

Full Length Research Paper

Evaluation of essential trace metals in female type 2 diabetes mellitus patients in Nigerian population

Samuel Oyewole Oyedeji^{1*}, Adeyemi Adeleke Adesina², Olusegun Taiwo Oke³ and Yetunde Olufunmilayo Tijani⁴

¹School of Medical Laboratory Science, Chemical Pathology, Department, Obafemi Awolowo University Teaching Hospital Complex, P.M.B.5538, Ile-Ife, Osun State, Nigeria.

²Chemical Pathology Department, Obafemi Awolowo University Teaching Hospital Complex, P.M.B.5538, Ile-Ife, Osun State, Nigeria.

³School of Medical Laboratory Science, Haemathology Department, Obafemi Awolowo University Teaching Hospital Complex, P.M.B.5538, Ile-Ife, Osun State, Nigeria.

⁴General Hospital, Ifaki-Ekiti, Ekiti State, Nigeria.

Received 21 May, 2012; Accepted 26 February, 2014

Diabetes mellitus (DM) has been reported to be associated with derangement of micronutrients. This study was to investigate the plasma levels of antioxidant elements (zinc, selenium, copper) haemopoietic elements (chromium and iron) and magnesium in female type 2 diabetes mellitus (DM) patients. Fifty (50) female type 2 DM patients (test subjects) aged 20 to 50 years and 35 healthy, non-diabetic age-matched females (control subjects) were recruited for the study. Fasting blood plasma levels of the elements were determined using atomic absorption spectrophotometer (AAS) after acid digestion. Blood glucose level was determined by glucose oxidase/peroxidase method to confirm the status of the test and the control subjects. Body mass index was determined from weight and height of the subjects. The mean values of plasma zinc, selenium, magnesium and chromium were significantly lower in the diabetic patients than in non-diabetics ($P < 0.002$, $P < 0.001$, $P < 0.001$ and $P < 0.001$ respectively). The mean values of glucose, BMI, iron and copper was observed to be significantly higher in diabetic patients than control subjects ($P < 0.002$, $P < 0.05$, $P < 0.002$ and $P < 0.001$ respectively). A significant positive correlation existed between glucose and BMI, copper and iron ($r = 0.717$; $P < 0.0001$, $r = 0.717$; $P < 0.05$, $r = 0.721$; $P < 0.05$) whereas an inverse relationship was observed between iron and chromium, selenium and magnesium ($r = -0.448$, -0.703 and -0.651 ; $p < 0.004$, 0.000 , 0.000 respectively). Negative association was also observed between copper and zinc ($r = -0.716$; $P < 0.01$). These findings revealed that DM is associated with significant alteration in the concentrations of essential trace metals and significant increase in BMI. This may contribute to various metabolic complications and increased mortality from cardiovascular diseases in DM patients in the rural locality as it is observed among diabetics in urban settings.

Key words: Trace metals, diabetes mellitus, copper, chromium, Ekiti state.

INTRODUCTION

Diabetes mellitus (DM) represents today a disease with massive spreading with medico-social consequences.

Recent research has shown a close relationship between some specific micronutrients and this disease, with impli-

cations for the pathogenesis of this disease and its vascular complications. The relationship between nutrition and diabetes was suspected as early as 1674 and over the last 20 years, numerous studies have found alterations in micronutrients status of patients with diabetes mellitus (Mooradian and Morley, 1987). There are accumulating evidences that the metabolism of several trace metals are altered in diabetes mellitus and these micronutrients might have specific roles in the pathogenesis and progression of the disease (Akinloye et al., 2010). In particular, DM has been shown to be associated with abnormalities in the metabolism of zinc, chromium, copper, magnesium and manganese (Retnam and Bhandarkar, 1981). People with type 2 DM have greater excretion and lower tissue levels of chromium than non-diabetic control (Musad et al., 2004). Evidence that systematic iron overload could contribute to abnormal glucose metabolism was first derived from the observation that the frequency of diabetes is increased in classic hereditary hemochromatosis (HH) (Sundararaman et al., 2007). Zinc is involved in the regulation of insulin receptor-initiated signal transduction mechanism. Zinc deficiency is associated with a number of metabolic disturbances including impaired glucose tolerance, insulin degradation, and reduced pancreatic insulin content (Nsonwu et al., 2006). Copper level has been found to be high in people with diabetes compared to those without. The higher the copper level, the more likely the complications from diabetes (Kumar et al., 2007). Magnesium is an essential cofactor for multiple enzymes involved in glucose metabolism and is hypothesized to play a role in glucose homeostasis, insulin action and in the development of type 2 diabetes (Larsson and Wolk, 2007).

The objective of this study was to investigate the levels of essential trace metals (Mg, Cr), antioxidant related trace metals (Zn, Cu, and Se) and iron (Fe) in type II diabetes mellitus.

MATERIALS AND METHODS

Study location

The study was carried out in general hospital, Ikole-Ekiti, Ekiti state, South Western region of Nigeria. The hospital is a secondary health institution provided by the Ekiti state government in Nigeria to serve as a health care providing centre in this town and the adjoining towns and villages. Ikole-Ekiti is a rural town with the majority of the inhabitants and surrounding towns being farmers and traders.

Study population

The study population was known as female type II DM patients attending the hospital and apparently healthy non-diabetic age-matched individuals in the same locality.

Study design

The study was a stratified randomized study designed to investigate the status of trace metals in female type 2 DM patients in rural community.

Exclusion criteria

These included proteinuria, pregnancy, lactating mothers and alcoholism. Patients on prolonged medication that could alter measured metals such as chelating agents, D-penicillamine, or oral contraceptive pills were excluded from the study.

Subjects population

The subjects were recruited from patients attending general hospital, Ikole-Ekiti, Ekiti State, Nigeria. 110 women who gave their consent after adequate education on purpose of the study were recruited for this study. 85 women participated in the study. The subjects were grouped into two categories comprising of 50 female type 2 DM patients (test subjects) and 35 apparently healthy non-diabetic women tagged control subjects. Questionnaires were administered among the subjects to obtain socio-demographic information.

Determination of body mass index (BMI)

This was done by measurement of height in metre (m) and weight in kilogramme (Kg) using standard methods. The BMI was calculated from the average height and weight as follow:

$$\text{BMI} = \text{Weight (kg)} / \text{Height}^2 (\text{m}^2).$$

Blood sample collection

After an overnight fast of 10 to 12 h, venous blood was collected from the cubital vein of the participants using sterile hypodermic syringe into lithium heparin sample bottles for trace metals estimation, and fluoride oxalate bottles for fasting blood glucose. The sample in lithium heparin bottles were centrifuged, the plasma was separated and stored frozen at -20°C till analysis was carried out.

Biochemical analysis

Blood glucose was determined using standard enzymatic spectrophotometric method.

Trace metals determination

Concentrations of the trace metals were estimated using flame atomic absorption spectrophotometry after deproteinization of the plasma by adding 2 ml of the sample diluted to 10 ml with dilute hydrochloric acid. The diluted sample was then centrifuged at 3000 revolution /min for 30 seconds and the supernatant aspirated directly into the flame. The principle is based on dissociation of the element (by the flame) from its chemical bonds. This is then placed

*Corresponding author. E-mail: oyedejis1214@yahoo.com. Tel: +2348033822818.

Table 1. Characteristics of parameters in test and control subjects (Mean±SEM) and comparison of mean using students' t-test.

Parameter	Test group (N=50)	Control group (N=35)	T-test	P-value
	Mean ± SEM	Mean ± SEM		
Zinc (µmol/L)	18.15 ± 0.33	21.86 ± 0.06	40.607	P < 0.002
Selenium (µmol/L)	0.47 ± 0.03	1.02 ± 0.01	127.75	P < 0.001
Iron (µmol/L)	28.83 ± 0.45	22.27 ± 0.02	68.121	P < 0.002
Magnesium (µmol/L)	0.55 ± 0.01	1.13 ± 0.02	109.78	P < 0.001
Copper (µmol/L)	1.81 ± 0.02	0.61± 0.30	112.00	P < 0.001
Chromium (µmol/L)	0.96±0.08	5.39 ± 0.84	132.80	P < 0.001
Glucose (mmol/L)	13.41 ± 0.66	4.34 ± 0.20	61.33	P < 0.002
BMI (kg/m ²)	25.52 ± 0.49	21.66 ± 0.81	15.89	P < 0.05

BMI-Basal metabolic rate.

in unexcited or ground state (neutral atom). The neutral atom at low energy level is capable of absorbing radiation at a very narrow bandwidth corresponding to its own line spectrum (Kaneka, 1990).

Statistical analysis

The data obtained were processed statistically using statistical package for social sciences (SPSS Inc., Chicago, IL software, version 14.3). Differences were significant when the p-value was <0.05. Data obtained from the study were expressed as means and standard error of mean. The differences in the means were compared using student t-test and Pearson's correlation was used to determine the association between variables.

RESULTS

Table 1 shows the mean ± standard error of mean of all the parameters estimated and the result of student t-test comparing mean of the test and the control subjects. The mean plasma fasting blood glucose and BMI of test subjects were significantly higher than that of the control subjects (P<0.002 and P<0.05 respectively). The plasma concentrations of zinc, selenium, magnesium and chromium were all significantly lower in test subjects than control subjects. (P<0.002, P<0.001, P<0.001, P<0.001, and P<0.001 respectively). However, the mean plasma concentrations of iron and copper in diabetic patients were found to be significantly higher (P<0.002) than that in control subjects. Table 2 shows the result of Pearson's correlation of parameters with one another. There was strong positive relationship between fasting blood glucose and iron (r=0.721, P<0.001), copper (r=0.815, P<0.001) and BMI (r=0.717, P<0.001). Negative correlation was observed between fasting blood glucose and zinc (r= -0.479, P<0.000), selenium (r= -0.669, P<0.001), magnesium r=-0.584, P<0.001). Zinc, selenium, magnesium were positively correlated with one another. But they had inverse relationship with iron.

DISCUSSION

This study showed that there was derangement in the

levels of trace metals and increased BMI in type 2 diabetes mellitus (DM). Some trace metals act as antioxidants preventing the deleterious activities of oxidants on membranes while others act directly as co-factors in metabolism of macro molecules such as glucose. Diabetes has been associated with abnormalities in the metabolism of these trace metals (Schlienger et al., 1988). In this study, zinc level was found to be significantly lower in DM patients (P< 0.002) than in healthy individuals. Zinc has been found to have insulin-like effects in that it enhances glucose up take by inhibiting glycogen synthesis (Diwan et al., 2006). The findings of past studies on zinc level in type 2 DM has been mixed. Some have reported low Zinc levels in diabetes (Nakamura et al., 1991), some have reported higher level (Zargar et al., 2002) and some others reported no significant difference (Babalola et al., 2007). The present study corresponds with those that have reported lower levels. Selenium is known to act as antioxidant and peroxynitrite scavenger when incorporated into selenoprotein (Beytht and Akasakai, 2003). It is the main element in glutathione peroxidase which acts as an active antioxidant enzyme that reduces formation of free radicals and peroxides of lipoproteins. Selenium level was found to be significantly lower in diabetic than in non-diabetic group in this study. This finding corresponds with the report of Burt (2007); Akinloye et al. (2010). Selenium has strong positive correlation with zinc and magnesium in this study. Iron is an essential element for wide varieties of metabolic processes but it also has the potential to cause deleterious effects by formation of toxic oxygen radicals that can attack all biological molecules (Halliwell and Gutteridge, 1999). There are suggestive evidences that iron plays pathogenic roles in diabetes mellitus and its complication such as micro angiopathy and arteriosclerosis (Swaminathan et al., 2007). The concentration of plasma iron of diabetics in this study was found to be significantly higher than in control subjects (P< 0.002) and has strong positive correlation with copper, glucose and BMI. Our finding suggests that one of the causes of oxidative stress in type 2 diabetes mellitus, this may be

Table 2. Pearson correlation showing level of association in the BMI and biochemical parameters among female type 2 DM patients.

Parameter	Zn	Se	Fe	Mg	Cu	Glucose	BMI
Zinc	1	0.635**	-0.450*	0.558**	-0.717**	-0.479**	-0.331**
Selenium	0.635*	1	-0.703*	0.544**	-0.844**	-0.669**	-0.440*
Iron	-0.430*	-0.703**	1	-0.651**	0.811**	0.721**	0.458*
Magnesium	0.558*	0.544**	-0.651**	1	-0.698**	-0.584**	-0.427*
Copper	-0.716*	-0.884**	0.811*	-0.898**	1	0.815**	0.581*
Glucose	-0.479*	-0.669**	0.721*	-0.584**	0.815**	1	0.717*
BMI	-0.333*	-0.440**	0.458*	-0.427**	0.581**	0.717**	1

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level. BMI = Basal metabolic rate.

the accumulation of plasma iron and copper which can be involve in Fenton reaction.

This study shows a significantly lower concentration ($P < 0.001$) of magnesium level in the test subjects when compared with the control subjects. This finding correlates with the earlier studies (Purvis and Movahed, 1992). Magnesium is needed for the synthesis and secretion of insulin, it also enhance cells uptake of insulin and therefore keep up their insulin sensitivity. The reduced insulin levels in diabetes leads to hyperglycemia (Jerry and Nadler, 2000). Hypermagnesuria in diabetics have been attributed to osmotic diuresis. Glycosuria, which accompanies diabetic state, impairs renal tubular reabsorption of magnesium from the glomerular filtrate (Garland, 1992) and likely contributes to high frequency of hypomagnesaemia in poorly controlled diabetics (Nsonwu et al., 2006). Copper is an important trace metal for various metabolic enzymes. Its deficiency has been attributed to anemia and low level HDL-cholesterol (Sreedhar et al., 2004). Serum copper concentration in this study was found to be significantly higher in DM patients ($P < 0.001$) than in non diabetes. The finding correlates with that of Walter and his colleagues (1991) but at variance with that of Babalola et al. (2007). Diabetes has been shown to be associated with abnormalities in the metabolism of chromium and its impairment has been reported as aggregating factor in the progression of the disease. It is a cofactor in the action of insulin so it controls the action of insulin (Kimura, 1996). In this study, there was significantly higher concentration of chromium in non diabetic individuals than that in the diabetes patients ($P < 0.001$). This report corresponds to the finding of Morris et al. (1999). The body mass index (BMI) of the diabetics was found to be significantly higher ($P < 0.05$) than that of the non diabetes and has strong positive correlation with iron, copper, and glucose. Increased BMI has been shown to be associated with type 2 DM. The observation in this study correlates with that of Anjum and Arbab (2010). The study established that DM patients are often overweight (with BMI > 25) and this is independent of the socioeconomic status.

In conclusion, this study demonstrate significant reduc-

tion of the essential trace (zinc, selenium and magnesium) metals in type 2 DM patients and significant increase in copper, chromium, iron, glucose and BMI. These alterations may be responsible for different metabolic disturbances, oxidative stress and other complications associated with diabetes mellitus.

REFERENCES

- Akinloye O, Ogunleye K, Oguntibeju OO (2010). Cadmium, lead, arsenic and selenium levels in patients with type2 diabetes mellitus. *Af. J. Biotech* 9:(32) 5189-5195
- Anjum H, Arbab SS (2010). Comparison of body mass index and waist circumference in predicting incident diabetes. *Pak. J. Physiol.* 6:1-5
- Babalola OO, Ojo LO, Akinleye AO (2007). Status of the levels of lead and selected trace elements in type 2 diabetes mellitus patients in Abeokuta, Nigeria. *Afr. J. Biochem. Res.* 1(7):127-131
- Burt RK (2007). Antologus non myeloablative haematopoietic stem cell transplantation in newly diagnosed type 1 diabetic mellitus *JAMA* 297:14
- Diwan AG, Fradhan AB, Lingojar D (2006). Serum Zinc, Chromium and magnesium levels in Type 2 diabetes. *Int Diabetes Devel Countries* 26:122-123.
- Garland HO (1992). Magnesium deficiency in diabetes. *Magnes Res* 5:193-202
- Halliwel B, Gutteridge JMC (1991). Role of free radicals and catalytic metals ions in human disease: an over view. *Meth. Enzymol.* 186:81-85.
- Kaneka JJ (1990). *Chemical Biochemistry of animal.* 4th edition JJ Kaneko (ed) Acad Press Inc New York p 932.
- Kimura K (1996). Role of essential trace elements in the disturbance of carbohydrate metabolism. *Nippon Rinsho* 54:79-84.
- Kumar N, Butz JA, Burrit MF (2006). Clinical significance of the laboratory determination of low serum copper in adult. *Chin. chem. Lab. Med.* 45(10):1402-1410.
- Larsson SC, Wolk A (2007). Magnesium intake and risk of type 2 diabetes: a meta-analysis. *Intern Med.* 262: 208-214.
- Mooradian AD, Moley JE (1987). Micro nutrient status in diabetes mellitus. *Am. J. Clin.* 45:877-895.
- Morris BW, Mac MS, Hardistry TA, Hellter S, Grey TA (1999). Chromium homeostasis in patient with type II (NIDDM) diabetes. *Am. Clin. Nur.* 62:425-1532.
- Musad AG, Abd-Allah Y (2004). Evaluation of some biochemical changes in diabetic patients. *Clinical Climica. Acta.* 364:161-70
- Nakamura T, Higgashi A, Nisinyama S (1991). Kinetics of Zinc Status in children with IDDM. *Diabetic Care* 14:553-557.
- Nsonwu AC, Usoro CAC, Etukudo MH, Usoro IM (2006). Glycemic control and serum and urine levels of Zinc and Magnesium in diabetics in Calabar, Nigeria. *Pak. J. Nutri.* 6 (5):75-78.

- Purvis JR, Movahed A (1992). Magnesium disorder and cardiovascular disease. *Rev. Clin. Cardio.* 5:556-568.
- Retnam VJ, Bhandarkar SD (1981). Trace elements in diabetes mellitus. *J. Postgrad. Med.* 27:129.
- Schlienger JL, Grunenberger F, Maier EA (1988). Disorders of plasma trace elements in to blood glucose equilibrium. *Press e Med.* 17:1076-1079.
- Sreedhar B, Subra MR, Nair KM (2004). A protective role of zinc on intestinal peroxidative damage during oral iron repletion. *Biochem Biophys. Res. Commun.* 318:992-997.
- Sundararaman S, Vivian A, Muhammed GA, Surhir VS(2007) .The role of iron in diabetes and its complications. *Diabetes Care* 430 (7):1926-1933.
- Swaminathan S, Fonseca VA, Alam MG, Shah SV (2007). The role of iron in diabetes and its complications. *Diabetes Care* 30(12):1923-33.
- Walter RM, Unm-hard JY, Olim KL, Oster MH, Anawalt BD, Critchfield JW, Keen CL (1991). Copper, Zinc, Manganese and magnesium status and complications of diabetes mellitus. *Diabetes Care* 14(11) 1050-1056.