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DETERMINATION OF TOXIC AND ESSENTIAL ELEMENTS IN THE SCALP HAIR OF PATIENTS WITH TYPE 2 DIABETES

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ABSTRACT

Diabetes is one of the most common chronic diseases in the word. Many factors such trace elements contributing to the pathophysiology of type II Diabetes mellitus (DM). Certain elements have been identified as essential trace elements that play an important role in the genesis and progression of such diseases like diabetes. The aim of the present investigation was to evaluate lead, cadmium, copper, zinc, iron, molybdenum, nickel, cobalt and chromium, contents in scalp hair samples of patients having diabetes mellitus type-2 from Tehran (Iran). The hair samples were collected during 2009 – 2010, from 108 patients having diabetes mellitus type-2 age ranged (35-55), for a comparative study 250 age matched non-diabetics, healthy subjects of the same city were selected. Prior to analysis, samples were washed with 1% (w/v) sodium diethyldithiocarbamate (DDTC), 0.1M HCl, and deionized water. The hair samples were digested afterward in a mixture of HNO_3, and H_2O_2. Concentrations of all metals were assessed by flame and graphite furnace atomic absorption spectrophotometer. The type 2 diabetes was corresponding to elevated hair Pb, Cd, Cu, Ni, Mo, Al and decreased Fe, Cr, Co and Zn.

Keywords: Diabetes mellitus; Human hair, Trace elements, Spectrophotometry, Atomic absorption

INTRODUCTION

If we look upon the statistics, it is revealing that diabetes diagnoses are increasing at an alarming rate worldwide. Many factors such as age, diet, sex, obesity, life style, genetics and stress are involved in the etiology of DM. Among these, obesity stands as the foremost risk factor for diabetes type 2. Clinical research suggests that body's balance of trace elements and minerals can be disrupted by DM. Diabetes and diabetes-related kidney disease are serious health problems which are the causes of growing concern in many parts of the world (1,2,3). DM is a very common metabolic disorder with the potential to cause devastating chronic complications (4). The incidence of disturbed glucose tolerance and diabetes mellitus type 2 has been increasing among middle-aged and elderly people (5). The increased oxidative stress is considered as an important factor in the development of diabetes (6). Several studies have emphasized diabetes as a risk factor for many geriatric syndromes (7). Diabetes mellitus has been defined as a cardiovascular disease of metabolic origin (8). There is accumulating evidence that the metabolism of several trace elements is altered in diabetes mellitus (9). Interest in the importance of trace elements to human health has considerably increased during the last decades (10). Trace element deficiencies mostly occur in combination with chronic diseases (11).

Deficiency and efficiency of some essential trace elements may play a role in the development of diabetes mellitus. The status of trace elements in diabetic patients is also influenced by diet, drugs and, to a large extent, environmental factors (12).

It's important to determine the status of trace elements in diabetic patients. Blood, scalp hair and urine may be used bio indicators for this purpose (13). Interest in human hair as a clinical sample for analysis of trace element has increased (14). With development of analytical techniques of great power and sensitivity, the significance of the levels of trace elements in human hair has attracted researchers and many authors have reported that human hair is a good indicator of environmental pollution (15).

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Several factors contribute to the choice of hair: simplicity of the matrix, higher concentrations of the trace elements, and easier sample collection. Hair is an inert and chemically homogeneous sample (16, 17).

Recently, certain elements have been identified as essential trace elements which are significant in the genesis and progression of several diseases like diabetes (18). Some investigations show the statue of some elements is changed in diabetes mellitus. Karahan reported that plasma copper levels were higher in diabetic patients than in normal subjects (19). People with diabetes type 2 have higher circulating ferrite levels (20). Several epidemiological studies have reported a positive association between elevated ferrite levels, and the risk of developing diabetes type 2 (21, 22). It was reported that diabetes could increase the risk of cadmium induced renal damage, especially tubular dysfunction (23). It was consistent with the previous study that diabetic patients may be more susceptible to the toxic effect of cadmium on the renal proximal tubule (24). Zinc (Zn) is an essential trace element which is found in the entire body. Several complications of DM may be mediated through oxidative stress that is amplified in part by zinc deficiency (25). The influence of the concentrations of specific metals to various diseases has been explored for a long time. It is predicted that the number of diabetics will exceed 360 million in the years 2007–2025 (26).The age of diabetic's patient decreased and the number of diabetic women is more than diabetic men. In Iran, metal pollution is more than other countries so the status of toxic elements in diabetic patients in Iran is more than any other country. It triggers a lot of investigation. The aim of the present paper is to associate hair elemental content and diabetes type 2.

EXPERIMENTAL

Instrumentation

An Atomic Absorption Spectrometer, Varian model Spectra AA-220, was used for flame atomic absorption analysis of Fe, Cu and Zn. For graphite furnace analysis of cd, pb, Co, Cr, Ni and Mo the apparatus was equipped with a GTA-100 graphite furnace atomizer, deuterium lamp as a background corrector, and a Varian programmable sample dispenser. The spectrometer parameters are shown in table 1.

Reagents

All reagents used in the present study were analytical grade and deionized water of high purity (from a Millipore ultra pure water system) was used.

Table1. Spectrometer operating conditions and techniques

Elements	*Wavelength (nm)*	*Spectral bandwidth (nm)*	*Lamp current (mA)*	*Technique*
Cd	228.8	0.5	4.0	ETAAS[a]
Cr	357.9	0.2	7.0	FAAS[b]
Co	240.7	0.2	7.0	FAAS
Cu	324.8	0.5	4.0	FAAS
Fe	248.3	0.2	5.0	FAAS
Al	309.3	0.5	10	FAAS
Mn	279.5	0.2	5.0	ETAAS
Mo	313.3	0.5	7.0	ETAAS
Ni	232.0	0.2	4.0	ETAAS
Zn	213.9	1.0	5.0	FAAS

[a]Electrothermal atomic absorption spectrometry.
[b] Flame atomic absorption spectrometry.

Concentrated nitric acid 65% and 30% hydrogen peroxide purchased from Merck (Darmstadt, Germany) were checked for possible trace metal contamination. Standard solutions of Cr, Co, Fe, Cu, Ni, Zn, Mo, Cd, Pb and Al were prepared by dilution of certified standard solutions (1000 ppm) FlukaKamica (Bush, Switzerland). Laboratory glassware and nalgene bottles were are cleaned in 5%

(V/V) nitric acid before used. Prior to analysis, hair samples were washed with 1% (w/v) (DDTC), 0.1M HCl and deionized water.

Sample Collection

During 2009-2010 totally 250 women 33-35 years old, living mainly in Tehran (Iran), were investigated. They were n=250, divided in two groups: control (group 1, n=142) and diabetic women (group 2, n=108). They answer to some questions to obtain information about age, sex, food ingestion, source of drinking water, smoking habits, alcohol consumption, health condition, medication and workplace. The hair samples were cut from the nape of the neck of each person, as close to the scalp as possible. All samples were stored individually in sealed plastic bags at room temperature.

Influence of Analytical Parameter

The influence of various analytical parameters including the amount of human hair sample,digestion procedures and washing factors (concentration and volume of washing solution), was investigated and after finding the optimum situation of analyze, all experiments runs and the uncertainty of analytical result estimated.

Washing procedures

Hair samples were washed with 1% (w/v) (DDTC), 0.1M HCl and deionized water. The hair samples were firstly washed with HCL then one time with deionized water then with acetone and again one time with deionized water. Afterwards, the hair sample dried in oven at 70° C for 8 hours and then digested the next day (27).

Hair digestion procedures

Dry ashing digestion procedure was carried out. Typical ashing temperatures are 450 to 550 °C. Magnesium nitrate is commonly used as an ashing aid. For this purpose, hair samples were ashed in a muffle furnace (Fisher “Isotemp” muffle furnace, Model 184) for 10 h at 550 °C after oven-drying at 250 ° C for 2 h. After they had cooled for 1 h, we removed the samples from the furnace and added five drops of HNO_3. One hour later, we returned the samples to the muffle furnace, where they were heated at 250 ° C for 2 h, then ashed at 450° C for an additional 8 h (28). We again let the samples cool for 1 h, then added 1 mL of $HN0_3$, in 4 mL of water, to each beaker. After 30 min, we decanted the samples into 250 mL volumetric flasks, washed the beakers with de-ionized water, and used the combined washings to bring the samples to volume.

Data analysis

The values of the metal concentrations in human hair of the subjects were presented as arithmetic mean (µg g^{-1}) with standard error (±SE). The statistical significance of concentrations of heavy metals between different parameters was determined by ANOVA as appropriate. The level of significance was set at $P<0.05$. All calculations were performed using statistical packages SPSS (VERSION 14).

RESULT AND DISCUSION

The present study provides data on toxic and essential element concentrations in scalp hair, obtained from the diabetics and normal people. In the hair of diabetic group Cd, Pb, Cu, Mo, Ni and Al elements are observed more than control group, but the level of Zn, Cr, Co and Fe elements are less (Table 2).

In comparison diabetic group with the control group, significantly elevated hair Cd, Pb and decreased Zn and Co levels. Hair Al and Mo levels were significantly higher in type 2 diabetic patients as compared to the control group. The concentration of elements in the samples studied varies widely among individuals. Thus, a significantly large number of samples from population need to be analyzed if the results were treated statistically for meaningful correlation. Various factors (e.g. stress, nutrition, drugs, etc.) influence the concentration of trace elements in human organism, thus the interpretation of the obtained results must be done with great care.

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Table 2. Trace element contents in hair of diabetic women as compared to control women

Element	*Element concentration (µg g -1)*			
	Group 1 (Control ; n =142)		*Group 2 (Diabetic ; n =108)*	
	Mean[a]	*Median*	*Mean*	*Median*
Cd	0.076±0.6[0.025-0.240]	0.061	0.192±1.53[0- 0.932]	0.046
Cr	1.505±1.33[0.130-9.620]	0.619	0.777±1.8[0-7.437]	0.452
Co	0.559±1.38[0.050-3.510]	0.158	0.534±1.34[0.026-2.400]	0.313
Cu	2.648±1.3[0.100-12.395]	0.685	4.745±1.24[0.069-24.200]	2.404
Fe	22.962±0.6[3.481-77.590]	20.586	10.571±0.5[2.306-27.849]	9.865
Al	95.998±0.7[44.272-198.285]	131.250	152.721±0.6[76.830-377]	151.70
Mo	0.457±1.11[0.180-2.426]	0.248	0.606±0.4 [0.470-1.250]	0.936
Ni	1.851± 0.7[0.535-6.137]	1.354	2.023±1.07[0-7.370]	0.917
Pb	1.711±0.4[0.660-3.946]	1.622	2.256±1.00[0-7.733]	1.583
Zn	55.753±0.6[4.678-129.166]	52.396	40.678±0.4[16.625-94.350]	33.887

[a]Mean value in bold (± SD) and the range of values in square brackets.

There are negative correlations between Zn content in the human body and insulin resistance, type 2 diabetes. The excretion of Zn was higher in diabetic patients than that of the controls in both genders. In addition to zinc, chromium seems to be essential for insulin secretion. Chromium was reported to make insulin more effective by helping the insulin in transporting sugar into the cells (29, 30).Elevated Ni was observed in scalp hair of female diabetics. Excess iron has been implicated in the pathogenesis of diabetes and its complications.

An increase in Cu concentration has been linked with disorders in the structure of the arterial walls, stress, infection, and diabetes mellitus. We found a negative correlation between levels of Cu and Zn in patients with diabetes mellitus.

Our investigation showed that the altered Pb and Cd metabolism may be one key in the pathogenesis of diabetes mellitus. It is important to devote more attention of how trace elements affect metabolic processes in different organ cells in both physiological and pathological conditions. The results obtained from the comparison of concentration elements in diabetic group with the control group are shown in Fig.1.

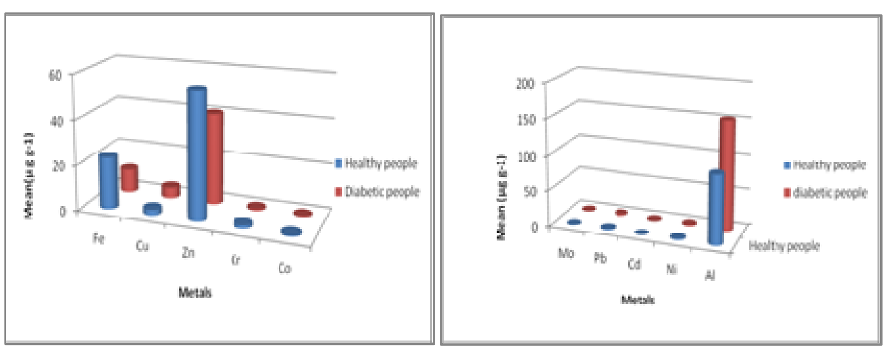

Figure 1. Mean concentration of metals in healthy and diabetic people

CONCLUSION

We discuss in that, variation in the trace elements levels in body affect human health and using hair as a clinical sample has some advantages. While the idea of measuring trace elements in hair is attractive, our findings suggest that the use of this biomarker is appropriate for some specific elements.

In conclusion, diabetes mellitus can be regarded as a multifactorial disease with various contributing factors. Trace elements could have a role as cofactors in the pathogenesis of type II DM. It can be concluded that impaired trace-element metabolism may have a role in the pathogenesis and progression of type-2 diabetes mellitus.

The data obtained showed that there was no significant relationship between the concentrations of iron or zinc ions in the studied and control group. The Zn deficiency and imbalance can play an important role in pathogenesis of type 2 diabetes. Also, the result showed that compared with healthy people, the concentration of Pb, Cd, Cu, Ni, Al and Mo contents in hair of diabetic women increased, but the concentration of Co, Fe, Cr and Zn contents had a decreasing trend.

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