

Estimation of toxic elements in the samples of different cigarettes and their impact on human health of Irish hypertensive consumers

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ABSTRACT

Background: 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 hypertension incidence in population living in Dublin, Ireland is investigated. **Methods:** The different brands of cigarette (filler tobacco, filter and ash) consumed by the studied population were analyzed for cadmium (Cd), nickel (Ni), and lead (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. **Results:** The filler tobacco of different branded cigarettes contains Cd, Ni and Pb concentrations in the ranges of 1.73-2.02, 0.715-1.52 and 0.378-1.16 pg/cigarette, respectively. The results of this study showed that the mean values of Cd, Ni and Pb were significantly higher in scalp hair and blood samples of hypertensive patients in relation to healthy controls, while the difference was significant in the case of smoker patients ($p < 0.001$). The levels of all the three TEs were 2-3 folds higher in scalp hair and blood samples of non-hypertensive smoker subjects as compared to nonsmoker controls. **Conclusion:** The high exposure of toxic metals as a result of cigarette smoking may be synergistic with risk factors associated with hypertension.

Keywords: Scalp hair, Blood, Different brands of cigarette, Cigarette smokers, Toxic elements, Inductive coupled plasma atomic, emission spectrophotometer,

1. Introduction

Hypertension (HT) is an increasingly important medical and public health issue. The prevalence of HT increases with advancing age (60-90 years) [1]. But today, the age criteria have been changed and even people below 30 years of age have HT problems because of the lack of exercise, fast foods, smoking, coffee and alcohol consumption [2]. Genetic effect may also be a factor [3]. Smoking, however, is an important source of exposure to toxic elements (TEs) such as cadmium (Cd), nickel (Ni) and lead (Pb), which have been proposed as causative agents of cigarette smoke-induced physiological disorders [4-6]. 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 [6]. Cigarette design has evolved considerably over the last few decades with the incorporation of new tobacco processes, papers, filters and several ingredients (flavor, humectants and casing materials), which either alone or in combination have the potential to modify the quantity and/or the quality of the smoke yielded [7].

The tobacco plant absorbs TEs most probably from the soil, from fertilizers or from pesticides [8]. Other environmental factors that may influence the uptake of TEs by tobacco plants include the pH of soil, contaminated irrigated water and sewage sludge used as fertilizers. Tobacco smoking delivers 87 organic carcinogens to the lungs, in addition to TEs [9], which may partition into the smoke phase on combustion [10]. Some of these (Cd, Ni and Pb) readily pass into the bloodstream and may accumulate in specific organs, such as the kidney and liver [11]. There are a few studies that have reported on the large variations of heavy metal/TEs in the compositions of commercial tobacco products, which have tried to link smoking-related diseases with TEs derived from tobacco combustion [12]. The intake of trace and TEs may promote hypertensive and atherosclerosis 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 blood pressure levels [13].

The deficiency of essential nutrients, lack of homeostatic control or an excess intake of some TEs causes chronic physiological disorders, such as HT and cardiovascular disease [14]. 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 [15]. In the majority of cases, whole blood, serum, plasma, and urine were analyzed [16]. 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, multi-element determinations. The sensitivity of ICP-AES is lower than that of either inductive coupled plasma mass spectrophotometer (ICP-MS) or atomic absorption graphite tube atom-izer (AA-GTA), but ICP-AES can handle higher levels of total dissolved solids (TDS) than ICPMS and is much faster than AA-GTA [17,18]. 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 [19]. The aim and objective of our present study was to assess the concentrations of Cd, Ni and Pb in the scalp hair and blood samples of smoker and hypertensive patients. For a comparative study, 54 non-hypertensive individuals (smoker and nonsmokers) of the same age group (ranged 30-50 years), socioeconomic status, localities and dietary habits were

selected as controls. The understudy elements were analyzed by inductive coupled plasma atomic emission spectrophotometer, after microwave-assisted acid digestion. Presently, we also evaluated and compared the status of toxic metals (TEs) (Cd, Ni and Pb), in different pre-smoking and post-smoking components (filler tobacco, filter and ash) of various imported branded cigarettes existing in Ireland.

2. Materials and methods

2.1. 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 $0 < 50$ and $65 \mu\text{m}$ 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 1800 grooves/mm holographic grating used in up to 4 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 instrumental conditions are shown in Table 1. A Hinari Life style (Elstree) 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 inductive coupled plasma atomic emission spectroscopy Liberty 220 ICP-AES.

Parameters	Cd	Ni	Pb
Wavelength (nm)	226.5	231.6	220.5
Height (mm)	3	5	3
<i>Common parameters</i>			
Windows (nm) (above the coil)	0.027	Nebulizer type	V-groove
Scan (nm)	0.040	Nebulizer pressure	150 kPa
Integration (s)	3	Stabilization time	10 s
Replicates	3	Sample delay time	30 s
Sample uptake (s)	30	Rinse time	10 s
PMT (V)	650	Pump-tube	Orange-orange (inlet) Blue-blue (outlet)
Power (kW)	1.10	Snout purge	off
Plasma flow (l/min)	15.0	Fast pump	On
Auxiliary flow (l/min)	1.50	Background mode	Dynamic
Pump speed (rpm)	15	Max curve order	1
C.C. Limit	0.995		

2.2. Reagents and glass wares

Ultrapure water obtained from ELGA Lab Water system was used throughout the work. Concentrated nitric acid (65%) and hydrogen per-oxide (30%) were from Merck and checked for possible trace metal con-tamination. Working standard solutions of Cd, Ni and Pb were prepared immediately prior to their use, by stepwise dilution of certified standard solutions (1000 ppm) Fluka Kamica (Buchs), with 0.5 mol/l HNO₃. All solutions were stored in polyethylene bottles at 4 °C. For the accuracy of methodology, the certified reference material (CRM), human hair NCSZN 81002b, Clincheck® control-lyophilized human whole blood and Virginia tobacco leaves (ICI-ITJ-cta-VTL-2) 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.

2.3. Sample collection and pretreatment

2.3.1. Cigarette pretreatment

Five different commercially available branded cigarettes (BCs) were purchased from local market of Dublin (Ireland) during July and August 2010 (Table 2). The samples were in their original packaging, and placed in pre-washed dried plastic bags separately and stored at 4 °C until test-ed. The weight of each cigarette after dried at 80 °C was determined. A duplicate 4 composite samples of each branded cigarette (n = 10) were taken randomly from 4 different batches (packed on different dates). For analysis of TES in cigarette tobacco, we separated all compo-nents of cigarette, tobacco, filter and wrapping paper of 5 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 0 65 µm. The remaining 5 cigarettes of each corresponding composite batch of all branded ciga-rettes 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 carrying 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 2
Information of branded cigarettes.

Sample code	Sample name	Description	Wt/cigarette (g)
BC1 ^a	Dunhill	International, filter deluxe UK	0.731 ± 0.008
BC2	Pine	Benhson and hedges	0.548 ± 0.005
BC3	Marlboro	Filter class A cigarettes (USA)	0.869 ± 0.015
BC4	Silk cut	Japan tobacco	0.715 ± 0.009
BC5	John Player blue	Nottingham, England.	0.692 ± 0.013

^a Branded cigarette.

2.32. Biological samples pretreatment

Before the start of this study, all controls and HT patients of both genders, age range 30-50 years, were informed through a consent form by the administration about the aim of study, and all agreed to participate and signed the form. A questionnaire was also administered to them to collect details regarding physical data, ethnic origin, health and duration of smoking, frequency of smoking, dietary habits, age and consent.

The patients were grouped according to their habits, non-smoker patients (PNS) and cigarette smoker patients (PS). While control group are also divided into 2 groups, first group nonsmoker (CNS) and cigarette smoker (CS), as shown in Table 3. 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 controls, anthropometric parameters including weight, height and waist circumference were measured using the standard protocols (Table 4).

Table 3
Characteristics of study subjects (30-50) age groups.

Parameters	Referents		Hypertensive patients	
	Male	Female	Male	Female
<i>Occupation</i>				
Labor	25	20	25	22
Office workers	18	21	25	15
Not working	15	18	19	20
<i>Habit</i>				
Smoking tobacco	40	35	48	40
Nonsmoking tobacco	18	24	21	17

Table 4
Clinical and biochemical characteristics of hypertension patients and controls.

Parameters	Controls		HT patients	
	CNS ^a	CS ^b	PNS ^c	PS ^d
<i>Male</i>				
Height (cm)	180.3 ± 2.5	181.6 ± 0.9	179.6 ± 1.32	179.8 ± 0.83
Weight (kg)	79.4 ± 1.3	82.1 ± 1.13	80.3 ± 2.54	82.6 ± 2.68
Waist circumference (cm)	76.4 ± 1.19	79.3 ± 1.21	85.8 ± 1.02	87.3 ± 1.16
BMI (kg/m ²) ^e	24.4 ± 2.0	24.9 ± 0.85	24.9 ± 1.6	25.5 ± 1.34
Systolic BP (mm Hg)	119.3 ± 3.1	122.7 ± 2.4	152.2 ± 2.8	160.5 ± 1.06
Diastolic BP (mm Hg)	79.9 ± 2.7	83.2 ± 0.92	96.5 ± 1.42	99.2 ± 0.62
<i>Female</i>				
Height (cm)	164.2 ± 1.28	165.2 ± 1.0	165.1 ± 0.95	164.3 ± 0.82
Weight (kg)	60.5 ± 1.26	62.3 ± 0.72	62.2 ± 1.73	64.7 ± 0.55
Waist circumference (cm)	63.4 ± 1.06	65.1 ± 1.30	64.2 ± 0.85	65.6 ± 0.63
BMI (kg/m ²)	22.4 ± 1.57	22.8 ± 1.65	22.8 ± 1.57	23.9 ± 1.74
Systolic BP (mm Hg)	119.1 ± 1.2	120.7 ± 0.5	150.4 ± 1.92	158.5 ± 1.21
Diastolic BP (mm Hg)	80.1 ± 0.93	83.5 ± 0.61	95.2 ± 1.25	96.5 ± 0.47

^a Control nonsmokers.

^b Control smokers.

^c Patient nonsmokers.

^d Patient smokers.

^e BMI = body mass index.

There were no statistically significant differences between both groups of patients and controls with regard to height and weight. Among controls and patients smokers, the range of consumption was of 10-15 cigarettes/day. The 62% hypertensive patient used antihypertensive drugs. The study protocol was approved by the local ethics committee of Dublin city university, Ireland. The hypertensive patients, who had blood pressure exceeding 130/95 mm Hg (systolic/diastolic), were admitted for their uncontrolled HT and had earlier histories of high blood pressure.

The criteria of healthy subjects included no history of symptoms of hypertension and any coronary disease documented in their medical notes, and no family history of heart disease was defined by a first-degree relative with a myocardial infarction (MI), or cardiac death before the age of 55 years. All control subjects underwent a routine medical examination including MI test. 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. There is no difference in the dietary habits of Irish controls and hypertensive patients of both genders. The dietary habits of Irish people (elder age group) depend mainly upon meat (chicken, mutton, lamb) and plants (vegetables and beans) consumption.

The socioeconomic status of understudied Irish people is average. The >65% of understudied controls and hypertensive people reported earning more or equal than €320 per week. All understudied older people had achieved at least secondary education. The elderly have greater susceptibility of increased or decreased levels of elements than younger. Gender is also a factor to consider due to differences in the intake of energy, essential elemental status, or hormonal influences, which can affect the bioavailability of trace elements.

2.4. Collection of blood and scalp hair samples

Venous blood samples (5 ml) were collected by using 7 mm heparinized lithium Vacutainer® tubes (Becton Dickinson). About 2 ml of venous blood samples were stored at — 20 °C until elemental analysis [19]. 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 [19]. The samples were then 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 procedure was carried out, in order to achieve a shorter digestion time. Replicate six samples of each certified and triplicate samples of filler tobacco (FT) of each cigarette brand (0.2 g), while filter and ash (obtained from each cigarette), were weighed in PTFE flasks (25 ml in volume), added 2.0 ml mixture of concentrated HNO₃-H₂O₂ (2:1, v/v) to tobacco leaves and filter, while acids mixture HNO₃-HCl (1:3, v/v) was used for ash of cigarette, kept all flasks at room temperature for 10 min. Placed flasks in a PTFE container close it and subjected to at 80% of total microwave energy (800 W). After cooling, the contents of each flask were heated on electric hot plate to semi dried 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 de-ionized water as stock sample solutions. Duplicate samples of scalp hair (200 mg) and 0.5 ml of blood samples of each hypertensive patients and control subjects individuals were directly placed into Teflon PFA flasks. Two milliliters of a freshly prepared mixture of concentrated HNO₃-H₂O₂ (2:1, v/v) were added to each flask, 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 mol/l 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 5) [20].

Table 5
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
<i>Certified sample of human hair (µg/g)</i>					
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
<i>Certified sample of whole blood (µg/l)</i>					
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
<i>Virginia tobacco leaf (µg/g)</i>					
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: $\frac{MWD}{CDM} \times 100$.

^c Means in percentage, values in () are CV.

2.6. 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 Cd, Ni, and Pb standards. Sensitivity (m) was the slope value obtained by least-square regression analysis of calibration curves based on absorbance signals. The limit of detection, equal to 0.0003 ng/mg, 0.01 ng/mg and 0.0003 ng/mg for Cd, Ni, and Pb respectively, was de-fined as 3 SD/m, corresponding to 10 blank injections and 'm' the slope of the calibration graph. The quantification limits, defined as 10 SD/m were calculated as: 0.0009 ng/mg, 0.05 ng/mg and 0.001 ng/mg for Cd, Ni and Pb respectively.

3. Result

3.1. Toxic metals in different components of cigarettes

The analysis of 5 different IBCs for three toxic in different components of cigarette (filler tobacco, filter) pre-smoked and (filter and ash) post-smoked were determined by ICP-AES. The TE metals (Cd, Ni and Pb) obtained from filler tobacco, ash and filter of IBC origin, showed a wide variation with regard to concentration levels of three TEs. The information about cigarettes are given in Table 2. The mean values of each TE were calculated on the basis of weight of each studied cigarette brands (Table 2). The results of TEs in different component of BCs were expressed as mean \pm SD as shown in Table 6.

The filler tobacco of different BCs of different batches contains Cd, Ni and Pb concentrations in the ranges of 1.73-2.02, 0.715-1.52 and 0378-1.16 $\mu\text{g}/\text{cigarette}$, respectively (Table 6). It was observed that the understudy analytes were not detected in filter of cigarette before smoking.

Table 6
Concentration of cadmium, nickel and lead in filler tobacco (FT), filter (F) and ash of different branded cigarettes (IBCs) (result based on mean $(\bar{x} \pm s)$ $\mu\text{g}/\text{cigarette}$, n = 10).

Codes	Filler tobacco (FT)	Filter (F)	Ash (A)	Smoke concentration = FT - F + A ^a	Estimated metals $\mu\text{g}/10$ cigarette smoke
Cd					
BC1	2.02 \pm 0.054	0.359 \pm 0.014 (17.8) ^b	0.562 \pm 0.025 (27.8)	1.09 \pm 0.015 (54.0)	10.9
BC2	1.78 \pm 0.109	0.264 \pm 0.016 (14.8)	0.479 \pm 0.022 (26.9)	1.03 \pm 0.071 (57.9)	10.3
BC3	1.83 \pm 0.097	0.361 \pm 0.013 (19.7)	0.398 \pm 0.019 (21.7)	1.07 \pm 0.065 (58.5)	10.7
BC4	1.97 \pm 0.135	0.348 \pm 0.015 (17.7)	0.425 \pm 0.011 (21.6)	1.19 \pm 0.111 (60.4)	11.9
BC5	1.73 \pm 0.082	0.241 \pm 0.012 (13.9)	0.406 \pm 0.016 (23.5)	1.08 \pm 0.054 (62.4)	10.8
Ni					
BC1	0.916 \pm 0.069	0.0687 \pm 0.006 (7.5)	0.389 \pm 0.027 (42.5)	0.458 \pm 0.036 (50.0)	4.58
BC2	0.715 \pm 0.059	0.0760 \pm 0.004 (10.6)	0.237 \pm 0.016 (33.1)	0.402 \pm 0.039 (56.2)	4.02
BC3	1.06 \pm 0.073	0.0935 \pm 0.001 (8.82)	0.387 \pm 0.011 (36.51)	0.579 \pm 0.061 (54.6)	5.79
BC4	0.919 \pm 0.076	0.0752 \pm 0.0035 (8.2)	0.367 \pm 0.015 (39.9)	0.477 \pm 0.057 (51.9)	4.77
BC5	1.52 \pm 0.065	0.0552 \pm 0.005 (3.6)	0.583 \pm 0.016 (38.4)	0.882 \pm 0.044 (58.0)	8.82
Pb					
BC1	0.935 \pm 0.048	0.0609 \pm 0.007 (6.5)	0.362 \pm 0.013 (38.7)	0.512 \pm 0.028 (54.8)	5.12
BC2	0.378 \pm 0.034	0.0289 \pm 0.004 (7.6)	0.158 \pm 0.013 (41.8)	0.191 \pm 0.017 (50.6)	1.91
BC3	0.603 \pm 0.037	0.0418 \pm 0.008 (6.93)	0.238 \pm 0.010 (39.5)	0.323 \pm 0.019 (53.6)	3.23
BC4	0.846 \pm 0.052	0.0516 \pm 0.018 (6.10)	0.315 \pm 0.008 (37.2)	0.479 \pm 0.028 (56.6)	4.79
BC5	1.16 \pm 0.062	0.0635 \pm 0.007 (5.47)	0.535 \pm 0.019 (46.12)	0.561 \pm 0.036 (48.4)	5.61

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

^b Values in parenthesis is (%) of toxic metals in different components of cigarette with related to total contents in filler tobacco.

After smoking the percentage of Cd, Ni and Pb absorbed and trapped by filter of different branded cigarettes were found in the ranges of 13.9-19.7, 3.6-8.82 and 5.47-7.60 $\mu\text{g}/\text{cigarette}$, respectively, of total metals content observed in FT (Table 6). The percentage of Cd, Ni and Pb in ash of all studied cigarettes, were observed in the ranges of 21.6-27.8, 33.1-42.5 and 37.2-46.12 $\mu\text{g}/\text{cigarette}$, of total contents of FT, respectively (Table 6). The concentration of TEs in FT were higher than those in the ash, these results are consistent with other study [21]. 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 1.73 to 2.02 $\mu\text{g}/\text{cigarette}$ (Table 6). The minimum amount of Cd was observed in IBC5 (John Player blue), while highest amount was also observed in IBC1 (Dunhill).

As compared with the reported results for Cd in the United Kingdom (0.90 $\mu\text{g}/\text{g}$) and Korean cigarettes (1.02 $\mu\text{g}/\text{g}$), the average Cd contents in all cigarette brands are 1.74-2.20 times higher than those of United Kingdom and Korea, respectively [22], but lower than some branded cigarettes of Jordan [23]. The levels of Pb in 5 branded cigarette was found in the range of 0.378-1.16 $\mu\text{g}/\text{cigarette}$ corresponding to 0.676-1.67 $\mu\text{g}/\text{g}$ of filler tobacco. The average Pb contents in studied cigarette of different brands are comparable with literature reported values of Pb [22], while 3 fold lower than those results of Pb in tobacco of cigarette reported by Massadeh et al. (2.10 to 3.23 $\mu\text{g}/\text{g}$) [23]. The resulted data of toxic elements indicated that by smoking 10 cigarettes of different brands in a day, inhaled 10.3-11.9, 4.02-8.82 and 1.91-5.61 μg of Cd, Ni and Pb, respectively, by the smoker or spreads into the environment.

3.2. Toxic metals in biological samples of controls and HT patients

In the study population, more than 50% of controls and hypertensive patients were smokers. Blood pressure of controls and patients were measured according to standard protocol, in the sitting position after a 5-min rest. A patient was diagnosed as hypertensive if systolic blood pressure was 150 mm Hg, and diastolic pressure was 90 mm Hg. The other physical parameters of both groups of patients and con-trols were obtained by standard methods as shown in Table 4. The weight and body mass index of HT patients were not significantly different as compared to controls of both gender ($p > 0.05$). The blood pressure (systolic and diastolic blood pressure) of HT patients was significantly higher than those in non-hypertensive controls of same age group ($p < 0.05$).

The elemental contents in the biological (scalp hair & 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 TEs.

Table 7
Concentrations of trace and toxic metals in scalp hair and blood samples of referent and hypertensive subjects.

Parameters	Controls		HT Patients	
	CNS ^a	CS ^b	PNS ^c	PS ^d
<i>Scalp hair (µg/g)</i>				
<i>Male</i>				
Cd	0.65 ± 0.14	1.49 ± 0.19	2.17 ± 0.14	2.62 ± 0.18
Ni	1.78 ± 0.29	3.32 ± 0.34	3.89 ± 0.38	6.20 ± 0.44
Pb	3.38 ± 0.35	4.85 ± 0.29	5.31 ± 0.25	7.45 ± 0.33
<i>Female</i>				
Cd	0.56 ± 0.08	1.37 ± 0.10	2.03 ± 0.17	2.48 ± 0.15
Ni	1.62 ± 0.41	3.50 ± 0.47	3.40 ± 0.43	5.62 ± 0.33
Pb	3.26 ± 0.28	4.47 ± 0.18	5.19 ± 0.20	7.30 ± 0.27
<i>Blood (µg/l)</i>				
<i>Male</i>				
Cd	3.53 ± 0.61	5.23 ± 0.28	5.79 ± 0.62	8.86 ± 0.74
Ni	1.81 ± 0.39	3.70 ± 0.48	3.62 ± 0.29	6.25 ± 0.74
Pb	192 ± 15.8	271 ± 18.4	373 ± 20.6	516 ± 17.5
<i>Female</i>				
Cd	3.36 ± 0.45	5.16 ± 0.42	5.54 ± 0.49	8.65 ± 0.70
Ni	1.64 ± 0.18	3.59 ± 0.27	3.53 ± 0.24	6.11 ± 0.58
Pb	183 ± 15.6	257 ± 15.7	358 ± 17.8	502 ± 20.6

^a Control nonsmokers.

^b Control smokers.

^c Patient nonsmokers.

^d Patient smokers.

The mean concentrations with standard deviations for each element in biological samples, as shown in Table 7, indicate that the concentrations of TEs (Cd, Ni and Pb) were altered in the scalp hair and blood samples of smoker hypertensive patients. An elevated level of Cd content was observed in the scalp hair of male and female CS. The ranges of Cd in the scalp hair samples of male control nonsmokers (CNS) and control smokers CS were found at 95% confidence intervals (CI: 0.57-0.72 and CI: 1.40-1.60 µg/g, respectively),

whereas those in male patient nonsmokers (PNS) and patient smokers (PS) have CI: 2.07-2.25 µg/g and CI: 2.53-2.73 µg/g, respectively ($p < 0.002$) (Table 7). The concentrations of Cd in the blood samples of male CNS and CS were significantly lower (CI: 3.25, 3.80 and CI: 5.09, 5.37 µg/l, respectively), compared with those in male PNS and PS (CI: 5.48, 6.10 and CI: 8.54, 9.23 µg/l, respectively), with $p < 0.003$. The same trend was observed in female cases (Table 7).

The Pb concentrations in the scalp hair samples of male and female CNS and CS were found to be CI: 3.20, 3.53 and CI: 4.70, 4.98, and CI: 3.13, 3.39 and CI: 4.40, 4.58 µg/g, respectively whereas in the male and female PNS and PS, the Pb levels were in the range of CI: 5.16, 5.45 and CI: 7.27, 7.62, and CI: 5.10, 5.29 and CI: 7.16, 7.40 µg/g, respectively (Table 7). Similarly, a higher levels of Pb was observed in the blood samples of male and female PNS and PS (CI: 363, 382 and CI: 507, 525, and CI: 350, 365 and CI: 492, 510 µg/l respectively) than in male and female CNS and CS (CI: 185, 200 and CI: 263, 280, and CI: 175, 189 and CI: 249, 264 µg/l, respectively) ($p < 0.001$) (Table 7).

The levels of Ni in the scalp hair samples of male and female PNS and PS were found to be CI: 3.70, 4.07 and CI: 6.00, 6.43, and CI: 3.22, 3.62 and CI: 5.48, 5.84 µg/g, respectively.

While male and female CNS and CS, the Ni levels were in the range of CI: 1.64, 1.95 and CI: 3.15, 3.50, and CI: 1.41, 1.80 and CI: 3.27, 3.72 $\mu\text{g/g}$, respectively (Table 7).

The resulted data indicated that the scalp hair of smoker controls and hypertensive patients have two fold higher level of Ni, as compared to healthy and nonsmoker patients ($p < 0.001$). The ranges of Ni concentration in the blood samples of male and female CNS and CS (CI: 1.62, 1.99 and CI: 3.46, 3.95, and CI: 1.45, 1.74 and CI: 3.45, 3.75), were found to be lower as compared to male and female PNS and PS (CI: 3.48, 3.77 and CI: 5.90, 6.53, and CI: 3.41, 3.65 and CI: 5.83, 6.40 $\mu\text{g/l}$, respectively) ($p > 0.002$) (Table 7). The unpaired student t test at different degrees of freedom between hypertensive patients and controls 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 TEs in controls and hypertensive patients ($p < 0.001$).

4. Discussion

This study provides data on TEs (Cd, Ni and Pb) in scalp hair and blood samples obtained from smoker hypertensive and non-hypertensive controls of both genders of age group (30-50 years). There are many causes of high blood pressure, but cigarette smoking is also an important risk factor, considering that 60% of all the studied patients were smokers (Table 4). Tobacco-related disease originates from the biological consequences of repeated inhalation exposure to numerous toxic constituents including TEs 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 [24]. TEs uptake by tobacco plants depends on the concentration of these toxicants in the soil, soil amendments with sewage sludge and soil pH [25].

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 [26,27]. 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 [26]. 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 [28]. 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 [29].

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 mg/cigarette [30]. The Pb may also be present in high concentrations in tobacco smoke. Smokers have considerably higher blood Pb levels than nonsmokers [31]. 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 [32]. The levels of Cd, Pb and Ni passed to the smokes of 10 cigarettes of different brands were estimated to be 103-11.9, 4.02-8.82 and 1.91-5.61 [µg/10 cigarettes respectively (Table 4), either passed into mainstream or side stream smoke. It was investigated that one pack of cigarettes deposits 2-4 µg Cd, 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 [28].

Our resulted data indicated that smoker HT patients and controls have increase levels of all three TEs in blood and scalp hair samples, which were may be associated with increasing prevalence of hypertension in both genders. Some epidemiologic studies have found positive associations between body Cd levels and elevated blood pressure or hypertension [33-36]. It was reported in literature that both active and passive smoking [37] are associated with the development of several clinical disorders, that alters LDL (low-density lipoprotein) [38], reducing the endothelium-dependent relaxation induced by acetylcholine. TEs 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 [39].

It was reported in previous study that TEs have antagonistic effect with essential trace elements like zinc (Zn) and calcium (Ca). It was investigated that the accumulation of Cd in the human body may replace Zn in the arteries, which contributes to arteries becoming brittle and inflexible. Once the arteries become inflamed and brittle, the body may coat them with Ca and fatty plaques to prevent their rupture [40]. This plaque unfortunately reduces the interior diameter of the arteries, resulting in more pressure being required to force the blood through the smaller diameter arteries, which in turn raises blood pressure [41]. The Pb may also be present in high concentrations in tobacco smoke. Smokers have considerably higher blood Pb levels than nonsmokers. It was observed that smoker patients and controls have considerably higher levels of Pb in their blood and scalp hair than do nonsmokers, these results are consisted with previous study [42].

The children are more sensitive to the toxic effects of Pb compared with adults and passive smoking plays an important role in exposure of children to Pb [43]. Other possible health consequences of Pb accumulation are HT and peripheral arterial diseases. The Pb may also replace Zn and Ca, contributing to the severity of HT problems [41]. In vitro and in vivo studies suggested that Pb-induced oxidation contributes to red blood cell damage [44]. The Pb and Cd may also replace Zn and Ca contributing to the severity of HT problems due to accumulate in kidneys, which damage their ability to regulate the water balance in the body. This can lead to water retention, salt retention and high blood pressure [42]. These both TEs may also stimulate the production of inflammatory cytokines and may induce endothelial damage by down regulating the production of nitric oxide [45].

It was observed in our study that the level of Ni was significantly ($p < 0.001$) higher in smoker hypertensive patients and controls than in nonsmoker study population (Table 7). Besides this, the inhalation of vapors of Ni carbonyl obtained from burning of tobacco and from certain occupations (welding, fitting and so on) may also cause elevated Ni levels in biological samples [46]. As is the case with Cd, tobacco plants absorb Ni from the soil and

concentrate it in the leaves [43]. The Ni has been examined either alone or in combination with Cd [47]. The Ni as a trace element that is 'probably' essential, given its role in Ni-containing enzymes found in plants and microorganisms.

However, evidence that Ni has similar functions in humans is not currently available. In contrast, Ni compounds can display tumor promoting capability via a number of mechanisms including inhibition of intercellular communication, the induction of DNA deletions and aberrations, production of DNA-protein cross-links, oxidative damage, inhibition of nucleotide excision repair and an increase in DNA methylation leading to inactivation of gene expression [48]. Ni has long been known to produce nasal, prostate and lung cancers in relation to its high occupational exposure [32]. In studied area, a survey study by us confirmed that the rate of hypertension incidence is prevalent in smokers.

5. Conclusions

This study provided a new data for the health authorities in Ireland. The results of toxic elements (Cd, Ni, Pb) in different branded cigarettes consumed in Ireland, confirmed that tobacco is a notable source of their exposure to the general population. In the present study we only demonstrate that there was a significant association between toxic elements in blood and scalp hair of smoker and nonsmoker hypertensive patients and controls but the prevalence being more in smoker patients. The higher levels of Cd, Pb and Ni, correlated well with the consequences of hypertension. This study provides some support for the hypothesis that dietary intake of toxic elements most probably through smoking cigarette, may increase the risk of hypertension and related physiological disorders, which indicates that the causal link may be stronger among cigarette smokers. It was recommended that the TEs measurements may be performed on patients reaching in the emergency department, to test whether their concentration may serve not only as markers of hypertension and its remedies but also as predictors of adverse outcomes. Further studies may be useful to infer a causal relationship between TEs exposure from tobacco smoke in addition to other sources (food, water and atmosphere) and hypertension.

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