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Antioxidant Status in Diabetic Patients Attending the Federal Teaching Hospital Gombe- Nigeria

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Abstract*: Fasting blood samples were collected from 120 diabetes mellitus subjects attending medical clinic at Federal teaching hospital Gombe and apparently healthy subjects. The level of glycated hemoglobin was obtained for the samples and samples with glycated hemoglobin ≥7.0 were further analysed for antioxidant enzymes of superoxide dismutase, catalase, and glutathione peroxidase.Level of lipid peroxidation marker; malondialdehyde was also determined.Malondialdehyde levels (µmol/L) assessedwere significantly increased (p< 0.05) in all diabetic groups compared to apparently healthy individuals (1.94± 0.22µmol/L), the results showed the level of antioxidant enzymes of SOD (u/ml) and CAT (u/ml) were decreased in the diabetics compared to apparently healthy individuals. However, the level of GPx determined in type 1 diabetics was significantly increased compared to apparently healthy individuals, but lower in type 2 and gestational diabetics. The results in this study confirmed that increased oxidative stress due to decreased SOD and CAT activities seen in diabetics which may also enhance lipid peroxidation as seen in high level of malondialdehyde.*
Keywords*: Diabetes Mellitus, Malondialdehyde, Oxidative Stress, Antioxidant Enzymes.*

I. Introduction

The development of a wide range of diseases such as diabetes, malaria, cardiovascular and Parkinson's diseases etc have been attributed to oxidative stress [1]. Free radicals which are the product of oxidative stress [2] are elevated in these disease states leading to decrease in the antioxidant defence system.

The term 'oxidative stress' is used when the body's natural defence mechanism are exceeded by the production of deleterious reactive oxygen species (ROS), resulting in damage to susceptible cell components such as DNA, proteins and lipids. Features of the brain that cause it to be particularly sensitive to oxidative stress include a high rate of oxidative metabolic activity, relatively low levels of antioxidant enzymes (e.g. catalase, glutathione peroxidase), a high concentration of unsaturated fatty acids, large iron and copper stores, and a low mitotic index [3].

Superoxide dismutase is one of the key antioxidant enzymes which provide an essential defence against oxygen toxicity to the cell. Superoxide dismutase is the antioxidant enzyme that catalyses the conversion of $O_2^{\cdot -}$ to O_2 and to the less reactive species H_2O_2.There are three forms of SOD in humans [4]; Cystolic Cu,Zn-SOD, Mitochondrial Mn-SOD, Extracellular SOD EC- SOD.

Catalase is a heme containing redox enzyme, found in high concentration in the peroxisomes. The enzyme is present in the cells of plants, animals and aerobic bacteria [5].The enzyme very efficiently promotes the conversion of H_2O_2 to water and molecular oxygen.

Glutathione metabolism is one of the most essential of antioxidative defence mechanisms. GPx acts in conjunction with the tripeptide glutathione (GSH), which is present in cells in high concentration (micro molar). The substrate for the catalytic reaction of GPx is H_2O_2, or organic peroxide ROOH. GPx decomposes peroxides to water (or alcohol) while simultaneously oxidising GSH.

Reactive oxygen species can be produced from both endogenous and exogenous substances. Potential endogenous sources include mitochondria, peroxisomes, Cytochrome P_{450} metabolism and inflammatory cell activation [6]. Mitochondria have long been known to generate significant quantities of hydrogen peroxide. The hydrogen peroxide molecule does not contain an unpaired electron and thus is not a radical specie.

Diabetes occurs either because of a lack of insulin or because of the presence of factors that oppose the action of insulin. The result of insufficient action of insulin is an increase in blood glucose concentration (hyperglycaemia). Many other metabolic abnormalities occur, notably an increase in ketone bodies in the blood when there is a severe lack of insulin [7]. The diagnosis of diabetes must always be established by a blood glucose measurement made in an accredited laboratory.

Diabetes is rapidly emerging as a global health care problem that threatens to reach pandemic levels by 2030; the number of people with diabetes worldwide is projected to increase from 171 million in 2000 to 366 million by 2030 [8]. This increase will be most noticeable in developing countries, where the number of people with diabetes is expected to increase from 84 million to 228 million [9]. It has also become known that free radicals play a significant role in the aetiology of many diseases, and that these diseases are as a result of oxidative stress. However, the several antioxidant enzymes present in the body have been found to play major role in protecting the human body against oxidative stress induced by these reactive free radicals. In response to this, this research determined the level of antioxidant enzymes in Type I, II and gestational diabetic patients.

II. Materials And Methods

A. Study Area

This study was conducted in Gombe State. It is one of Nigeria's 36 states with an area of 20,265 km² and a population of around 2,353,000 people as of 2006; having its state capital Gombe. The State nicknamed the 'Jewel in the Savannah' was curved out in October 1996 from part of the old Bauchi State by the Late Gen. Sani Abacha. The state is being located in the northeastern zone; right within the expansive savannah allows the state to share common borders with the states of Borno, Yobe, Taraba, Adamawa and Bauchi states.

B. Study Subjects and Selection Criteria

The subjects for this study were drawn from Diabetic patients attending the Medical clinic of Federal Teaching Hospital Gombe (FTHG). The subjects were registered with the clinic and their informed consent was sought. The patients were previously diagnosed with diabetes for more than a year, and they were treated for the disease but still had their glycated haemoglobin above normal range. They were free from other diseases and chronic diabetic complications. The institutional ethical board granted the approval of the research protocols. The subjects were drawn from Type1, Type2, and Gestational diabetic patients.

C. Sample Size

Thirty (30) Types 1, 2, and gestational diabetic patients were used for the research with a 50:50 ratio on both sexes. Thirty (30) patients who did not have diabetes or any other chronic illnesses were used as control.

D. Methodology

Plasma malondialdehyde was determined by the method of [10]. Glycated Hemoglobin was determined as described by [11]. Superoxide dismutase was measured according to method of [12], catalase according to the method of [13]. Glutatahione peroxidase activity was measured according to the method described by [14].

E. Ethical Clearance

Before the commencement of this research, an ethical clearance was obtained from the board of the Federal Teaching Hospital, Gombe. Also, consent was sought from all the subjects employed in the study.

III. Results

Table 1: Levels of % Glycated Hemoglobin of Diabetic and apparently healthy Subjects

Condition	No. of Subjects	Mean± Std Dev
Type 1	30	7.070± 0.09[b]
Type2	30	7.448±0.03[a]
GDM	30	7.209±0.57 [a]
Control	30	4.64±0.56[c]

Values are mean ± standard deviation
N= number of samples
Values along a column that do not share a letter are significantly different at $p< 0.05$.

Table 2 shows the mean± standard deviation level of antioxidant enzymes and malondialdehyde in diabetic and apparently healthy individuals. From the results, the mean serum level in type 1 (0.189± 0.018 u/ml) and type 2 (0.17± 0.023 u/ml) diabetics was significantly lower compared to apparently healthy individuals (0.210± 0.012 u/ml) ($p <0.05$), while the level of SOD in GDM subjects (0.207± 0.018) was decreased compared to apparently healthy individuals but no to a significant level.

Level of the antioxidant enzyme catalase was significantly lower in all groups of diabetic subjects compared to apparently healthy individuals (60.59± 5.88u/ml) ($p <0.05$), with type 2 diabetics (30.60± 9.08u/ml) having the least catalase level, followed by type 1 diabetics (39.57± 10.26u/ml) and gestational diabetics (50.28± 6.83u/ml) with the highest level among the diabetics subjects (table 2).

The mean± standard deviation level of glutathione peroxidase, an antioxidant enzyme was significantly higher in type 1 diabetics (135± 22.4nmol/min/ml) compared to apparently healthy individuals (87±10.81

nmol/min/ml) ($p<0.05$), but the level of the enzyme was significantly lower in both type 2 (69± 20.73 nmol/min/ml) and gestational diabetics (71± 11.05 nmol/min/ml) as compared to apparently healthy individuals ($p<0.05$) (table 2). The mean± standard deviation of malondialdehyde level (table 17) in apparently healthy individuals (1.94± 0.22 µmol/L) was significantly lower compared to type 1 (5.61± 1.32 µmol/L), type 2 (12.50±3.12 µmol/L) and gestational diabetics (11.25±1.71 µmol/L) ($p<0.05$).

Table 2: Serum levels of Antioxidant Enzymes and Malondialdehyde in Diabetic and Apparently Healthy Subjects

Condition	SOD (u/ml)	CAT(u/ml)	GPx(nmol/min/ml)	MDA(µmol/L)
Type (n=30)	10.189^{b} ± 0.01	839.57^{c} ± 10.26	135^{b}± 22.45	$.61^{c}$± 1.32
Type (n= 30)	20.17^{c} ± 0.02	330.60^{d} ± 9.08	69^{c}± 20.73	12.50^{a}±3.12
GDM (n= 30)	0.207^{a} ± 0.01	850.28^{b} ± 6.83	71^{d}± 11.05	11.25^{b}±1.71
Control (n= 30)	0.210^{a}± 0.01	260.59^{a} ± 5.88	87^{a}±10.81	1.94^{d}± 0.22

Values are mean ± standard deviation
n= number of samples
Values along a column that do not share a letter are significantly different at $p< 0.05$.

IV. Discussion

Oxidative stress is known to increase in a system where the rate of free radical production is increased and /or the antioxidant mechanisms are impaired. In recent years, the oxidative stress-induced free radicals have been implicated in the pathology of diabetes mellitus [15].

The mean± standard deviation of MDA in type 1 and type 2 diabetic patients (table 2) was significantly higher ($p<0.05$) compared to apparently healthy individuals.This is in accordance with the work of [16]. There was also significantly increased level of MDA in gestational diabetics as compared to apparently healthy individuals ($p<0.05$) which also agrees with the work of [17]. The increased level of MDA in the diabetic patients may be due to oxidative stress in the individuals, or compositional changes in LDL which leads to conformational changes, possibly resulting in a different exposure of fatty acids to free radicals that enhance a faster rate of lipid peroxidation [16]. Also increased level of copper, a transition metal that catalyses lipid peroxidation, may enhance oxidation of LDL causing increased level of MDA in diabetics as reported by [18].

The result (table 2) revealed that hyperglycaemia produced marked oxidant impact as demonstrated in the lower levels of antioxidant enzymes of superoxide dismutase and catalase level in all diabetic patients compared to apparently healthy individuals ($p<0.05$). The decrease in level of SOD and catalase enzymes in gestational diabetics as compared to control is in agreement with the work reported by [19]. [20] also reported decreased level of antioxidant enzymes in gestational diabetics. Decreased level of these enzymes in type 1 diabetic subjects was reported by [21]. [16] also reported a significant decrease in the level of antioxidant enzymes in type 2 diabetic. The decrease in superoxide dismutase and catalase enzyme in diabetic subjects as compared to apparently healthy individuals may be due to deficiency of blood zinc, observations have shown that zinc, copper and magnesium have antioxidant activities because not only do they constitute the active sites and/or stabilize the conformation of several antioxidant enzymes, but they also compete for iron- and copper-binding sites and can provide protection against transition metal-mediated and free radical-induced injury [16].

Decrease in SOD might be attributed to hyperglycaemia which activates various biochemical pathways such as glucose autoxidation, non- enzymatic glycation of proteins and activation of protein kinase C, which in turn, overproduce oxidants such as superoxide and hydroxyl radicals as well as hydrogen peroxide [22]; or due to the increased glycosylated SOD that leads to the inactivation of superoxide dismutase [23]. The decrease in SOD might also be due to loss of its two factors Zn^{2+} and Cu^{2+}[24].

The decrease in catalase enzyme activity may be due to the decreased level of H_2O_2 generated by SOD [16].

Glutathione peroxidase works in parallel with SOD to protect cell proteins and cell membranes against oxidative damage. From the results (table 2), there is significant increase in the antioxidant enzyme in type 1 diabetic patients as compared with the apparently healthy individuals ($p<0.05$) which agrees with the work of [20]. However, there is significant decrease in the level of glutathione peroxidase between type 2 diabetic patients, gestational diabetics compared to apparently healthy individuals ($p<0.05$) which is in accordance with the work of [21]. The low GPx activity could be directly explained by either low GSH content or enzyme inactivation under severe oxidative stress. [25] reported that glibenkilamide and metformin down-regulate the activity of GPx in streptozotocin-induced diabetic rats.

V. Conclusion

Decreased activiti.es of superoxide dismutase and catalase enzymes seen in the diabetics may be due to marked oxidant impact as a result of hyperglycaemia, which may enhance the rates of lipid peroxidation as seen in high level of malondialdehyde in the diabetic patients.

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