ORIGINAL ARTICLE

Antioxidant status of type 2 diabetic patients in Port Harcourt, Nigeria

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Abstract

Context: Oxidative stress has been implicated in the pathogenesis of type 2 diabetes mellitus (T2DM) and its complications.
Aims: This study was conducted to determine and compare total antioxidant status (TAS), vitamin C and E levels in T2DM patients and healthy control subjects.
Settings and Design: Fifty-five previously diagnosed DM patients aged between 34 years and 70 years and 50 control subjects aged between 35 years and 69 years were consecutively recruited into this study.
Materials and Methods: Blood pressure (mmHg), body mass index (kg/m^2), concentrations of plasma glucose (mmol/l), lipid profile (mmol/l), TAS (mmol/l), vitamins C (μmol/l), and E (μmol/l) were determined in all participants.
Statistical Analysis Used: Statistical Package for Social Sciences (SPSS) version 11.0 was used for statistical analysis.
Results: The mean plasma TAS (1.18 ± 0.27 mmol/l), vitamin C (26.59 ± 7.39 μmol/L) and vitamin E (15.33 ± 4.05 μmol/l) of T2DM patients were significantly lower (P=0.0001 for all) than those of controls (1.58 ± 0.28 mmol/l, 43.56 ± 6.86 μmol/l, 31.22 ± 6.20 μmol/l respectively). TAS had a positive correlation with vitamin E (r=0.588; P=0.013) but no correlation with vitamin C (r=−0.387; P=0.139) among diabetics.
Conclusions: TAS, vitamin C and E levels are reduced in T2DM patients compared with those of controls.

Key words: Total antioxidant status, type 2 diabetes mellitus, vitamin C, vitamin E

Introduction

Type 2 diabetes mellitus (T2DM) is characterized by peripheral insulin resistance, impaired insulin secretion and excessive hepatic glucose production.[1-3] Oxidative stress may contribute to the pathogenesis of DM through impairment of insulin action, injury to pancreatic β-cells, increased lipid peroxidation, and vascular endothelial damage.[4-7]

Antioxidants like vitamins C and E provide a defense system against free radical-induced damage.[2,8]

Earlier studies suggest that diabetics have lower plasma TAS, vitamin C and E concentrations than those without DM.[2-4,9] This study was conducted to determine and compare TAS, vitamin C and E levels in T2DM patients and healthy controls.

Materials and Methods

The study was conducted after obtaining approval from the hospital's ethical committee.

Diabetic patients were previously diagnosed T2DM patients on dietary and oral hypoglycemic (Metformin and Glibenclamide) therapy attending the Metabolic and

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Medical Outpatient Clinics of the hospital and control subjects were selected from among the hospital workers. Fifty-five consenting patients between 34 and 70 years and 50 apparently healthy controls between 35 and 69 years were consecutively recruited into this study. Participants with diagnosed cardiovascular disease, kidney disease, liver disease, and those on vitamin C and E supplements were not included.

After obtaining informed written consent, questionnaires were initially administered to participants to determine inclusion and exclusion criteria. Wearing light clothing, the waist circumferences of participants were measured with a flexible but nonstretchable tape just above the iliac crest to the nearest 0.5 cm.[5] Their weights and heights were measured with a bathroom weighing scale (Camry) and a stadiometer (Surgifriend Medicals, UK) respectively.[6] Body mass index (BMI) was then calculated for each participant by dividing the weight in kilograms by the square of the height in meters squared (kg/m^2).[7]

Blood pressure was obtained from the right upper arm after 10 minutes of rest and with the participant in a sitting position, using a standard mercury sphygmomanometer (Accoson, UK) with appropriate cuff sizes. Systolic blood pressure was taken to correspond to the appearance of Korotkoff sounds (phase I) and diastolic blood pressure corresponded to the disappearance of Korotkoff sounds (phase V). Two consecutive measurements were taken and the average of the two measurements was used.[2]

After an overnight fast 10 ml of venous blood was drawn from participants and 3 ml was put into ethylenediaminetetraacetic acid (EDTA) bottles for fasting lipid analysis and 1 ml into fluoride oxalate bottles for fasting plasma glucose analysis. The remaining 6 ml was put into a lithium heparin bottle for analysis of vitamins C, E, and TAS.

Plasma was separated from blood cells within 30 minutes of collection by centrifugation at 2500 g for 15 minutes and transferred into plain bottles with Pasteur pipettes. Samples for all the assays were batch analyzed; fasting plasma glucose was measured within 24 hours of collection of blood. Samples for the lipid profile, vitamin C, vitamin E, and TAS analysis were stored frozen and analyzed within 2 weeks of collection.

Plasma glucose concentration was determined using the glucose oxidase method (Randox kit).[10] Plasma triglyceride was determined using the enzymatic method of analysis (Randox kit).[11] Plasma total cholesterol and HDL were determined using the enzymatic method of analysis (Randox kit).[12,13] The LDL concentration was calculated from the total cholesterol, HDL and triglyceride concentrations according to the equation by Friedewald *et al.*[14]

Plasma vitamins C and E were determined using the high performance liquid chromatography (HPLC) method (Agilent HPLC 1100 series).[15,16] Plasma total antioxidant status was analyzed using the ABTS (2,2′-Azino-di-[3-ethylbenzthiazoline sulphonate]) spectrophoto-metric method (Randox kit).[17] Randox quality control samples were included in every batch during the within-run and between-run analysis of samples.[17]

Statistical analysis of the data generated from the study was done using the Statistical Package for Social Sciences (SPSS) version 11.0. Values were expressed as mean±standard deviation. The means of continuous variables were compared using the unpaired Student *t*-test. Correlation statistics were computed with Pearson coefficients. *P* values less than or equal to 0.05 were taken to be significant.

Results

Fifty-five T2DM patients made up of 18 males and 37 females (male to female ratio: 1:2) with a mean age of 54.33 ± 9.09 years and 50 controls consisting of 20 males and 30 females (male to female ratio: 1:1.5) with a mean age of 53.71 ± 10.26 years were studied. There was no significant difference between the mean ages of patients and controls (*P*=0.745).

Patients were more obese, had higher mean blood pressure, higher fasting plasma glucose, and a more dyslipidemic lipid profile than control subjects. The mean duration of diabetes was 7.0 ± 3.95 years. The physical and biochemical parameters of patients compared with those of controls using student's *t*-test are summarized in Tables 1 and 2 respectively.

The mean total antioxidant status (1.18 ± 0.27 mmol/l), vitamin C (26.59 ± 7.39 μmol/l) and vitamin E (15.33 ± 4.05 μmol/l) of T2DM patients were significantly lower (*P*=0.0001 for all) than those of controls (1.58 ± 0.28 mmol/l, 43.56 ± 6.86 μmol/l, 31.22 ± 6.20 μmol/l respectively) [Table 3].

From the correlation statistics Table 4, TAS had a positive correlation with vitamin E (*r*=0.588; *P*=0.013) but no correlation with vitamin C (*r*=−0.387; *P*=0.139) among diabetics.

Discussion

Increased superoxide production, and thus increased oxidative stress, contributes to the pathogenesis of diabetes and its complications by activating four major molecular mechanisms and these include increased polyol pathway influx, increased formation of advanced glycation end-products, activation of protein kinase C isoforms, and increased hexoseamine pathway activity.[18] These mechanisms exacerbate insulin resistance and may lead to

Table 1: Physical attributes of diabetics and controls

Parameter	Diabetics (n=55) Mean±SD	Controls (n=50) Mean±SD	P value
Age (years)	54.33 ± 9.09	53.71 ± 10.26	0.745
Waist circumference (cm)	99.33 ± 10.85	83.58 ± 10.03	0.0001*
Body mass index (kg/m²)	28.72 ± 5.12	25.25 ± 4.23	0.0001*
Systolic blood pressure (mmHg)	138.77 ± 22.05	113.56 ± 9.87	0.0001*
Diastolic blood pressure (mmHg)	87.28 ± 12.18	75.38 ± 7.20	0.0001*

*Statistically significant (P<0.05)

Table 2: Biochemical parameters of diabetics and controls

Parameter	Diabetics (n=55) Mean±SD	Controls (n=50) Mean±SD	P value
Fasting plasma glucose (mmol/l)	7.56 ± 2.17	4.06 ± 0.70	0.0001*
Triglyceride (mmol/l)	1.24 ± 0.58	0.71 ± 0.30	0.0001*
High density lipoprotein (mmol/l)	1.37 ± 0.62	1.68 ± 0.31	0.0001*
Low density lipoprotein (mmol/l)	2.76 ± 0.90	2.43 ± 0.81	0.031*
Total cholesterol (mmol/l)	4.70 ± 1.19	4.22 ± 0.83	0.012*

*Statistically significant (P<0.05)

Table 3: Antioxidant levels of diabetics and controls

Antioxidant	Diabetics (N=55) Mean±SD	Controls (N=50) Mean±SD	P value
Vitamin C (µmol/l)	26.59 ± 7.39	43.56 ± 6.86	0.0001*
Vitamin E (µmol/l)	15.33 ± 4.05	31.22 ± 6.20	0.0001*
Total antioxidant status (mmol/l)	1.18 ± 0.27	1.58 ± 0.28	0.0001*

*Statistically significant (P<0.05)

Table 4: Correlation between total antioxidant status and vitamins of patients

Vitamin	N	r-value	P value
Vitamin E	55	0.588	0.013*
Vitamin C	55	−0.387	0.139

r = Pearson correlation, P = Significance (two-tailed). *Statistically significant (P<0.05)

diabetic complications including cardiovascular disease, retinopathy, nephropathy, and neuropathy.

In this study, significantly lower concentrations of vitamins C and E and TAS were observed in diabetic patients compared to controls. There was a direct correlation between TAS and vitamin E but no correlation between TAS and vitamin C. Most of the evidence from previous research demonstrated that blood TAS, vitamins C and E concentrations in DM patients were significantly lower than concentrations in persons without DM.[2,4,19,20]

Fasting plasma glucose, total cholesterol, triglyceride, and low-density lipoprotein concentrations were observed to be higher and high density lipoprotein concentration lower in diabetic patients compared to controls in this study. Insulin resistance and impaired insulin secretion in diabetes result in hyperglycemia and dyslipidemia.[2] Mitochondrial metabolism of the excessive glucose and free fatty acids results in increased superoxide production and oxidative stress.[18]

Antioxidants include vitamins like vitamin C (ascorbic acid), vitamin E (alpha-tocopherol) and beta-carotene, enzymes like catalase, superoxide dismutase, and glutathione peroxidase, and transition metal binding proteins like caeruloplasmin. In addition to albumin and urate, vitamins C and E have been reported to be major contributors to serum total antioxidant activity.[2,8,21] Vitamin E is the major lipid-soluble antioxidant vitamin present in cell membranes and lipoproteins. Vitamin C is the most important aqueous phase chain breaking antioxidant.[8]

Antioxidants in combination produce a better and more effective action against ROS than that when working individually because of the synergistic interactions between them. Plasma total antioxidant status encompasses all the antioxidants in plasma including those not yet identified or not easily measured and is a quantitative measurement of the state of balance of these various components under specified reaction conditions. It is also a more meaningful estimate of plasma antioxidant capacity.[22]

It has been suggested by data from previous research that low levels of antioxidants may be due to their increased consumption during the process of combating excessive free radicals generated in diabetes.[2,6] As a result there is depletion of antioxidant reserves which include vitamins C and E.

Other factors that have been associated with low plasma antioxidant levels include low intake of antioxidant-rich foods (particularly fruits and vegetables), poor health status, cigarette smoking, and low physical activity.[2,9] 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 ambulant and clinically stable. Participants with significant history of cigarette smoking and current smokers were not included in this study. Seventy-five percent (75%) of patients included businessmen and women, farmers, and artisans who engaged in activities that were physically demanding.

An inverse association has been demonstrated between plasma TAS and fasting plasma glucose in diabetes, suggesting that hyperglycemia-induced oxidative damage may have contributed to low antioxidant levels.[19,20]

Previous research has shown that vitamins C and E act synergistically to reduce lipid peroxidation, inhibit leukocyte adhesion, enhance endothelial function (thereby decreasing atherogenesis), and improve insulin action.[3,7] They have also been found to normalize many parameters of oxidative stress and delay development of complications in diabetics.[1,6] Therefore reduced levels of vitamins C and E in diabetics may aggravate the degree of oxidation and increase susceptibility to oxidative damage and development of diabetic complications.[2]

It is suggested that supplementation studies be done with these antioxidants to see if they will improve the health outcome of diabetics.

In conclusion, this study showed that T2DM patients in Port Harcourt had significantly lower plasma levels of TAS, vitamins C and E compared to healthy control subjects, which may be as a result of oxidative stress. Increased intake of antioxidant-rich foods or supplements may confer both cardiovascular and metabolic benefits in T2DM.

Acknowledgments

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