Estimation of toxic elements in the samples of different cigarettes and their effect on the essential elemental status in the biological samples of Irish smoker rheumatoid arthritis 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 rheumatoid arthritis incidence in population living in Dublin, Ireland, is investigated. The trace {zinc (Zn), copper (Cu), manganese (Mn), and selenium (Se)} and toxic elements arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) were determined in biological (scalp hair and blood) samples of patients diagnosed with rheumatoid arthritis, who are smokers living in Dublin, Ireland. These results were compared with age and sex-matched healthy, nonsmoker controls. The different brands of cigarette (filler tobacco, filter, and ash) consumed by the studied population were also analyzed for As, Cd, Hg, and Pb. The concentrations of trace and TEs in biological samples and different components of cigarette were measured by inductively coupled plasma mass spectrophotometer after microwave-assisted acid digestion. The validity and accuracy of the methodology were checked using certified reference materials. The recovery of all the studied elements was found to be in the range of 96.4–99.8 % in certified reference materials. The filler tobacco of different branded cigarettes contains Hg, As, Cd, and Pb concentrations in the ranges of 9.55–12.4 ng, 0.432– 0.727 μg, 1.70–2.12 μg, and 0.378–1.16 μg/cigarette, respectively. The results of this study showed that the mean values of As, Cd, Hg, and Pb were significantly higher in scalp hair and blood samples of rheumatoid arthritis patients as compare to healthy controls, while Zn, Cu, Mn, and Se concentrations were found to be lower in rheumatoid arthritis patients, the difference was significant in the case of smoker patients (p<0.001). The levels of four toxic elements were 2–3-folds higher in scalp hair and blood samples of nonrheumatoid arthritis 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 rheumatoid arthritis.

Keywords

Biological samples · Different brands of cigarette · Cigarette smokers · Toxic elements,

Inductively coupled plasma mass spectrophotometer

Introduction

Rheumatoid arthritis (RA) is a long-term disease that leads to inflammation of the joints and surrounding tissues. The RA is a major public health problem in elderly persons. Among the many contributing agents that have been proposed to take part in the pathogenesis of this condition, trace and toxic elements have also been investigated (Yazar et al. 2005; Helgeland et al. 2000; Ala et al. 2009; Silverio Amancio et al. 2003). A variety of trace elements are found in bone including iron (Fe), copper, selenium, zinc, manganese, fluoride, strontium, and boron (Cashman and Flynn 1998). The participation of trace elements, especially copper (Cu), manganese (Mn), and zinc (Zn), in the normal development and maintenance of the skeleton is, at least in part, related to their catalytic functions in organic bone matrix synthesis or in the functioning of cells of bone or cartilage (Grynpas 1990).

Selenium (Se), Zn, Cu, and Fe are essential trace elements, and the plasma contents of these nutrients change during the course of most infection and inflammation (Mastousek et al. 1993). However, it is not exactly known yet whether the causes of these changes are as a result of specific deficiency from dietary inadequacies and imbalances or a part of inflammatory response of an organism that is regulated by some cytokines. Despite progress in the research, there is also relatively little known regarding the pathogenesis of RA diseases. Several investigators have found depressed plasma or serum Se values in RA (O'Dell et al. 1991; Aaseth et al. 1978; Tarp 1990, 1995; Köse et al. 1996), whereas Peretz et al. (1987) and Gambhir and Lali (1999) have reported normal plasma Se concentrations. The role of Zn in healthy aging is particularly important as it prevents neoplastic cell growth, is involved in mitotic cell division, DNA, and RNA repair. Zinc plays an important role in nucleic acid synthesis, transcription, and translation as a cofactor for some of the enzymes involved and may therefore participate in a broad range of metabolic activities in the bone. Zinc has also been shown to be required by enzymes which have specific functions in bone metabolism. Zinc constitutes a structural element of alkaline phosphatase (ALP), with four of its atoms being present in the enzyme. Zinc also stimulates ALP synthesis in osteoblasts and plays an important role in bone mineralization (Heath and Shaw 2001). Copper deficiency during fetal and postnatal development has been shown to produce skeletal abnormalities and fragility in various experimental animals including the rat, chick, pig, horse, and rabbit (Dollwet and Sorenson 1988). It has been investigated that Cu deficiency can impair the cross-linking of collagen and elastin in the organic bone matrix (Jonas et al. 1993). Through this mechanism, Cu deficiency may lead to diminished tensile strength of the bone (Jonas et al. 1993). The deficiency of Cu and Zn reduces the antioxidant activity of Znand Cu-containing proteins and enzymes such as metallothioneins, ceruloplasmin, and Cu-Zn superoxide dismutase (Milanino et al. 1993). Kuo (1999) study has investigated that Cu, Zn, and Mn are key components of the two major superoxide dismutase enzymes which have been shown to fight against the reactive intermediaries that are linked to the joint damage in arthritis (Soylak and Kirnap 2001; Kuo 1999). Evans and Halliwell (2001) reported that mitochondrial Mn superoxide dismutase (Mn-SOD) is the primary cellular defense against damaging superoxide radicals generated by aerobic metabolism and as a consequence of inflammatory disease. Elevated levels of Mn-SOD provide potent cytoprotective advantage during acute arthritic inflammation.

It has been reported in literature that reactive oxygen species play a key role in the etiology of RA (Cerhan et al. 2003; Sarban et al. 2005, 2007; Fautrel and Bourgeois 2000; Piotrowska-Jastrzębska et al. 2002) and that one of them is the superoxide radical, which is eliminated by

superoxide dismutase—an enzyme containing zinc in its molecule (Tapiero and Tew 2003). It has also been found that over 90 % of this trace element present in erythrocytes is bound with carbonic anhydrase and superoxide dismutase (Mierzecki et al. 2011).

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 (Kazi et al. 2008a, 2008b). 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 (Kazi et al. 2008b). 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, genotoxic, mutagenic, and carcinogenic properties (Husgavfel-Pursiainen 2004). Tobacco plant (*Nicotiana tabacum*) is well known for its capacity to concentrate TEs from its growing environment, correspondingly higher levels in the tobacco leaves and in the smoke particulate (Lugon-Moulin et al. 2006). Other environmental factors may influence TEs uptake by tobacco plants including soil pH and toxic elements— containing sludge or fertilizers applied to crops (Jung et al. 1998). The possible sources of TEs 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 (Lugon-Moulin et al. 2006; Jung et al. 1998).

Thus, different cigarette brands could yield markedly different smoke particulate levels of TEs 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 (Jung et al. 1998). Although there are global and brand variations in the TEs compositions of commercial tobacco products (Jung et al. 1998), several TEs found in tobacco smoke such as Cd, Cr, Pb, and Ni also accumulate in tissues and fluids through smoking (Jung et al. 1998). The TEs present in tobacco smoke and contribute substantially to cancer risk indices (Fowles and Dybing 2003). Toxic elements (Cd, Hg, Ni, Pb, and As) 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 (Goyer 1996). The intake of trace and TEs may promote RA 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 (Nawrot et al. 2002). The deficiency of essential nutrients, lack of homeostatic control, or an excess intake of some TEs causes chronic physiological disorders, such as RA, diabetic mellitus, and cardiovascular disease (Nawrot et al. 2002).

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 (Afridi et al. 2008; Tuzen and Soylak 2007). 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 (Divrikli et al. 2006; Soylak et al. 2007). 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 (Afridi et al. 2006; Soylak et al. 2001).

The aim and objective of our present study was to assess the concentrations of trace essential

(Cu, Mn, Zn, and Se) and TEs (As, Pb, Cd, and Hg) in the scalp hair and blood samples of smoker and nonsmoker RA patients. For a comparative study, 49 non-RA individuals (smoker and nonsmokers) of the same age group (ranged 42–56 years), socioeconomic status, localities, and dietary habits were selected as controls. The understudy elements were analyzed by inductively coupled plasma mass spectrophotometer, after microwaveassisted acid digestion. Presently, we also evaluated and compared the status of TEs (As, Pb, Cd, and Hg), in different presmoking and postsmoking components (filler tobacco, filter, and ash) of various imported branded cigarettes existing in Ireland.

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. An Agilent 7500i (Santa Clara, California, USA) inductively coupled plasma mass spectrometer with the axially viewed plasma was used for the analysis. The 7500i was designed specifically to handle complex, high matrix samples. A robust 27.12-MHz plasma, low sample uptake rate, cooled spray chamber, and proven small orifice interface protect the octo-pole reaction system from contamination by undissociated sample matrix. A novel ion optic, mounted outside the high vacuum region for easy access, further protects the reaction cell, which features an octo-pole for optimum ion transmission. The octo-pole is mounted off-axis to minimize random background levels. The instrumental conditions are shown in Table 1. A Hinari Lifestyle (Elstree, Hertfordshire, England) domestic microwave oven (maximum heating power of 800 W) was used for digestion of the scalp hair, blood, and different cigarette component samples. Acid-washed polytetrafluoroethylene (PTFE) vessels and flasks were used for preparing and storing the solutions.

Reagents and glass wares

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 As, Cu, Cd, Hg, Se, Mn, Pb, and Zn were prepared immediately prior to their use, by stepwise dilution of certified standard solutions (1000 ppm) Fluka Kamica (Buchs, Switzerland), with 0.5 mol/l HNO_3. All solutions were stored in polyethylene bottles at 4 °C. For the accuracy of methodology, the certified reference 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.

Table 1 Measurement conditions for inductively coupled plasma-mass spectrometer Agilent 7500i

Inductively coupled plasma-mass spectrometer Agilent 7500i (Santa Clara, California, USA)	
Sample introduction	
Nebulizer	Babinton-type
Flow injection	Integrated Sample Introduction *System* (ISIS)
Plasma	
ICP torch injector	Quartz, 2.5 mm
Rf generator	Frequency: 27 MHz, power outlet: 1500 W
Ar flow rate, l/min	Plasma: 15, auxiliary: 1, carrier: 1.17
Spray chamber	Glass, double pass
Spray chamber temp	2 °C
Solution uptake rate	0.4 ml/min
Sampling depth	8 mm
Interface	
Sampler cone	Nickel, T-mode
Skimmer cone	Nickel, T-mode
Data acquisition	Dwell time 0.33 s, points/mass 3, No. of replicates 3
Internal standard	^{140}Ce, ^{205}Tl
Analytical isotopes of elements	^{55}Mn, ^{63}Cu, ^{66}Zn, ^{75}As, ^{82}Se, ^{111}Cd, ^{202}Hg, ^{208}Pb
Monitored isotopes for appropriate correction of interference	^{23}Na, ^{24}Mg, ^{27}Al, ^{31}P, ^{34}S, ^{39}K, ^{43}Ca, ^{47}Ti, ^{56}Fe, ^{95}Mo, ^{13}C, ^{37}Cl

mm millimeter, *MHz* megahertz, *W* watt, *ml/min* milliliter/minute, *s* second

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 2). 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 being dried at 80 °C was determined. A duplicate 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 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 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
BC 7	More	Menthol filter Class A Cigarettes (USA)	0.947±0.04

[a]Branded cigarette

Biological sample pretreatment

Before the start of this study, all referents and RA patients of both genders, age range 42–56 years, were informed through a consent form by the administration about the aim of study, and all agreed to participate and signed the form. A questionnaire was also administered to them to collect details regarding physical data, ethnic origin, health, duration and frequency of smoking, dietary habits, age, and consent. The RA patients were grouped according to their habits nonsmokers (PNS) and smoker patients (PS). While control groups were also divided into two groups, referents nonsmokers (CNS) and smokers (CS) as shown in Table 3.

Table 3 Characteristics of study subjects (42–56) age groups

Parameters	Referents (52)		Arthritis patients (53)	
	Male	Female	Male	Female
Occupation				
Labor	12	11	12	14
Office workers	15	14	15	12
Habits				
Smokers	14 (52 %)	12 (48 %)	15 (56 %)	15 (58 %)
Nonsmokers	13 (48 %)	13 (52 %)	12 (44 %)	11 (42 %)

All the patients had active disease defined by the following criteria: erythrocyte sedimentation rate (ESR) of at lease 30 mm/h, six or more tender joints, three or more swollen joints and morning stiffness of at least 30-min duration. Thirty patients were IgM rheumatoid factor positive. None of the patients had been treated with steroids, immunosuppressives, or penicillamine in the 3 months before the study. They all were receiving nonsteroidal anti-inflammatory drugs (NSAIDs) (diclofenac sodium, 100 mg/day).

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 4). 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.

Table 4 Biochemical characteristics of rheumatoid arthritis and referent subjects

Parameters	Male			
	CNS[a]	CS[b]	PNS[c]	PS[d]
Height (cm)	177.3±1.15	178.6±1.03	177.0±0.82	176.6±1.37
Weight (kg)	75.3±1.24	76.7±1.36	73.9±1.03	76.3±0.94
Waist circumference (cm)	77.4±0.92	74.4±0.76	76.9±1.16	73.9±1.09
BMI (kg/m^2)	23.9±0.60	24.0±0.47	23.6±0.56	24.5±1.02
Systolic BP (mmHg)	119.9±0.62	120.4±0.49	120.3±0.33	120.5±0.45
Diastolic BP (mmHg)	79.9±1.17	80.2±0.68	80.3±0.49	80.5±0.34
	Female			
Height (cm)	163.8±0.72	163.5±0.58	163.6±1.21	163.5±1.06
Weight (kg)	60.9±1.13	61.3±1.38	60.1±0.52	60.2±0.94
Waist circumference (cm)	63.3±1.09	63.2±1.12	63.4±0.69	63.8±0.61
BMI (kg/m^2)	22.7±1.14	22.9±0.72	22.4±0.57	22.5±1.04
Systolic BP (mmHg)	120.1±0.46	120.4±0.32	120.2±0.51	120.7±1.01
Diastolic BP (mmHg)	80.4±0.42	80.5±0.58	81.2±0.35	80.9±0.48

[a]Control nonsmokers
[b]Control smokers
[c]Patient nonsmokers
[d]Patient smokers
BMI body mass index

The criteria of healthy subjects included no history of symptoms of any coronary disease documented in their medical notes. All control subjects underwent a routine medical examination. All patients and controls/referents were requested to complete an interviewer-administered questionnaire, concerning their demographic characteristics, age, health history, lifestyle habits, and diet. They gave written consent to participate in the study.

Collection of blood and scalp hair samples

Venous blood samples (5 ml) were collected by using 7mm heparinized lithium Vacutainer® tubes (Becton Dickinson). About 2 ml of venous blood samples was stored at −20 °C until elemental analysis (Afridi et al. 2008). 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 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 0.5cm-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 (Afridi et al. 2008). The samples were then dried in an oven at 75±5 °C for 2 h. Dried samples were stored separately in polyethylene bags.

Microwave-assisted acid digestion

A microwave-assisted digestion 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 HNO_3–H_2O_2 (2:1, *v*/*v*) to tobacco leaves and filter, while acid mixture HNO_3–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 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 hypertensive patients and control subject individuals were directly placed into Teflon PFA flasks. Two milliliters of a freshly prepared mixture of concentrated HNO_3–H_2O_2 (2:1, *v*/*v*) was 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 were checked with certified values of human hair NCSZC 81002b, Clincheck® control-lyophilized human whole blood, Virginia tobacco leaves (ICHTJ-ctaVTL-2) and with those obtained from conventional wet acid digestion method (Table 5; Afridi et al. 2006).

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

Elements	Conventional digestion method CDM	Microwave digestion method MWD	T value[a]	% recovery[c]	Certified values
Cd	0.0716±0.003 (4.19)	0.0714±0.006 (8.40)	0.305	99.7	0.072±0.010
Cu	33.5±1.92 (5.73)	33.0±1.58 (4.79)	0.193	98.5	33.6±2.3
Mn	3.79±0.34 (8.97)	3.76±0.28 (7.45)	0.727	99.2	3.83±0.39
Pb	3.80±0.37 (9.74)	3.72±0.35 (9.41)	0.081	98.05	3.83±0.18
Zn	191±7.28 (3.81)	187±9.53 (5.09)	0.648	97.9	191±16
Se	1.92±0.15 (6.25)	1.85±0.12 (8.10)	0.0203	96.4	2.00±0.08
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
Cu	13.87±1.02 (7.35)	13.69±0.89 (6.5)	0.72	98.7	13.9±2.7
Mn	24.80±1.8 (7.26)	24.67±1.3 (5.27)	0.0017	99.5	25±5
Pb	33.29±1.21 (3.63)	32.56±1.18 (3.62)	0.096	97.8	33±1.2
Zn[b]	2.25±0.09 (4.00)	2.20±0.08 (3.64)	0.091	97.77	2.27±0.06
Se	114.6±2.47 (2.15)	113.5±3.68 (3.24)	0.247	99.0	116±23.5
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	96.4	22.1±0.0772
Hg	0.0475±0.004 (8.42)	0.0474±0.003 (6.25)	0.071	99.8	0.048±0.009
As	0.967±0.08 (8.27)	0.964±0.09 (9.34)	0.858	99.7	0.969±0.072

[a] Paired t test between CDM and MWD, DF=9, T (critical) at 95 % CI=2.262, p=0.05

[b] Means in percentage, values in ( ) are RSD

[c] % recovery was calculated according to [MDM]× 100, ( )* indicates in mg/l [CDM]

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 As, Cu, Cd, Hg, Mn, Pb, Se, 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, equal to 0.002, 0. 01, 0.0003, 0.09, 0.0003, 0.052, 0.0003, and 0.01 ng/mg for As, Cu, Cd, Hg, Mn, Se, Pb, and Zn, respectively, was defined as 3 SD/m, corresponding to 10 blank injections and Bm^ the slope of the calibration graph. The quantification limits, defined as 10 SD/m, were calculated as follows: 0.007, 0.03, 0.0009, 0.26, 0.009, 0.16, 0.009, and 0.03 ng/ mg for As, Cu, Cd, Hg, Mn, Se, Pb, and Zn, respectively.

Result

Toxic metals in different components of cigarettes

The analysis of seven different branded cigarettes for six toxic in different components of cigarette (filler tobacco, filter) presmoked and (filter and ash) postsmoked was determined by ICP-AES. The toxic elements (As, Cd, Hg, 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. The information about cigarettes is given in Table 2. The mean values of toxic elements were calculated on the basis of weight of each studied cigarette brands (Table 2). The results of toxic elements in different component of BCs were expressed as mean ± SD as shown in Table 6. The filler tobacco of different BCs of different batches contains As, Hg, Cd, and Pb concentrations in the ranges of 0.432–0.727 μg, 9.55–12.4 ng, 1.70–2.12 μg, and 0.378–1.16 μg/cigarette, respectively (Table 6). It was observed that the understudy analytes were not detected in filter of cigarette before smoking. After smoking, the percentage of As, Hg, Cd, and Pb absorbed and trapped by filter of different branded cigarettes were found in the ranges of 9.3–16.0 μg, 14.1– 18.5 ng, 13.9–19.7 μg, and 5.47–7.60 μg/cigarette, respectively, of total metal content observed in FT (Table 6). The percentage of As, Hg, Cd, and Pb in ash of all studied cigarettes were observed in the ranges of 30.7–42.4 μg, 36.3–42.1 ng, 14.9–27.8 μg, and 37.2– 47.2 μg/cigarette, of total contents of FT, respectively (Table 6). The concentration of toxic elements in FT was higher than that in the ash; these results are consistent with other study (Ebisike et al. 2004). Cigarette ash plays an important role in terms of toxic metal distribution toward 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 6). 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 (Jung et al. 1998), but lower than some branded cigarettes of Jordan (Adnan et al. 2005). The levels of Pb in seven branded cigarette were 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 (Jung et al. 1998), while 3-fold lower than those results of Pb in tobacco of cigarette reported by Massadeh et al. (2.10 to 3.23 μg/g) (Adnan et al. 2005). The resulted data of toxic elements indicated that by smoking 10 cigarettes of different brands in a day, inhaled 2.16–3.76 μg, 10.3–11.9 μg, 43.5–53.1 ng, and 1.91–5.61 μg of As, Cd, Hg, and Pb, respectively, by the smoker or spreads into the environment.

Table 6 Concentration of cadmium, lead, arsenic, and mercury in filler tobacco (FT), filter (F), and ash of different imported branded cigarettes (IBC) (result based on x±s, μg/cigarette, $n=10$)

Codes	Filler tobacco	Filter	Ash	Smoke concentration =FT−F+A[a]	Estimated metals/10 cigarette smoke
Cadmium					
BC1	2.02±0.054	0.359±0.014 (17.8) [b]	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
Lead					
BC 1	0.935±0.048	0.0609±0.007 (6.5)	0.362±0.013 (38.7)	0.512±0.028 (54.8)	5.12
BC 2	0.378±0.034	0.0289±0.004 (7.6)	0.158±0.013 (41.8)	0.191±0.017 (50.6)	1.91
BC 3	0.603±0.037	0.0418±0.008 (6.93)	0.238±0.010 (39.5)	0.323±0.019 (53.6)	3.23
BC 4	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
BC 6	0.986±0.055	0.0520±0.005 (5.3)	0.384±0.015 (38.9)	0.550±0.045 (55.8)	5.50
BC 7	1.14±0.029	0.0689±0.007 (6.04)	0.538±0.041 (47.2)	0.534±0.037 (46.8)	5.34
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±0.0 78	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] Values in parenthesis is % of toxic metals in different component of cigarette with related to total contents in filler tobacco

Trace and toxic elements in biological samples of controls and RA patients

In the study population, approximately 56–58 % of RA patients and 48–52 % of referents subjects of both genders were smokers. The physical parameters of RA patients and referents were obtained by a standard method as shown in Table 7. RA and healthy subjects were similar in age, height, body weight, and body mass index (BMI), as seen in Table 7.

Table 7 Essential trace and toxic elements in scalp hair and blood samples of referent and rheumatoid arthritis subjects

Parameters	Controls		RA patients	
	CNS[a]	CS[b]	PNS[c]	PS[d]
Scalp hair (μg/g)				
Male				
Se	1.70±0.12	1.22±0.10	0.95±0.16	0.68±0.08
Zn	203±7.53	178±5.28	135±9.42	122±4.63
Cu	10.4±1.23	10.9±0.93	7.34±0.61	8.09±0.82
Mn	3.41±0.65	3.24±0.47	1.93±0.28	1.89±0.31
Hg	1.05±0.03	1.23±0.05	1.65±0.05	2.19±0.09
Cd	0.68±0.07	0.94±0.12	2.13±0.37	3.35±0.61
As	0.85±0.07	1.37±0.11	1.96±0.17	2.78±0.15
Pb	3.36±0.41	3.75±0.28	4.55±0.34	5.62±0.87
Female				
Se	1.65±0.14	1.29±0.07	0.92±0.12	0. 64±0.06
Zn	211±8.36	167±5.82	126±8.59	117±7.28
Cu	10.6±0.84	10.7±0.99	7.66±0.75	7.95±0.95
Mn	3.39±0.58	3.15±0.32	1.82±0.48	1.74±0.25
Hg	0.98±0.05	1.18±0.05	1.59±0.06	2.15±0.08
Cd	0.59±0.09	0.83±0.18	1.73±0.17	2.62±0.13
As	0.79±0.09	1.25±0.09	1.89±0.12	2.75±0.11
Pb	3.24±0.15	3.59±0.16	4.39±0.26	5.42±0.51
Blood (μg/l)				
Male				
Se	228±7.65	203±9.58	138±5.08	112±6.20
Zn[e]	10.5±1.95	8.75±1.09	7.17±0.75	5.25±0.60
Cu[e]	1.82±0.30	1.50±0.25	0.87±0.13	0.68±0.15
Mn	62.8±2.16	48.5±4.24	38.4±2.50	25.7±3.08
Hg	0.85±0.06	1.16±0.08	1.74±0.05	2.25±0.11
Cd	3.59±0.37	5.36±0.29	5.84±0.55	8.96±0.33
As	1.74±0.12	2.19±0.10	3.58±0.29	4.39±0.38
Pb	198±12.7	285±14.6	382±16.8	530±16.9
Female				
Se	220±9.15	197±6.96	125±9.59	108±7.21
Zn[e]	9.95±1.58	8.26±0.91	6.82±0.36	5.10±0.38
Cu[e]	1.90±0.19	1.48±0.27	0.95±0.15	0.70±0.20
Mn	58.5±1.78	43.8±1.41	31.8±2.79	27.3±2.05
Hg	0.80±0.05	1.09±0.06	1.68±0.08	2.28±0.11
Cd	3.48±0.28	5.27±0.31	5.76±0.45	8.84±0.51
As	1.70±0.10	2.15±0.16	3.46±0.36	4.28±0.25
Pb	189±13.9	278±12.3	378±15.7	526±14.6

[a] Control nonsmokers

[b] Control smokers

[c] Patient nonsmokers

[d] Patient smokers

[e] mg/l

The mean concentrations with standard deviations for essential trace (Cu, Mn, Se, and Zn) and toxic elements (As, Cd, Hg, Pb) in biological samples, as shown in Table 7, 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 RA 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 were CI 1.65–1.78, 1.17–1.28 and CI 1.58–1.73, 1.25– 1.33 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 0.89–1.02, 0.65–0.71 and CI 0.88–1.03, 0.61–0.68 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 225–232, 198–206 and CI 216–225, 194–201 μg/L, which were significantly higher than male and female PNS and PS patient CI 136–141, 108–115 and CI 120–131, 104–112 μg/L (p<0.001), respectively (Table 7).

The concentrations of Zn in the scalp hair samples of male CNS and CS were significantly higher at 95 % confidence interval (CI) 199, 206 and 175, 18 μg/g, respectively, compared with those in PNS and PS, CI 131, 140 and CI: 119, 125 μg/g, respectively, with p<0.001. The Zn levels in the blood samples of CNS and CS, CI 9.80, 11.3 and CI 8.21, 9.21 mg/l, respectively, were found to be higher than those in PNS and PS, CI 6.74, 7.50 and CI 4.97, 5.53 mg/ l, respectively (p=0.001–0.002). The same trend was observed in female patients and referents (Table 7).

The concentrations of Cu in the scalp hair and blood samples of males and females CNS and CS were 25.7– 29.4 and 48.5–52.1 % higher than those values obtained from PNS and PS patients (p<0.01–0.006), respectively (Table 7). The levels of Mn in scalp hair samples of male and female Irish CNS and CS were CI 3.09–3.74, 3.00–3.50 and CI 3.10–3.67, 3.01–3.28 μg/g, respectively, but the mean values of Mn in the scalp hair samples of male and female nonsmokers (PNS) and patient smokers (PS) patient were found in the range of CI 1.78–2.12, 1.75– 2.06 and CI 0.88–1.03, 0.61–0.68 μg/g, respectively, which were significantly lower than the control subjects of the same age group (p<0.001). The concentrations of Mn in the blood of male and female Irish CNS and CS were observed in the range of CI 61.7–63.8, 46.3–50.8 and CI 57.6–59.5, 43.1–44.5 μg/l, which were significantly higher than male and female PNS and PS patient, CI 37.2–39.6, 23.2–27.3 and CI 30.4–33.2, 26.3– 28.4 μg/l (p<0.001), respectively (Table 7).

It was observed that the level of Cu, Mn, Se, and Zn did not vary significantly in the scalp samples of referent smokers and nonsmokers indicating that the alteration of these trace elements in scalp hair samples was mainly due to disease state of the patients.

An elevated level of Cd content was observed in the scalp hair and blood samples of male CNS and CS. The ranges of Cd in the scalp hair and blood samples of CNS and CS were CI 0.64–0.72, 0.88-1.00 μg/g and CI 3.40– 3.80, 5.21–5.49 μg/l, respectively, whereas those in PNS and PS were CI 1.95–2.40, 3.07–3.63 μg/g and CI 5.61–6.07, 8.79–9.12 μg/l, respectively (p<0.001). The same trend was observed in female cases (Table 7). The level of Cd in blood samples was statistically significantly higher (p<0.01) in smoker RA patients of both genders (Table 7).

The Pb concentration in the scalp hair samples of male CNS and CS was found at 95 % CI 3.17, 3.57, 3.61–3.90 μg/g, whereas in the PNS and PS, the Pb level was in the range of CI 4.38–5.84, 5.18–6.43 μg/g (Table 7). 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 and CS (p<0.001). The same trend was observed in females (Table 7).

The levels of As and Hg in scalp hair and blood samples were statistically significantly higher (p<0.01) in both groups of RA patients (PNS, PS) compared with referent groups (CNS, CS) of both genders (Table 7).

The unpaired Student's t test at different degrees of freedom between RA 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 RA 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 €3000 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 in outdoor areas (European Commis- sion 2013). 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 10 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 EUwide ban on cigarettes and cigars by 2025 (EU Observer 2008). As of July 2009, it is prohibited to advertise cigarettes and sell 10 packs of cigarettes in retail outlets. Additionally, as of February 2013, any tobacco product placed on the market must have graphic warnings (Roche 2013). There is legislation being made to introduce plain cigarette packets and make Ireland the second country to do so, after Australia (Irish Times 28 May 2013).

This study provides data on essential trace (Cu, Mn, Se, and Zn) and toxic elements (As, Cd, Hg, and Pb) in scalp hair and blood samples obtained from Irish smoker RA and nonhypertensive controls of both genders of age group (42–56 years). RA is an autoimmune disease, a disorder in which the body attacks its own healthy cells and tissues. When someone has arthritis, the membranes around his or her joints become inflamed and release enzymes that cause the surrounding cartilage and bone to wear away. In severe cases, other tissues and body organs also can be affected.

The skeleton is a major bone store of Zn, and in humans, approximately 30 % of total body Zn is found in the bone, probably bound to hydroxyapatite (Sauer and Wuthier 1990). Zn deficiency is associated with delayed bone growth, but few studies have been done to elucidate its potential role in bone turnover regulation. An increased Zn urinary excretion with unchanged Zn content in bone has been reported in postmenopausal osteoporosis (Honkanen et al. 1991). It has been proposed that, since urinary Zn excretion is almost uninfluenced by variation in diet, urinary Zn excretion may be used as a marker of changes in bone metabolism (Relea et al. 1995). Zn supplementation was reported to decrease periarticular osteoporosis in RA patients (Honkanen et al. 1991). Defects in skeletal development have been reported in man due to zinc deficiency and also due to the acrodermatitis enteropathica, an inherited congenital disorder of zinc absorption (Alegre et al. 1984).

It has been reported that forearm bone mineral content is correlated with zinc intake in premenopausal women, suggesting a possible role for zinc in the maintenance of bone mass (Angus et al. 1988). ß-Alanyl-Lhistidinato Zn has been shown to have a more potent effect than zinc sulfate on bone metabolism in experimental animals and this Zn chelate has been proposed as a possible treatment for osteroporosis (Yamaguchi 1990).

In our study, scalp hair and blood Se concentrations in the smoker and nonsmoker RA patients were significantly lower than those in the healthy CNS and CS subjects (p<0.002). This can be a sign of depletion or redistribution of Se from the plasma pool into the other tissues as a defense mechanism that it might be modulated by proinflammatory and immunoregulatory cytokines. Our

result that lower plasma Se level is in agreement with most other studies previously published (O'Dell et al. 1991; Aaseth et al. 1978; Tarp 1990; Köse et al. 1996; Svenson et al. 1985; Naveh et al. 1997).

Our result showed that the mean concentration of Cu and Mn was found to be lower in the scalp hair and blood samples of RA patients of both genders with related to referents (Table 7). Forestier was among the first to report that a Cu complex, Cupralene, was effective in the treatment of RA. Based on open studies, he concluded in 1949 that Cu salts are effective in the treatment of arthritis. They give better results than gold salts in the early stages of the disease (Forestier 1949). The clinical treatment with copper-containing agents, the clinical use of the anti-inflammatory copper-dependent metalloenzyme superoxide dismutase (SOD), should also be commented upon. Bovine SOD has been shown to reduce inflammation when given intraarticularly into the joints of arthritis patients. Ceruloplasmin and therapeutic Cu complexes have been shown to possess SOD-like activity. Hence, the demonstrated physiological rise of ceruloplasmin in arthritis is suggested to represent a protective response. Consistent with this, a lack of rise of ceruloplasmin may increase the risk of chronic disease, as seen in Cu-deficient animals with adjuvant arthritis (Conlan et al. 1990). There is some evidence for a role of Cu deficiency in age-related osteroporosis. Serum copper levels of 46 elderly patients with fractures of the femoral neck were reported to be significantly lower than those of a group of controls matched for age and sex (Jorde et al. 2010). A significant positive correlation between serum Cu concentration and bone mineral density at the lumbar spine has been reported in a cross-sectional study in postmenopausal women (Conlan et al. 1990). EatonEvans et al. (1996) have recently shown that Cu supplementation (3 mg/day for 2 days) reduced the rate of loss of bone mineral density at the lumbar spine in 46to 56year-old women. This indicates that inadequate dietary copper intake may be a contributory factor to age-related bone loss in this population group.

Apps et al. (1992) have studied that Mn deficiency is unlikely but the effects of it are likely to be on the skin and bones primarily. The rareness of deficiency may be due to the fact that Mg can readily substitute for Mn in many reactions when the latter is not available. Effects of Mn deficiency in humans are not well defined. Limited information indicates that dermatitis, and possibly decreased levels of clotting proteins, decreased serum cholesterol, reddening of black hair, and slowed growth of hair and nails may be consequences of Mn deficiency (Murray et al. 2000). Effects of Mn deficiency in animals include impaired growth, skeletal abnormalities, testicular degeneration in males, impaired reproductive function in females, ataxia, altered carbohydrate, and lipid metabolism. Its deficiency also increased oxidation of mitochondrial membranes and reduced high-density lipoprotein (ATSDR 2001).

There are many causes of arthritus, 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 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 (Reilly 2002). Toxic element uptake by tobacco plants depends on the concentration of these toxicants in the soil, soil amendments with sewage sludge, and soil pH (Mulchi et al. 1992). 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. (Csalari and Szantai 2002; Hecht 2003). Tobacco leaves naturally accumulate and concentrate relatively high levels of toxic heavy metals, and therefore, smoking of tobacco is an important source of these metal exposure for smokers (Csalari and Szantai 2002; Hecht 2003).

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 (Kazi et al. 2009). 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 (Lugon-Moulin et al. 2006). 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 (Satarug and Moore 2004). The Pb may also be present in high concentrations in tobacco smoke. Smokers have considerably higher blood Pb levels than nonsmokers (Chiba and Masironi 1992).

Arsenic in mammals causes lipid peroxidation, protein and enzyme oxidation, and glutathione depletion (Rosen 1995). It was also reported in literature the interaction of arsenate with glutathione, 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 As [$(GS)_3As$] (Savory et al. 1996). This As–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 the lungs, resulting in tissue-damaging effects.

Lead can increase osteoporosis, and it may disrupt the normal formation of calcium hydroxyapatite, thus critically weakening the bone (Skinner 2000). Tandon et al. (2001) had been reported that effects of Pb on humans include anemia, abdominal colic, and gum wastage, while Cd alters calcium and phosphorus metabolism, thus contributing to arthritis, osteoporosis, and neuromuscular diseases. These effects may have been common in ancient times in such a severely polluted landscape. Lead has an exceptionally long half-life in bone compared to other elements (Aufderheide 1989). The many TEs, As, Cd, cobalt, antimony, and mercury (Hg) can deposit in the bone from respiratory exposure (Oakberg et al. 2000). Toxic elements (As, Cd, Hg, and Pb) 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 (Goyer 1996).

The levels of As, Cd, Hg, and Pb passed to the smokes of 10 cigarettes of different brands were estimated to be 2.16–3.76 μg, 10.3–11.9 μg, 43.5–53.1 ng, and 4.02–8.82 μg/10 cigarettes, respectively (Table 6), either passed into mainstream or side stream smoke. 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 metal exposures for smokers (Kazi et al. 2009). The total amount of carcinogens in cigarette smoke ranges from 1 to 3 μg per cigarette (Csalari and Szantai 2002).

The country of origin and type of the product play major roles in determining the chemical composition of cigarette tobacco. It was investigated that one pack of cigarettes deposits 0–1.4 μg As, 2–4 μg Cd, 0.46 to 6.5 ng Hg, and 1–2 μg Pb into the lungs of a smoker, whereas some of the smoke passes into the air to be inhaled by smokers and nonsmokers alike (Kazi et al. 2009). It was also consistent with another study that smokers generally exhibit significantly higher As, Cd, Hg, and Pb body burdens than nonsmokers (Hecht 2003). The results suggested that As, Cd, Hg, and Pb pose a hazard effects on essential trace element homeostasis of various organs, coexposure can pose a major threat, while consumption of ethanol may absorb much more Cd and Pb than their unexposed counterparts (Sharma et al. 1991).

In the past few years, increasing consideration has been given to interactions occurring in the organism between TEs and bio-elements essential for life. These interactions are complex and

involve bio-elements such as Zn, Cu, Fe, Mn, Se, calcium (Ca), and TEs, including Cd (Brzoska et al. 1997). The basis of Cd toxicity is its negative influence on enzymatic systems of cells, resulting from substitution of other essential elemental ions (mainly Zn, Cu, and Ca) in metalloenzymes and its very strong affinity to biological structures containing SH groups, such as proteins, enzymes, and nucleic acids (Stohs and Bagchi 1995). The relevance of As, Cd, Hg, Pb, Ni, Cu, and Fe–Zn and Se interactions should be considered in the light of the general population exposure to TEs (Waalkes et al. 1992) and common deficiency of essential trace elements in the world, mainly due to nutritional factors (Lonnerdal 1993; Sandstead et al. 1998).

Conclusion

This study provided a new data for the health authorities in Ireland. The results of toxic elements (As, Cd, Hg, 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 arthritis patients for the determination of essential trace (Cu, Mn, Se, and Zn) and TEs (As, Cd, Hg, Pb).

It can be concluded that impaired trace element metabolism may have a role in the pathogenesis and progression of arthritis. The really overlooked issue here is the dramatic impact of the TEs on human health. The excessive body burden of TEs may create the symptoms manifest in the form of muscle and joint complaints. The As, Cd, Hg, and Pb interfere with the normal biochemical processes of Mn, Cu, Se, Zn, and other nutrients in the cells of human body. When these essential minerals in the body are disrupted by TEs, musculoskeletal symptoms such as muscle and joint pain commonly occur. The TEs impair the immune system, cause abnormal cell responses, and may aggravate the sign and symptom of arthritis and other types of arthritis commonly called as Brheumatoid arthritis.^ It is necessary to add essential trace elements via food supplements. The results of this study provided guidance to clinicians and other professional investigating deficiency of essential trace elements and excessive level of TEs in biological samples of healthy and arthritis patients. This study also provides some support for the hypothesis that dietary intake or inhalation of TEs (As, Cd, Hg, and Pb), most probably through smoking cigarette, may increase the risk of RA and related disorders, which indicates that the causal link may be stronger among cigarette smokers. We propose that essential and toxic elemental measurements may be performed on patients reaching in the emergency department, to test whether the concentration of it may serve not only as markers of RA and its remedies but also as predictors of adverse outcomes.

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Conflict of interest
The authors declare no conflict of interest.

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