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Full Length Research Paper

Trace elements in the hair of normal and chronic arsenism people

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The analysis of trace elements in hair has been widely used for several decades. The non-invasiveness of the method, ease of sample collection, and storage as well as advances in modern analytical techniques are great advantages of hair for determining the levels of various major, trace and toxic elements in animals and humans. To evaluate scalp hair as a possible bio-indicator of trace element, the concentrations of some trace micronutrients (Ca, Fe, Cu and Zn) and pollutants (As and Pb) in scalp hair of 124 normal subjects (71 males and 53 females, average age 29 years) and 110 chronic arsenism patients (62 males and 48 females, average age 27 years) were examined by energy dispersive x-ray fluorescence spectrometry in this study. The results showed the patient group has lower contents of trace micronutrients than that of normal group and also differences found between the two sex groups. Micronutrients concentrations in females were higher than those in males. Concentrations of As in hair of patients and normal groups, 13.2 mg/kg and <3.0 mg/kg respectively, were significantly different while male patients contain higher As concentration (14.7 mg/kg) than female (11.7 mg/kg). The frequency distributions of the elements with respect to age, sex, diseased state and locality are presented here and the results are compared with the data reported in the literature.

Keywords: Trace elements, hair, chronic arsenism, human.

INTRODUCTION

Since 1929, human's salp hair has been used to assess toxic levels of different elements (Althausen *et al.* 2006). Hair is widely accepted for assessing toxic element exposures and measured by most clinical laboratories capable of making trace element measurements. Using hair to assess essential elements is more controversial, yet researchers have found many correlations of essential elements to diseases, metabolic disorders, environmental exposures, and nutritional status (Leclair and Quig 2001, Werbach 1993, Foo *et al.* 1993, Bosque *et al.* 1991, Man *et al.* 2006, Huang *et al.* 1991, Donma *et al.* 1990, Aharoni *et al.* 1992, Suzuki *et al.* 1993, Chattopadhya *et al.* 1990, Katz and Chatt 1988, Druyan *et al.* 1998). Compared to other types of clinical specimens, hair has different uses and even advantages over blood or urine (Bass 2001).

Hair has a unique potential to reveal retrospective information about the nutritional status and exposure of subjects (Sela *et al.* 2007). It grows approximately 1 cm a month, and trace element composition in hair reflects blood levels at the time the hair was generated (Gellein *et al.* 2008). Trace elements are incorporated into hair during the growth process and reflect the composition of trace elements in blood plasma at the time of formation (Benner and Levin 2005, Yukawa *et al.* 1984). Blood and urine analysis on the other hand, reflects the trace

element status only at the time the sample was obtained. Hair has also been reported to be a valuable indicator of environmental pollution (Phelps *et al.* 1980, Weiss *et al*. 1972, Giovanoli-Jakubczak and Berg 1974) and has important information in several historical or forensic cases has been obtained from hair analyses (Shamberger 2002).

Hair is a biological tissue that indicates concentration profiles of elements in an individual at a particular time period and the concentrations of elements are affected by different factors that are also specific to certain regions and subjects. Many causal factors causing change in the hair levels of elements are grouped under four main categories: biological factors, personal factors, environmental factors, and analytical and methodological factors. (Sukumar 2002).

Trace elements play an important role in human health and nutrition. Deficiencies of certain essential elements like calcium, iron, copper, zinc, and vitamins may lead to increased body burden of toxic elements such as arsenic, lead, cadmium, mercury and the supplementation of former may mitigate the toxic effects of the later. It is an established fact that there exist interaction between nutrients (Ca, Fe, Cu, Zn, etc.) and environmental pollutants (As, Pb, Cd, Hg, etc).

There are known instances of inter-element interactions of Pb and anaemia due to iron deficiency, Hg and Fe, As and Se, Se and I, Cd and Zn, among others. It is thus very clear that nutrition; especially micronutrient nutrition may has a very important role in the detoxification of arsenic toxicity. Nutrition priorities are identified separately from pollution under various initiatives, but until now there is very little information available on nutrition-pollution interrelationships. The area of nutrition-pollution interactions and the deleterious health effects resulting from these interactions are in its state of rapid growth, which needs extensive research to be matured.

Currently, millions of Bangladeshis are suffering from arsenicosis- the clinical syndrome of varying degrees ranging from spotted melanosis (skin pigmentation at the primary stage) and keratosis (thickening of skin of palm/foot at the secondary stage) up to the gangrene and cancer at the last stage. In rural areas of Bangladesh, the intake of a number of essential trace elements (Ali 1995) and vitamins are below the recommended levels. The severity of the arsenic poisoning situation among the rural poor in Bangladesh may likely to be aggravated due to their existing poor nutrition and infection. However, there is no study to obtain data about the situation, particularly reflecting the interaction of trace micronutrients (Ca, Fe, Cu and Zn) and arsenic poisoning in the country.

The aim of the present work is to report levels of some micronutrients in human scalp hair of Bangladesh normal rural population to establish baseline information and then to find out how they correlate their levels in chronic arsenism conditions.

MATERIALS AND METHODS

Selection of subjects

Three clinical types of 110 chronic arsenism patients (62 males and 48 females, average age 27 years) confirmed by hair arsenic concentration greater than 3.0 mg/kg were selected for this study. 124 apparently healthy adults (71 males and 53 females, average age 29 years) were selected as control, matching socio-economic status with the study population residing in arsenic hot spot areas in rural Bangladesh. Screening was done by history of drinking arsenic contaminated water for more than 6 months. The patients were clinically diagnosed by skin manifestations such as melanosis, leukomelanosis, keratosis, hyperkeratosis, etc. and confirmed by hair arsenic contents >3.0 mg/kg. Pregnant women and lactating mothers were excluded from the study.

Sampling and sample preparation

The samples were collected from individuals to be at risk of arsenic exposure residing in arsenic hot spot areas of Bangladesh. The peoples from arsenic hot spot areas visited to different hospitals in Dhaka were referred to our laboratory for diagnosis of chronic arsenicosis before any treatment was initiated. After filling a questionnaire regarding socio-demographic information, the scalp hair was collected from each of them. About, 5 g of hair was cut from the closest distance of the scalp and from different sites around the head with stainless steel scissors. The samples were kept in clean paper envelopes in a vacuum desiccator until preparation for analysis.

All peoples, both male and female with history of exposure to arsenic contaminated water and arsenical skin manifestations including hair arsenic concentration >3.0 mg/kg were grouped as patient and the normal group was free from clinical features of chronic arsenicosis with normal hair arsenic contents. The hair samples were washed according to the procedure described by the International Atomic Energy Agency (IAEA) (Ryabukhin 1978) to remove external contaminants. The washed samples are then charred in an electric oven at 180^{o}C for about 1 hour and finely powdered them in an aluminum carbide mortar according to the prescribed procedure reported earlier[6].

From the homogeneously mixed powdered material of both samples and standards (Orchard leaves, NIST-SRM 1571; Human hair, GBW 09101; Human hair, IAEA-086), a 100-mg portion of the fine residue was pressed into 1-mm thick and 1 cm diameter pellet with hand press pellet maker for x-ray analysis. The ready made pellets from each sample and standard were then put into clean dry small plastic petridishes and preserved in a vacuum desiccator until irradiation.

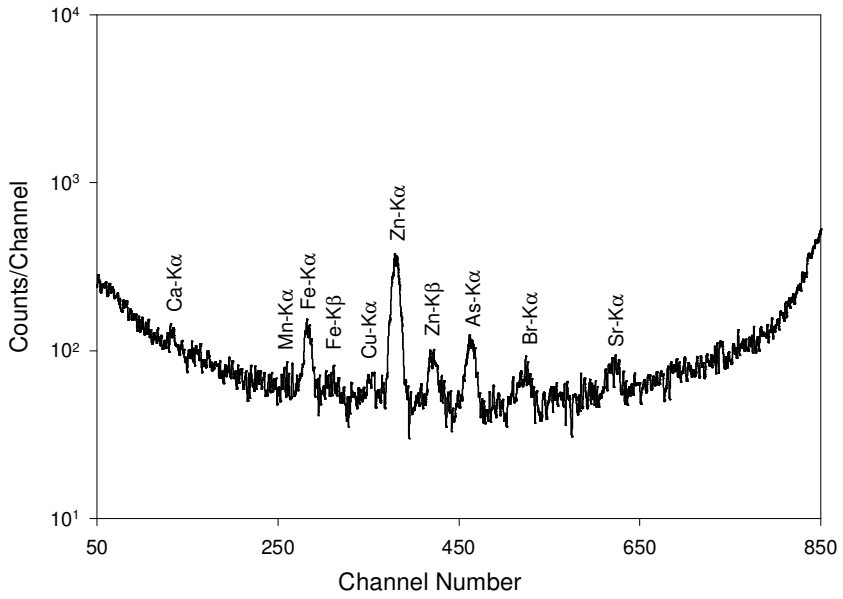


Figure 1. A typical RIXRF spectrum of scalp hair sample of an arsenic patient

Analytical method

The components of an energy-dispersive radioisotope induced x-ray spectrometer developed in AECD laboratory for this purpose consists of a primary x-ray source, sample holder, an x-ray detector, a multi-channel analyzer (MCA) and associated NIM electronics for data acquisition and processing. A microcomputer is dedicated to this system for on-line XRF data analysis.

The source of primary x-rays for excitation of characteristic x-rays is a high intensity ^{109}Cd annular sealed x-ray source. The sample holder is simply a receptacle to hold the sample. The excitation source is a ring type construction, which is ideal for the Si (Li) detector. The sample is directly irradiated from the source. The details of the method have been reported earlier[5]. A typical RIXRF spectrum of scalp hair sample obtained from an arsenic patient is shown in Figure 1.

RESULTS AND DISCUSSION

Concentration calibration and validity of the method

In order to determine concentration of elements in hair samples, a calibration curve as shown in Fig. 2 was constructed from the NIST orchard leaves standard (SRM-1571). The calibration curve as shown in Figure 2 was constructed from the average peak areas obtained from the irradiation of five 100 mg standard pellets prepared in the same way as the sample pellets. Using this curve, the concentration of an element 'C_E' in mg/kg in a given hair sample is obtained from the expression: $C_E = (Y_E / S_E) \times 1/D$; where, C_E = Concentration of arsenic in mg/kg; Y_E = X-ray yield of an element in sample in counts/second; S_E = Calibration factor (sensitivity) of an element in counts/ppm/second; D = Ratio of wet weight to the dry weight of the sample and $(1/D)$ = Conversion factor of the estimated concentration from dry state of the sample to the wet state.

The standard orchard leaf material was used as such without further treatment. The treatment of the samples at 180 ± 5^0C as has been mentioned earlier in sample preparation is not expected to affect at least the concentration of elements studied in this work. The hair sample material has moisture content of about 12%. The mortar blank was negligible. In the present calibration, the matrix of the standard was assumed to be similar to that of the sample on the ground that in all biological matrices, the predominant components are H, C, O, N, S, etc. This assumption has been verified experimentally[5-6] where it has been shown that the multi-element orchard leaf standard can be used to analyze different biological specimens to obtain results with about 10% accuracy. From the above discussion, we may conclude that the use of this x-ray yield curve as shown in Figure 2 for the analysis of elements in hair tissues is justified and correct.

Minimum detection limit (MDL)

The minimum detection limit of this method depends generally on matrix elements and experimental conditions.

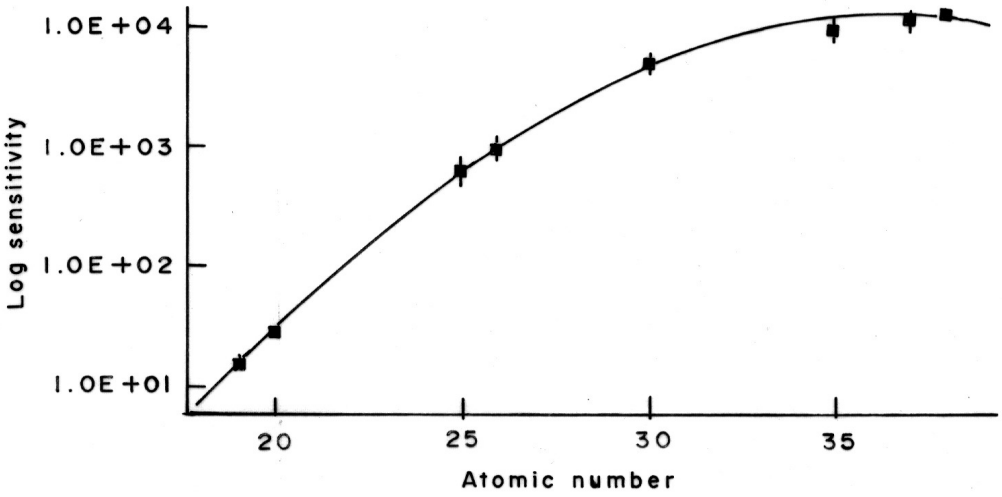


Figure 2. X-ray yield curve for concentration calibration constructed from NIST orchard leaves standard SRM 1571.

The detection limit of XRF is determined by the relation Ip $\geq 3(I_B)^{1/2}$; where Ip is the counts of a characteristic x-ray peak and I_B is the counts of continuum x-rays covered by FWHM of the peak. This relation means that a characteristic x-ray peak should be larger than three times the background fluctuation to be identified as a characteristic x-ray peak. Using this relation, the minimum detection limit (MDL) of elements in hair matrix is estimated from the relation of MDL = $[(3 \times I_B^{1/2}) / I_P] \times C$, where, C is the concentration of the element, I_P is the net peak area of the element, and I_B is the net background area under the element photo peak. Under the present experimental conditions, EDXRF offers the detection limit of Ca: 395, Fe: 9.5, Cu: 4.1, Zn: 3.0, As: 2.5, and Pb: 3.5 mg/kg in this study without pretreatment of the sample material. The results on the MDL of the technique indicates that a fairly reliable estimate of elements can be made without employing any pre-concentration technique except oven drying of the sample for a short period of time.

Distributions of trace elements

The concentrations of Ca, Fe, Cu, Zn, As and Pb in scalp hair of different subject groups are given in Figure 3 which states the following order: Ca > Zn > Fe > Cu > Pb > As in all investigated subjects except in chronic arsenism male subjects in which the concentration of As was higher level than Pb, showing the following order: Ca > Zn > Fe > Cu > As > Pb. Both normal and patient female contains higher amount of Ca, Fe, Cu and Zn micronutrients. The reverse is true in case of arsenic concentrations. This finding indicates that our rural females are better in trace element nutritional status than males irrespective of age. It is evident from comparison between normal and patient group that there is correlation of diseased conditions with nutritional status. Calcium and zinc nutrition has been found lower under chronic arsenism conditions (Figure 3).

Arsenic

The frequency distribution of arsenic in hair of Bangladeshi population under chronic arsenism conditions is shown in Figure 9. Males suffering from chronic arsenism are significantly higher than female. It is evident from Figure 3 that the estimated arsenic level in the hair of arsenic patients ranges from 3.0 mg/kg to 53 mg/kg, the normal levels (Ali 1995, Maidul et al. 1996, Ryabukhin et al. 1978) are somewhere below 3.0 mg/kg. According to Guinn and Demiralp (1993) arsenic concentrations above 100 mg/kg are often encountered in hair sections of arsenic poisoning cases. From the present study, the average concentration of arsenic in patient hair is 13.2 mg/kg that indicates alarming public health hazards in Bangladesh arising from drinking arsenic contaminated water. The average concentration of arsenic in patient hair of Bangladesh (13.2 mg/kg) is observed much higher than West Bengal, India mean value of 8.44 mg/kg[12].

Out of a large number of contaminated districts only 7 districts have patients with arsenic pollution in this study. From the present findings it has been observed that the arsenic affected patients are more in the districts of Noakhali > Chandpur > Laxmipur > Faridpur > Munshiganj compared to those of other districts (Figure 9). Frequency distribution of age among arsenic patients is given in Figure 3. The age distribution shows that, the arsenic affected patients are found to be more within the

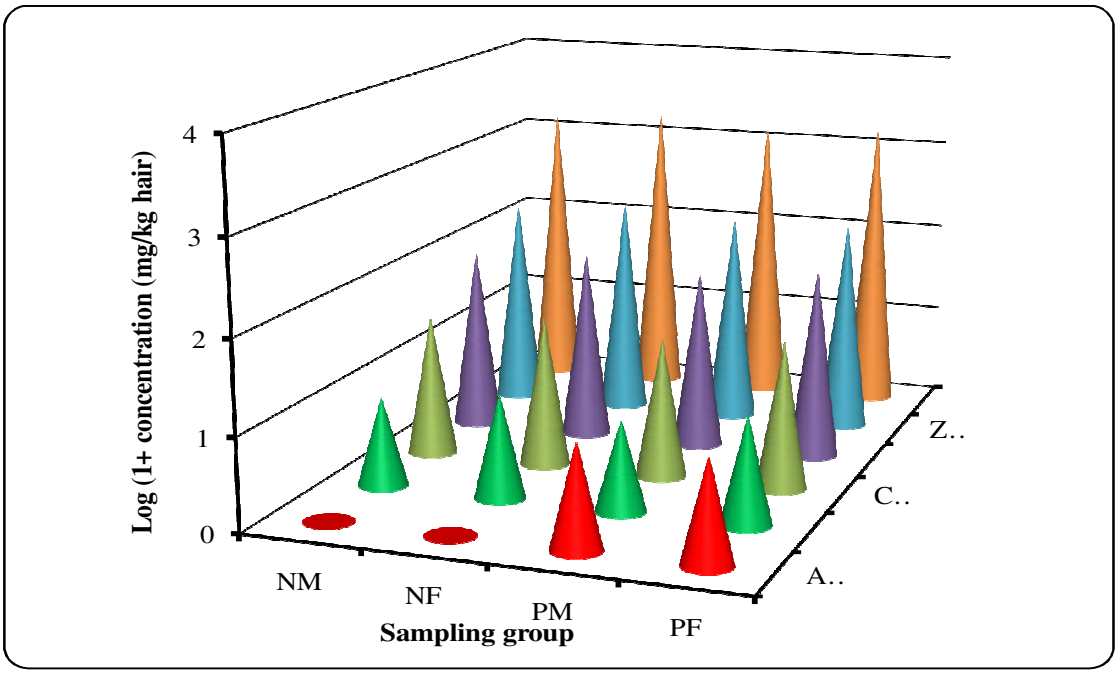


Figure 3. Concentration of different trace elements obtained from four sampling group. NM: Normal male; NF: Normal female; PM: Patient male and PF: Patient female

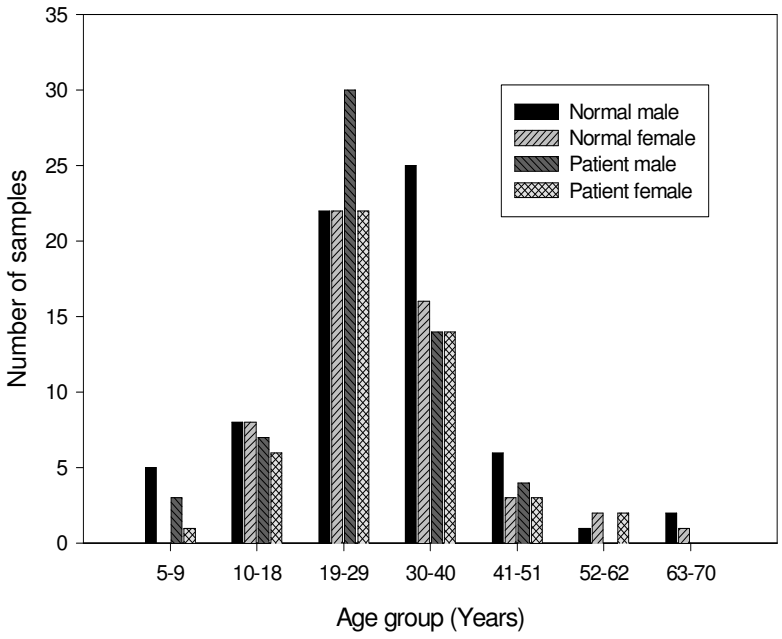


Figure 3. Grouping of hair sample according to age in normal and chronic arsenism subjects

adult age group of 19 to 29 years compared to children, adolescent and other adult age groups. The males are more affected to arsenical diseases (60%) compared to those of the females (40%) so far investigated. This may be due to higher consumption of drinking water by this group than others. The major health hazards due to arsenic toxicity in Bangladesh are from drinking arsenic contaminated groundwater.

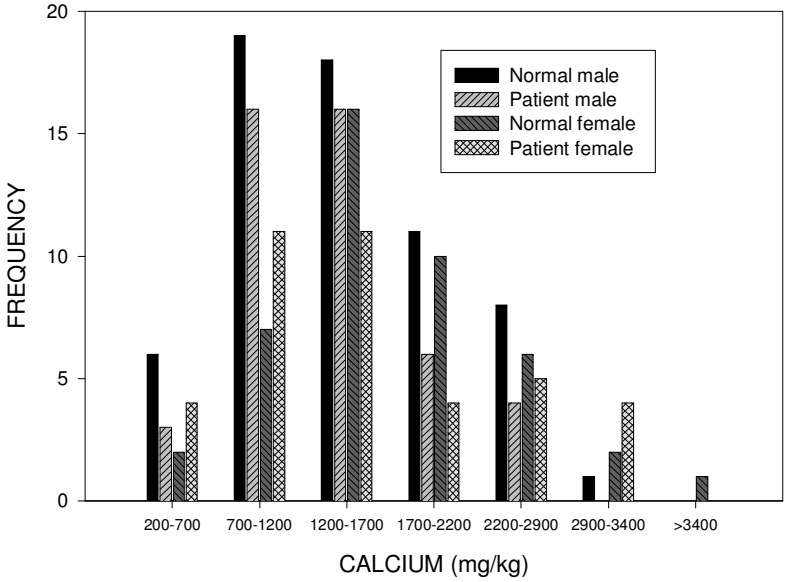


Figure 4. Frequency distribution of calcium in hair of normal and chronic arsenism subjects

Lead

The mean concentrations of Pb in normal and patient hair are found to be 10.6 and 10.7 respectively. These are within the reference value (IAEA 1994) (20-30 mg/kg) indicating that the population studied here are free from the influence of Pb contamination in excess of the normal level. Similar result were obtained in other parts of the world such as Pb contained in hair is 7.0 (Eltayeb and Grieken 1990), 10.6 (Ashraf et al. 1998), 5.1 (Barker et al. 1976). Our value is more than in South Poland 4.99 mg/kg in (Chojnacka et al. 2005, Nowak, 1998, and 0.96 in Sweden (Rodushkin and Axelsson, 2000) but less than in Rio de Janeiro city where it is 119 mg/kg (Miekeley et al. 1998).

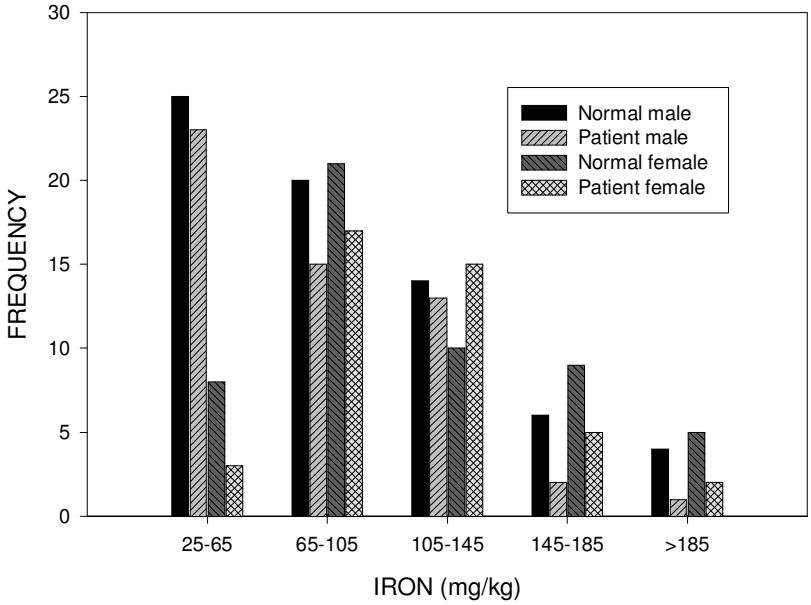
The levels of lead are higher in male than in female both in normal and arsenism subject (TS1) but it did not exceeded normal level. The reason for the higher levels of lead in male than in female could be due to the atmospheric pollution coupled with the level of occupational exposure (Phelps et al. 1980) because in our country males are mainly involved in occupational works that cause more exposed to open environment than female. Lead would most probably be of environmental origin (Valkovic et al. 1975, AShraf et al. 1998). No significant difference was obtained when compare normal people to arsenism. It indicates that As content does not influence the Pb uptake in human hair.

Calcium (Ca)

The hair calcium (Ca) concentration has been used as an indicator of disorders in Ca and bone metabolism (Miekeley et al. 2001) and as a predictor of risk of coronary heart disease (MacPherson and Bacsó 2000). It is also are more reliable indicators of spine bone mineral density than its concentration in serum in premenopausal women (Song et al. 2007).

Female hair contained higher amount of Ca than that of male in both cases normal and arsenism subject (TS1) and exceeded the normal level (According to MineraLab data[9] the normal range is 200-600 mg/kg). Figure 4 shows the frequency distribution of the concentration of calcium in hair of normal and chronic arsenism male and female subjects. Our results showed higher amount of Ca than the other parts of the world, 1042 mg/kg in (Chojnacka et al. 2005, Nowak, 1998) in South Poland, 750 mg/kg in Sweden (Rodushkin and Axelsson, 2000) and 802mg/kg in Rio de Janeiro city (Miekeley et al., 1998). Similar pattern was also observed in Poland described by Chojnacka et al. (2005) and referred that 2.5times higher level than in Rio de Janeiro, 1.5-times higher level than in Sweden.

The reason of higher Ca concentration in hair of female than male may be intake of vitamin D and other factors like the presence of promoters or inhibitors of its absorption in the food consumed, additional host-related factors such as overall health, hormonal status, physical activity, and coexisting diseases as well as the use of medications that might affect Ca absorption (Fishbein 2004). Female takes medicine and vitamin more frequently than male in Bangladesh. The very high hair Ca level in woman may be due to a lower rate of hair growth resulting in higher accumulation of minerals (Jeruszka-Bielak and Brzozowska 2011). Calcium levels



Figures 5. Frequency distribution of iron in hair of normal and chronic arsenism subjects

in hair were affected by all three factors, age, sex, treatment and highest calcium levels were observed in treated female hair taken from persons under age 45(Vance et al. 1998).

Iron (Fe)

Toxicity of iron in humans has been found to bring about vomiting, cardiovascular collapse and diarrhoea. While iron deficiency may lead to failure of blood clotting (WHO 1995). The mean concentration of iron among healthy controls was 102 mg/kg and arsenism people it was 96.7 mg/kg. These values are higher than that of other parts of the world, whereas it was 45.7 mg/kg in (Chojnacka et al. 2005, Nowak, 1998) in South Poland, 9.6 mg/kg in Sweden (Rodushkin and Axelsson, 2000) and 20.8 mg/kg in Rio de Janeiro city (Miekeley et al., 1998) but did not exceeded the normal range (15-175 mg/kg).[10] Similar pattern was reported by Chojnacka et al. (2005) in Poland, Fe content is 2-times higher level than in Rio de Janeiro, 2-times higher level than in Sweden.

The frequency distribution of iron data showed in Figure 5 present that most of the normal and patient group falls in the concentration range of 25-65 mg/kg. Most of the normal male and female group belongs to the concentration range of 65 -105 mg/kg group. Higher Fe concentration was found in woman than man although it is not affected by any of the sex (Vance et al. 1998).

Copper (Cu)

Copper is a common environmental metal and is essential in cellular metabolism but at high concentrations it can be highly toxic to fish (Kabata, H and Pendias, 1993). Copper is an essential substance to human life, however, in high concentrations, it can cause anaemia, liver and kidney damage, stomach and intestinal irritation (WHO 1995). The frequency distribution of copper between the normal and patient groups (Figure 6) indicate that most of the normal and patient groups belong to the concentration ranges between 25-40 mg/kg that did not cross the normal level 7 to 40 mg/kg (10). These values are true for other people in world, such as 22.7mg/kg in Poland whose level were 2-times lower than in Rio de Janeiro and Sweden (Chojnacka et al. 2005).

The higher concentration of copper was found in female than male irrespective of arsenism. Though, sex does not influence Cu (14.89±0.89 µg/g and 15.26±0.79 µg/g hair for males and females, respectively) but age influences Cu concentrations, but only significantly in females: Cu levels decrease over 60 y of age (Bertazzo et al. 1996). So our results varied due to hair color influences Cu concentrations in both males and females. In males, white hair containes less Cu than black hair; in females, white hair's Cu levels are significantly lower than those of dark blond, red, light brown, and brown hair (Bertazzo et al. 1996).

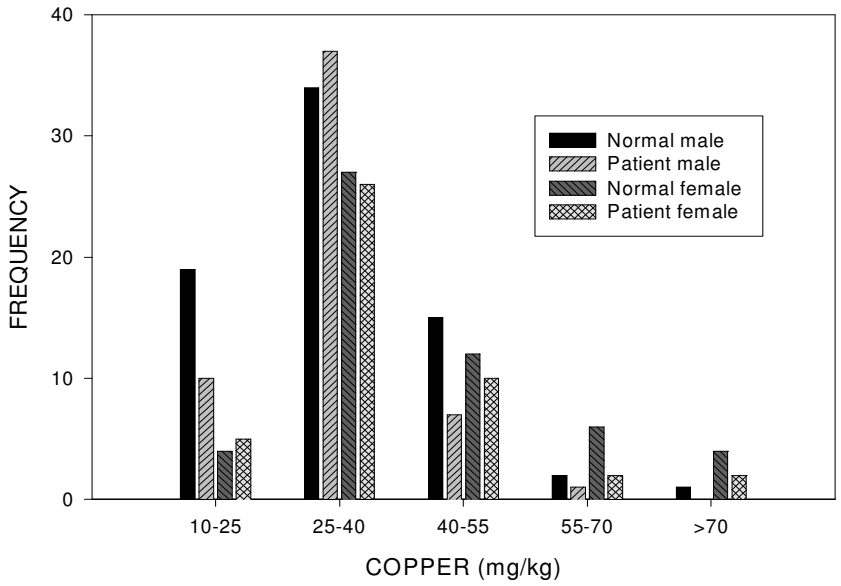


Figure 6. Frequency distribution of copper in hair of normal and chronic arsenism subjects

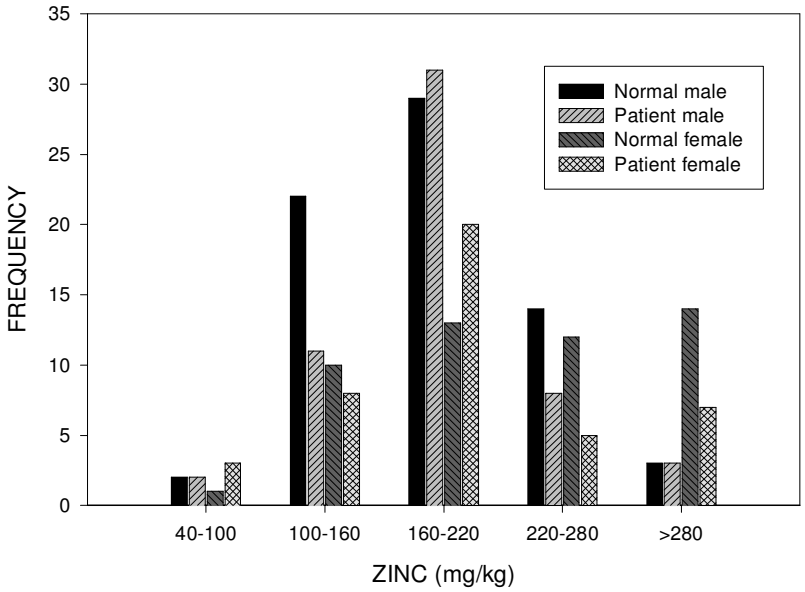


Figure 7. Frequency distribution of zinc in hair of normal and chronic arsenism subjects

Zinc (Zn)

Zinc is an important element in protein synthesis and gene expression involving the immune and endocrinological systems (Yenigun et al. 2004). Zinc deficiency may play a crucial role in some of the pathological manifestations associated with Down syndrome such as infections and malfunctioning of the thyroid gland (Bjorksten et al, 1980; Sustrova, & Strbak, 1994). The mean concentration of hair zinc is 215 mg/kg in control group whereas it is 193 mg/kg in patient subjects. There is small difference between the two values indicating low in patient group. The normal zinc hair value is 150-250 mg/kg (IAEA 1994) indicating our values are in normal range.

The frequency distribution of zinc in hair of normal and patient groups (Figure 7) showed that both normal and patient male and and patient female have higher populations in concentrations between 160-220 mg/kg. In South Poland, it was found 179mg/kg (Chojnacka et al.

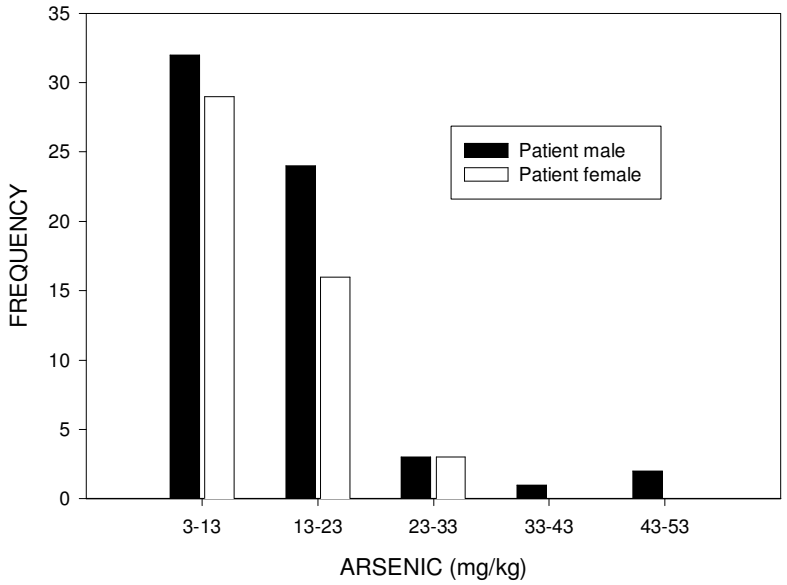


Figure 8. Frequency distribution of arsenic in hair of Bangladeshi population under chronic arsenism condition

Figure 9. Frequency distribution of arsenic patients among different districts in Bangladesh

2005), 119 mg/kg in (Nowak, 1998) 142 in Sweden (Rodushkin and Axelsson, 2000) and 156 in Rio de Janeiro city (Miekeley et al., 1998). Our values showed higher than that of other parts of the world. It may be occurred due to environmental pollution. Our country is becoming more polluted than the referred country. The content of Zn in female hair is higher than that of male due to the effect of sex (Bertazzo et al. 1996). There are no significant differences in Zn concentrations with respect to different hair colors, in either males or females

and age influences Cu and Zn concentrations, but only significantly in females (Bertazzo et al. 1996). Age, sex and body mass index have been reported to be some of the influencing factors that affect the concentration of zinc in the hair (Chen et al., 1985; Mateo et al. 2000). Another reason for different concentration obtained from our study than other parts of the world is food habit. Food habits and frequency of intake of different products also influence zinc concentration in hair (Schlegel-Zawadzka et al., 2002; Deeming and Weber, 1978). Also can be different for location subjects. The higher levels of zinc observed in the Ibadan City population could be attributed to the nutritional characteristics of the two populations (Adekola et al. 2004).

CONCLUSIONS

The results of this study will contribute to better understanding of the interactions of elevated arsenic with Ca, Fe, Cu and Zinc nutrition and to develop proper remedial measures of chronic arsenic poisoning. To the best of our knowledge, work on hair trace elements of normal and chronic arsenism patients in Bangladesh population has not yet been reported.

In this study, a significantly higher level of arsenic in scalp hair of chronic arsenism people was observed compared with that of normal healthy subjects. But the differences in the levels of Ca, Fe, Cu and Zn between the two groups were not found significant. This may be due to non-suitability of hair tissue as a biomarker for changes of these elements under chronic arsenism conditions although it is an established bio-indicator for chronic exposure of heavy metals. Although chronic arsenism is a disease of long-term arsenic toxicity, the roles of Ca and possibly of Fe, Cu and Zn in arsenic toxicity deserve further attention.

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REFERENCES

Adekola FA, Dosumu 00, Olaleye GA (2004). Comparative study of the age and location dependence of some heavy metals in human hair from two nigerian citieS. Toxieo!. and Environ. Chelfl., 2004, 86, (1-4):189-194.

Aharoni A, Tesler B, Paltieli Y (1995). Hair chromium content of women with gestational.

Ali M (1995) X-ray Analytical Studies of Trace Elements in Food and Drink: A Nutritional Assessment in Bangladesh, Ph.D. Thesis, Jahangirnagar University, Dhaka.

Althausen TL, Gunther L. Acute arsenic poisoning: a report of seven cases and a study.

Antonella B, Carlo C, Monica B, Graziella A, Girolamo C, Giuseppe P (1996). Determination of copper and zinc levels in human hair. Biological Trace Element Research. 52: 37-53.

Ashraf W, Jaffar M, Anwer K, Ehsan U (1998). Environ. Pollut., 87: 61-64.

Barker DH, Rencher AC, Mittal BM, Shanbhag SV, Sharma LS (1976). In: Hemphill, D. (Ed.), Trace Substances in Environmental Health. University of Missouri Columbia, MO.

Benner BA, Levin BC (2005). Hair and human identification. In: Tobin DJ (ed) Hair in toxicology: an important bio-monitor. RSC, Cambridge, 127–159.

Biomedical and Environmental Sciences. New York, NY: VCH Publishers; 1988:105-109.

Bosque MA, Domingo JL, Llobet JM, Corbella J (1992). Cadmium in hair of school children diabetes compared with nondiabetic pregnant women. Am J Clin Nutr 55:104-107.

Chattopadhyay PK, Joshi HC, Samaddar KR (1990). Hair cadmium level of smoker and non-smoker human volunteers in and around Calcutta City. Bull Environ Contam Toxicol 45:177180.

Chojnacka K, orecka HG (2005). Inter-element interactions in human hair. Environmental Toxicology and Pharmacology 20 (2005) 368–374.

Donma O, Gunbey S, Tas MA, Donma MM (1990). Zinc, copper, and magnesium concentrations in hair of children from southeastern Turkey. Biol Trace Elem Res 24:39-47.

Druyan ME, Bass D, Puchyr R (1993). Determination of reference ranges for elements in Arch Occup. Environ Health 65:S83-S86.

Eltayeb MAH, Grieken RE (1990). Sci. Total Environ., 95: 157-165.

Fishbein L (2004). Multiple sources of dietary calcium—some aspects of its essentiality. Regul Toxicol Pharmacol. 39:67–80.

Foo SC, Khoo NY, Heng A, Metals in hair as biological indices for exposure. Int.

Giovanoli-Jakubczak T, Berg GG (1974). Measurement of mercury in human hair. Arch Environ Health 28:139–144.

Guinn VP, Demiralp R (1993). Arsenic in Hair by INAA – A. Major Recent Murder Case. J Radioanal Nucl Chem 168: 249 – 256.

Huang BX, Lin SQ, Chen SY (1991). Hair chromium levels in patients with vascular diseases. Biol Trace Elem Res 29:133137.

Human scalp hair. Biol Trace Elem Res 1998;62:183-197.

IAEA (1994) Application of hair as an indicator for trace element exposure in man. A review. NAHRES 22, IAEA Vienna 1994.

Kabata, H and Pendias, A (1993). Trace Elements in Soil and Plants, 2nd Edn., Boca Boca Raton FL, USA, 365, Lewis, 1993.

Katz SA, Chatt A. The significance of hair analysis. In: Hair Analysis: Applications in the

LeClair JA, Quig DW. Mineral status, toxic metal exposure and children's behaviour. J

living in Tarragona Province, Spain. Relationship to age, sex, and environmental factors. Biol Trace Elem Res 1991;28:147-155.

MacPherson A, Bacsó J. Relationship of hair calcium concentration to incidence of coronary heart disease. Sci Total Environ. 2000;255:11–19.

Maidul AZM, Momin A, Akramullah SM, Zakir A, Afsar S, Salahuddin A, Tarafdar SA, Ali M (1996). Bangladesh J Dermatol Venereol Leprol 13(1): 1

Man AC, Zheng YH, Mak PK (1996). Bio. Trace Elem. Res., 53, 241-247.

Man AC, Zheng YH, Mak PK (2011).Trace elements in scalp hair of professional drivers and Marta Jeruszka-Bielak and Anna Brzozowska. Relationship Between Nutritional Habits and Hair Calcium Levels in Young Women. Biol Trace Elem Res. 2011 December; 144(1-3): 63–76.

Miekeley N, de Carvalho Fortes LM, Porto da Silveira CL (2001). Elemental anomalies in hair as indicators of endocrinologic pathologies and deficiencies in calcium and bone metabolism. J Trace Elem Med Biol. 15:46–55

Miekeley N, Dias Carneiro, MTW, Porto da Silveira, CL, (1998). How reliable are human hair reference intervals for trace elements? Sci. Total Environ. 218, 9.

MINERALAB, Inc., 22455 Maple Court, Hayward, CA 94541, USA.

Nowak B (1998). Contents and relationship of elements in human hair for a non-industrialized population in Poland. Sci. Total Environ. 209, 59.

Phelps RW, Clarkson TW, Kershaw TG, Wheatley B (1980). Interrelationships of blood and hair mercury concentrations in a North American population exposed to methylmercury. Arch Environ Health 35:161–168.

Rodushkin I, Axelsson MD (2000). Application of double focusing sector field ICP-MS for multielemental characterization of human hair and nails. Part II. A study of the inhabitants of northern Sweden. Sci. Total Environ. 262, 21.

Ryabukhin YS (1978). Activation Analysis of Hair as an Indicator of Contamination of Man by Environmental Trace Element Pollutants, Report, IAEA/RL/50.

Sela H, Karpas Z, Zoriy M, Pickhardt C, Becker JS (2007). Biomonitoring of hair samples by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Int J Mass Spectrom 261:199– 207.

Shamberger RJ (2002). Validity of hair mineral testing. Biol Trace Elem Res 87:1–28

Song CH, Barrett-Connor E, Chung JH, Kim SH, Kim KS (2007). Associations of calcium and magnesium in serum and hair with bone mineral density in premenopausal women. Biol Trace Elem Res. 118:1–9.

Sukumar A (2002). Factors influencing levels of trace elements in human hair.

Suzuki T, Hongo T, Yoshinaga J (1993). The hair-organ relationship in mercury concentration in contemporary Japanese. Arch Environ Health 48;221-229.

university teachers in Hong Kong. Biol Trace Elem Res 1996;53:241-247.

Valkovic V, Rendlc D, Phillips GC (1975). Environ. Sci. Tech., 9, 1150-1152

Vance DE, Ehmann WD, Markesbery WR (1998). Trace Element ontent in Fingernails and Hair of a Nonindustrialized US Control Population. Biological Trace Element Research. 17:109-121.

Weiss D, Whitten B, Leddy D (1972). Lead content of human hair (1871–1971). Science 178:69–70

Werbach MR (1993). Lab methods for nutritional evaluation. In: Nutritional Influences on Illness, 2nd ed. Tarzana, CA: Third Line Press 642-654.

WHO, World Health Organization, (1995). Environmental Health Criteria 165: Inorganic Lead, Geneva: International Programme on Chemical Safety. World Health Organization, Geneva.

Yenigun A, Ozkinay F, Cogulu O, Coker C, Cetiner N, Ozden G, Aksu O, Ozkinay C (2004). Hair zinc level in Down syndrome. *Down Syndrome Research and Practice*. 9(2);53-57.

Yukawa M, Suzuki-Yasumoto M, Tanaka S (1984). The variation of trace element concentration in human hair: the trace element profile in human long hair by sectional analysis using neutron activation analysis. Sci Total Environ 38:41–54