A Comparative Study on Serum Level Concentration of Micronutrients Like Zinc, Copper and Chromium Status in Type 2 Diabetic Patients in Diabetes & Endocrinology Unit, Tikur Anbessa Specialized Hospital, Ethiopia

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Abstract: *Background*: Diabetes mellitus (DM) is a chronic metabolic disorder, characterized by an absolute or relative deficiency of insulin and insulin resistance. Many studies have reported an association between diabetes and alterations in the metabolism of several micronutrients. In Ethiopia the study in the relationship between micronutrients (Zn, Cu and Cr) status and type 2 diabetes (T2DM) is scanty. The aim of this study was to assess and compare the concentration of the fasting serum zinc, copper and chromium status in T2DM and control subjects.

Method: A cross-sectional comparative study, conducted on 108 human subjects divided in to two groups: 54 subjects with the diagnosis of T2DM and the other 54 subjects were grouped as the control. After demographic and anthropometric information gathered, the blood sample was collected for the biochemical analysis. Fasting serum glucose was measured by glucose oxidase methods. The serum concentration of micronutrients namely zinc, copper and chromium were determined by using atomic absorption spectrophotometer. Data were analyzed using SPSS version 16 software.

Results: Compared with control groups, T2DM patients had greater BMI ($p<0.001$); higher WHR ($p<0.001$); elevated SBP ($p<0.001$); and higher diastolic DBP ($p<0.001$). Fasting serum glucose level of T2DM (196.4 ± 86.77mg/dl) was significantly higher than control (90 ± 14.39mg/dl). Mean serum levels of zinc in T2DM (0.744 ± 0.211mg/l) was significantly ($p<0.003$) lower than control (1.099 ± 0.502mg/l), chromium (0.679 ± 0.413 mg/l) was also significantly ($p<0.0001$) lower than control (1.064±0.483mg/l) and Cu (0.502±0.148mg/l) had significantly ($p<0.0001$) higher than control (0.340±0.137mg/l). In this study, the fasting serum glucose were found negatively correlated with serum levels of Zn ($r= -0.290$, $p=0.033$), and Cr ($r=-0.012$, $p<0.0001$) of diabetic subjects. Non-significant positive relationship was observed between concentrations of serum glucose and Cu($r = 0.438$, $p =0.113$).

Conclusion: Findings of this study indicated a lower serum zinc and chromium concentrations and higher copper status were found in type 2 diabetics of Ethiopian subjects. The study suggests that another research should be conducted in the effect of the supplementation of micronutrients on controlling of type 2 diabetic mellitus.

Keywords: Type 2 diabetic, zinc, copper, chromium, atomic absorption spectrophotometer.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder, characterized by absolute or relative deficiencies in insulin secretion or insulin action associated with chronic hyperglycemia [1]. Several pathogenic processes are involved in the development of diabetes mellitus. These range from an autoimmune destruction of the β-cells of the pancreas with a consequent absolute insulin deficiency to abnormalities that result in resistance to insulin action and insulin secretory defect [2]. Diabetes mellitus (DM) is a chronic disease characterized by chronic micro vascular complications such as retinopathy, neuropathy and nephropathy. Moreover, the risk for cardiovascular disease is considerably elevated in patients with type 2 diabetes mellitus compared to the general population [3].

Recently the WHO [1] delineated diabetes mellitus into categories including type 1, type 2, gestational and other specific types. Type 1 diabetes mellitus (T1DM) is typically a result of an autoimmune-mediated process in which pancreatic β-cells are destroyed resulting in little or absent insulin production and known as juvenile-onset or insulin-dependent DM. Type 2 diabetic mellitus (T2DM) is characterized by insulin resistance with relative insulin deficiency. It is accounts for 90-95 % of all diabetic cases and formerly called adult- onset diabetes or non-insulin dependent DM [3].

Incidence and prevalence of diabetes mellitus are rapidly increasing worldwide. The WHO estimated the

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number of adults with diabetes to increase from 366 million in 2011 to 550 million in 2030 [4]. Type 2 diabetes mellitus is now a common and serious global health problem, which, for most countries, has evolved in association with rapid cultural and social changes, increasing urbanization, dietary changes, reduced physical activity [3]. In Ethiopia, according to WHO estimated the number of adults with diabetes to increase from 1.4 million in 2011 to 2.6 million in 2030, with the prevalence predicted to increase from 3.5 % in 2011 to 3.8 % in 2030 [4].

Micronutrients are accepted as essential for optimum human health, because of their diverse metabolic characteristics and functions. They serve a variety of catalytic, structural and regulatory functions, in which they interact with macromolecules such as enzymes, pro-hormones and biological membranes. Chronic hyperglycemia may cause significant alterations in the status of some micronutrients and on the other hand, these nutrients can directly modulate glucose homeostasis [5]. Deficiencies of certain micronutrients such as zinc, copper and chromium have been shown to predispose a person to glucose intolerance and to promote the development of DM complications. In particular, diabetes has been shown to be associated with abnormalities in the metabolism of zinc, chromium and copper [6].

Zinc serves an essential role as a cofactor for more than 200 metal enzymes and regulates the metabolism of carbohydrates, lipids and proteins. It is required for insulin synthesis and storage and insulin is secreted as zinc crystals, it maintains the structural integrity of insulin [7]. Zinc is also considered as an integral component of Cu-Zn SOD, which acts as anti-oxidant and protects from free radicals [8]. A number of studies have reported correlation between diabetes and zinc [9].

Copper is the third most abundant essential trace mineral in the body and has an important role in the body, being component of two of our most important anti-oxidant enzymes; Cu-Zn SOD and ceruloplasmin [10]. Both increased and decreased copper levels were found in diabetic patients [11].

Chromium is an essential micronutrient required for normal carbohydrate and lipid metabolism. It enhances the insulin binding by increasing the number of insulin receptors and activation of insulin receptor tyrosine kinase, thus leading to increased insulin sensitivity and facilitates action of insulin [12]. Chromium deficiency results in glucose intolerance and insulin resistance [13].

In view of these facts, it is important to determine the micronutrient concentrations in serum samples of type 2 diabetes patients to monitor and assess their impact on human health. Although the status of serum micronutrients like zinc, copper and chromium concentrations in T2DM were studied for population in several countries, there was no reported data for Ethiopia. Therefore, the aim of the present study was to assess the serum micronutrients levels of zinc copper and chromium concentration status in type 2 diabetic patients attending a diabetic clinic at the Tikur Anbesssa Specialized Hospital.

MATERIALS AND METHODS

Patients and Study Design

Type 2 diabetic patients attending the diabetic clinic of the Tikur Anbessa Specialized Hospital and healthy non-diabetic subjects who selected from postgraduate students and staffs of the faculty were included in the study control. Informed consent was obtained from subjects before recruitment into the study. The ethical committee of departments of Medical Biochemistry and Internal Medicine has approved the study protocol. The inclusion criteria for the study group of age from 20-76 years at the time of the study and type 2 diabetic patients for the past two years. Inclusion criteria for the control group of age were 20-76 years old healthy subjects included in the study. The exclusion criteria included the pregnant or lactating women and subjects who had other chronic disease in both groups, which would interfere with the blood analysis and interpretation of the data.

Selection of Study Populations

A total of 108 study participants, 54 type 2 diabetes patients' subjects taking as study group and 54 taking as control were recruited to participate in the study. All of them were willing to respond the investigator based administered questionnaires, give the required volume of blood and signed the consent form**.**

Body weight and height, waist and hip circumferences were measured and used to calculate the BMI and WHR respectively which were used as a measure of relative body weight/or central obesity. Blood pressures of subjects were taken prior to sample collection. A structured questionnaire was used to obtain the data on occupation, physical activity, lifestyle pattern as smoking and alcohol consumption, past and present illness and medication.

Sample Collection

About 5ml of venous blood samples were collected aseptically from overnight fasting type 2 diabetes patients by a vaccutainer system in SS^{TM} metal free test tube. The specimen was allowed to clot for 30 minutes. The serum separated by centrifuging at 3000 rpm for 10 minutes. Then after, serum samples were aliquot into nunc tubes aseptically by using a micropipette with disposable sterile blue tips. Finally, serum glucose was analysized immediately and the aliquots were stored at -70 C^0 until the time of serum zinc, copper and chromium determination.

Methods

Fasting serum glucose was determined using the glucose oxidase method (photometer 5010, Robert Reile GmbH and Co KG, Berlin, Germany) and serum zinc, copper and chromium levels were determined using the atomic absorption spectrophotometer (AA Analyst 600 USA model) with graphite furnace using argon gas.

Statistical Analysis

The significance of difference between the groups was assessed by student's t-test analysis. The interrelationship between serum levels of glucose with serum levels of zinc, copper and chromium in type 2 diabetic and control subjects were tested by using Pearson's correlation. P value <0.05 was considered statistically significant.

RESULTS

Basic Clinical Characteristic of the Study Populations

A total of 108 study participants, 54 type 2 diabetes patients' subjects taking as study group and 54 taking as control were recruited to participate in the study. All of them were willing to respond the investigator based administered questionnaires, give the required volume of blood and signed the consent form. The age, body mass index, waist circumference, waist to hip ratio, systolic blood pressure and diastolic blood pressure are shown in Table **1**. The data presented are mean ± SD and P –value of the unpaired t-test.

Table **1** shows basic clinical characteristic values of type 2 diabetes and non-diabetic control subjects. The mean age of the T2DM patients from Table **1** was 55.8 ±12.1 years where as the non-diabetic subjects was 45±9.1 years in respectively. Furthermore, the age difference between the two groups were highly statistically significant (p<0.001). The mean value of BMI among the T2DM patients was 25.41±3.93 kg/m^2 that was higher than that observed among the non diabetic control group (21.19 ± 3.08 kg/m^2). There was a significant difference at (p< 0.001) in the BMI of T2DM subjects when compared to non-diabetic control subjects. As the results indicated that, a higher proportion of subjects in diabetes patients 21 (38.9 %) was found to be overweight when compared to the control subjects 9(16.7%) and 10(18.5 %) of T2DM patients was obese, while none of the non- diabetic control group was found in the obese cutoff values.

As the mean value of waist to hip of T2DM patients were found to be 0.90 ± 0.107 which was greater than that of (0.85 ± 0.26) of the control subjects according to Table **1**. As mentioned above the mean difference between the two groups were statistically significant at (P< 0.001). The mean value of systolic blood pressure (SBP) in T2DM patients was (132 ± 25.08 mmHg) whereas a control group was (120 ± 2.7mmHg). As a mean value indicated above Table **1** the difference between T2DM and control groups had statistically significant at (p <0.001). The mean value of diastolic blood pressure (DBP) of T2DM patients was (84 ± 6.2mmHg), which was a higher than control groups (80 ± 7.83mmHg), there was statistically significant at (p<0.0001).

Table 1: Basic Clinical Characteristic Values of Type 2 Diabetes and Non-Diabetic Control Subjects

Parameters	Type 2 diabetes patients (n=54)	Non-diabetic subjects (n=54)	P-value< (unpaired t-test)
Age (years)	55.81±12.12	45±9.1	0.001
BMI (kg/m^2)	25.4±3.93	21.2±3.1	0.001
WHR	0.902±0.108	0.85±0.03	0.001
SBP (mmHg)	132 ± 25.08	120 ± 2.7	0.001
DBP (mmHg)	84±6.20	80.0 ±7.83	0.0001

Data presented as mean ± standard deviation, BMI-body mass index, WHR-waist-hip ratio, SBP-systolic blood pressure, DBP-diastolic blood pressure, **p<0.0001.

Table 2: Biochemical Variables in the T2DM Patients and Control Subjects

Biochemical Variables	T2DM patients (n=54)	control subjects (n=54)	P-value< (unpaired t-test)
FSG (mg/dL)	196.4±86.8	89.74±15	0.0001
Zinc (mg/l)	0.744 ± 0.211	1.099 ± 0.502	0.0001
Copper (mg/l)	0.526 ± 0.148	0.343 ± 0.137	0.0001
Chromium (mg/l)	0.731± 0.504	1.059 ± 0.545	0.0001

Data presented as mean ±standard deviation, FSG-fasting serum glucose, **P<0.0001.

The Biochemical Variables of The Study Populations

Table **2** shows the serum concentrations of glucose, zinc, copper and chromium in type 2 diabetes patients and healthy control subjects. The data presented are mean ± SD and P –value of the unpaired t-test.

Table **2** indicates biochemical variables in the T2DM patients and control subjects. The mean values of fasting serum glucose in the T2DM patients was elevated (196.4 ± 86.77mg/dl) when compared to control subjects (90.01 ± 14.39mg/dl). The mean difference was highly significant at (p < 0.0001). In T2DM patients, significantly lower (P<0.0001) serum levels of zinc has been found as compared to control subjects. T2DM subjects had significantly lower serum concentrations of Zn (0.744 ± 0.211mg/l), as compared with the control subjects (1.099 ± 0.502mg/l). The mean fasting serum level concentration of copper in T2DM (0.526 ± 0.148 mg/l) and control subjects (0.343 ± 0.137 mg/l). In T2DM patients, significantly higher (P<0.0001) serum levels of copper was noticed as compared to control subjects. The results shown from the Table **2**, the serum concentration level of chromium in T2DM patients was (0.467 ± 0.195mg/l) that was lower than control subjects (1.064±0.483mg/l). There was statistically highly significant difference between the two groups at (p <0.0001).

Table **3** shows the relationship of gender with the serum micronutrient concentration in T2DM and non-diabetic control subjects. The serum zinc and chromium levels were found higher in diabetic females than diabetic males and serum copper level was higher diabetic males than diabetic females. However, the difference was not statistically significant (P >0.05). There were also no significant difference observed in the serum levels of zinc, copper and chromium of both females and males of the non-diabetic subjects (P >0.05).

Figure **1** shows correlation of fasting serum glucose with serum zinc, copper and chromium concentration in T2DM patients. As the correlation curves for zinc, copper and chromium with fasting serum glucose levels shown that, the fasting serum glucose levels negatively correlated with the serum concentrations of Zn(r=-0.290, p=0.033), and Cr(r= -0.102, p< 0.0001). And a non- significant positive relationship (P >0.05) has been found between fasting serum glucose and copper concentrations in T2DM patients (r = 0.218, P = 0.113).

Table 3: Relationship of Gender with the Serum Zinc, Copper and Chromium Concentration in Type 2 Diabetes Mellitus and Non-Diabetic Subjects

Subjects	Serum zinc	Serum copper	Serum chromium
Type 2 Diabetic males n=27	0.733 ± 0.211	0.517± 0.172	0.443 ± 0.178
Type 2 Diabetic females n=27	0.756 ± 0.214	0.545 ± 0.119	0.494 ± 0.215
p-value	0.690	0.499	0.344
Non-Diabetic males n=32	0.972 ± 0.485	0.346 ± 0.160	1.016 ± 0.515
Non-Diabetic females n=22	1.223 ± 0.497	0.338 ± 0.108	1.120 ± 0.448
p-value	0.069	0.836	0.429

Data Presented as Mean ±Standard Deviation, *p<0.05.

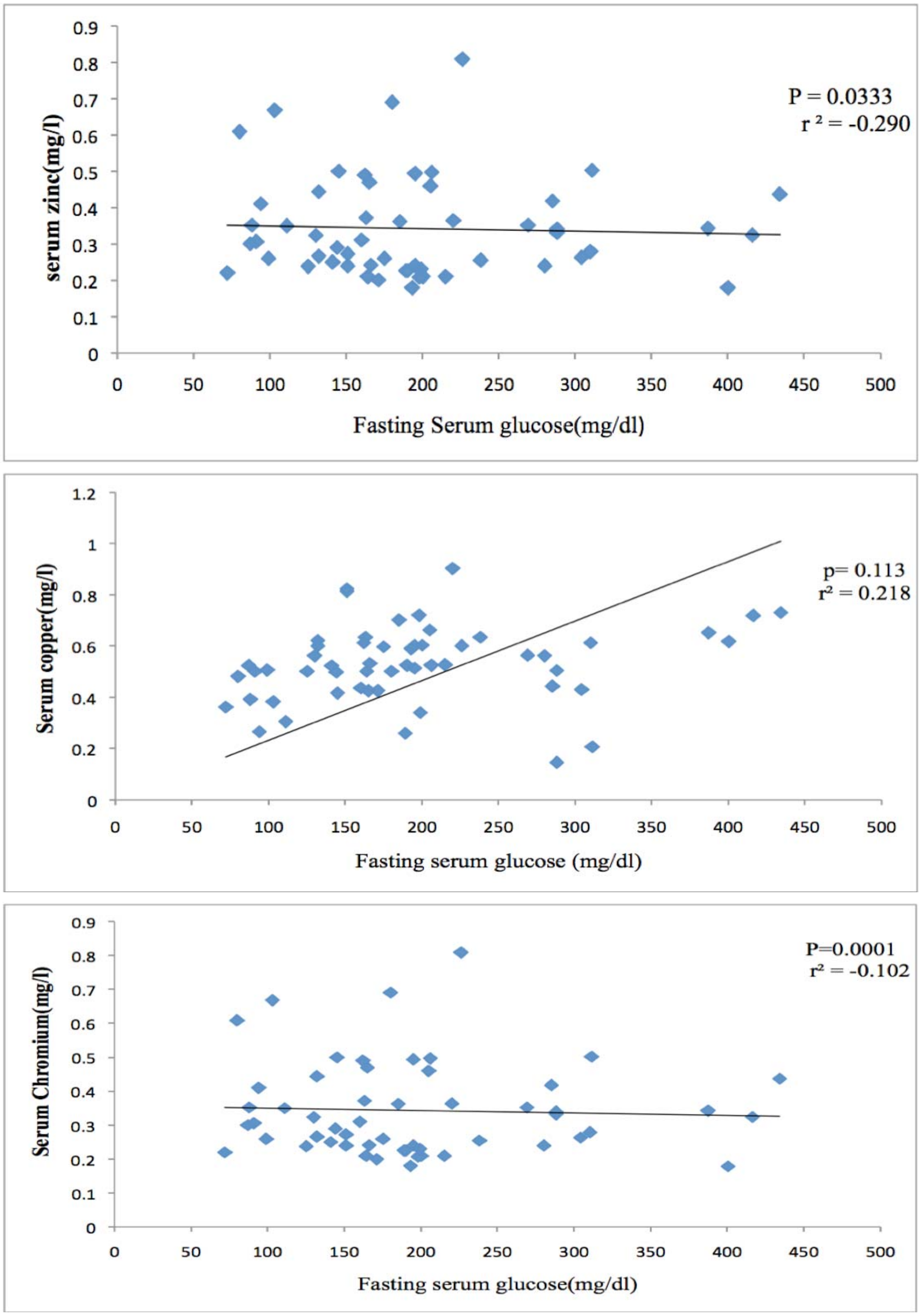

Figure 1: Correlation of fasting serum glucose with serum zinc, copper and chromium concentration in type 2 diabetic patients.

Tables **4** and **5** shows the correlation between serum zinc, copper and chromium SBP and DBP respectively. The above serum micronutrients negatively associated with SBP of T2DM patients Zn(r= -0.143), Cu(r= -0.209), Cr(r= -0.081). However, there was not a significant association of serum zinc, copper and chromium with SBP of T2DM patients (P > 0.05). The serum zinc and chromium levels of the T2DM subjects were negatively correlated and serum copper was positively correlated with DBP of T2DM groups, but the association of these micronutrients with DBP in T2DM subjects had not statistically significant (P> 0.05).

DISCUSSION

Micronutrients have been identified for a long time as potential candidates for improving metabolic disorders like pre-diabetes (insulin resistance, obesity, metabolic syndrome) or diabetes [5]. For example, Zn is an essential mineral that is directly involved in the

Table 4: Correlation between Systolic Blood Pressure with Serum Zinc, Copper and Chromium in Type 2 Diabetes Mellitus Subjects

Systolic blood pressure of T2DM	Correlation Coefficient(r)	Serum zinc	Serum copper	Serum chromium
		- 0.143	-0.209	- 0.081
	p-value	0.301	0.130	0.523

Data presented as mean ±standard deviation, *p<0.05.

Table 5: Correlation between Diastolic Blood Pressure with Serum Zinc, Copper and Chromium in the Type 2 Diabetic Subjects

Diastolic blood pressure of T2DM	Correlation Coefficient(r)	Serum zinc	Serum copper	Serum chromium
		-0.125	0.116	-0.091
	p-value	0.367	0.404	0.515

Data presented as mean ±standard deviation, *p<0.05.

synthesis, storage and secretion of insulin, as well as antioxidant properties [14]. Copper is also an essential mineral present in the body combined with enzymes to form metallo-enzymes such as ceruloplasmin, SOD, these enzymes play major role in redox-reactions, such as superoxide dismutase which plays key role in antioxidant defense. It has been postulated that copper possesses insulin like activity and promotes lipogenesis [15]. The chromium increases insulin binding to cells by increasing insulin receptors on membrane or activation of insulin receptor signaling and it may lead to increased insulin sensitivity, glucose utilization and β-cell activity [16].

In prospective studies patients with type 2 diabetes mellitus, demonstrate that diabetic patients have abnormal metabolism of serum zinc [14], copper [15] and chromium concentrations [17]. In the present study, fasting serum glucose level was significantly higher in type 2 diabetic patients than non diabetic control groups. It has now been established by different studies [18] that diabetic patients have significantly higher levels of fasting serum glucose than those of the non- diabetic control groups.

In this study no significant (P >0.05) gender related differences in serum zinc, copper and chromium levels were found in the type 2 diabetic subjects of the study with the females having higher serum levels of zinc and chromium than the males and serum copper level was higher in diabetic males than diabetic females, this study also agree with other studies [19]. The gender related differences in micronutrient levels in diabetics might be attributed to hormonal imbalance with the diabetic state [19]. There was no significant difference observed in the serum levels of zinc, copper and chromium of both females and males of the non-diabetic subjects (P >0.05).

In the current study, the serum zinc level of type 2 diabetes mellitus patients is significantly (P <0.0001) lower than non-diabetic control groups. This has been proposed that the decreased serum zinc level is due to its complex formation with insulin and hence the lack of free insulin enhances the appearance of the symptoms of diabetes that occurred in these patients [13]. According to [20], Zinc is involved in many biological processes including catalysis, regulation of gene expression, influences many metabolic functions and has insulin-like effects that enhance glucose uptake by inhibiting glycogen synthesis.

The low serum level concentration of zinc in type 2 diabetic patients is in accordance with those reported by several studies [21]. Lower gastrointestinal absorption and high urinary excretion of Zinc in diabetic patients may explain hypozincemia seen in the diabetic mellitus patients [20]. Hyperglycemia has been postulated to interfere with the active transport of zinc back into the tubular cells [20]. However, other studies failed to find significant differences with non-diabetic control groups [22]. In correlation analysis, our study showed that there has been a significant (P<0.017) negative correlation was found between serum zinc concentration and fasting serum glucose in the type 2 diabetic patients. A similar negative association result was also observed by another study [23].

Copper is the most abundant essential micronutrients in the body. It is present in the body combined with enzymes to form metalloenzymes such as ceruloplasmin and Cu/Zn-SOD, known as oxidant defense enzymes [24]. Copper also possesses insulin–like activity and promotes lipogenesis [25]. Human studies demonstrate that diabetic patients may have abnormal levels of serum copper [26].The current study also revealed that serum copper concentration levels was significantly ($p<0.0001$) increased in type 2 diabetes patients compared to non-diabetic control subjects. The increased levels of copper in type 2 diabetic patients compared with normal human subjects agree with other studies [26]. In this study, an association was established between the serum copper and fasting serum glucose. There was no significant ($P > 0.05$) positive correlation between fasting serum glucose and serum copper concentrations have been found. A similar positive correlation results were also observed [15].

The present study indicated that there was a significant ($P<0.0001$) decrease in serum concentrations of chromium in type 2 diabetic patients than control groups. This decreased serum chromium levels in type 2 diabetic patients than controls has been well described in earlier similar studies [27]. Chromium is a cofactor in the action of insulin and it potentiates the action of insulin [21]. One study has been reported that administration of 500mg chromium two times per day for 2 months resulted in a significant improvement of glycosylated hemoglobin (HbAlc) values and has a positive effect on serum glucose levels [12]. Our study that demonstrated highly significant ($P<0.0001$) negative correlation of fasting serum glucose with chromium levels in type 2 diabetic patients. Similar associations between fasting serum glucose and serum chromium have been reported by different studies [23].

Blood pressure is the force of the blood pushing against the walls of the arteries [15]. Minerals regulates blood pressure; any imbalances of dietary intake of these minerals namely Zn and Cu will affect the blood pressure and lead to the development of hypertension and vascular disease [28].

Zinc acts as components of Cu-Zn SOD, which is essential for the strength and the integrity of the heart and blood vessels, so decrease the amount of zinc will lead to decrease in the SOD activity, which leads to impaired heart functions [29]. Copper is essential both for its role in the antioxidant enzyme, like Cu-Zn SOD and ceruloplasmin, which are essential for the strength and the integrity of the heart and blood vessels [30]. In this current study, there is no significant ($p>0.05$) negative association between systolic blood pressure with serum zinc and chromium have been found in type 2 diabetic subjects. While serum zinc and chromium negatively but serum copper had non-positively significant ($p>0.05$) correlated with diastolic blood pressure in type 2 diabetic subjects.

CONCLUSION

From our findings, we conclude that the serum concentration of zinc and chromium were significantly lower in patients with T2DM when compared to control groups, whereas serum copper concentration was significantly higher in the T2DM groups than in the control groups. This current study indicated that there was a significant negative correlation between the serum level concentration of zinc and chromium and the fasting serum levels of glucose in the T2DM groups. A non-significant positive correlation was found between fasting serum glucose and serum copper concentration in T2DM subjects. There was no association of serum level concentration of these micronutrients with gender and blood pressure in T2DM patients. Therefore, lower serum zinc and chromium concentrations and higher copper status are found in type 2 diabetics of Ethiopian subjects, which may affect their diabetic control. The study suggests that another research should be conducted in the effect of the supplementation of micronutrients on controlling of T2DM.

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REFERENCES

[1] Whitney EN. Nutrition for health and health care. Wadsworth, Belmont, USA 2011: 76.

[2] Wiernsperger NF. Oxidative stress as a therapeutic target in diabetes: revisiting the controversy. Diabetes Metab 2003; 29: 579-585. http://dx.doi.org/10.1016/S1262-3636(07)70072-1

[3] American Diabetes Association. Diabetes care, volume 35, Supplement January 2012.

[4] Klevay LM. Cardiovascular disease from copper deficiency - a history. J Nutr 2003; 130: 489S-492S.

[5] Zargar AH, Bashir MI, Masoodi SR, *et al*. Copper, zinc and magnesium levels in type- 1 diabetes mellitus. Saudi Med J 2002; 23: 539-542.

[6] Chinyere NA, Opara UCA, Henrieta EM, Nathanie UI. Serum and urine levels of Cr and Mg in type 2 diabetics in Calabar, Nigeria. Mal J Nutr 2005; 11: 133-242.

[7] Chen MD, Lin PY, Tsou CT, Wang JJ, Lin WH. Selected metals status in patients with noninsulin-dependent diabetes mellitus. Biol Trace Elem Res 1995; 50: 119-124. http://dx.doi.org/10.1007/BF02789414

[8] Syed M. Farid. Serum trace elements in type II diabetes. Medical Journal of Islamic World Academy of Sciences 2012; 20(4): 130-139.

[9] Nsonwu AC, Usoro CAO, Etukudo MH, Usoro IN. Influence of age, gender and duration of diabetes on serum and urine levelsof zinc, magnesium, selenium and chromium in type-2 diabetics in Calabar, Nigeria. Turk J Biochem 2006; 31: 107-14.

[10] Serdar MA, Bakir F, Hasimi A, Celik T, Akin O, Kenar L, *et al*. Trace and toxic element patterns in nonsmoker patients with noninsulin-dependent diabetes mellitus, impaired glucose tolerance, and fasting glucose. Int J Diabetes Dev Ctries 2009; 29: 35-40. http://dx.doi.org/10.4103/0973-3930.50713

[11] Joshi YK. Basics of clinical nutrition. Jaypee. New Delhi, India 2003; 130-132.

[12] Anderson RA, Cheng N, Bryden NA, *et al*. Elevated intakes of supplemental chromium improve glucose and insulin variables with type 2 diabetes. Diabetes 2004; 46: 1786. http://dx.doi.org/10.2337/diab.46.11.1786

[13] Chausmer AB. Zinc, Insulin and diabetes. J Am Coll Nutr 1998; 17: 109-114. http://dx.doi.org/10.1080/07315724.1998.10718735

[14] International Diabetes Federation (WHO). IDF Diabetes Atlas, International Diabetes Federation, Brussels, Belgium 2011.

[15] Yahya H, Yahya KM, Saqib A. Minerals and type 2 diabetes mellitus level of zinc,magnesium and chromium in diabetic and non diabetic population. Jumdc 2011; 2(1): 34-35.

[16] Quilliot D, Dousset B, Guerci B, Dubois F, Drouin P, Ziegler O. Evidence that diabetes mellitus favors impaired metabolism of zinc, copper, and selenium in chronic pancreatitis. Pancreas Apr 2001; 22(3): 299-306. http://dx.doi.org/10.1097/00006676-200104000-00012

[17] World Health Organization Report (WHO). Diagnosis and classification of diabetes mellitus 1999.

[18] Olaniyan OO, Osadolor HB, Felicia AA, Abisoye AI. Low serum chromium in Nigerian Type 2 diabetes. Tanzania Journal of Natural and Applied Sciences 2011; 2(2): 367-372.

[19] Olaniyan MAM, Awonuga AF, Ajetunmobi IA, Adeleke OJ, Fagbolade KO, Olabiyi B, Oyekanmi A, Osadolor HB. Serum copper and zinc levels in Nigerian type 2 diabetic patients. African Journal of Diabetes Medicine 2012; 20: 36-38.

[20] EL Zebda GA. Significance of serum levels of copper and zinc in Type II diabetic hypertensive, and diabetic hypertensive patients in Gaza City 2006; 1427: 2-31.

[21] Becker KL, Bilezikian JP, Humg W, Robertson GL, Wartofasky L. In principle of endocrinology & metabolism, J.B. lippincott company 1990.

[22] Tan LK, Chua KS, Toh AK. Serum magnesium, copper and zinc concentration in acute myocardial infarction. Circulation 1992; 86: 803. http://dx.doi.org/10.1002/jcla.1860060513

[23] Rathore S, Gupta A, Batra HS, Rathore R. Comparative study of trace elements and serum ceruloplasmin level in normal and pre-eclamptic pregnancies with their cord blood. Biomedical Research 2011; 22(2): 207-210.

[24] Singh RB, Niaz MA, Rastogi SS, Bajaj S, Gaoli Z, Shoumin Z. Current zinc intake and risk of diabetes and coronary artery disease and factors associated with insulin resistance in rural and urban populations of North India. J Am Coll Nutr 1998; 17: 564-570. http://dx.doi.org/10.1080/07315724.1998.10718804

[25] Rukgauer M, Neugebauer RJ, Plecko T. The relation between selenium, zinc and copper concentration and the trace element dependent ant oxidative status. J Trace Elem Med Biol 2001; 15: 73-78. http://dx.doi.org/10.1016/S0946-672X(01)80046-8

[26] de Fatima Campos PL, Franciscato CSM. Metabolic and functional alterations of copper in diabetes mellitus. Brazilian J Nutr 1999; 12(3): 213-224.

[27] Adewumi MT, Njoku CH, Saidu Y, Abubakar MK, Shehu RA, Bilbis LS. Serum Cr, Cu, and Mn levels of diabetic subjects in Katsina, Nigeria. Asian J Biochem 2007; 2: 284-288. http://dx.doi.org/10.3923/ajb.2007.284.288

[28] Mosaad A, Abou-Seif G, Abd-Allah Y. Evaluation of some biochemical changes, in diabetic patients 2004.

[29] Mumayun M, Khalid A, Ali A, Ahmed S, Javed A. To study levels of serum Cr, Cu, Mg and Zn in patients with diabetes mellitus type 2. Pak J Med Health Sci 2011; 5: 34-38.

[30] Behets GJ, Dams G, Damment S, Debwe ME. An assessment for the effects of lanthanum on bone in a chronic renal failure (CRF) rat model. J Am Soc Nephrol 2001; 12: 740-750.

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