

Full Length Research Paper

Levels of antioxidant vitamins in newly diagnosed cases of type 2 diabetes mellitus in South Eastern Nigeria

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Free radicals have important roles in pathogenesis of diabetes mellitus. It has been well documented that there is a link between oxidative stress and secondary complications of diabetes mellitus. However, humans are well endowed with antioxidant defences, primarily by free radical scavengers, such as Vitamins A, C, E and some trace elements. Deficiencies of these micronutrients may increase susceptibility to this disease and the associated complications. In this study, serum antioxidant vitamins (Vitamin A, C and E) were estimated in 50 Type 2 diabetic patients using standard procedures, and the results obtained were compared with those of apparently healthy, non-diabetic subjects of comparable age and social status. Serum glucose level of the diabetic subjects (11.47 ± 1.67 mmol/L) was significantly higher ($P < 0.05$) than the value obtained for the non-diabetic subjects (4.16 ± 0.46 mmol/L). Vitamin A (14.38 ± 7.59 μ g/L), C (0.66 ± 0.17 mg/dl) and E (0.51 ± 0.19 mg/dl) concentrations were significantly lower ($P < 0.05$) in diabetic patients relative to the levels of Vitamin A (44.12 ± 11.79 μ g/L), C (0.97 ± 0.23 mg/dl) and E (0.68 ± 0.13 mg/dl) in control subjects. About 30, 36 and 12% of the diabetic subjects had severe Vitamins A, C and E deficiencies, respectively. These deficiencies may be contributing factor to the complications of type 2 diabetes mellitus. The outcome of the inclusion of Vitamin A, C and E supplements in the therapeutic regimen for Type 2 diabetics in Nigeria should be studied so that health care providers could be advised.

Key words: Nigerians, antioxidants, vitamins, diabetics.

INTRODUCTION

Recent research has shown that free radicals have important roles in pathogenesis of diabetes and a relationship between oxidative stress and secondary complications of diabetes exist (Singal et al., 2001; Mercuri et al., 2000). Patients with type 2 diabetes are subjected to chronic oxidative stress (Ghiselli, 1995). Oxidation reactions can produce free radicals which start chains of reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reaction, and as a result serve as reducing agents (Sies, 1997).

Despite the inhibition of free radicals by antioxidants, there are several potential sources of free radical production in diabetes, including auto-oxidation of plasma glucose (1), leucocyte activation, and increased transition metal bioavailability (Wolfs, 1991) which could overwhelm the body's antioxidant defences. The total antioxidant status (TAS) in Type 1 or 2 diabetes mellitus is lower than that of age-matched controls, a finding which might be attributable to lower levels of Vitamin C, Vitamin E (Vericel, 2004) or other factors including micronutrients in blood (Cunningham, 1998).

It has been proposed that oxidant vitamins can be used to block formation of free radicals and hence, prevent development of diabetes (Anderson, 2001; Tezuka, 1991), while superoxide radicals are cleansed by 2 enzymatic dismutase, antioxidants clean free radicals in

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Table 1. Some anthropometric characteristics of the type 2 diabetics and normal subjects in apparent good health.

Subject	n	Weight (kg)	Height (m)	BMI (kg/m ²)	Age (years)
Normal					
Male	26	66.65 ± 21.30	1.69 ± 0.20	23.54 ± 1.13	47.54 ± 11.3
Female	24	65.28 ± 15.73	1.52 ± 0.12	25.34 ± 0.93	43.29 ± 10.7
Total	50	66.62 ± 17.42	1.57 ± 0.17	24.24 ± 1.43	45.37 ± 9.7
NIDDM					
Male	25	79.52 ± 2.78	1.64 ± 0.13	29.79 ± 1.58	45.12 ± 13.3
Female	25	73.11 ± 13.73	1.56 ± 0.51	28.77 ± 1.72	44.64 ± 11.4
Total	50	76.27 ± 10.22	1.60 ± 0.13	29.24 ± 2.34	44.63 ± 8.9

Values are expressed as mean ± SD for 'n' subjects. BMI, Body mass index; NIDDM, non-insulin dependent (Type 2) diabetes mellitus. BMI classification: Under-weight: 16.5 to 18.5; Normal: 18.5 to 25; Over-weight: 25 to 30; Obese: 30 to 40.

organisms (Preuss, 1998). Glutathione is a very non-enzymatic antioxidant together with antioxidant Vitamins A, C and E (Ueno, 1998). It has been proposed that in diabetic patients, several abnormalities related with absorption develop in the absence of antioxidant vitamins (Urmila, 2004). Absolute or relative deficiency of antioxidant defence may lead to a situation of increased oxidative stress, especially in case of obesity, dyslipidemia and insulin resistance, and this may be associated with both the causes and consequences of a variety of disorder (Mayes, 1996). Thus, this investigation was designed to report the levels of antioxidant vitamins (A, C and E) in newly diagnosed type 2 diabetes yet to be treated.

MATERIALS AND METHODS

Subjects

The subjects selected for this study were 50 consenting diabetic patients (25 males and 25 females) who were newly diagnosed in the diabetic clinic, Federal Medical Centre (FMC), Owerri, Nigeria. Information on the age, weight, height and nature of occupation of the subjects were obtained. Fifty (50) apparently healthy, non-diabetic subjects (26 males and 24 females) of similar socioeconomic status, who were members of the hospital community were recruited to serve as control. The consent of all subjects were sought and obtained. The study was approved by the Research and Ethics Committee inaugurated by the Hospitals Board.

Specimen collection and preservation

Fasting blood samples were collected into labelled centrifuge tubes after 8 to 12 h overnight fast from the subjects by veno-puncture using 21 gauge sterile hypodermic syringe and needle. The blood samples were centrifuged at 2000 rpm for 10 min using a desktop centrifuge, and the serum was separated and kept in labelled sample bottles at 20°C until required.

Specimen analysis

Blood glucose was measured by the glucose oxidase method (Trinder, 1964) using commercial test kit supplied by Randox Laboratories, Ardmore, UK.

Vitamin A concentration in serum was determined by the Carr-Price reaction. Levels of serum Vitamin C were measured spectrophotometrically. Serum vitamin E level was determined by a colorimetric method (Quaife et al., 1949). The reagents for determining the amounts of antioxidant vitamins (A, C and E) in serum were AnalaR grade chemicals supplied by BDH, Poole, England.

Statistical analysis

Results are presented as mean ± standard deviation and were separated on the basis of gender. Serum levels of the trace antioxidant vitamins were correlated with serum glucose levels of both the diabetic and control subjects and correlation coefficients determined. The student's t-test was used to determine the level of significance which was set at $P < 0.05$.

RESULTS

The diabetic patients had higher weight measures than the control subjects (Table 1). The result of the body mass index (BMI) indicates that the diabetic subjects were over-weight, while the healthy, non-diabetic control subjects were within the reference range for normal weight.

From Table 2, there was a significant difference ($P < 0.05$) between serum glucose level of the Type 2 diabetic and non-diabetics. When separated on the basis of gender, the result still shows significant differences ($P < 0.05$). Serum levels of antioxidant vitamins were significantly lower ($P < 0.05$) in diabetic subjects when compared with normal control individuals.

From Table 3, prevalence of hypovitaminosis among diabetics is widely spread in the study area. As indicated (Table 3), 30, 36 and 12% of the subjects had severe Vitamins A, C and E deficiencies, respectively.

Table 2. Serum glucose and antioxidant vitamin levels of the type 2 diabetes and normal subjects.

Subject	n	FBS (mmol/L)	Vitamin A ($\mu\text{g/dl}$)	Vitamin C (mg/dl)	Vitamin E (mg/dl)
Normal					
Male	26	4.22 \pm 0.42	43.03 \pm 12.32	0.97 \pm 0.16	0.67 \pm 0.13
Female	24	4.08 \pm 0.51	46.23 \pm 12.02	0.98 \pm 0.24	0.68 \pm 0.14
Total	50	4.16 \pm 0.46	44.12 \pm 11.79	0.97 \pm 0.23	0.68 \pm 0.13
NIDDM					
Male	25	10.23 \pm 1.04	14.42 \pm 7.42	0.66 \pm 0.18	0.53 \pm 0.21
Female	25	12.62 \pm 2.20	14.12 \pm 7.98	0.64 \pm 0.15	0.49 \pm 0.17
Total	50	11.47 \pm 1.67*	154.38 \pm 7.59*	0.66 \pm 0.17*	0.15 \pm 0.19*

Values are expressed as mean \pm SD for 'n' subjects. *Value differs significantly ($P < 0.05$) from the comparable control value. FBS, Fasting blood sugar; NIDDM, non-Insulin dependent (Type 2) diabetes mellitus.

Table 3. Percentage of subjects with severe vitamins A, C and E deficiencies (hypovitaminosis).

Subjects	Severe deficiency			Marginal deficiency		
	Vitamin A ($<10 \mu\text{g/dl}$)	Vitamin C ($<0.5 \text{ mg/dl}$)	Vitamin E ($<0.5 \text{ mg/dl}$)	Vitamin A ($<20-29 \mu\text{g/dl}$)	Vitamin C ($<0.6-0.69 \text{ mg/dl}$)	Vitamin E ($<0.6-0.69 \text{ mg/dl}$)
Normal (%)						
Male	0.0	0	0	8	5	2.8
Female	0.0	0	0	1.6	5.1	7
Total	0.0	0	0	6	5	2.1
NIDDM (%)						
Male	28	32	20	22	20	13
Female	36	36	28	14	20	16
Total	30	36	12	18	20	6

Vitamin A based on IVACG (1996) Criteria, Vitamin C and E based on WHO/MONICA (1992) Criteria (Dosso et al., 2001).

DISCUSSION

The primary need of a diabetic patient is to attain and sustain normoglycaemia. His potential problems are largely the complications that could develop as a result of poor management of the disease. Management and treatment of diabetes is difficult due to poor level of education and health care facilities in developing countries. Therefore, reported increase of diabetes in Nigeria has been of great concern (Bakari et al., 1999).

The WHO/MONICA dual study of antioxidants and that of Cunningham indicated that normal serum concentration of vitamin A ranged from 40 to 70 $\mu\text{g/dl}$ (Cunningham, 1998). Lower acceptable levels are 30 $\mu\text{g/dl}$ for children and 20 $\mu\text{g/dl}$ for adults. Serum concentrations of less than 10 $\mu\text{g/dl}$ for adults are indicative of deficiency and severe depletion of liver stores of vitamin A (IVACG, 1996). High levels are usually found in individuals receiving diets high in vitamin A (Cunningham, 1998). Low serum levels of vitamin A are also found in gastrointestinal, pancreatic and hepatic disease and in infections like fever and

chronic nephritis (Sankale et al., 1992). However, women taking steroids or oral contraceptives have been reported to have slightly elevated serum concentrations of vitamin A (Packer, 2000). Vitamin A activity is very important for maintaining health and thus, humans and other animals have developed the capacity to store it. Storage lessens the need for regular intake of the vitamin, since it can be mobilized from these stores as retinal bound to retinol binding protein (Wongsiriroj and Blaner, 2004). Liver is the major storage organ for vitamin A, though other tissues, such as lungs, eyes adipose tissue and skin can also store it (Wongsiriroj and Blaner, 2004).

In the current work, mean serum levels of vitamin A recorded for the non-insulin dependent (type 2) diabetes mellitus (NIDDM) and control subjects were 14.38 \pm 7.59 and 44.12 \pm 11.79 $\mu\text{g/dl}$, respectively.

Concentration of vitamin A in NIDDM subjects were significantly lower than the values obtained for the control subjects. This is an indication that NIDDM subjects in the study area are vitamin A deficient. Further analyses of the results revealed that 28 and 36% of the male and female

diabetic patients, respectively had less than 10 µg/dl of vitamin A, a condition considered as severe deficiency state. Additionally, 40% of the male and 36% of the female diabetic patients have moderate vitamin A deficiency (10 to 19 µg/dl). Furthermore, 22% of the male and 14% of the female diabetic patients had serum vitamin A concentrations within the range of 20 and 29 µg/dl; a marginal deficient state. Only about 2% male and 1.2% of the female control subjects had moderate vitamin A deficiency (10 to 19 µg/dl). There was no recorded cases of severe deficiency (that is <10 µg/dl) among the control subjects.

An individual is considered Vitamin C deficient when his/her serum Vitamin C concentration is less than 0.8 mg/dl. In the present study, 80% of male diabetic subjects and 30% of female diabetics had serum Vitamin C concentrations below 0.8 mg/dl. Acceptable normal range for vitamin E is 0.80 to 1.2 mg/dl (Cunningham, 1998). In the present study, reference range obtained for Vitamin E was 0.50 to 1.3 mg/dl. The mean serum Vitamin E obtained for the diabetic patients was significantly lower ($P < 0.05$) than the values obtained for the control subjects. Further analysis of the results indicates that 68 and 62% of female and male diabetic patients, respectively were Vitamin E deficient.

Several diseases are associated with free radical production, these diseases include among others, diabetes mellitus, atherosclerosis and cancer. Consumption of fruits and vegetables has been associated with reduced risk of free radicals production (Yochum et al., 2000). The potential protective effects of these foods may be due to their antioxidant vitamin contents. B-carotene, one of the dietary sources of Vitamin A, C and E, the free radical scavengers have been shown to quench singlet oxygen, superoxide, hydroxy radical and peroxy radicals (Stahl and Sies, 1997). The implication of these to the diabetic subjects cannot be overemphasized. In the current work, a direct negative correlation between serum antioxidant vitamins and fasting blood sugar was observed.

Hyperglycaemia is a recognized pathogenic factor of long-term complications in diabetes mellitus. It does not only generate reactive oxygen species, but also alternatives antioxidant mechanism, creating a state of oxidative stress (Catherwood et al., 2002). The beta cells are sensitive to oxidative stress, because their intracellular antioxidant defense mechanisms are weak (Rasilainens et al., 2002). It is believed that one of the mechanisms responsible for secondary complications of diabetes involve non-enzymatic glycosylation of proteins by glucose auto-oxidation (Cariello et al., 1992). Non-enzymatic glycosylation is a spontaneous chemical reaction between glucose and the amino groups of proteins resulting in the formation of reversible schiff's bases and Amadon products. These products have been reported to generate free radicals, causing oxidative stress and tissue damage (Baynes, 1991). Antioxidant

vitamins, such as Vitamins C and E have been shown to reduce protein glycosylation and act as scavengers of free radicals generated by glycosylated proteins (Cariello et al., 1992). Cardiovascular complication of diabetes is in part due to small vessel damage by oxidized low density lipoprotein (LDL). Vitamin E was found to lower LDL oxidation, thus lowering the risk of diabetic cardiovascular complications (Fuller et al., 1996).

CONCLUSION AND RECOMMENDATION

It is apparent from the current study that subjects with Type 2 diabetes in the study area have low levels of serum antioxidant vitamins. The deficiencies of these vitamins have been implicated in the development of diabetic late complications, such as cataract, nephropathy and neuropathy (Packer, 2000). Some studies suggest that people with diabetes have elevated levels of free radicals and lower levels of antioxidants (Schneider et al., 2000). Some other studies suggested further that Vitamin E, biotin and Vitamin B₆ supplementation may improve symptoms of diabetes and reduce the risk of associated complications (Schneider et al., 2000). In a similar study, Vitamin E was found to reduce lipid peroxidation stress in Type 2 diabetic subjects with retinopathy (Chung et al., 1998). The study further showed that nutrients including Vitamin E, Vitamin C, magnesium, chromium, lipoic acid and vanadium, all have beneficial effects on the symptoms or complications associated with diabetes. Many of these nutrients appear to be closely associated with insulin metabolism and help maintain proper blood glucose levels (Chung et al., 1998).

In view of the significant reduced antioxidants concentrations in NIDDM subjects obtained in this study and significant negative correlations between serum antioxidant vitamins and fasting blood glucose level of the subjects, it may not be out of place to recommend the inclusion of antioxidant vitamins in therapeutic regimens for the management of NIDDM in particular and diabetes mellitus in general in the study area. This could assist in reducing or delaying the risk of diabetic late complications. Therefore, the effect of supplementing the therapeutic regimens for NIDDM management with antioxidants vitamins on diabetic complications needs to be studied and scientific facts obtained so that health care providers could be convincingly advised.

REFERENCES

- Anderson RA (2001). Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus. *J. Am. Coll. Nutr.*, 20: 212–218.
- Bakari AG, Onyemekujwe GC, Sani BG, Hassan SS, Aliyu TM (1999). Prevalence of diabetes mellitus in suburban Northern Nigeria: Results of public screening survey. *Diabet. Int.*, 9: 59-60.
- Baynes JW (1991). Role of Oxidative stress in development of

- complications of diabetes. *Diabetes*, 40: 405–412.
- Cariello A, Quatraro A, Giugliano D (1992). New insight on non enzymatic glycosylation may lead to therapeutic approaches for the prevention of diabetic complications. *Diabet. Med.*, 9: 297–299.
- Catherwood MA, Powell LA, Anderson P, McMaster D, Sharpe PC, Trimble ER (2002). Glucose-induced oxidative stress in mesangial cells. *Kidney Int.*, 6: 599–608.
- Cunningham JJ (1998). Micronutrients as nutraceutical interventions in diabetes mellitus. *Am. Coll. Nutr.*, 17: 7-10.
- Dosso DK, Rana SV, Offe-Amoyaw D, Tete D, Maddy SQ (2001). Total antioxidant status in non-insulin-dependent diabetes mellitus patients in Ghana. *West Afr. J. Med.*, 20: 148-186.
- Fuller CJ, Chandalia M, Garg A (1996). RRR-Alpha tocopheryl acetate supplementation at pharmacologic doses decreases low-density-lipoprotein oxidative susceptibility but not Protein glycation in Patients with diabetes mellitus. *Am. J. Clin. Nutr.*, 63: 753–759.
- Ghiselli AA (1995). Fluorescence-based method for measure total plasma antioxidant capability. *Free Radic. Biol. Med.*, 18: 29–36.
- IVACG (1996). *IVACG Policy Statement on Vitamin A, Diarrhea and Measles*, Washington, USA, pp. 5-28.
- Mercuri F, Quagliari L, Ceriello A (2000). Oxidative stress evaluation in diabetes. *Diabet. Technol. Ther.*, 2(94): 589-600.
- Mayes PA (1996). Lipids of Physiological significance. In: *Harpers biochemistry*. Murray RK, Grammer, DK., Mayes PA., Rodwel VW eds. 24 (Ed) United States. Appleton and Lange, pp. 147-157.
- Packer L (2002). Alpha lipoic acid as biological antioxidants. *J. Free Rad. Biol. Med.*, 20: 1020-1032.
- Preuss HG (1998). Comparative effects of chromium, vanadium and gymnema sylvestre on sugar-induced blood pressure elevations in SHR. *J. Am. Coll. Nutr.*, 17: 116– 123.
- Quaif ML, Scrimshaw NS, Lowry OH (1949). A micromethod for assay of total tocopherols in blood and serum. *J. Biol. Chem.*, 180: 1229–1235.
- Sies H (1997). Oxidative stress: oxidants and antioxidants. *Exp. Physiol.*, 82(2): 291–5.
- Singal PK, Bello-klein A, Farahmand F, Sandhawatia V (2001). Oxidative stress and functional deficite in diabetic cardiomyopathy. *Adv. Exp. Med. Biol.*, 498: 213-220.
- Stahl W, Sies H (1997). Antioxidant defence: Vitamin E and C and Carotenoids. *Diabetes*, 6: S14 – 17.
- Tezuka M (1991). Chromium (III) decreases carbon tetrachloride originated trichloromethyl radical in mice. *J. Inorg. Biochem.*, 44: 261–265.
- Trinder P (1964). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem.*, 6: 24-27.
- Ueno S (1998). Effects of chromium in lipid peroxidation in isolated hepatocytes. *Jpn. J. Sci.*, 50:45–52.
- Urmila AS (2004) Anti-diabetic activity and Mechanism of action of chromium chloride. *Exp. Clin. Endocrinol. Diabet.*, 112: 248–252.
- Vericel E (2004). Diabetic patients without vascular complications display enhanced basal latelet activation and decreased antioxidant status. *Diabetes*, 53: 1046–1051.
- WHO/MONICA (1992). The role of antioxidants in prevention of diabetes mellitus. *WHO Technical Report Series. Geneva. 727*.
- Wolf SP (1991). Protein glycosylation and oxidative stress in diabetes mellitus and aging. *Free Radic. Biol. Med.*, 10: 339–352.
- Wongsiriroj N, Blaner WS (2004). Vitamin A storage and action in the liver. *Sight and Life Newsletter*, 2: 4-10.
- Yochum LA, Folsom AR, Kushi LH (2000). Intake of antioxidant vitamins and risk of death from stroke in postmenopausal women. *Am. J. Clin. Nutr.*, 72: 476-481.