Comparative metal distribution in scalp hair of Pakistani and Irish referents and diabetes mellitus patients

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ABSTRACT

Background: The essential metals, chromium (Cr), magnesium (Mg), manganese (Mn) and zinc (Zn), are necessary for many metabolic processes and their homeostasis is crucial for life. The toxic metals, cadmium (Cd) and lead (Pb), have no beneficial role in human metabolism. The aim of this study was to investigate the levels of Cd, Cr, Mg, Mn, Pb, and Zn in scalp hair samples of type 2 diabetes mellitus patients of both genders, ages ranging from 30 to 50 y, and belong to urban areas of Ireland and Pakistan. For comparison purposes, age matched non-diabetic subjects of both countries were selected as referents. Methods: The concentrations of metals in scalp hair samples were measured by inductively coupled plasma atomic emission spectrophotometer and atomic absorption spectrophotometer after microwave-assisted acid digestion. The validity and accuracy of the methodology were checked by conventional wet-acid-digestion method and using certified reference materials. Results: The mean values of Cd and Pb were significantly higher in scalp hair samples of both Pakistani and Irish diabetic patients as compared to referents of both countries ($P<0.001$). In contrast, lower Cr, Mg, Mn, and Zn ($P<0.01$) concentrations were detected in scalp hair derived from patients with type 2 diabetes versus healthy subjects of both countries. Conclusion: This study showed that, increased toxic elements and decreased essential elements are associated with diabetes mellitus. Therefore, these elements may play a role in the development and pathogenesis of diabetes mellitus.

Keywords: Scalp hair, Magnesium, Zinc, Chromium, Toxic metals, Type 2 diabetes mellitus.

1. Introduction

Diabetes mellitus (DM) is a global disease, which prevails all over the world, though the prevalence rate differs from country to country [1]. Clinical research suggests that the balance of essential and toxic elements in the human body can be disrupted by diabetes mellitus [2]. It was reported that abnormalities in the metabolism of zinc (Zn), chromium (Cr), magnesium (Mg) and manganese (Mn) have been associated with diabetes mellitus [3]. Chronic hyperglycemia can cause significant alterations in the status of some micronutrients and on the other hand, some of these nutrients can directly modulate glucose homeostasis [4,5].

Magnesium is a necessary cofactor in >300 enzymatic reactions, phosphorylation processes, and in all reactions that involve the utilisation and transfer of ATP, including cellular

responses to growth factors and cell proliferation [6]. Epidemiologic studies showed a high prevalence of hypomagnesaemia and lower intracellular Mg concentrations in diabetic subjects [7]. Mg depletion provoked a deleterious effect on glucose metabolism due to an impairment of both insulin secretion and action [7]. Deficiencies of Mg in diet and serum have been associated with an increased risk of developing glucose intolerance and diabetes [8,9]. While increased Mg intake is associated with a significant decline in the incidence of type 2 diabetes [10].

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 [11]. Lower levels of Zn in the body may affect the ability of islet cells of the pancreas to produce and secrete insulin, particularly in type 2 diabetes [12]. In diabetes mellitus patients, the lower intake of Zn increases the risk of coronary heart disease by a factor of 2-4 times [13].

It was intensively investigated that chromium acts as a blood-sugar modulator that could guard against glucose imbalances [14,15]. Trivalent Cr was proposed as a structural part of glucose tolerance factor and it was reported that Cr participates in the stimulation of insulin signalling [16]. The vast majority of studies have now regularly demonstrated the beneficial effects of Cr supplementation on glucose tolerance in children, adults and elderly with compromised glucose metabolism. Insufficient dietary Cr intake has also been implicated as a possible risk factor for the development of diabetes [17]. Manganese plays an important role in a number of physiologic processes as a constituent of some enzymes and an activator of different enzymes [18].

Mn maintains the blood glucose level in normal range and hence is useful in treating diabetes and hypo-glycemia [19]. The recommended Mn levels required for the develop-ment of normal insulin synthesis and secretion are 2.5 to 5 mg/day [20]. The toxic metals, lead (Pb) and cadmium (Cd), are ubiquitous en-vironmental toxins that are related to a broad range of physiologic, biochemical, and behavioural dysfunctions [21]. Cd is a widespread environmental pollutant that accumulates in the pancreas and exerts diabetogenic effects in animals [22]. It can cause high blood glucose, damage beta cells, and cause diabetes in rodents [23].

Many epidemiological studies indicated that Cd may exacerbate the harmful renal effects of diabetes and vice versa [24]. The effects of Pb poisoning in diabetic subjects have been recognised [25]. Epidemiologic studies in animals have reported Pb toxic effects at high levels of exposure may contribute to the progression of diabetes complications in dia-betic patients [26]. In view of these facts, it is important to determine the essential trace and toxic elemental concentrations in biological samples of dia-betes mellitus patients and to monitor and assess their impact on human health. In the majority of cases, whole blood, serum, plasma, and urine were analysed [27]. Hair can provide a more permanent record of trace and toxic elements associated with normal and abnor-mal metabolism as well as their assimilation from the environment.

In addition, hair is easily collected, conveniently stored, and easily treated. Therefore, the analysis of human hair has become an impor-tant way to understand any quantitative change in certain elements inside the body [28]. One of the most widely used analytical tech-niques for different elements of determination in biological and envi-ronmental materials is inductively coupled plasma atomic emission spectrometry (ICP-AES) due to its advantages over other analytical methods: to simultaneously determine many elements of interest, freedom from different chemical interferences and high detection power. The sensitivity of ICP-AES is lower than that of either induc-tively coupled plasma mass spectrophotometer (ICP-MS) or electro-thermal atomic absorption spectrometer (ETAAS). The ICP-AES can handle higher levels of total dissolved solids than ICPMS and is much faster than ETAAS. Since ICPAES is able to analyse samples with higher TDS, more concentrated solutions can be prepared allowing trace ele-ments to be measured. The main advantage of microwave-assisted samples pretreatment is it requires a small amount of mineral acids and a reduction in the production of nitrous vapours. 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 [29].

2. Materials and methods

2.1. Apparatus

In Ireland, the analysis of understudy elements was carried out by means of Varian Liberty 220 (Mulgrave, Victoria, Australia) inductively coupled plasma atomic emission spectrometer with the axially viewed plasma used for the analysis. The Liberty Series II ICP features a 40 MHz free running RF generator and a 0.75 m Czerny-Turner mono-chromator with 1800 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 Table 1. A Hinari Lifestyle (Elstree, Hertfordshire, England) domestic microwave oven (maximum heating power of 800 W) was used for digestion of the scalp hair samples.

The analysis of elements in Pakistan was carried out by a double beam Perkin-Elmer atomic absorption spectrometer model 700 (Norwalk, CT), equipped with a flame burner and graphite furnace HGA-400, a pyrocoated graphite tube with an integrated platform and an autosampler AS-800 (Perkin Elmer). The instrumental parameters are shown in Table 2. Mg and Zn were measured under optimised operating conditions using FAAS with an air-acetylene flame, whereas Cd, Cr, Mn and Pb were determined using ETAAS.

Table 1
Measurement conditions for inductively coupled plasma atomic emission spectroscopy Liberty 220 ICP-AES.

Parameters	Cd	Cr	Mn	Mg	Pb	Zn
Wavelength (nm)	226.5	267.72	259.37	279.806	220.553	213.8
Height (mm)	3	5	5	5	3	5
Windows (nm) (above the coil)	0.027	0.040	0.027	0.027	0.027	0.027
Scan (nm)	0.040	0.060	0.040	0.040	0.040	0.040
Common parameters	Nebuliser type (V-groove), nebuliser pressure (150 kPa), stabilisation 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), integration (3s), replicates 3, sample uptake (s) 30, PMT (V) 650, power (kW) 1.10, plasma flow (l/min) 15.0, auxiliary flow (l/min) 1.50, pump speed (rpm) 15, background mode (dynamic), max curve order (1)					

Key words: kPa = kilo Pascal, s = seconds, PMT = photo multiplier tube, kW = kilo watt, rpm = rotation per minute.

Table 2
Measurement conditions for electrothermal atomization AAS 700.

	Electrothermal AAS				Flame AAS	
Parameters	Cd	Cr	Mn	Pb	Mg	Zn
Lamp Current (MA)	6.0	7.5	7.5	8.0	7.5	7.5
Wavelength (nm)	228.8	357.9	279.5	283.3	285.2	214
Slit-width (nm)	0.7	0.7	0.2	0.7	0.7	0.7
Dry temperature (°C)/ramp/hold (s)	140/15/5	140/15/5	140/15/15	140/15/5	Common parameters:	
Ashing temperature(°C)/ramp/hold (s)	850/10/20	1400/10/20	1400/10/20	700/10/20	Burner height (mm) (7.5)	
Atomization temperature(°C)/ramp/hold (s)	1650/0/5.0	2500/0/5.0	2200/0/5.0	1800/0/5.0	Oxidant (air) l/min (17.0)	
Cleaning temperature (°C)/ramp/hold (s)	2600/1/3	2600/1/3	2600/1/3	2600/1/3	Fuel (acetylene) l/min (2.0)	
Chemical modifier	$Mg(NO_3)_2 + Pd(NO_3)_2$	$Mg(NO_3)_2$	$Mg(NO_3)_2$	$Mg(NO_3)_2$		
Sample volume (10 µl), cuvette = cup, carrier gas = (200 ml/min) Background correction (D_2 lamp) used for all elements						

Signals were measured as absorbance peaks in the flame absorption mode, whereas integrated absorbance values (peak area) were determined in the graphite furnace. A Pel (PMO23, Osaka, Japan) domestic microwave oven (maximum heating power of 900 W) was used for digestion of scalp hair samples. Acid-washed PTFE (polytetrafluoroethylene) vessels (Kartell, Milan, Italy) and flasks were used for preparing and storing solutions.

2.2. Reagents and glass wares

Ultrapure water obtained from ELGA LabWater 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, Mg, Mn, Pb and Zn were pre-pared immediately prior to their use, by stepwise dilution of certified standard solutions (1000 ppm) Fluka Kamica (Buchs, Switzerland), with 0.5 mol/l HNO_3. All solutions were stored in polyethylene bot-tles at 4 °C. For the accuracy of methodology, the certified reference materials (CRMs) of human hair NCS ZC 81002b (Beijing, China) and certified reference materials (CRMs) of human hair BCR 397 (Brussels, Belgium) were used (Table 3). All glassware and plastic materials used were previously soaked for 24 h in 5 mo1/1 nitric acid, washed with distilled and finally rinsed with ultrapure water, dried, and stored in a class 100 laminar flow hoods.

Table 3
The number of diabetic patients and referent.

Countries	Referents	DM-2	Referents	DM-2
Pakistan	59	53	62	41
Occupation	Office worker	Office worker	Housewives	Housewives
	34 (58%)	27 (51%)	37 (59%)	20 (48%)
	Shopkeeper	Shopkeeper	Office worker	Office workers
	25 (42%)	26 (49%)	25 (41%)	21 (52%)
Ireland	31	29	25	22
Occupation	Office worker	Office worker	Office worker	Office worker
	21 (69%)	21 (74%)	20 (81%)	17 (78%)
	Teachers	Teachers	Teachers	Teachers
	10 (31%)	8 (26%)	5 (19%)	5 (22%)

2.3. Sample collection and pretreatment

The study was carried out in 2 phases on patients who have type 2 diabetes mellitus and with ages ranging from 30 to 50 y related to non-diabetic subject as referents. Phase 1 study was carried out from January 2006 to June 2006 in Hyderabad, Pakistan, while phase 2 was conducted from July 2010 to October 2010 in Dublin, Ireland. The temperatures of both cities are observed in the range of 9.0-17.5 °C and 10-38 °C for Dublin and Hyderabad, respectively. The Institutional Review Board of both countries approved the protocol, and all subjects signed the informed consent. The details of the demographics of patients and referents of both countries are given in Table 3. Type 2 diabetes mellitus patients of both countries were free from serious complications of diabetes (Table 3).

All patients have not used insulin to control their diabetes, and were under treatment with different oral medicines. All referents and patients were nonsmokers. A questionnaire was also administered to them in order to collect de-tails concerning physical data, ethnic origin, duration of diabetes, dietary habit, gender and age.

The details related to a donor's identity, residence, health condition, socio-economic status, smoking, alcohol drinking and education were also recorded. For all subjects, anthropometric parameters including weight, height, and waist circumference were measured using standard protocols. Blood pressure, glycohaemoglobin, fasting plasma glucose, fasting plasma insulin, serum total cholesterol, serum HDL cholesterol, serum LDL cholesterol, and serum triglycerides were measured using standard methods (Table 4).

The mean duration of the disease was 5.8 ± 3.5 y (2-10 y). The criteria for the selection of diabetic patients was already diagnosed as DM-2 and confirmed by biochemical tests such as fasting plasma glucose and fasting plasma insulin. DM patients with kidney disorders, hypertension, heart disease and thyroid disease were not included in the study. The inclusion criteria of referent subjects of both genders had no history of DM and any other disease, documented in their medical notes. All control subjects underwent a routine medical examination in basic health care unit.

Table 4
Characteristics of referent and type 2 diabetes mellitus patients of both countries.

Parameters	Pakistan				Ireland			
	Male		Female		Male		Female	
	Referents	Diabetics	Referents	Diabetics	Referents	Diabetics	Referents	Diabetics
Height (cm)	176.2 ± 1.9	173.5 ± 2.2	149.7 ± 6.3	152.6 ± 8.73	179.2 ± 1.34	180.2 ± 1.44	164.0 ± 1.03	164.8 ± 1.52
Weight (kg)	75.8 ± 2.3	78.9 ± 6.3	67.8 ± 7.4	70.6 ± 6.28	78.7 ± 1.25	80.7 ± 1.36	60.4 ± 1.13	62.5 ± 1.36
Waist circumference (cm)	76.4 ± 9.9	83.2 ± 3.1	72.5 ± 7.89	76.9 ± 8.21	75.9 ± 1.25	85.9 ± 1.45	63.1 ± 0.51	63.7 ± 1.52
BMI (kg/m^2)	24.4 ± 1.6	26.2 ± 2.9	30.3 ± 3.6	30.2 ± 4.1	24.5 ± 1.59	24.8 ± 1.28	22.5 ± 1.32	23.0 ± 0.62
Systolic BP (mm Hg)	124.2 ± 6.8	121.4 ± 5.1	122 ± 5.28	121.9 ± 8.94	119.8 ± 2.46	120.9 ± 1.57	119.6 ± 1.09	120.2 ± 1.36
Diastolic BP (mm Hg)	79.7 ± 6.2	82.5 ± 4.73	79.8 ± 4.2	82.8 ± 3.62	79.6 ± 2.3	85.4 ± 1.05	79.9 ± 1.42	80.3 ± 1.16
Fasting plasma glucose (mmol/l)	(90, 99)	(132, 189)	(88, 95)	(123, 182)	(90, 99)	(131, 187)	(85, 99)	(135, 192)
Fasting plasma insulin (mmol/l)	4.35	6.5	4.32	5.96	4.29	6.65	4.36	6.53
Serum total cholesterol (mg/dl)	162 ± 37	193 ± 35	171 ± 28.2	197 ± 38.6	158 ± 25.8	195 ± 28.2	165 ± 19.6	196 ± 27.6
Serum HDL cholesterol (mg/dl)	37.5 ± 11.5	29 ± 9.9	38.6 ± 6.2	28.5 ± 3.6	35.4 ± 6.95	28.5 ± 4.72	38.6 ± 9.38	27.8 ± 7.8
Serum LDL cholesterol (mg/dl)	98 ± 34	246 ± 36	93.5 ± 9.86	255 ± 23.6	96.7 ± 25.8	238 ± 25.9	97.2 ± 23.9	238 ± 26.6
Serum triglycerides (mg/dl)	155 ± 88.1	234 ± 52.8	153 ± 25.3	258 ± 19.5	148 ± 69.6	240 ± 36.7	152 ± 70.6	243 ± 38.7

Diabetic patients and referents were physically active persons, and no statistically significant differences were observed regarding height and weight. The dietary habits of Pakistani people depend upon animals (chicken, mutton, and beef), plants (vegetables and beans) and grain (wheat, rice and others),while the Irish people mostly used chicken, vegetables, grains and beans.

2.4. Collection of scalp hair samples

The hair samples (-1.0 g each) were taken from the nape of the neck. The collected hair samples were kept in separate plastic envelopes for each participant and marked with identification number. 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, then subsequently washed with diluted Triton X-100, distilled water and deionised water.

The samples were then rinsed three times with acetone [29], and dried in an oven at 75 ± 5 °C for 2 h. Dried samples were stored separately in polyethylene bags.

2.5. 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 DM-2 patient and referent were directly placed into PTFE flasks (25 ml in capacity). Two millilitres of a freshly prepared mixture of concentrated HNO3-H202 (2:1, v/v) was 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 programme at 80% of total power (800 W). Complete digestion of scalp hair samples required 5-8 min. After digestion, the flasks were left to cool and the resulting solution was evaporated to semidried mass to remove the excess acid.

About 5 ml of 0.1 mol/1 nitric acid was added to the residue, filtered through a Whatman no. 42 filter paper and made volume up to 10.0 ml in volumetric flasks with 0.1 mol/1 nitric acid.

Blank extractions were carried out through the complete procedure. Blanks and standard solutions were prepared in a similar acid matrix. The validity and efficiency of the MWD meth-od were checked with certified values of human hair NCS ZC 81002b, certified human hair CRM 397 and with those values obtained from conventional wet acid digestion method as reported in our previous work [29].

2.5.1. Statistical analysis

Statistical analyses were performed using computer programme Excel XL State (Microsoft Corp., Redmond, WA) and Minitab 13.2 (Minitab Inc., State College, PA). The Student's t-test was used to as-sess the significance of the differences in obtained and certified values of elements in CRMs. Differences were significant when the P-value was <0.05. Pearson's correlation was used to link the difference in concentration of elements in patients and referents.

2.6. Analytical figures of merit

The linear range of calibration curve of understudy elements was reached from the detection limit up to 100 µg/l. The detection limit (LOD) was defined as 3s/m, where s is the standard deviation corresponding to 10 blank injections and m is the slope of the calibration graph. The LODs of 0.05, 0.55, 0.41, 0.845, 6.54 and 5.04 pg/l were calculated for Cd, Pd, Cr, Mn, Mg and Zn, respectively. The validity and efficiency of the microwave assisted digestion method were checked with those obtained from conventional wet acid digestion method [27-29]. The indicative values for both protocols were calculated as the mean values of six replicates of certified human hair sample (BCR 397) and NCS ZC 81002b were close to that of the certified values, which confirmed the reliability of the methods (Table 5).

The precision of the methods, expressed as the variances of 8 independent analyses of the same sample, provided valuesb10% for both techniques.

Table 5
Determination of trace elements in certified sample of human hair (CRM) by conventional (CDM) and microwave digestion method (MDM) (n = 10).

Elements	CDM	MDM	T value[a]	% recovery[b]	Certified values
Certified human hair reference material (NCS ZC 81002b) (µg/g)					
Cd	0.0716 ± 0.003 (4.19)[b]	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
Mn	3.79 ± 0.34 (8.97)	3.76 ± 0.28 (7.45)	0.727	99.2	3.83 ± 0.39
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
Mg	247.4 ± 13.6 (5.50)	246.5 ± 10.8 (4.38)	0.820	99.6	248 ± 14
Certified human hair material CRM 397 (µg/g)					
Cd	0.53 ± 0.025 (4.72)	0.524 ± 0.024 (4.58)	0.2256	98.87	0.52 ± 0.024
Cr	90.94 ± 5.99 (6.59)	89.23 ± 6.53 (7.32)	0.0023	98.12	91.0 ± 10*
Mn	11.09 ± 0.85 (7.66)	10.88 ± 0.95 (8.73)	0.0012	98.11	11.2 ± 0.3*
Pb	33.29 ± 1.21 (3.63)	32.56 ± 1.18 (3.62)	0.096	97.8	33 ± 1.2
Zn	197 ± 12.8 (6.2)	194 ± 11.3 (5.7)	0.0345	98.6	199 ± 5
Mg	199.0 ± 13.5 (6.79)	198.2 ± 12.3 (6.2)	0.838	99.6	200 ± 5*

% recovery was calculated according to: $\frac{MDM \times 100}{CDM}$.
[a] Paired *t*-test between CDM and MWD, DF = 9, T (critical) at 95% CI = 2.262, P = 0.05, means in percentage.
[b] Values in ( ) are RSD.
* Informative value.
* Indicative value.

3. Results

Table 6 shows the descriptive data as mean values with standard deviation (SD) and P-values for each element in diabetic and referent subjects. Significantly lower concentrations of Zn, Cr, Mn and Mg were found in diabetic patients as compared to healthy subjects (unpaired t-test, Pb0.05).

Table 6
Concentrations of essential trace and toxic metals in scalp hair samples of Pakistani and Irish referent and diabetes mellitus patients (μg/g).

	Cadmium	Chromium	Manganese	Magnesium	Lead	Zinc
Referents						
Pakistani						
Male	1.63 ± 0.25	3.7 ± 0.32	3.8 ± 0.63	71.0 ± 16.3	5.93 ± 0.35	224 ± 10.6
Female	1.54 ± 0.17	3.9 ± 0.37	4.3 ± 0.49	72.8 ± 15.1	5.12 ± 0.52	236 ± 9.8
Irish						
Male	0.68 ± 0.15	3.86 ± 0.18	3.93 ± 0.21	73.9 ± 21.6	3.63 ± 0.37	252 ± 13.6
Female	0.62 ± 0.07	3.94 ± 0.33	4.35 ± 0.15	74.8 ± 20.6	3.52 ± 0.21	245 ± 10.5
Diabetes mellitus patients						
Pakistani						
Male	3.19 ± 0.3	2.1 ± 0.22	2.3 ± 0.42	43.5 ± 19.6	9.98 ± 0.52	163 ± 8.6
Female	2.85 ± 0.33	2.2 ± 0.17	2.4 ± 0.3	46.9 ± 19.8	9.31 ± 0.83	176 ± 9.32
Irish						
Male	2.26 ± 0.32	2.58 ± 0.14	2.10 ± 0.22	41.8 ± 17.5	6.42 ± 0.50	157 ± 9.3
Female	1.76 ± 0.06	2.65 ± 0.21	2.46 ± 0.34	42.6 ± 18.4	6.36 ± 0.47	148 ± 12.9

The concentrations of Mg in the scalp hair samples of male and female Irish referents were significantly higher at 95% confidence interval (CI: 72.8, 74.9) and (73.9, 75.0) μg/g, respectively, as compared to male and female Irish DM patients (P>0.001). The Mg levels in the scalp hair samples of male and female Pakistani referents (CI: 70.3, 71.8) and (CI: 72.0, 73.5) pg/g, respectively, were found to be higher than those values observed for DM patients of both genders (P> 0.001).

The concentrations of Zn in the scalp hair samples of male and female Irish referents were significantly higher (CI: 245, 259) and (239, 250) μg/g, respectively, as compared to DM patients (CI: 153, 162) and (CI: 141, 155) μg /g, for male and female respectively (P<0.001). The same trend was observed for Zn levels in scalp hair samples of male and female Pakistani referents and DM-2 patients (P> 0.001). It was observed that the level of Zn in scalp hair samples of Irish referent was 10-11% higher than Pakistani referents (P= 0.35). The concentrations of Cr in the scalp hair samples of male and female Irish DM patients were significantly lower (CI: 2.50, 2.66) and (2.55, 2.76) μg, respectively, as compared to referent males and females (P<0.01).

The same difference was observed between Pakistani DM patients and referents of both genders. The concentrations of Mn in the scalp hair samples of male and female Irish referents were significantly higher (CI: 3.81, 4.04) and (4.27, 4.43) pg/g, respectively, as compared to male and female Irish DM patients (P<0.01). The Mn levels in the scalp hair samples of male and female Pakistani referents (CI: 3.52, 4.15) and (CI: 4.05, 4.55) μg/g, respectively, were found to be higher than those values observed for DM patients of both genders (P> 0.01). For both categories of donors, the levels of Cd and Pb in scalp hair samples of DM patients were higher as compared to referent subjects. The concentrations of Cd in the scalp hair samples of male and female Irish DM patients were significantly higher at 95% confidence interval (CI: 2.10, 2.42) and (CI: 1.72, 1.79) pg/g, respectively, as compared to male and female referents (CI: 0.61, 0.76) and (CI: 0.58, 0.66) pg/g, respectively, with P<0.001 (Table 5). The Cd levels in the scalp hair samples of Pakistani

male and female referents (CI: 1.50, 1.75) and (CI: 1.46, 1.62) µg /g, respectively, were found to be significantly lower than those in male and female Pakistani DM patients (P< 0.001) (Table 6). The levels of Cd in scalp hair samples of Pakistani patients and referents were two to three folds higher than the Irish referents and DM-2 patients.

The ranges of Pb in the scalp hair samples of male Irish and Pakistani referents were (CI: 3.44-3.80) and (CI: 5.78-6.10) µg /g, respectively, whereas in Irish and Pakistani DM male patients were found to be (CI: 628-6.67) and (CI: 9.72-10.2) pg/g, respectively (P<0.001). The same trend was observed in female cases (Table 6). The positive correlation was observed between Zn, Cr and Mg levels in scalp hair of both genders (r= 0.632 to 0.829, P <0.001), indicating that these essential elements have beneficial roles in normal carbohydrate metabolism. While negative correlation was achieved between essential (Mg, Mn, Cr and Zn) and toxic elements (Cd and Pb) in scalp hair of understudied populations belonging to both countries (r = — 0.587-0.812, P <0.001).

4. Discussion

The analytical results of hair provide a more permanent record of elemental contents than blood and urine analyses [30]. Clinical research suggests that the homeostasis of elements can be disturbed by DM. Conversely, researches also suggest that the early imbalances of specific elements may play an important role in upsetting normal glucose and insulin metabolism. With regard to essential elements, the main clinical interest and majority of publication focus on deficiencies of a single element or certain combinations of elements [2]. In the present study, the correlation of essential and nonessential elements in scalp hair of DM-2 patients of both genders related to non-diabetic subjects belonging to Pakistan and Ireland was carried out. The results presented in Table 4 for healthy subjects and DM-2 patients confirm higher levels of Cd and Pb while lower values of Mg, Mn, Zn and Cr are associated with diabetes, which are consistent with other studies [31-34]. The results showed that the level of Mg was significantly lower in scalp hair samples of DM-2 patients as related to referents (P <0.001). Many studies suggest an inverse association between serum or plasma

Mg levels and risk of type 2 diabetes, indicating a potential role of Mg status in its pathogenesis [35,36]. Mg is a critical cofactor for several enzymes in carbohydrate metabolism, and involved in energy producing phosphorylation reactions [37]. Magnesium deficiency in type 2 diabetes mellitus is frequently associated with both extracellular and intracellular Mg depletion.

Epidemiologic studies have found high prevalence of hypomagnesaemia in subjects with type 2 diabetes, especially in those with poorly controlled glycemic control [38]. The present study shows that diabetic patients have low levels of Zn in scalp hair samples, which is consistent with other study [39]. Zn levels in female diabetics and referents of both countries were found to be higher than male diabetic and referent subjects, which are consistent with other investigation [40]. Zn deficiencies in diabetes are associated with excess free radical activity and increased oxidation of fats (lipids). When fats become oxidised, they are believed

to become more reactive and damaging to the heart, arteries, and other integral parts of the vascular system [41].

The resulted data indicated that the scalp hair of DM-2 patients of both countries have significantly lower levels of Cr as compared to referents. Cr is an essential element required for normal carbohydrate and lipid metabolism. Many scientists have demonstrated that a severe deficiency in Cr led to fasting hyperglycemia, glucosuria and impaired growth [42]. Our results are in good agreement with other investigation, which reported that the administration of Cr has a beneficial role on diabetes mellitus patients [43,44].

In our study, DM patients of both genders have lower levels of Mn in scalp hair samples than normal healthy groups of both genders. It was reported that urinary Mn excretion was slightly higher in DM patients as compared to referents [45]. Lower Mn concentrations were detected in lymphocytes derived from DM patients versus healthy subjects [46]. Ekmekcioglu et al. showed that insulin resistant diabetic patients responded well to oral doses of Mn [46]. It was investigated that appropriate Mn levels are required for normal insulin synthesis and secretion [47]. The findings of the present study clearly demonstrate that the concentration of toxic metals increased in the scalp hair samples of diabetic patients as compared to referents (Table 5). Although the understudy populations were non-smokers, but the potential health impact from smoking cigarettes that deliver high levels of toxic metal is not limited to active smokers. In indoor environments, Cd, Pb, As and organic carcinogens from side stream smoke are readily available for passive exposure [48]. An epidemiological study sug-gests a positive association between exposure to Cd and the incidence and severity of diabetes [49].

A significant association between elevated levels of urinary Cd and increases in fasting blood glucose was observed in a number of individuals diagnosed with type 2 diabetes [49]. A previous study has shown that exposure of experimental animals to Cd compounds increased the blood glucose concentration [50]. An epidemiological investigation has also indicated that the in-creased blood glucose level and decreased serum insulin level were shown in Cd-exposed workers as compared with the nonexposed subjects [51].

However, the detailed effects and mechanisms of Cd on insulin secretion/utilisation and blood glucose regulation are still unclear. Cd-induced cellular toxicity has been described in various targets including metalloenzyme interferences, thiol protein alterations, inhibition of energy metabolism, DNA and membrane structure/function alterations, and excessive oxidative damage [52]. Several studies have shown that Cd-induced hyperglycemia was associated with increased lipid peroxidation, decreased insulin release, increased activation of gluconeogenic enzymes and impaired insulin receptor [53,54]. The high Pb burden is a predictor of prospective increases in complexity of diabetes. Environmental or occupational Pb exposures create high prevalence (and growing incidence) of type 2 diabetes to occur in the general population [55]. An increase in bone resorption is a characteristic of age-ing in both men and women, ageing-associated release of bone Pb into the circulation is a potentially important source of soft-tissue Pb expo-sure and toxicity in diabetes [56].

This is the first study with comprehensive data on toxic and essential elements in the scalp hair samples of diabetic and referent subjects of two countries (Pakistan and Ireland). The

concentrations of essential trace and toxic elements in scalp hair samples of the Irish referent subjects were close to those reported for other European [57–65], American [66–69] and Australian [70] countries (Table 7).

The concentrations of Cd and Pb in Pakistani referents were almost higher than in European countries, which are in agreement with the studies carried out in Asia [71-85], and African countries [86-90] (Table 7).

Table 7
Comparison of different elemental contents (μg/g) in scalp hair of people from various parts of the world.

Countries/Authors	Elements	Age (y)	N	x ± s/range (μg/g)
Europe				
Sweden				
Rodushkin and	Pb	1–75	114	0.22–7.26
Axelsson [57]	Cd			0.010–0.356
	Zn			68–198
	Mn			0.080–2.41
	Cr			0.046–0.527
England				
Reilly and Harrison [58]	Zn	16–25	215	210–235
Germany				
Seifert et al. [59]	Cr	25–69	2524	0.111–0.117
	Cd			0.045–0.048
	Pb			0.93–0.99
	Mg			29.5–31.4
	Zn			154–158
Poland				
Nowak and	Pb	25–39 y	624	4.8–5.7
Chmielnicka [60]	Cd			0.56 ± 2.3
	Zn			132.7 ± 135.7
	Cr			1.1 ± 1.6
Trojanowski et al. [61]	Pb	26–50	109	3.71 ± 0.29
	Cd			0.401 ± 0.035
	Pb	51–75	121	3.88 ± 0.35
	Cd			0.260 ± 0.022
Suliburska et al., [62]	Mg	25–65	40	88.9 ± 45.5
	Zn			211.3 ± 66.0
Italy				
Sturaro et al. [63]	Zn	21–60 y	50	171–314
	Pb			6.5–8.7
France				
Goulle et al. [64]	Zn	40–60 y	45	129–209
	Pb			0.13–4.57
Netherlands				
Iyengar and	Zn	21–60 y	50	176 ± 38
Wolttlez [65]				
South America				
Nagra et al. [66]	Cd	22–59 y	50	31.6 ± 38
Douglas et al. [67]	Zn	20–55 y	42	108–357
North America				
Saiki et al., 2008 [68]	Zn	50–70 y	50	45–162
	–	71–87 y	50	30–202
DeAntonlo et al. [69]	Zn	15–35 y	67	90–294
Australia				
McKenzie [70]	Zn	16–56 y	118	189 ± 24
Asia				
Pakistan				
Pasha et al. [71]	Pb	15–94 y	86	14.62 ± 8.01 (0.577–31.8)
	Cd			2.13 ± 1.74 (0.196–9.17)
	Zn			154.2 ± 117.1 (12.4–729.2)
	Mn			1.69 ± 1.02 (0.10–4.83)
	Cr			2.61 ± 1.60 (0.495–7.375)
	Mg			153.4 ± 75.15 (46.5–341)
Pasha et al. [72]	Pb	37–65 y	37	15.50 ± 8.11
	Cd			1.675 ± 1.13
	Zn			140.7 ± 79.5
	Cr			2.345 ± 1.03
Shah et al. [73]				
(Pak)[a]	Pb	3–54 y	62	15.97 ± 5.56
(Lib)[b]				24.95 ± 8.69
(Pak)	Cd			0.38 ± 0.186
(Lib)				0.53 ± 0.26
(Pak)	Zn			226 ± 53.7
(Lib)				190 ± 34.0
(Pak)	Mn			1.93 ± 0.94
(Lib)				1.73 ± 1.09
(Pak)	Cr			3.3 ± 2.549
(Lib)				3.93 ± 2.46
Khalique et al. [74]	Cd	31–40 y	10	5.799 ± 5.639
		41–50 y		0.318 ± 0.150
	Zn			248.6 ± 62.4
				318.8 ± 59.1
	Mn			15.38 ± 12.6
				10.521 ± 4.875
	Cr			3.682 ± 1.362
				5.027 ± 1.714
	Mg			327.0 ± 319
				418.2 ± 124.2
India				
Vishwanathan	Pb	36 ±	25	24.8 ± 5.92
et al. [75]	Cd	1.23 y		5.12 ± 3.41
	Zn			265.2 ± 17.3
	Cr			35.2 ± 3.62
Sukumar and	Pb	31–45 y	17	8.9 ± 1.9
Subramanian [76]		46–60 y	11	4.5 ± 2.8
	Cd			1.5 ± 0.3
				1.9 ± 0.5
	Zn			87.0 ± 1.9
				112.8 ± 25.3
	Mn			1.3 ± 0.3
				1.4 ± 0.6
	Cr			1.0 ± 0.6
				0.4 ± 0.2
Mehra and Juneja [77]	Pb	1–30 y	50	7.60 ± 6.44
	Cd			0.32 ± 0.21
	Zn			182.4 ± 45.2
	Mn			6.71 ± 3.38
	Cr			68.6 ± 35.4
Rao et al. [78]	Cd	17–60 y	20	0.12–0.61
	Zn			45.44–123.5
	Pb			0.75–4.1
Bangladesh				
Ashrafur et al. [79]	Zn	Adults	30	199.16 ± 27.85
	Cd			0.47 ± 0.32
Turkey				
Sasmaz et al. [80]	Pb	Adults	26	3.06 ± 1.42
	Cd			0.67 ± 0.33
Ulvi et al. [81]	Zn	47.8 ±	29	176.96
	Cr	13.1 y		42.74
	Mg			121.4
Iran				
Faghihian and	Zn	14–67 y	100	36–329
Rahbarnia [82]				
Hong Kong				
Man and Zheng [83]	Pb	20–50 y	30	12.04 ± 7.0
	Zn			184.85 ± 60.89
Man et al. [84]	Zn	30–69 y	95	355–503
China				
Sandstead et al. [85]	Zn	7–25 y	662	109–155
Africa				
Nigeria				
Nnorom et al. [86]	Pb	1–30 y	46	63.6
	Cd			1.0
	Zn			128.6
Saudi Arabia				
Hashem and Abed [87]	Cd	20–25 y	20	0.035 ± 0.007
Syria				
Khuder et al. [88]	Pb	21–59 y	281	10.7 ± 8.9
	Zn			260 ± 113
Sudan				
Eltayeb and	Zn	30–50 y	35	89–170
Van-Grieken [89]	Pb			3–17
Egypt				
Mortada et al. [90]	Pb	28–40 y	93	1.8–9.7
	Cd			0.08–0.82

[a] Pak = Pakistan; [b] Lib = Libya.

There are a number of factors contributing to the higher levels of Cd and Pb in congested areas of Pakistan and other Asian countries. In Asian countries, there are many areas which represent a typical urban environment with heavy traffic load, high population density, and industrial activities. In addition, open burning of plastics and brick-making among other activities contribute to this higher level of toxic elements.

The study population in Hyderabad, Pakistan may be affected more by domestic and industrial exposures as compared to those residing in Dublin, Ireland [91]. Once taken up by fish, plants, and animals, Cd stays in the body for a long time [92]. Humans are also affected by Cd through smoking and consumption of foods and beverages. Rice is the main source of Cd in rice-eating countries. Human Pb exposure is mainly through air and food. The presence of lead in fuels has contributed too much of the current human exposure [93]. In most developed countries, the fuel content of Pb has been controlled but still remains an issue of immediate consideration in developing countries, including Pakistan. Other sources of lead expo-sure include lead-based paints, lead pipelines in water supply systems, and ceramics. Lead-based products, including paints and food containers, are not completely banned in Pakistan [94].

5. Conclusion

The observed variations in essential trace and toxic metal concentrations in scalp hair of referents and DM-2 patients of two donor groups (Pakistan and Ireland) reflected are collectively affected by individual variability and metabolic activity. In addition, environmental exposure emerged as a critical covariate that was found to overload the city atmosphere of Hyderabad, Pakistan, due to Cd and Pb pollution arising from automobile exhaust emissions. The results of this study revealed that DM patients have different patterns of essential trace and toxic elements in their scalp hair samples than do controls/ referents. However, higher levels of Cd, and Pb, as well as lower levels of Mg, Zn, Cr and Mn, correlated well with the consequences of DM. The deficiency of the essential elements, Mg, Zn, Cr and Mn, which are replaced by toxic elements (Cd and Pb), may result in abnormal physiology disorders, and, in addition to other factors, this may have a role in diabetes mellitus disease. The analysis of essential trace and toxic metals in biological samples of DM-2 patients related to healthy referents provides knowledge to physicians about the importance of alteration in metabolism of elements. This information may be used for diagnostic and therapeutic purposes.

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