Copper, zinc and magnesium levels in type-1 diabetes mellitus

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ABSTRACT

Objective: Alterations in plasma concentrations of several trace elements have been reported to occur in type-1 diabetes mellitus. These micronutrients are suspected to have a role in pathogenesis and progression of the disease.

Methods: In a comparative analysis, the plasma concentration of copper, zinc and magnesium was estimated in 37 patients with type-1 diabetes mellitus and 25 healthy non-diabetic subjects at Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Kashmir, India. Trace elements were estimated using a GBC 902 double beam atomic absorption spectrophotometer.

Results: Mean plasma concentrations of copper and magnesium were comparable between diabetic patients and control subjects. Plasma zinc levels were significantly higher (P=0.022) in diabetic patients (17.78 ± 0.6 µmol/L) as compared to controls (15.80 ± 0.75 µmol/L). Glycemic control and presence of microalbuminuria did not influence the plasma levels of copper, zinc and magnesium.

Conclusion: Plasma zinc levels are significantly higher in type-1 diabetes mellitus patients, while plasma copper and magnesium levels are not significantly altered. No effect of sex, glycemic control or presence of microalbuminuria could be demonstrated on plasma concentration of trace elements in type-1 diabetes mellitus patients.

Keywords: Type-1 diabetes mellitus, copper, zinc, magnesium.

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Many trace elements are important for optimum human metabolic function. These micronutrients serve a variety of functions including catalytic, structural and regulatory activities in which, they interact with macromolecules such as enzymes, prohormones, presecretory granules and biological membranes.[1] There is accumulating evidence that the metabolism of several trace elements are altered in type-1 diabetes mellitus (DM) and that these nutrients might have specific roles in the pathogenesis and progression of this disease.[2] Plasma copper levels has been found to be elevated in type-1 DM patients, while the urinary excretion of copper has been found to be affected by DM.[2,3] Zinc deficiency has been shown to increase the risk of diabetes in diabetes prone experimental animals.[4] Increase in plasma zinc levels has been reported in diabetic patients who were previously treated with insulin.[5] Hypomagnesemia has been reported in both type-1 and type-2 DM patients.[6,7] This study evaluated the plasma levels of copper, zinc and magnesium in patients with type-1 DM and the influence of glycemic control, microalbuminuria and sex.

Methods. Thirty-seven patients with type-1 DM attending the diabetic clinic of Sher-i-Kashmir

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Institute of Medical Sciences, Srinagar, Kashmir, India, and 25 healthy non-diabetic volunteers (with normal oral glucose tolerance test) constituted the study population. The diagnosis of type-1 DM was made in patients who 1. had DM according to the World Health Organization (WHO) study group criteria[8] 2. were lean, thin and aged < 30 years, at the time of diagnosis 3. had stormy onset of osmotic symptoms. 4. had history of diabetic ketoacidosis either at the onset of diabetes or on withdrawal of insulin later on. 5. required insulin therapy for control of hyperglycemia/prevention of ketoacidosis from the diagnosis of diabetes and 6. had no pancreatic calculi documented either on plain x-ray abdomen or on ultrasonography (US). Type-1 DM patients with overt proteinuria or azotemic nephropathy were excluded from the study; however, patients with microalbuminuria (urinary albumin excretion 30-300mg/day) were included. Age, sex and anthropometric parameters [height, weight, body mass index (BMI) and waist to hip ratio (WHR)] were recorded in all subjects. In diabetic patients' notes were made of the duration of diabetes, history of diabetic ketoacidosis, type and dose of insulin used and evidence of neuropathy, nephropathy or retinopathy. All diabetic patients were asked to stop insulin 48 hours prior to taking blood samples for trace element estimation. None of the subjects were receiving any trace element supplementation. After an overnight fast venous blood samples were drawn in the morning for estimation of blood counts, glucose, urea, creatinine, calcium, phosphorous, albumin, total proteins, cholesterol, fructosamine and trace elements (copper, zinc and magnesium). In addition, urine examination, 24-hour urinary protein estimation, creatinine clearance, 12 lead electrocardiogram and chest x-ray were carried out in all diabetic patients. First void morning urine sample was collected for estimation of microalbuminuria using Micral type II test strips from Boehringer Mannheim Diagnostica, Germany. Serum fructosamine levels were estimated to assess the glycemic control by reduction test with nitroblue tetrazolium (NBT), based on the principle of conversion of NBT to formazan by fructosamine. The reaction was catalyzed by uricase, and carbonate buffer was used to attain an alkaline pH of 10.3. Color changes, thus observed are proportional to serum fructosamine concentration. Measurements were made against a standard using quality control by precinorm fructosamine for normal range and precipath fructosamine for pathological range. Estimations of copper, zinc and magnesium levels were performed by a Graeme B. Chappel 902 double beam atomic absorption spectrophotometer (a scientific equipment in Victoria, Australia). Heparinized zinc and copper free, polypropylene tubes were used to collect venous blood for estimation of trace elements. Plasma was separated after centrifugation at 1500g for 10 minutes. Plasma was diluted with an equal volume of trichloroacetic acid to precipitate proteins, and the precipitate was kept at zero temperature for 10 minutes. The supernatant was directly aspirated in to the spectrophotometer. The instrument was calibrated using standards from Sigma, St. Louis, MO, United States of America (USA). For magnesium estimation, plasma was diluted to one in 200 with distilled water and aspirated into the spectrophotometer for analysis. Analytic reliability was determined by quality control sera obtained from Boehringer Mannheim Diagnostica, Germany. Statistical analysis was carried out with Statistical Package for Social Sciences (SPSS) version 6 on an IBM compatible PC with windows 98 as operating system. All data are expressed as mean ± standard error of mean (SEM). A 2 tailed p-value was calculated and a p-value of less than 0.05 taken as significant.

Results. Thirty-seven patients with type-1 DM (22 men and 15 women, mean age ± SEM 21.78 ± 1.22 years) and 25 healthy non-diabetic volunteers (16 men and 9 women, mean age ± SEM 35.08 ± 1.43 years) were studied. The mean duration of DM were 4.99 ± 0.75 years. The mean BMI and WHR were 17.59 ± 0.40 kg/m^2 and 0.84 ± 0.01 in the diabetic group and 23.34 ± 0.75kg/m^2 and 0.95 ± 0.009 in the control group. Mean plasma concentration of copper, zinc and magnesium in type-1 DM patients and non-diabetic controls are depicted in **Table 1**. Type-1 DM did not significantly influence plasma copper and magnesium levels, however, plasma concentration of zinc was significantly elevated in type-1 DM patients (p=0.022). The plasma levels of copper, zinc and magnesium were comparable in patients with well-controlled (fructosamine ≤285 µmol/L) and uncontrolled (fructosamine >285 µmol/L) type-1 DM (**Table 2**). Presence of microalbuminuria did not influence the plasma concentration of copper, zinc

Table 1 - Comparison of plasma levels of copper, zinc and magnesium in control subjects and patients with type-1 diabetes mellitus.

Parameter	Controls (N=25)	Type-1 DM (N=37)	P-value
Copper (µmol/L)	13.91 ± 0.55	15.10 ± 0.60	0.163
Zinc (µmol/L)	15.80 ± 0.75	17.78 ± 0.60	0.022
Magnesium (mmol/L)	0.92 ± 0.04	0.98 ± 0.06	0.124

N - number,
DM - diabetes mellitus,
data are mean ± standard error of mean.

and magnesium (**Table 3**). Sex did not influence the trace element concentration either in type-1 DM patients or controls (**Table 4**).

Discussion. Interest in the biochemical and clinical consequence of trace element metabolism has been steadily increasing over the last 3 decades. Trace elements have important physiological effects when present at concentrations other than those associated with classical toxicity or with extreme deficiency.[1] Many studies have reported significant though variable alteration in trace element concentration in type-1 and type-2 DM patients.[2,3,5,6] In a recent study, we documented that plasma copper, zinc and magnesium levels were comparable between patients with fibrocalculous pancreatic diabetes and normal control subjects.[9] Copper, an essential trace element, plays an important role in cytochrome oxidase function in the mitochondria. Copper deficiency results in swelling and subsequent disruption of the mitochondria of metabolically active tissues like hepatocytes and pancreatic acinar cells.[1] In our study, copper levels were comparable between type-1 DM patients and non-diabetic controls. Glycemic control and presence of microalbuminuria did not affect the plasma copper levels. Earlier, we have documented elevated plasma copper levels in type-2 DM patients[10]. Conflicting results have been reported regarding the copper levels in type-1 DM, both elevated as well as decreased plasma copper concentrations had been reported.[11,12] Studies on zinc status in type-1 DM have shown contradictory results. Zinc is essential for many enzymes involved in the human metabolism and plays a role in the biosynthesis and storage of insulin in the ß-cells. Significantly, low plasma zinc levels have been reported earlier.[11,13] In fact, one study has suggested that low zinc consumption through drinking water is associated with later development of childhood onset DM.[14] Surprisingly, we found significantly elevated levels of zinc in our type-1 DM patients compared to controls. Increased serum zinc levels have been documented in patients with type-1 DM previously treated with insulin.[3] Our results are in accordance with this study as all our patients were receiving insulin. Glycemic control and presence of microalbuminuria did not influence plasma zinc levels in our patients significantly. 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.[15,16] The cellular physiology of magnesium homeostasis is not

Table 2 - Laboratory parameters in controlled and uncontrolled type-1 diabetes mellitus patients.

Parameter	Controlled (N=10)	Uncontrolled (N=27)	P-value
Hemoglobin (g/L)	119.00 ± 3.8	124.00 ± 4.2	0.447
Total cholesterol (mmol/L)	3.94 ± 0.24	3.91 ± 0.17	0.941
Albumin (g/L)	37.80 ± 1.9	40.80 ± 1.4	0.262
Calcium (mmol/L)	2.04 ± 0.04	2.14 ± 0.03	0.113
Fructosamine (µmol/L)	249.50 ± 11.29	405.67 ± 27.82	0.002
Creatinine clearance (ml/minute)	81.50 ± 7.60	75.63 ± 5.34	0.571
Copper (µmol/L)	15.73 ± 1.05	14.86 ± 0.74	0.530
Zinc (µmol/L)	17.18 ± 0.86	18.02 ± 0.53	0.413
Magnesium (mmol/L)	0.94 ± 0.12	1.00 ± 0.07	0.672

data are mean ± standard error of mean, N - number

Table 3 - Plasma copper, zinc and magnesium levels in type-1 diabetes mellitus patients with and without microalbuminuria.

Parameter	With microalbuminuria (N=17)	Without microalbuminuria (N=20)	P-value
Copper (µmol/L)	15.18 ± 0.90	15.02 ± 0.83	0.899
Zinc (µmol/L)	17.95 ± 0.55	17.63 ± 0.71	0.728
Magnesium (mmol/L)	1.02 ± 0.08	0.95 ± 0.09	0.590

data are mean ± standard error of mean, N - number

Table 4 - Relationship of sex with levels of copper, zinc and magnesium in control subjects and type-1 diabetes mellitus patients.

Parameter	Males	Females	P-value
Copper (µmol/L)			
Controls	13.82 ± 0.70	14.13 ± 0.85	0.842
Type-1 DM	14.24 ± 0.72	16.35 ± 0.99	0.087
Zinc (µmol/L)			
Controls	15.94 ± 0.92	15.47 ± 1.33	0.724
Type-1 DM	17.55 ± 0.60	18.10 ± 0.72	0.558
Magnesium (mmo/L)			
Controls	0.96 ± 0.04	0.89 ± 0.05	0.192
Type-1 DM	0.96 ± 0.08	1.02 ± 0.09	0.590

data are mean ± standard error of mean, DM - diabetes mellitus

fully understood. It is not clear whether DM is a state of magnesium deficiency. Intracellular magnesium depletion with significant hypomagnesemia has been reported in type-1 DM patients.[17] Isbir et al[11] had reported low serum magnesium levels in patients with type-1 DM. We did not find any significant alteration in plasma magnesium levels in our type-1 DM patients compared to controls. Glycemic control did not influence magnesium levels in our patients; however, an earlier study has reported lower magnesium levels in type-1 DM patients with poor glycemic control.[18] Contrary to earlier studies reporting hypomagnesemia in type-1 DM patients with microalbuminuria and clinical proteinuria,[19] microalbuminuria did not influence plasma magnesium levels in our study. Low levels of zinc and magnesium in type-1 DM had been reported to be associated with development of insulin resistance.[11] Improvement in glycemic control is expected to occur with trace element therapy. Dietary zinc supplementation has been reported to improve hyperglycemia in animal models (db/db mice).[20] In a study from India, oral zinc supplementation was shown to achieve better glycemic control and improve severity of peripheral neuropathy in diabetic patients.[21] Diabetes mellitus as such has been reported to alter copper, zinc and magnesium status, although changes in trace elements occurring as a result of diabetes had not been confirmed.[2]

From this study, we conclude that plasma copper and magnesium levels are not significantly altered in stable type-1 DM patients. Plasma zinc levels are significantly higher in type-1 DM patients who have been treated with insulin. Presence of microalbuminuria, glycemic control and sex did not influence the plasma concentration of any of these trace elements.

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