (ELISA).*Current protocols in molecular Biology* II.

Poljsak, B. and Milisav, I. (2013). Aging, oxidative Stress and antioxidants. In: J.A. Morales Gonzalez (Ed). *Oxidative stress and chronic degerative diseases: A role of antioxidants*. Rijeka, Croatia: InTech. PP345.

Prechl, J., Szaleczky, E., Pusztai, P., kocsis, I., Tulassay, Z. And Somogyi, A. (1997). Effect of clinical duration of diabetic mellitus on various antioxidants in blood. *Medical Science Monitor*. **3**: 167 – 170.

Seshosai, S.R., Keptoge, S., Thompson, D. Anelantonia, E., Gao, P. *et al* (2011).Diabetes mellitus, fasting glucose and risk of causing specific death. *New England Journal of Medicine* **3641** (a):824-841.

Soedamah-muthu, S.S. , Fuller, J.H. , Mulmien, H. E. , Raleigh, V.S. , lawrenson, R.A. and Co. houn H.M (2006) All cause mortality rates in patients with type 1 diabetic population from the UK general practise research database (1992-1999). *Diabetologies* **49**(4): 660-666.

Turk, H.M., Servinc, A., Canci, C., Cigli, A., Buyukberber, s. Sarli, H. *et al*(2002). Plasma, Lipid Peroxidation products and antioxidant enzymes activities in patients with type 2 Dm. *Acta Diabetologica* **39**(3): 117-122.

Uotila, m., Ruosiahti, E. and Engrall, E.(1981) Enzymes Linked Immino assays. *Journal of Immunological Methods* **42**: 11-15.

World Health Organization (2011). Use of glycated haemoglobin (HbAic) in the diagnosis of diabetes mellitus. *Abbreviated Reportof a WHO consultation Geneva*.