

## **Trace element analysis of hair, nail, serum and urine of diabetes mellitus patients by inductively coupled plasma atomic emission spectroscopy**

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### **Abstract**

**Background:** Trace elements play important roles in carbohydrates and lipids metabolism. According to some studies, trace elements concentrations are different in serum and urine of diabetic and healthy population. In this work, for the first time six trace elements (Zn, Cu, Mg, Mn, Cr, Se) concentration of scalp hair, nail, urine and serum of diabetes mellitus patients and control group were analyzed at the same time. Due to the very low concentration of some trace elements (e.g. Cr, Se), measurements were carried out by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

**Methods:** In this study diabetic patients (n=150) and healthy control (n=151) were enrolled in Tehran University of Medical Sciences Hospital. Their biological samples (scalp hair, nail, urine and serum) were analyzed by ICP-AES.

**Results:** In serum samples zinc (Zn), magnesium (Mg), copper (Cu) and chromium (Cr) concentration of diabetic patients and control group were significant ( $p<0.05$ ). In urine Zn, Mg, Se, and Mn had significant difference ( $p<0.05$ ). In hair difference between amount of Zn, Mn and Cr in diabetic patients and control group were significant ( $p<0.05$ ). The difference in Zn, Se and Cr content of nail were significant ( $p<0.05$ ).

**Conclusion:** The obtained results showed that scalp hair and nail samples are the best biological samples for trace element analysis especially in case of Cr, Se, and Mn due to the high accumulation of these elements in hair and nail which causes a better detection.

**Keywords:** Trace elements, Diabetes Mellitus, Inductively Coupled Plasma Atomic Emission Spectroscopy

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## Introduction

Trace element in view of analytical chemistry is an element in a sample that has an average concentration of less than 100 parts per million (ppm) measured in atomic count, or less than 100 micrograms per gram. In biochemistry, a trace element, known also as “micronutrients”, is a chemical element that is needed in small quantities for the proper growth, development, and physiology of the organism (1).

Trace elements form part of daily diet, which are well known to play vitally important roles in the maintenance of health (2). Now days, composition of our diet has changed considerably which causes greatly to increasing incidence of many different diseases such as diabetes mellitus (3). Diabetes is estimated to afflict about 170 million people world-wide (4) and this represents about 2% of the world's population (5). In Iran, about 6% of the population is afflicted, with over 90% of these being non-insulin dependent (6).

Literature survey shows that some trace elements as chromium, magnesium, vanadium, zinc, manganese, molybdenum and selenium play important role in insulin action (7) including activation of insulin receptor sites (8), serving as cofactors or components for enzyme systems involved in glucose metabolism (9), increasing insulin sensitivity and acting as antioxidants preventing tissue per oxidation (10). Alternatively, homeostasis of trace elements can be disrupted by diabetes mellitus (11-13). Deficiencies of certain minerals such as Mg, Zn, and Cr predispose to glucose intolerance and promote the development of diabetic complications (14) such as retinopathy, thrombosis and hypertension (15-17), impaired repair of tissues and wound healing (18,19), and diabetic angiopathy (16,20). Reduction of trace elements stores might be responsible for various adverse clinical effects even with normal serum trace element concentration (21-25).

According to the above mentioned, it is important to determine the essential trace element concentrations in biological samples of diabetes mellitus patients. Biological samples such as serum, scalp hair, nail, urine, and other body fluids can be used as indicators

for this purpose. Each sample type can provide a distinctive view into the status of these important elements in diabetic and non-diabetic individuals.

There are several modern techniques for determination of very low concentrations of elements in biological samples (26, 27). Methods approved by Association of Official Analytical Chemists (AOAC) are the best procedures available for specific analyses under controlled conditions (28). The AOAC has approved trace elements determination by techniques such as fluorometry (FL), hydride generation atomic absorption spectroscopy (HG-AAS), inductively coupled plasma-mass spectrometry (ICP-MS), and inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (29). However, ICP-AES has become the most appropriate technique for trace element determination. ICP-MS has the detection power to determine trace elements at sub- $\mu\text{g L}^{-1}$  levels. Previously reports have shown that AAS is one of the most widely used methods for trace elements analysis in biological samples. However, in comparison to AAS, ICP technique has advantages of accurate, high sensitivity, a wide range of sample types, need for small sample sizes, and especially lower detection limit for trace elements having very low concentration in various biological samples (30-35).

In this study, trace elements concentrations (zinc (Zn), copper (Cu), magnesium (Mg), selenium (Se), manganese (Mn) and chromium (Cr)) in biological samples (serum, urine, scalp hair and nail) of diabetic and non-diabetic individuals were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

## Methods

### *Study Design and patients*

The study was designed to assess the serum, urine, nail and hair levels of zinc, manganese, selenium, copper, chromium and magnesium in known type 2 diabetic patients attending the diabetic clinic of Tehran University of Medical Sciences (Dr Shariati Hospital) and non-diabetic

individuals selected from apparently healthy population who they were referred to lab for check-up. Informed consent was sought and obtained from individuals before enrollment into the study. The ethics committee of Tehran University of Medical Sciences approved the study protocol. The inclusion criteria for the study were as follows: Aged 35-60 years, known as type 2 diabetic patient for the past five years, and non-diabetic considering to glucose tolerance test or FBS <90 mg/dL. Exclusion criteria were as follows; pregnancy in diabetic group and control, presence of renal complications and hypertension, chronic illness, medication including diuretics and nutritional supplement. A total of 301 people were recruited for the study. 150 known type 2 diabetic patients (53 males and 80 females) and 40 non-diabetic individuals (50 males and 25 females) were used as control. Body weight and height were measured and used to calculate the body mass index (BMI), which was used as a measure of relative body weight. A structured questionnaire was used to obtain data on, alcohol consumption, history of hypertension, past and present illness and medication (Table 1).

### **Sample collection**

After an overnight fast, 10 mL blood was taken in the morning from each individual for fasting plasma glucose, hematocrit, serum zinc, manganese, selenium, copper, chromium and magnesium determination. Assay for serum urea and creatinine were also done to test for renal function. Fasting spot urine samples were also collected into sterile chemically clean universal container. Serum and urine were collected in to metal free plastic tube and a certain amount of nitric acid was added to the tubes. Urine trace elements concentrations are expressed per gram of creatinine. Toe nail clippings from all 10 toes were collected within 8 weeks of inclusion in the study and were stored in small plastic bags at room temperature. Scalp hair samples were obtained using stainless-steel scissors from the occipital region. The hair samples were cut into approx 3 cm pieces in length.

### **Sample Preparation**

Solid sample of hair and nail were washed immediately with distilled water and then alcohol dried and stored. The certain amounts of nail and hair samples were weighed carefully and transfer in to a beaker and added 10 mL of concentrated nitric acid. Then, the solutions were heated for complete digestion. Next, the resulted solution diluted in a volumetric flask with distilled water. Certain amount of blood and urine samples were also mixed with concentrated nitric acid to remove interfering of organic compounds, the resulted solution were then centrifuged. The trace elements amount of each samples were measured by ICP-AES instrument using calibration method.

### **Instrumentation**

A Varian (model: VISTA-MPX) inductively coupled plasma atomic emission spectrometer (ICP--OES) was used for analysis. The operation conditions are summarized in Table 1. The pH values were measured with a Metrohm pH-meter (model: 713, Herisau, Switzerland) supplied with a glass-combined electrode.

### **Statistical analysis**

The significance of difference in trace elements level in samples between two groups was tested using t-test analysis. Association between variables was determined using the Pearson's correlation analysis on Microsoft excel and SPSS soft ware 16.0 version (California Inc.). A two sided P value <0.05 was considered statistically significant for the t-test and Pearson correlation analysis respectively.

### **Results**

General characteristics and laboratory finding of the population studied are shown in Table 1. The mean age diabetic patient was 51.6 vs. 49.6 years in non-diabetic individuals with an approximately equal number of men and women.

The mean age, sexes of the population were statistically similar. However, there was significant difference in the mean BMI of the diabetic patient when compared with the

control group. Fasting plasma glucose, Hb A1c, serum and urine creatinine) was significantly ( $p>0.05$ ) higher in diabetics than non-diabetic subjects.

In type 2 diabetic group, serum zinc, magnesium and chromium levels were found to be significantly low and serum copper level was significant higher in diabetics as compared to the non-diabetic group ( $p<0.05$ ), whereas there was no significant difference in serum manganese and selenium levels in diabetics and non diabetics (Table 2).

The urine zinc, magnesium, selenium and manganese level was significantly ( $p<0.05$ ) higher in diabetics than non-diabetics. The

urine chromium level was higher in diabetics than in non-diabetics, but these differences were not statistically significant ( $p>0.05$ ) (Table 3).

As shown in table 4 and 5, nail and hair zinc and chromium concentrations were significant lower in diabetic than non-diabetics. Nail selenium and hair manganese concentration was significant difference in two groups.

A significant relationship was not observed between fasting blood sugar, Hb A1C, and trace elements concentration in biological samples. The elements also showed no significant correlations with each other.

**Table 1. The descriptive data and laboratory finding of diabetics and non-diabetic subjects**

Data	Diabetics (n=150)	Non-diabetics (n=151)
Age	51.6±6.7	49.6±6.9
Sex(male/female)	31.5%-68.5%	38.4%-61.6%
BMI*	28.07±4.22	26.38±3.86
Hb A1C *	8.02±1.73	5.17±0.49
FBS*	178.2±68.6	90.71±11.56
BUN*	14.51±6.23	10.85±3.32
Creatinine*	0.99±0.26	0.90±0.26
Urine creatinine*	119.90±61.93	145.16±70.55

\* Significant difference in the mean

**Table 2. The mean values (µg/ml) of zinc (Zn), magnesium (Mg), copper (Cu), selenium (Se), chromium (Cr) and manganese (Mn) in serum sample of diabetic and non-diabetic subjects**

Group	Zn(µg/ml)	Mg(µg/ml)	Cu(µg/ml)	Se(µg/ml)	Mn(µg/ml)	Cr(µg/ml)
Diabetics	0.68±0.27	0.16±0.4	0.39±0.12	0.0139±0.007	0.0109±0.005	0.0013±0.0004
Non-diabetics	0.98±0.3	0.19±0.6	0.31±0.12	0.0148±0.005	0.0110±0.007	0.0018±0.0014
p-value	$p\leq 0.05^*$	$p\leq 0.05^*$	$p\leq 0.05^*$	$p\geq 0.05$	$p\geq 0.05$	$p\leq 0.05^*$

\* Significant difference in the mean

**Table 3. The mean values (µg/ml) of zinc (Zn), magnesium (Mg), copper (Cu), selenium (Se), chromium (Cr) and manganese (Mn) in urine sample of diabetic and non-diabetic subjects**

Group	Zn(µg/ml)	Mg(µg/ml)	Cu(µg/ml)	Se(µg/ml)	Mn(µg/ml)	Cr(µg/ml)
Diabetics	1.38±0.63	7.18±2.15	0.04±0.04	0.061±0.035	0.0039±0.0003	0.010±0.006
No-diabetics	0.63±0.32	7.03±2.38	0.04±0.04	0.058±0.027	0.0018±0.0001	0.009±0.007
p-value	$p\leq 0.05^*$	$p\leq 0.05^*$	$p\geq 0.05$	$p\leq 0.05^*$	$p\leq 0.05^*$	$p\geq 0.05$

\* Significant difference in the mean

**Table 4. The mean values (µg/g) of zinc (Zn), magnesium (Mg), copper (Cu), selenium (Se), chromium (Cr) and manganese (Mn) in hair sample of diabetic and non-diabetic subjects**

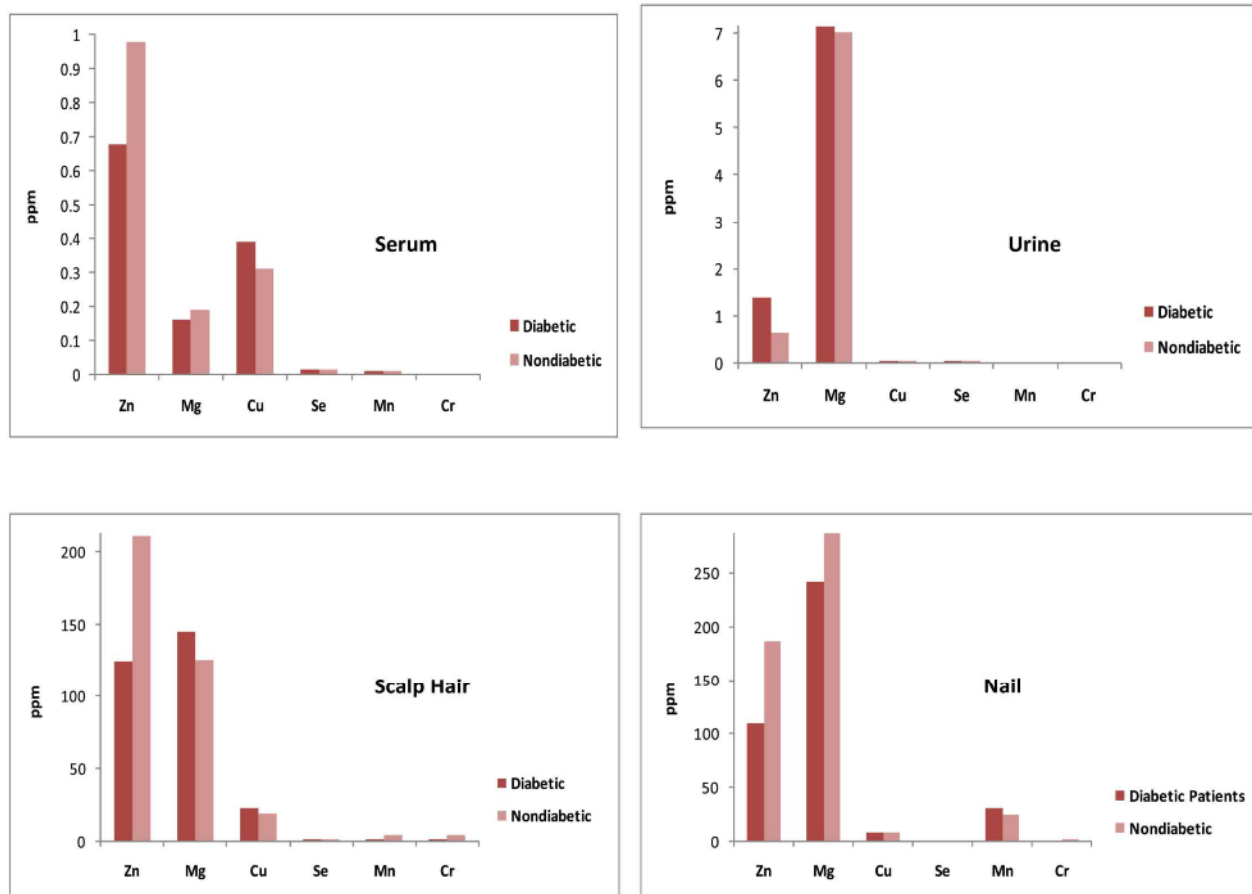
Group	Zn(µg/g)	Mg(µg/g)	Cu(µg/g)	Se(µg/g)	Mn(µg/g)	Cr(µg/g)
Diabetics	124.59±5.73	145.64±8.99	22.55±4.54	1.45±0.56	1.67±1.03	1.21±0.97
No-diabetics	211.41±11.53	126.1±20.65	19.56±5.32	1.57±0.74	4.60±1.42	4.36±1.03
p-value	$p\leq 0.05^*$	$p\geq 0.05$	$p\geq 0.05$	$p\geq 0.05$	$p\leq 0.05^*$	$p\leq 0.05^*$

\* Significant difference in the mean

**Table 5. The mean values ( $\mu\text{g/g}$ ) of zinc (Zn), magnesium (Mg), copper (Cu), selenium (Se), chromium (Cr) and manganese (Mn) in nail sample of diabetic and non-diabetic subjects**

Group	Zn( $\mu\text{g/g}$ )	Mg( $\mu\text{g/g}$ )	Cu( $\mu\text{g/g}$ )	Se( $\mu\text{g/g}$ )	Mn( $\mu\text{g/g}$ )	Cr( $\mu\text{g/g}$ )
Diabetics	109.78 $\pm$ 35.84	242.22 $\pm$ 58.0	8.14 $\pm$ 4.54	0.30 $\pm$ 0.16	30.72 $\pm$ 15.67	1.10 $\pm$ 0.34
No-diabetics	186.56 $\pm$ 40.38	286.5 $\pm$ 89.9	8.19 $\pm$ 4.71	0.82 $\pm$ 0.20	24.54 $\pm$ 12.42	2.37 $\pm$ 0.64
p-value	$p\leq 0.05^*$	$p\geq 0.05$	$p\geq 0.05$	$p\leq 0.05^*$	$p\geq 0.05$	$p\leq 0.05^*$

\* Significant difference in the mean



**Figure 1. Mean values (ppm) of zinc (Zn), magnesium (Mg), copper (Cu), selenium (Se), chromium (Cr) and manganese (Mn) in serum, urine, scalp hair and nail samples of diabetic and non-diabetic subjects.**

## Discussion

Trace elements have been accepted as essential for optimal health. Some studies have confirmed that patients with diabetes in the initial phase have disturbances in the metabolism of zinc, magnesium, copper, selenium, manganese and chromium.

## Zinc

Zinc is involved in a many of biological processes including catalysis, stabilization of cell membranes, regulation of gene expression and influence many metabolic functions (36). Zinc has insulin-like effects which causes

enhance glucose up-take by inhibiting glycogen synthesize (37).

The present study shows that diabetes is associated with low serum, hair and nail zinc concentration in diabetic patients.

Low serum level of zinc and higher urine values for zinc in diabetic patient are in accordance with those reported by several research groups (38-43). The low gastrointestinal absorption and high urinary excretion of zinc in diabetic patients may explain hypozincemia seen in diabetic's population. Hyperzincuria may be as a result of hyperglycemia than any specific effect of endogenous or exogenous insulin on the renal tubules. Hyperglycemia has been postulated to

interfere with the active transport of zinc back into the tubular cells (44-46).

Zargar et al. showed that no significant difference between hair zinc concentration level in healthy groups and diabetic patients. However, in our study hair and zinc concentrations were lower in diabetic patients than controls that is consistent with the works of (Kazi et al, 2007), who demonstrated low scalp hair zinc concentration in diabetics; this may be that there is long term zinc deficiency in our patients (47, 48).

### **Magnesium**

In the studied population, serum Mg levels were found significantly low as compared to healthy controls and urine excretion of Mg was showed higher in diabetics. As the same results have been reported by others studies (37, 40, 41, 46, 47, 49). Hypermagnesuria in diabetics have been attributed to osmotic diuresis. Glycosuria, which accompanies diabetic state, impairs renal tubular reabsorption of magnesium from the glomerular filtrate (Garland, 1992) and likely contributes to high frequency of hypomagnesemia in poorly controlled diabetics (46-49). Though serum Mg may not accurately reflect the level of total body Mg stores (50). The results of ICP-AES analysis depicted that hair and nail concentration of Mg were low in the diabetic group.

### **Copper**

Clinical studies of type 1 and type 2 diabetes have shown alterations in copper metabolism in these diseases (40, 51-53). ICP-AES analysis of biological samples revealed significant higher serum copper level in diabetic individuals. Our result showed no significant difference between blood copper levels in diabetics and control groups which is however inconsistent with the findings of Smith et al. Ito et al. and Babalola et al (37, 53, 54). It is not yet known that the abnormalities in copper metabolism noted in these subjects are a consequence of the disease or they play a role in the progression of the disease. Although alterations in tissue concentrations of copper have not been documented in diabetes studies in humans,

studies in animal models have reported increased tissue copper concentrations in diabetes. The results of these studies suggest altered copper transport at the intestinal brush border in diabetic animals (55, 56).

### **Selenium**

Selenium concentration in plasma depends largely on selenium intake and varies widely geographically (56-60). The baseline plasma selenium concentrations observed in this study were lower than plasma selenium concentrations reported in other area study (61). Selenium status in diabetics lower than in healthy groups, the results of this study have been consistent with the finding of S. Rajpathak et al and J. Bleys et al (61,62). Urine selenium concentration was observed to be significantly higher in diabetics than non-diabetics population studied. Low plasma selenium concentrations in diabetic populations may be due to low intake, undernutrition, high selenium urine excretion, increased requirements or metabolic changes.

### **Manganese**

Manganese is a cofactor for a number of enzymatic systems and manganese deficiency results glucose intolerance in some animal species (63,64). There is evidence that manganese may play a role in the pathogenesis of diabetes (40, 42). In our study serum manganese level did not differ significantly between diabetics and non-diabetics subjects. High urinary manganese excretion and decreased concentrations of hair manganese were observed in diabetic individuals. These results are consistent with results obtained by some other studies (48, 64). Kazi, et al. have reported the diabetic patients had lower blood and hair levels of manganese as compared to control group (47). It have not determined whether diabetes causes high manganese urinary excretion and low serum and hair level of manganese or manganese deficiency contribute to the development of the glucose intolerance (64).



## Chromium

The biological activity of chromium depends on its valence and chemical complexes which it forms. Glucose tolerance factor (GTF) is a trivalent form of chromium that has high biological activity. This is required for optimal glucose consume by the cells (65-67). In the present study, lower chromium concentration in serum, hair and nail were observed in the diabetics compared to the non-diabetic population. Hyperglycaemia and high levels of insulin increase chromium excretion (68), thus, low serum levels of chromium seen in the diabetics has been attributed to insulin resistance, hyperglycaemia and osmotic diuresis resulting from glycosuria, which increase urine chromium excretion (68,69). The Cr levels in the urine of controls were lower than those of patients, but the difference was not significant. A similar observation was made by Nsonwu (46), however this results is inconsistent with the finding of Bahijiri (70), who demonstrated increased urinary excretion of chromium and zinc in elderly diabetics. Insulin administration and hyperglycaemia enhances urinary chromium loss. The reason for the disparity in the two findings is not known and may be attributed to low baseline serum chromium concentration.

There was a highly significant correlation between chromium levels in hair and serum for both males and females. The results indicated hair chromium levels are valid additions to the serum chromium level in the assessment of chromium status in humans (71).

In conclusion, due to the very low concentration of some trace elements in biological samples (e.g. Cr, Se), Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) was used for trace element analysis. This study observed no significant correlation between the serum trace elements concentration and urinary trace elements concentration in diabetic subjects. The obtained results showed that scalp hair and nail samples are the best biological samples for trace element analysis especially in case of Cr, Se, and Mn due to the high accumulation of these elements in hair and nail which causes a better detection.

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