Serum levels of selected trace metals and their association with body mass index and c-reactive protein in type 2 diabetes mellitus patients in Oredo Local Government Area, Benin City

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Abstract

Background: Diabetes is a major worldwide health problem, characterized by chronic hyper glycaemia resulting from diverse aetiologies. It is associated with increased metabolic processes and oxidative stress. There is accumulating evidence that the metabolism of several trace elements is altered in type 2 diabetic patients.

To determine serum levels of copper, manganese, selenium and chromium in type 2 diabetes mellitus patients, and their association with body mass index and C Reactive protein.

Methods: Forty six type 2 diabetic subjects, both male and female, in the age range 38 to 83 years and 23 age matched controls were involved in the study. Fasting plasma glucose, glycated haemoglobin, trace elements (copper, chromium selenium, manganese), body mass index, C Reactive protein levels were determined.

Results: Fasting plasma glucose, glycated haemoglobin, , C reactive protein levels were significantly elevated in the diabetics (7.9 ± 0.4 mmol/L, 8.3 ± 2.2, 155.4 ± 29.9mg/L) than in the controls (4.5 ± 1.1mmol/L, 4.5 ± 1.0%, , 10.3 ± 2mg/L) P<0.05. There was no statistically significant difference between the body mass index in the diabetics (27.6 ± 5.8 kg/m^2) and the controls (23.2 ± 1.0 kg/m^2) p > 0.05. The mean serum levels of manganese and selenium were significantly lower in the diabetic subjects (122.3 ± 44.7 µmol L, 244.9 ± 15.8µmol /L) than in the controls (170.9 ± 39.4 µmol /L, 353.4 ± 10.9 µmol /L) respectively P<0.05. Mean serum copper and chromium levels were significantly higher in the diabetic subjects (242.5 ±68.0 mmol, 9 ± 1.2µmol/L) than in the controls (198.3 ± 22.0 µmol /L, 0.0 µmol/L) p < 0.05. Serum copper showed a significant positive correlation with body mass index, r = 0.440, p <0.05, and showed a weak positive correlation with C Reactive protein which was not significant.Serum chromium, showed a non significant positive correlation with body mass index and C Reactive protein.

Conclusion:Decreased serum levels of selenium and manganeese in type 2 diabetes mellitus may be suggestive of the possible roles deficiency of these micronutrients play in aietio pathogenesis of type 2 diabetes mellitus. The significantly elevated serum copper levels and its positive correlation with body mass index, is suggestive that elevated copper levels may play a role in aietio pathogenesis of type 2 diabetes mellitus and the mechanism by which it does this could be multiple.

Key words: Type 2 diabetes mellitus, Trace metals, Body mass index, C-reactive protein

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Introduction:

Diabetes mellitus is a metabolic syndrome of heterogenous aetiology, with hyperglycemia as a common denominator, due to absolute or relative insulin deficiency[1].

Trace elements are essential nutrients with resulting immunologic, antioxidant functions resulting from their action as essential components or cofactors of enzymes throughout metabolism.[2] Trace elements and minerals influence the pathogenesis of obesity and diabetes and their complications, mainly through their involvement in peroxidation and inflammation[3]. Disturbances in trace element status and increased oxidative stress in diabetes, may contribute to insulin resistance and the development of diabetes and diabetic complications.[1,4] Trace elements like chromium, selenium, magnesium, molybdenum, zinc play essential role in insulin action[5].

Copper is an essential mineral, needed for several biological functions. It is required for catalytic activity of superoxide dismutate that participates in the protection of cells from superoxide radicals[6]. Disturbances in copper levels in various biofluids and tissues are associated with abnormalities implicated in metabolic pathway of diabetes and it's complications[7].

Manganese acts as a cofactor in several enzymes including those involved in bone marrow production and metabolism of carbohydrates, proteins, fats[7,8].

The biological activities of chromium depends on its valence state and the chemical complexes it forms[9]. Trivalent form of chromium has high biological activity which is required for optimal glucose uptake by cells[10].

Selenium is involved in the complex system of defense against oxidative stress through selenium dependent perioxidases and other selenoproteins. Due to it's antioxidant properties, selenium might be preventing the development of diabetes. In addition, selenate an

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inorganic form of selenium, mimics insulin activity in experimental models.

Several studies[11,12,13] have reported different findings on trace metal status in diabetics. While some have reported low zinc, manganese, selenium and high copper levels[12] in type 2 diabetics, others have reported high zinc levels[13] and in some cases, no significant difference in serum levels of these trace metals[11]. Some studies have also described association between some trace metals and body mass index and C Reactive protein[14,15].

In this study, serum levels of copper, manganese, chromium, and selenium was determined in type 2 diabetics and their association with body mass index and C Reactive protein evaluated.

Materials and methods

The study was a cross sectional study, carried out in the state specialist hospital, Benin City, Edo State, Nigeria, between September 2012 and April 2013.

Consent for the study was obtained from the ethical committee of the hospital and informed consent obtained from the subjects and controls.

Forty six type 2 diabetic patients, both male and female were recruited for the study. They were already diagnosed as type 2 diabetic patients and attending the outpatient endocrine clinic. They were recruited consecutively at presentation, after obtaining their informed consent.

Structured questionnaires were used to obtain vital information from the patients, which included duration of disease, type of medications they are on, history suggestive of complications. General physical examination was performed on the patients and parameters such as height and weight were measured with a stadiometer and weighing scale respectively. Body mass index was calculated using the formula weight(kg)/height (m^2). Blood pressure was measured with a sphygmomanometer. Twenty-three age and gender matched controls were recruited for the study from the same Local Government Area as the subjects. They were not known diabetics.

Inclusion-criteria – type 2 diabetes mellitus subjects.

Exclusion criteria – History of smoking, history suggestive of immunologic diseases or malignancies.

Sample collection

Ten ml of fasting venous blood was collected from the patients in the morning, and dispensed in plain bottles for serum manganese, selenium, copper, chromium, and C-Reactive protein estimation, flouride oxalate bottle for glucose assay, ethylene diamine tetracetate bottle for glycated haemoglobin assay.

Samples in EDTA bottle were refrigerated immediately at 4^0c, while that in the flouride oxalate bottle was centrifuged at 3000 rpm for 5 minutes and plasma separated into clean plain tubes and stored in an ultradfrizer at -80^0c until time of analysis.

Samples in plain bottle was allowed to clot and centrifuged for 5 minutes, serum separated and dispensed in clean plain tubes, refrigerated in an ultradfrizer until time of analysis.

Biochemical Assay

Plasma glucose was assayed by the glucose oxidase method. C-Reactive protein by the immuno-turbidometric method, Glycated haemoglobin by Boronate Affinity method (Bio Rad). Serum levels of copper, maganeese, selenium and chromium was estimated using atomic absorption spectrophotometry.

Results

A total of 46 subjects and 23 age and gender matched controls participated in the study. The subjects have been diabetic for a mean duration of 5 years and 3 months. Forty percent of the controls were males and 60% females, with a mean age 56.1±1 years, while 38.1% of the subjects were males and 61.9% females, with a mean age of 58.3±1.2 years. (Table 1)

Table 1: Demographic and Biochemical Parameters of Subjects and Control

Parameters	Diabetic	Control	Value
Age (years)	58.6 ± 11.7	56.1 ± 1.1	0.32
Male (%)	38.1	40	
Female (%)	61.9	60	
Diabetes duration			
<5 years	(53.3)		
>5 years	(36.7)		
Body mass index (kg/m2)	27.6 ± 5.8	23.2 ± 1	.06
Fasting plasma glucose (mmol/L)	7.9 ± 0.4	4.5 ± 1.1	0.001
Hb A1c (%)	8.3 ± 2.2	4.5 ± 1	<0.001
C Reactive Protein (mg/l)	155.4 ± 29.8	10.3 ± 2.0	.005
Copper (μmol/L)	242.5 ± 68.0	198.3±22.0	0.05
Maganeese (μmol/L)	122.3 ± 44.7	170 ± 39.4	.004
Chromium (μmol/L)	9 ± 1.2	.0±	0.02
Selenium	244.9 ± 15.8	353.4 ±10.9	0.052

Mean fasting plasma glucose and glycated haemoglobin was significantly higher in the diabetics (7.9±0.4 mmol/l 8.3±2.2%) than in the controls (4.5±1.1 mmol/l, 4.5±1.0%) p=0.001, p=<0.001 respectively. Mean and C Reactive protein was significantly higher in the diabetics (155.4±29.9 mg/L) than in the controls (10.3±2.0 mg/l) p=0.005. (Table 1) Mean body mass index was 27.6 ± 5.8 kg/m^2 in the diabetics and 23.2± 1.0 kg/m^2 in the controls. Difference was not statistically significant p = 0.06.

Mean serum manganese, and selenium were significantly lower in the diabetic subjects (122.3±44.7 μmol/l, 244.9±15.8 μmol/l) than in the controls(170 ±39.4μmol/l,353.4±10.9 μmol/l) P=0.004,p=0.05 respectively.) (Table 1).

Table 2: Correlation between Body Mass Index and Copper, Maganeese, Selenium Chromium in the diabetic subjects

	R	P
copper	0.440	0.01*
Selenium	0.196	0.309
Chromium	0.310	0.102
Mageneese	0.100	0.302

*Copper showed a statistically significant correlation with body mass index

Serum copper and chromium levels were significantly higher in the diabetics (242.5 ± 68.0 mmol/L, 9 ± 1.2 μmol) than the controls (198.3±22.0 μmol/L, 0.0 чmol/L) p=0.05, p=0.02 respectively (Table1).

Table 3: Correlation between C Reactive Protein and Copper, Selenium, Chromium, Mageneese in the diabetic subjects.

	R	p
copper	0.059	0.758
Selenium	0.122	0.520
Chromium	0.087	0.647
Mageneese	0.195	0.302

Serum copper showed a statistically significant positive correlation with body mass index in the subjects r=0.440, p=0.01 (Table2). It showed a weak positive correlation with C-reactive protein which was not statistically significant r= 0.059, p=0.758. Serum maganese, Chromium and Selenium did not show any statistically significant correlation with body mass index and C-Reactive protein.

Body mass index correlated positively with C-reactive protein in the subjects but was not statistically significant (r=0.401, p=0.071).

Discussion:

Prevalence of diabetes mellitus is on the increase worldwide, type 2 diabetes mellitus being the commonest. Altered trace metal metabolism has been reported in various studies worldwide[7,11,12,13].

In this study, mean serum level of manganese and selenium were significantly lower in the diabetic subjects than the controls. Similar findings was reported by Onah CE et al[15] in a male type 2 diabetic population in Nnewi South East Nigeria. Other studies[16,17] in different parts of the world have also reported lower levels of manganese and selenium in type 2 diabetics. These metals play essential roles in insulin action. These findings support the need to look into micronutrient supplementation in type 2 diabetic patients.

Serum chromium levels was significantly elevated in the subjects. In this study, though other studies reported decreased levels[18,19].

Mean serum copper was significantly elevated in the diabetic subjects. Several studies have reported significantly elevated levels of serum copper in type 2 diabetics. Olaniyan O[20] and co-authors reported serum copper levels to be significantly elevated in their study in a type 2 diabetic population in Nigeria. Saha Roy[21] and co-authors reported similar findings amongst type 2 diabetic patients in India and Dosa MD and co-authors[22] in Romania. Tanaka Ayoko et al[23] in their study highlighted, that production of reactive oxygen species (ROS) is facilitated in the presence of copper ions, and that ROS are induced in diabetic states and likely associated with the development of type 2 diabetes.

Evans JL[24] in their review article, reported that excessive ROS lead to damage of proteins, lipids, DNA[24,25] and it plays a significant role in activating stress sensitive signaling pathways that regulate gene expression resulting in cellular damage.[22,26] One major target of ROS is NF-Kβ which plays a critical role in mediating immune and inflammatory responses and apoptosis. The aberrant regulation of NF-Kβ is associated with a number of chronic diseases including diabetes and atherosclerosis[24]. Hence, this could be one of the mechanisms by which elevated serum copper level plays a role in aietiopathogenesis of type 2 diabetes mellitus. Also Alessandro Sinopoh and co-authors[27] reported that copper has been implicated in the aggregation process of Amylin, a peptide hormone produced by the isled β cells of pancreas. The formation of these aggregates is said to be strongly associated with β cell degeneration in type 2 diabetes[27]. These various reports highlight the fact, that there could be various mechanisms and pathways by which increased serum copper levels play a role in aietiopathogenesis of type 2 diabetes mellitus.

Furthermore, serum copper showed a strong positive correlation with body mass index in the diabetic subjects in this study. Other studies have reported similar findings. Yer likaya HF[28] and co-authors reported significantly higher levels of copper in obese non-diabetic and obese diabetic women than in the controls in their study. They also demonstrated a strong positive correlation between Copper and Body Mass Index. Omar S et al[29] reported high serum copper levels in obese

subjects and observed that serum copper levels rise with increasing body mass index. Body mass index =30kg/m^2 according to WHO criteria[30] is classified as obesity. Could increased or increasing copper levels lead to increased body mass index and hence obesity? Olusi S and co-authors[31] in their study in a healthy adult population in Kuwait, reported a positive correlation between serum leptin level and copper levels, even after co-founding effects of age, gender has been removed. Increased serum leptin levels is found in obesity. The question therefore arises, whether elevated copper levels can predispose to obesity?

Obesity is known to be a strong risk factor for development of type 2 diabetes mellitus, as it decreases insulin stimulated glucose transport and metabolism in adipocytes and skeletal muscle. It also impairs suppression of hepatic glucose output[32].

H E Bays[33] in their study reported that increased BMI was associated with increased prevalence of diabetes mellitus. Could raised copper levels, leading to increased body mass index and therefore obesity, which could cause insulin resistance and hence diabetes, be a possible mechanism in which copper may be involved in aietiopathogenesis of type 2 diabetes mellitus? These are areas for further research studies.

Copper showed a weak positive correlation with C-reactive protein in this study, which was not statistically significant. Yer likaya HF[28] in their study also reported a non-significant positive correlation of copper with C-reactive protein. Onah A[34] reported that diabetes was significantly predicted by C Reactive Protein levels in women in Turkish population. Other studies[35,36] have also demonstrated a strong positive correlation between C Reactive Protein and body mass index. C-reactive protein is made by the liver in response to inflammatory cytokines such as interleukin (1L-6) and Tumour Necrosis Factor, and adipose tissue is a major source of these inflammatory cytokines. With studies reporting positive correlation between body mass index and C-reactive protein, which in some cases is a strong correlation[37]; and Body mass index showing a positive correlation with C-reactive protein in our study, though not statistically significant, we hypothesize that another possible mechanism in which raised copper level may play a role in aetiopathogenesis of type 2 diabetes mellitus could be; increase in serum copper levels cause altered lipid metabolism and therefore increased Body Mass Index which in turn causes raised C reactive protein levels, and predispose to pathogenesis of type 2 diabetes mellitus apart, from development of complications.

From various studies already reported and in our own study too, altered copper metabolism seems to play an important role in aetiopathogenesis of type 2 diabetes mellitus, and possibly its complications. There seems to be multiple mechanisms by which it does this; ranging from the direct effect of raised copper levels on the β cells of the pancreas, to its influence on body mass index and C-Reactive protein levels, which independently and jointly can predispose to development of type 2 diabetes mellitus. The study needs to be carried further.

Conclusion

The significantly lower levels of manganese and selenium in type 2 diabetics in this study, highlights the role deficiency of these micronutrients may play in pathogenesis of type 2 diabetes mellitus. Significantly raised serum copper levels and it's positive correlation with body mass index, suggests that altered copper metabolism may play a role in pathogenesis of type 2 mellitus, and the mechanism by which it does this, may be multiple.

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