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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.

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

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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 hypoglycemia [19]. The recommended Mn levels required for the development of normal insulin synthesis and secretion are 2.5 to 5 mg/day [20].

The toxic metals, lead (Pb) and cadmium (Cd), are ubiquitous environmental 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 diabetic patients [26].

In view of these facts, it is important to determine the essential trace and toxic elemental concentrations in biological samples of diabetes 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 abnormal 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 important way to understand any quantitative change in certain elements inside the body [28]. One of the most widely used analytical techniques for different elements of determination in biological and environmental 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 inductively coupled plasma mass spectrophotometer (ICP-MS) or electrothermal 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 elements 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 monochromator 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.

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.

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. 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 prepared 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 bottles 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 mol/l nitric acid, washed with distilled and finally rinsed with ultrapure water, dried, and stored in a class 100 laminar flow hoods.

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