Association between essential trace and toxic elements in scalp hair samples of smokers rheumatoid arthritis subjects

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Abstract
The incidence of rheumatoid arthritis (RA) has been increased among people who possess habit of tobacco smoking. In the present study, zinc (Zn), copper (Cu), manganese (Mn), lead (Pb) and cadmium (Cd) were determined in scalp hair samples of smokers and nonsmokers RA patients, residents of Dublin, Ireland. For comparison purposes scalp hair samples of age and sex matched healthy smokers and nonsmokers were also analyzed. The concentrations of understudied elements were measured by inductive coupled plasma atomic emission spectrophotometer, prior to microwave assisted acid digestion. The validity and accuracy of methodology was checked using certified reference material (NCS ZC 81002b) and by the conventional wet acid digestion method on the same certified reference material and on real samples. The mean hair Zn, Cu and Mn contents were significantly lower in smokers and nonsmokers RA patients as compared to healthy individuals ($p = 0.01$-0.001). Whereas the concentrations of Cd and Pb were significantly higher in scalp hair samples of RA patients of both group ($p < 0.001$). The referent smokers have high level of Cd and Pb in their scalp hair samples as compared to those had not smoking tobacco ($p < 0.01$). The ratio of Cd and Pb to Zn, Cu and Mn in scalp hair samples was also calculated. The Cd/Zn ratio was higher in smoker RA patients with re-lated to nonsmoker RA and referents. This study is compelling evidence in support of positive associations between toxic elements, cigarette smoking, deficiency of essential trace elements and risk of arthritis.

Keywords: Rheumatoid arthritis, Scalp hair, Cigarette smokers, Elements, Inductive coupled, plasma atomic emission, Spectrophotometer.

1- 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, copper, zinc, manganese, fluoride, strontium and boron (Sandstrom and Walter, 1998). The participation of trace elements, especially copper, manganese and zinc, 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).

The role of Zn in healthy aging is particularly important as it pre-vents neoplastic cell growth, is involved in mitotic cell division, DNAand RNA repair. Zinc plays an important role in nucleic acid synthesis, transcription and translation as a cofactor for some of the enzymes in-volved and may therefore participate in a broad range of metabolic activities in 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 syn-thesis 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 bone (Jonas et al., 1993). The deficiency of Cu and Zn reduces the antioxidant activity of zinc- and copper-containing proteins and en-zymes such as metallothioneins, ceruloplasmin and Cu-Zn-superoxide dismutase (Milanino et al., 1993).

Kuo (1999) study has investigated that 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; Fautrel and Bourgeois, 2000; Piotrowska-Jastrzcbska et al., 2002; Sarban et al., 2007) 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., DOI 10.1007/ s12011-010-8952-2).

Cigarette design has evolved considerably over the last few de-cades 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 (Kozlowski and Connor, 2002). The tobacco plant absorbs toxic elements most probably from the soil, fertilizers or pesticides (Wagner, 1993).

Other environmental factors that may influence the uptake of toxic elements by tobacco plants include the pH of soil, contaminated irri-gated water and sewage sludge used as fertilizers. Tobacco smoking delivers 87 organic carcinogens to the lungs, in addition to toxic ele-ments (Chiba and Masironi, 1992), which may partition into the smoke phase on combustion (Chiba

and Masironi, 1992). Some of the toxic metals (Cd, Ni and Pb) readily pass from inhaled smoke to the bloodstream and may accumulate in specific organs, such as the kidney and liver (Csalari and Szantai, 2002). There are a few studies that have reported on the large variations of heavy metal/toxic ele-ments in the compositions of commercial tobacco products, which have tried to link smoking-related diseases with toxic elements de-rived from tobacco combustion (Klaus et al., 2001). The intake of trace and toxic elements may promote rheumatoid arthritis disorders by increasing oxidative stress, by catalyzing the production of reactive oxygen species or inhibiting their degradation, due to the deficiency of antioxidant elements (Zn, Cu and Mn). The deficiency of essential nutrients, lack of homeostatic control or an excess intake of some toxic elements causes chronic physiological disorders, such as hypertension, cardiovascular disease and rheumatoid arthritis (Witte et al., 2005).

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 (Aydin and Soylak, 2007; Soylak and Dogan, 1996). In the majority of cases, whole blood, serum, plasma, and urine were analyzed (Kazi et al., 2008). Hair can provide a more permanent record of trace and toxic elements associated with normal and abnormal metabolism as well as toxic elements assimilated from the environment. In addition, hair is easily collected, conveniently stored, and easily treated. Therefore, the analysis of human hair has become an important way to understand any quantitative change in certain elements inside the body (Afridi et al., 2008). One of the most widely used analytical technique for different elements determination in biological and environmental materials is inductively coupled plasma atomic emission spectrometry (ICP-AES) due to its advantages over other analytical methods: simultaneous determination of many elements of interest, freedom from different chemical interferences and high detection power. The sensitivity of ICP-AES is lower than that of either Inductive coupled plasma mass spectrophotometer (ICP-MS) or atomic absorption graphite tube atomizer (AA-GTA), but ICP-AES can handle higher levels of total dissolved solids (TDS) than ICP-MS and is much faster than AA-GTA. The main advantage of microwave-assisted samples 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 (Afridi et al., 2006).

As seen in the literature, the alterations in trace and toxic element concentrations in the scalp hair samples of RA patients are inconsistent and, to our knowledge, there are no available reports of the understudy elemental levels in smoker and nonsmokers RA patients. The aim and objective of our present study was to assess the concentrations of Cd, Cu, Mn, Pb and Zn in the scalp hair samples of smoker RA patients. For comparative purposes, 26 non-rheumatoid arthritis individuals (smoker and nonsmoker) of the same age group (ranged 42-56 years), socioeconomic status, localities and dietary habits were selected as referents. The elements under study were analyzed by inductive coupled plasma atomic emission spectrophotometer, after microwave-assisted acid digestion.

2- Materials and methods

2.1. Apparatus

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 fea-tures a 40 MHz free running RF generator, a 0.75 m Czerny-Turner monochromator with 1800 grooves/mm holographic grating used in up to four orders. The resolution of the spectrometer is typically 0.018 nm in 1st order, 0.009 nm in 2nd order, 0.007 nm in 3rd order and 0.006 nm in 4th order. The instrument was controlled with a Dig-ital Equipment Corporation (DEC) Venturis computer with an Intel Pentium processor and Varian Plasma 96 software running under Microsoft Windows 95 operating system. The instrumental condi-tions are shown in Tables 1a-lb. A Hinari Life style (Elstree, Hertford-shire, England) domestic microwave oven (maximum heating power-of 800 W) was used for digestion of the scalp hair samples. Acid-washed polytetrafluoroethylene (PTFE) vessels and flasks were used for preparing and storing solutions.

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

Parameters	Cu	Zn	Mn	Pb	Cd
Wavelength (nm)	327.396	213.8	259.373	220.553	226.502
Height (mm)	5	5	5	3	3
Windows (nm) (above the coil)	0.027	0.027	0.027	0.027	0.027
Scan (nm)	0.040	0.040	0.040	0.040	0.040
Integration (s)	3	3	3	3	3
Replicates	3	3	3	3	3
Sample uptake (sec)	30	30	30	30	30
PMT (V)	650	650	650	650	650
Power (kW)	1.10	1.10	1.10	1.10	1.10
Plasma flow (L/min)	15.0	15.0	15.0	15.0	15.0
Auxiliary flow (L/min)	1.50	1.50	1.50	1.50	1.50
Pump speed (rpm)	15	15	15	15	15
Background mode	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic
Max curve order	1	1	1	1	1
C.C. Limit	0.995	0.995	0.995	0.995	0.995

Table 1b
Liberty 220 common parameters.

Nebulizer type	V-groove
Nebulizer pressure	150 kPa
Stabilization time	10 s
Sample delay time	30 s
Rinse time	10 s
Pump-tube	Orange–orange (inlet) Blue–blue (outlet)
Snout purge	Off
Fast pump	On

Key: s = seconds, kPa = kilo Pascal, L = liter, min = minutes, nm = nanometer, V = volt.

2.2. Reagents and glass wares

Ultrapure water obtained from ELGA Lab Water system (Bucks, UK) was used throughout the work. Concentrated nitric acid (65%) and hydrogen peroxide (30%) were obtained from Merck (Darmstadt, Germany), and checked for possible trace metal contamination. Working standard solutions of Cd, Cu, Mn, Pb and Zn were prepared immediately prior to their use, by stepwise dilution of certified stan-dard solutions (1000 ppm) Fluka Kamica (Buchs, Switzerland), with 0.5 M 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 (Beijing, China), was used (Table 2). All glassware and plastic materials used were previously soaked for 24 h in 5 M nitric acid, washed with distilled and finally rinsed with ultrapure water, dried, and stored in a class 100 laminar flow hoods.

Table 2
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^	% recovery$	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

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

2.3. Sample collection and pretreatment

Before the start of this study, all referents and rheumatoid arthritis 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 ad-ministered 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 non-smokers (NSRA) and smoker patients (SRA). While control groups were also divided into two groups, referents nonsmokers (RNS) and smokers (RS) as shown in Table 3.

All the patients had active disease defined by the following criteria: erythrocyte sedimentation rate (ESR) of at least 30 mm/h, six or more tender joints, three or more swollen joints and morning stiff-ness 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 non-steroidal anti-inflammatory drugs (NSAIDs) (diclofenac sodium, 100 mg/day).

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%)

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 pa-rameters including weight, height and waist circumference were mea-sured 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	RNS	RS	NSRA	SRA
Male				
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 (mm Hg)	119.9 ± 0.62	120.4 ± 0.49	120.3 ± 0.33	120.5 ± 0.45
Diastolic BP (mm Hg)	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 (mm Hg)	120.1 ± 0.46	120.4 ± 0.32	120.2 ± 0.51	120.7 ± 1.01
Diastolic BP (mm Hg)	80.4 ± 0.42	80.5 ± 0.58	81.2 ± 0.35	80.9 ± 0.48

Key: RNS = referent nonsmokers, RS = referent smokers, NSRA = nonsmokers Rheumatoid arthritis patient, SRA = smokers Rheumatoid arthritis patient, 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.

2.4. Collection of scalp hair samples

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: samples were then rinsed with distilled water and then with deionized water. The samples were then rinsed three times with acetone (Afridi et al., 2006). The sam-ples 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 (MWD) procedure was carried out, in order to achieve a shorter digestion time. Duplicate samples of scalp hair (200 mg) of each hypertensive patients and control 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 and kept for 10 min at room temperature then placed in a covered PTFE container. This was then heated following a one-stage digestion program at 80% of total power (800 W). Complete digestion of scalp hair samples required 5-8 min. After the digestion, the flasks were left to cool and the resulting solution was evaporated to semidried mass to remove excess acid. About 5 ml of 0.1 M nitric acid was added to the residue and filtered through a Whatman no. 42 filter paper and diluted with deionized water up to 10.0 ml in volumetric flasks. Blank extractions were carried through the complete procedure. Blanks and standard solutions were prepared in a similar acid matrix. The validity and efficiency of the MWD method was checked with certified values of human hair NCSZC 81002b and with those obtained from conventional wet acid digestion method (Kazi et al., 2008).

2.6. Analytical figures of merit

Statistical analyses were performed using computer program Excel XL State (Microsoft Corp., Redmond, WA) and Minitab 13.2 (Minitab Inc., State College, PA). 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, Cu, Fe, Ni, Pb and Zn standards. Sensitivity (m) was the slope value obtained by least-square regression analysis of calibration curves based on absorbance signals. The equation (n= 5) for the calibration curves was as follows:

$Y= (1.28x10^{-3} \pm 8.60\ x10^{-5})(Cd) + (1.30x10^{-3} \pm 1.23x10^{-4})$, r = 0.999
$Y = (4.38\ x10^{-2} \pm 7.11\ x10^{-3})\ (Cu) + (4.39\ x10^{-2} \pm 7.25\ x10^{-3})$, r =0.999
$Y = (7.12x10^{-3} \pm 3.\ 1\ x10^{-5})\ (Mn) + (6.98x10^{-3} \pm 2.91x10^{-5})$, r2= 0.999
$Y = (1\ .875\ x10^{-2} \pm 7.40\ x10^{-4})\ (Pb) + (1.91x10^{-2} \pm 5.83x10^{-3})$. r = 0.999
$Y = (7.83\ x10^{-2} \pm 1.18\ x10^{-2})\ (Zn) + (8.51x10^{-2} \pm 1.03x10^{-2})$, r = 0.999

where Y is the integrated absorbance, r is the regression and the con-centration range of As, Cd, and Pb for calibration curve reached from the detection limits up to 100 μgL. The limit of detection, equal to 0.0003 ng/mg, 0.01 ng/mg, 0.01 ng/mg, 0.0003 ng/mg and 0.01 ng/mg for Cd, Cu, Mn, Pb, and Zn respectively, was defined as 3 s/m, 's' being the standard deviation

corresponding to ten blank injections and 'rn' the slope of the calibration graph. The quantification limits, defined as 10 s/m were calculated as: 0.0009 ng/mg, 0.03 ng/mg, 0.03 ng/mg, 0.001 ng/mg and 0.04 ng/mg for Cd, Cu, Mn, Pb, and Zn respectively.

3-Results

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 4. Rheumatoid arthritis and healthy subjects were similar in age, height, body weight, and body mass index (BMI), as seen in Table 4. The concentrations of Zn in the scalp hair samples of male RNS and RS were 33.5-38.2% higher as compared with male smokers and nonsmokers arthritis patients ($p = 0.005$-0.001). In the female cases, Zn levels in the scalp hair samples of NSRA and SRA patients were found to be 60-70% lower than those in referents of both group ($p<0.001$). The concentrations of Cu in the scalp hair samples of females and males RNS and RS were 25.7-29.4% higher than those values obtained from NSRA and SRA patients ($p <0.01$-0.006) (Table 5). The concentrations of Mn in the scalp hair samples of male RNS and RS were 43.4-47.0% higher as compared with Mn values obtained in scalp hair samples of NSRA and SRA patients ($p<0.001$). The same trend was observed in female case.

It was observed that the level of Cu, Mn 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 of female NSRA and SRA patients ($p = 0.01$-0.001). The same trend was observed in male cases (Table 5). The Pb concentration in the scalp hair samples of male referents of both group was found to be 24-29% lower than patients (<0.05). The same trend was observed in females (Table 5). The unpaired student t test at different degrees of freedom between RA patients and referents of both genders were calculated at different probabilities. Our calculated t ,alue exceeds that of t -critical value at 95% confidence intervals, which indicated the significant dif-ferences between mean values of understudy elements in referents and RA patients ($p <0.001$). The ratio of essential trace elements to toxic elements (Zn, Cu, Mn/Cd and Pb) was varied in the scalp hair samples of referents as compared to the RA patients of both genders (Table 6).

Table 5
Essential trace and toxic elements in scalp hair samples of referent and Rheumatoid Arthritis subjects (μg/g).

Parameters	RNS	RS	NSRA	SRA
Male				
Copper	10.4 ± 1.23	10.9 ± 0.93	7.34 ± 0.61	8.09 ± 0.82
	8.2–11.7*	9.95–11.9	6.6–7.95	7.15–8.96
	9.58, 11.1^	10.4–11.5	7.02–7.72	7.56–8.55
Manganese	3.41 ± 0.65	3.57 ± 0.47	1.93 ± 0.28	1.89 ± 0.31
	2.89–4.10	3.06–4.15	1.58–2.36	1.54–2.29
	3.09, 3.78	3.29, 3.83	1.79, 2.08	1.67, 2.05
Zinc	203 ± 7.53	178 ± 5.28	135 ± 9.42	122 ± 4.63
	194–211	172–185	124–145	118–128
	196, 207	175, 182	131, 138	120, 125
Cadmium	0.68 ± 0.07	0.94 ± 0.12	2.13 ± 0.37	3.35 ± 0.61
	0.59–0.78	0.81–1.05	1.79–2.56	2.53–4.08
	0.64, 0.72	0.87, 1.01	1.95, 2.31	2.96, 3.64
Lead	3.36 ± 0.41	3.75 ± 0.28	4.55 ± 0.34	5.62 ± 0.87
	2.95–3.85	3.49–4.05	4.12–4.91	4.75–6.45
	3.15, 3.56	3.60, 3.89	4.38, 4.71	5.22, 6.08
Female				
Copper	10.6 ± 0.84	10.7 ± 0.99	7.66 ± 0.75	7.95 ± 0.95
	9.74–11.4	9.84–11.9	6.95–8.46	6.96–8.92
	10.2, 11.0	10.4, 11.2	7.31, 8.10	7.48, 8.43
Manganese	3.39 ± 0.58	3.55 ± 0.62	1.82 ± 0.48	1.74 ± 0.25
	2.81–3.96	2.89–4.21	1.34–2.34	1.46–2.05
	3.10, 3.67	3.22, 3.83	1.76, 2.08	1.56, 1.90
Zinc	211 ± 8.36	167 ± 5.82	126 ± 8.59	117 ± 7.28
	201–220	159–173	118–135	108–125
	206, 216	163, 172	123, 131	113, 122
Cadmium	0.59 ± 0.09	0.83 ± 0.18	1.73 ± 0.37	2.62 ± 0.33
	0.48–0.69	0.69–1.04	1.53–2.22	2.28–2.98
	0.54, 0.63	0.73, 0.92	1.56, 1.93	2.46, 2.79
Lead	3.24 ± 0.15	3.59 ± 0.16	4.39 ± 0.26	5.42 ± 0.51
	3.06–3.42	3.32–3.78	4.12–4.69	4.87–5.96
	3.17, 3.31	3.51, 3.67	4.25, 4.53	5.17, 5.67

Key: RNS = referent nonsmokers, RS = referent smokers, NSRA = nonsmokers Rheumatoid arthritis patient, SRA = smokers Rheumatoid arthritis patient.
* Range.
^ 95% confidence interval.

Table 6
Zn, Cu, Mn vs. Cd and Pb Mole Ratios in scalp hair samples of referents and arthritis patients of both genders.

Mole ratio	Genders	RNS	RS	NSRA	SRA
Zn/Cd	Male	513.1	325.4	108.9	62.8
	Female	614.6	345.8	125.2	76.7
Zn/Pb	Male	191.4	151.2	94.0	68.8
	Female	206.3	147.4	90.9	68.4
Cu/Cd	Male	27.1	20.5	6.1	4.3
	Female	31.8	22.8	7.8	5.4
Cu/Pb	Male	10.1	9.5	5.3	4.7
	Female	10.7	9.7	5.7	4.8
Mn/Cd	Male	10.3	7.8	1.9	1.2
	Female	11.8	8.8	2.2	1.4
Mn/Pb	Male	3.8	3.6	1.6	1.3
	Female	3.9	3.7	1.6	1.2

Key: RNS = referent nonsmokers, RS = referent smokers, NSRA = nonsmokers Rheumatoid arthritis patient, SRA = smokers Rheumatoid arthritis patient.

4- Discussion

This study provides data on the essential trace element (Cu, Mn and Zn) and toxic elements (Cd and Pb) in scalp hair samples obtained from smoker, nonsmokers RA patients and referents of both genders with age ranged (42-56) years.

Rheumatoid arthritis is an autoimmune disease, a disorder in which the body attacks its own healthy cells and tissues. When some-one has arthritis, the membranes around his or her joints become in-flamed 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 result shows that the level of Zn in scalp hair samples of rheumatoid arthritis patients was lower than referents. The skeleton is a major bone store of Zn and in humans approximately 30% of total body Zn is found in 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 supple-mentation was reported to decrease periarticular osteoporosis in rheumatoid arthritis 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 con-genital disorder of zinc absorption (Alegre et al., 1984; Swann et al., 1981; Tudor et al., 2001).

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). p-Alanyl-L-histidinato 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 osteoporosis (Yamaguchi, 1990). Our result showed that the mean concentration of Cu and Mn was found to be lower in the scalp hair samples of rheumatoid ar-thritis patients of both genders with related to referents (Table 5). Forestier (1949) was among the first to report that a Cu complex, Cupralene, was effective in the treatment of rheumatoid arthritis. 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. 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 re-duce 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 repre-sent 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 osteoporosis. 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). Eaton-Evans 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 46- to 56-year-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). The mean levels of Pb and Cd in the biological samples of referents of both genders were found to be lower than those recorded in arthritis patients (Table 5). 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 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 toxic elements, Arsenic, Cd, Cobalt and Antimony can deposit in bone from respiratory exposure (Oakberg et al., 2000). Toxic elements (Cd, Pb and Ni) 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).

Tobacco leaves naturally accumulate and concentrate relatively high levels of Cd, Ni, Pb, Fe, Cu, and therefore smoking of tobacco is an important source of these metals exposure for smokers (Kazi et al., 2009). The total amount of carcinogens in cigarette smoke ranges from one to 3 pg 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 2-4 iLig Cd, 1-2 pg 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 (Hecht, 2003). It was also consistent with another study that smokers generally exhibit significantly higher Cd, Ni and Pb body burdens than non-smokers (Hecht, 2003). The results suggested that Cd, Ni, Pb pose a hazard effects on essential trace elements homeostasis of various organs, co-exposure 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 toxic elements and bio-elements essential for life. These interactions are complex and in-volve bio-elements such as zinc, copper, iron, selenium, calcium and

toxic elements, including cadmium (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 bio-logical structures containing — SH groups, such as proteins, enzymes and nucleic acids (Stohs and Bagchi, 1995). The relevance of Cd, Pb, Ni, Cu and Fe-Zn interactions should be considered in the light of the general population exposure to toxic elements (Waalkes et al., 1992) and common deficiency of Zn in the world, mainly due to nutritional factors (Lonnerdal, 1993).

5- Conclusion

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 toxic elements on human health. The excessive body burden of toxic elements may create the symptoms manifest in the form of muscle and joint com-plaints. The Cd and Pb interfere with the normal biochemical processes of Mn, Cu, Zn and other nutrients in the cells of human body. When these essential minerals in the body are disrupted by toxic elements, musculoskeletal symptoms such as muscle and joint pain commonly occur. The toxic elements 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 "rheumatoid 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 toxic elements in biological samples of healthy and arthritis patients. This study also provides some support for the hypothesis that dietary intake or inhalation of toxic elements (Cd and Pb), most probably through smoking cigarette, may increase the risk of rheumatoid arthritis 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 rheumatoid arthritis and its remedies but also as predictors of adverse outcomes.

The concentrations of essential trace and toxic elements in scalp hair samples of the Irish referent subjects were close to those reported from other European (Rodushkin and Axelsson, 2000; Reilly and Harrison, 1979; Nowak and Chmielnicka, 2000; Trojanowski et al., 2010; Sturaro et al., 1993; Goulle et al., 2005), American (Nagra et al., 1992; Ryan et al., 1978; Saiki et al., 2008; DeAntonlo et al., 1982) and Australian (McKenzie, 1979) countries (Table 7). The ele-mental concentrations of Cd and Pb in Pakistani referents were al-most higher than in European countries, which is in agreement with the studies which were done in Asian (Pasha et al., 2008, 2010; Shah et al., 2006; Khalique et al., 2005; Vishwanathan et al., 2002; Sukumar and Subramanian, 2003; Mehra and Juneja, 2005; Rao et al., 2002; Ashrafur et al., 2009; Sasmaz et al., 2003; Ulvi et al., 2002; Faghihian and Rahbarnia, 2002; Man and Zheng, 2002; Man et al., 1996; Sandstead et al., 1998), African (Nnorom et al., 2005; Hashem and Abed, 2007; Khuder et al., 2008; Eltayeb and Van-Grieken, 1989; Mortada et al., 2002) countries (Table 7).

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

Authors	Elements	Age (years)	N	x ± s (μg/g)
Europe				
Sweden				
Rodushkin and Axelsson(2000)	Pb	1–75	114	0.22–7.26
	Cd	1–75	114	0.010–0.356
	Zn	1–75	114	68–198
	Cu	1–75	114	8.5–96
	Mn	1–75	114	0.080–2.41
England				
Reilly and Harrison (1979)	Zn	16–25	215	210–235
Poland				
Nowak and Chmielnicka(2000)	Pb	25–39 years	624	4.8–5.7
	Cd	25–39 years	624	0.56 ± 2.3
	Zn	25–39 years	624	132.7 ± 135.7
	Cu	25–39 years	624	7.2 ± 6.2
	Mn	25–39 years	624	2.5 ± 2.2
Trojanowski et al.(2010)	Pb	26–50	109	3.71 ± 0.29
	Cd		109	0.401 ± 0.035
	Pb	51–75	121	3.88 ± 0.35
	Cd		121	0.260 ± 0.022
Italy				
Sturaro et al.(1993)	Cu	21–60 years	50	10–21
	Zn	21–60 years	50	171–314
	Pb	21–60 years	50	6.5–8.7
France				
Goulle et al.(2005)	Cu	40–60 years	45	9.0–61.3
	Zn	40–60 years	45	129–209
	Pb	40–60 years	45	0.13–4.57
	Cd	40–60 years	45	0.004–0.17
Netherland				
Iyengar and Wolttlez (1988)	Zn	21–60 years	50	176 ± 38
South America				
Nagra et al.(1992)	Cd	22–59 years	50	31.6 ± 38
Ryan et al.(1978)	Zn	20–55 years	42	108–357
North America				
Saiki et al.(2008)	Zn	50–70 years	50	45–162
	Zn	71–87 years	50	30–202
DeAntonlo et al.(1982)	Zn	15–35 years	67	90–294
Australia				
McKenzie(1979)	Zn	16–56 years	118	189 ± 24
Asia				
Pakistan				
Pasha et al.(2008)	Pb	15–94 years	86	14.62 ± 8.01 (0.577–31.8)
	Cd	15–94 years	86	2.13 ± 1.74 (0.196–9.17)
	Zn	15–94 years	86	154.2 ± 117.1 (12.4–729.2)
	Cu	15–94 years	86	22.35 ± 12.9 (3.27–73.6)
	Mn	15–94 years	86	1.69 ± 1.02 (0.10–4.83)
Pasha et al.(2010)	Pb	37–65 years	37	15.50 ± 8.11
	Cd	37–65 years	37	1.675 ± 1.13
	Zn	37–65 years	37	140.7 ± 79.5
	Cu	37–65 years	37	21.1 ± 5.73
Shah et al.(2006)	Pb	3–54 years (Pakistan)	62	15.97 ± 5.56
		3–54 years (Libya)	62	24.95 ± 8.69
	Cd	3–54 years (Pakistan)	62	0.38 ± 0.186
		3–54 years (Libya)	62	0.53 ± 0.26
	Zn	3–54 years (Pakistan)	62	226 ± 53.7
		3–54 years (Libya)	62	190 ± 34.0
	Cu	3–54 years (Pakistan)	62	17.2 ± 4.2
		3–54 years (Libya)	62	17.2 ± 4.2
	Mn	3–54 years (Pakistan)	62	1.93 ± 0.94
		3–54 years (Libya)	62	1.73 ± 1.09
Khalique et al.(2005)	Cd	41–50 years (M)	18	0.283 ± 0.126
		51–60 years		0.697 ± 0.467
		41–50 years (F)	21	0.318 ± 0.150
		51–60 years		0.732 ± 0.714
	Zn	41–50 years (M)	18	228.2 ± 29.6
		51–60 years		153.6 ± 37.0
		41–50 years (F)	21	319 ± 59.1
		51–60 years		257 ± 47.9
	Cu	41–50 years (M)	18	15.38 ± 2.71
		51–60 years		14.20 ± 5.53
		41–50 years (F)	21	14.5 ± 4.59
		51–60 years		26.2 ± 8.69
	Mn	41–50 years (M)	18	5.946 ± 1.37
		51–60 years		5.364 ± 1.05
		41–50 years (F)	21	10.52 ± 4.87
		51–60 years		7.169 ± 2.44
India				
Vishwanathan et al.(2002)	Pb	36 ± 1.23 years	25	24.8 ± 5.92
	Cd	36 ± 1.23 years	25	5.12 ± 3.41
	Zn	36 ± 1.23 years	25	265.2 ± 17.3
	Cu	36 ± 1.23 years	25	9.70 ± 1.98
Sukumar and Subramanian(2003)	Pb	31–45 years	17	8.9 ± 1.9
		46–60 years	11	4.5 ± 2.8
	Cd	31–45 years	17	1.5 ± 0.3
		46–60 years	11	1.9 ± 0.5
	Zn	31–45 years	17	87.0 ± 1.9
		46–60 years	11	112.8 ± 25.3
	Mn	31–45 years	17	1.3 ± 0.3
		46–60 years	11	1.4 ± 0.6
	Cu	31–45 years	17	27.8 ± 7.5
		46–60 years	11	17.2 ± 4.2
Mehra and Juneja (2005)	Pb	1–30 years	50	7.60 ± 6.44
	Cd	1–30 years	50	0.32 ± 0.21
	Zn	1–30 years	50	182.4 ± 45.2
	Cu	1–30 years	50	8.48 ± 2.06
	Mn	1–30 years	50	6.71 ± 3.38
Rao et al.(2002)	Cd	17–60 years	20	0.12–0.61
	Cu	17–60 years	20	4.90–22.54
	Mn	17–60 years	20	0.62–6.94
	Zn	17–60 years	20	45.44–123.5
	Pb	17–60 years	20	0.75–4.1
Bangladesh				
Ashrafur et al. (2009)	Zn	–	30	199.16 ± 27.85
	Cd	–	30	0.47 ± 0.32
Turkey				
Sasmaz et al.(2003)	Pb	–	26	3.06 ± 1.42
	Cd	–	26	0.67 ± 0.33
Ulvi et al.(2002)	Zn	47.76 ± 13.11 years	29	176.96
Iran				
Faghihian and Rahbarnia, (2002)	Zn	14–67 years	100	36–329
Hong Kong				
Man and Zheng (2002)	Pb	20–50 years	30	12.04 ± 7.0
	Zn	20–50 years	30	184.85 ± 60.89
Man et al. (1996)	Zn	30–69 years	95	355–503
China				
Sandstead et al. (1998)	Zn	7–25 years	662	109–155
Africa				
Nigeria				
Nnorom et al.(2005)	Pb	1–30 years	46	63.6
	Cd	1–30 years	46	1.0
	Zn	1–30 years	46	128.6
	Cu	1–30 years	46	121.0
Saudi Arabia				
Hashem and Abed (2007)	Cd	20–25 years	20	0.035 ± 0.007
Syria				
Khuder et al.(2008)	Pb	21–59 years	281	10.7 ± 8.9
	Zn	21–59 years	281	260 ± 113
	Cu	21–59 years	281	15.6 ± 5.7
Sudan				
Eltayeb and Van-Grieken(1989)	Zn	30–50 years	35	89–170
	Pb	30–50 years	35	3–17
Egypt				
Mortada et al.(2002)	Pb	28–40 years	93	1.8–9.7
	Cd	28–40 years	93	0.08–0.82

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