Study of Serum Zinc and Copper levels in Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: Diabetes Mellitus is a metabolic disease characterised by hyperglycemia due to defective insulin secretion or action. Levels of trace elements like Copper and Zinc have been found to be altered in this disorder. Theses might have some role in progression of this disease. Purpose of the study was to estimate serum zinc and copper levels in type 2 diabetes mellitus patients with and without micro-vascular complication and to compare with that of healthy individuals. And also, to identify the inter-relationship among these.

Material and methods: A cross sectional study was carried out in the Department of Biochemistry in collaboration with Department of Medicine, Regional Institute of Medical Sciences, Imphal, Manipur. Eighty randomly selected cases of confirmed type 2 diabetes mellitus (T2DM) patients diagnosed for more than one year and another forty, age and sex matched, healthy controls were included in this study. Serum copper and zinc levels were estimated colorimetrically in the serum of these patients using commercially available kit.

Results: Serum Zn levels were lower for T2DM cases with complication (89.65 ± 4.21) than cases without complications (92.32 ± 5.15) and controls (95.40 ± 3.90), while serum Copper was highest among cases with complication (164.05 ± 9.32) than cases without complication (161.40 ± 6.43) and controls (131.85 ± 7.92).

Conclusion: Altered levels of trace elements Zn and Cu are found to be an important predisposing factors for diabetic patients for developing complications.

Keywords: Diabetes mellitus, Zinc, Copper

INTRODUCTION

Diabetes Mellitus is most common endocrine disease. It is a group of metabolic disease which is characterized by hyperglycaemia, various clinical manifestations and systemic complications and is caused by either deficiency in the secretion or action of insulin or both. The metabolic derangement is frequently associated with permanent and irreversible functional and structural changes in the cells of the body, those of the vascular system, being particularly most susceptible. The chronic hyperglycaemia of diabetes is associated with long term damage, dysfunction and failure of different organs, and these changes in turn lead to development of well-defined clinical entities, the so called complications, which may affect especially the eyes, kidneys, heart, blood vessels, the skin and the nervous system.[1]

Interest in trace elements has been steadily increasing over the last 25 years. Trace elements are accepted as essential substances 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, pre secretory granules and biological membranes.[2]

Direct association of minerals, trace elements and vitamins in the pathogenesis and natural course of both type 1 and 2 diabetes mellitus has been observed in many research studies. An alteration in the metabolism of these minerals and vitamins has been demonstrated. Diabetes mellitus is a heterogeneous disease associated with an absolute or relative deficiency of minerals as well as insulin resistance.[3] Some trace elements act as antioxidants, prevent membrane peroxidation while others act directly on glucose metabolism. It is generally agreed that disturbed concentration of Zinc (Zn) and Magnesium (Mg) in the body are often found in patients of diabetes mellitus.

Among the trace elements Copper (Co) and Zn are of particular interest.[4] In subjects with Insulin Dependent Diabetes Mellitus (IDDM), Zn concentrations have been demonstrated to be lower in leucocytes and erythrocytes than in serum, while no such alteration has been found with copper.[5] Cu is involved in oxidation – reduction and has a dominant role in diverse proteins such as cytochrome oxidase and cytoplasmic superoxide dismutase.

Zinc another essential trace element, is a component of many enzymes, and plays an important role in the maintenance of several tissue functions including the synthesis, storage and release of insulin.[6,7] Zn plays an important role in glucose metabolism.[8] It has been found to enhance effectiveness of insulin in vitro and it has been postulated that its deficiency may aggravate the insulin resistance in non-insulin dependent diabetes mellitus (NIDDM).[9]

Aims and objectives of the research were to estimate serum zinc and copper levels in type 2 diabetes mellitus patients with and without micro-vascular complication and to compare with that of healthy individuals. And also, to identify the inter-relationship among these components in healthy controls, type 2 diabetes mellitus with and without complications.

MATERIAL AND METHODS

This cross sectional study was carried out in the Department

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of Biochemistry in collaboration with Department of Medicine, Regional Institute of Medical Sciences, Imphal, Manipur, India during the period from September 2011 to August 2013. The study group comprised of eighty randomly selected cases of confirmed T2DM patients diagnosed with T2DM for more than one year among patients coming from different areas of Manipur and attending diabetic clinic and/or admitted in the Medicine ward, irrespective of sex, religion and socio-economic status. Cases were divided into two groups. (i)- Forty confirmed type 2 diabetic mellitus patients without complication who were under treatment with insulin or oral hypoglycemic agents and/or diabetic diet. (ii)- Forty confirmed T2DM cases with complication like nephropathy, retinopathy or neuropathy etc under treatment. Forty age and sex matched apparently healthy individuals were selected as controls.

All cases and controls were aged 18 years and above. Each individual enrolled in study underwent a detailed history, clinical examination and laboratory examination designed for the study. Type 2 DM patients with and without complication were diagnosed on the basis of history, physical examination, biochemical investigations and according to revised criteria for diagnosing DM issued by consensus panel expert from the National Diabetes Group and World Health Organization.

Patients suffering from carcinoma, any chronic systemic disease, smokers, alcoholics, pregnant, lactating mothers, with history of acute infections and thyroid dysfunction were excluded from this study.

Five ml of blood was collected from each individual. Four ml was collected in sterile plain vial for examination of Zn and Cu. It was processed within 1hr. of collection.

Serum copper and zinc levels were estimated colorimetrically using Di-Br-PAESA method using commercially available kit of CREST BIOSYSTYEM, GOA.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS version 16. Datas were expressed in Mean ± SD. Statistical tests like $\chi 2$-test, independent t-test, ANOVA (F-test) and correlation coefficient 'r' were applied whenever found suitable and necessary. The P-value less than 0.05 was considered significant. Study was approved by the institutional ethical committee. Informed consent was obtained from all individuals and nature of the study was explained to them.

RESULTS

Table -1 shows that in both the case groups i.e. diabetics with complication and diabetics without complications, the number of females were more than males. However insignificant test value (P = 0.670) indicates that a visible variation of number of males and females over the three groups, is considered, negligible and therefore the three groups are compatible in the sense that sex is matched.

Zinc level was clubbed into two groups i.e., 81-90 µg/dl and 91 – 100 µg/dl for the purpose of analysis, and the distribution pattern of the values among the three groups is being shown in this table-2. Majority of the diabetics with complications (55%) and diabetics without complications (70%) had Zn level in the range of 81-90 µg/dl and majority of the controls had Zn level in the range of 91-100 µg/dl.

Copper level was distributed into 6 groups as shown in table 3. It is observed that majority of diabetic cases with complications (45%) and majority of diabetics without complications (57.5%) had Cu level in the range 156-165 µg/dl, whereas majority of controls (37.5%) had Cu level in the range of 116-125 µg/dl.

sex	Types of group					
	controls	%	Case with complications	%	Case without complications	%
male	21	52.5	19	47.5	17	42.5
female	19	47.5	21	52.5	23	57.5
total	40	100	40	100	40	100

Table-1: Groups and sex wise distribution

Zn µg/dl	Types of group					
	Control	%	Cases with complication	%	Cases without complication	%
81-90	6	15	22	55	28	70
91-100	34	85	18	45	12	30
total	40	100	40	100	40	100

Table-2: Groups and distributions of Zn level

Cu in µg/dl	Types of Group					
	controls	%	Cases with complications	%	Cases without complications	%
116-125	15	37.5	-	-	-	-
126-135	17	42.5	1	2.5	-	-
136-145	7	17.5	1	2.5	-	-
146-155	1	2.5	12	30	7	17.5
156-165	-	-	18	45	23	57.5
166-175	-	-	8	20	10	25
total	40	100	40	100	40	100

Table-3: Groups and distribution of Cu level

Table-4 shows distribution of mean ± SD of trace elements i.e. Zn and Cu among the three study groups. In case of Zn, it is found to be highest in the control group followed by diabetics without complications and then diabetics with complications. For Cu, diabetics with complications have the highest level and control the lowest. The variation of mean for each trace elements was found to be highly significant ($p < 0.001$) statistically.

In order to evaluate better understanding of multiple comparison Post Hoc test is done and the findings are shown in Table 5. The pair-wise mean comparison is performed for Zn, Cu. It was observed that for Zn, all comparisons are statistically significant. Nevertheless, insignificant difference is observed between the two diabetic groups.

DISCUSSION

In recent years, chronic diseases such as diabetes and hypertension have been shown to be major causes of death worldwide.[10] The prevalence of diabetes in developed countries has reached immense proportions which represent a major public health problem.

In current study number of females were found to be more than males in both groups i.e. in diabetics with complication and diabetics without complications.This finding is consistent with the statement that Type 2 DM is more common in women.[11,12]

In this study, zinc levels in diabetics – both with complications and without complications, were lower than the control group. This finding was in agreement with the findings of Schlienger JL et al[13] and Pai LH,[14] but this finding was contradictory with the findings of Osman E et al,[15] D'Ocon C et al[16] and Mateo MC et al.[17] In the mammalian pancreas, Zinc is essential for the correct processing, storage, secretion, and action of insulin in beta (β)-cells. Insulin is stored inside secretory vesicles or granules, where two Zn^{++} ions coordinate six insulin monomers to form the hexameric-structure on which maturated insulin crystals are based.[18] It is also known that like, most other chronic disorders, diabetes increases the excretion of minerals.[19] Hyperglycemia in diabetes is usually associated with hyperzincuria and increased urinary loss of Zn^{++}, which is responsible for decreases in total body Zn^{++}.[20-22] Zinc has antioxidant properties; thus it can stabilize macromolecules against radical induced oxidation.[23] Zinc is a component of the important antioxidant enzyme superoxide dismutase (Cu-ZnSOD).[24] Thus the protection of this antioxidant against free radicals generated in the disease[25] will be diminished. It is also very important to note that Zn concentration regulates the metabolism of other very important members of the antioxidant defence system. Vitamin A (an antioxidant) is dependent on adequate zinc level for its release from the storage site in the liver and metabolism.[24,25] Similarly the potent antioxidant, vitamin E and zinc have a number of functions in common, including membrane stabilization, antioxidant functions and modulation of prostaglandin metabolism.[26] Furthermore, zinc deficiency produces high vulnerability to lipoprotein oxidation in experimental models.[27] Hyperglycemia and hyperinsulinemia increases the production of free radicals and there is evidence that lipid peroxidation is increased in type 2 diabetes mellitus patients.[28]

Although some investigators suggest that decreased serum Zn levels can be prevented by oral Zn replacements, later researches[29-31] indicated that different representations of serum Zn level are independent of diet.

Present study has shown increased Cu levels in diabetic patients- both with complication and without complications, than the controls. Similar finding has been observed by other studies as Di-Silvestro RA et al[32] and Zagar AH et al.[33] Urinary excretion of copper has been found to be affected by diabetes mellitus.[34,35] Previous studies proved involvement of copper to cause oxidative stress. Majority of plasma copper is transported bound to ceruloplasmin (>95%); rest is bound to albumin, transcuprein and copper-amino acid complexes.[36] Ceruloplasmin is an acute phase reactant, has ferro-O2-oxidoreductase (pro-oxidant) activity directed towards ferrous ion stimulated lipid peroxidation and formation of hydroxyl radical in Fenton reaction.[36] Copper is toxic in its unbound form, causes redox imbalance due to it's highly redox active nature, which leads to activation of stress sensitive intracellular signaling pathways through Haber-Weiss reaction.[36,17] The increase in Cu ion levels in patients with diabetes mellitus may be attributed to hyperglycaemia that may stimulate glycation and release of copper ions and this accelerates the oxidative stress, so that, Advanced Glycation End products are formed[38], that are involved in the pathogenesis of diabetic complications.Transition metal like copper has affinity

	Mean value ± SD			P value
	controls	Cases with complications	Cases without complications	
Zn	95.40 ± 3.90	89.65 ± 4.21	92.32 ± 5.15	< 0.001
Cu	131.85 ± 7.92	164.05 ± 9.32	161.40 ± 6.43	< 0.001

Table-4: Distribution of mean ± SD of Zn and Cu among the three study groups

variable	(I)	(II)	Mean difference* (I-II)	p-value
Zn	Control	Case with complication	5.75000	0.001
		Case without complication	3.07500	0.008
	Case with complication	Case without complication	-2.67500	0.025
Cu	Control	Case with complication	-29.55500	0.001
		Case without complication	-32.20000	0.001
	Case with complication	Case without complication	-2.64500	0.423

*The mean difference is significant at the .05 level

Table-5: Pair-wise (group) comparison of Mean of trace elements

to bind with proteins that have been glycated. Copper in its free form is a potent cytotoxic element because of its redox chemistry it readily participates in Fenton and Heiber-Weiss reactions to generate ROS.[39] 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.[40]

Considering all of the findings together it was implied that the ratio of Cu/Zn levels, instead of serum Zn levels alone provides more useful information.[41,42] In present study there exist antagonistic relationship in the levels of Cu and Zn in diabetes. Thus the role of trace elements in diabetes mellitus become important.[43,44]

CONCLUSION

Altered levels of trace elements Zn, Cu are found to be important predisposing factors for diabetic patients for developingcomplications. From the present study it may be concluded that altered levels of trace elements like zinc and copper may have a role in the pathogenesis and progression of T2DM. The decreased blood levels of Zn and increased blood levels of Cu as have been found in present study can-be utilized for the screening, diagnosis and management of diabetes mellitus. However this observation requires further study.

Because of important role of trace elements like zinc and copper in diabetes mellitus, it is suggested that an adequate supply of these substances in the diet of diabetic patients can be beneficial in the long term management of diabetic patients, and further studies in this field are recommended.

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