Effect of Trace and Toxic Elements of Different Brands of Cigarettes on the Essential Elemental Status of Irish Referent and Diabetic Mellitus Consumers

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Abstract

Cigarette smoking interferes with the metal homeostasis of the human body, which plays a crucial role for maintaining the health. A significant flux of heavy metals, among other toxins, reaches the lungs through smoking. In the present study, the relationship between toxic element (TE) exposure via cigarette smoking and diabetic mellitus incidence in population living in Dublin, Ireland is investigated. The trace [zinc (Zn) and selenium (Se)] and toxic elements arsenic (As), aluminum (Al), cadmium (Cd), nickel (Ni), mercury (Hg), and lead (Pb) were determined in biological (scalp hair and blood) samples of patients diagnosed with diabetic mellitus, who are smokers living in Dublin, Ireland. These results were compared with age and sex matched healthy, nonsmokers controls. The different brands of cigarette (filler tobacco, filter, and ash) consumed by the studied population were also analyzed for As, Al, Cd, Ni, Hg, and Pb. The concentrations of TEs in biological samples and different components of cigarette were measured by inductively coupled plasma atomic emission spectrophotometer after microwave-assisted acid digestion. The validity and accuracy of the methodology were checked using certified reference materials (CRM). The recovery of all the studied elements was found to be in the range of 96.4–99.7 % in certified reference materials. The filler tobacco of different branded cigarettes contains Hg, As, Al, Cd, Ni, and Pb concentrations in the ranges of 9.55–12.4 ng/cigarette, 0.432–0.727 μg/cigarette, 360–496 μg/cigarette, 1.70–2.12 μg/cigarette, 0.715–1.52 μg/cigarette, and 0.378–1.16 μg/cigarette, respectively. The results of this study showed that the mean values of Al, As, Cd, Hg, Ni, and Pb were significantly higher in scalp hair and blood samples of diabetic mellitus patients in relation to healthy controls, while the difference was significant in the case of smoker patients ($p<0.001$). The levels of all six toxic elements were twofolds to threefolds higher in scalp hair and blood samples of nondiabetic mellitus smoker subjects as compared to nonsmoker controls. The high exposure of toxic metals as a result of cigarette smoking may be synergistic with risk factors associated with diabetic mellitus.

Keywords

Biological samples · Different brands of cigarette · Cigarette smokers · Toxic elements · Inductively coupled plasma atomic emission spectrophotometer

Introduction

Diabetes mellitus (DM), a global disease, prevails all over the world, but its prevalence rate differs from country to country [1]. The clinical research suggests that the body's balance of trace elements and minerals can be disrupted by diabetes mellitus [2]. Conversely, research also suggests that early imbalances of specific elements may play an important role in upsetting healthy glucose and insulin metabolism. With regard to essential trace elements, the main clinical interest and the majority of publications focus on deficiencies in 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; on the other hand, some of these nutrients can directly modulate glucose homeostasis [3, 4]. The deficiencies of certain minerals, such as magnesium, zinc (Zn), selenium (Se), vanadium (V), and chromium (Cr), have been shown to predispose a person to glucose intolerance and to promote the development of diabetic complications [5].

Smoking, however, is an important source of exposure to toxic elements (TEs), such as aluminum (Al), arsenic (As), cadmium (Cd), mercury (Hg), nickel (Ni), and lead (Pb), which have been proposed as causative agents of cigarette smoke-induced physiological disorders [6–8]. In fact, a study showed that serious symptoms (strong urges to smoke, feeling anxious, or unsuccessful attempts at not smoking) appeared in youth within weeks or only days after the initial start of smoking [8]. The use of tobacco products constitutes the most significant cause of morbidity and mortality in the world. Tobacco-related disease originates from the biological consequences of repeated inhalation exposure to numerous toxic constituents in cigarette smoke, which are produced by pyrosynthesis or liberated during combustion. Tobacco smoke has toxic [9], genotoxic [10], mutagenic [11], and carcinogenic properties [12]. Tobacco plant (*Nicotiana tabacum*) is well known for its capacity to concentrate toxic elements (TEs) from its growing environment, with corresponding higher levels in the tobacco leaves and in the smoke particulate [13]. Other environmental factors may influence toxic elements uptake by tobacco plants including soil pH and toxic elements containing sludge or fertilizers applied to crops [14]. The possible sources

of toxic elements for plants (tobacco leaves) presumably include surface contamination by industrial activities, natural uptake from soil, and even the use of arsenical pesticides in countries where these are still permitted [14, 15].

Thus, different cigarette brands could yield markedly different smoke particulate levels of toxic elements depending on where the tobacco was grown. Cigarette design has been largely evolved over the last decades with the incorporation of new tobacco processes, papers, filters, and several ingredients (flavors and casing materials), which either alone or in combination have the potential to modify the quantity and/or the quality of the smoke yielded [16, 17]. However, there are global and brand variations in the TEs compositions of commercial tobacco products [18, 19]. Several TEs found in tobacco smoke, such as Cd, Pb, and Ni, also accumulate in tissues and fluids through smoking [20]. The toxic elements are present in tobacco smoke and contribute substantially to cancer risk indices [21]. Aluminum is one of the most abundant elements present in tobacco [9]. The most prominent early pathological change associated with aluminum toxicity is the accumulation of neurofibrillar tangles in many regions of the brain. Aluminum also competes with and alters calcium metabolism in several organ systems including the brain. In tobacco smoke, Cd is a "strong carcinogens" with Ni, and As currently classified "carcinogenic to humans" by the International Agency for Research on Cancer (IARC) among 87 mainly organic carcinogens [22]. Toxic elements (cadmium, mercury, nickel, lead, and arsenic) may deplete glutathione and protein-bound sulfhydryl groups, resulting in the production of reactive oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radical [23].

The intake of trace and toxic elements may promote diabetic mellitus disorders by increasing oxidative stress (for example, by catalyzing the production of reactive oxygen species or inhibiting their degradation) due to the deficiency of an antioxidant element and by increasing glucose levels [24]. The deficiency of essential nutrients, lack of homeostatic control, or an excess intake of some toxic elements causes chronic physiological disorders such as hypertension, diabetic mellitus, and cardiovascular disease [24].

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 [25]. In the majority of cases, whole blood, serum, plasma, and urine were analyzed [26]. One of the most widely used analytical techniques for different element determinations in biological and environmental materials is inductively coupled plasma atomic emission spectrometry (ICP-AES) due to its advantages over other analytical methods: before all a possibility of simultaneous determination of many elements of interest, freedom from different chemical interferences, and high detection power. ICP-AES also offers rapid, multielement 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 [27, 28]. Since ICP-AES is able to analyze samples with higher TDS, more concentrated solutions can be prepared allowing trace elements to be measured. The main advantage of microwave-assisted sample pretreatment 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 [29].

The aim and objective of our present study was to assess the concentrations of trace essential (Zn, V, and Se) and toxic (Al, As, Cd, Hg, Ni, and Pb) elements in the scalp hair and blood samples of smoker nonsmoker and diabetic mellitus patients. For a comparative study, 117 nondiabetic mellitus individuals (smoker and nonsmokers) of the same age group (ranged 30–50 years), socioeconomic status, localities, and dietary habits were selected as controls. We also evaluated and compared the status of toxic elements (TEs) (Al, As, Cd, Hg, Ni, and Pb), in different presmoking and postsmoking components (filler tobacco, filter, and ash) of various imported branded cigarettes existing in Ireland. The understudy elements were analyzed byinductively coupled plasma atomic emission spectrophotometer, after microwave-assisted acid digestion.

Materials and Methods

Apparatus

Agate ball mixer mill (MM-2000 Haan, Germany), was used for grinding the cigarette tobacco, filter, and ash. Sieves made of nylon with mesh sizes of Ø<50 and 65 μm were used to study the influence of particle size on extraction. 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 first order, 0.009 nm in second order, 0.007 nm in third order and 0.006 nm in fourth order. The instrumental conditions are shown in Tables 1 and 2. A HINARI Lifestyle (Elstree, Hertfordshire, England) domestic microwave oven (maximum heating power of 800 W) was used for digestion of the

scalp hair, blood, and different cigarette component samples. Acid-washed polytetrafluoroethylene (PTFE) vessels and flasks were used for preparing and storing the solutions.

Table 1 Measurement conditions for inductively coupled plasma atomic emission spectroscopy

Parameters	Cd	As	Hg	Al	Se	Ni	Pb	Cr	V	Zn
Wavelength (nm)	226.502	193.692	253.652	308.216	196.026	231.604	220.553	267.716	292.402	213.8
Height (mm)	3	3	3	5	3	5	3	5	5	5
Windows (nm) (above the coil)	0.027	0.040	0.027	0.027	0.027	0.027	0.027	0.040	0.040	0.027
Scan (nm)	0.040	0.060	0.040	0.040	0.040	0.040	0.040	0.060	0.060	0.040
Integration (s)	3	3	3	3	3	3	3	3	3	3
Replicates	3	3	3	3	3	3	3	3	3	3
Sample uptake (s)	30	30	30	30	30	30	30	30	30	30
PMT (V)	650	650	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	1.10	1.10
Plasma flow (l/min)	15.0	15.0	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	1.50	1.50
Plump speed (rpm)	15	15	15	15	15	15	15	15	15	15
Background mode	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic
Max curve order	1	1	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	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

Reagents and Glasswares

Ultrapure water obtained from ELGA Lab Water system was used throughout the work. Concentrated nitric acid (65 %) and hydrogen peroxide (30 %) were from Merck (Darmstadt, Germany) and checked for possible trace metal contamination. Working standard solutions of Al, As, Cd, Cr, Hg, Se, Ni, Pb, V, and Zn were prepared immediately prior to their use, by stepwise dilution of certified standard solutions (1, 000 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 material (CRM), human hair NCSZN 81002b (Beijing, China), Clincheck® control-lyophilized human whole blood (Recipe, Munich, Germany), and Virginia tobacco leaves (ICHTJ-cta-VTL-2) (Dorodna, Warszawa, Poland) were used. 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.

Sample Collection and Pretreatment

Cigarette Pretreatment

Seven different commercially available branded cigarettes (BCs) were purchased from local market of Dublin (Ireland) during July and August 2010 (Table 3). The samples were in their original packaging, and placed in prewashed dried plastic bags separately and stored at 4 °C until tested. The weight of each cigarette after dried at 80 °C was determined. A duplicate of four composite samples of each branded cigarette (n=10) were taken randomly from four different batches (packed on different dates). For analysis of trace and toxic elements in cigarette tobacco, we separated all components of cigarette, tobacco, filter, and wrapping paper of five cigarettes of each composite samples and dry it in a sterilized glass beaker for 48 h at 80 °C; the dried tobacco were ground with agate ball mixer mill and sieved through nylon sieves with mesh sizes of Ø=65 μm. The remaining five cigarettes of each corresponding composite batch of all branded cigarettes understudy were used for smoking by a volunteer to collect ash of cigarette in cleaned PTFE beaker separately at room temperature (30– 35 °C). Cigarette smoking

termination was carried out when the burning line reached the butt length (different according to different brands). Care was taken to avoid any source of contamination, and this preparation was done in a clean room.

Table 3 Information of branded cigarettes

Sample code	Sample name	Description	Weight/cigarette (g)
BC1[a]	Dunhill	International, filter deluxe (UK)	0.731±0.008
BC2	Pine	Benhson and hedges	0.548±0.005
BC3	Marlboro gold	Filter class A cigarettes (USA)	0.869±0.015
BC4	Silk cut blue	Japan tobacco	0.715±0.009
BC5	John Player blue	Nottingham, England	0.692±0.013
BC6	Silk cut purple	Japan tobacco	0.702±0.005
BC7	More	Menthol filter class A cigarettes (USA)	0.947±0.04

[a] Branded cigarette

Biological Samples Pretreatment

An epidemiological cross-sectional survey was conducted among 140 referents and 117 DM subjects of both genders, age ranged 35–55 years, lived in urban area of Dublin, Ireland (Table 4). Before the start of this study, all referents and DM patients of both genders, 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 of alcohol, frequency of smoking, dietary habits, age, and consent. 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, not smoking patients (PNS), and cigarette smoking patients (PS). While control group are also divided into two groups: not smoking referents (CNS) and cigarette smoker referents (CS) 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. 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, and only patients who have type 2 diabetes were selected for study. In our survey, 23 % of patients had documented vascular disease (9 % having a history of cardiovascular disease, 12 % have hypertension and 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 other 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.

Table 4 Characteristics of study subjects (30–50) age groups

Parameters	Referents		Diabetic mellitus patients	
	Male	Female	Male	Female
Occupation				
Labor	25	20	32	25
Office workers	18	21	25	19
Not working	15	18	13	16
Habit				
Smoking tobacco	40	35	45	37
Nonsmoking tobacco	18	24	25	23

Collection of Blood and Scalp Hair Samples

Venous blood samples (5 ml) were collected by using 7 mm heparinized lithium Vacutainer® tubes (Becton Dickinson, Rutherford, NJ, USA). About 2 ml of venous blood samples were stored at −20 °C until elemental analysis [26]. 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 subsampling of the hair specimen. After cutting, each sample was washed with diluted Triton X-100, then samples were rinsed with distilled water and then with deionized water. The samples were then rinsed three times with acetone [29]. The samples were then dried in an oven at 75±5 °C for 2 h. Dried samples were stored separately in polyethylene bags.

Table 5 Clinical and biochemical characteristics of Diabetic mellitus patients and controls

Parameters	Controls		DM patients	
	CNS[a]	CS[b]	PNS[c]	PS[d]
Male				
Height (cm)	179.2±1.34	179.3±1.2	180.2±1.44	178.3±1.25
Weight (kg)	78.7±1.25	81.8±1.06	80.7±1.36	83.3±1.47
Waist circumference (cm)	75.9±1.25	78.7±1.23	85.9±1.45	86.2±1.35
BMI (kg/m^2)	24.5±1.59	25.4±1.39	24.8±1.28	26.2±1.15
Systolic BP (mmHg)	119.8±2.46	124.3±1.9	120.9±1.57	124.5±1.22
Diastolic BP (mmHg)	79.6±2.3	80.3±1.42	85.4±1.05	83.9±1.37
Fasting plasma glucose (mmol/l)	(90, 99)	(92, 100)	(131, 187)	(143, 192)
Fasting plasma insulin (mmol/l)	4.29±0.13	4.42±0.45	6.65±0.24	7.49±0.63
Diabetes duration (year)	–	–	9.2±0.32	10.6±0.57
Female				
Height (cm)	164.0±1.03	162.9±1.2	164.8±1.52	163.7±1.24
Weight (kg)	60.4±1.13	62.8±0.54	62.5±1.36	63.9±1.72
Waist circumference (cm)	63.1±0.51	65.3±1.17	63.7±1.52	64.3±0.76
BMI (kg/m^2)	22.5±1.32	23.7±1.31	23.0±0.62	23.8±1.09
Systolic BP (mm Hg)	119.6±1.09	119±0.76	120.2±1.36	122.4±1.03
Diastolic BP (mm Hg)	79.9±1.42	81.8±0.73	80.3±1.16	82.9±1.25
cFasting plasma glucose (mmol/l)	(85, 99)	(92, 100)	(135, 192)	(140, 195)
Fasting plasma insulin (mmol/l)	4.36±0.25	4.40±0.37	6.53±0.47	7.47±0.54
Diabetes duration (year)	–	–	10.4±0.59	11.4±1.24

BMI body mass index

[a] Control nonsmokers

[b] Control smokers

[c] Patient nonsmokers

[d] Patient smokers

Microwave-Assisted Acid Digestion

A microwave-assisted digestion procedure was carried out, in order to achieve a shorter digestion time. There were six samples of each certified replicate and triplicate samples of filler tobacco (FT) of each cigarette brand (0.2 g). Filter and ash (obtained from each cigarette) were weighed in PTFE flasks (25 ml in volume) and added 2.0 ml mixture of concentrated HNO_3–H_2O_2 (2:1, v/v) to tobacco leaves and filter, while acids mixture HNO_3–HCl (1:3, v/v) was used for ash of cigarette and all flasks were kept at room temperature for 10 min. Flasks were placed in a PTFE container close to it and were subjected to 80 % of total microwave energy (800 W). After cooling, the contents of each flask were heated on electric hot plate to semidried mass and dissolved in 5 ml of 1.0 M nitric acid and filtered through Whatman filter paper 42; the final volume was made up to 10 ml with deionized water as stock sample solutions.

Duplicate samples of scalp hair (200 mg) and 0.5 ml of blood samples of each DM patients and control subjects individuals were directly placed into Teflon PFA flasks. 2 ml of a freshly prepared mixture of concentrated HNO_3–H_2O_2 (2:1, v/v) were

added to each flask, left for 10 min. After this period, the flasks were placed in a covered PTFE container. This was then heated following a one-stage digestion program at 80 % of total power (800 W), during 2–3 min for blood, and 5–8 min for hair samples. After the digestion, the flasks were left to cool and the resulting solution was evaporated to semidried 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, Clincheck® control-lyophilized human whole blood, Virginia tobacco leaves (ICHTJ-cta-VTL-2) and with those obtained from conventional wet acid digestion method (Table 6) [29].

Table 6 Determination of trace elements in certified sample of human hair, blood, and Virginia tobacco leaf by conventional (CDM) and microwave digestion method (MWD) (n=10)

Elements	CDM	MWD	T value[a]	% Recovery[b]	Certified values
Certified sample of human hair (μg/g)					
Cd	0.0716±0.003 (4.19)	0.0714±0.006 (8.40)	0.305	99.7	0.072±0.010
Ni	5.71±0.51 (8.93)	5.67±0.43 (7.58)	0.339	99.4	5.77[c]
Pb	3.80±0.37 (9.74)	3.72±0.35 (9.41)	0.081	98.05	3.83±0.18
Se	1.92±0.15 (6.25)	1.85±0.12 (8.10)	0.0203	96.4	2.00±0.08
Zn	191±7.28 (3.81)	187±9.53 (5.09)	0.648	97.9	191±16
V	–	–	–	–	–
Cr	8.72±0.73 (8.37)	8.67±0.49 (5.65)	0.902	99.4	8.74±0.97
Al	13.26±0.94 (7.09)	13.2±0.62 (4.62)	0.145	99.5	13.3±2.3
Hg	12.2±0.21 (1.72)	11.9±0.23 (1.93)	0.146	97.5	12. 3±0.874
As	197.3±6.21 (3.15)	196.6±8.25 (4.20)	0.058	99.6	198±23
Certified sample of whole blood (μg/l)					
Cd	0.53±0.025 (4.72)	0.524±0.024 (4.58)	0.2256	98.87	0.52±0.024
Pb	33.29±1.21 (3.63)	32.56±1.18 (3.62)	0.096	97.8	33±1.2
Ni	46.07±1.41 (3.06)	45.75±1.38 (3.02)	0.9242	99.3	46.0±1.4
Se	114.6±2.47 (2.15)	113.5±3.68 (3.24)	0.247	99.0	116±23.5
Zn[d]	2.25±0.09 (4.00)	2.20±0.08 (3.64)	0.091	97.77	2.27±0.06
Cr	1.98±0.11 (5.55)	1.940±0.09 (4.64)	6.25×10^{-7}	97.98	2.00±0.5
Al	–	–	–	–	–
V	–	–	–	–	–
Hg	3.31±0.31 (9.06)	3.27±0.25 (7.64)	0.225	98.8	3.4±1.0
As	–	–	–	–	–
Virginia tobacco leaf (μg/g)					
Cd	1.52±0.102 (6.71)	1.50±0.0781 (5.20)	0.158	98.68	1.52±0.171
Pb	22.3±0.352 (1.57)	21.5±0.381 (1.77)	0.0311	96.41	22.1±0.0772
Ni	1.97±0.201 (10.2)	1.95±0.181 (9.28)	0.275	98.98	1.98±0.212

[a] T (critical) at 95 % CI=2.262, p=0.05

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

[c] Means in percentage, values in parentheses are CV

[d] In mg/l

Analytical Figures of Merit

Statistical analyses were performed using Minitab 13.2. The Student's t test was used to assess the significance of the differences in concentrations of elements among study subjects. Calibration was performed with a series of Al, As, Cr, Cd, Hg, Ni, Se, Pb, V, and Zn standards. Sensitivity (m) was the slope value obtained by least square regression analysis of calibration curves based on absorbance signals. The limit of detection, which is equal to 0.90, 0.002, 0.0003, 0.0003, 0.09, 0.003, 0.052, 0.0003, 0.0003, and 0.01 ng/mg for Al, As, Cr, Cd, Hg, Ni, Se, Pb, V, and Zn, respectively, was defined as 3 SD/m, corresponding to ten blank injections, and m is the slope of the calibration graph. The quantification limits, defined as 10 SD/m were calculated as: 2.5, 0.005, 0.0009, 0.0009, 0.26, 0.009, 0.16, 0.0009, 0.009, and 0.03 ng/mg for Al, As, Cr, Cd, Hg,

Ni, Se, Pb, V, and Zn, respectively.

Result

Toxic Metals in Different Components of Cigarettes

The analyses of seven different branded cigarettes for six toxins in different components of cigarette (filler tobacco, filter) presmoked and (filter and ash) postsmoked were determined by ICP-AES. The toxic elements (Al, As, Cd, Hg, Ni, and Pb) obtained from filler tobacco, ash, and filter of branded cigarettes origin showed a wide variation with regard to concentration levels of six toxic elements. Table 3 contains information about these cigarettes. The mean values of toxic elements were calculated on the basis of weight of each studied cigarette brands (Table 3). The results of toxic elements in different components of BCs were expressed as mean±SD as shown in Table 7. The filler tobacco of different BCs of different batches contains Al, As, Hg, Cd, Ni, and Pb concentrations in the ranges of 360–510 μg/cigarette, 0.432–0.727 μg/cigarette, 9.55–12.4 ng/cigarette, 1.70–2.12 μg/cigarette, 0.715–1.52 μg/cigarette, and 0.378–1.16 μg/cigarette, respectively (Table 7). It was observed that the understudy analytes were not detected in filter of cigarette before smoking. After smoking, the percentage of Al, As, Hg, Cd, Ni, and Pb absorbed and trapped by filter of different branded cigarettes were found in the ranges of 2.4–6.61 μg/cigarette, 9.3–16.0 μg/cigarette, 14.1–18.5 ng/cigarette, 13.9–19.7 μg/cigarette, 3.6–10.6 μg/cigarette, and 5.47–7.60 μg/cigarette, respectively, of total metals content observed in FT (Table 7). The percentages of Al, As, Hg, Cd, Ni, and Pb in ash of all studied cigarettes were observed in the ranges of 93.15–97.3 μg/cigarette, 30.7–42.4 μg/cigarette, 36.3–42.1 ng/cigarette, 14.9–27.8 μg/cigarette, 33.1–42.5 μg/cigarette, and 37.2–47.2 μg/cigarette of total contents of FT, respectively (Table 7). The concentration of toxic elements in FT were higher than those in the ash; these results are consistent with another study [30]. Cigarette ash plays an important role in terms of toxic metal distribution towards human health and environmental pollution.

The changes in the composition of tobacco, ash, and filter of cigarettes of various brands are associated with peculiarity of tobacco plant varieties and tobacco processing. There is no significant difference in average concentration of Cd in all branded cigarettes tested, ranging from 1.70 to 2.12 μg/cigarette (Table 7). The minimum amount of Cd was observed in BC7 (More), while the highest amount was also observed in BC6 (silk cut purple). As compared with the reported results for Cd in the UK (0.90 μg/g) and Korean cigarettes (1.02 μg/g), the average Cd contents in all cigarette brands are 1.74–2.20 times higher than those of UK and Korea, respectively [19], but lower than some branded cigarettes of Jordan [31]. The levels of Pb in seven branded cigarette was found in the range of 0.378–1.16 μg/cigarette corresponding to 0. 676–1.67 μg/g of filler tobacco. The average Pb contents in studied cigarette of different brands are comparable with literature reported values of Pb [19], while threefolds lower than those results of Pb in tobacco of cigarette reported by Massadeh et al. [31] (2.10 to 3.23 μg/g). The resulted data of toxic elements indicated that by smoking ten cigarettes of different brands in a day, 5.28–15.0 μg, 2.16– 3.76 μg, 10.3–11.9 μg, 43.5–53.1 ng, 4.02–8.82 μg, and 1.91–5.61 μg of Al, As, Cd, Hg, Ni, and Pb, respectively, were inhaled by the smoker or spreads into the environment.

Table 7 Concentration of cadmium, nickel, and lead in filler tobacco (FT), filter (F), and ash of different imported branded cigarettes [result based on $x \pm s$ (μg/cigarette), n=10]

Codes	Filler tobacco	Filter	Ash	Smoke concentration=FT – F+A[a]	Estimated metals/ten cigarette smoke
Cadmium					
BC1[b]	2.02±0.054	0.359±0.014 (17.8)[c]	0.562±0.025 (27.8)	1.09±0.015 (54.0)	10.9
BC2	1.78±0.109	0.264±0.016 (14.8)	0.479±0.022 (26.9)	1.03±0.071 (57.9)	10.3
BC3	1.83±0.097	0.361±0.013 (19.7)	0.398±0.019 (21.7)	1.07±0.065 (58.5)	10.7
BC4	1.97±0.135	0.348±0.015 (17.7)	0.425±0.011 (21.6)	1.19±0.111 (60.4)	11.9
BC5	1.73±0.082	0.241±0.012 (13.9)	0.406±0.016 (23.5)	1.08±0.054 (62.4)	10.8
BC6	2.12±0.104	0.392±0.014 (18.5)	0.546±0.033 (25.7)	1.18±0.102 (55.7)	11.8
BC7	1.70±0.120	0.259±0.014 (15.2)	0.253±0.035 (14.9)	1.19±0.124 (70.0)	11.9
Nickel					
BC1	0.916±0.069	0.0687±0.006 (7.5)	0.389±0.027 (42.5)	0.458±0.036 (50.0)	4.58
BC2	0.715±0.059	0.0760±0.004 (10.6)	0.237±0.016 (33.1)	0.402±0.039 (56.2)	4.02
BC3	1.06±0.073	0.0935±0.001 (8.82)	0.387±0.011 (36.51)	0.579±0.061 (54.6)	5.79
BC4	0.919±0.076	0.0752±0.0035 (8.2)	0.367±0.015 (39.9)	0.477±0.057 (51.9)	4.77
BC5	1.52±0.065	0.0552±0.005 (3.6)	0.583±0.016 (38.4)	0.882±0.044 (58.0)	8.82
BC6	0.764±0.042	0.0721±0.002 (9.44)	0.259±0.010 (33.9)	0.433±0.045 (56.7)	4.33
BC7	0.978±0.087	0.0735±0.005 (7.51)	0.385±0.009 (39.4)	0.519±0.023 (53.1)	5.19
Lead					
BC1	0.935±0.048	0.0609±0.007 (6.5)	0.362±0.013 (38.7)	0.512±0.028 (54.8)	5.12
BC2	0.378±0.034	0.0289±0.004 (7.6)	0.158±0.013 (41.8)	0.191±0.017 (50.6)	1.91
BC3	0.603±0.037	0.0418±0.008 (6.93)	0.238±0.010 (39.5)	0.323±0.019 (53.6)	3.23
BC4	0.846±0.052	0.0516±0.018 (6.10)	0.315±0.008 (37.2)	0.479±0.028 (56.6)	4.79
BC5	1.16±0.062	0.0635±0.007 (5.47)	0.535±0.019 (46.12)	0.561±0.036 (48.4)	5.61
BC6	0.986±0.055	0.0520±0.005 (5.3)	0.384±0.015 (38.9)	0.550±0.045 (55.8)	5.50
BC7	1.14±0.029	0.0689±0.007 (6.04)	0.538±0.041 (47.2)	0.534±0.037 (46.8)	5.34
Aluminum					
BC1	372±17.8	10.5±0.419 (2.82)	361±22.7 (97.04)	0.528±0.011 (0.14)	5.28
BC2	408±10.5	9.95±0.038 (2.4)	397±9.85 (97.3)	1.22±0.07 (0.30)	12.2
BC3	384±14.8	11.5±0.041 (3.00)	371±0.037 (96.6)	1. 5±0.05 (0.4)	15.0
BC4	360±20.8	12.2±0.35 (3.39)	347±8.92 (96.3)	0.79±0.02 (0.22)	7.9
BC5	465±36.2	18.6±0.98 (4.00)	445.8±20.9 (95.88)	0.60±0.05 (0.12)	6.0
BC6	510±40.8	25.1±2.75 (4.92)	584±30.7 (94.9)	0.90±0.007 (0.18)	9.0
BC7	496±24.8	32.8±3.65 (6.61)	462±11.5 (93.15)	1.20±0.09 (0.24)	12.0
Arsenic					
BC1	0.559±0.051	0.052±0.002 (9.3)	0.212±0.04 (37.9)	0.295±0.021 (52.8)	2.95
BC2	0.432±0.009	0.060±0.005 (13.9)	0.156±0.031 (36.1)	0.216±0.034 (50.0)	2.16
BC3	0.727±0.025	0.078±0.009 (10.7)	0.273±0.028 (37.6)	0.376±0.015 (51.7)	3.76
BC4	0.658±0.057	0.082±0.002 (15.5)	0.279±0.040 (42.4)	0.297±0.024 (45.1)	2.97
BC5	0.627±0.093	0.106±0.008 (17.0)	0.228±0.051 (36.4)	0.293±0.045 (44.6)	2.93
BC6	0.664±0.078	0.095±0.013 (14.31)	0.232±0.045 (34.94)	0.337±0.062 (50.75)	3.37
BC7	0.593±0.035	0.095±0.006 (16.0)	0.182±0.032 (30.7)	0.316±0.08 (53.3)	3.16
Mercury (ng)					
BC1	11.3±0.09	1.59±0.03 (14.1)	4.76±0.08 (42.1)	4.95±0.05 (43.8)	49.5
BC2	12.4±0.08	2.05±0.07 (16.5)	5.09±0.12 (41.1)	5.26±0.09 (42.4)	52.6
BC3	9.55±0.05	1.73±0.06 (18.1)	3.47±0.15 (36.3)	4.35±0.08 (45.6)	43.5
BC4	10.7±0.09	1.82±0.07 (17.0)	4.12±0.21 (38.5)	4.76±0.11 (44.5)	47.6
BC5	11.6±0.13	2.15±0.06 (18.5)	4.62±0.27 (39.9)	4.83±0.20 (41.6)	48.3
BC6	10.9±0.24	1.99±0.09 (18.3)	4.21±0.33 (38.6)	4.70±0.18 (43.1)	47.0
BC7	12.2±0.16	2.09±0.14 (17.2)	4.80±0.29 (39.3)	5.31±0.12 (43.5)	53.1

[a] Concentration of TMs in smoke obtained from total content in filler tobacco minus concentration of filter and ash values of same cigarette

[b] Branded cigarette

[c] Values in parenthesis is % of toxic metals in different component of cigarette with related to total contents in filler tobacco

Trace and Toxic Metals in Biological Samples of Controls and DM Patients

In the study population, more than 50 % referents and DM patients of both genders were smokers. 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 referents weighed more than nonsmoker referents ($p=0.042$). The elemental contents in the biological (scalp hair and blood) 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 toxic elements.

The mean concentrations with standard deviations for essential trace (Se, V, and Zn) and toxic elements (Al, As, Cd, Hg, Ni, and Pb) in biological samples, as shown in Table 8, indicate that the concentrations of essential trace elements were found to be lower, while the toxic elemental levels were higher in the scalp hair and blood samples of smoker diabetic mellitus patients.

The concentrations of Se in scalp hair samples of male and female Irish control nonsmokers (CNS) and control smokers (CS) at the 95 % confidence interval (CI) were (CI 1.65–1.78, 1.30–1.40) and (CI 1.63–1.75, 1.29–1.40) μg/g, respectively, but the mean values of Se in the scalp hair samples of male and female nonsmokers (PNS) and patient smokers (PS) patient were found in the range of (CI 0.73–0.86, 0.54–0.67) and (CI 0.72–0.86, 0.51–0.60) μg/g, respectively, which were significantly lower than the control subjects of the same age group ($p<0.001$). The concentrations of Se in the blood of male and female Irish CNS and CS were observed in the range of (CI 229–242, 205–218) and (CI 224–235, 174–185) μg/l, which were significantly higher than male and female PNS and PS patient, (CI 121–129, 106–110) and (CI 122–131, 94.8–100) μg/l ($p<0.001$), respectively, (Table 8). The concentrations of Zn in the scalp hair samples of male CNS and CS were significantly higher at 95 % confidence interval (CI 245, 260) and (CI 180, 190) μg/g, respectively, compared with those in PNS and PS, (CI 153, 162) and (CI 128, 135) μg/g, respectively, with $p<0.001$. The Zn levels in the blood samples of CNS and CS, (CI 9.50, 11.6) and (CI 8.25, 10.1) μg/g, respectively, were found to be higher than those in PNS and PS, (CI 5.59, 6.98) and (CI 4.52, 5.89) μg/g, respectively, ($p=0.001–0.002$). The same trend was observed in female patients and referents (Table 8).

Table 8 Concentrations of trace and toxic metals in scalp hair and blood samples of referent and diabetic mellitus patients

Parameters	Controls		Diabetic mellitus patients	
	CNS[a]	CS[b]	PNS[c]	PS[d]
Scalp hair (μg/g)				
Male				
Se	1.72±0.13	1.35±0.10	0.82±0.18	0.60±0.11
Zn	252±13.6	186±9.5	154±9.3	132±7.52
Cr	3.86±0.18	3.52±0.24	2.58±0.14	2.30±0.20
V	0.048±0.008	0.032±0.01	0.025±0.007	0.016±0.005
Al	6.24±0.48	8.86±0.55	13.5±1.05	22.9±1.99
As	0.86±0.08	1.37±0.12	1.89±0.23	2.79±0.15
Hg	1.02±0.07	1.26±0.09	1.69±0.08	2.25±0.09
Cd	0.68±0.15	1.52±0.23	2.26±0.32	2.93±0.29
Ni	3.42±0.36	3.56±0.93	4.95±0.67	6.34±0.62
Pb	3.63±0.37	4.51±0.18	6.42±0.50	7.62±0.28
Female				
Se	1.70±0.13	1.34±0.06	0.79±0.12	0.56±0.08
Zn	245±10.5	197±9.32	148±12.9	115±7.92
Cr	3.94±0.33	3.65±0.35	2.65±0.21	2.26±0.18
V	0.046±0.009	0.031±0.006	0.023±0.008	0.014±0.007
Al	5.82±0.70	8.57±0.39	12.8±0.69	19.9±0.74
As	0.76±0.09	1.28±0.06	1.79±0.09	2.58±0.15
Hg	0.98±0.03	1.13±0.06	1.78±0.07	2.15±0.09
Cd	0.62±0.07	1.56±0.12	1.76±0.06	2.72±0.15
Ni	3.35±0.47	5.32±0.24	4.42±0.36	6.51±0.52
Pb	3.52±0.21	4.35±0.47	6.36±0.47	7.48±0.27
Blood (μg/l)				
Male				
Se	235±12.2	208±11.0	125±6.74	108±5.08
Zn[e]	10.8±1.05	8.92±0.81	6.09±1.21	4.68±0.98
Cr	78.2±5.29	65.9±4.86	45.9±5.89	34.8±4.25
V	0.26±0.02	0.19±0.03	0.12±0.04	0.09±0.03
Al	8.15±0.97	11.8±0.92	17.9±0.71	22.5±1.38
As	1.83±0.19	2.38±0.22	3.17±0.19	3.90±0.27
Hg	0.85±0.08	1.28±0.07	1.92±0.07	2.55±0.09
Cd	3.53±0.68	5.24±0.27	5.94±0.53	7.24±1.06
Ni	1.83±0.26	2.78±0.38	3.57±0.32	6.74±0.65
Pb	186±14.8	275±18.7	378±19.3	545±29.4
Female				
Se	229±9.31	179±8.31	126±9.42	97.3±5.45
Zn[e]	9.69±1.39	8.22±1.07	5.41±1.08	4.10±0.73
Cr	76.9±6.38	64.7±4.31	41.9±3.57	30.9±2.82
V	0.25±0.03	0.17±0.04	0.10±0.03	0.08±0.02
Al	8.06±0.72	11.4±0.95	17.3±0.62	21.9±1.09
As	1.79±0.18	2.17±0.33	2.96±0.25	3.63±0.49
Hg	0.85±0.04	1.24±0.07	1.79±0.06	2.41±0.09
Cd	3.32±0.47	5.19±0.26	5.72±0.51	6.93±0.74
Ni	1.64±0.18	2.59±0.27	3.53±0.24	6.11±0.98
Pb	178±12.4	252±18.9	349±19.3	495±22.7

[a] Control nonsmokers
[b] Control smokers
[c] Patient nonsmokers
[d] Patient smokers
[e] In mg/l

The concentrations of Cr in the scalp hair samples of female CNS and CS were significantly higher at 95 % confidence interval (CI 3.78, 4.01) and (CI 3.48, 3.83) μg/g, respectively, compared with those in PNS and PS, (CI 2.54, 2.77) and (CI 2.17, 2.34) μg/g, respectively, with $p<0.001$. The Cr levels in the blood samples of CNS and CS, (CI 73.6, 79.9) and (CI 62.8, 66.9) μg/g, respectively, were found to be higher than those in PNS and PS, (CI 39.7, 43.5) and (CI 29.5, 32.4) μg/g, respectively, ($p<0.001$). The same trend was observed in male patients and referents (Table 8). The levels of V in the scalp hair samples of male and female Irish CNS and CS were significantly higher at 95 % confidence interval, (CI 0.046–0.049, 0.031–0.033) and (CI 0.044–0.048, 0.030-0.033) μg/g, respectively, compared with those in male and female PNS and PS

patients, (CI 0.022–0.027, 0.014–0.018) and (CI 0.023–0.025, 0.012–0.016) μg/g, respectively, with $p<0.002$. The V levels in the blood of male and female CNS and CS, (CI 0.25–0.27, 0.18–0.20) and (CI 0.23–0.27, 0.15–0.19) μg/l, respectively, were found to be higher than those in male and female PNS and PS patients, (CI 0.10–0.14, 0.08– 0.10) and (CI 0.08–0.12, 0.07–0.09) μg/l, respectively, ($p= 0.001–0.002$; Table 8).

An elevated level of Cd content was observed in the scalp hair of male CNS. The ranges of Cd in the scalp hair samples of CNS and CS were (CI 0.61–0.75) and (CI 1.41–1.74) μg/g, respectively, whereas those in PNS and PS were (CI 2.10– 2.42) and (CI 2.78–3.09) μg/g, respectively ($p<0.001$). The same trend was observed in female cases (Table 8). The level of Cd in blood samples were statistically significantly higher ($p<0.01$) in smoker diabetic patients of both genders (Table 8).

The Pb concentration in the scalp hair samples of male CNS was found at 95 % CI (3.44, 3.72) μg/g, whereas in the PNS, the Pb level was in the range of (CI 6.18–6.67) μg/ g (Table 8). Similarly, a higher level of Pb was observed in the blood samples of male PNS (CI 370–390) μg/g and PS (CI 530–560) μg/g than in CNS ($p<0.001$). The same trend was observed in females (Table 8).

The levels of Ni in the scalp hair samples of female RNCS and RCS were found to be lower, (CI 3.13–3.58) and (CI 5.20–5.45) μg/g, respectively, compared with those in PNS and PS, (CI 4.25–4.59) and (CI 6.24–6.76) μg/g, respectively, (Table 8). The same trend was observed in males (Table 8; $p>0.002$). The level of Ni in blood samples were statistically significantly higher ($p<0.01$) in smoker diabetic patients of both genders (Table 8). The levels of As and Hg in scalp hair and blood samples were statistically significantly higher ($p<0.01$) in both groups of DM patients (PNS, PS) compared with referent groups of both genders (Table 8).

The unpaired Student's t test at different degrees of freedom between diabetic patients and referents of both genders were calculated at different probabilities. Our calculated t_{value} exceeds that of $t_{critical}$ value at 95 % confidence intervals, which indicated the significant differences between mean values of understudy elements in referents and diabetic patients ($p<0.001$).

Discussion

Smoking in workplaces in Ireland was banned on 29 March 2004, making Ireland the first country in the world to institute an outright ban on smoking in workplaces, with fines of up to €3,000 on the spot. From 29 March 2004, under the *Public Health (Tobacco) Acts*, it has been illegal to smoke in all enclosed workplaces. The ban is strictly enforced and includes bars, restaurants, clubs, offices, public buildings, company cars, trucks, taxis, and vans—and within a 3-m radius to the entrances of these locations. However, it is permitted in designated hotel rooms and there is no ban in residential care, prisons, and outdoor areas [32]. Premises must display a sign to inform patrons of the ban in any of the nation's two official languages, and the contact person for any complaints. Ireland also banned in-store tobacco advertising and displays of tobacco products at retail outlets and a ban on the sale of packets of ten cigarettes in the second half of 2009. The same bill also started new controls on tobacco vending machines. On 18 July 2008, Irish Fine Gael MEP Avril Doyle proposed in a committee in the European Parliament that she would like to see an EU-wide ban on cigarettes and cigars by 2025 [33]. As of July 2009, it is prohibited to advertise cigarettes and sell ten packs of cigarettes in retail outlets. Additionally, as of February 2013, any tobacco product placed on the market must have graphic warnings [34]. There is legislation being made to introduce plain cigarette packets and make Ireland the second country to do so, after Australia [35].

This study provides data on essential trace (Cr, Se, V, and Zn) and toxic elements (Al, As, Cd, Hg, Ni, and Pb) in scalp hair and blood samples obtained from Irish smoker diabetic mellitus type 2 patients and nondiabetic controls of both genders of age group (30–50 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 [36]. 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 [37]. Tobacco-related disease originates from the biological consequences of repeated inhalation exposure to numerous toxic constituents including toxic elements in cigarette smoke, which are produced by pyrosynthesis or liberated during combustion. According to World Health Organization (WHO), every 10 s, another person dies as a result of tobacco use in the world [38].

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 [39]. The evidence that smoking is an independent risk factor for the development of diabetes is still considered preliminary [40]. Some studies have shown a dose–response association between smoking and incidence of diabetes [39, 40]. Also, some earlier prospective research failed to find an increased risk of diabetes among tobacco users [40]. 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 [41]. 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 [42]. 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 the lung and other organs. The carcinogens, oxidants, and a number of toxic substances have direct or indirect, modulatory or damaging effects on DNA, membrane lipids, cell signaling proteins, and various macromolecules [43]. 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, a type of hormone, are produced in greater quantity in smokers and act as an antagonist to insulin action [44].

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 [45]. The total amount of metal carcinogens in cigarette smoke ranges from 1 to 3 μg/cigarette [46]. Toxic elements uptake by tobacco plants depends on the concentration of these toxicants in the soil, soil amendments with sewage sludge, and soil pH [45]. It is likely that cigarettes made from tobacco grown in various geographical regions or under different agricultural conditions will have different levels of the heavy metals in the tobacco filler and thus generate different levels in the smoke [37, 38]. Tobacco leaves naturally accumulate and concentrate relatively high levels of toxic heavy metals, and therefore, smoking of tobacco is an important source of these metals exposure for smokers [46].

The investigated data indicates that smokers could receive significantly higher exposures to TEs (Cd, Pb, and Ni) from different BCs. The country of origin and type of the product play major roles in determining the chemical composition of cigarette tobacco [47]. Tobacco plants have a profound ability to absorb Cd from the soil and accumulate it in high concentrations in the leaves and can lead to human exposure to this carcinogenic metal [46]. The Cd is the best studied metal from cigarette smoke, and smoking is the main source of Cd intake by humans. Although the Cd amounts varied, the average Cd content per cigarette lies between 0.5 and 1.5 μg/cigarette [22]. The Pb may also be present in high concentrations in tobacco smoke. Smokers have considerably higher blood Pb levels than nonsmokers [45]. The Ni reacts with carbon monoxide in tobacco smoke to form a highly toxic carbonyl compound, which is believed to be a potential carcinogen. The amount of Ni in the tobacco plant lies between 0.640 and 1.15 μg/g and varies greatly in cigarettes of different brands [15]. Arsenic in mammals causes lipid peroxidation, protein and enzyme oxidation, and glutathione depletion [48]. It was also reported in literature of the interaction of arsenate with glutathione that several enzyme systems are involved, including arsenate reductase and a glutathione *S*-transferase, with the resultant formation of a complex consisting of three molecules of glutathione with a single atom of arsenic [$(GS)_3As$] [49]. This arsenic–glutathione complex undergoes rapid biliary excretion. The lack of glutathione is believed to result in the occurrence of an oxidative stress due to the decrease in adequate antioxidant protection within cells. Therefore, arsenate in cigarette smoke may contribute to the oxidative stress that is produced in lungs, resulting in tissue-damaging effects. Aluminum concentration was high in all BCs, one would imagine that smoking could be one source of aluminum exposure. The most prominent early pathological change associated with aluminum toxicity is the accumulation of neurofibrillar tangles in many regions of the brain. Aluminum also competes with and alters calcium metabolism in several organ systems including the brain [50]. Aluminum has a fixed oxidation number and, therefore, cannot participate in redox reactions. However, as previously noted, aluminum can displace iron from binding sites and, therefore, result in an increase in catalytically active iron [51]. Furthermore, aluminum has been shown to accelerate iron-stimulated peroxidation of membrane lipids and may cause rearrangement of membrane lipids [52]. Thus, aluminum in tobacco smoke may enhance iron-dependent free radical induced tissue damage via an indirect mechanism [53–55].

53.1 The levels of Al, As, Cd, Hg, Pb, and Ni passed to the smokes of ten cigarettes of different brands were estimated to be 5.28–15.0 μg, 2.16–3.76 μg, 10.3–11.9 μg, 43.5– ng, 4.02–8.82 μg, and 1.91–5.61 μg/ten cigarettes, respectively (Table 7), either passed into mainstream or sidestream smoke. It was investigated that one pack of cigarettes deposits 1–3 μg Al, 0–1.4 μg As, 2–4 μg Cd, 0.46 to 6.5 ng Hg, and 1–2 μg Pb and Ni into the lungs of a smoker, whereas some of the smoke passes into the air to be inhaled by smokers and nonsmokers alike [47].

Our resulted data indicated that smoker DM patients and controls have increased levels of toxic elements (Al, As, Cd, Hg, Pb, and Ni) while they have the lowest value of Cr, Se, V, and Zn in blood and scalp hair samples (Table 8), which were maybe associated with increasing prevalence of diabetic mellitus in both genders. This study revealed that the level of Zn was low in scalp hair samples of diabetic smokers and alcohol drinker patients. 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 [56]. In diabetic individuals, enteric neuropathy and microvascular disease can alter intestinal absorption of carbohydrates, amino acids, and minerals [57]. 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 [58].

Analysis of Cr in the scalp hair samples of DM subjects were found significantly lower than patients (p>0.001). Our

results are extensively consistent with other investigations such as Anderson [59, 60] who elucidated the action 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 [61]. Our older patients have lower values of Cr in scalp hair samples, which is consistent with the study of Davies et al. [62], who found age-related decreases of chromium in hair as compared to matched normal subjects. 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 [63] have indicated that Cr supplementation increases muscle gain and fat loss associated with exercise and improves glucose metabolism and the serum lipid profile in patients with or without diabetes. Most of the patients under study have cardiac problems, and they are also consistent with other studies [64, 65], which reported that the low Cr concentrations and the associated impairments in insulin, glucose, and lipid metabolism may also result in increased cardiovascular risk.

In our study, the DM patients of both genders have higher level of Ni in scalp hair and blood 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 [66, 67].

Type 2 diabetes is associated with oxidative stress attributable to the production of excess levels of reactive oxygen species in hyperglycemia [68]. As pancreatic β-cells are poorly protected by intrinsic enzymatic antioxidants and are very susceptible to oxidative injury, it might be thought that the antioxidant selenoenzyme GPx could protect against that stress [68]. Indeed overexpression of GPx in islets provided enhanced protection against oxidative stress [68], whereas selenium, as selenite or selenate, at physiological levels was able to stimulate pancreatic β-cell gene expression and enhance islet function [69]. Whether cause or consequence, plasma GPx3 concentration was decreased in newly diagnosed, drug-naive diabetic patients compared to subjects with normal or impaired glucose tolerance and was lower in db/db than in normal mice [70]. Another selenoprotein, SEPS1, seems to protect the β-cell against endoplasmic reticulum stress and oxidative stress and may protect against β-cell apoptosis [70].

In the present study, the mean scalp hair and blood V level in smoker and nonsmoker diabetic mellitus patients showed a significant decrease as compared to controls; this may be due to the glomerular hyperfiltration in diabetes [71]. In the earlier study, V in lymphocytes was significantly higher in diabetic patients as compared to control [72]. However, there is limited information about the metabolic effects of vanadium in humans [73]. It has been studied that vanadyl sulfate, which is the biologically significant form of V, improves hepatic and muscle insulin sensitivity in type 2 diabetes [74].

The findings of the present study clearly demonstrate that the concentration of toxic metals varied in the scalp hair samples of smoker and alcohol drinker diabetic patients as compared to smoker and referents, i.e., Cd and Pb concentrations increased in scalp hair and blood were found as shown in Table 8. Metallic carcinogenicity is generally thought to generate of free radicals, and thus, some metals were reported to play a role in lung tumorigenesis. The potential health impact from smoking cigarettes that deliver high levels of toxic metal is not limited to active smokers. In indoor environments, cadmium, lead, arsenic, and organic carcinogens from sidestream smoke are readily available for passive exposure [75]. Cadmium exposure from smoking cigarettes may be a more serious health concern than cadmium in food. Smokers may double their daily intake of cadmium compared with nonsmokers. Each cigarette may contain from 1 to 2 mg of cadmium, and 40–60 % of the cadmium 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 cadmium into their body per day from each pack of cigarettes smoked. Smoke from other people's cigarettes probably does not cause nonsmokers to take in much cadmium. Aside from tobacco smokers, people who live near hazardous waste sites or factories that release cadmium into the air have the potential for exposure to cadmium 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 nonsmoker workers [76].

There is rare 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 [77]. The stimulatory effect of Cd on glucose transport was also confirmed in cell culture model and again, no effects on GLUT4 protein were observed [78]. It seems that aforementioned findings on Cd-induced glucose transport could explain previously described in vitro insulin mimetic effect of cadmium on glucose lipogenesis and glucose oxidation [78] in rat adipocytes. In pancreatic islets of obese hyperglycemic mice, low cadmium concentration evoked basal and glucose stimulated insulin response [78]. In contrast, high Cd concentration significantly inhibited the secretory response to glucose [78]. In vivo rat intake of cadmium resulted in lower glycemia accompanied with higher serum insulin value [78]. Further discrepancies in cadmium effects on glucose homeostasis and insulin levels are results of hyperglycemia and inhibition of insulin release from rat pancreas in rats exposed to cadmium [78]. Incompatibility of literary data on cadmium 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 the 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 accumulated exposure and lead burden [79]. In addition, bone lead also serves as an endogenous source of lead exposure for individuals with increased bone turnover [80]. Therefore, 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. Given that 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 softtissue 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) [80] and is well known as an independent predictor of accelerated decline in kidney function.

Both in vitro and in vivo studies [81, 82] found that MeHg exposure, at a level similar to that in some seafood, significantly decreased cell viability in the pancreatic β-cell line and caused pancreatic islet β-cell dysfunction, which may lead to diabetes development [83]. In particular, laboratory studies demonstrated that islet β-cell function and survival was affected by mercury exposure through an oxidative stress pathway [81, 82]. In mouse models, even low-dose mercury exposure caused pancreatic islet β-cell dysfunction by inducing oxidative stress and phosphatidylinositol 3-kinase activation [82]. Also, MeHg was observed to induce oxidative stress-triggered β-cell apoptosis and death [81]. In addition, a study found that 8-hydroxy-2′-deoxyguanosine, a biomarker of oxidative DNA damage, was significantly higher in urine samples of mercury- exposed persons compared with control subjects [84]. Human data relating mercury exposure to diabetes are sparse, and the results were contradictory. A study conducted in Japan reported that mercury levels in hair from patients including patients with diabetes were considerably higher than that of healthy people of the same age groups [85]. This finding was in agreement with results from studies conducted in Turkey [86] and Mexico [87], but was not consistent with another Japanese survey in which the prevalence of diabetes among people living in an MeHg-polluted area was not increased [88]. In a recent study conducted in Taiwan [89], blood mercury concentrations were significantly associated with HOMA-IR and HOMA of β-cell function index. In addition, a study conducted in Korea reported that hair mercury levels in patients with metabolic syndrome were significantly higher than those in normal control subjects [90]. Data from Western countries are not available. Our investigation provides evidence from a human study supporting that mercury exposure at young adulthood may be longitudinally associated with increased risk of diabetes and pancreatic islet β-cell dysfunction.

Conclusion

This study provided a new data for the health authorities in Ireland. The results of toxic elements (Al, As, Cd, Hg, Ni, and Pb) in different branded cigarettes consumed in Ireland confirmed that tobacco is a notable source of their exposure to the general population. So, we had analyzed the biological samples (scalp hair and blood) of referent and diabetic mellitus type 2 patients for the determination of essential trace (Cr, Se, V, and Zn) and toxic elements (Al, As, Cd, Hg, Ni, and Pb). The results of this study revealed that diabetic mellitus type 2 patients have a different pattern of essential trace and toxic elements in their biological samples than do controls/referents, with the prevalence being more in smoker patients. The impaired trace element metabolism of the present work may have a role in the pathogenesis and progression of DM, where the increase of Al, As, Cd, Hg, Ni, and Pb and the decrease of Cr, Se, V, and Zn concentrations in biological samples of DMs may disturb the secretion and action of insulin. The high levels of Al, As, Cd, Hg, Ni, and Pb may disturb the antioxidants and enhance the lipid peroxidation. Oxidative modification of lipoproteins, particularly LDL, may be at least one cause of vascular complications of DM. The DM patients of both genders have lower levels of Zn in biological sample possibly due to a loss of Zn due to hyperzincuria. A decreased Cr content in biological samples reflected a reduced Cr supply in diabetics type 2. The poorer the metabolic control of diabetics type 2, the lower was the Se, V, and Cr status. Thus, these patients— especially type 2 diabetics with metabolic disorders—should consider supplementation of these minerals. The deficiency of the essential elements, Cr, Se, V, and Zn, which are replaced by trace and toxic elements (Al, As, Cd, Hg, Pb, and Ni), may result in abnormal physiology disorders, and, in addition to other factors, this may have a role in diabetic mellitus type 2. Smoking and tobacco consumption further aggravates the problem by increasing the levels of toxic elements (Al, As, Cd, Hg, Pb, and Ni). It was recommended that the trace and toxic elemental measurements may be performed on patients reaching in the emergency department, to test whether their concentration may serve not only as markers of diabetic mellitus and its remedies but also as predictors of adverse outcomes. Further studies may be useful to infer a causal relationship between toxic elements exposure from tobacco smoke in addition to other sources (food, water, and atmosphere) and diabetic mellitus.

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