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Study of the role of Copper, Zinc and Magnesium in Diabetic Nephropathy

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ABSTRACT

Diabetic nephropathy is a complication of diabetes mellitus. A copper, zinc and magnesium important nutrient that is involved in various physiological metabolisms. This present study investigates the relation of copper, zinc and magnesium in diabetic nephropathy cases to establish possible results. Thirty healthy no diabetic subjects were studied for comparative analysis. Thirty diabetic Blood samples were collected from both cases and controls for determination of FBS, PPBS, HbA1c, copper, zinc and magnesium levels. The mean concentrations of FBS, PPBS, HbA1c, cases were significantly higher than that of controls. The mean magnesium levels of cases (1.80 ± 0.29 meq/L) were significantly lower than controls 2.40 ± 0.20 meq/L ($p < 0.05$). But the mean copper levels of cases, 150.42 ± 5.40 $\mu\text{g/dl}$, shows no significant difference with controls, 156.6 ± 5.70 $\mu\text{g/dl}$, ($p > 0.05$). The mean zinc levels of cases, 55.45 ± 33.53 $\mu\text{g/dl}$, shows no significant difference with controls, 60.73 ± 12.3 $\mu\text{g/dl}$ ($p > 0.05$). The findings in the present study suggest that hypomagnesaemia may be linked with development of diabetic nephropathy.

Keywords: Copper, Zinc, Magnesium, Diabetic Nephropathy and Hyperglycemia

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INTRODUCTION

The prevalence of diabetes mellitus is increasing worldwide. Recent studies showed that India has got large number of diabetic patients. Diabetic nephropathy (DN) develops in 30% to 40% of patients with type 1 diabetes mellitus and in 10% to 20% of patients with type 2 diabetes mellitus. Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) worldwide ^[1]. Diabetic Vasculopathy (DV) is a broad subject comprising micro-vascular complications of Diabetes Mellitus such as diabetic neuropathy, diabetic nephropathy (DN), diabetic retinopathy and macro-vascular complications of Diabetes Mellitus such as cardio-vascular disease (CVD), cerebro-vascular disease and peripheral vascular disease (PVD). Micro-vascular and macro-vascular complications are equally important because they are the major cause of mortality and morbidity in patients with DM. The most important cause of mortality in patients with DM is CVD. The micro-vascular and macro-vascular complications do not occur in isolation and often may co-exist. The presence of one may be indicative of the presence of the other and requires further investigation to assess the status of the other systems. Diabetic nephropathy in type 2 DM is similar to nephropathy in type 1 DM with similar pathology, response to interventions of glucose control and anti-angiotensin II therapy, and progression to chronic renal failure [2]. Hypomagnesaemia leads to collagen and ADP induced platelet agreeability and also decreased function of magnesium dependent enzymes, kinases, and channels regulating insulin action leading to complications. In Diabetes mellitus because of hyperglycemia increased production of Reactive Oxygen Species is seen, to counter this there will be increased cellular anti oxidant defense mechanisms. Hyperglycemia induce the expression of Super oxide dismutase, Catalase & Glutathione peroxidase, all these enzymes contain magnesium, copper and zinc. The present study is done to observe the status of elements like Copper, Zinc and Magnesium in diabetic nephropathy cases which is a complication of chronic hyperglycemia. Zinc effectively ameliorates diabetes-related complications in various animal models [3]. Several complications of diabetes may be related to increased intracellular oxidants and free radicals associated to decreases in intracellular Zn and Zn-dependent antioxidant enzymes⁴. No definite relationship has been described between copper concentrations and the clinical status of patients with diabetes mellitus⁵. Magnesium plays an important role in the activities of various enzymes involved in glucose oxidation, and may play a role in the release of insulin^{6, 7}. Copper, a transition metal with an atomic mass of 63.54 Daltons, has 2 stable isotopes, Cu, and Cu with natural abundances of 69.2 and 30.8% respectively (there are 7 radio isotopes of copper, most with a ½ life of seconds (or) minutes⁸. One explanation for this interaction is that high dietary zinc induces intestinal MT. Copper does not play an important role in the induction of MT, but it has a stronger affinity for MT than zinc. Copper displaces zinc in intestinal MT and is trapped. Copper depletion was observed in human study subjects when supplements of 50mg (280 µmol) (or) more of zinc were given for extended periods ⁹.

MATERIAL AND METHODS

A hospital based study carried out at Narayana Medical College & Hospital, Nellore, AndhraPradesh. Venous blood samples from the subjects were collected in the morning after an overnight fast, into special blood collection tubes. There were used blood vacutainers with sodium heparin for the measurement of zinc, copper, magnesium in

plasma. Plasma concentrations were determined through spectrophotometric method, using Randox kits, with plasma reference materials and controls, normal and abnormal level. Measurements were made by means of an Rx Daytona analyzer. Atomic absorption spectrophotometer (AAS) is the reference method for the determination of the cations in biological specimens, but it is not a usual method in clinical laboratories. The colorimetric methods used in clinical laboratories, especially for magnesium which is widely used, are fairly accurate and precise with a good correlation ($r= 0.986$) compared to AAS. Heparinized samples were centrifuged at 1500 g for 10 min to separate plasma from erythrocytes. Plasma was used for estimation of extracellular magnesium (without deproteinization), while trichloroacetic acid was added to precipitate proteins, the supernatant being used for analysis of zinc and copper. The measurements were initially made before the administration of metformin, and after 3 months of therapy. To assess the Status of the levels of selected Trace elements like Copper, Zinc & Magnesium in Diabetic Nephropathy cases. Thirty (30) Diabetic Nephropathy cases (Age: 45-70yrs, 22 males, 8 females) who had been clinically diagnosed participated in the study voluntarily, while Thirty (30) age matched apparently healthy subjects served as controls. In addition to clinical examination, careful history pertaining to diabetes was taken and FBS, PPBS, HbA1c, Trace elements Copper, Zinc & Magnesium were analyzed.

LABORATORY METHODS

Copper concentrations were determined by using Atomic Absorption spectrophotometer, the reference values are 100-200 $\mu\text{g/dl}$ ¹¹. Zinc concentrations were determined by as Nitro-PAPS (Phosphor adenosyl phosphor sulfate) method, the reference values are 60-120 $\mu\text{g/dl}$ ¹². Magnesium concentrations were determined by Xylidyl blue method using Daytona analyzer (Random) with reference values 1.5-2.6 mg/dl.

Method for the determination of serum copper by atomic absorption spectrophotometry [11]

Principle: five diluted serum sample are aspirated into the atomic absorption flame. The copper concentration is determined by comparing the signal from diluted serum with the signal from aqueous calibrations, which are prepared in a diluted glycerol matrix (5ml/dl) to stimulates the viscosity of diluted plasma

Specimen: precautions must be taken throughout all procedures to avoid copper contamination during sample collection or from reagents. Collect at least 3ml of blood by venipuncture as described previously under the section sample collection and testing. Anticoagulants may be, but they must be free of copper contamination. After centrifugation, use copper free pipettes to transfer the serum to clean polypropylene tubes. Hemolysis must be avoided because erythrocytes contain at least 10 times more copper than serum. The specimens may be stored in the refrigerator for several days, but specimens should be frozen at -200 c for long-term storage.

Reagents: Glycerol diluents. Dilute 50 ml of reagent grade glycerol to 1000ml with deionized water. Calibrators: Stock calibrator: use a commercially available 1g/L aqueous copper calibrator. Alternatively, dissolve 1.0000g pure copper metal in 100ml of 10-fold diluted nitric acid and dilute to 1L with deionized water.

Working calibrator: copper 100, 200, 300 and 400 $\mu\text{g/L}$. deliver 1mL of 1 $\mu\text{g/L}$ copper calibrator into a 100mL volumetric flask and dilute to volume with glycerol diluents. Mix by inverting at least 10 times. Place 1-, 2-, 3-, and 4-mL aliquots of this intermediate solution into four 100mL volumetric flasks and dilute to volume with the glycerol diluents. The calibrator, 10, 20, 30, and 40 μg copper /L, correspond to plasma copper concentrations of 50,100,150, and 200 $\mu\text{g/L}$, respectively. The calibrators are stable in polypropylene bottles at room temperature and does not need to be prepared freshly with each test run.

Instrumental conditions: Instrument settings. Wavelength, 213.8nm; readout, absorbance; slit width, 0.7nm. Burner gas mixture. Air-acetylene, fuel rich, luminescent flame.

Procedure: allow serum to come to room temperature and then mix by gently inverting the tubes. Deliver 0.5 mL of specimen or control into a 16-mm plastic test tube. Add 2mL of deionizer water and immediately mix the solution thoroughly. Establish instrumental and gas flow settings and aspiration rate to optimize signal and minimize background noise. Check instrument manual for specific instrumental settings. Aspirate glycerol diluents into the flame and set the baseline to read zero absorbance. To correct for baseline fluctuations, aspirate the glycerol diluents before and after each aspiration of calibrators and specimen and reset the baseline to zero as required. Aspirate the copper working calibrators sequentially from the most to the most concentrated, aspirating until the reading is stable (within $\pm 0.004A$). The resulting values are used to establish the calibration curve by use of a least-square regression fit. Aspirate the specimens and the serum control. Calculate specimen concentration form absorbance readings by interpolation from the calibration curve.

STATISTICAL ANALYSIS

The data was analyzed by basic measures of central tendency and dispersion (mean and standard deviation). Correlation is seen for magnesium and copper with FBS, PPBS and HbA1c by using Karl Persons correlation coefficient. The significance of the study was tested by using student's t-test. Means were considered significantly different where $p < 0.05$.

RESULTS

The FBS, PPBS, HbA1c, concentrations are statistically higher as expected in the Diabetic Nephropathy cases than controls. The Mean blood magnesium levels in Diabetic Nephropathy cases 1.62 ± 0.32 are significantly lower ($p < 0.005$) than those found in control subjects 2.142 ± 0.16 . The mean zinc levels in Diabetic Nephropathy cases 55.45 ± 33.53 were not significantly different from those found in controls subjects 60.73 ± 12.3 $\mu\text{g/dl}$ ($p > 0.05$). However, the mean blood copper levels in Diabetic Nephropathy cases 165.42 ± 5.71 were not significantly different from those found in control subjects 166.6 ± 5.48 . (Table 1 & Figure-1, 2). Diabetes as such has been reported to alter copper, zinc and magnesium status, although differences in trace element levels occurring as a result of diabetes have not been confirmed¹⁰. (figure1-2 show)

Table: FBS, PPBS, HbA1c, Copper, Zinc & Magnesium concentrations in all subjects (60)

Serial No:	Parameters	Diabetic Nephropathy	Controls	p Value	Reference Value
1	FBS(mg/dl)	170.23±40.61	92.8±9.14	<0.005	70-110
2	PPBS(mg/dl)	265.70± 38.66	128.72± 6.8	<0.005	90-140
3	HbA1C (%)	9.31± 1.82	4.90± 0.68	<0.005	4-6%
4	Magnesium(mg/dl)	1.80 ± 0.29	2.40 ± 0.29	<0.005	1.5 – 2.6
5	Copper(μ/dl)	150.42± 5.40	156± 5.70	>0.05	100-200
6	Zinc(μ/dl)	55.45 ± 33.53	60.73±12.3	> 0.05	60-120

Figure:1 Correlation between the cases and controls of FBS, PPBS and HbA1 levels

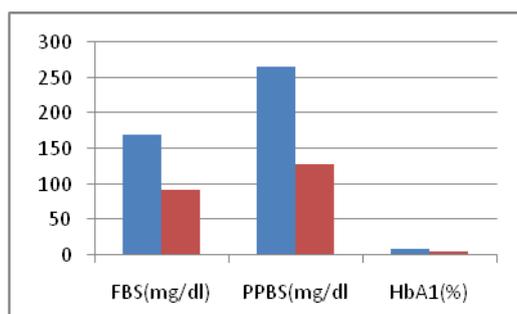
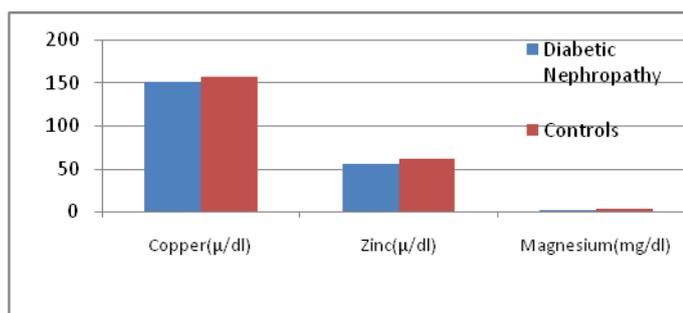


Figure:2 Variation of the cases and controls of Trace elements Copper, Zinc and magnesium



The role of copper and Zinc in glucose homeostasis is not well defined, but impairment of glucose tolerance can be secondary to copper, zinc deficiency. However in contrary to this, the present study revealed no significant relation between Diabetic Nephropathy cases and the controls. So, in Diabetic nephropathy cases because of chronic hyperglycemia there will be more Oxidative stress which can be the result with depletion of micro and cellular elements like magnesium with anti oxidative properties. The best known interaction in trace element metabolism is the reported antagonism between copper and zinc.¹⁴ Excessive dietary zinc is reported to induce copper deficiency by several mechanisms,

all involving induced synthesis of an intracellular binding protein, metallothionein. Excessive intake of zinc is thought to induce synthesis of the protein, resulting in sequestering of both metals, with subsequent excretion when cells are sloughed into the intestinal lumen. Thus, the protective mechanism preventing zinc toxicity also results in copper deficiency. Hormonal influences may also lead to apparently antagonistic zinc copper interactions. Carbohydrate-active steroids and a mononuclear phagocyte produced hormone, interleukin-1, enhance intracellular zinc accumulation while increasing intracellular copper efflux as caeruloplasmin [11].

CONCLUSION

The present study revealed that magnesium had a significant negative relation with Diabetic Nephropathy cases, but the other trace element copper and Zinc has no relation. We conclude that magnesium depletion reduces insulin sensitivity and by increasing oxidative stress, may contribute to the development of vascular complications in diabetic patients. It may be prudent in clinical practice to periodically monitor serum magnesium concentrations in diabetic patients to observe risk of complications.

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