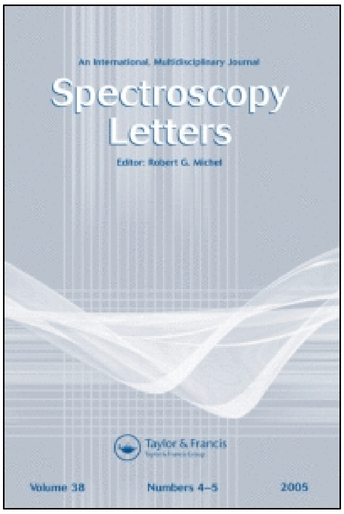

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Analysis of Heavy Metals in Scalp Hair Samples of Hypertensive Patients by Conventional and Microwave Digestion Methods

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Analysis of Heavy Metals in Scalp Hair Samples of Hypertensive Patients by Conventional and Microwave Digestion Methods

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Abstract: The abnormal metabolism of metal ions plays an important role in health and disease conditions, and studies about them have been attracting significant interest. The aim of our study was to assess the heavy metals (cadmium, copper, iron, nickel, chromium, lead, and zinc) in scalp hair samples of 200 hypertensive (HT) patients of an urban population together with 215 non-hypertensive male subjects in the age group of 30–60 years. Hair samples were digested with conventional wet ashing and microwave digestion. Analyses of both digests were done by flame and graphite furnace atomic absorption spectrometry. The validity of methodology was checked by use of the certified reference material (CRM 397) hair, provided the Community Bureau of Reference, Commission of the European Communities. According to a statistical evaluation of the results, the microwave digestion method was a valid alternative to the conventional acid digestion method, p value >0.05, but it gave a faster

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digestion. The overall metal recoveries were 96–98% of those obtained with microwave digestion. Among the toxic elements determined, levels of nickel, cadmium, copper, chromium, and lead in scalp hair samples of hypertensive patients were significantly higher compared normal male subjects, whereas the essential elements such as iron and zinc were found to be low compared age-matched non-hypertensive subjects.

Keywords: Scalp hair, urban population, hypertension, wet digestion, method, microwave digestion, atomic absorption spectrometry

INTRODUCTION

Analysis of hair tissue for minerals is a sophisticated laboratory measurement used in preventive medicine for assessing mineral imbalances and toxicities in the human body. Using this general diagnostic screening technique, information regarding the following nutritional minerals and toxic metals can be assessed with greater accuracy than by any other diagnostic methods.[1–5] During the past few decades, human hair has been extensively studied in clinical practice as a noninvasive tool for determination of trace elements in the body. The advantage of hair analysis is that, besides being noninvasive, it gives an average concentration of trace elements over a period of time.[6]

Hypertension (HT) is an increasingly important medical and public health issue. The prevalence of hypertension increases with advancing age to the point where more than half of the people 60–69 years of age and approximately three-fourths of those 70 years of age and older are affected.[7] But nowadays the age criteria have been changed and even people under 30 have HT problems due to lack of exercise, fast foods, coffee consumption, smoking, and alcohol use.[8–11]

Toxic metals play a vital role in diseases of unknown etiology. Many researchers have studied the relationship of cadmium, lead, zinc, and copper to hypertensive status using hair as a biopsy material.[12–15] The exposure of cadmium occurred mainly through cigarette smoking, inhalation of airborne cadmium in ambient air (usually higher near coal-fired power plants and municipal waste incinerators), or consumption of some foods (highest levels in shellfish, and liver and kidney meats).[16]

In urban areas, the use of concrete and the combustion of oil, coal, and gasoline represent the major potential sources of environmental pollution for toxic metals,[17,18] and toxic metals containing particles may thus easily find their way into the respiratory tract.[19] Certain metals may promote hypertensive and atherosclerosis by increasing oxidative stress (e.g., by catalyzing the production of reactive oxygen species or inhibiting their degradation) and increasing the blood pressure levels.[20–22]

The aim of our study was to assess the trace, essential, and toxic elements in a biological sample (scalp hair) of hypertensive patients and normal male subjects of matching age groups. We evaluate the increase or decrease of these elements in hypertensive patients related to age and habits. The preparation of hair samples by two digestion methods was also evaluated.

EXPERIMENTAL

Collection of Scalp Hair Samples

Scalp hair samples were taken from 215 healthy, drug-free cases (male), age range between 30 and 60 years, taken as normal control, and from hypertensive patients (200) of the same age groups visiting the Basic Health Units (BHUs) for health check-ups during the period between September 2003 and August 2004, three times in a year, all occupants of site areas of Hyderabad (Pakistan). Before the start of this study, all the control subjects and HT patients were informed about the aim of the study by being administered a form, and all agreed to participate and signed the form. A questionnaire was administered to them in order to collect details concerning physical data, ethnic origin, health, and dietary and smoking habits. Physical examinations were performed to measure participants' weight, height, blood pressure, and biochemical data. There were no statistically significant differences between normal and hypertensive patients regarding height, weight, or comparable aspects of family.

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. The total number of normal and hypertensive subjects is shown in Table 1.

Washing

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

Table 1. The number of subjects as control and hypertensive patients (male)

Age groups	Normal	Hypertensive patients
30–45	120	97
46–60	95	103
Total	215	200

acetone.[23–25] The samples were then dried in an oven at $75 \pm 5°C$. Dried samples were stored separately in polyethylene bags.

Reagents and Glassware

Ultrapure water obtained from a Milli-Q purifier system (Millipore Corp., Bedford, MA, USA) was used throughout the work. Nitric acid and hydrogen peroxide were analytical reagent-grade from E. Merck. Standard solutions of zinc, copper, lead, and cadmium were prepared by dilution of certified standard solutions (1000 ppm, Fluka Kamica, Switzerland) of corresponding metal ions. All glassware and plastic material used was previously treated for a week in 2 N suprapure nitric acid and rinsed with double-distilled water and then with ultrapure water.

Apparatus

A Milestone microwave oven (Rotar MPR-300/12S, pressure 35 bar, with maximum temperature 300°C, Italy) was used. Metals were determined in acid digests using either a flame atomic absorption spectrophotometer (FAAS) or electrothermal atomic absorption spectrophotometer (ETAAS) of Hitachi Ltd., Japan (Model 180-50, S.N.5721-2), with a deuterium lamp back corrector, linear (least square) mode equipped with a graphite furnace G-03, and a Hitachi Model 056 recorder was used for recording analytical data of the metals under investigation. Hollow cathode lamps (Hitachi) operating at recommended current were used for all cases.

Digestion Methods

Triplicate samples from each subject (1200 mg each) and replicate five samples of CRM 397 were weighed in Pyrex flasks, then 5–10 mL HNO_3 was added in a digesting flask. The flasks were covered and heated on electric hot plate to decompose the organic matter in samples at 60–70°C for 1–2 hr. The digests were then treated with additional 5 mL nitric acid and a few drops of H_2O_2 and heated on hot plate at about 80°C until the color of the digestion solution become clear transparent yellow, then cooled and diluted to a volume of 25 mL in volumetric flasks with 2 N nitric acid.[26–28]

For comparative sample preparation method, a microwave-assisted digestion procedure was applied in order to achieve a shorter digestion time (Table 2): 200 mg of duplicate dried samples of scalp hair and replicate three sample of CRM 397 were weighed and placed in a PTFE reactor, then 6 mL of HNO_3 and 2 mL of 30% H_2O_2 were added. The reactor was sealed and stood for 2 hr. Then it was heated following a one-stage digestion

Table 2. Determination of metals in CRM 397 by microwave and wet acid digestion methods (μg/g)

Elements	Wet digestion method	% RSD	% Recovery	Microwave digestion method	% RSD	% Recovery	Certified values
Cadmium	0.54 ± 0.03	5.4	103	0.51 ± 0.03	7.6	98.0	0.52 ± 0.024
Chromium	90.12 ± 6.2	6.8	99	88.6 ± 9.4	10.6	97.3	91.0 ± 10[a]
Copper	108.4 ± 6.7	6.1	98	105.8 ± 7.2	7.3	95.4	110 ± 5[b]
Iron	575.0 ± 22.0	3.8	99	565.8 ± 24.0	4.2	97.5	580.0 ± 10[a]
Nickel	45.2 ± 2.0	4.5	98	44.7 ± 3.4	7.6	97.1	46.0 ± 1.4[b]
Lead	32.2 ± 2.2	6.8	97.5	31.8 ± 2.5	8.0	95.0	33 ± 1.2
Zinc	197.2 ± 12.8	6.2	99.0	194.5 ± 11.3	5.7	97.7	199 ± 5

[a]Indicates informative value.
[b]Indicates indicative value.

program (250 W, 30 min). After cooling, sample digests were filtered through Whatman no. 42 filter paper, transferred into a 25 mL flask, and brought to volume with Milli Q water. Blank extractions (without sample) were carried through the complete procedure. The accuracy and precision were examined by analyzing a certified reference material of hair (CRM 397) (Table 2). All of the analytical results obtained by the proposed method were in good agreement with the certified values. The relative standard deviation (RSD) was less than 8%.

Blank and standard solutions were prepared in a similar acid matrix. For copper, iron, and zinc, all of these digests and a series of standard solutions of these elements were atomized in air-acetylene flame with a Hitachi 180-50 Atomic Absorption Spectrophotometer under conditions shown in Table 3. For cadmium, chromium, nickel, and lead, ETAAS was used under conditions shown in Tables 4 and 5. High-purity argon (99.99%) was used as a purge gas.

Statistical Analysis

The values of metal levels in scalp hair are presented as arithmetic mean in μg/g with standard deviation and tabulated to illustrate concentration profile over each group. The statistical significance of mean values between different groups has been determined by applying Student ± test calculated with Minitab 13.2, an Excel computer software statistical program (Microsoft Corp., Redmond, WA, USA). The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The analytical results of hair provide a more permanent record of the trace elements than blood and urine analysis.[29] Its values, therefore, are valuable in forensic studies, environmental investigation, nutritional status, and clinical diagnosis.

Table 3. Measurement conditions for AAS

Elements	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Burner height (mm)	Oxidant (air) (kg/cm^2)	Fuel (acetylene) (kg/cm^2)
Zn	214.0	1.3	7.5	7.5	1.60	0.2
Cu	325.0	1.3	8.0	7.5	1.60	0.2
Fe	248.5	0.2	7.5	7.5	1.60	0.3

Table 4. Measurement conditions for ETAAS

Parameters	Pb	Cd	Cr	Ni
Lamp current (mA)	7.5	7.5	7.5	10
Wavelength (nm)	283.3	228.8	357.9	232.0
Slit width (nm)	1.3	1.3	1.3	0.2
Background correction	D_2 lamp	D_2 lamp	—	D_2 lamp
Cuvette	Cup	Cup	Tube	Tube
Carrier gas (mL/Min)	200 mL	200 mL	200 mL	200 mL
Sample volume (μL)	10	10	10	10

Analytical results of the CRM 397 obtained by both digestion methods were close to that of the certified values, which confirmed the reliability of the methods (Table 2). The analysis of normal and patient scalp hair samples is reported as mean values with standard deviation (SD) and p values reported for each element (Table 6).

The mean values of iron in the scalp hair samples of hypertensive patients of both age groups were lower as compared with normotensive subjects of matched groups, as shown in Table 6. Iron is important in the transportation of oxygen from the lungs to the whole body. Iron deficiency is probably the most frequent nutritional deficiency disorder in the world. A recent estimate based on World Health Organization (WHO) criteria indicated that around 600–700 million people worldwide have a marked iron deficiency anemia.[30] Whole-wheat flour has a high content of phytate and strongly inhibits iron absorption. Phytates strongly inhibit iron absorption in a dose-dependent fashion and even small amounts of phytates have a marked effect.[31,32]

The concentrations of zinc in the scalp hair samples of the hypertensive patients of both age groups were lowered (Table 6). Zinc deficiency causes the arteries to become hard, brittle, and often inflamed instead of soft and flexible. This loss of flexibility will raise the blood pressure, in particular the systolic pressure.[33,34] In the case of hypertensive patients, vegetarian foods have high phytate, and their intake may result in reduced availability of iron and zinc for intestinal absorption.[35]

Table 5. Temperature program (min/sec)

	Pb	Cd	Cr	Ni
Dry	80–120/15	80–120/15	80–120/15	80–120/15
Ash	300–600/15	300–600/15	300–700/15	300–700/15
Atomization	2000–2100/5	1500–1800/5	2600–2700/5	2500–2600/5
Cleaning	2100–2400/2	1800–2000/2	2700–2900/2	2600–2800/2

Table 6. Determination of trace and toxic metals in scalp hair samples of normal and hypertensive male subjects (μg/g)

	Male		
Age groups	Normal	Hypertensive	p value
Iron			
30–45	39.82 ± 1.68	24.5 ± 0.4	0.003
46–60	31.9 ± 1.43	20.7 ± 1.41	0.011
Zinc			
30–45	224.0 ± 7.07	125.14 ± 5.49	0.002
46–60	214.14 ± 8.2	112.0 ± 4.32	0.001
Chromium			
30–45	3.89 ± 0.16	5.64 ± 0.28	0.007
46–60	3.25 ± 0.28	4.38 ± 0.4	0.015
Copper			
30–45	12.85 ± 1.0	16.84 ± 1.68	0.002
46–60	11.45 ± 0.85	17.01 ± 1.36	0.000
Nickel			
30–45	6.79 ± 1.87	13.14 ± 2.41	0.013
46–60	5.37 ± 0.92	7.87 ± 0.78	0.000
Cadmium			
30–45	1.68 ± 0.31	2.98 ± 0.2	0.002
46–60	1.25 ± 0.31	2.64 ± 0.14	0.001
Lead			
30–45	8.08 ± 0.52	14.45 ± 1.7	0.02
46–60	7.47 ± 0.38	14.0 ± 1.44	0.005

The concentration of chromium in the scalp hair samples of hypertensive patients of both age groups was higher, but the differences were not significant (Table 6). The high Cr concentration obtained may be attributed to the Cr content in tobacco leaves, which might get incorporated in the leaves from the soil. High levels of Cr in smokers have also been reported.[36]

The Cu, Cd, and Ni in the occupational hypertensive persons from urban areas were significantly higher[37] as compared with normal subjects. Smoking was found to be a contributing factor to higher bioaccumulation of Cd as also reported by other researchers.[10] Workers with chronic headache and dizziness have higher levels of Cr and Pb in the scalp hair samples, such as in those working in a fireworks factory.[38]

In the current study, it was observed that the level of copper concentration was significantly higher in scalp hair samples when compared with those of age-matched male subjects (Table 6). High plasma Cu levels may be associated with heart failure and rhythm disorders; Cu inhibits phosphodiesterase activity and manganese inhibits andenylate cyclase activity thus exerting an influence on the contractility of cardiomyocites and of smooth muscle cells in coronary arteries.[39] The possible source of Cu in smokers may be due to insecticidal sprays on soil-producing tobacco. Beef and meat contain the highest amount of Cu and Cd as compared with vegetarian food.[40]

It was observed in our studies that the level of nickel was significantly higher in hypertensive subjects as compared with normotensive age-matched subjects (Table 6). Significant Ni levels in smokers compared with nonsmokers have also been reported at significant level of $p < 0.005$.[11] Besides this, the inhalation of vapors of nickel carbonyl certain occupations (welding, fitting, etc.) also causes elevated Ni levels in biological samples.[41,42]

Cadmium, which enters the environment from mining, industry, vehicles, and household waters, binds strongly to soil particles or dissolves in water.[43] Once taken up by fish, plants, and animals, cadmium stays in the body for a long time.[44] Humans are also affected by cadmium through smoking and consumption of foods and beverages. Rice is the main source of cadmium in rice-eating countries.

Human lead exposure is mainly through air and food. The presence of lead in fuels has contributed to much of the current human exposure.[45] In most developed countries, the fuel content of lead has been controlled but still remains an issue of immediate consideration in developing countries, including Pakistan. Other sources of lead exposure include lead-based paints, lead pipelines in water supply systems, and ceramics. Lead-based products, including paints and food containers, are not completely banned in Pakistan.[46]

A study was conducted on rural and urban populations residing in villages near New Delhi, India. The males living in urban areas had higher levels of cadmium and lead than those living in nonindustrial areas.[38]

There are a number of factors contributing to the higher levels of cadmium and lead in congested areas like Hyderabad City. Hyderabad City represents a typical urban environment with heavy traffic load, high population density, and industrial units. In addition, open burning of plastics and brick-making among other activities contribute to this higher level of toxic elements. Our study revealed that the levels of cadmium and lead in hair were higher in Hyderabad City, suggesting that dust containing these heavy metals is attached to hair samples due to a typical urban environment with heavy traffic load, high population density, and industrial activities.

The accumulation of cadmium in the human body may replace zinc in the arteries, which contributes to arteries becoming brittle and inflexible. Cadmium accumulates in the kidneys, resulting in high blood pressure and kidney disease. Therefore, cadmium is known to cause arterial hypertension. Death rates in hypertensive heart disease are closely correlated with cadmium in air and milk.[47] Cadmium causes significant increases in blood pressure.[48] High levels of lead have been identified in significant numbers of patients with high blood pressure.[8,9] Lead may also replace zinc and calcium, contributing to this cause of hypertension.[49] Once the arteries become inflamed and brittle, the body may coat them with calcium and fatty plaques to prevent rupture of the arteries. This plaque unfortunately reduces the interior diameter of the arteries, which in turn raises blood pressure. More pressure is required to force the blood through the smaller diameter arteries. Toxic substances can build up within the kidneys and damage their ability to regulate water balance in the body. This can lead to water retention, salt retention, and high blood pressure.

CONCLUSIONS

The deficiency of essential metals that are replaced by toxic metals may result in abnormal physiology, and in addition to other factors this may have a role in hypertension disease. The smoker hypertensive patients have a high level of toxic metals as compared with the nonsmoker hypertensive patients.

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