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Evaluation of Plasma Glucose and Glycated Haemoglobin Levels of 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 concentrations of glycated haemoglobin (HbA1C) and fasting blood glucose (FBG) as indices of glycaemic control 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. FBG was measured using glucose oxidase method and HbA1C using micro column method. The mean activities of FBG, HbA1C were higher ($p < 0.05$) in diabetic patients when compared to non-diabetic controls. These findings confirm that diabetic patients have high levels of FBG and HbA1C compared to the non-diabetic control subjects. Periodic assay of the activities of FBG and HbA1C in diabetic patients as adjunct biochemical analytes in monitoring is suggested.

Key words: Plasma Glucose, Glycated Haemoglobin Levels, 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 *et al.*, 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 in 2000 (World Health Organization, 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 (Aragno *et al.*, 1999; Oberley, 1988; Bonnefont-Rousselot *et al.*, 2000). 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, 2004; Weyer *et al.*, 1999; 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, 1999). 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 cross-sectional 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 out-patient 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 who were monitored as controls. The controls were recruited from the population of Staff and Students of Usmanu Danfodiyo University (UDUS) and UDUTH, Sokoto.

Inclusion Criteria

Individuals with established type- 2 diabetes mellitus who consented to participating in the study 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 patients and subjects before inclusion using approved protocol.

Ethical Approval

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

Sampling Techniques

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, 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 was collected for the biochemical analysis using a sterile syringe and needle from antecubital vein (venepuncture) after disinfecting the site with methylated spirit (70%) and allowing it 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 2 ml 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 non-haemolyzed plasma. The plasma was harvested into a sterile separation tubes and kept frozen at -20 °C before assay.

Chemicals and reagents

All the chemicals and reagents were of analytical grade.

Analytical Methods

Plasma glucose was measured using enzymatic method of Trinder (1969). GHbA1c was

measured using micro column method of Trivelli *et al.* (1971).

Results

The results obtained in the current study are presented in the following tables 1-13. 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/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 fasting blood glucose and glycated haemoglobin levels of diabetic patients and controls. The mean fasting glucose concentration in diabetic patients (7.29± 0.29mmol/L) was significantly higher (p<0.05) than the value in non-diabetic control subjects (4.12± 0.07mmol/L). The mean value of glycated haemoglobin in the diabetic patients (7.56±0.13%) was significantly higher (p<0.05) than that of controls (5.21±0.08%).

Table 3 shows the reference values of plasma antioxidant enzymes, fasting blood glucose and glycated haemoglobin in control subjects with upper and lower limits.

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: Fasting blood glucose and glycated haemoglobin levels (mean ± SEM) of diabetic patients and controls

Subjects	n	FBG (mmol/l)	HbA1c (%)	Duration (Years)
Patients	165	7.29 ± 0.29 ^a	7.56± 0.13 ^a	6.7±5.0
Controls	101	4.12± 0.07 ^b	5.21 ± 0.08 ^b	-

^{ab} means with different superscripts differ significantly (P< 0.05) FBG= Fasting Blood Glucose; HbA1C= Glycated Haemoglobin, n = number of subjects; SEM = Standard error of mean



Table 3: Reference values of plasma antioxidant enzymes, fasting blood glucose and glycated haemoglobin in control subjects (2.5 and 97.5 percentile levels).

Subjects	FBG (mmol/l)	HbA _{1c} (%)
Upper Limit	5.6	6.7
Lower Limit	3.0	3.8

GPX= Glutathione Peroxidase; CAT=Catalase; SOD= Superoxide Dismutase, FBG=Fasting Blood Glucose; HbA_{1c}=Glycated Haemoglobin.

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 and meeting the cost of management of complications is a challenge which increasingly confronts diabetic patients and health facilities in developing countries (Baynes, 1991).

The present study had demonstrated that diabetic patients have higher levels of fasting blood glucose and glycated haemoglobin as compared to values in the control subjects. These observations are in agreement with reports from other investigators (Hisalkar *et al.*, 2012; Ebenezer *et al.*, 2003; Rahbani – Nobar *et al.*, 1999). These results are expected in uncontrolled or poorly controlled diabetic patients' due to insulin resistance. Since most of these patients are undergoing treatment, it is expected that the glucose and glycated haemoglobin should be normal or near normal. The fact that values are significantly high may indicate poor glycaemic control (both current and past), several reasons could account for these. The patients may not have enough money to meet with the high cost of their drugs. On the other hand, the patients may not adhere strictly to the clinician's prescriptions.

Conclusions

Mean levels of glycated haemoglobin and fasting blood glucose were significantly higher in diabetic patients than in controls.

Recommendations

Periodic assays of CAT, GPX and SOD in known diabetic patients on treatment are suggested. Strict monitoring of glucose and patients' compliance to treatment to ensure normoglycaemia is recommended.

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