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Original Research Article

A Hospital Based Analytical Comparative Assessment of Serum Levels of Zinc, Copper and Magnesium in Type 2 Diabetes Mellitus

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Abstract

Aim: The aim of the study was to estimate and compare serum zinc, copper and magnesium in Type 2 DM patients with nondiabetic controls and to correlate the serum zinc, copper and magnesium with HbA1c levels in Type 2 DM.

Material & Methods: This study was a cross-sectional study conducted in Department of Pathology, NMCH, Patna, Bihar, India. The sample collection and analysis was carried out between 12 months. Ethical clearance for the study was obtained from the Institutional Ethics Committee (IEC). Written informed consent was obtained from all Participants.

Results: In controls, deficiency of zinc was not observed. However, 04 subjects had decreased serum levels of both copper and magnesium. Individual serum copper and serum magnesium deficiency were noted in 10 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. Pearson's correlation analysis showed that the association between zinc, copper and magnesium with HbA1c was random. Although, the correlation is not statistically significant.

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 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]

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]

Study regarding these elements has been continuously increasing during last two decades, they are known as essential for health of human being because of their various functions in metabolism and cell structure. They have an important role in functions of cell membrane, as enzymes and hormones. [6] In between Copper (Co), Zinc (Zn) and magnesium (Mg) are of. Important [7] copper has insulin–like activity and increasing lipogenesis. [8]

The aim of the study was to estimate and compare serum zinc, copper and magnesium in Type 2 DM patients with nondiabetic controls and to correlate the serum zinc, copper and magnesium with HbA1c levels in Type 2 DM.

Material & Methods

This study was a cross-sectional study conducted in Department of Pathology, NMCH, Patna, Bihar, India. The sample collection and analysis was carried out between 12 months. Ethical clearance for the study was obtained from the Institutional Ethics Committee (IEC). 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. [9]

Inclusion Criteria: Fifty 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 50 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. [10]

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 [11]

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 [12]

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 [13]

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. [14] 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%. [10]

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.

Result

Table 1: Comparison of mean between type 2 diabetes mellitus patients and healthy controls

Variable	Type 2 Diabetes Mellitus (n=50)	Controls (n=50)	t-value	p-value
HbA1c (%)	8.30±1.80	5.5±0.40	-8.489	<0.0001
Zinc (µg/dL)	93.47±46.94	121.79±37.13	2.587	0.010
Copper (µg/dL)	144.71±62.11	119.21±53.23	-1.707	0.090
Magnesium (mg/dL)	1.98±0.22	1.97±0.11	-0.308	0.759

In controls, deficiency of zinc was not observed. However, 04 subjects had decreased serum levels of both copper and magnesium. Individual serum copper and serum magnesium deficiency were noted in 10 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.

Table 2: Pearson's correlation of HbA1c with zinc, copper and magnesium in type 2 diabetes mellitus

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

Pearson's correlation analysis showed that the association between zinc, copper and magnesium with HbA1c was random. Although the correlation is not statistically significant.

Discussion

Complications associated with diabetes mellitus are increasing during last two decades and increasing the rate of mortality in developing and developed countries around the globe. [15] The role of trace elements are still not clear in diabetic patients, especially copper, Zinc and Magnesium. [7,16]

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. [17,18] The decrease in serum zinc is probably due to its increased excretion in urine and disturbed mechanism of intestinal absorption. [19,20] The increased excretion of zinc in the urine is attributable to the high osmotic effect of glycosuria. [21] 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. [22]

The current study 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. [23,24] Studies conducted by Basaki M et al., and Ahmed AM et al., showed that copper was significantly lower in patients with diabetes mellitus. [25,26] 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. [27]

In this study, we found that there was no significant correlation between serum zinc and HbA1c which is similar to the findings of Dorre F et al. [28] 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. [23] The correlation of magnesium with HbA1c was not significant which is at par with the findings by Tiwari D et al. [29] 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. [30]

Conclusion

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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