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Original Article

Biochemistry Section

A Cross-sectional Study of Serum Levels of Zinc, Copper and Magnesium in Type 2 Diabetes Mellitus in South Indian Urban Population

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ABSTRACT

Introduction: Variability in the levels of these trace elements may reflect altered insulin metabolism and poor glycaemic control in the background of elevated oxidative stress. Mineral metabolism is another entity that may be disrupted by diabetes mellitus. Conversely, there are studies implicating early imbalances of trace elements in upsetting glucose homeostasis and insulin metabolism.

Aim: To estimate and compare serum zinc, copper and magnesium in Type 2 Diabetes Mellitus (DM) patients with non diabetic controls and to correlate the serum zinc, copper and magnesium with Glycated Haemoglobin levels in Type 2 DM.

Materials and Methods: This cross-sectional study was conducted at the Sapthagiri Institute of Medical Sciences and Research Center, Bengaluru, Karnataka, India. The study included 30 Type 2 DM patients and 30 healthy, age and gender matched controls without Type 2 DM. Their serum levels of zinc, copper and magnesium were measured and compared. Statistical Package for the Social Sciences (SPSS) version 16 software was used to perform the statistical analysis. The data obtained was subjected to descriptive statistical analysis.

Results: Mean±SD of serum zinc in Type 2 DM and controls was 93.44±46.99 µg/dL and 121.74±37.15 µg/dL, respectively. Serum zinc was significantly decreased in Type 2 DM. However, there was no significant alteration with respect to serum copper and magnesium. Pearson's correlation analysis showed that the association between HbA1c with zinc (r=0.069, p=0.718), copper (r= -0.094, p=0.622) and magnesium (r=0.116, p=0.543) was random.

Conclusion: Zinc deficiency noticed in Type 2 DM patients may be due to increased excretion in urine. Zinc oral preparations are cheap and easily available. Considering these, it can be further explored if micronutrient supplementation would help to improve the glycaemic variability in Type 2 DM.

Keywords: Glucose homeostasis, Mineral metabolism, Trace elements

INTRODUCTION

Diabetes Mellitus (DM) is a major health problem which has gained pandemic proportions causing increased morbidity and mortality [1]. Hyperglycaemia is associated with increased oxidation of glucose and subsequent increased free radical formation. The gamut of consequences of increased free radicals include changes in antioxidant enzymes, vitamins, trace elements, lipid peroxidation, nitrite concentration, non-enzymatic glycation of proteins and oxidative degradation of proteins. The role of trace elements in human health has diversified facets in which they serve a variety of catalytic, structural and regulatory functions. Variability in the levels of these trace elements may reflect altered insulin metabolism and poor glycaemic control in the background of elevated oxidative stress. Mineral metabolism is another entity that may be disrupted by DM [2].

Conversely, there are studies implicating early imbalances of trace elements in upsetting glucose homeostasis and insulin metabolism. Zinc is involved in the synthesis, storage and release of insulin. Previous studies have implicated the participation of copper in free radical generation and its pro-oxidant property. In mineral metabolism, antagonism between copper and zinc is well known. Type 2 DM may aggravate oxidative stress through a maladaptive interplay of this antagonism. There are studies which show alteration in serum magnesium in Type 2 DM [3,4].

Research comparing trace elements between Type 2 DM patients and healthy individuals have reported inconsistent results. However, a meta-analysis by Sanjeevi N et al., shows lower zinc status accompanied by increased copper and ferritin levels in patients with type 2 diabetes when compared to controls [4]. Chu A et al., conducted the first systematic review of prospective cohort studies assessing the relationship between zinc status and risks of cardiovascular disease with Type 2 DM, showed the protective effect of zinc in the development of cardiometabolic diseases and the vulnerability of the Type 2 DM with zinc deficiency [5]. Though there are studies exploring the same, but there is paucity of data with respect to South Indian Urban population. Hence, this cross-sectional study was designed to contribute towards increasing knowledge database on serum zinc, copper and magnesium in Type 2 DM. The aim was to estimate and compare serum zinc, copper and magnesium in Type 2 DM patients with nondiabetic controls and correlate the serum zinc, copper and magnesium with HbA1c levels in Type 2 DM.

MATERIALS AND METHODS

This study was a cross-sectional study conducted in Sapthagiri Institute of Medical Sciences and Research Center, Bengaluru, Karnataka, India. The sample collection and analysis was carried out between two months from July 2019 to August 2019. Ethical clearance for the study was obtained from the Institutional Ethics Committee (IEC) vide reference IEC NO. SIMS&RC/IECC/1/2019 dated 22/01/2019. Written informed consent was obtained from all Participants.

Purposive sampling was carried out. Sample size was estimated by taking the mean difference of serum zinc levels between type 2 diabetics and healthy controls from a study by Yadav C et al., at a confidence interval of 95%, power 90% and ratio of cases to controls 1:1 using OpenEpi, Version 3 open source calculator and

the number was 10 in each group. However, a sample size of 30 in each group was considered [6].

Inclusion Criteria: Thirty already diagnosed cases of Type 2 DM of either gender attending Medicine Outpatient Department or presenting for blood work up at the Central Clinical Chemistry laboratory and 30 healthy, age and gender matched controls without Type 2 DM were enrolled for the study. Age group of subjects between 30-70 years were included in the study. The diagnosis of Diabetes Mellitus was based on American Diabetes Association criteria (ADA) and data was collected from patients past records of Fasting Blood Sugar (FBS), Post prandial blood glucose and HbA1c. Patients who had HbA1c >6.5% were considered as diabetic [7].

Exclusion Criteria: Diabetic patients with complications, coronary heart disease, thyroid dysfunction, renal disorders, malignancy, fever or clinical signs of infection, pregnant and postmenopause were excluded from the study.

Method of collection: Universal safety precautions were taken while collecting the blood samples. Sterile disposable needle and vacutainer was used for sample collection. Correct procedure was followed at every step such as site for venepuncture and pressure used to transfer into vacutainer; on the whole the occurrence of haemolysis can be prevented by this.

After obtaining informed consent, about 4 mL of venous blood was drawn under aseptic precautions in EDTA lavender top, plain red top containing vacutainers and processed accordingly. Plain red top containing vacutainers centrifuged at 3000 rpm for 15 minutes and the serum was obtained.

Serum sample was analysed for the following parameters in Microlab 300 semi auto analyser using commercially available kits from Tulip Diagnostics (P) Ltd., India (zinc and copper) and Lab Care Diagnostics (India) Pvt. Ltd (magnesium):

- Serum zinc by Nitro-PAPS method [8]

  In an alkaline medium, zinc reacts with Nitro-PAPS forming a purple coloured complex. The colour developed is read at 578 nm after 5 minutes of addition of reagent but before 20 minutes.

  Reference range: Serum 60-120 µg/dL
- Serum copper by 4-(3,5-dibromo-2-pyridylazo) N-ethyl-N-sulfopropylaniline method [9]

  In an acidic medium, copper reacts with 4-(3,5-dibromo-2-pyridylazo) N-ethyl-N-sulfopropylaniline forming a coloured complex and the intensity of colour formed is directly proportional to the concentration of copper in serum.

  Reference range: Serum

  Males: 80-140 µg/dL, Females: 80-155 µg/dL
- Serum Magnesium by Xylidyl blue method [10]

  Magnesium reacts with Xylidyl blue in an alkaline medium forming a coloured complex and the optical density reading is used to calculate the concentration of serum magnesium.

  Reference range: Serum 1.9-2.5 mg/dL

Mispa i3 auto analyser was used to estimate the HbA1c levels in the EDTA samples by nephelometry [11]. Nephelometry is based on the antigen and antibody interaction where the HbA1c calibration curve is constructed from the amount of agglutination measured.

Reference normal value (NGSP certified method): 4.6%-6.2% [7].

STATISTICAL ANALYSIS

The values of the parameters collected were tabulated. Mean and standard deviation was obtained and Independent t-test was performed to compare the mean values of the parameters. Pearson's correlation was done to find the association of serum zinc, copper and magnesium with HbA1c in Type 2 DM. The p-value <0.05 was considered to be statistically significant. SPSS version 16 software was used to perform the statistical analysis.

RESULTS

In the present study, the age group of the subjects was between 30-70 years. Authors have observed the subjects with a mean age of 48.83±17.0, 48.86±12.42 in healthy controls and Type 2 DM, respectively. Percentage of gender distribution amongst Type 2 DM was 60% males (n=18) and 40% females (n=12). The average duration of diabetes mellitus was 9.5 years. Percentage of gender distribution amongst controls was 63% males (n=19) and 37% females (n=11).

In diabetics, two patients were having decreased levels of all three elements. Two patients were deficient of both serum copper and magnesium. Individual element deficiencies were noted in diabetics as decreased zinc only in 06 patients and decreased magnesium only in 06 patients. However, decreased serum copper levels alone was not observed in diabetics.

In controls, deficiency of zinc was not observed. However, 02 subjects had decreased serum levels of both copper and magnesium. Individual serum copper and serum magnesium deficiency were noted in 07 subjects each. Comparison of means between Type 2 DM patients and healthy controls using Independent t-test demonstrated that serum zinc was significantly decreased in Type 2 DM. However, there was no significant alteration with respect to serum copper and magnesium as depicted in [Table/Fig-1].

Variable	Type 2 Diabetes Mellitus (n=30)	Controls (n=30)	t-value	p-value
HbA1c (%)	8.32±1.84	5.4±0.38	-8.489	<0.0001**
Zinc (µg/dL)	93.44±46.99	121.74±37.15	2.587	0.012*
Copper (µg/dL)	144.71±62.11	119.21±53.23	-1.707	0.093
Magnesium (mg/dL)	1.98±0.22	1.97±0.11	-0.308	0.759

[Table/Fig-1]: Comparison of mean between type 2 diabetes mellitus patients and healthy controls.
*p<0.05 statistically significant; **p<0.0001 statistically highly significant

Pearson's correlation analysis [Table/Fig-2] showed that the association between zinc, copper and magnesium with HbA1c was random. Although the correlation is not statistically significant, the magnitude of the diabetes mellitus in pandemic proportions warrants the need for future interventional studies.

HbA1c	Zinc	Copper	Magnesium
Pearson's correlation			
r-value	0.069	-0.094	0.116
p-value	0.718	0.622	0.543

[Table/Fig-2]: Pearson's correlation of HbA1c with zinc, copper and magnesium in type 2 diabetes mellitus.

DISCUSSION

In the current study, the alterations of serum zinc, copper and magnesium was studied with respect to Type 2 DM. The study demonstrates significantly decreased serum zinc levels in Type 2 DM when compared to healthy controls (p=0.012). This finding is at par with the findings of Al-Maroof RA and Al-Sharbatti SS, Król E et al., [12,13]. The decrease in serum zinc is probably due to it's increased excretion in urine and disturbed mechanism of intestinal absorption [14,15]. The increased excretion of zinc in the urine is attributable to the high osmotic effect of glycosuria [16].

In a study by Saha-Roy S et al., serum zinc levels were significantly low (p<0.001) in diabetic subjects compared to controls. There were no significant differences in serum magnesium between groups. Fasting plasma glucose level has significant positive correlation with serum level of copper (r=0.567; p<0.001), while zinc has negative correlation (r= -0.311; p<0.047), but there is no significant

correlation of plasma glucose level with serum magnesium level. Diabetic patients have significantly lower mean serum zinc levels and significantly higher serum copper concentration compared with healthy controls, respectively [17].

The current study shows shows increased copper levels in serum of type 2 diabetics when compared to healthy controls but this finding was not statistically significant (p=0.093). In studies conducted by Atari-Hajipirloo S et al., and Wolide AD et al., serum copper was found to be elevated in Type 2 DM [18,19]. Studies conducted by Basaki M et al., and Ahmed AM et al., showed that copper was significantly lower in patients with diabetes mellitus [20,21]. This discrepancy in serum copper levels observed in various studies could be due to difference in lifestyles and dietary habits in different populations.

Magnesium levels in serum in this study subjects with Type 2 DM was elevated when compared to healthy controls. However, this increase was statistically not significant (p=0.759). This is similar to the findings of Walter RM et al., [22]. In contrast to these, Wolide AD et al., Ahmed AM et al., Sundaram G et al., found that serum magnesium was significantly decreased in Type 2 DM [19,21,23]. In diabetes mellitus, impairment of tubular reabsorption of magnesium by glycosuria and hyperglycaemia might contribute towards hypomagnesemia. Disturbance in insulin levels also might affect the cellular uptake of magnesium [21]. In a study by Prabhu G et al., it was noticed in patients with increasing duration of diabetes mellitus the serum magnesium levels decreased proportionately. Among the 19 patients who had hypomagnesaemia, 18 patients had HbA1c >7% [24]. Simialrly, in the present study out of the 07 patients who had hypomagnesaemia, 05 patients had HbA1c >7%.

In this study, Authors found that there was no significant correlation between serum zinc and HbA1c which is similar to the findings of Dorre F et al., [25]. Wolide AD et al., observed a negative correlation of serum zinc and magnesium with fasting blood glucose [19]. There was no significant correlation of serum copper with HbA1c in this study which is contrast with the findings of Atari-Hajipirloo S et al., who observed that copper positively correlated with HbA1c [18]. The correlation of magnesium with HbA1c was not significant which is at par with the findings by Tiwari D et al., [26]. High urinary excretion, hyperglycaemia, oxidative stress and other health related factors might be contributing factors to the disturbed trace elements and micronutrient status in Type 2 DM.

Limitation(s)

Considering that in T2DM there is glucotoxicity and lipotoxicity; estimation of lipid profile and correlating with the trace elements would have added to better understanding of the role of trace elements in T2DM.

CONCLUSION(S)

The present study shows the evidence of increasing database of knowledge of zinc deficiency in Type 2 DM. There was no significant alteration in the serum levels of copper and magnesium. Zinc deficiency noticed in Type 2 DM patients may be due to increased excretion in urine. Zinc oral preparations are cheap and easily available. Considering these it can be further explored if micronutrient supplementation would help improve the glycaemic variability in Type 2 DM.

Further prospective studies with larger sample size is needed for categorising the diabetics into HbA1c subgroups based on their glycaemic control and using ANOVA to assess the maladaptive interplay of individual trace element in uncontrolled diabetes mellitus.

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