Interaction between essential trace and toxic elements in the scalp hair samples of smokers and alcohol user diabetics

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Abstract

In the present study, trace and toxic elements were determined in the Scalp Hair (SH) samples of patients diagnosed with diabetes mellitus (DM) who were smokers and habitual alcohol drinkers living in Dublin, Ireland. The concentrations of elements were measured by inductively coupled plasma atomic emission spectrophotometer after microwave-assisted acid digestion. The validity and accuracy of the methodology was checked using Certified Reference Material (CRM) (NCS ZC 81002b) and by the conventional wet acid digestion method on the same CRM. The results of this study showed that the mean values of cadmium, copper, iron, nickel and lead were significantly higher ($P<0.001$), in scalp hair samples of diabetic patients as compared to referents of both gender. While the smokers and alcohol drinker referents and DM patients have two to three time higher values of these elements than those subjects who were not smokers and teetotallers. The concentrations of zinc, chromium and manganese were lower in the scalp hair samples of diabetic patients as compared to referents. The deficiency of zinc, chromium and manganese, while the high exposure of cadmium, lead and nickel, as a result of cigarette smoking and alcohol consumption, may be synergistic with risk factors associated with diabetes.

Keywords

Diabetes mellitus • Scalp hair • Cigarette smokers • Alcohol drinking • Trace and toxic elements Inductively coupled plasma atomic emission spectrophotometer

Introduction

Clinical research suggests that the body's balance of trace elements and minerals can be disrupted by diabetes mellitus [1, 2]. Conversely, research also suggests that early imbalances of specific elements may play an important role in upset-ting healthy glucose metabolism and insulin action. With regard to essential trace elements, the main clinical interest and the majority of publications focus on deficiencies of a single element or a combination of elements. Trace element deficiencies mostly occur in combination with chronic diseases and malabsorption. Chronic hyperglycemia can cause significant alterations in the status of some micronutrients and furthermore, some of these nutrients can directly modulate glucose homeostasis [3, 4]. The deficiencies of certain minerals such as magnesium (Mg), zinc (Zn) and chromium (Cr) have been shown to predispose a person to glucose intolerance and to promote the development of diabetic complications [5]. It was reported that Zn is involved in

the synthesis, storage, secretion and conformational integrity of insulin, Zn and insulin monomers assemble to a dimeric form for storage and secretion as crystalline insulin [6]. Lower level of Zn in body may affect the ability of the islet cells of the pancreas to produce and secrete insulin, particularly in type-2 diabetes [7]. Many epidemiological studies reported the decreased plasma Zn and intracellular Zn concentrations, and increased urinary Zn excretion compared to non- diabetic subjects. The metabolic disorder type 2 diabetes mellitus increases the risk of coronary heart disease by a factor of two to four times and is a major cause of mortality among diabetic patients [8-10].

It was intensively investigated that chromium (Cr) acts as a powerful blood glucose modulator that can help guard against glucose imbalances [11]. It tends to lower glucose response in individuals with elevated levels and heighten glucose response in those with insufficient levels. Insufficient dietary Cr intake has also been implicated as a possible risk factor for the development of diabetes [12]. The metabolisms of other micronutrients such as copper (Cu), iron (Fe) and manganese (Mn) have been reported to be altered in diabetes. Manganese is a cofactor for a number of enzymatic systems including arginase which has been found to be elevated in diabetic rats [13]. Mn levels are required for development of normal insulin synthesis and secretion [14, 15]. Diabetes mellitus is associated with altered iron homeostasis in both human and animal diabetic models. Iron is a metal oxidant, capable of generating reactive oxygen species and has been postulated to contribute to diabetic nephropathy.

Excess iron has been implicated in the pathogenesis of diabetes and its complications [16, 17]. Cigarette manufacturing design has evolved considerably over the last few decades with the incorporation of new tobacco processes, papers, filters and several ingredients (flavour, humectants and casing materials), which either alone or in combination have the potential to modify the quantity and/or the quality of the smoke yielded [18]. The tobacco plant absorbs toxic elements most probably from the soil, fertilizers or from pesticides [19, 20].

Other environmental factors that may influence the uptake of toxic elements by tobacco plants include the pH of soil, contaminated irrigated water and sewage sludge used as fertilizers. Smoking delivers heavy metals (the term is used for some lighter metals and metalloids) to the lungs [18], particularly the more volatile metals such as cadmium (Cd) and mercury that partition preferentially into the smoke phase on combustion [21]. Some of these readily pass into the blood-stream and may accumulate in specific organs [22].

Indeed smoking has long been considered a major source of several heavy metals in blood and various organs, and Cd in particular is regarded as one of the "strong carcinogens" in tobacco smoke [23] with nickel (Ni) and arsenic (As) are currently classified "carcinogenic to humans" by the Inter-national Agency for Research on Cancer (IARC) among 87 mainly organic carcinogens. Intake of alcohol is a generally accepted behavior, but it has a significant impact on health. Cross sectional investigations have demonstrated a high prevalence of diabetes mellitus in drinkers, suggesting a possible contributory role for alcohol intake in the development of diabetes. From some cross-sectional population studies, it was reported that diabetes could augment the risk of Cd induced renal damage, especially tubular dysfunction [24].

It was consistent with a previous study that diabetic patients may be more susceptible to the toxic effect of Cd on the renal proximal tubule [25]. Several experimental studies have demonstrated an increased susceptibility toward Cd nephrotoxicity [26] in spontaneously diabetic mice and hamsters, when compared with normal animals of the same strain. Streptozotocin-induced diabetic rats are more susceptible to Cd nephrotoxicity than are normal rats when they are exposed subchronically to Cd chloride in drinking water [27].

Determinations of trace elements in human tissues and fluids were used to obtain information on nutritional status for diagnosis of diseases, indication of systemic intoxication, and to obtain information on environmental exposure. In the majority of cases, whole blood, serum, plasma, and urine were analyzed [28]. Hair can provide a more permanent record of trace and toxic elements (TEs) associated with normal and abnormal metabolism as well as TEs assimilated from the environment. In addition, hair is easily collected, conveniently stored, and easily treated. Therefore, the analysis of human hair has become an important way to understand any quantitative change in certain elements in-side the body [29].

One of the most widely used analytical technique for different elements determination in biological and environmental materials is inductively coupled plasma atomic emission spectrometry (ICP-AES) due to its advantages over other analytical methods; i.e. the possibility of simultaneous determination of many elements of interest, freedom from different chemical interferences and high detection power. ICP-AES also offers rapid, multi-element determinations. The sensitivity of ICP-AES is lower than that of either inductive coupled plasma mass spectrophotometer (ICP-MS) or atomic absorption graphite tube atomizer (AA-GTA), but ICP-AES can handle higher levels of total dissolved solids (TDS) than ICPMS and is much faster than AA-GTA. Since ICP-AES is able to analyze samples with higher TDS, more concentrated solutions can be pre-pared allowing trace elements to be measured. The main advantage of microwave-assisted samples pre-treatment is its requirement of small amount of mineral acids and a reduction in the production of nitrous vapors. Microwave systems keep blank levels low because only small volumes of reagents are required and allow more samples to be processed per hour than conventional digestion systems [30].

The aim and objective of our present study was to assess the concentrations of Cd, Cu, Cr, Fe, Mn, Ni, Pb and Zn in the scalp hair samples of smoker and alcohol user diabetic patients. For a comparative study, 88 non- diabetic individuals (smoker and alcohol user) of the same age group (ranged 35-55 years), belongs to same socioeconomic status, localities and dietary habits were selected as referents.

The elements under study were analyzed by inductively coupled plasma atomic emission spectrophotometer, prior to microwave-assisted acid digestion.

Materials and methods

Apparatus

A Varian Liberty 220 (Mulgrave, Victoria, Australia) Inductively Coupled Plasma Atomic Emission Spectrometer with the axially viewed plasma was used for the analysis. The Liberty Series II ICP features a 40 MHz free running RF generator, a 0.75 m Czerny-Turner monochromator with 1,800 grooves/mm holographic grating used in up to four orders. The resolution of the spectrometer is typically 0.018 nm in 1st order, 0.009 nm in 2nd order, 0.007 nm in 3rd order and 0.006 nm in 4th order. The instrument was controlled with a Digital Equipment Corporation (DEC) Venturis computer with an Intel Pentium processor and Varian Plasma 96 software running under Microsoft Windows 95 operating system. The instrumental conditions are shown in Tables 1 and 2. A Hinari Life style (Elstree, Hertfordshire, England) domestic microwave oven (maxi-mum heating power of 800 W) was used for digestion of the scalp hair samples. Acid-washed polytetrafluoroethylene (PTFE) vessels and flasks were used for preparing and storing solutions.

Reagents and glass wares

Ultrapure water obtained from ELGA Lab Water system (Bucks, UK) was used throughout the work. Concentrated nitric acid (65 %) and hydrogen peroxide (30 %) were obtained from Merck (Darmstadt, Germany), and checked for possible trace metal contamination. Working standard solutions of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were prepared immediately prior to their use, by stepwise dilution of certified standard solutions (1,000 ppm) FlukaKamica (Buchs, Switzerland), with 0.5 M HNO3. All solutions were stored in polyethylene bottles at 4 °C. For the accuracy of methodology, the certified reference material (CRM), human hair NCSZN 81002b (Beijing, China), was used (Table 3).

All glassware and plastic materials used were previously soaked for 24 h in 5 M nitric acid, washed with distilled and finally rinsed with ultrapure water, dried, and stored in a class 100 laminar flow hoods.

Sample collection and pretreatment

An epidemiological cross-sectional survey was conducted among 150 referents and 129 DM subjects of both genders, age ranged 35-55 years, living in urban area of Dublin, Ireland (Table 4).

Before the start of this study, all referents and diabetic patients of both genders, age range 35–55 years, were informed through a consent form by the administration about the aim of study, and all agreed to participate and signed the form. A questionnaire was also administered to them to collect details regarding physical data, ethnic origin, health, duration of smoking and drinking the alcohol, frequency of smoking and drinking alcohol, dietary habits, age, and consent.

Table 1 Measurement conditions for inductively coupled plasma atomic emission spectroscopy

Parameters	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Wavelength (nm)	226.502	267.716	327.396	238.204	259.373	231.604	220.553	213.8
Height (mm)	3	5	5	5	5	5	3	5
Windows (nm) (above the coil)	0.027	0.040	0.027	0.027	0.027	0.027	0.027	0.027
Scan (nm)	0.040	0.060	0.040	0.040	0.040	0.040	0.040	0.040
Integration (s)	3	3	3	3	3	3	3	3
Replicates	3	3	3	3	3	3	3	3
Sample uptake (sec)	30	30	30	30	30	30	30	30
PMT (V)	650	650	650	650	650	650	650	650
Power (kW)	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Plasma flow (L/min)	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Auxiliary flow (L/min)	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Pump speed (rpm)	15	15	15	15	15	15	15	15
Background mode	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic
Max curve order	1	1	1	1	1	1	1	1
C.C. limit	0.995	0.995	0.995	0.995	0.995	0.995	0.995	0.995

Table 2 Liberty 220 common parameters

Nebulizer type	V-groove
Nebulizer pressure	150 kPa
Stabilization time	10 s
Sample delay time	30 s
Rinse time	10 s
Pump- tube	Orange- orange (inlet)
	Blue- blue (outlet)
Snout purge	off
Fast pump	On

Table 3 Determination of trace elements in certified sample of human hair (CRM) by conventional (CDM) and microwave digestion method (MWD) (n=10) (μg/g)

Key: ^Paired t-test between CDM and MWD, DF=9, T (critical) at 95 % CI=2.262, ***P*=0.05,** * means in percentage, Values in ( ) are RSD

$ % recovery was calculated according to: $\frac{[MDM]}{[CDM]} \times 100$

Elements	Conventional digestion method CDM μg/g	Microwave digestion method MWD μg/g	T value^	% recovery$	Certified values μg/g
Cd	0.0716±0.003 (4.19)	0.0714±0.006 (8.40)	0.305	99.7	0.072±0.010
Cr	8.72±0.73 (8.37)	8.67±0.49 (5.65)	0.902	99.4	8.74±0.97
Cu	33.5±1.92 (5.73)	33.0±1.58 (4.79)	0.193	98.5	33.6±2.3
Fe	158±6.73 (4.26)	154±8.72 (5.66)	0.182	97.5	160±16
Mn	3.79±0.34 (8.97)	3.76±0.28 (7.45)	0.727	99.2	3.83±0.39
Ni	5.71±0.51 (8.93)	5.67±0.43 (7.58)	0.339	99.4	5.77*
Pb	3.80±0.37 (9.74)	3.72±0.35(9.41)	0.081	98.05	3.83±0.18
Zn	191±7.28 (3.81)	187±9.53 (5.09)	0.648	97.9	191±16

Table 4 Characteristics of study subjects (35–55) age groups

Parameters	Referents		Diabetic mellitus patients	
	Male (N=89)	Female (N=61)	Male (N=70)	Female (N=59)
Occupation				
Labour	38	24	32	24
Office workers	35	20	25	19
Not working	16	17	13	16
Habits				
Smoking tobacco	19 (21.4 %)	13 (21.3 %)	15 (21.4 %)	13 (22.0 %)
Alcohol drinkers	18 (20.2 %)	10 (16.4 %)	11 (15.7 %)	15 (25.4 %)
Smoking tobacco +Alcohol drinkers	16 (18.0 %)	12 (19.7 %)	13 (18.6 %)	13 (22.0 %)
Non smoking tobacco and alcohol drinkers	36 (40.4 %)	26 (42.6 %)	31 (44.3 %)	18 (30.5 %)

The criteria for the diagnosis of diabetes mellitus by a positive glucose tolerance test showing fasting blood glucose >140 mg/dl (>7.7 mmol/L) and postprandial blood glucose >200 mg/dl (>11.1 mmol/L) 2 h after 75 g of oral glucose. The patients were grouped according to their habits, non-smoking or nonalcohol users patients (PNACS), cigarette smoking patients (PCS), alcohol users patients (PAD), and patients who had both habits (PACS). While control group are also divided into four groups: non-smoking or non-alcohol users referents (RNACS), cigarette smoker referents (RCS), alcohol users referents (RAD) while last group had alcohol consumption with cigarette smoking (RACS) as shown in Table 4. Physical examinations were carried out in a basic health unit of Dublin, Ireland to measure participant's weight, height, blood pressure and biochemical data. For all patients and referents, anthropometric parameters including weight, height and waist circumference were

measured using the standard protocols (Table 5). There were no statistically significant differences between both groups of patients and referents with regard to height and weight.

Table 5 Clinical and biochemical characteristics of diabetic patients and referents

	Referents				Patients			
	RNACS	RACS	RCS	RAD	PNACS	PACS	PCS	PAD
Male								
Height (cm)	179.2±1.34	180±0.54	179.3±1.2	177.3±1.25	180.2±1.44	179.5±1.24	178.3±1.25	182.0±1.24
Weight (kg)	78.7±1.25	82.4±1.53	81.8±1.06	82.6±1.48	80.7±1.36	82.9±1.76	83.3±1.47	83.8±1.73
Waist circumference (cm)	75.9±1.25	79.5±1.34	78.7±1.23	82.7±1.27	85.9±1.45	81.3±1.17	86.2±1.35	87.9±1.52
BMI (kg/m^2)	24.5±1.59	25.4±1.28	25.4±1.39	26.3±1.42	24.8±1.28	25.7±1.86	26.2±1.15	25.3±1.47
Systolic BP (mmHg)	119.8±2.46	125.9±1.3	124.3±1.9	126.9±1.31	120.9±1.57	128.2±1.69	124.5±1.22	129.9±1.39
Diastolic BP (mmHg)	79.6±2.3	79.4±1.28	80.3±1.42	81.2±1.53	85.4±1.05	88.2±1.46	83.9±1.37	86.5±1.16
Fasting plasma glucose (mmol/l)	(90, 99)	(95, 106)	(92, 100)	(93, 102)	(131, 187)	(149, 213)	(143, 192)	(145, 202)
Fasting plasma insulin (mmol/l)	4.29±0.13	4.87±0.24	4.42±0.45	4.75±0.09	6.65±0.24	8.34±0.22	7.49±0.63	7.87±0.48
Diabetes duration (year)	–	–	–	–	9.2±0.32	11.5±0.64	10.6±0.57	11.8±1.28
Female								
Height (cm)	164.0±1.03	163.7±0.7	162.9±1.2	163.8±1.35	164.8±1.52	162.9±0.81	163.7±1.24	163.1±0.79
Weight (kg)	60.4±1.13	63.6±1.27	62.8±0.54	63.9±1.08	62.5±1.36	64.7±1.52	63.9±1.72	65.3±1.12
Waist circumference (cm)	63.1±0.51	63.6±1.34	65.3±1.17	64.6±1.32	63.7±1.52	64.9±0.65	64.3±0.76	65.3±0.63
BMI (kg/m^2)	22.5±1.32	23.7±1.18	23.7±1.31	23.8±1.69	23.0±0.62	24.4±1.35	23.8±1.09	24.5±1.35
Systolic BP (mmHg)	119.6±1.09	120.2±1.2	119±0.76	120.6±1.18	120.2±1.36	121.7±1.16	122.4±1.03	124.5±1.26
Diastolic BP (mmHg)	79.9±1.42	81.3±1.05	81.8±0.73	82.1±0.83	80.3±1.16	82.5±1.08	82.9±1.25	84.3±1.05
Fasting plasma glucose (mmol/l)	(85, 99)	(94, 103)	(92, 100)	(93, 105)	(135, 192)	(146, 202)	(140, 195)	(141, 198)
Fasting plasma insulin (mmol/l)	4.36±0.25	4.85±0.16	4.40±0.37	4.68±0.22	6.53±0.47	8.52±0.19	7.47±0.54	7.93±0.42
Diabetes duration (year)	–	–	–	–	10.4±0.59	10.6±0.73	11.4±1.24	10.5±1.72

BMI body mass index, *RNACS* non-smoking or non-alcohol users referents, *RACS* referent who were cigarette smokers and alcohol users, *RCS* referent cigarette smokers, *RAD* referent alcohol drinkers, *PNACS* non-smoking or non-alcohol users patients, *PACS* patient who were cigarette smokers and alcohol users, *PCS* patient cigarette smokers, *PAD* patient alcohol drinkers

The study protocol was approved by the local ethics committee of Dublin city university, Ireland. In diabetic patients, the duration of diabetes was 8-12 years. 23 % of patients in our survey had documented vascular disease (9 % having a history of cardiovascular disease, 12 % had hypertension and were receiving antihypertensive therapy). 50 % of the patients in this survey were obese while 50 % of patients also received insulin. The criteria of healthy subjects included no history of symptoms of diabetes and any coronary disease, documented in their medical notes. All control subjects underwent a routine medical examination. All patients and controls/referents were requested to complete an interviewer-administered questionnaire, concerning their demographic characteristics, age, health history, lifestyle habits, and diet. They gave written consent to participate in the study.

Collection of scalp hair samples

The hair samples (-1.0 g each) were taken from the nape of the neck. Hair samples were put into separate plastic envelopes for each participant, on which the identification (ID) number of the participant was indicated. The plastic envelope of each subject was tightly sealed and attached to a questionnaire. Before analysis, each individual hair sample was cut into approximately 0.5 cm long pieces and mixed to allow a representative sub-sampling of the hair specimen. After cutting, each sample was washed with diluted Triton X-100: samples were then rinsed with distilled water and then with deionized water. The samples were then rinsed three times with acetone [28]. The samples were then dried in an oven at 75±5 °C for 2 h. Dried samples were stored separately in polyethylene bags.

Microwave-assisted acid digestion

A microwave-assisted digestion (MWD) procedure was carried out, in order to achieve a shorter digestion time. Duplicate samples of scalp hair (200 mg) of each diabetic patients and control individuals were directly placed into Teflon PFA flasks. Two milliliters of a freshly prepared mixture of concentrated HNO3–H2O2 (2:1, v/v) were added to each flask and kept for 10 min at room temperature then placed in a covered PTFE container. This was then heated following a one-stage digestion program at 80 % of total power (800 W). Complete digestion of scalp hair samples required 5–8 min. After the digestion, the flasks were left to cool and the resulting solution was evaporated to semi-dried mass to remove excess acid. About 5 ml of 0.1 M nitric acid was added to the residue and filtered through a Whatman no. 42 filter paper and diluted with deionized water up to 10.0 ml in volumetric flasks. Blank extractions were carried through the complete procedure. Blanks and standard solutions were prepared in a similar acid matrix. The validity and efficiency of the MWD method was checked with certified values of human hair NCSZC 81002b and with those obtained from conventional wet acid digestion method [29, 30].

Analytical figures of merit

Data processing and statistical analysis were conducted by using the computer program EXCEL (XP 2002; Microsoft Corp., Redmond,WA) and Minitab 13.2 Minitab Inc., (State College, PA) software packages. Normally distributed data were expressed as mean ± SD,

Student's t-test and Mann–Whitney test were used to assess the significance of the differences in concentrations of elements among study subjects. All tests were two-sided and a P value of <0.05 was considered significant.Calibration was performed with a series of Cd, Cr, Cu,Fe, Mn, Ni, Pb and Zn standards. Sensitivity (m) was the slope value obtained by least-square regression analysis of calibration curves based on absorbance signals. The equation (N05) for the calibration curves was as follows:

$$Y = (1.28 \times 10^{-3} \pm 8.60 \times 10^{-5})(Cd) + (1.30 \times 10^{-3} \pm 1.23 \times 10^{-4}),\ r = 0.999$$
$$Y = (1.198 \times 10^{-3} \pm 5.7 \times 10^{-5})(Cr) + (1.167 \times 10^{-3} \pm 4.59 \times 10^{-4}),\ r = 0.999$$
$$Y = (4.38 \times 10^{-2} \pm 7.11 \times 10^{-3})(Cu) + (4.39 \times 10^{-2} \pm 7.25 \times 10^{-3}),\ r = 0.999$$
$$Y = (1.38 \times 10^{-3} \pm 9.41 \times 10^{-4})(Fe) + (1.40 \times 10^{-3} \pm 8.07 \times 10^{-4}),\ r = 0.999$$
$$Y = (7.12 \times 10^{-3} \pm 3.1 \times 10^{-5})(Mn) + (6.98 \times 10^{-3} \pm 2.91 \times 10^{-5}),\ r = 0.999$$
$$Y = (1.66 \times 10^{-2} \pm 2.24 \times 10^{-3})(Ni) + (1.73 \times 10^{-2} \pm 2.92 \times 10^{-3}),\ r = 0.999$$
$$Y = (1.875 \times 10^{-2} \pm 7.40 \times 10^{-4})(Pb) + (1.91 \times 10^{-2} \pm 5.83 \times 10^{-3}),\ r = 0.999$$
$$Y = (7.83 \times 10^{-2} \pm 1.18 \times 10^{-2})(Zn) + (8.51 \times 10^{-2} \pm 1.03 \times 10^{-2}),\ r = 0.999$$

where Y is the integrated absorbance, r is the regression and the concentration range of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn for calibration curve reached from the detection limits up to 500 μg/L. The limit of detection, equal to 0.0003 ng/mg,0.0004 ng/mg, 0.01 ng/mg, 0.01 ng/mg, 0.01 ng/mg, 0.01 ng/mg, 0.0003 ng/mg and 0.01 ng/mg for Cd, Cr, Cu, Fe,Mn, Ni,Pb, and Zn respectively. 3 σ/m 'σ' being the standard deviation corresponding to ten blank injections and 'm' the slope of the calibration graph. The quantification limits, defined as 10 σ/m were calculated as: 0.0009 ng/mg, 0.0013 ng/mg, 0.03 ng/mg,0.03 ng/mg, 0.03 ng/mg, 0.05 ng/mg, 0.001 ng/mg and 0.04 ng/mg for Cd, Cr, Cu, Fe,Mn, Ni, Pb, and Zn respectively.

Result

In the study population, ~26 % DM patients and ~35 % referents of both genders were smokers, whilst 24–28 % DM patients and 24–34 % referents of both genders were alcohol drinkers. The physical parameters of both groups of patients and referents were obtained by a standard method as shown in Table 5. The weight, body mass index, and blood pressure (systolic and diastolic blood pressure) levels of DM patients were higher than those in healthy referents, but there is no significant difference (P<0.05).

The smoker and alcohol drinker referents weighed more than nonsmoker referents (P00.042).The elemental contents in the scalp hair samples varied widely among individuals; thus, a significantly large number of samples were required for statistical interpretation of the data to achieve a meaningful correlation between physiological disorders and concentrations of trace and TEs.

The mean concentrations with standard deviations of each element in scalp hair samples shown in Table 6, indicate that the concentrations of the essential trace element (Cr, Mn and Zn) were lower in the scalp hair samples of all groups of DM patients, whilst the level of Cd, Cu, Fe, Ni and Pb were elevated (not significantly) in the scalp hair samples of DM patients, but significant difference was found in smoker and alcohol drinker DM patients with respects to the referents.

In the case of essential trace elements,Cr, Mn, Zn, no significant difference was observed for RCS with respect to the RNACS, although the levels of toxic elements were higher in their scalp hair samples.The concentrations of Zn in the scalp hair samples of male RNACS and

RCS were significantly higher at 95 % (CI: 3.58, 4.25) and (CI:3.46, 3.82) μg/g, respectively, compared with those in PNACS and PCS, (CI: 152, 161) and (CI: 131, 140) μg/g, respectively, with P<0.001. The Zn levels in the scalp hair samples of RACS and RAD, (CI:155, 169) and (CI: 136, 145) μg/g, respectively, were found to be higher than those in PACS and PAD, (CI: 99.7, 112) and (CI: 112, 125) μg/g, respectively, (P00.001–0.002).

The same trend was observed in female patients and referents(Table 6).The concentrations of Cr in the scalp hair samples of female RNACS and RCS were significantly higher at 95 % (CI: 3.82, 4.04) and (CI: 2.51, 2.83) μg/g, respectively,compared with those in PNACS and PCS, (CI: 2.54, 2.77) and (CI: 2.17, 2.34) μg/g, respectively, with P<0.001. The Cr levels in the scalp hair samples of RACS and RAD, (CI:2.95, 3.13) and (CI: 3.27, 3.42) μg/g, respectively, were found to be higher than those in PACS and PAD, (CI: 1.84, 2.02) and (CI: 2.05, 2.16) μg/g, respectively, (P<0.001). The same trend was observed in male patients and referents (Table 6).

Table 6 Concentrations of trace and toxic metals in scalp hair samples of referent and Diabetic mellitus subjects (μg/g)

Elements	Referents				Patients			
	RNACS	RACS	RCS	RAD	PNACS	PACS	PCS	PAD
Male								
Cadmium	0.68±0.15*	1.95±0.15	1.52±0.23	1.73±0.22	2.26±0.32	3.79±0.39	2.93±0.32	3.48±0.46
Copper	11.6±1.25	14.7±1.37	12.9±1.06	13.5±1.63	14.7±1.05	17.8±1.31	15.2±1.13	16.6±1.37
Iron	19.6±2.51	26.9±1.74	23.9±2.32	25.7±1.05	22.3±1.37	28.1±1.49	24.7±2.31	27.3±1.69
Nickel	3.42±0.36	4.74±0.69	3.56±0.93	4.53±0.79	4.95±0.67	7.83±1.25	6.34±0.62	7.54±0.52
Lead	3.63±0.37	5.98±0.43	4.51±0.18	5.48±0.36	6.42±0.50	9.43±1.12	7.62±0.28	8.79±0.45
Chromium	3.86±0.18	3.10±0.07	3.52±0.24	3.33±0.14	2.58±0.14	1.92±0.17	2.30±0.20	2.12±0.08
Manganese	3.93±0.21	2.14±0.12	2.67±0.31	2.36±0.23	2.10±0.22	1.27±0.24	1.65±0.19	1.36±0.16
Zinc	252±13.6	164±14.8	186±9.5	141±7.52	157±9.3	105±12.4	136±11.5	118±12.9
Female								
Cadmium	0.62±0.07	2.04±0.16	1.56±0.12	1.87±0.16	1.76±0.06	3.63±0.19	2.72±0.15	3.05±0.32
Copper	10.7±1.03	17.9±1.07	16.5±1.28	16.8±1.31	17.3±0.85	25.7±1.06	23.2±1.01	26.1±0.46
Iron	19.9±1.64	25.7±0.57	23.3±0.92	24.7±0.97	25.2±0.83	30.7±3.28	27.4±1.36	29.2±0.53
Nickel	3.35±0.47	5.85±0.31	5.32±0.24	5.58±0.29	4.42±0.36	7.63±1.15	6.51±0.52	7.28±1.31
Lead	3.52±0.21	5.67±0.38	4.35±0.47	5.49±0.36	6.36±0.47	9.17±1.37	7.48±0.27	8.56±0.63
Chromium	3.94±0.33	3.04±0.16	3.65±0.35	3.42±0.28	2.65±0.21	1.49±0.25	2.26±0.18	2.03±0.19
Manganese	4.35±0.15	3.26±0.26	3.94±0.29	3.58±0.17	2.35±0.23	1.52±0.44	1.78±0.16	1.49±0.26
Zinc	245±10.5	143±7.52	197±9.32	152±8.56	148±12.9	54.3±6.42	115±7.92	65.7±7.12

The concentrations of Mn in the scalp hair samples of male RNACS and RCS were significantly higher at 95 % confidence interval (CI) (3.82, 4.04) and (2.51, 2.83) μg/g, respectively, compared with those in PNACS and PCS, (CI:1.99, 2.21) and (CI: 1.56, 1.75) μg/g, respectively, with P<0.001. The Mn levels in the scalp hair samples of RACS and RAD, (CI: 2.07, 2.19) and (CI: 2.24, 2.47) μg/g, respectively, were found to be higher than those in PACS and PAD,(CI: 1.15, 1.40) and (CI: 1.27, 1.43) μg/g, respectively,(P<0.001).
The same trend was observed in female patients and referents (Table 6). It was observed that the level of Cr,Mn and Zn did not vary significantly in the scalp samples of referent smokers and alcohol users, indicating that the alteration of these trace metals in scalp hair samples of smokers and alcohol drinker diabetic patients was mainly because of the disease state of the patients.

The levels of Cu and Fe in scalp hair samples were statistically significantly higher ($P<0.01$) in all groups of diabetic patients (PCS, PAD, PACS) compared with referent groups of both genders (Table 6). An elevated level of Cd content was observed in the scalp hair of smoker patients of both genders ($P<0.001$) (Table 6). The ranges of Cd in the scalp hair samples of female RACS and RAD were (CI:1.87, 2.03) and (CI: 1.62, 1.84) μg/g, respectively, whereas those in PACS and PAD were (CI: 3.61–3.97) μg/g and (CI:3.25, 3.71) μg/g, respectively, ($P<0.002$). The same trend was observed in male patients and referents ($P<0.001$) (Table 6).

The Pb concentration in the scalp hair samples of male RNACS was 3.45-3.81 μg/g, (95 % CI), whereas in the PNACS, the Pb level was (6.17, 5.26) μg/g (Table 6). Similarly, a higher level of Pb was observed in PACS (CI: 8.92,9.87) μg/g, PCS (7.46, 7.77) μg/g and PAD (CI: 8.56, 9.03) μg/g than in RNACS ($P<0.001$). The same trend was observed in females (Table 6). The levels of Ni in the scalp hair samples of female RNACS and RCS were found to be lower, (3.25, 3.59) and (CI: 5.18, 5.47) μg/g, respectively, compared with those in PNACS and PCS, (4.45, 4.80) μg/g and (6.34, 6.90) μg/g, respectively. The concentration of Ni in scalp hair samples of RACS and RAD were (5.68, 6.03) and (5.43, 5.74) μg/g, respectively, compared with those of PACS and PAD, (7.04, 8.15) and (6.68, 7.90) μg/g, respectively. The same trend was observed in males (Table 6)($P>0.001$).

Discussion

This study provides data on the essential trace element (Cu,Cr Fe, Mn and Zn) and TEs (Cd, Ni and Pb) in scalp hair samples obtained from smoker and alcohol user diabetics and referents subjects of both genders of age group (35–55 years).Trace elements are uniquely required for growth and maintenance of life and health. Lack or an inadequate supply of such nutrients produces a functional impairment or can result in disease [31]. There is accumulating evidence that the metabolism of several trace elements is altered in insulindependent DM and that these nutrients might have specific roles in the pathogenesis and progress of this disease[32].

Tobacco use has long been known to be a major risk factor for cardiovascular disease, and recent study has identified a positive association between smoking and incidence of diabetes [33]. The evidence that smoking is an independent risk factor for the development of diabetes is still considered preliminary [34]. Drinking too much alcohol can raise the levels of some fats in the blood (triglycerides). It can also lead to high blood pressure, heart failure, diabetes and an increased calorie intake. Some studies have shown a dose–response association between smoking and incidence of diabetes [33, 34]. Also, some earlier prospective research failed to find an increased risk of diabetes among tobacco users [34].

Several hypotheses have been proposed to link tobacco use and incidence of diabetes. Smoking has been linked to impaired response to glucose tolerance tests and insulin resistance [35]. Although smoking cessation can result in modest weight gain, smoking is related to a more unhealthy distribution of upper body weight and greater waist:hip ratio [36]. Smoking and dinking alcohol have also been associated with risk of chronic pancreatitis and

pancreatic cancer, suggesting that tobacco smoke may be directly toxic to the pancreas [37]. Tobacco smoke, which exists in two major phases, namely the gas phase and particulate (tar) phase, has a large number of chemical carcinogens and generates reactive oxygen species, which can lead to oxidative stress in thelung and other organs.

The carcinogens, oxidants, and anumber of toxic substances have direct or indirect, modulatory or damaging effects on DNA, membrane lipids, cell signaling proteins, and various macromolecules [38].People with diabetes are more likely to have high blood pressure and high levels of fats such as triglycerides. Several factors, including genetics and obesity, increase a person's risk of insulin resistance and smoking has also been shown to increase the risk of this condition. It is believed that catecholamines, are produced in greater quantity in smokers and act as an antagonist to insulin action [39].Tobacco leaves naturally accumulate and concentrate relatively high levels of Cd, Ni, Pb, Fe, Cu, and therefore smoking of tobacco is an important source of these metals exposure for smokers [40]. The total amount of metals carcinogens in cigarette smoke ranges from 1 to 3 μg per cigarette [41]. The country of origin and type of the product play major roles in determining the chemical composition of cigarette tobacco [20]. It was investigated that one pack of cigarettes deposits 2–4 μg Cd, 1–2 μg Pb and 0.96–1.34 μg

Ni into the lungs of a smoker, whereas some of the smoke passes into the air to be inhaled by smokers and nonsmokers alike [40]. It was also consistent with another study that smokers generally exhibit significantly higher Cd, Ni, Pb,Fe and Cu body burdens than non-smokers, while smoking with alcohol consumption enhance the Cd, Ni, Pb, Fe and Cu absorption and accumulation in all the tissues [23]. The results suggested that although these toxic elements (Cd, Ni,Pb) pose a hazard to essential trace metal homeostasis of various organs, co-exposure can pose a major threat, while consumption of ethanol may absorb much more Cd and Pb than their unexposed counterparts [42].

This study revealed that the level of Zn was low in scalp hair samples of diabetic smokers and alcohol consumers. Alteration of Zn homeostasis in diabetics is supported by a large body of experimental and clinical evidence. The low levels of Zn in diabetic patients may be due to excessive urinary output especially in patients with diabetic nephropathy or signs of infection during which Zn will act as a defense mechanism [43]. In diabetic individuals, enteric neuropathy and microvascular disease can alter intestinal absorption of carbohydrates, amino acids, and minerals [44]. Zinc deficiencies in diabetics are associated with excess free radical activity, and the increased oxidation of fats

(lipids). When fats become oxidized, they are believed to become more reactive and damaging to the heart, arteries, and other integral parts of the vascular system [7]. The concentration of copper and iron were higher in the scalp hair samples of smokers and alcohol drinker diabetic patients as compared to referents of both genders (Table 6). Excess iron has been implicated in the pathogenesis of diabetes and its complications [45]. Free iron serves as a catalyst for lipid and protein oxidation and the formation of reactive oxygen species. In addition, iron indices are correlated with obesity and insulin sensitivity [46]. In the presence of hyperglycemia and inflammation, iron may contribute towards the development and progression of oxidative injury. Iron may also negatively impact on glycemic control [16]. However, iron indices are strongly correlated with Hb, which represents an important

risk factor for morbidity and mortality in patients with diabetes, particularly in patients with established cardiovascular disease [47].

Increase in Cu concentration has been linked with disorders in the structure of the arterial walls, stress, and infection and also in diabetes mellitus [48]. The relationship between an increase in Cu concentration and the oxidation of lowdensity lipoproteins has been verified [49].Concentration of Cr in the scalp hair samples of diabetic subjects were found to be significantly lower than referents ($p>0.001$). Our results are consistent with other investigators,Anderson; 2002, 1998 [50, 51] who elucidated theaction of Cr in diabetes and showed that the administration of Cr may have beneficial effects on the disease. Cr is an essential element required for normal carbohydrate and lipid metabolism. Many scientists have demonstrated that a sever Cr deficiency led to fasting - hyperglycemia, glucosuria and impaired growth [52]. Our older patients had lower values of Cr in scalp hair samples which is consistent with other studies [53], where age-related decreases of Cr in hair as compared to matched normal subjects were described.

In general, based on observations from different groups of studies, in addition to impaired Cr utilization, age plays a major role in the status of Cr. Results from some trials [54] have indicated that Cr supplementation increases muscle gain and fat loss associated with exercise and improvesglucose metabolism and the serum lipid profile in patients with or without diabetes. Most of the patients under study have cardiac problems, which is consistent with other studies,who reported that low Cr concentrations and the associated impairments in insulin, glucose and lipid metabolism resulting in increased cardiovascular risk [55, 56]. Insulinresistant diabetic patients responded well to oral doses of Mn [57]. Appropriate Mn levels are required for development of the normal insulin synthesis and secretion [58]. In our study, the diabetic patients of both genders had lower level of Mn in scalp hair samples than normal healthy groups of both genders.

Recent studies with rats and humans indicate that nickel deprivation depresses growth, reproductive performance, and plasma glucose and alters the distribution of other elements in the body, including calcium, iron and zinc [59, 60].

Int J Diabetes Dev Ctries (July–September 2012) 32(3):151–162 159 Bonnefont-Rousselot [61] has investigated the use of minerals (vanadium, chromium, magnesium, zinc, selenium) and vitamins (tocopherol, ascorbic acid, nicotinamide,riboflavin) in diabetes, with a particular focus on the prevention of diabetic complications. It was also reported that dietary supplementation with micronutrients may be complementary to classical therapies for preventing and treating diabetic complications and the supplementation is expected to be more effective when a deficiency in these micronutrients exists [62].

The findings of the present study clearly demonstrate that the concentration of toxic metals (Cd, Pb) varied in the scalp hair samples of smoker and alcohol drinker diabetic patients as compared to smoker and referents (Table 6). Metallic carcinogenicity is generally thought to generate free radicals,and thus some metals were reported to play a role in lung tumorigenesis. The potential health impact from smoking cigarettes that delivers high levels of toxic metal is not limited to active smokers. In indoor environments, Cd,lead, arsenic and organic carcinogens from side stream smoke are readily available for passive exposure [63]. Cd exposure from smoking cigarettes may be a more serious health concern than Cd in food. Smokers may double their daily intake of Cd compared with non-smokers. Each cigarette

may contain from 1 to 2 mg of Cd, and 40–60 % of the Cd in the inhaled smoke can pass through the lungs into the body.This means that smokers may take in an additional 1–3 μg of Cd into their body per day from each pack of cigarettes smoked. Smoke from other people's cigarettes probably does not cause non-smokers to take in much Cd. Aside from tobacco smokers, people who live near hazardous waste sites or factories that release Cd into the air have the potential for exposure to Cd in air. It was reported in our previous study that the steel mill workers who smoked had significantly high level of Cd in scalp hair and blood samples as compared to the unexposed and non-smoker workers [64].

There is scarce information on Cd effects on insulin receptors and insulin action in adipose tissue. Addition of Cd (1 mM) to intact rat adipocytes did not affect the insulin receptor kinase activity, but stimulated glucose transport without changing the amount of glucose transporter in crude plasma membranes [65]. The stimulatory effect of Cd on glucose transport was also confirmed in cell culture model and again, no effects on GLUT 4 protein were observed [66]. It seems that aforementioned fmdings on Cd-induced glucose transport could explain previously described in vitro insulin-mimetic effect of Cd on glucose lipogenesis and glucose oxidation [66] in rat adipocytes. In pancreatic islets of obese hyperglycemic mice low Cd concentration evoked basal and glucose stimulated insulin response [67]. In con-trast, high Cd concentration significantly inhibited the secretory response to glucose [67]. In vivo rat intake of Cd resulted in lower glycemia accompanied with higher serum insulin value [67].

Further discrepancies in Cd effects on glucose homeostasis and insulin levels are results of hyper-glycemia and inhibition of insulin release from rat pancreas in rats exposed to Cd [67]. Incompatibility of literary data on Cd effect is based on both, experimental approach (in vivo vs. in vitro studies) and the various metal concentrations used. Low doses of Cd used in experiments mimic low or moderate levels of environmental contamination. Lead in blood is present almost entirely in the cells. Bone lead, which comprises >95 % of adult body lead burden and has a biologic half-life ranging from years to decades, is a better biologic marker for studying chronic toxicity of ac-cumulated exposure and lead burden [68]. In addition, bone lead also serves as an endogenous source of lead exposure for individuals with increased bone turnover [69]. There-fore, bone lead may be a risk factor for impaired renal function either by serving as a dosimeter of cumulative exposure of the kidney to lead or a measure of the major endogenous source of blood lead that, in turn, may affect the kidney. An increase in bone resorption is a characteristic of aging in both men and women, aging-associated release of bone lead into the circulation is a potentially important source of soft-tissue lead exposure and toxicity. Another factor associated with aging that may increase the nephrotoxicity of lead is diabetes. The more prevalent form, type-2 diabetes, affects approximately 10 % or more of the general population (with substantially higher rates at >55 years of age) [70] and is well known as an independent predictor of accelerated decline in kidney function.

Conclusion

The results of this study revealed that diabetics have a different pattern of essential trace and toxic elements in their scalp hair samples than controls/referents, with the prevalence being more in smokers and alcohol users. However, higher levels of Cd, Cu, Fe, Pb and Ni, as well

as a lower level of Cr, Mn, Zn, correlated well with the consequences of DM. The impaired trace element metabolism of the present work may have a role in the pathogenesis and progression of DM where the increase of Fe, Cd, Cu, Ni and Pb and decrease of Zn, Cr and Mn concentration in scalp hair samples of diabetics may disturb the secretion and action of insulin, the high level of Cu, Cd, Fe and Pb may disturb the antioxidants, and enhance the lipid peroxidation. Smoking and alcohol consumption further aggravates the problem by increasing the level of toxic elements (Cd, Pb, Ni).

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