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Evaluation of Serum Antioxidant Vitamins in Type 2 Diabetic Nigerians

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Abstract:
Background: *Patients with type 2 diabetes mellitus are more prone to diabetic complications and oxygen free radicals are known to contribute to the development of complications but there are conflicting reports regarding antioxidant status in type 2 diabetic patients.*
Methodology: *The current cross sectional study was designed to evaluate the serum antioxidant vitamins in type 2 diabetic patients and age matched control subjects in ABUTH Shika, Zaria. Also to assess their correlation with clinical parameters and biochemical analytes. 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, glycatedhaemoglobin ($GHbA_{1c}$) using micro column method while vitamins C and E using Moeslinger and Dahot methods respectively.*
Results: *The reference values of vitamins C and E are 0.2 – 7.0 Mg/ml and 3.7- 119.3 g/L respectively. The mean values of vitamin C (3.1 ± 0.1 Mg/ml) and vitamin E (53.2 ± 2.2g/L) was significantly lower ($p < 0.05$) in diabetic patients than corresponding in control subjects (3.6 ± 0.2 Mg/ml) and (61.5 ± 2.9g/L) respectively. While on the other hand the mean values of FBG and $GHbA_{1c}$ in diabetic patients (7.0 ± 0.3 and 8.2 ± 0.2mmol/L) were significantly higher ($p< 0.05$) than corresponding values in controls (4.1 ± 0.8 and 5.0 ±0.1mmol/L) respectively. The mean values of vitamin E was significantly lower ($p< 0.05$) among diabetic patients with poor glucose control (41.7±2.8g/L) than good glucose control (44.5 ± 2.7g/L). Similarly, the mean values of vitamin E was significantly lower ($p< 0.05$) in diabetic patients with complications than those without complications (42.5 ± 2.1g/L versus 49.5 ± 7.0 g/L). In diabetic patients, non- significant negative correlation exist between $GHbA_{1c}$ with vitamins C and E ($r= -0.073$, $p > 0.05$ and $r=0.052$, $p > 0.05$) respectively.*
Conclution: *These results suggest that type 2 diabetic patients of the study area have low serum level of antioxidant vitamins C and E.*
Keywords: Blood glucose, glycatedhaemoglobin, DM, Vitamins C and E.

I. Introduction

Diabetes mellitus is a chronic and a multisystem disease that is widely spread throughout the world, affecting carbohydrate, protein and lipid metabolisms.[1] Abnormally high glucose levels can be harmful to individual's health and can lead to serious complications. A number of studies have suggested that enhanced oxidation is the underlying abnormality responsible for some of the complications of DM.[2]Antioxidants are free radical scavengers, while DM is a free radical associated disease. Antioxidants have the power to help prevent DM as well as help manage it. Studies has shown that a number of antioxidants participate in the protection of human body against free radical pathology and its consequences.[1]

It has been suggested that overproduction of reactive oxygen and nitrogen species may be involved in the initiation and development of vascular complications in DM.[2 3] Epidemiological studies revealed that there is indisputable evidence from a shift in the equilibrium between ROS and antioxidants in favour of oxidative stress in DM.[4 5 6] These abnormalities can be seen in pre-diabetic state and therefore early intervention can be beneficial.[7]

Several studies have shown that DM can worsen antioxidant status hence deficiencies in some vitamins such as vitamins C and E can aggravate several complications of DM.[8] Medical nutrition therapy (MNT) guidelines acknowledge the need to identify deficiencies of antioxidant vitamins. However, the guidelines observe that such identification is difficult. Thus there is evidence that available clinical laboratory tests for antioxidant vitamins C and E are not been carried out in clinical practice.[9]

Vitamins C and E tests can be used to ascertain the levels of antioxidant vitamins.[9] Since DM is associated with increased lipid peroxidation, this has been implicated in the pathogenesis of DM complications. To control these, several antioxidant protective mechanism like vitamins C and E exist.[10] Ascorbic acid is of great importance in biochemicSal reactions as a reducing agent. For example, recycling of antioxidants such as vitamin E by ascorbic acid has been shown to be protective against oxidative stress.[11]

Glucose and vitamin C have similar structure and so vitamin C can replace glucose in many chemical reactions hence prevent non-enzymatic glucosylation of proteins.[12] In addition Vitamin C has been studied in pigs and found out to act as regulator of catabolism of cholesterol and bile acid hence vitamin C is an important factor in lipid regulation.[12]

Several studies revealed that vitamin C is decreased in DM patients.[12 13] In India, the level of vitamin C is significantly reduced in diabetic patients with complications than those without complications.[13] Study also shows that vitamin C as an antioxidant may help in importing plasma glucose by reducing insulin resistance hence lowering oxidative stress in type 2 diabetic patients.[14] Vitamin C is found to protect, prevent and reduce the extent of oxidative destruction of cellular tissue.[14] Furthermore, study shows that vitamin C was found to prevent the onset of type 2 DM.[14]

Vitamin E is an antioxidant vitamin and it is also known as α-tocopherol. However, vitamin E has been shown to play a role in preventing DM.[14 15]Vitamin E protects, prevents or reduces extend of oxidative destruction of cellular tissues.[14] Vitamin E is considered as the most important lipid-soluble exogenous antioxidant in humans. It is regenerated by Vitamin C in the system.[16]

If the level of vitamin E is reduced in the system, it leads to increase free radical production which later mediates tissue injury in DM.[14] Adults with metabolic syndrome have been shown to have suboptimal concentrations of vitamin E.[14] Diabetes mellitus is associated with production of high levels of lipid peroxidation products like malondialdehyde (MDA) and reduced glutathione (GSH). Vitamin E increases GSH and reduces MDA hence reduce microvascular and macrovascular complications.[14]

Oxidative stress and associated tissue damage represent a common end point of chronic diseases such as DM. The potential contribution of increased oxidative stress to the development of complications of DM is of growing interest, [17 18]especially in developing countries where nutrition is generally poor. It is therefore important to evaluate the levels of vitamins C and E 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 vitamins C and E in type 2 diabetic patients. [1 9 10]The aim of the present study was to evaluate the serum vitamins C and E 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 glycatedhaemoglobin 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 .[19] $GHbA_{1c}$ was measured using micro column method of Trivelliet al.[20] Serumvitamin C was determined by spectrophotometric method of Moeslingeret al.[11]Serum vitamin E (tocopherol) was measured by spectrophotometric method of Dahotet al[21].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 FBG, $GHbA_{1c,}$vitamins C and E in diabetic patients and controls are shown in Table 4. The mean value of vitamins C and E were 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 goodand poor glucose control

ENTS	N	AGE (years)	BMI (Kg/m^2)	DODM (Years)	SBP (mmHg)	DBP (mmHg)
l)	92	54± 1.0	28.7±0.5	4± 0.4*	118 ±0.9	77±0.7
)l)	89	52± 1.2	28.1±0.5	6±0.6*	118± 0.9	78±0.8
e		> 0.05	>0.05	<0.05	>0.05	>0.05

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

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

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

SUBJECTS	N	FBG (mmol/L)	$GHbA_{1c}$ (%)	Vit. C (Mg/ml)	Vit E (g/l)
Patients	181	7.0±0.3*	8.2± 0.2*	3.1±0.1*	53.2±2.2*
Controls	100	4.1±0.8*	5.0±0.1*	3.6±0.2*	61.5±2.9*
p-value		<0.05	<0.05	<0.05	<0.05

n=Number of subjects, FBG=fasting blood glucose, $GHbA_{1c}$ = glycated haemoglobin, Vit C= vitamin C, Vit E = vitamin E and SEM= standard error of mean.* statistically significant (p< 0.05).

IV. Discussion

The importance of normal levels of serum vitamins C and E cannot be over emphasized. 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.[1]The present study examined the serum levels of vitamins C and E, 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 al [1] and Benrebaiet al[8] 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 al[22]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, antioxidant vitamins C and E were significantly lower in diabetic patients than controls. Similar findings were reported by other workers Sundaramet al,[23]; Maxwell et al,[24]; Nuttalet al, 1999[25]; Cerielloet al,[26];Sief and Youseef[27];Aliyu et al,[1]; Afkahami-Ardekan and Shojoeldiny-Adekami[12];Benrebaiet al[8] . Nwose, [12] noted a significant decrease of vitamins C and E in diabetic patients when compared with controls. The low levels of these antioxidant vitamins may account for the low level of Total Antioxidant Status as seen in some studies.

Vitamins C and E are major contributors to Total Antioxidant Status. However, Sirivatsanet al [28] in Iran noted similar values of vitamin C and E in patients and controls. However, vitamin C and E were found to be non significantly correlated with $GHbA_{1c.}$ This is in agreement with the findings of Dogun and Ajala**[29],** that reported non significant correlation between vitamins C and E with the levels of FBG and $GHbA_{1c.}$ On the other hand, Sawantet al[30] discovered highly significant inverse correlation between vitamin C and $GHbA_{1c}$.

Vitamin C lowers sorbitol level, which is harmful to the eyes and kidneys in patients with DM. Besides, it decreases protein loss through the urine and improves glucose tolerance in type 2 DM. In the present study, the decrease in the level of vitamin C could be due to the fact that it functions as an important component of cellular defense against oxygen toxicity and lipid peroxidation caused by free radicals. This indicates that poor diabetic control is associated with reduced serum antioxidant activities which were revealed in significantly lower mean levels of vitamin E in diabetic patients with poor glycaemic control.

In diabetic patients, there is always increased loss of water soluble vitamin C in urine. There could also be impaired transport and dietary deficiency as well as increase demand of vitamin C in diabetic patients,Sawantet al[30]. The increased demand is to remedy the increased oxidative stress. It has been discovered that vitamin C is involved in the regeneration of vitamin E. These may be a contributing factor to the decrease in the levels of vitamin C observed in the diabetic patients of the present study. The results of the present study indicated negative correlations between $GHbA_{1c}$ and each of the antioxidant vitamins. Similar study by Aliyuet al[1] and Sawantet al [30] revealed that mean blood glucose and $GHbA_{1c}$ levels correlated negatively with vitamins C and E.

The current study revealed that, mean values of vitamin E was significantly higher in those with good glycaemic control than in those with poor glycaemic control. Kanetoet al[3]and Gupta and Chari [13] reported that improved glycaemic control leads to decrease in macrovascular diseases. This is in agreement with the studies of Ruhe and McDonald[31], Aliyuet al[1] and Chertow[32] who established that there is a strong correlation between high glucose concentration and poor prognosis. This study reflects that there is low level of vitamins C and E in type 2 diabetic patients but it could not be concluded if the low level 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 vitamins C and E were found to be significantly lower, while those of FBG and $GHbA_{1c}$ levelswere significantly higher in type 2 diabetic patients than in controls. Therefore it can be recommended from these findings that measurements of vitamins C and E 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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