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Evaluation of Plasma Antioxidant Enzymes in Type 2 Diabetic Patients in Sokoto, Nigeria

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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 defense. 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 cross-sectional study, 165 type 2 diabetic patients and 101 non-diabetic controls 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

colorimetric enzymatic techniques. The mean activities of CAT, GPX and SOD were significantly lower ($p < 0.05$, < 0.001 and < 0.001 respectively in diabetic patients when compared to non-diabetic controls respectively). These findings confirm that diabetic patients have low activities of the antioxidant enzymes compared to the non-diabetic controls. Periodic assay of the activities of these enzymes in diabetic patients as adjunct biochemical analytes in monitoring is suggested.

Key words: Evaluation, Plasma Antioxidant Enzymes, Type 2 Diabetes, Sokoto, Nigeria

Introduction

Diabetes mellitus, often simply referred to as diabetes, is a metabolic disorder of multiple aetiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion and/or insulin action, and is often accompanied by glycosuria, polydipsia and polyuria (American Diabetes Association, 2005; Sailaja.,2003; Celik *et al.*, 2002). 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 (2000). 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 (Bonfont-Rousselot *et al.*, 2000; Aragno *et al.*, 1999; Oberley, 1988). 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 defense 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/or 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 defenses (Robertson, 2000; West, 2000; Lenzen *et al.*, 1996; Grodsky *et al.*, 1989).

Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems, i.e. increased free radical production or reduced activity of antioxidant defenses or both. 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 (Mullarkey *et al.*, 1990), impaired glutathione metabolism (McLennan *et al.*, 1991), alteration in antioxidant enzymes (Strain, 1991). In addition, there are other defense mechanisms against free radicals like the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) whose activities contribute to eliminate superoxide, hydrogen peroxide and hydroxyl radicals (Soto *et al.*, 2003).

Materials and Methods

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.

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 Danfodiyo University Teaching Hospital, Specialist Hospital, Sokoto and Maryam Abacha Women and Children Hospital, Sokoto, and 101 apparently healthy individuals serving as controls. The control participants were recruited from the population of Staff and Students of Usmanu Danfodiyo University (UDUS) and UDUTH, Sokoto.

Sample Size Determination

The sample size is calculated using the formula below

$$n = \frac{(z_{1-\alpha})^2(p)(1-p)}{d^2}$$

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.

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.

Informed Consent

Verbal informed consent was obtained from both subjects and controls before inclusion using approved protocol.

Ethical Approval

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

Sampling Techniques

Arrangement was made with the clinicians, whereby subjects who gave consent and satisfy the study inclusion criteria were selected. Questionnaires were administered to the study population. Information including the name, age, gender, occupation, duration of disease, height, weight, BMI, was obtained through personal interviews while type of DM, drugs taken and complications was obtained from the Physician in the clinic, and these was followed by specimen collection. The findings were documented in the proforma.

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 antecubital vein (venepuncture) after disinfecting 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

Chemicals

All the chemicals and reagents were of analytical grade.

Analytical Methods

GPx activity was measured using enzymatic method of Ursini *et al.* (1985). Catalase activity was measured using enzymatic method of Johansson *et al.* (1988). Superoxide dismutase activity was measured using enzymatic method of Malstrom *et al.* (1975).

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.

clear non-haemolyzed plasma. The plasma was harvested into a sterile separation tubes and kept frozen at -20 °C before assay.

The mean values of BMI in diabetic patients and the non-diabetic controls were 28.50± 0.37 kg/m² and 24.61±0.51kg/m² 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, catalase and superoxide dismutase activity in diabetic patients (60.43±0.62 nmol/min/ml), (28.10±0.57 nmol/min/ml) and (4.47±0.24 U/ml) respectively were significantly lower than value in non-diabetic controls (74.89±1.61nmol/min/ml), (36.46±0.84 nmol/min/ml) and (8.15±0.47 U/ml) (p<0.001, <0.05 <0.001) respectively.

Table 3 shows the antioxidant enzymes of diabetic patients based on gender. The results of all the three enzymes show no significant difference in males and females (p>0.05).

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 Antioxidant Enzymes Diabetic Patients based on Gender (Mean ± SEM)

| Subjects | N | GPX (nmol/min/ml) | CAT (nmol/min/ml) | SOD (U/ml) |
|----------|-----|-------------------|-------------------|-------------|
| Male | 102 | 60.92 ± 0.83 | 28.16 ± 0.72 | 4.45 ± 9.29 |
| Female | 63 | 59.63 ± 0.89 | 28.06 ± 0.91 | 4.35 ± 0.40 |

P values of all the antioxidant are statistically non-significant at P (>0.05). GPX = Glutathione Peroxidase; CAT = Catalase; SOD =Superoxide Dismutase N = number of subjects; SEM = Standard error of mean

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 (Gupta and Chari, 2006). Once these complications occur it may be irreversible. Meeting the cost of management of complications is a challenge which increasingly confronts diabetic patients and health facilities in developing countries (Baynes, 1991). 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 (Hisalkar *et al.*, 2012; Marica *et al.*, 2004; Mosaad *et al.*, 2004; Alberti *et al.*, 1997; Domingues *et al.*, 1989).

In diabetes mellitus, there is an accumulation of hydrogen peroxide which is formed from autoxidation of glucose which inactivates SOD (Fajens, 2001). This could be one of the reasons for decrease activities of SOD in diabetic patients. Another reason could be as a result of glycosylated SOD that may lead to the inactivation of the enzymes (Aria *et al.*, 1989) or the decrease may be due to the loss of its two factors Zn^{2+} and Cu^{2+} (Lin, 1996; Zbronska *et al.*, 1995).

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 predominates, 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.* (1990). However, some authors found no difference between GPX activity in diabetic and non-diabetic controls (Jain and Buist, 1994). 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 (Alberti *et al.*, 1997). The inactivation of the enzyme as a result of severe oxidative stress could also be attributed to low levels of the enzymes (Condell and Tappel, 1983). The inactivation of the enzymes may occur through glycation as a result of hyperglycaemia (Arai *et al.*, 1987).

The decrease in CAT as an inducible enzyme may be due to the decrease level of H_2O_2 generated by SOD (McCord *et al.*, 1971).

Conclusions

Mean antioxidant enzymes are significantly lower in diabetic patients compared to controls. Periodic assays of CAT, GPX and SOD in known diabetic patients on treatment are suggested.

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