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Research Article
Levels of Trace Elements and Antioxidant Vitamins in Type 2 Diabetic Patients in Ile-Ife, Nigeria

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Abstract

Background: Levels of Zn, Cu, Mg, vitamin E and vitamin C were measured in 65 Nigerian diabetic subjects with and without micro vascular complications and compared with controls, in order to ascertain their association with the disease and its complications. **Methods:** Fasting blood glucose, glycated hemoglobin, Zn, Cu, Mg, vitamin E and vitamin C were assayed according to standard procedures. Statistical analysis was carried out using SPSS version 17.0. **Results:** Mean values of Zn, Mg and vitamin E were significantly higher ($p<0.05$) in control groups than diabetic subjects. The Zn levels correlated negatively with fasting blood glucose ($p = 0.000$, $R = -0.443$) and glycated hemoglobin ($p = 0.030$, $R = -0.269$) while Mg and vitamin E correlated negatively with fasting blood glucose ($p = 0.000$. $R = -0.405$ and $p = 0.000$, $R = -0.524$, respectively). The Zn, Mg and vitamin E levels correlated positively with each other. Diabetic subjects with retinopathy and nephropathy had significantly lower levels of vitamin E than others. **Conclusion:** Alterations in trace elements and vitamin status were observed in Nigerian diabetes mellitus subjects. The pattern differs from previous studies in respect of Cu and vitamin C levels. The alterations appear to be more as a result of diabetes itself, rather than being involved in the progression of the disease.

Key words: Diabetes mellitus, fasting blood glucose, glycated hemoglobin, trace elements, antioxidant vitamins

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nigeria has the highest incidence of type 2 diabetes mellitus and impaired glucose tolerance in Africa, with approximately 3,921,500 (4.99%) affected individuals as of 2013[1]. Diabetes therefore, constitutes an enormous disease burden in this country.

Oxidative stress from free radicals and reactive oxygen species which are produced during normal metabolism, cause damage when not being quenched by the body's antioxidant systems. Oxidative stress may result either from an overproduction of reactive oxygen species or from the inactivation of antioxidants, thus shifting the oxidative stress/reactive oxygen species balance in favour of stress. It is well known that hyperglycaemia increases the production of free radicals, resulting in oxidative stress. Increased oxidative stress is a widely accepted factor in the development and progression of type 2 diabetes and its complications and has been proposed as the root cause underlying the development of insulin resistance, β-cell dysfunction and impaired glucose tolerance. It has also been implicated in the progression of long term diabetes complications, including micro vascular and macro vascular dysfunction[2,3]. The antioxidant defense system of the body consists of both enzymatic and non-enzymatic components. Antioxidant enzymes require various metal ion activators including copper, zinc, manganese, magnesium and iron[4] hence, these low-abundance metals are referred to as antioxidant trace elements, even though they have no antioxidant actions on their own. The level of these trace elements critically influences the susceptibility of various tissues to oxidative stress. Other antioxidant agents include the vitamins C and E. These vitamins are diet-derived and detoxify free radicals directly. Vitamin E has been reported to protect membranes from lipid per oxidation and its deficiency is concurrent with increased peroxides and aldehydes in many tissues[5]. Ascorbic acid is known to reduce or neutralize reactive oxygen species such as hydrogen peroxide[6]. It is also a substrate for the redox enzyme ascorbate reductase.

There are few reports on the role of trace elements and vitamins in the pathophysiology of diabetes in African subjects. Consequently, medical management of these patients in regard to supplementation with trace elements and vitamins is largely based on information from studies on other populations. This is less than ideal given that racial and country specific differences should can be expected based on differences in genetics, diet and environment. In addition, previous reports on these studies are rather conflicting and inconsistent[7-9]. It remains unclear, for instance whether the differences in trace elements and vitamin levels in diabetics are a simple consequence of the disease itself or if they instead somehow contribute to the clinical expression of the disease, especially to the development of complications. This study there fore attempts to provide data on trace elements status and levels of vitamins C and E in type 2 diabetes patients in Nigeria and to test their possible association with glycaemic control and micro vascular complications.

MATERIALS AND METHODS

Sixty five test subjects (33 males, 32 females) and 50 control subjects (25 males, 25 females) were involved in the study. Test subjects are diabetic patients of the Obafemi Awolowo University Teaching Hospital (OAUTH) who regularly attended the weekly diabetes clinic of the institution while control subjects comprise volunteers among members of staff of the Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, who were matched with the test subjects for age and sex. Convenience sampling design was employed. Ethical clearance was obtained from the Ethics and Research Committee of OAUTH. All subjects signed informed consent forms which explained the purpose and procedure of the study. Patients on vitamin or mineral supplements as well as pregnant women were excluded from the study. Control subjects were screened for diabetes using an ACCU-CHECK glucometer, product of Roche, Mannheim, Germany. Subjects with fasting blood glucose >100 mg dL^{-1} were excluded. Structured questionnaires were administered to all subjects to obtain information on socio-demographic parameters as well as medical and drug history.

Fasting venous blood (10 mL) was collected from the antecubital vein before 9 am and dispensed into lithium heparin tubes (for Cu, Zn, Mg, vitamins E and C assay) and fluoride oxalate bottles (for glucose assay). Samples were centrifuged at 6,000 rpm for 20 min. Plasma aliquots collected from the lithium heparin bottles were kept frozen at -80°C until time of analysis whereas those from the fluoride tubes were used for glucose measurement on the same day. This was carried out using commercial kits supplied by Randox Laboratories, United Kingdom.

Assay of Cu and Zn were carried out spectro photometrically using commercial kits obtained from CENTRONIC Germany while the kit used for Mg assay was obtained from AGAPPE Diagnostics, Switzerland. Amount of Cu in the sample was estimated using the method of Abe *et al.*[10]. Zinc was determined by the method of Johnsen and Eliasson[11].

Assay of Mg is based on its reaction with xylidyl blue, which produces a colored compound in alkaline solution.

Table 1: Comparison of antioxidant parameters between diabetic and control subjects

Parameters	Status	N	Mean±SD	Range	p-value
Age (years)	Control	50	61.05±12.5	30-88	0.262
	Test	65	58.44±11.7	37-90	
Fasting blood sugar (mmol L^{-1})	Control	50	4.77±0.50	4.11-5.80	0.000
	Test	65	9.12±3.90	6.50-20.30	
Zinc (μM L^{-1})	Control	50	15.01±4.30	5.67-23.06	0.000
	Test	65	6.61±4.10	0.38-22.47	
Cu (μM L^{-1})	Control	50	16.89±6.30	1.03-27.65	0.145
	Test	65	19.06±8.90	2.56-37.77	
Mg (mmol L^{-1})	Control	50	0.76±0.04	0.66-0.820	0.000
	Test	65	0.64±0.08	0.41-0.810	
Vitamin E (μmol L^{-1})	Control	50	111.22±32.5	58.74-224.3	0.000
	Test	65	34.13±25.5	1.39-116.56	
Vitamin C (μM L^{-1})	Control	50	72.67±15.3	44.85-103.90	0.215
	Test	65	77.79±24.9	46.55-143.08	

Table 2: Comparison of antioxidant parameters between subjects with good glycaemic control (HbA1c<7.0%) and those with poor glycaemic control (HbA1c≥7.0%)

Parameters	Glycaemic control	N	Mean±SD	Range	p-value
Age (years)	Good	13	58.69±11.25	39-79	0.418
	Poor	52	61.61±11.91	37-90	
Zinc (μM L^{-1})	Good	13	7.69±4.40	2.85-19.11	0.332
	Poor	52	6.33±4.00	0.37-21.90	
Cu (μM L^{-1})	Good	13	21.61±11.3	7.83-37.32	0.206
	Poor	52	17.15±9.00	2.56-37.68	
Mg (mmol L^{-1})	Good	13	0.65±0.05	0.58-0.760	0.555
	Poor	52	0.64±0.07	0.41-0.810	
Vitamin E (μmol L^{-1})	Good	13	41.54±15.5	18.34-67.80	0.111
	Poor	52	32.15±27.3	1.39-116.50	
Vitamin C (μM L^{-1})	Good	13	88.7±17.50	61.32-121.51	0.034
	Poor	52	75.2±25.80	46.56-143.09	

Vitamin E was estimated using the method of Emmerie and Engel as modified by Rutkowski *et al.*[12]. Vitamin C was estimated by redox titration with 2,6-dichloro phenolindophenol[13].

Glycated hemoglobin was measured in diabetic patients using clover A1c self-system kits. HbA1c was used as index of glycaemic control. Good glycaemic control was defined as HbA1c<7.0% and poor glycaemic control[14] as HbA1c≥7.0%. Biochemical tests were not carried out to ascertain the presence duration of nephropathy and neuropathy in test subjects. Rather, information was obtained from the patient's case notes. For retinopathy screening, patients had dilated fundoscopy using volks +78D lens and Carl Zeiss slit lamp biomicroscope. Each patient had binocular indirect ophthalmoscopy using +20D volks lens and appassamy indirect ophthalmoscope.

Statistical analysis was carried out using SPSS version 17. The $p<0.05$ was taken as significant.

RESULTS AND DISCUSSION

Mean age of diabetic and control subjects was 61.03±1.45 and 58.44±1.77 years, respectively, fasting blood glucose were 9.12±0.47 and 4.77±0.06 mmol L^{-1}, respectively for the two groups. Mean HbA1c for the diabetic group was 8.83±0.42%. Thirteen of the diabetic subjects (20%) had good glycaemic control (HbA1c<7.0%) while 52 (80%) had poor glycaemic control (HbA1c≥7.0%). Thirteen diabetic patients were being managed for diabetic retinopathy, 9 for nephropathy and 15 for neuropathy. Twenty-four (64.8%) of diabetic subjects with complications had poor glycaemic control. All the subjects with complications were diagnosed with diabetes ≥5 years ago. Mean values of zinc, Mg and vitamin E were significantly higher ($p<0.05$) in control groups than diabetic subjects while there were no significant differences in the Cu and vitamin C levels of the 2 groups, although the diabetic groups had higher levels of these parameters (Table 1).

When the good and poor glycaemic control groups were compared, significant difference was only found in the levels of vitamin C (Table 2). The Zn levels correlated negatively with fasting blood glucose ($p = 0.000$, $R = -0.443$) and glycated haemoglobin ($p = 0.030$, $R = -0.269$) while Mg and vitamin E correlated negatively with fasting blood glucose ($p = 0.000$. $R = -0.405$ and $p = 0.000$, $R = -0.524$. respectively). The Zn, Mg and vitamin E levels correlated positively (Table 3). Diabetic

Table 3: Correlation of glycated hemoglobin, fasting blood glucose and antioxidant parameters in all subjects

Parameters	Age	HbA1c	FBG	Zinc	Copper	Magnesium	Vitamin E	Vitamin C
Age								
Pearson correlation	1	0.095	-0.021	-0.091	-0.141	0.038	-0.209	0.029
p-value		0.451	0.826	0.333	0.133	0.687	0.025	0.761
HbA1c								
Pearson correlation	0.095	1	0.286	-0.269	-0.135	0.052	-0.113	-0.097
p-value	0.451		0.021	0.030	0.285	0.679	0.368	0.443
FBG								
Pearson correlation	-0.021	0.286	1	-0.443	0.002	-0.405	-0.524	-0.030
p-value	0.826	0.021		0.000	0.982	0.000	0.000	0.754
Zinc								
Pearson correlation	-0.091	-0.269	-0.443	1	-0.017	0.522	0.589	-0.056
p-value	0.333	0.030	0.000		0.860	0.000	0.000	0.552
Copper								
Pearson correlation	-0.141	-0.135	0.002	-0.017	1	-0.056	-0.186	0.083
p-value	0.133	0.285	0.982	0.860		0.555	0.046	0.378
Magnesium								
Pearson correlation	0.038	0.052	-0.405	0.522	-0.056	1	0.571	0.114
p-value	0.687	0.679	0.000	0.000	0.555		0.000	0.227
Vitamin E								
Pearson correlation	-0.209	-0.113	-0.524	0.589	-0.186	0.571	1	-0.149
p-value	0.025	0.368	0.000	0.000	0.046	0.000		0.111
Vitamin C								
Pearson correlation	0.029	-0.097	-0.030	-0.056	0.083	0.114	-0.149	1
p-value	0.761	0.443	0.754	0.552	0.378	0.227	0.111	

Table 4: Comparison of antioxidant parameters in diabetic subjects with and without complications

	Retinopathy			Nephropathy			Neuropathy		
Parameters	Yes (N = 13)	No (N = 52)	p-value	Yes (N = 9)	No (N = 56)	p-value	Yes (N = 15)	No (N = 50)	p-value
Zinc (μM L^{-1})	5.520±0.74	6.87±0.600	0.291	4.960±0.86	6.87±0.570	0.199	4.420±0.68	7.26±3.935	0.018
Copper (μM L^{-1})	20.77±2.32	18.64±1.260	0.448	15.33±2.74	19.67±1.190	0.180	19.22±1.97	19.02±1.320	0.940
Magnesium (mmol L^{-1})	0.640±0.02	0.65±0.008	0.739	0.630±0.03	0.65±0.008	0.379	0.670±0.02	0.64±0.008	0.134
Vitamin E (μM L^{-1})	0.670±0.07	1.67±0.160	0.003*	0.660±0.11	1.59±0.150	0.017*	0.650±0.09	1.71±0.150	0.001*
Vitamin C (μM L^{-1})	76.10±7.98	78.35±3.400	0.793	74.94±6.24	78.35±2.830	0.699	1.590±0.15	74.38±2.830	0.028*

*p<0.05

subjects with retinopathy and nephropathy had significantly lower levels of vitamin E than others while those with neuropathy had significantly lower levels of vitamin E and C zinc than those without the complication (Table 4).

One of the main findings of this study is that diabetic patients had significantly lower levels of zinc than control subjects. There was also significant negative correlation of fasting blood glucose and glycated hemoglobin with zinc levels (Table 3). This agrees with previous studies[15,16] which linked the low levels of plasma zinc in the patients with increased zinc excretion. However, this finding differs from a study carried out in Nigeria[17] which found no significant correlation of zinc with either FBG or HbA1c. Apart from plasma levels, type 2 diabetic patients have also been shown to have lower concentrations of zinc compared with controls in lymphocytes, granulocytes and platelets[18] improvement in immune response was demonstrated when zinc supplements were administered to these subjects[19]. The observation of significantly lower levels of zinc in diabetic subjects with neuropathy compared with their counterparts without this complication (Table 4) also suggests that zinc deficiency may play a role in the development of diabetic neuropathy. Hayee *et al.*[20] found that zinc therapy may help in achieving improvement in peripheral neuropathy in diabetic subjects. Zinc appears to modulate the overall excitability of the brain via effects on glutamate and probably GABA receptors.

We did not observe any significant difference in the levels of plasma Cu in diabetic and control subjects. There were also no differences in the levels of copper in diabetic subjects with micro vascular complications compared with those without these complications. This is at variance with most previous studies which found higher levels of this trace element in diabetic subjects[21-23]. High plasma copper or ceruloplasmin has also been found in other disease states such as lymphatic leukaemia, inflammation, atherosclerosis and hypertension in the absence of diabetes in humans and experimental animals.

Magnesium levels were also found in this study to be lower in diabetic subjectsand negatively correlated with fasting plasma glucose levels. However, there was no association between magnesium and glycated haemoglobin levels and no difference in Mg levels of diabetic subjects with micro vascular complications and those without these complications. This suggests that alteration in Mg levels is associated with the development of DM but may not play a significant role in micro vascular complications. Most previous studies like ours found hypomagnesaemia in diabetic subjects[24,25]. One study associated the low Mg level with retinopathy[26].

Vitamin E levels were also significantly different in control and diabetic groups. It is also notable that subjects with micro vascular complications had significantly lower levels of vitamin E than those without these complications. It is reported that high dose of vitamin E is effective in normalizing retinal hemodynamic abnormalities and improving renal function without inducing a significant change in glycaemic control, thus suggesting that vitamin E supplements may provide an added benefit in reducing risk of developing diabetic retinopathy or nephropathy[27]. Moreover, some studies suggest that vitamin E supplements may lower HbA1c levels in patients with inadequate glycaemic control[28].

Vitamin C levels were not different between diabetic and control groups. Significance difference was however obtained also observed in the levels of this vitamin among subjects with good glycaemic control versus poorglycaemic control as well as diabetic subjects with complications versus those without complications. Low vitamin C levels reported appeared to be a consequence of the disease itself and not due to inadequate dietary intake of vitamin C. The typical Nigerian diet contains a lot of vitamin C rich food and vitamin C deficiency is not very common. Further studies should therefore focus more on dynamics of vitamin C metabolism in diabetes.

CONCLUSION

Although there are alterations in trace element and vitamin status in Nigerian type 2 diabetes mellitus subjects, the pattern differs slightly from what has been reported in other countries, especially in respect of Cu and vitamin C levels. It is desirable to carry out mega studies on trace element status in the six different geo-political zones in Nigeria. From the findings of this study, we suggest that zinc and vitamin E tablets could be beneficial to type 2 diabetes patients in Nigeria.

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