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COMPARATIVE STUDY OF SERUM ZINC, MAGNESIUM AND COPPER LEVELS AMONG PATIENTS OF TYPE 2 DIABETES MELLITUS WITH AND WITHOUT MICROANGIOPATHIC COMPLICATIONS

Meenakshi Puri, Uma Gujral, S.B. Nayyar

Department of Biochemistry, Govt. Medical College, Amritsar, Punjab, India.

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Corresponding Author:
Meenakshi Puri,
Department of Biochemistry, Govt. Medical College, Amritsar, Punjab, India.

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ABSTRACT

A number of studies have shown that patients with type 2 diabetes mellitus are more prone to develop microangiopathic complications. The patients of clinically diagnosed type 2 diabetes mellitus were divided into 2 groups, based on the presence or absence of at least 2 out of 3 microangiopathic complications, namely, diabetic retinopathy, diabetic nephropathy and peripheral neuropathy. Then the patient in each group (n=50) were subjected to tests for estimation of Zinc, Magnesium and Copper concentration in serum. Hypozincaemia was seen in all diabetic subjects, but the decrease in levels was more in the group with microangiopathic complications (81.16±24.34 vs. 92.01±20.17; $p<0.05$). Hypomagnesaemia was also more significant in the group of patients with microangiopathic complications (1.64±0.67 vs. 2.09±0.56; $p<0.001$). Hypercupremia was seen in all the patients, but the increase in levels was more significant in the group with microangiopathic complications (140.64±33.61 vs. 116.77±26.22; $p<0.001$). Hypozincaemia and hypomagnesaemia in type 2 diabetes is said to be due to hyperglycaemia that promotes increased excretion of these trace elements in urine. The glycated proteins seen in type 2 diabetes mellitus patients have an increased affinity for copper, leading to hypercupremia. This bound copper is redox active and leads to production of free radicals that cause oxidative stress which, plays some role in the development and progression of microangiopathic complications of type 2 diabetes mellitus.

INTRODUCTION

Type 2 diabetes mellitus is on the track to become one of the major global health challenges of the 21st century (1). Diabetes mellitus is characterized by hyperglycaemia due to absolute or relative deficiency of insulin (2), leading to impaired metabolism of carbohydrates, proteins, fats, water and electrolytes. The persistence of these metabolic disturbances lead to permanent and irreversible functional and structural changes in the cells of the body which in turn lead to the development of "diabetic complications", characteristically affecting, the cardiovascular system, eye, kidney and nervous system mainly (3).

Chronic complications of diabetes mellitus can be divided into vascular and nonvascular complications. The vascular complications of DM are further subdivided into microvascular (retinopathy, neuropathy, nephropathy) and macrovascular complications [coronary artery disease (CAD), peripheral arterial disease (PAD), cerebrovascular disease] (4). Several of the complications of diabetes may be related to increased intracellular oxidants and free radicals associated with decrease in intracellular zinc and zinc dependent antioxidant enzymes (5). Low serum magnesium levels may contribute to the evolution of diabetic complications such as retinopathy, abnormal platelet function, cardiovascular disease and hypertension via reduction in the rate of inositol transport and subsequent intracellular depletion (6). Patients with severe diabetic retinopathy have lower magnesium levels than do diabetic patients with minimal retinal changes, which suggests that hypomagnesaemia may be a risk factor in development of diabetic retinopathy (7). Abnormal copper metabolism is associated with human and experimental diabetes. Diabetic rats have elevated Cu concentration in plasma, liver and kidney compared to controls (8). 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 (9) , that are involved in the pathogenesis of diabetic complications.

Impaired insulin release, altered insulin action and increased glucose intolerance in experimental animals and human subjects with diabetes mellitus have been linked to a deficit in the cellular availability of magnesium as well as other minerals including chromium, selenium, vanadium

and zinc. A deficiency of magnesium, which is involved in more than 300 enzymatic reactions throughout the body, would be expected to negatively impact essential biochemical processes (10). Diabetes and poor glycaemic control alters the metabolism of zinc and magnesium by increasing their urinary excretion and lowering serum zinc and magnesium levels (5). Zinc is critical for the function of a number of metalloproteins, including members of oxido-reductase, hydrolase, ligase, lyase family and has co-activating functions with copper in superoxide dismutase and phospholipase C (11).

According to Grafton and Baxter (12), hypomagnesaemia leads to reduction of inositol transport and subsequent inositol depletion that might enhance the development of diabetic complications. Hypomagnesaemia has also been postulated as a possible risk factor in the development and progression of diabetic retinopathy (13,14). Glycated proteins which are higher in the diabetic patients, have an increased affinity for transition metals such as copper (15,16). The bound copper can be redox active and participate in oxidation-reduction reactions including the production of free radicals that in turn can contribute to increased oxidative stress in diabetes (17).

Thus levels of serum zinc, magnesium and copper are affected in patients suffering from type 2 diabetes mellitus. The present study was designed in order to estimate the alterations in the levels of these elements in type 2 diabetes mellitus patients with microangiopathic complications in comparison to those without these complications and there relation with duration of disease and glycaemic control.

MATERIAL AND METHOD

In the present study, 50 patients (Group A) in the age group of 31 – 70 years, diagnosed as type 2 diabetes mellitus with microangiopathic complications, on the basis of history, clinical symptoms and duration of disease were compared with another 50 patients (Group B), age and sex matched from the same population, suffering from type 2 diabetes mellitus without microangiopathic complications. Patients suffering from any 2 out of the 3 microangiopathic complications namely – diabetic retinopathy, diabetic nephropathy and diabetic neuropathy were included in this study. Subjects suffering from renal disease, hepatic disease, severe congestive heart failure and those taking trace elements were excluded from the study. Informed consent was obtained from all the participants of the study and the protocol was approved by the ethical committee.

Hemolysed samples were excluded from the study. Fasting blood samples were collected in sterile, dry and acid washed vials. Fasting blood glucose was estimated by GOD/POD method (18). Serum zinc and magnesium were estimated by colorimetric kit method (19,20). Serum copper was also estimated by colorimetric kit method (21). Also a spot urine sample was collected from each patient to estimate urinary creatinine and protein. Urinary creatinine was estimated by colorimetric kit methods (22). Urinary protein was estimated by colorimetric kit method utilizing pyragallol red. Protein creatinine ratio (PCR) was then calculated which is commonly used index to assess diabetic nephropathy. Diabetic retinopathy and neuropathy were diagnosed by fundoscopy and general physical examination of each patient.

STATISTICAL ANALYSIS

Statistical significance was analyzed by students 't' test and correlation between variables were studied by using Pearson's correlation coefficient test. p values less than 0.05 were considered significant.

RESULT

As seen in Table 1, all the diabetic patients with microangiopathic complications (Group A) had significantly lower levels ($p<0.05$) of serum zinc than in diabetic patients without microangiopathic complications (Group B). The variation in zinc levels ($p<0.05$) in patients of 41 to 50 yrs of age in both Group A and B was not statistically significant. Also upon comparison, the level of zinc ($p>0.05$) among patients of 51 to 60 years of age in Group A and B were statistically insignificant. As from Table 1, it was clear that, alteration in zinc levels ($p>0.05$) in accordance with duration of disease among the patients of the 2 groups was insignificant.

In Table 2, all the diabetic patients in Group A had significantly lower levels ($p<0.05$) of magnesium than in patients of Group B. The variation in magnesium levels ($p<0.05$) in patients of 41 to 50 yrs of age in both Group A and B was not statistically significant. Also upon comparison, the level of magnesium ($p>0.05$) among patients of 51 to 60 years of age in Group A and B were statistically insignificant. In Table 2, the alteration in magnesium levels ($p>0.05$) were not statistically significant among patients of Group A and B in accordance with duration of disease.

In Table 3, the levels of copper were significantly increased ($p<0.001$) in patients of Group A in comparison to Group B. However, the variation in copper levels ($p<0.05$) in patients of 41 to 50 yrs of age in both Group A and B was not statistically significant. Also upon comparison, the level of copper ($p>0.05$) among patients of 51 to 60 years of age in Group A and B were statistically insignificant. Further, the alteration in copper levels ($p>0.05$) were not statistically significant among patients of Group A and B in accordance with duration of disease.

As seen in Table 4, the fasting blood glucose levels ($p<0.01$) were significantly increased in patients belonging to Group A as compared to those in Group B. Microproteinuria is also significantly higher ($p<0.001$) in patients of Group A, in comparison to Group B. However creatininuria is statistically insignificant ($p>0.05$), upon comparison among patients in both the groups. The rise in Protein Creatinine Ratio (PCR), is highly significant ($p<0.001$) in patients belonging to Group A than in those belonging to Group B.

Table 1: Comparative analysis of serum zinc levels among patients of type 2 diabetes mellitus with and without microangiopathic complications.

	Group A (n=50)	**Group B (n=50)**
Serum zinc (µg/dl)	81.16 ± 3.44	92.01 ± 2.85*
Serum zinc levels in different age group		
Group I	---	102.00 ± 5.95
Group II	105.48 ± 12.23	90.49 ± 2.85**
Group III	86.21 ± 5.16	88.06 ± 6.76**
Group IV	75.02 ± 4.59	---
Serum zinc levels according to duration of disease		
1 to 3 yrs	81.33 ± 8.16	91.55 ± 3.30**
4 to 6 yrs	83.11 ± 7.91	92.41 ± 5.79**
7 yrs and above	80.37 ± 4.45	92.72 ± 8.44**

*Values are mean ± SE, Values in parenthesis represent sample size. Statistical comparison was done among patients of type 2 diabetes mellitus with (Group A) and without (Group B) microangiopathic complications. Group I – 31 to 40 yrs, Group II – 41 to 50 yrs, Group III – 51 to 60 yrs, Group IV – 61 to 70 yrs. * p<0.05, ** p>0.05. Normal range of serum zinc : 50 to 150 µg/dl.*

Table 2: Comparative analysis of serum magnesium levels among patients of type 2 diabetes mellitus with and without microangiopathic complications.

	Group A (n=50)	Group B (n=50)
Serum magnesium (mg/dl)	1.64 ± 0.09	2.09 ± 0.08*
Serum magnesium levels in different age groups		
Group I	---	2.34 + 0.13
Group II	2.09 + 0.75	2.05 + 0.11**
Group III	1.72 + 0.13	2.00 + 0.16**
Group IV	1.55 + 0.14	---
Serum magnesium levels according to duration of disease		
1 to 3 yrs	1.79 + 0.21	2.14 + 0.11**
4 to 6 yrs	1.52 + 0.23	2.05 + 0.13**
7 yrs and above	1.64 + 0.12	2.02 + 0.22**

*Values are mean ± SE, Values in parenthesis represent sample size. Statistical comparison was done among patients of type 2 diabetes mellitus with (Group A) and without (Group B) microangiopathic complications. Group I – 31 to 40 yrs, Group II – 41 to 50 yrs, Group III – 51 to 60 yrs, Group IV – 61 to 70 yrs. * p<0.001, ** p>0.05. Normal range of serum magnesium : 1.8 to 3.0 mg/dl.*

Table 3: Comparative analysis of serum copper levels among patients of type 2 diabetes mellitus with and without microangiopathic complications.

	Group A (n=50)	Group B (n=50)
Serum copper (µg/dl)	140.64 ± 4.75	116.77 ± 3.71*
Serum copper levels in different age groups		
Group I	---	123.91± 5.74
Group II	130.23 ± 15.83	130.31 ± 5.39**
Group III	170.13 ± 7.67	106.37 ± 6.27**
Group IV	147.18 ± 5.88	---
Serum copper levels according to duration of disease		
1 to 3 yrs	144.45 ± 9.71	115.76 ± 5.33**
4 to 6 yrs	146.02 ± 9.98	117.24 ± 6.96**
7 yrs and above	125.71 ± 6.35	116.63 ± 8.16**

*Values are mean ± SE, Values in parenthesis represent sample size. Statistical comparison was done among patients of type 2 diabetes mellitus with (Group A) and without (Group B) microangiopathic complications. Group I – 31 to 40 yrs, Group II – 41 to 50 yrs, Group III – 51 to 60 yrs, Group IV – 61 to 70 yrs. * p<0.001, ** p>0.05. Normal range of serum copper: 100 to 200 µg/dl.*

Table 4: Comparative analysis of fasting blood glucose, Microproteinuria, creatininuria and protein- creatinine ratio(UP/UC) among patients of type 2 diabetes mellitus with and without microangiopathic complications.

	Group A (n = 50)	Group B (n = 50)
Fasting blood glucose (mg/dl)	183.39 ± 7.70	154.84 ± 6.90*
Microproteinuria (mg/dl)	18.55 ± 2.22	1.86 ± 0.23***
Creatininuria (mg/dl)	86.29 ± 8.23	73.41 ± 5.21**
Protein creatinine ratio (µg/mg of creatinine)	208.67 ± 12.66	24.77 ± 1.94***

*Values are mean ± SE, Values in parenthesis represent sample size. Statistical comparison was done among patients of type 2 diabetes mellitus with (Group A) and without (Group B) microangiopathic complications. * p < 0.01, ** p > 0.05, *** p <0.001.*

DISCUSSION

Diabetes mellitus is an endocrinological disease having metabolic and oxidative stress in high quantity. Findings show that oxidative stress has the greatest role in development of the complications (23). Zinc, an essential trace element, is useful in synthesis, storage and secretion of insulin (24). The predominant effect on zinc homeostasis of diabetes is hypozincaemia which may be the result of hyperzincuria or decreased gastrointestinal absorption of zinc or both (5). Zinc is necessary factor in a variety of "antioxidant" enzymes, particularly superoxide dismutase, catalase and peroxidase, alterations of zinc metabolism such that adequate zinc is unavailable for these enzymes might be expected to contribute to the tissue damage observed in diabetes (25). Zinc has antioxidant properties; thus it can stabilize macromolecules against radical induced oxidation (26). Hyperglycemia and hyperinsulinemia increases the production of free radicals and there is evidence that lipid peroxidation is increased in type 2 diabetes mellitus patients (27). In diabetic patients, zinc supplementation decreased lipid peroxidation (28). The present study was undertaken to ascertain whether zinc levels were altered to a greater degree in patients of type 2 diabetes mellitus with microangiopathic complications in comparison to patients without microangiopathic complications. As seen in this study, Zn levels were lower (Table 1) in patients of type 2 diabetes mellitus with microangiopathic complications than those without these complications. Similar results were reported by Walter RM et al in their study (29). However contradictory findings have been observed in other studies according to which there was no significant difference in serum zinc levels among the type 2 diabetic patients (30). The different results in the above mentioned studies indicate that further research is required, with greater number of patients.

Magnesium acts as a cofactor in the glucose transporting mechanism of the cell and also plays an important role in glucose metabolism by acting as a critical cofactor for the activities of various enzymes involved at multiple steps in insulin secretion, binding and activity (31). Hypomagnesemia defined by low serum magnesium concentration has been reported to occur in 13.5 to 14.7% of non-hospitalised patients with type 2 diabetes compared with 2.5 to 15% among their counterparts without diabetes(32). Not only has hypomagnesemia been associated with type 2 diabetes, but also numerous studies have reported an inverse relationship between glycaemic control and serum magnesium levels (33). Diabetic hypomagnesaemia may be attributed to 2 factors, namely, the osmotic action of glucosuria and the hyperglycemia per se, the latter being known to depress the net tubular reabsorption in normal man (34). Low serum magnesium levels may contribute to the evolution of diabetic complications such as retinopathy, abnormal platelet function, cardiovascular disease and hypertension via reduction in the rate of inositol transport and subsequent intracellular depletion (35).

The link between hypomagnesaemia and diabetic retinopathy was reported in two cross- sectional studies that involved both "insulin dependent" patients and patients with type 2 diabetes. Not only did patients with diabetes have lower serum Mg levels compared with their counterparts without diabetes, but also the serum Mg levels among the cohort with diabetes had an inverse correlation with the degree of retinopathy (36). The kidney plays a major role in magnesium homeostasis and in maintenance of magnesium concentration (37). In addition to osmotic action of glucosuria, hypomagnesemia may also occur following insulin therapy for diabetic ketoacidosis and may be related to the anabolic effects of insulin driving magnesium back into cells (38) In a comparative study that involved 30 patients who had type 2 diabetes mellitus without microalbuminuria, 30 with microalbuminuria, and 30 with overt proteinuria, Corsonello et al (39) observed a significant decrease in serum ionized Mg in both the microalbuminuria and overt proteinuria groups compared with the non- albuminuric group. There are also data to suggest the association between hypomagnesmia and other diabetic complications including neurological abnormalities and dyslipidaemia (40). As seen in this study, magnesium levels were lower (Table 2) in patients of type 2 diabetes mellitus with microangiopathic complications in

comparison to patients without these complications. Similar results were reported by previous studies (41).

Transition metal like copper has affinity to bind with proteins that have been glycated. Generally, serum concentration of copper and ceruloplasmin is elevated in type 2 diabetes mellitus patients (42). 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 (43). Furtermore the incubation of ceruloplasmin with glucose reportedly causes fragmentation and time dependent release of its bound Cu^{2+} , which then appears to participate in a Fenton type reaction to produce hydroxyl radicals (44). The redox active metal ions (Cu^{2+} and Fe^{3+}) have been implicated in catalyzing the autoxidation of glycoaldehyde and generation of hydroxyl radical, leading to production of glyoxal and associated α - oxoaldehyde derived AGE (Advanced glycosylation end products) formation (45). A wealth of experimental evidence supports the hypothesis that AGES formed from glyoxal, methylglyoxal and 3- deoxy glucosone have an etiological role in the development of diabetic complications and other diseases (46). As seen in this study, the copper levels were higher (Table 3) in type 2 diabetes mellitus patients with microangiopathic complications as compared to those patients without these complications. In another study undertaken by a group of investigators, elevated serum copper levels were not correlated with the duration of diabetes, but levels were higher in older patients and in those with complications (47), thus supporting this study. However , studies showing contradictory findings, emphasizing no alteration in serum copper levels in diabetics are present (48). Copper, bound to glycated proteins, may blunt normal EDRF dependent relaxation of diabetic arteries and provide a rationale for the use of transition metal chelators in therapy of diabetic vasculopathy and neuropathy (15).

In addition to the above mentioned findings, as shown in Table 5, it was also observed that, peripheral neuropathy was present in 94% patients of Group A and in 56% patients of Group B. Diabetic retinopathy was seen in 96% patients of Group A and in 12% patients of Group B. Results also showed that diabetic nephropathy was present in 100% patients of Group A and only in 8% patients of Group B.

Table 5: Periphral Neuropathy, Diabetic Retinopathy and Diabetic Nephropathy in patients of type 2 diabetes mellitus with and without microangiopathic complications.

Microangiopathic Group B	Positive cases in Group A	Positive cases in
Complication	**(n = 50)**	**(n = 50)**
Periphral Neuropathy	47 (94.0%)	28 (56.0%)
Diabetic Retinopathy	48 (96.0%)	6 (12.0%)
Diabetic Nephropathy	50 (100.0%)	4 (8.0%)

TABLE – 6Distribution of patients of Group A and B into subgroups based on age group of patients (Group I to IV) and based on duration of disease:

Number of subjects		
1.Age Group	**Group A**	**Group B**
Group I (31-40 yrs)	nil	10
Group II (41-50 yrs)	2	24
Group III (51-60 yrs)	22	16
Group IV (61-70 yrs)	26	nil
2.Duration of disease	**Group A**	**Group B**
1 to 3 yrs	10	27
4 to 6 yrs	11	12
7 & above	29	11

In conclusion, whether the above mentioned alterations are the cause or consequence of diabetes mellitus remains yet to be ascertained but its strong association with type 2 diabetes mellitus and its complications signifies the role played by zinc and magnesium in glucose disposal. The free radical damage caused by increased copper levels in patients of type 2 diabetes mellitus also contributes in worsening of the complications. All these observations suggest that serum zinc, magnesium and copper estimation should be a part of the screening panel in the risk detection and progression of diabetic complications. It has also been documented that zinc and magnesium supplementation plus chelation therapy for copper, in addition to other nutritional treatment, may prove beneficial in delaying the further progress of diabetic complications.

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