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## Research Paper

# A clinical study on trace elements and hyperglycemia levels in diabetics with allied complications

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## Abstract

Diabetes Mellitus is a major endocrine and metabolic disease worldwide. Its related complications are neuropathy, nephropathy, macro and micro-angiopathy. Angiopathic complications are believed to be a major cause for morbidity. Hyperglycemia may lead to alterations in zinc and magnesium concentrations, availability for metabolic and homeostasis in body. The present study was done to understand the effect of glycemic control on zinc and magnesium availability in diabetics with angiopathic complications and non-diabetics with no complications from south Indian population were determined. Fasting blood glucose (FBS), Glycated Hemoglobin (HbA1c), serum and urine Creatinine levels, serum and urine Zinc (Zn), serum and urine Magnesium (Mg), were determined in 500 diabetic subjects with macro and micro-angiopathic complications (aging in between 35-60 years) and 100 non-diabetic subjects with no complications (over 40 years) in south Indian population. The FBS, HbA1c, serum and urine Creatinine, urine Zn and Mg levels were significantly ( $p < 0.05$ ) higher in diabetics than non-diabetic subjects of both sexes. There was significant ( $p > 0.05$ ) differences in urine Zn and Mg levels in diabetics with poor glycemic control (HbA1c  $> 8.0\%$ , Male-Zn =  $8.65 \pm 0.57$ , Mg =  $13.65 \pm 0.67$  and Female-Zn =  $8.77 \pm 0.47$ , Mg =  $13.98 \pm 0.70$ ) and those with good glycemic control (HbA1c  $< 8.0\%$ , Male-Zn =  $7.25 \pm 0.49$ , Mg =  $12.15 \pm 0.57$  and Female-Zn =  $7.88 \pm 0.70$ , Mg =  $12.58 \pm 0.17$ ), there was significant elevation observed in the urinary Zn and Mg levels in diabetics with poor glycemic control in comparison to the non-diabetics. A correlation was observed between HbA1c control and elevation in urine Creatinine Zn and Mg levels of the diabetics (both males and females) of the study indicating the risk and enhancement in angiopathic conditions. The results obtained in the study indicated that the female subjects both diabetics and non-diabetics were losing Zn and Mg in urine slightly more than the male subjects. Diabetes and poor glycemic control alters the concentration Zn and Mg in human body and lowering the availability of Zn and Mg for several enzyme activities and metabolisms as the Zn and Mg is lost in urine and contributing to angiopathic complications in those with low Zn and Mg levels.

**Keywords:** Urinary zinc and magnesium, diabetes mellitus, HbA1c, Cardiomyopathic complications.

## Introduction

The world's population is succumbing to diabetes due to urbanization, aging, high fat food diet, obesity and physical inactivity. The rate of pre-diabetes is rising with every year passing. Diabetes mellitus is considered as major cause of morbidity and mortality worldwide, the number of diabetics has increased from 108 million in 1980 to 422 million in 2014<sup>1</sup>, and expected to double by the year 2030 especially in developing countries. 80% of the world's population is living in developing countries. 62 million people are suffering from diabetes in India and among which 6.3% population is from Telangana, South India<sup>2</sup>. Diabetic complications such as cataract, nephropathy,

neuropathy, heart attacks due to macro- and micro-vascular complications (coronary heart disease, peripheral arterial occlusive disease and cerebrovascular insufficiency), stroke, renal failures, diabetic foot syndrome and lower limb amputation<sup>3</sup>. 85-90% of diabetics have type-2 diabetes, which is a metabolic disorder characterized by lack of insulin, high sugar levels and insulin resistance<sup>4,5</sup>. Insulin exists as a hexamer containing two Zinc ions ( $Zn^{2+}$ ) in pancreatic cells and released into portal veins during de-granulation of cells<sup>6,7</sup>. The suppression of inherent amyloidogenic properties of monomeric insulin is due to co-secretion of Zn and insulin.

Among the trace elements required for body metabolism Zinc is the most important element as it plays vital role in the function of more than 300 enzymes and it is involved in cellular processes like cell division and apoptosis<sup>8</sup>. Hence, the concentration of zinc in the human body is tightly regulated and disturbances of zinc homeostasis have been associated with several diseases including diabetes mellitus<sup>9</sup>. Zinc plays a pivotal role in insulin action and carbohydrate metabolism. In the pathogenesis of diabetes, the oxidative stress has implicated as one of factors<sup>10</sup>. Zinc acts as an anti-oxidant agent, whose supplementation has decreased oxidative stress in cell culture and animal models<sup>11</sup>. Superoxide dismutase is the major anti-oxidant enzyme present in our body and for its function to be intact Zn acts as a key component and in case of its deficiency could only lead to increased oxidative stress due impaired synthesis of superoxide dismutase<sup>11</sup>. Literature review suggests that hypozincemia and hyperzincuria is associated with diabetes<sup>4,12</sup>. In developing countries where diabetes incidence is on rise is due to the deficiency of zinc.

Zinc supplementation in mice has shown improvement in fasting insulin level and fasting. Similarly Zinc supplementation has shown beneficial properties in both type-1 and type-2 diabetes in humans<sup>8</sup>. However, there are several contradictory reports being published from randomized and individual studies. De Sena et al. (2005)<sup>14</sup> reported the negative effects of Zinc supplementation on glucose homeostasis in Type 1 diabetes studies. The bias generated due to individual studies can be reduced by the help of systematic reviews as they compare the treatment effects shown in individual studies as a result provide the most accurate overall assessment of an intervention<sup>15</sup>.

Magnesium (Mg) is another trace element required for glucose homeostasis and glucose metabolisms as there is a complex interplay exists in between them. Mg is involved in functioning of various enzymes which are required for glucose oxidation, and release of insulin<sup>16-18</sup>. Insulin stimulates the uptake of Mg intracellular<sup>19</sup>. The reduction of inositol transport and depletion is due to the deficiency of Mg. The deficiency Mn of tend to alter the activity of membrane bound  $Na^{+}-K^{+}$  ATPases<sup>20,21</sup>, which play a role in glucose transport by providing  $Na^{+}$  and  $K^{+}$  ions. Low secretion of insulin in pancreas is due to deficiency of Mg<sup>22,23</sup>.

Serum creatinine is a breakdown metabolite of creatine, which is primarily located in skeletal muscle. Monitoring the levels of Creatinine in the serum and urine will help in tracking the progression diabetic nephropathy. 0.8 to 1.4 mg/dL is considered as normal Creatinine value, whereas it is found to be less in females (0.6 to 1.2 mg/dL) than males, as the females have less muscle mass<sup>23</sup>. The plasma Creatinine concentration is found to be consistent (unit of Creatine/skeletal muscle is equal to breakdown of creatine)<sup>24</sup>. Harita et al (2009)<sup>25</sup>, suggested that the incidence rate of type 2 diabetes could be due to lower serum Creatinine and lower skeletal muscle mass. The insulin majorly targets the skeletal muscle tissue and lesser mass of skeletal muscle means less target sites for insulin leading to insulin resistance and development of type 2 diabetes<sup>26</sup>. Type 2 diabetes pathogenesis could be due to the lower serum Creatinine levels in animal body.

It is the objective of this study to compare and determine the concentrations of trace elements such as Zinc and Magnesium in serum and urine of Chronic diabetic with angiopathies and non-diabetics and in different states of glycemic controls to know the status of Zn and Mg in diabetics (both sexes) in Telangana population (South India), India.

Statistical analysis of results was performed on Graph pad prism software.

## Materials and Methods

500 diabetic subjects (both males and females) who were admitted as in-patients (Cardiac wing, Sunshine Heart institute) in Sunshine Hospitals were selected for the study were aging between 35-60 years and the patients consent was taken from each subject before enrolling them into the study and

prior human ethical committee approval (SS/2015/1EC125) was obtained before enrollment of subjects. Their medical history, personal data and other parameters as weight and height were obtained. The patients enrollment for the study was done based on the laboratory findings such as fasting plasma glucose levels greater than 126mg/dL or 7.00mmol/L on two consecutive days, or if the postprandial plasma glucose levels greater than 140mg/dL or 11.00mmol/L on two consecutive days<sup>27,28</sup>.

The non-diabetics (100 subjects-males and female) enrolled for the study were over 40 years with no clinical history of any kind and these subjects were working within sunshine hospitals were selected as controls in the study. Their medical history and personal data were obtained via consent cum questionnaires. The blood samples was collected from the subjects via vein puncture for fasting plasma glucose, glycated hemoglobin (HbA1c), serum(Creatinine, Zn and magnesium (Mg))were determination or quantified. Morning urine samples were collected into sterile, chemically clean containers for quantification of Creatinine, zinc and magnesium in urine. Fasting plasma glucose was estimated using the glucose oxidase-peroxidase method, glycated hemoglobin, serum and urine Magnesium estimated on an automatic bioanalyzer (Beckman Coulter. Inc) Serum and urine Creatinine was estimated by Jaffes reaction method<sup>29</sup>, while serum and urine zinc was quantified using an Abcam's Zinc Quantification Kit and absorbance was checked at 560nm.

## Results and Discussion

The mean levels of fasting blood glucose (FBS), HbA1c, urine Creatinine, urine Zinc and urine Magnesium of diabetics (both sexes) were observed to be significantly higher than the non-diabetics (Table 1) and the levels of serum Creatinine, serum Zinc and serum Magnesium of diabetics there was noticeable difference observed in the levels of FBS, HbA1c, serum and urine Creatinine, serum and urine Zinc, serum and urine magnesium in between male and female subjects.

Zinc and Magnesium concentration in serum and urine was expressed in  $\mu\text{mol/L}$ , the blood and urine samples were collected once in a day, the sample from diabetics was collected in morning and non-diabetics were collected randomly and subjects blood samples were also collected in similar fashion. Analysis of urine Zinc and Magnesium levels was done in different states of glycemic control in diabetic patient's i.e. good and poor management of diabetes in both sexes (Table 2).

The comparison of urine Zinc and Magnesium concentrations were done in between poor glycemic control and non-diabetic controls of both sexes (Table 3) and it is observed that the female subjects were losing more zinc and magnesium in urine in comparison to male subjects.

According to WHO (1996)<sup>27</sup> dysfunction of trace element metabolism is believed in causing the acquired deficiency states in a wide range of ailments including diabetes mellitus, anemia, cardiovascular disease, cancer etc<sup>13</sup>. Earlier research studies have established the correlation between trace elements (Zinc, Magnesium, Molybdenum, Chromium, Selenium, Vanadium, and Manganese) in carbohydrate metabolism and insulin action, but actual role of these trace elements in pathogenesis of diabetes still remain mystery<sup>4,31-33</sup>. Srivastava et al.(1993)<sup>34</sup> suggests that hyperglycemia and increased protein glycosylation are the reasons for the alterations in the levels of trace elements in diabetics.

The comparison of FBS, HbA1c, urine Creatinine, urine Zinc and Magnesium levels in diabetic and non-diabetic subjects showed significant loss of Zn and Mg in diabetics than non-diabetics and in our study the females showing higher glycemic index and losing more Zn and Mg in comparison to males (Table 1). The serum and urine levels of zinc and magnesium in diabetics and in different states of glycemic control as determined by glycated hemoglobin levels were determined in between male and female subjects in the study (Table 2).

A study done by Chausmer (1998)<sup>6</sup> suggested that hypozincemia is due to hyperzincuria or diminished gastrointestinal absorption of zinc in the diabetic's population. Even though, there are reports about zinc excretion are uniform, but the data doesn't give lucidness about the diminished the data absorption of zinc.

**Table 1: Fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), serum and urine Creatinine, serum and urine zinc, serum and urine magnesium in diabetics and non-diabetic subjects of both sexes**

Subjects	FBS (mg/dL)	HbA1c (mmol/L)	Serum- Creat (mg/dL)	Urine- Creat (mg/dL)	Serum- Zn (µmol/L)	Urine- Zn (µmol/L)	Serum- Mg (mg/dL)	Urine- Mg (mg/dL)
Diabetic N= 250 males	138.73± 34.96	7.9±2.13	1.23±0.61	2.57±0.27	4.47±0.57	10.37±0.57	6.87±0.57	11.70±0.71
Non-diabetic N= 50 males	78.3± 13.05	5.68±0.40	0.83±0.09	0.23±0.19	8.43±0.21	3.25±0.89	10.25±0.49	2.28±0.29
p -value	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
Diabetic N= 250 females	145.73± 44.96	8.00±2.13	1.06±0.861	2.27±0.27	4.23±0.57	10.78±0.77	6.78±0.77	12.08±0.77
Non-diabetic N= 50 females	80.3± 13.05	5.78±0.48	0.79±0.19	0.21±0.12	8.13±0.25	3.45±0.49	10.45±0.09	2.45±0.19
p -value	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

**Table 2: Zinc and Magnesium levels in serum and urine different states of glycemic control in diabetic subjects of both sexes**

Subjects	Serum Zn (µmol/L)	Urine- Zn (µmol/L)	Serum- Mg (mg/dL)	Urine-Mg (mg/dL)
Poor HbA1c (>8.00 mmol/L) N= 162males	3.65±0.52	8.65±0.57	6.65±0.67	13.65±0.67
Good HbA1c (6.25-8.00 mmol/L) N= 88 males	3.95±0.41	7.25±0.49	8.65±0.57	12.15±0.57
p -value	p<0.05	p<0.05	p<0.05	p<0.05
Poor HbA1c (>8.00 mmol/L) N= 160males	3.88±0.70	8.77 ±0.47	6.25±0.47	13.98±0.70
Good HbA1c (6.25-8.00 mmol/L) N= 90 females	4.05±0.59	7.88±0.70	8.85±0.77	12.58±0.17
p -value	p<0.05	p<0.05	p<0.05	p<0.05

**Table 3: Urine zinc and magnesium levels in diabetics with poor glycemic control and non-diabetic subjects of both sexes**

Subjects	Urine- Zinc ( $\mu\text{mol/L}$ )	Urine- Magnesium (mg/dL)
Poor HbA1c (>8.00mmol/L) N= 162 males	8.65 $\pm$ 0.57	13.65 $\pm$ 0.67
Non-diabetic N=25 males	3.25 $\pm$ 0.49	2.28 $\pm$ 0.29
p -value	p<0.05	p<0.05
Poor HbA1c (>8.00mmol/L) N= 160 females	8.77 $\pm$ 0.77	13.98 $\pm$ 0.70
Non-diabetic N= 25 females	3.45 $\pm$ 0.79	2.45 $\pm$ 0.19
p -value	p<0.05	p<0.05

Zinc and Magnesium concentration in urine was observed to be significantly higher in diabetics than in non-diabetics population studied. Our findings are coherence with El-Yazigi et al. (1991)<sup>35</sup> work, which mentioned about excretion of zinc in urine, is high in patients suffering from diabetes and related disorders, and demonstrated the loss of zinc in urine of the diabetic patients. The hyperglycemia condition leads to hyperzincuria may be not due to the presence of endogenous or exogenous insulin on the renal tubules. According to Chausmer (1998)<sup>6</sup> high glycemic conditions cause hindrance in the active transport of zinc back into the tubular cells.

The results obtained in this study clearly showed that there is correlation between HbA1c and Creatinine, zinc and magnesium levels (serum and urine) in diabetics of both sexes, and it is found that the female's subjects were losing more zinc and magnesium in comparison to male diabetic subjects and similar observation made even in non-diabetic subjects, the reason for this phenomenon is not clear why females are losing more zinc and magnesium to that of males. The glycemic index is also seen to be more in females than to males.

### Conclusion

From our observations, we therefore conclude that diabetes and poor glycemic control alters the availability of zinc and magnesium in the diabetes patients and increasing the incidence of allied complications such as Angiopathy, cardiomyopathies, neuropathies, nephropathies etc. This study has documented previous history and food intake details of all subjects, and gives a scope in management of Diabetes and allied complications in poor diabetics through counselling and proper diet suggestions.

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