

# ORIGINAL RESEARCH ARTICLE

## Evaluation of zinc, vitamin C, and vitamin E levels in male subjects with type 2 diabetes mellitus

### ABSTRACT

**Aim:** The aim of this study was to determine the serum/plasma levels of zinc, vitamin C and E in male subjects with type 2 diabetes mellitus to establish their concentration pattern.

**Place and Duration of Study:** Medical outpatient clinic, Chemical Pathology Laboratory of Enugu State University of Science and Technology Teaching Hospital, between January and December 2016.

**Methodology:** This prospective cohort study enrolled 40 male individuals with type 2 diabetes mellitus (DM) and 40 apparently healthy control, within the age range of 45 - 75 years. Zinc, vitamin C, vitamin E, and fasting blood glucose levels (FBG) were determined at pre-treatment, six months and 12 months into treatment.

**Results:** The mean values of zinc, vitamin E and FBG were significantly higher at pre-treatment compared to apparently healthy control values (147.76 +/- 32.95 vs 114.31 +/- 15.58 µg/dl, 11.88 +/- 3.13 vs 3.42 +/- 0.21 mg/dl and 8.08 +/- 3.22 vs 5.25 +/- 0.35 mmol/l respectively) (p=<0.001, <0.001, and <0.001 respectively). At 6 month into treatment in comparison to pre-treatment values, there were significant decreases in vitamin C (3.90 +/- 0.97 vs 5.15 +/- 1.43 mg/dl) (p= 0.04). At 12 month into treatment in comparison to pre-treatment values, there were significant decreases in vitamin C (3.25 +/- 0.16 vs 5.15 +/- 1.43 mg/dl) (p= <0.001). At pre-treatment, vitamin C significantly decrease from 6.49 +/- 0.96 mg/dl in age group 45-64 years to 4.10 +/- 0.76 mg/dl in age >65 years (p=0.01).

Keywords: vitamin, diabetes mellitus, month, treatment

### 1. INTRODUCTION

Diabetes mellitus is a present-day public health disease affecting people worldwide both in low income and developed countries, and giving rise to a

major socio-economic challenge. The commonness of diabetes in the age groups between 20 to 70 years worldwide was approximated to be 8.3% in 2013 and 10.1% in 2035. The absolute number of adult with diabetes is estimated to rise from 382 million in 2013 to 592 million in 2035. In 2013, a calculated 5.1 million people died from consequences of hyperglycemia. More than 80% of diabetes deaths occur in low and middle-income countries [1]. Zinc (Zn) is one of the vital trace elements which are included in the integrating, depository, production and building integrity of insulin. Lack of Zn is associated with many chronic diseases [2]. Zinc takes part in a crucial role in glucose metabolism. It assists in the usage of glucose by muscle and fat cells. It is needed as a cofactor for the function of intracellular enzyme that may be included in protein, lipid and glucose metabolism[3]. Zinc may be needed in the control of insulin body structure-initiated signal transduction mechanism and insulin anatomical structure synthesis [3]. Zinc is a fundamental part of crucial antioxidant enzymes such as superoxide dismutase, and zinc shortage damage their synthesis, leading to high oxidative stress. Other antioxidant agents are the vitamins C and E. These vitamins are diet-obtained and get rid of free radicals directly. Vitamin E has been shown to protect membranes from lipid peroxidation and its lack is coexisting with high peroxides and aldehydes in several tissues. Ascorbic acid is known to decrease or neutralize reactive oxygen species such as hydrogen peroxide. It is also a basis for the redox enzyme ascorbate reductase [4]. Also antioxidants, vitamins C and E protect against the destroying effects of increase oxidative stress due to non enzymatic glycosylation, autoxidative glycosylation, and metabolic stress in persons with diabetes. Lower serum levels of these micronutrients have been reported in the diabetic state [4]. The aim of the present study was to evaluate the serum levels of Zinc, plasma levels of vitamin C and vitamin E in male patients with type 2 DM.

## **2. MATERIALS AND METHODS**

Prospective cohort study was conducted at medical outpatient clinic and chemical pathology laboratory both at Enugu State University of Science and Technology Teaching Hospital (ESUTH), during January 2016 to December 2016, 40 male patients with type 2 (aged between 45-75 years)

were enrolled. After an overnight fasting, 6 ml of peripheral blood was taken (2ml in fluoride oxalate container, 2ml in plain container and 2ml in ethylenediaminetetracetate (EDTA) bottle). The blood samples were centrifuged at 3000 rpm for 10 min and serum stored at  $-20^{\circ}\text{C}$ . Blood samples for plasma vitamin C, E, FBG and serum zinc, were obtained from each patient before they were placed on diabetes treatment, 6 months into treatment and 12 months of treatment. Ethical approval was obtained from Research Ethics Committee of Enugu State University of Science and Technology Teaching Hospital (ESUTH) Enugu, informed consent was taken from each participant after the full explanations about the study.

## **2.1 ESTIMATION OF VITAMIN C**

Vitamin C was determined by spectrophotometric method described by Roe and Keuther [5]. Standard ascorbate 0.1ml (1mg/ml) of the supernatant was taken. The volume was made up to 1.0ml with 4% trichloroacetic acid (TCA). Two, 4-Dinitrophenylhydrazine (DNPH) reagents (0.5ml) were added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at  $37^{\circ}\text{C}$  for 3 hours resulting in the formation of osazane crystals. The crystals were dissolved in 2.5ml of 85% sulphuric acid. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. The tubes were cooled in ice and the absorbance was read at 540 in a spectrophotometer.

### **2.1.1 ESTIMATION OF VITAMIN E**

Vitamin E was determined by futter-mayercolorimetric method of the association of vitamin chemists [6, 7]. 0.1ml of the sample was mixed with 10ml of ethanoic sulphuric acid and boiled gently under reflux for 30 minutes. It was transferred to a separating funnel and treated with 3x30ml diethyl ether and recovering ether layer each time, the ether extract was transferred to a dessicator and dried under for 30 minutes and later evaporated to dryness at room temperature. The dried extract dissolved in 10ml of pure ethanol. 1ml of the dissolved extract and equal volume of standard vitamin E were transferred to separate tubes. After continuous addition of 5ml of absolute alcohol and 1ml of concentrated nitric acid solution, the mixtures were allowed to stand for 5minutes and the respective absorbance measured in a spectrophotometer at 410nm with blank reagent at zero.

## 2.1.2 ESTIMATION OF ZINC BY ATOMIC ABSORPTION SPECTROPHOTOMETER

Principle: Atomic absorption spectrometer (AAS) is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample. For determination of zinc 1ml of serum diluted with 4ml of distil water, at wave length 213.9 nm.

## 2.1.3 ESTIMATION OF GLUCOSE

Glucose was determined by glucose oxidase enzymatic method. Ten microliters of sample were pipetted into test tubes, 10 $\mu$ l of standard was pipetted into another test tube. One thousand microliters of reagent blank were pipetted into another test tube. Mixed, incubated for 10 minutes at 37<sup>0</sup>C. The absorbance of the standard (A standard) was measured and the absorbance of sample (A sample) was measured against the reagent blank within 60 minutes.

Calculation

$$\text{Glucose concentration (mmol/l)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{standard concentration (mmol/l)}$$

## 2.1.4 STATISTICAL ANALYSIS

The statistical analysis of data generated from this study was done by computer software Statistical Package for Social Sciences (SPSS) version 21.0. Values are expressed as a mean $\pm$ standard deviation. The data is analyzed using Analysis of variance (ANOVA).  $p < 0.05$  is considered significant.

## 3. RESULT

Table 1 showed baseline values of zinc, vitamin C, E and FPG of male individuals with type 2 DM before treatment and control subjects. Before treatment, serum zinc levels of DM patients were significantly higher (147.76 $\pm$ 32.95  $\mu$ g/dl) compared with control (114.31 $\pm$ 15.58  $\mu$ g/dl)

( $p < 0.001$ ), Plasma vitamin E levels of DM patients were significantly higher (11.88 $\pm$ 3.13 mg/dl) compared with control (3.42 $\pm$  0.21 mg/l) ( $p < 0.001$ ). Before treatment, fasting blood sugar levels of DM patients were significantly higher (8.08 $\pm$  3.22 mmol/l) compared with control (5.25 $\pm$  0.35 mmol/l) ( $p = 0.01$ ). Table 2 showed mean  $\pm$  SD of zinc, vitamin C, E and FBG of male diabetic patients before treatment, 6 months of treatment and 12 months of treatment. After initiating treatment, zinc significantly reduced from 147.76 $\pm$  32.95  $\mu$ g/dl to 39.55 $\pm$  2.43  $\mu$ g/dl at 6 months ( $p < 0.001$ ), vitamin C significantly reduced from 5.15 $\pm$ 1.43 mg/dl to 3.90  $\pm$  0.97 mg/dl (0.04) at six months. At 12 month into treatment in comparison to pre-treatment values, there were significant decreases in vitamin C (3.25 $\pm$ 0.16 vs 5.15 $\pm$ 1.43 mg/dl) ( $p = < 0.001$ ). The FBG of male patients before treatment (8.08 $\pm$  3.22 mmol/l) were significantly higher compared with its level at 6 months of treatment (6.05 $\pm$  0.57mmol/l) and 12 months of treatment (5.04 $\pm$  1.17 mmol/l) respectively ( $F = 6.00$ ;  $p = 0.01$ ). Table 3 showed mean  $\pm$ SD of zinc, vitamin C, E and FBG in male patients with type 2 DM between age group 45-64 years and  $> 65$  years before treatment, 6 months into treatment and 12 months of treatment. Before treatment, vitamin C significantly reduced from 6.49 $\pm$  0.96 mg/dl in age group 45-64 years to 4.10 $\pm$  0.76 mg/dl in age  $> 65$  years ( $p = 0.01$ ), zinc significantly increase from 111.58 $\pm$  32.61  $\mu$ g/dl in age group 45-64 years to 138.96 $\pm$  21.14  $\mu$ g/dl in age  $> 65$  years at ( $p = 0.02$ ). At 12 months of treatment, vitamin E significantly increase from 1.29 $\pm$  0.04 mg/dl in age group 45-64 years to 3.30 $\pm$  0.04 mg/dl in age  $> 65$  years ( $p < 0.001$ ).

**Table 1 Comparison of Baseline Values of zinc, vitamin C, E and FBG between Individuals with Type 2 DM and Apparently Healthy Control**

	Zn ( $\mu$ g/dl)	Vit C (mg/dl)	Vit E (mg/dl)	FBG (mmol/l)
BT	147.76 $\pm$ 32.95	5.15 $\pm$ 1.43	11.88 $\pm$ 3.13	8.08 $\pm$ 3.22
C	114.31 $\pm$ 15.58	4.66 $\pm$ 0.28	3.42 $\pm$ 0.21	5.25 $\pm$ 0.35
P value	$< 0.001$	0.28	$< 0.001$	0.01

\*Key =  $p < .05$

Abbreviation: BT= before treatment, C=control, vit C = vitamin C, vit E= vitamin E

**Table 2 Mean values of zinc, vitamin C, E and FBG of male subjects with type 2 DM (across treatment periods)**

	Zn (µg/dl)	Vit C (mg/dl)	Vit E (mg/dl)	FBG (mmol/l)
BT	147.76±32.95	5.15±1.43	11.88±3.13	8.08±3.22
T6	39.55±2.43	3.90±0.97	12.17±2.83	6.05±0.57
T12	131.72±17.93	3.25±0.16	1.30±0.22	5.04±1.17
F (P) value	98.96 (<0.001)	9.26 (<0.001)	50.21 (<0.001)	6.00 (0.01)
BT VS T6	<0.001	0.04	0.93	0.19
BT VS T12	0.24	< 0.001	<0.001	0.03
T6 VS T12	<0.001	0.19	<0.001	0.03

\*Key =  $p < .05$

Abbreviation: T6= 6 months into treatment, T12= 12 months into treatment

**Table 3 Comparison of zinc, vitamin C, E and FBG in age groups of Individuals with Type 2 DM**

	Zn (µg/dl)	Vit C (mg/dl)	Vit E (mg/dl)	FBG (mmol/l)
Before treatment				
45-64 yrs	111.59±32.61	6.49±0.96	12.02±2.91	9.94±4.84
>65 yrs	138.96±21.14	4.10±0.76	8.93±2.71	8.25±2.50
p value	0.02	0.01	0.12	0.53
6 month				
45-64 yrs	145.63±20.46	2.53±0.55	11.53±3.58	6.07±0.79
>65 yrs	144.54±9.00	4.18±2.19	11.71±1.47	6.06±0.53
p value	0.90	0.16	0.91	0.99
12 month				
45-64 yrs	125.20±7.12	3.15±0.05	1.29±0.04	4.62±0.28
>65 yrs	126.60±6.77	3.11±0.01	3.30±0.04	4.51±0.42

p value	0.60	0.10	<0.001	0.68
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Abbreviation: yrs= years

#### 4. DISCUSSION

Diabetes mellitus is a complex metabolic disorder affecting metabolism of carbohydrates, lipids and proteins. Higher mean serum zinc level of male diabetic patients in comparison to healthy controls was noticed in this study. This finding contrast with study carried out in Nnewi, in which they found that the concentrations of serum Zn in male patients with T2D are significantly lower than control subjects [8]. This may suggest that profiles differs among populations residing in different states, it may be related to variation in dietary composition according to state of residence. After initiating treatment, Zn significantly reduced at six months in male diabetic patients. This finding also contrast with report of previous study which stated that determination of serum Zn after 3 months treatment with metformin revealed that there were not significant differences compared to the initial moment [9]. Deficiencies in vitamins C are re-occurring due to high consumption of processed foods and reduced fresh foods in the Nigerian diet, as well as to an indoor sitting lifestyle away from sun exposure. These dietary and lifestyle factors also expose one to diabetes and metabolic syndrome. In this study, after initiating treatment, vitamin c significantly reduced at six months in male diabetic patients. This is nearly identical with a review of 23 observational studies that looked at the vitamin C status of people with diabetes published between 1935 and 1996 found that people with diabetes have at least 30% lower vitamin C concentrations than do people without diabetes [10]. Study had shown that individuals with Type 2 diabetes have lower vitamin E levels compared to controls [11]. In this study, higher mean plasma level of vitamin E in male diabetic patients in comparison to healthy controls was observed. This disagrees with Fagbohun *et al.*, (2017) who reported that vitamin E were significantly higher in control groups than diabetic subjects [11].

#### CONCLUSION

Plasma vitamin C level of the study participants were low at six month, lower at twelve months and also plasma vitamin C levels were also low in patients greater than 65 years.

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