

SERUM MANGANESE IN CHILDREN WITH DIABETES MELLITUS TYPE 1

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Short title: SERUM MANGANESE IN DIABETES MELLITUS

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SUMMARY

Type 1 (insulin dependent) diabetes mellitus is an autoimmune disease leading to the development of diabetic vascular complications. The aim of the study was to investigate the correlation between serum manganese concentration and development of microvascular complications in diabetic children. The manganese concentration was measured by a highly sensitive catalytic method. The study included 59 diabetic children (mean age 13.2±3.3 years, diabetes duration 7.1±2.1 years), 25 of them with microvascular complications (group 1) and 34 without microvascular complications (group 2). Control group included 17 healthy children (mean age 13.1±4.1 years). Diabetic children showed statistically nonsignificantly higher manganese concentrations than control subjects (0.67±0.14 vs. 0.64±0.12 ng/ml; $p>0.05$). Group 1 showed statistically significant lower concentrations than controls (0.57±0.09 vs. 0.64±0.12 ng/ml; $p=0.045$). Group 2 showed nonsignificantly higher manganese concentrations than controls (0.69±0.10 vs. 0.64±0.12 ng/ml; $p=0.157$). Manganese concentration correlated with HbA1c ($r=0.45$; $p=0.008$), microvascular complications ($r=0.40$, $p=0.04$), and diabetes duration

($r=0.48$, $p=0.03$). We suggest that decreased manganese concentrations are associated with the development of microvascular complications in diabetic children.

INTRODUCTION

Type 1 (insulin-dependent) diabetes mellitus is a common chronic disease of childhood, which presents with acute, sometimes life-threatening, symptomatic hyperglycemia (1). Recently, epidemiologic studies have shown that type 1 diabetes is at least as great a risk factor for cardiovascular mortality as type 2 (non-insulin dependent) diabetes, and that this can also occur at a young age (2). Thus, the detection and treatment of risk factors for cardiovascular disease in type 1 diabetes are warranted. In diabetic children mainly microvascular complications, microalbuminuria and retinopathy, are present.

Manganese (Mn) plays an important role in a number of physiologic processes as a constituent of some enzymes and an activator of different enzymes (3). These manganese-activated enzymes play important roles in the metabolism of carbohydrates, amino acids and cholesterol.

Manganese superoxide dismutase (MnSOD) is the principal antioxidant enzyme of mitochondria. Because mitochondria consume over 90% of the oxygen used by cells, they are especially vulnerable to oxidative stress.

The superoxide radical is one of the reactive oxygen species produced in mitochondria during ATP synthesis. MnSOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, which can be reduced to water by other antioxidant enzymes (4).

Manganese deficiency has been observed in a number of animal species. Signs of manganese deficiency include impaired growth, impaired reproductive function, skeletal abnormalities, impaired glucose tolerance, and altered carbohydrate and lipid metabolism. In humans, demonstration of a manganese deficiency syndrome has been less clear (3,5). Low dietary manganese or low levels of manganese in blood or tissue have been associated with several chronic diseases like osteoporosis, epilepsy and diabetes mellitus.

As oxidative stress is a reason for development of diabetic complications it would be important to study the concentrations of manganese in the sera of diabetic patients. Therefore, the sera of 59 children with type 1 diabetes mellitus were tested and data were compared with those of control healthy children.

MATERIAL AND METHODS

Subjects

The baseline study population consisted of 59 patients (27 boys and 32 girls) with type 1 diabetes mellitus (mean age 13.2 ± 3.3 years) diagnosed according to the WHO definition. The control group consisted of 17 healthy, sex- and age-matched children (13.1 ± 4.1 years) with no family history of diabetes, atherosclerosis and nephropathy. All patients were treated by human insulin of Novo Nordisk Industri, Copenhagen, Denmark. None of the patients had a diagnosis of renal disease unrelated to diabetes.

The mean duration of diabetes was 7.1 ± 2.1 years. Twenty-five of 59 diabetics had microvascular complications.

Serum manganese concentration was measured by a highly sensitive catalytic method of Mutaftchiev *et al.* (6). Microalbuminuria was defined as a persistent urinary albumin excretion rate (AER) in the range from

20 to 200 $\mu\text{g}/\text{min}$ in sterile urine. Glycated hemoglobin A1c (HbA1c), total serum cholesterol, triglycerides and AER were measured as described earlier (7).

Ethical approval was obtained from the local research ethics committee and the parents of all subjects gave their informed written consent.

Statistical analysis

All values are expressed as mean \pm SD. Statistical analyses were done using the computer programs Excel and Statgraphics plus for Windows. The Student's t-test and ANOVA were used to assess differences between study groups. The correlation analysis was also used. The level of significance was set at $p < 0.05$.

RESULTS

Clinical data of the study patients are presented in Table 1. The patients were divided into two groups according to the presence (group 1) or absence (group 2) of microvascular complications. Insulin dosage and HbA1c were significantly higher in group 1 than in group 2 ($p < 0.05$). The concentrations of total cholesterol and triglycerides were higher in group 1 than in group 2, however, the difference did not reach statistical significance ($p > 0.05$).

Table 1. Clinical characteristics of 59 diabetic children divided into two groups: with microangiopathy (group 1, n=25) and without microangiopathy (group 2, n=34)

	Group 1	Group 2	p
Boys/girls	12/13	15/19	
Age (yrs)	13.2 ± 3.1	12.9 ± 3.6	NS
Diabetes duration (yrs)	6.9 ± 2.9	6.6 ± 2.5	NS
Mean glycated hemoglobin (%)	10.5 ± 1.1	9.3 ± 1.5	< 0.05
Triglycerides (mmol/l)	1.5 ± 0.55	1.4 ± 0.64	NS
Cholesterol (mmol/l)	5.2 ± 1.7	4.9 ± 1.2	NS
Systolic blood pressure (mm Hg)	117 ± 14	112 ± 11	NS
Diastolic blood pressure (mm Hg)	73 ± 9	70 ± 8	NS
Insulin dose (U/kg/24 h)	1.03 ± 0.25	0.77 ± 0.24	< 0.05
Microalbuminuria	13	0	
Retinopathy	7	0	
Microalbuminuria + Retinopathy	5	0	

Values are mean \pm SD

Manganese concentrations were nonsignificantly (0.67 ± 0.14 vs. 0.64 ± 0.12 ng/ml, $p > 0.05$) higher in diabetic than in control subjects. Twenty-five patients had diabetic microvascular complications (Table 1). Group 1 showed lowest manganese concentrations compared with group 2 ($p < 0.001$) and healthy controls ($p = 0.045$) (Table 2). Group 2 showed nonsignificantly higher manganese concentrations than healthy subjects (0.69 ± 0.10 vs. 0.64 ± 0.12 ng/ml, $p = 0.117$).

Manganese concentration correlated with HbA1c ($r = 0.45$; $p = 0.008$), microvascular complications ($r = 0.40$, $p = 0.04$), and diabetes duration ($r = 0.48$, $p = 0.03$).

Table 2. Serum manganese concentrations in diabetic children (N=59) with microangiopathy (group 1, n=25) and without microangiopathy (group 2, n=35), and in healthy controls (group 3, n=17)

Group	Mn concentration (ng/ml)	Comparison with other groups		
		1	2	3
1	0.57 ± 0.09	–	<0.05	<0.001
2	0.69 ± 0.10	<0.05	–	NS
3	0.64 ± 0.12	<0.001	NS	–

Values are mean \pm SD; NS = nonsignificant

DISCUSSION

Manganese is a trace mineral that is essential for human health. It works with enzymes, which are proteins that aid in the biochemical reactions in the cell. Manganese assists these enzymes in many reactions in the body. Some of the functions include: (i) making and activating superoxide dismutase – an antioxidant enzyme that helps protect the cell membranes and tissues from degeneration and disruption; (ii) helping the body break down carbohydrates, fats and proteins; and (iii) assisting in energy production and blood sugar.

Most of our patients with vascular complications had microalbuminuria. In patients with both retinopathy and microalbuminuria, the development of retinopathy followed the onset of microalbuminuria. The prevalence of microalbuminuria in type 1 diabetic patients is a sensitive predictor of future development of diabetic nephropathy. Microalbuminuria is a

recognized risk factor for increased mortality and renal failure in type 1 diabetes (8). Risk factors for the development of nephropathy include positive family history, male sex, poor glycemic control, hypertension, smoking, and presence of retinopathy and coronary artery disease.

Manganese helps the body metabolize glucose. People with diabetes may often have a serious manganese deficiency. Although results have been conflicting (9-11), some researchers suggest that people with diabetes have significantly lower levels of manganese in their bodies than people without diabetes. It is not clear, however, whether this is a cause or effect of the condition. In other words, researchers have yet to determine whether diabetes causes the levels of manganese to drop or deficiencies in this trace element actually contribute to the development of the metabolic disorder. In addition, diabetics with higher blood levels of manganese were better protected from oxidation of LDL cholesterol than those with lower levels of manganese. LDL oxidation contributes to the development of intra-arterial plaque, which can lead to heart attack and stroke (12).

Manganese deficiency results in glucose intolerance similar to diabetes mellitus in some animal species, but studies examining the manganese status of diabetic humans have reported inconsistent results. Whole blood manganese levels did not differ significantly between 57 type 1 or type 2 diabetics and 28 nondiabetic controls (9). There was no correlation between manganese and diabetic microvascular complications. However, urinary manganese excretion was slightly higher in 185 diabetics compared to 185 nondiabetic controls (10). Lower manganese concentrations were detected in lymphocytes derived from 53 patients with type 2 diabetes versus 50 healthy nondiabetic subjects (11). On the other hand, the activity of the antioxidant enzyme, MnSOD, was lower in the white blood cells of diabetics than of nondiabetic controls, and neither 15 mg nor 30 mg of oral manganese improved glucose tolerance in diabetics or nondiabetic controls when given at the same time with oral glucose challenge (13,14). Although manganese appears to play a role in glucose metabolism, there is

little evidence that manganese supplementation improves glucose tolerance in diabetic or nondiabetic individuals.

Our results obtained by analyzing a group of diabetic children compared with healthy subjects who donated blood confirmed that manganese concentrations were not different significantly between 59 diabetic children and 17 healthy subjects. However, diabetic children with microvascular complications showed a significantly lower serum manganese level than either diabetics without microvascular complications or healthy controls. According to our knowledge, this is the first report of an association between diabetic microvascular complications in children and serum manganese level. On the other hand, the lower serum manganese in this group of patients correlated with HbA1c and diabetes duration. The level of serum

manganese was nonsignificantly increased in diabetic children without microvascular complications as compared with healthy subjects.

In the present study we investigated manganese concentrations in the sera of diabetic children with and without microvascular complications. The contradiction with our results could be explained by the fact that the age, type of diabetes and diabetes duration of the study subjects were all different.

In conclusion, the results of this pilot study showed the serum manganese concentrations to be decreased in diabetic children with microvascular complications. The role of the finding in the development of diabetic micro- and macrovascular complications is, however, yet to be established.

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