

Serum Total Antioxidant Status In Type 2 Diabetic Nigerians

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Abstract: Background and Objectives: Patients with type 2 diabetes mellitus (DM) are more prone to diabetic complications and oxygen free radicals are known to contribute to the development of complications of DM. However, there are conflicting reports regarding antioxidant status in type 2 diabetic patients. The present cross sectional study was designed to evaluate the serum total antioxidant status (TAS) in type 2 diabetic patients and age matched control subjects in ABUTH Shika, Zaria.

Subjects and Methods: A total of 281 subjects were recruited for the study. These comprised of 181 type 2 diabetic patients and 100 controls. Fasting blood glucose (FBG) was measured using glucose oxidase method, glycated haemoglobin (GHbA1c) using micro column method and TAS using Mosmann method.

Results: The reference range of TAS is 15.7 – 63.1 mmol/L. The mean value of TAS (33.3 ± 0.5 mmol/L) was significantly lower ($p < 0.05$) in diabetic patients than in control subjects (39.4 ± 0.8 mmol/L). On the other hand the mean values of FBG and GHbA1c in diabetic patients (7.0 ± 0.3 and 8.2 ± 0.2) were significantly higher ($p < 0.05$) than corresponding values in controls (4.1 ± 0.8 and 5.0 ± 0.1) respectively. The mean values of TAS were however similar ($p > 0.05$) among diabetic patients with good and poor glucose control (33.3 ± 0.7 mmol/L versus 32.2 ± 0.7 mmol/L). Also the mean values of TAS were similar ($p > 0.05$) among diabetic patients with good and poor GHbA1c control (33.4 ± 0.6 mmol/L versus 33.1 ± 0.6 mmol/L). Similarly, the mean values of TAS in diabetic patients with complications and those without complications (32.8 ± 0.6 mmol/L versus 34.4 ± 1.1 mmol/L) were not significantly different ($p > 0.05$).

Conclusion: These results suggest that type 2 diabetic patients of the study area have low serum level of TAS.

Keywords -: Blood glucose, glycated haemoglobin, TAS and DM.

I. Introduction

DM is a syndrome that is characterized by chronic hyperglycaemia which is due to dynamic interactions between varying defects of insulin secretion and resistance.¹ Worldwide, an estimated 150 million people are affected by DM and this number is likely to reach 300 million by the year 2025 resulting in approximately 450,000 deaths a year, if successful strategies for its prevention and control are not implemented.^{1,2} In Nigeria, the national prevalence of DM was estimated to be 2.7%, while the northern part of Nigeria has a prevalence of 1.6%.³

Antioxidants are protective substances because they fight oxidative stress by preventing cell damage caused by charged particles. These charged particles are known as reactive oxygen species. This oxidative stress is thought to add to the progression of type 2 DM.^{4,5} Every second, tens of thousands of free radicals are created in the body.⁶ These free radicals lead to an increase in oxidizing response above a certain threshold which in the absence of concomitant rise in antioxidant/reducing response, leads to oxidative stress which is associated with the complications of DM.⁷ The prime targets of these radical reactions are the unsaturated fatty acids which have a role to play in membrane fluidity, receptor alignment and when compromised cellular lyses follow.

The potential contribution of increased oxidative stress to the development of complications of DM is of growing interest.⁸ Metabolic stress arising from changes in energy metabolism, alteration in sorbitol pathway activity, changes in the levels of inflammatory mediators and the status of antioxidant defense systems all contribute to the oxidative state in DM.⁹

Oxidative stress and associated tissue damage represent a common end point of chronic diseases such as DM.⁷ Hence the need to evaluate the levels of TAS in diabetic patients owing to their numerous advantages in preventing diabetic complications. Similarly, it is well established that there is paucity of data on the levels of TAS in type 2 diabetic patients.^{10,11,12}

The aim of the present study was to evaluate the serum total antioxidant status in type 2 diabetic patients.

II. Materials And Methods

A total of 281 subjects were recruited for the study. These consist of 181 known diabetic patients attending Medical Out-Patient Department (MOPD) clinic of ABUTH, Shika, Zaria and 100 apparently healthy individuals as controls. The approval of the study was obtained from the Ethical Committee of the Faculty of

Medicine, Ahmadu Bello University, Zaria in accordance with Helsinki declaration. Arrangement was made with the clinicians whereby subjects who satisfy the study inclusion criteria were selected.

Structured questionnaires were administered to the study population and this was followed by measurement of blood pressure and anthropometric parameters and then specimen collection. Glycaemic control was assessed by glucose and glycated haemoglobin measurements. Blood specimen for the biochemical measurements was collected from peripheral vein into plain tubes using sterile technique. The coagulated whole blood was then spun using a centrifuge. The chemicals used for the determinations were procured from Randox Company Limited.

Serum glucose was measured using enzymatic method of Trinder.¹⁴ GHbA_{1c} was measured using micro column method of Trivelli et al.¹⁵ Serum TAS was measured using rapid colourimetric 3-[4,5-dimethylthiazol-2-yl]-2,5-dimethyl tetrazolium bromide (MTT) assay by Mosmann.¹⁶ The data obtained were treated accordingly using Statistical Program for Social Sciences (SPSS 13.0) for windows (SPSS Inc., Chicago, 16). The data obtained from diabetic patients were compared with those of apparently healthy individuals (controls) using the two tailed student's t- test. A p-value of equal to or less than 0.05 ($p \leq 0.05$) was considered statistically significant.

III. Results

The mean values of clinical parameters in diabetic patients and controls are shown in Table 1. The mean values of BMI and DBP in diabetic patients were significantly higher ($p < 0.05$) than corresponding values in the control subjects. However, the value of SBP in diabetic patients was similar to that of controls ($p > 0.05$). The mean value of duration of diabetes mellitus (DODM) in diabetic patients with poor control was significantly higher ($p < 0.05$) than that of good control as shown in tables 2 and 3. The mean values of other parameters in both good and poor glucose controls were similar ($p > 0.05$). The mean values of TAS, GHbA_{1c} and FBG in diabetic patients and controls are shown in Table 4. The mean value of TAS was significantly lower ($p < 0.05$) in diabetic subjects than in controls. However, the mean values of FBG and GHbA_{1c} were significantly higher ($p < 0.05$) in diabetic patients than control subjects.

Table 1: Clinical parameters (Mean±SEM) in diabetic patients and controls

| SUBJECTS | n | Age (years) | BMI (Kg/m ²) | DODM (years) | SBP (mmHg) | DBP (mmHg) |
|----------|-----|----------------|-----------------------------|-----------------|---------------|---------------|
| Patients | 181 | 53 ± 0.8 | 28.4 ± 0.4* | 5 ± 0.4 | 118 ± 0.6 | 78 ± 0.5* |
| Controls | 100 | 52 ± 0.8 | 22.8 ± 0.3* | — | 117 ± 0.8 | 74 ± 0.8* |
| p-value | | <0.05 | <0.05 | — | >0.05 | <0.05 |

**n=Number of subjects, BMI=body mass index, DODM =duration of diabetes mellitus, SBP= systolic blood pressure, DBP= diastolic blood pressure, SEM=standard error of mean and NA=not applicable.
* statistically significant ($p < 0.05$).**

Table 2: Clinical parameters (Mean±SEM) in diabetic patients with good and poor glucose control

| PATIENTS | n | AGE (years) | BMI (Kg/m ²) | DODM (Years) | SBP (mmHg) | DBP (mmHg) |
|----------------------|----|-------------|--------------------------|--------------|------------|------------|
| Good control (≤ 6.0) | 92 | 54± 1.0 | 28.7±0.5 | 4± 0.4* | 118 ±0.9 | 77±0.7 |
| Poor Control (> 6.0) | 89 | 52± 1.2 | 28.1±0.5 | 6±0.6* | 118± 0.9 | 78±0.8 |
| p-value | | > 0.05 | >0.05 | <0.05 | >0.05 | >0.05 |

n=Number of subjects, BMI=body mass index, DODM=duration of diabetes mellitus, SBP- systolic blood pressure, DBP- diastolic blood pressure and SEM=standard error of mean. Good control = values below mean -3SD while poor control = values above mean -3SD
* statistically significant (p< 0.05).

Table 3: Clinical parameters (Mean±SEM) in diabetic patients with good and poor glycated haemoglobin control

| PATIENTS | n | AGE (years) | BMI (Kg/m ²) | DODM (Years) | SBP (mmHg) | DBP (mmHg) |
|----------------------|-----|-------------|--------------------------|--------------|------------|------------|
| Good control (≤ 7.0) | 59 | 54± 1.3 | 28.8± 0.6 | 4± 0.6* | 118±1.1 | 77±1.0 |
| Poor Control (> 7.0) | 122 | 53± 1.0 | 28.2±0.5 | 6± 0.4* | 119±0.8 | 79± 0.6 |
| p-value | | >0.05 | >0.05 | <0.05 | >0.05 | >0.05 |

n=Number of subjects, BMI =body mass index, DODM =duration of diabetes mellitus, SBP= systolic blood pressure, DBP= diastolic blood pressure and SEM=standard error of mean. Good control = values below mean -3SD while poor control = values above mean -3SD, * statistically significant (p< 0.05).

Table 4: Biochemical analytes (Mean±SEM) in diabetic patients and controls

| SUBJECTS | n | TAS (mmol/L) | FBG (mmol/L) | GHbA _{1c} (%) |
|----------|-----|--------------|--------------|------------------------|
| Patients | 181 | 33.3± 0.5* | 7.0±0.3* | 8.2±0.2* |
| Controls | 100 | 39.4± 0.8* | 4.1±0.8* | 5.0±0.1* |
| p-value | | <0.05 | <0.05 | <0.05 |

n=Number of subjects, TAS = total antioxidant status, FBG=fasting blood glucose, GHbA_{1c} = glycated haemoglobin and SEM=standard error of mean.* statistically significant (p< 0.05).

IV. Discussion

Diabetes mellitus usually begins gradually and progresses slowly. The early symptoms of untreated DM are related to elevated blood glucose levels and loss of glucose in the urine. The primary aim in the management of diabetic patients is to attain and sustain normoglycaemia. The problems are largely on the complications that could develop as a result of poor management of the disease. Management of diabetes mellitus is difficult due to poor levels of education and health care facilities in developing countries. Therefore, reported increase in the number of diabetic patients in Nigeria has been of great concern.¹⁰ The present study examined the serum levels of TAS, FBG and GHbA_{1c} in diabetic patients and control subjects. Clinical parameters such as age, body mass index (BMI), duration of diabetes mellitus (DODM), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were also studied.

The mean values of FBG and GHbA_{1c} were significantly higher in diabetic patients than in controls. However, 49.2 % of the patients were in poor glucose control and 67.4 % were in poor GHbA_{1c} control. This is consistent with earlier findings of Aliyuet al¹⁰ and Benrebaïet al¹⁶ who found significantly higher levels of serum FBG and GHbA_{1c} in diabetic patients. FBG levels significantly correlated with GHbA_{1c}. This is in agreement with the study of Awojobiet al¹⁷ who reported that there is a direct correlation between GHbA_{1c} and blood glucose levels. Higher percentage of patients with poor GHbA_{1c} control in this current study is suggestive of the reliability of GHbA_{1c} since it gives a better view of what is happening over time.

In the present study, TAS was significantly lower in diabetic patients than in control subjects. This is in agreement with earlier findings of Vaddeand Rama¹⁸ who reported significantly lower levels of serum TAS in type 2 diabetic patients than in controls. Benrebaïet al¹⁶ reported a very drastic decrease in serum TAS in diabetic patients than control subjects.¹⁶ Also, Akinosun and Bolajoko¹⁹ in Ibadan reported decrease in serum TAS in type 2 diabetic patients than controls. Similarly, in Brazil, a study by Medina et al⁷ revealed lower plasma antioxidant activity in diabetic patients than in healthy individuals. Studies revealed that TAS in type 2 DM is low and this could be attributed to low levels of antioxidant vitamins enzymes and other micro-nutrients in the blood. Benrebaïet al¹⁷ and Lai.²⁰ The decrease could be as a result of poor glycaemic control probably because of poor compliance rate of the diabetic patients.

Total antioxidant status represents the extrinsic (micronutrients) trace elements, vitamins and intrinsic factors including group of organic antioxidants such as enzymes-catalase, glutathione peroxidase, superoxide dismutase and non- enzymatic anti- oxidants and others like flavonoids, bilirubin and uric acid.¹⁶ Other factors that have been associated with low serum TAS levels include low intake of antioxidant-rich foods like fruits and vegetables, poor health status, cigarette smoking, and low physical activity.²¹ However, the diabetic patients in this study had been exposed to extensive counseling on dietary and lifestyle modification. This included a generous intake of fruits and vegetables to which most of them claimed compliance though the actual amount consumed could not be ascertained. All the patients were clinically stable. Cigarette smokers were not included in this study. Most of the patients included farmers, businessmen and women who engaged in activities that were physically demanding.

Kanetoet al⁵ Gupta and Chari¹¹ reported that improved glycaemic control leads to decrease in macrovascular diseases. This is in agreement with the studies of Aliyuet al¹⁰ Ruhe and McDonald²² and Chertow²³ who established that there is a strong correlation between high glucose concentration and poor prognosis. Also a study in Iran shows that there is a significant correlation between TAS and poor glycaemic control, hence the suggestion that measurement of TAS in DM could be used as an index of glycaemic control and development of diabetic complications.¹ This study reflects that there is low levels of TAS in type 2 diabetic patients but it could not be concluded if the low levels of TAS in this group of patients is due to poor glycaemic control.

V. Conclusions

It can be concluded from the findings of the present study that the mean serum TAS was significantly lower, while those of FBG and GHbA_{1c} levels were significantly higher in type 2 diabetic patients than in controls. Therefore it can be recommended from these findings that measurement of TAS be included as one of the test menu in the evaluation of patients with type 2 diabetes mellitus. This could improve the management of diabetic patients hence reduce morbidity and mortality from DM.

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