Original Research Article

CORRELATION OF ANTIOXIDANTS ENZYMES ACTIVITY WITH FASTING BLOOD GLUCOSE IN DIABETIC PATIENTS IN SOKOTO, NIGERIA

ABSTRACT

Several studies have reported the presence of oxidative stress in diabetes mellitus as a result of increased generation of reactive oxygen species and diminished antioxidant defence. The current study evaluated the plasma activities of three antioxidant enzymes- catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) in type 2 diabetic patients and non-diabetic control subjects. A total of 266 subjects were recruited for the study, 165 type 2 diabetic patients and 101 non-diabetic control subjects from Usmanu Danfodiyo University Teaching Hospital, Maryam Abacha Women and Children Hospital, and Specialist Hospital, Sokoto. All the three antioxidant enzyme activities were assayed in plasma using colourimetric enzymatic techniques. FBG was measured using glucose oxidase method. The mean activities of CAT, GPX and SOD, were significantly lower ($p< 0.05$, $p< 0.001$ and $p<0.001$) in diabetic patients when compared to non-diabetic control subjects respectively. The mean activities of CAT, GPX and SOD were correlated with fasting blood sugar. The mean activity of SOD was positively correlated while CAT and GPX were negatively correlated with fasting blood sugar. These findings confirm that diabetic patients have low activities of the antioxidant enzymes compared to the non-diabetic control subjects. Periodic assay of the activities of these enzymes in diabetic patients as adjunct biochemical analytes in monitoring is suggested.

Keywords: Correlation, Antioxidants Enzymes, Fasting Blood Glucose, Diabetic Patients, Sokoto, Nigeria

1.0 INTRODUCTION

Diabetes mellitus, often simply referred to as diabetes, is a metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion and insulin action, and is often accompanied by glycosuria, polydipsia and polyuria [1,2,3]. Diabetes mellitus is divided into two major classes, the less common type 1 (known as insulin dependent diabetes mellitus) and the more common type 2 (known as non - insulin dependent diabetes mellitus). Other sub-classifications include those of genetic defects of β-cell function, insulin action, endocrinopathy, infections, drug, chemicals and gestational diabetes mellitus often classified as type 4. This classification was endorsed by WHO expert committee on diabetes in 2000 [4]. Diabetes mellitus is associated with metabolic, micro-vascular and macro-vascular complications. In diabetics, persistent hyperglycaemia causes increased production of free-radicals, especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation [5,6,7]. Free radicals are generated as by-products of normal cellular metabolism; however, several conditions are known to disturb the balance between ROS production and cellular defence mechanism. This imbalance can result in cell dysfunction and destruction resulting in tissue injury. The increase in the level of ROS in diabetics could be due to their increased production and decreased destruction by non-enzymic and enzymic catalase (CAT), glutathione peroxidase

(GPx), and superoxide dismutase (SOD) antioxidants. The level of these anti-oxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes mellitus. This is particularly relevant and dangerous for the beta islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defences [8,9,10,11].

Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems i.e. increased free radical production or reduced activity of antioxidant defences or both. The implication of oxidative stress in the pathogenesis of diabetes is suggested not only by oxygen free-radical generation but also due to non-enzymatic protein glycosylation, auto-oxidation of glucose [12], impaired glutathione metabolism [13], alteration in antioxidant enzymes [14]. Also, there are other defence mechanisms against free radicals like the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) whose activities contribute to eliminating superoxide, hydrogen peroxide and hydroxyl radicals [15].

2.0 MATERIALS AND METHODS

2.1 Study Area

The study was conducted at Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Specialist Hospital Sokoto and Maryam Abacha Women and Children Hospital Sokoto. The samples were analyzed in the Research Laboratory of the UDUS City campus.

2.2 Sampling

A total of 266 subjects were recruited for the study. These consist of 165 diabetic patients attending the outpatient department of the medical clinic in Usmanu Danfodio University Teaching Hospital, Specialist Hospital, Sokoto and Maryam Abacha Women and Children Hospital, Sokoto, and 101 apparently healthy individuals serving as controls. These control subjects were recruited from the population of Staff and Students of Usmanu Danfodiyo University (UDUS) and UDUTH, Sokoto.

2.3 Inclusion Criteria

Individuals with established type 2 diabetes mellitus who agreed to participate were included in the study. Apparently, healthy non-diabetic subjects were recruited in the study as controls.

2.4 Exclusion Criteria

Individuals with type 1 diabetes mellitus and gestational diabetes mellitus were excluded from the study. Those with debilitating diseases e.g. liver cirrhosis, heart failure etc. and all those who declined to give their consent were excluded from the study.

2.5 Informed Consent

Informed consent was obtained from both patients and subjects before inclusion using approved protocol.

2.6 Ethical Approval

The approval of the study was obtained from the Ethical Committee of the Usmanu Danfodiyo University Teaching Hospital, Sokoto (UDUTHS).

2.7 Sampling Techniques

The arrangement was made with the clinicians, whereby subjects who satisfy the study inclusion criteria were selected. Questionnaires were administered to the study population. Information including the name, age, sex, occupation, duration of disease, height, weight, BMI, was obtained through personal interviews while the type of DM, drugs taken and complications was obtained from the Physician in the clinic,

and these were followed by specimen collection. The findings were documented in the proforma.

2.8 Specimen Collection and Processing

From each selected subject, about 5ml of fasting blood sample for the biochemical analysis was collected using a sterile syringe and needle from an antecubital vein (venepuncture) after cleaning the site with methylated spirit (70%) and allowed to dry. About 3ml of the whole blood was dispensed into EDTA bottle for estimation of glycated haemoglobin, glutathione peroxidase, catalase and superoxide dismutase. The remaining 2ml of the blood sample was dispensed into a fluoride container for the estimation of blood glucose level. The EDTA samples after collection were centrifuge at 3000rpm for 5minutes to obtain clear unhaemolyzed plasma. The plasma was harvested into a sterile separation tubes and kept frozen at -20 ^{0}C before assay.

2.9 Chemicals and reagents

All the chemicals and reagents were of analytical grade.

2.10 Analytical Methods

Plasma glucose, GPx, Catalase and Superoxide dismutase were measured using the enzymatic method of Trinder [16], Ursini *et al*. [17], Johansson *et al*. 18] and Malstrom *et al*. [19] respectively.

3.0 RESULTS

The results obtained in the current study are presented in the following tables 1-3.

Table 1 shows the age and anthropometric parameters of the diabetic patients and controls. The mean age of the diabetic patients and controls were (48.03+1.07yrs) and (45.41+1.57yrs), respectively. The mean values of BMI in diabetic patients and the non-diabetic controls were 28.50± 0.37 kg/m^2 and 24.61±0.51kg/m^2 respectively. The mean BMI of the diabetic patients was statistically higher ($p<0.05$) when compared with that of controls.

Table 2 shows the plasma antioxidant enzymes levels of diabetic patients and controls. The mean value of glutathione peroxidase activity in diabetic patients (60.43±0.62mmol/min/ml) was significantly lower ($p<0.001$) than value in non-diabetic controls (74.89±1.61nmol/min/ml). The mean value of catalase in the diabetic patients (28.10±0.57nmol/min/ml) was significantly lower ($p<0.05$) than that of the control subjects (36.46±0.84nmol/min/ml). Also, mean value of superoxide dismutase activity of diabetic patients (4.47±0.24 U/ml) was significantly lower ($p<0.001$) than the value in non-diabetic controls (8.15±0.47 U/ml).

Table 3 shows the correlations of antioxidant enzymes activity with FBG. No significant correlations were observed with all the three enzymes.

Table 1: **Age and anthropometric parameters (Mean ± SEM) of the diabetic patients and controls**

Subjects	n	Age (Years)	BMI (Kg/m^2)	Height (m)	Weight (kg)
Diabetic Patients	165	48.03±1.07	28.50 ± 0.01^a	1.64 ± 0.01	77.17± 1.09^a
Controls	*101*	45.41 ± 1.57	24.61 ±0.51^b	1.63 ± 0.01	65.69 ± 1.37^b

^{ab} means with different superscripts differ significantly (P< 0 .05) BMI= Body Mass Index; n = number of subjects; SEM = Standard error of mean

Table 2: Plasma antioxidant enzymes levels (mean ± SEM) of diabetic patients and controls

Subjects	n	GPX (nmol/min/ml)	CAT (nmol/min/ml)	SOD (U/ml)
Patients	165	60.43 ± 0.62^{a}	28.10 ± 0.57^{a}	4.47 ± 0.24^{a}
Controls	101	74.89 ± 1.61^{b}	36.46 ± 0.84^{b}	8.15 ± 0.47^{b}

[ab] means with different superscript differs significantly ($p<0.001$) GPX=Glutathione Peroxidase CAT= Catalase; SOD= Superoxide Dismutase. n = number of subjects; SEM = Standard error of mean

Table 3: Correlation of antioxidants enzymes activity with fasting blood glucose (X = 7.29, SEM = 0.29) in diabetic patients (n= 165) (Spearman's test).

Parameters		GPX (nmol/min/ml)	CAT (nmol/min/ml)	SOD (U/ml)
FBG (mmol/L)	Coefficient of correlation	-0.09	-0.12	0.09
	P	0.25	0.11	0.27

P- Value GPX = Glutathione peroxidase; CAT=Catalase; SOD= Superoxide dismutase FBG= Fasting blood glucose

4.0 DISCUSSION

Diabetes mellitus leads to many complications as a result of long-term oxidative damage to tissues by free-radicals arising from glucose metabolism which later results in increased morbidity and mortality [20]. Once these complications occur it may be irreversible and meeting the cost of management of complications is a challenge which increasingly confronts diabetic patients and health facilities in developing countries [21]. Hence, this study was designed to evaluate the plasma activities of three antioxidant enzymes- CAT, GPX and SOD in type 2 diabetic patients and in non-diabetic control subjects.

The results of the present study indicate reduced antioxidant status in diabetic patients which may indicate the presence of oxidative stress. A significant decrease was observed in all the three antioxidant enzymes – CAT, GPX and SOD in diabetic patients when compared with non- diabetic control subjects. This is in agreement with reports of several studies, which have been carried out previously [22,23,24,25,26].

In diabetes mellitus, there is an accumulation of hydrogen peroxide which is formed from autoxidation of glucose which inactivates SOD [27]. This could be one of the reasons for decrease activities of SOD in diabetic patients. The decrease may be due to the loss of its two factors Zn^{2+} and Cu^{2+} [28].

The low activity of SOD observed in the present study may suggest that with longer duration of diabetes, SOD induction and consequently its activity may progressively decrease, since other causes of hydrogen peroxide productions like the non-enzymatic glycation may later predominate, hence further inhibition of the SOD may occur.

The present study showed that there is a significant decrease in the activity of glutathione peroxidase in diabetic patients when compared to non-diabetic controls. These results are in agreement with those obtained by Jos *et al.,* [29]. However, some authors found no difference between GPX activity in diabetic and non-diabetic controls [30]. The low activity of GPX could be directly attributed to the low content of GSH found in diabetic patients, since GSH is a

substrate and a cofactor of GPX [22]. The inactivation of the enzyme as a result of severe oxidative stress could also be attributed to low levels of the enzymes [31]. The inactivation of the enzymes may occur through glycation as a result of hyperglycaemia [32].

The decrease in CAT as an inducible enzyme may be due to the decrease level of H_2O_2 generated by SOD [33].

5.0 CONCLUSIONS

Mean antioxidant enzymes are significantly lower in diabetic patients compared to controls. Mean activities of CAT, SOD and GPX were correlated with fasting blood glucose. There was the significantly positive correlation between SOD and fasting blood sugar.

REFERENCES

1. Celik, I. E., Yegin, F., Odabasoglu, F. (2002). Effect of experimental diabetes mellitus on plasma lactate dehydrogenase and glutamic oxaloacetic transaminase levels in rabbits, Turkish J. Biol., 26, 151 - 154.
2. Sailaja, Y. R., Baskar, R., Saralakumari, D, (2003). The antioxidant status during maturation of reticulocytes to erythrocytes in type 2 diabetes, Free Radic. Biol. Med.,35(2), 133 – 139.
3. ADA (American Diabetes Association), (2005). Diagnosis and classification of Diabetes Mellitus. Diabetes Care, 28 (suppl.1), S37 – S43.
4. World Health Organisation,(2000). Definition, diagnosis and classification of diabetes mellitus and its complications. Geneva.
5. Oberley, L.W,(1988). Free Radicals and Diabetes. *Free Radical Biology and Medicine*; 5:113-124
6. Aragno, M.E., Tamagno, V. Gato, E. Brignarddello, S. Parola, O. Danni, G. Boccuzzi,(1999).
7. Dehydroepiandrosterone protects tissues of streptozotocin – treated rats against oxidative stress. Free Radic. Biol. Med., 26(11/12), 1467 – 1474.
8. Bonnefont-Rousselot, D., Bastard, J.P., Jaudon M.C.,Delattre, J. (2000). Consequences of the diabetic status on the oxidant/antioxidant balance. Diabetes Metab., 26: 163-176.
9. Grodsky, G.M., Anderson, C.E., Coleman, D.L., Craighead, J.E., Gerritsen, G.C, Hansen, C.T., Herberg, L., Howard C.F., Lernmark, A., Jr, Matschinsky, F.M., Rayfield, E., Riley, W.J., Rossini, A.A, (1989). Metabolism and underlying causes of diabetes mellitus, Diabetes,31, 45 –53.
10. Lenzen, S.J., Drinkgern, M., Tiedge, (1996). Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues, Free radic. Biol. Med., 20, 463 – 466.
11. West, I.C, (2000). Radicals and oxidative stress in diabetes. Diabetic. Med.,17,171 – 180.
12. Robertson, R.P, (2004). Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes, J. Biol. Chem., 279 (41), 42351 – 42354.
13. Mullarkey, C.J., Edelstein, D., Brownlee, L., (1990). Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes, Biochem. Biophys. Res. Comm., 173, 932 -939.
14. Mclennan, S.V., Heffernen, S., Wright, L, (1991). Changes in

hepatic glutathione metabolism in diabetes, Diabetes, 40, 344 – 348.
15. Strain, J.J, (1991). Disturbances of micronutrient and antioxidant status in diabetes, Proceedings of the Nutrition Society,50, 591 – 604.
16. Soto, C., Recoba, R., Barron, H., Alvarez, C., Favari, L, (2003). Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas, Comparative Biochemistry and physiology, 136, 205 – 212.
17. Trinder P. (1969). Annals of Clinical Biochemistry. 6: 24
18. Ursini. F., Maiorino. M., and Gregolin, C. (1985). The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. Biochim. Biophys. Acta 839, 62 – 70.
19. Johansson, L.H. and Borg, L.A.H (1988). A spectrophotometric method for determination of catalase activity in small tissue samples. Analytical Biochemistry; 174: 331-336.
20. Malstrom, B., Andreasson, L., and Reinhammer, B. (1975). The Enzymes. Boyer, P., editor. XIIB, Academic Press. New York, 533.
21. Gupta, M., Chari, S, (2006). Prooxidant and antioxidant status in patients with type 2 diabetes mellitus with IHD. Indian J. Clin Biochem, 21: 118 -122.
22. Baynes, J.W. (1991). Role of oxidative stress in the development of complications in diabetes.Diabetes. 40(4): 405 -12.
23. Domingues, C., Ruiz, E., Gussinye, M., Carroscosa, A.(1998). Oxidative Stress at onset and in early stages of type 1 diabetes in children and adolescents. *Diabetes Care*; (21): 1736-1742
24. Mosaad, A.A., Abd- Allah, Y. (2004). Evaluation of some biochemical changes in diabetics patients. Elsevier, Clinica Chimica Acta 346: 161 – 170.
25. Alberti KGMM, Zimmet, P., Defronzo R.A., Keen, H, (1997). International textbook of diabetes mellitus. Second edition, John Wiley and Sons.
26. Marica, J. B., Veljko, B., Jadranka, B., Zeljko, M., Ivan, J., Zeljko, R, (2005). Impact of glycemic Control on Antioxidant Enzyme Activity in Patients with Type 2 Diabetes Mellitus. Diabetologia Croatica 33 (4): 131- 134.
27. Hisalkar, P.J., Patne, A.B., Fawade, M.M., Karnik, A.C, (2012). Evaluation of plasma superoxide dismutase and glutathione peroxidase in type 2 diabetic patients. Biology and Medicine, 4 (2): 65 - 72
28. Zbronska, H., Grzeszezak, W., Jendryezko, A., Zbronski, R., Kuz-niewiez, R.(1995). The activity of superoxide dismutase in erythrocytes and leukocytes and levels of zinc and copper in blood of patients with diabetes. Effects of diabetic treatment on examined parameters. Pol Arch Med wew,94(3):228 -234.
29. Lin, J.(1996). The association between copper ions and peroxidative reaction in diabetic cataract. Nippon Ganka Gakkai Zasshi.100(9): 672 -679.
30. Jos, J., Rybak, M, Patin, P.H., Robert, J.J., Boitard, C., thevenin, R.(1990). Etude des enzymes antioxydantes dans le diabete insulin-independent de l'enfant et de

I'adolescent. Diabete Metab, 16: 498 503.
31. Jain, A. and Buist, N.R.M. (1994). Effect of Ascorbate or N-acetylcysteine treatment in a patient with hereditary glutathione synthetase deficiency. Journal of Pediatrics; 124(2): 229-233
32. Domingues, C., Ruiz, E., Gussinye, M., Carrascosa, A. (1998). Oxidative stress at onset and in stages of type I Diabetes in children and adolescents. Diabetes Care, 21: 1736 – 1742.
33. Condell, R.A., Tappel, A.L. (1983). Evidence for suitability of glutathione peroxidase as a protective enzyme: Studies of oxidative damage, restoration and proteolysis. Arch. Biochem. Biophys 233: 407 -416.
34. Arai, K., Maguchi, S.,Fujii, S.,Ishibashi, H., Oikawa, K., Taniguchi, N.(1987). Glycation and inactivation of human CuZn superoxide dismutase: Identification of the in vitroglycated sites. J. Biol. Chem., 262: 16969-16972.
35. McCord, Jr J.M., Keele, B.B., Fridovich, L. (1971). An enzyme based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. Proc. Natl. Acad. Sci USA, 34(8): 487 – 90.