

Determination of Arsenic in Human Scalp Hair and Fingernails of Healthy Individuals Resident in Karbala, Iraq by Using ICP-OES

تقدير الزرنيخ في شعر فروة الرأس والأظافر للأفراد الأصحاء المقيمين في كربلاء بواسطة تقنية ICP-OES

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البحث مستل

Abstract:-

The use of unconventional biological materials as biomarkers in trace element studies has increased in terms of published research studies. In this study, human scalp hair and fingernails were used to be a possible biomarker for arsenic in the human body as no study has been published in Karbala yet. Samples were obtained from 56 healthy individuals resident in Karbala, Iraq. The level of arsenic was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). The validity, precision and accuracy of the methodology were evaluated using a “pooled” sample for each media and certified reference materials. The validation methods provided acceptable levels of precision and accuracy with lower range of RSD (1.1%) and acceptable range of elemental recoveries (98.09 %), respectively. The results show that the arsenic levels of study sample are in general agreement with the literature ranges for scalp hair with exception reported for fingernails. It was found that there was no significant difference for arsenic between the two tissues (at $P < 0.05$). In addition, the value of correlation coefficient ($r = 0.804$, $P = 0.05$) was indicated that there is a significant positive correlations for arsenic level between scalp hair and fingernails.

Keywords: Human scalp hair. Fingernails, arsenic; ICP-OES; Karbala.

الخلاصة:-

تم استخدام المواد البيولوجية غير التقليدية كمؤشرات حيوية في دراسات العناصر النزرة من حيث الدراسات والأبحاث التي تم نشرها. في هذه الدراسة، تم استخدام شعر فروة الرأس البشري والأظافر كمؤشر بيولوجي محتمل للزرنيخ في الجسم البشري حيث لا توجد دراسة منشوره في كربلاء حتى الان. تم الحصول على عينات من 56 فرد سليم مقيمين في كربلاء، العراق. تم تقدير مستوى الزرنيخ باستخدام تقنية البلازما المقترنة بالحث مطيافية الانبعاث الضوئي (ICP-OES). كما تم تقييم فعالية الطريقة باستخدام الدقة والضبط باستخدام عينة متجانسة من الشعر ومادة قياسية مرجعية معتمدة. تشير النتائج الى ان الطريقة تميزت بمستوى مثالي من الضبط (RSD(1.1% ودقة مقبولة (98.09 %). أظهرت النتائج أن مستويات الزرنيخ في النماذج التي تم دراستها توافقت بشكل عام مع الأدبيات بالنسبة لعينات الشعر مستثنا بذلك بعض عينات الأظافر. تبين أنه لا يوجد اختلاف مهم في مستويات الزرنيخ بين النسيجين (عند $P < 0.05$). إضافة إلى ذلك، فإن قيمة معامل الارتباط ($r = 0.804$, $P = 0.05$) أشارت الى أن هناك علاقة إيجابية مهمة بين شعر فروة الرأس والأظافر بالنسبة لمستوى الزرنيخ.

Introduction

In the last few decades, human scalp hair and nails (finger & toe) have widely been used as a good biomarker in the assessment of exposure to various pollutants in an occupational and/or environmental setting, and in terms of assessing the metabolic state of humans, for essential and toxic trace elements^{1, 2, 3}. Hair and nail tissues have several advantages over blood and urine, including: non-invasive materials and easily sampled; potentially represent a long-term growth material; and several trace elements may accumulate in hair and nail tissues over a time frame of 2 to 18 months. These advantages may provide useful data in determining the health status of an individual over long periods, as the tissues remain isolated from other metabolic activities in the human body^{4, 5}. The use of human hair and nails as an excellent tool to assess changes in our bodies has received a great deal of attention for a few decades and become successful in different applications⁶. The connection between inorganic elements and human health has long been recognized. This is may be due to human scalp hair is a long-term growth material which may provide useful data in determining health status of an individual for long periods and several trace elements being accumulated in hair as well⁷.

Arsenic, similar to other elements, can become toxic if its concentration in the human body is too high. A level of 1 - 3 mg/Kg As is enough to be lethal in a human adult⁸. The toxicity of arsenic is strongly related to its oxidation state and chemical form⁹. It was found that inorganic arsenic is suggested to be more toxic than organic forms in terms of human health¹⁰. Most cases of arsenic-induced toxicity in humans are due to natural exposure to inorganic arsenic via air, water, soil, dust and food^{11, 12}. In recent decades many studies have reported that arsenic plays a significant role in a number of diseases, such as cancer and diabetes^{13, 7}. Chronic arsenic exposure has been suggested to have an etiologic role in diabetes development¹⁴, with more than one study in the USA reporting that arsenic in drinking water is associated with the onset of diabetes¹⁵. Another study in Bangladesh has shown that the risk of diabetes is increasing among people exposed to high levels (more than 100 µg/L) of arsenic through drinking water¹⁶.

A proportion of studies further displayed that As₂O₃ also prevents many other hematologic malignancies and solid tumors such as neuroblastoma, breast cancer, liver cancer, gastric cancer, lung cancer, esophageal cancer, cervical cancer and so on¹⁷. Arsenic trioxide is effective in treating promyelocytic leukemia, and laboratory studies establish that arsenic trioxide reasons apoptosis of human breast cancer cells¹⁸.

The main aim of this study is to determine the levels of arsenic in scalp hair and fingernails for healthy individuals by using ICP-OES.

Materials and methods

In this study the concentrated nitric acid (Aristar® 65%) (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK) was used for digestion procedure, whereas, the pure acetone (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK) and de-ionised water (DDW; 18.2 MΩ) were used for washing methods and dilution, respectively.

Study Population

Human scalp hair and fingernails samples were collected from individuals living in Karbala which is a city in Iraq located about 100 Km south west of Baghdad. The participants were clearly informed of all the study procedures before signing the informed consent form. Generally, volunteers were interviewed at the time of sampling to obtain some general information about their health status, lifestyle, typical diet and smoking habits. In total, 56 samples were collected (28 scalp hair, 28 fingernails) from individuals in relation to different forms of smoking (none, passive and active), varying in gender (male and female) and age from three to sixty years old. Generally, all study populations followed a similar diet programme comprising of rice, bread, cereals, vegetables, fruit, meat, oils, cheese, butter, cream and milk; and the main drinks were soft drinks, fruit juice and tea; prepared with household tap water.

Sample collection and preparation

Scalp hair

Scalp hair samples were collected from the same site of the head for all individuals, namely, from the back of the head, less than 1 cm from the scalp using acetone/distilled deionised water washed scissors. This pre-treatment was undertaken to prevent contamination introduced by the tool during sample collection. Generally, a sample (mass > 0.5 g) was collected and stored in a polyethylene bag at room temperature until the time of analysis^{19, 20}. Hair samples were cut into small pieces (~ 5 mm) using acetone/distilled deionised water washed scissors so as to make the sample more homogenous²⁰.

Fingernails

Fingernail samples were collected from all 10 fingers using acetone distilled de-ionised water washed clippers²¹. The majority of studies have used this method to obtain nail samples, but in some cases only thumb nails have been collected²². The main advantages to collect all fingers rather than one big finger are: sufficient sample mass, and an estimate of the complete hand of exposure²³. Fingernail samples were cleaned manually of any visible dirt (e.g. soil) on the surface of nails prior to application of the washing procedure²⁴.

Washed and digestion

In brief, the cut scalp hair and fingernail samples were washed using the sequential washing procedure (acetone-water-water-water-acetone) was utilized in this study¹⁹. Samples were dried in an oven overnight at 60°C then stored at room temperature in labelled polyethylene bags. The wet digestion method using a Kjeldahl™ tube was employed for the complete digestion of washed human scalp hair and fingernails²⁵.

Sample analysis

Instrumentation

The new JY 2000 – 2 ICP Optical Emission Spectrometer (ICP – OES) Horiba Scientific was used in this study. An echelle grating and the charge-coupled device (CCD) were used in the ICP-OES instrument.

Precision and accuracy

The levels of precision and accuracy for the ICP-OES instrument were confirmed by calculation of the relative standard deviation (%RSD) and percentage recoveries (%R) using ten replicate measurements of a "pooled" water sample, and certified reference materials (CRMs), In general, good levels of precision were obtained for most elements with perfect value of 1.1% RSD, as shown in Table 1. Measured CRM values obtained for the analysis of arsenic by ICP-OES were highly comparative to certified values. Analytical recovery value is 98% for arsenic determined, as reported in Table 1. The value of limit of detection (LOD) for arsenic under investigation was within expected range for ICP-OES (11.30 µg/L).

Element (n = 3)	Elemental level (µg/kg)			Precision
	Accuracy			
	Measured value mean ± SD	Certified value mean ± SD	Percentage recovery (%R)	%RSD
As	43.52 ± 2.07	44.00± 1.72	98.90	1.1

SD is standard deviation, RSD and R are relative standard deviation and recovery, respectively (quoted as a % in brackets).

Statistical analysis

Descriptive data analysis (arithmetic mean, standard deviation (SD), range and 95% confidence interval) was performed on concentration values obtained for washed scalp hair and fingernails. An F-test and a two tailed t-test tests were used to assess the significance of the variations in washed scalp hair and fingernails arsenic levels for healthy individuals. The Pearson product correlation coefficient (*r*) was determined for arsenic to evaluate if there was any significant correlation between washed scalp hair and fingernails for arsenic.

Results and Discussion

In total, 56 human scalp hair and fingernails samples were collected from healthy Iraqi individuals resident in Karbala in order to determine the arsenic levels of scalp hair and fingernails. This can be used to investigate whether human scalp hair and fingernails can play a significant role as a biomarker in the assessment of human health and environmental chemical exposure. Arsenic levels (mg/kg dry weight, d.w.) in washed scalp hair and fingernail samples for healthy individuals are summarised in **Tables 2**.

Table 2: Population data for arsenic levels (µg/kg) in washed scalp hair and fingernails for individuals resident in Karbala (Iraq), along with literature range.

Variable	Scalp Hair	Fingernails	Literature range	
			Scalp Hair*	Fingernails*
Mean ± SD	6122.60 ± 1948.16	5907.86 ± 2884.16	15 - 26000	65 – 1090
Range	2703.18 – 9701.10	353.17 – 11786.67		
Lower Quartile	4505.14	4226.57		
Upper Quartile	7429.76	8136.34		
Confidence interval at 0.05%	6844.20 – 5401.01	6976.14 – 4839.57		

SD is standard deviation, CI is confidence interval for mean, n is the number of samples, *²⁶.

Elemental Composition of Washed Scalp Hair and fingernails

The findings of arsenic are reported as the mean, standard deviation, range, 95% confidence interval for mean and the number of samples for hair and fingernails, as shown in Table 2. The highest (11.787 mg/kg d.w. As) and lowest (0.353 mg/kg d.w. As) arsenic levels were found in fingernail samples. The results of scalp hair are in general agreement with the literature ranges reported in **Table 2** (0.015 – 26.000 mg/kg d.w. As)²⁶. It was found that the elemental levels for most of the trace elements in washed scalp hair are higher than those reported in blood, tear drops and saliva²⁷. The possible explanation is that the scalp hair is considered as a long-term growth material. Therefore, most trace elements accumulate in the hair²⁸. As a result, scalp hair can provide some useful data, and reflect the body status over a long period of time²⁹.

In the case of fingernails, the levels of arsenic in fingernails are higher than those reported by other authors (65 – 1090 µg/kg d.w. As). A possible explanation is that the bioaccumulation of trace elements in fingernails is a complicated process influenced by several factors during hair growth, namely metabolic changes, age, gender and living environment quality^{24, 30}. Recently, human fingernail tissue has been recognised as an invaluable tissue for the assessment of exposure to various pollutants in an occupational and/or environmental setting. It provides a useful indication of exposure to many toxic and essential trace elements over a long period of time, as this material remains isolated from any metabolic activity in the human body. Thus, they are considered to be a finger-print of the body's trace element levels over a period of time, which is not possible with materials such as blood¹. Previous studies have reported that the levels of trace elements in fingernail tissue were found to be higher than those of body fluids and other accessible tissues²⁹. In addition, fingernail material has many useful advantages for trace element research than other biological media, namely: is a stable matrix; does not show storage changes from the period

between sampling and analysis; and the potential for external contamination is lower when compared with scalp hair³¹. A major problem involves the limited sample mass provided by conventional collection methods, particularly children’s samples.

Comparison of scalp hair and fingernail

In total 56 samples of both human scalp hair and fingernails were provided by the healthy individuals in order to test whether there were any significant differences between the levels of arsenic in both media from an individual. An F-test and a two-tailed t-test were used to compare the two mean values for arsenic, as shown in **Table 3**. The results show that there are significant differences for arsenic levels between scalp hair and fingernails ($t_{(55)} = 0.745$, $t_{crit} = 2.005$, $P < 0.05$), (where the number in brackets is the number of degrees of freedom and the critical value (t_{crit}) is determined at $P = 0.05$).

Mean \pm SD ($\mu\text{g}/\text{kg}$)		Significance tests at $P = 0.05$			
Scalp hair	Fingernails	F-test		t-test	
		Calculated value	Critical value	Calculated value	Critical value
6122.60 \pm 1948.16	5907.86 \pm 2884.16	0.046	1.905	0.745	2.005

SD is standard deviation, n_1 , n_2 are the number of samples for scalp hair and fingernails, respectively.

The Pearson product correlation coefficient (r) was determined for arsenic and the value of r was subjected to a significance test to evaluate if there was any significant correlation. Significant positive correlations were found between scalp hair and fingernails for As ($r = 0.804$, $P = 0.05$), as shown in Figure 1.

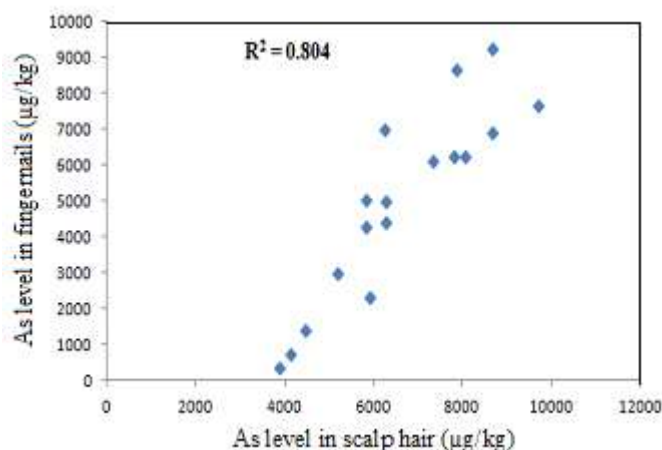


Figure 1: Correlation between arsenic levels in washed scalp hair and fingernails

Conclusion

In the light of these results, the use of washed scalp hair and fingernails as a potential biomarker for assessing human health status has been evaluated using several studies.

The present study is the first full study, to my knowledge to highlight the use of scalp hair and fingernails tissue as a biomarker for the level of arsenic in the human body. In addition, this study provides a preliminary assessment of the determination of arsenic levels in washed scalp hair and fingernails for Iraqi individuals in the province of Karbala, Iraq.

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