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Variations in some trace elements in various degrees of Diabetes mellitus

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Abstract: *Background:* Diabetes has been shown to be associated with abnormalities in the metabolism of trace elements especially chromium, zinc, copper, magnesium and manganese. Trace element abnormalities has played some role in several complications of diabetes mellitus. *Objective*: To evaluate serum zinc, copper, chromium and magnesium as indices of chronic complication and compare the parameters among subjects of varying degree of complications. Therefore this work would be evaluating trace element as predictors of chronic complications and establish their relationship with micro albuminuria. *Method:* It was designed to compare the serum concentration of zinc, copper, chromium and magnesium in the sera of 109 insulin-dependent diabetes mellitus (subjects with no complications, microvascular complications [retinopathy, nephropathy] only and a combination of micro vascular and macro vascular [cardiovascular and neuropathy] complications which were thirty six, thirty seven and thirty six respectively) and 100 non-diabetic healthy control subjects. The mean age of the diabetic patients was similar to that of control. The mean duration of the disease 7.4 +/- 5.8 years (1-15 years) and the mean HbA_1c is 9.41± 2.68 in age matched subjects and control. *Results:* Serum magnesium, and zinc were significantly reduced but copper was significantly increased on comparing with control. Zinc and magnesium has an inverse relationship with copper. Copper and zinc were significantly higher and lower respectively, when subjects with micro and macro vascular complication and micro vascular only are compared with patients without chronic complications. *Conclusion:* Trace element evaluation, supplementation as well as chelating agent may be helpful in management of Type 2 Diabetes Mellitus patients.

Keywords: Type 2 diabetes mellitus, Micro vascular complication, Macro vascular complication, trace elements.

Introduction

Micronutrients are essential nutrients that are required by the body in trace amounts or tiny quantities on a day-to-day basis in order to function properly. This includes four major classes: macro elements, trace elements, vitamins, and organic acids. Macro elements include chloride, calcium, phosphorous, magnesium, sodium, potassium, and iron. The trace elements include cobalt, boron, chromium, copper, sulfur, iodine, fluoride, selenium, manganese, zinc, and molybdenum. The macro elements such as calcium and magnesium have been associated with impaired insulin release, insulin resistance, and glucose intolerance in experimental animals and humans. A relationship was observed between diabetes mellitus and trace elements in many research studies [1]. In many cases, an alteration in the metabolism of these minerals was demonstrated. Insulin action was reported to be potentiated by some trace elements like chromium, magnesium, vanadium, zinc, manganese, molybdenum and selenium [2]. Proposed mechanisms of enhancement of insulin action by trace elements include activation of insulin receptor sites [3], serving as cofactors or components for enzyme systems which are involved in glucose metabolism [4], increasing insulin sensitivity and acting as antioxidants for preventing tissue peroxidation [5].

Magnesium activates enzymes, contributes to energy production and helps regulate calcium levels as well as copper, zinc, potassium, Vitamin D and other important nutrients in the

body. Hypomagnesaemia is frequently present in diabetic mellitus types 2 patients. Hypomagnesaemia is the commonest electrolyte abnormality in the ambulatory diabetic patient and is also a frequent finding in patients with diabetic ketoacidosis. Excessive urinary magnesium loss associated with glycosuria is probably the most important factor in the genesis of hypomagnesaemia in diabetic patient. The Clinical consequences of magnesium deficiency include impairment in insulin secretion, insulin resistance and increased macro vascular risk [6].

Zinc is required for over 300 different cellular processes including enzymes activity, protein synthesis and intracellular signaling [7]. It is involved in homeostasis, in immune responses, in oxidative stress, in apoptosis and in ageing. Zinc is required for normal immune function and taste acuity and enhances the invitro effectiveness of insulin. Impaired immune function and taste have been reported in diabetic subjects and decreased serum levels and hyperzincuria occur in some diabetic subjects and animals. Copper is present in the body in combination with enzymes to form metalloenzymes such as ceruloplasmin and superoxide dismutase (SOD). These enzymes play major roles in redox reactions and antioxidant defence. It has been postulated that copper possesses insulin like activity and promotes lipogenesis [8].

Chromium has been found to increase the number of insulin receptors present in target tissue as well as increase the binding of insulin to its receptors. Chromium's action on insulin receptors may be mediated via a chromium complex termed the 'glucose tolerance factor" while the exact identity of this factor has not been elucidated, chromium may be an essential component. This action of chromium is suspected to involve chromium's ability to regulate key reactions involving phosphorylation/DE phosphorylation which turn on and off insulin action. In the late 1950s studies in patients receiving total parental nutrition (TPN) demonstrated a severe deficiency of chromium, resulting in impaired glucose tolerance and subsequent hyperglycemia and glycosuria. Other signs of chromium deficiency include hyperinsulinemia especially in type 2 DM, decreased insulin receptors, decreased insulin binding and DM- associated neuropathies and vascular pathologies [9].

These trace element deficiencies appear to be an additional risk factor in the development and progress of disease and they contribute to the pathogenesis of diabetes mellitus and its complications. Impaired trace elements have been implicated in neurovascular complication of diabetes mellitus. Though the role of trace elements in some of the metabolic dysfunction and their contributions in the development of complication is not clear 10].

Material and Methods

Study design and population: This is a comparative cross-sectional descriptive study designed to investigate the levels of zinc, copper, chromium and HbA1c levels in DM subjects with or without complications and non-diabetic controls. This study was conducted from March to May 2013 at Federal Medical Centre which lies in the heart of the metropolitan town of Makurdi, Benue State, Nigeria. The study design was approved by the Ethics Committee of Federal Medical Centre Makurdi, Benue State.

A total of two hundred and nine (209) subjects were recruited for this study. One hundred and nine (109) type 2 DM patients (36 subjects with no complications, 37 subjects with micro vascular complications only and 36 subjects with a combination of micro vascular and macro vascular complications) were recruited by consecutive sampling method from diabetic clinics of Federal Medical Centre Makurdi, Benue State. Age and sex matched healthy controlswere recruited from the blood bank donors as well as non- diabetic subjects.

Male or female DM patient under treatment of age range 40-80yrs were included in the study. Pregnant women, non- diabetic patients, patients with proteinuria, hypoproteinemia, abnormal liver or kidney function, those treated with drugs affecting urinary albumin excretion (ACE inhibitors, angiotensin converting enzyme blocker) in the last 3 months were excluded from the study. Subjects that are diabetic, pregnant, has any form of chronic disease or less than or greater than 40-80 respectively were excluded from the control subjects.

Sample collection and Biochemical analysis: Whole blood sample were used for $HBA1_C$. HbA1c was determined using device by Bio-Rad HbA1c based on Boronate affinity chromatography described by [11] while estimation of Serum copper, zinc, magnesium and chromium were analyzed using FS240AA Agilent technology Atomic Absorption Spectrophotometer Atomic Absorption Spectrophotometry. HbA1c values<7% was considered normal.

Statistical Analysis: Data analysis was done using the statistical package for social sciences (SPSS) for windows version 20.Comparison was made using student t-test and Anova while Pearson correlations were used to determine correlations. Cross-tabulations was done using chi-square.

Results

Table-1: Mean levels of magnesium and trace elements between diabetes mellitus type 2 subjects and control

Parameters	Diabetic Subjects (N=109)	Control (N=100)	P value
Magnesium (mmol/L)	0.04 ± 0.004	0.95 ± 0.15	0.00**
Chromium (µg/dl)	0.11± 0.006	0.13 ± 0.21	0.59
Copper (µg/dl)	129 ± 12.06	65.84 ± 6.67	0.00**
Zinc (µg/dl)	53.41± 4.35	112.46 ± 9.23	0.00**

Values are expressed as mean ± SD. *Significant at P < 0.05. **Significant at P < 0.01

Table-2: Copper, Magnesium, Chromium and Zinc of diabetic subjects with varying complication (Mean ± SD)

Parameter	No complications	Microvascular Complications	Micro and Macrovascular complications	P-value
Copper (µg/l)	61.45±8.45	64.65±10.84 [a,c]	82.82±11.59 [a,b]	.006*
Magnesium (µg/l)	0.52±0.05	0.38±0.09	0.37±0.12	.237
Chromium (µg/l)	0.25±0.42	0.09±0.22	0.07±0.19	.115
Zinc (µg/l)	64.70±8.45 [b,c]	52.70±6.72[a]	49.28±7.52 [a,b]	.024*

* =Significant at <0.05. a = Significant at <0.05 when compared with No complication. b = significant at <0.05 when compared with micro vascular. c = significant < 0.05 when compared with both micro and macro vascular complication.

Table 2, depicts that for copper there was significant increase in concentration at p<0.01 in subjects with no complication than those with micro vascular disease. There was also a significant difference when subjects with micro vascular disease were compared with those with micro vascular and macro vascular disease.

Moreso, zinc is reduced significantly at P<0.05 when subjects with no complication and microvascular complications were compared with those with micro and macrovascular disease respectively.

Discussion

In this study serum magnesium, copper and zinc were significantly reduced (P< 0.01) compared to the control. This may be due to polyuria as seen in diabetes mellitus can affect corporal concentration of magnesium, developing hypomagnesaemia which may still be related directly with some micro vascular and macro vascular complications observed in diabetes mellitus. The magnesium is an essential ion involved in multiple levels in insulin's secretion and its binding and its activity; and it is also a critical cofactor of many enzymes in carbohydrate metabolism.

The magnesium plays an important role to improve insulin resistance. Our finding is consistent with that of Sales et al [6] who observed a linear relationship between hypomagneasemia and micro vascular disease. Comparison of serum magnesium of subjects with no complications and subjects with micro vascular and macro vascular complications, Mg was decreased in patients with complications. Also, this is consistent with Sharma *et al* [12] where Mg also decreased on comparing patient with complications with those without complications. Therefore, hypomagnesaemia seems to be associated with worsening chronic complication probably by worsening insulin resistance and decreased activity of enzymes catalyzing normal metabolic pathway leading to worsening hyperglycemia.

Secondary complications of diabetes most of the time results owing to alterations in vascular basement membrane composition as well as accumulation of glucose derived reaction products due to over utilization of glucose in insulin independent tissues. Furthermore, hyperglycemia increases the non- enzymatic glycation reaction glucose and free amino groups in proteins and therefore disturbs the biological function of various proteins. The products of non-enzymatic glycation such as AGE (Advanced glycation end product) are known to induce various cytokines in vascular cells. These factors are likely to play some role in the development of diabetic complications.

Zinc also, showed reduced concentration in the subjects compared with control subjects ($P< 0.01$). This could be attributed to hyperzincuria which has been reported from other works in diabetic patients. The complications of diabetes may be mediated, at least in part, through oxidative stress, and zinc plays a key role in the cellular anti oxidative defense. Therefore, an abnormal zinc metabolism may play a role in the pathogenesis of diabetes and some of its complications. The cause of decreased serum zinc levels in diabetes may be an increase in urinary loss. Hyperglycaemia has been postulated to interfere with the active transport of zinc back in to the tubular cells [13]. Other possible causes may be disturbed metabolisms of zinc metalloenzymes and an abnormal binding of zinc to tissue proteins, which cause hyperzincuria.

Zinc has been found to enhance the effectiveness of insulin in–vitro and hence, a zinc deficiency may aggravate the insulin resistance in type II diabetes. This is in line with the work of Nnodim et al [14]. This may cause complications [15]. Moreover Antioxidant enzymes such as superoxide dismutase, catalase and peroxidase require zinc. Therefore, reduced concentration of magnesium may contribute to imbalance between anti-oxidants and oxidative stress as a result of decrease in activity of enzymatic anti-oxidants.

Excessive copper in serum may promote development and progression of oxidative stress, altered immunity and altered insulin secretion or its action [16]. Studies on the complication of diabetes mellitus have revealed that altered immune response may be antecedent to complication of diabetes mellitus which may have a synergistic effect when they coexist with the host zinc deficiencies and elevated copper, which seems to be associated with an increased oxidative stress along with an altered immune response which could lead to various diabetic complications. The redox chemistry of Cu makes this both a powerful enzyme catalyst and a dangerous reactant that generates hydroxyl radical which may lead to increase consumption of available antioxidants in the body.

In this study copper was increased in subjects ($p<0.01$) with microvascular complications and macrovascular disease compared with those without complications. This is consistent with the discovery made by Mosaad *et al* [10] in his study there is hypercupriseamia in patients with microvascular complication on comparing with subjects without complications and control. Transition metal like copper has affinity to bind with proteins that have been glycated. Generally, serum concentration of copper and ceruloplasmin is elevated in type 2 diabetes mellitus patients. Ceruloplasmin and serum albumin are the main Cu binding proteins in plasma and there is some evidence that chronic hyperglycemia can damage the Cu binding properties of both [17].

Furthermore the incubation of ceruloplasmin with glucose reportedly causes fragmentation and time dependent release of it is bound Cu2+, which then appears to participate in a Fenton type reaction to produce hydroxyl radicals [18]. Moreso, The redox active metal ions (Cu2+ and Fe3+) have been implicated in catalyzing the autoxidation of glyceraldehyde and generation of hydroxyl radical, leading to production of glyoxalin and associated α-Oxoaldehyde derived AGE (Advanced glycosylation end products) formation . A wealth of experimental evidence supports the hypothesis that AGES formed from glyoxal, methylglyoxal and 3- deoxyglucosone have an etiological role in the development of diabetic complications and other diseases. Therefore, high concentration of serum copper may accelerate chronic complications of diabetes mellitus.

Conclusion

These trace element abnormalities appear to be an additional risk factor in the development and progress of disease and they contribute to the pathogenesis of diabetes mellitus and its complications. Critical attention to trace element abnormalities may be an effective therapeutic intervention in prevention of the progression of the diabetes and its complications, along with a glycemic control and control of other risk factors.

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