RESEARCH ARTICLE

Assessment of Arsenic Levels in Body Samples and Chronic Exposure in People Using Water with a High Concentration of Arsenic: a Field Study in Kutahya

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Abstract

Objective: This study aimed to evaluate the prevalence of skin lesions, which is a health effect of chronic arsenic (As) exposure, and determine the hair/blood arsenic concentrations of people living in Kutahya villages who are using and drinking tap water with a high concentration of arsenic. Materials and Methods: A total of 303 people were included in the present cross-sectional study. A prepared questionnaire form was used to collect the participants’ information and environmental history. Skin examination was performed on all participants. Hair, blood and water samples were analyzed using atomic absorption spectroscopy. The cumulative arsenic index (CAI) was calculated for all participants. Results: Villages were divided into two groups according to the arsenic level (<20 µg/L, Group I; >20 µg/L, Group II) in their water. The prevalence of skin lesions, hair and blood arsenic level, and CAI were found to be higher in the Group II participants. There was a positive association between body arsenic levels and CAI in the participants of each group. Conclusions: The number of skin lesions and arsenic concentrations in body samples were found to increase with the water arsenic level and exposure time. We hope that sharing this study’s results with local administrators will help accelerate the rehabilitation of water sources in Kutahya.

Keywords: Arsenic in water - arsenic in body samples - environmental exposure - field study - Turkey

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Introduction

Today, because environmental pollution and exposure to heavy metals are major public health problems in developing and underdeveloped countries, several relevant epidemiological studies have been conducted (Samanta et al., 2004; Anwar et al., 2005; Fasinu et al., 2013). Some of the heavy metals found intensely in the environment can easily be detected in the human tissues and have clear toxic effects (Chhabra et al., 2012). The update amount, entry route into the body and time of exposure to some heavy metals such as As determine the effects of heavy metals on human health (Jarup, 2004; Anwar et al., 2005). High levels of As exposure from drinking tap water causes a variety of skin lesions, neurological effects and various diseases, including cancer and hypertension. Toxic doses of exposure are evaluated using samples that are obtained from people, and every country determines its own toxic values (Rodrigues et al., 2008; Smith et al., 2009; Sela et al., 2013). Great importance is placed on these values in terms of planning and management of regional health services, establishing public health policy, and developing primary prevention programs (Rodrigues et al., 2008).

Because of its current geological structure, our country is among those with a high potential presence of inorganic arsenic in natural underground waters. Therefore, it is possible for inorganic arsenic to diffuse from geothermal resources and arsenic-rich rocks into underground water. In our country, the Western and Central Anatolia regions are at the highest risk of exposure. The province of Kutahya is located close to the Aegean and central Anatolia regions with a similar climate and geological nature and has been reported to have rich concentrations of minerals and heavy metals in its soil and water (Dogan et al., 2007; Gunduz et al., 2010). Several studies in certain regions of the province have found high levels of arsenic in the underground water and have investigated the health effects of arsenic exposure using various questionnaire forms (Dogan et al., 2005; Gunduz et al., 2014). However, there are a limited number of small studies in our country, and no population-based field studies exist that have evaluated the arsenic concentration in human body tissues.

This study aimed to evaluate to prevalence of skin lesions, a health effect due to chronic arsenic exposure...
and to determine the hair/blood arsenic concentration in people living in Kutahya villages who are using and drinking tap water with a high concentration of arsenic.

**Materials and Methods**

**Study population**

Forested land comprises 47.7% of the total area of the Kutahya province, and agricultural activities use 34% of this forested land. There are 13 towns and 511 villages throughout the province. The total population is 573,421, and of these, 248,054 are living in city centers. The remaining 198,154 (34.6%) are living in villages. Tap water in the villages is provided from underground sources.

In the “Regulation for the Water for Human Consumption” in our country, the maximum permissible limit of arsenic in the drinking water has been specified to be 10 µg/L.

The regions with water containing a high level of arsenic comprised the study area. To obtain the list of the villages and the results of water analyses in Kutahya in the last year, information was requested from the Provincial Directorate of Health. A total of 5 villages were randomly selected from the list. In these villages, Iğdeköy, Kirgil, Kayaarasi, Gumusköy and Kepez, there are a total of 1102 people aged 18 years or older. The prevalence of skin symptoms due to contact with arsenic has been reported to vary between 5% and 30% (Smith et al., 2009; Naujokas et al., 2013). Therefore, we targeted at least 285 people to obtain a prevalence of skin lesions of 10%, a 3% margin of error and 95% confidence interval. The field study was conducted in May-September 2014, and a total of 303 people were included in the study. The inclusion criteria were being aged 18 years or over, living in those villages for at least 5 years and agreeing to participate in the study. Pregnant women and those not meeting the inclusion criteria were excluded from the study.

All stages of the study were conducted in accordance with the Declaration of Helsinki and were approved by the Eskisehir Osmangazi University Clinical Research Ethics Committee (Number: 2014-80558721/170).

**Field study/sample collection**

In the field study, the pre-determined villages and the leaders of the villages were visited. The people in the villages were informed about how to protect themselves from environmental exposure.

A prepared questionnaire form was used to collect the data about sociodemographic variables, employment status, drinking and tap water sources, nutritional characteristics, smoking status, chronic diseases and deceased family members. Moreover, body height, body weight, and waist and hip diameters were measured. After 10 min of rest, blood pressure was measured in the sitting position using an ACURA AC-443 blood pressure monitor followed by a skin examination. In the skin examinations performed by a dermatologist, arsenic keratosis was defined as a large number of hard yellowish keratotic lesions with a symmetric localization in the palmarplantar region.

Hair/blood samples from the participants and samples from the water sources used in the villages were obtained to determine the arsenic level. Hair samples of approximately 1 cm were obtained from the suboccipital region. Blood samples of 4 ml were taken using a sterile disposable needle (Vacutainer Systems) and were collected according to international standards into vacuum EDTA-containing tubes. On the same day, samples were transferred to the Dumlupinar University Advanced Technology Research Center with a maintained cold chain.

**Sample analysis**

The samples were analyzed using the microwave oven (CEM Corporation, MARS 6, Matthews, USA) burning system and hydride system atomic absorption spectroscopy (Analytik Jena, ZEEnit 650P, Germany). Hair samples and blood samples were stored at +4 ºC and -80 ºC until analysis, respectively. Hair samples were washed in acetone-water-acetone. Hair samples of 0.1 g and blood samples of 1 ml were obtained, and solutions containing 2 ml H2O2 (Merck, 35%) and 4 ml HNO3 (Merck, 65%) were prepared. After incubating for 30 min, solutions were collected in teflon tubes and burned in the microwave oven. After cooling for 30 min, the burned samples were transferred into pivoted cap polypropylene tubes of 15 ml. To minimize the loss of samples, teflon tubes were washed with ultra-pure water and added to the polypropylene tube to yield a total volume of 15 ml. For the determination of arsenic concentration, the wavelength of the device was set at 193.696 nm. The stock solution used for the analysis was 1000 ppm arsenic standard (containing 4% HNO3, SCP Science). Standard concentrations for hair and water samples were 1.00, 2.00, 3.00, 4.00 and 5.00 ppb (µg/L), and those for blood samples were 1.00, 5.00, 10.00, 15.00 and 20.00 ppb (µg/L). These standards were used to derive a calibration curve, which was used to determine the arsenic concentration in each sample.

Chronic exposure was calculated in each participant according to the following formula: CAI=arsenic level in the water (µg/L) x daily drinking water intake (L/day) x duration of exposure (year) x 365.25

(where a is the mean water arsenic concentration measured in the last year and b is the duration of water use)

**Statistical analysis**

Data were analyzed using SPSS version 20 statistical package (SPSS, Inc., Chicago, IL, USA). A one-sample Kolmogorov-Smirnov test was used to assess whether the data were normally distributed. The Mann-Whitney U test was used to compare data that were not normally distributed and a t test was used to compare the remaining data. A Chi-square analysis was used to compare the frequencies.

The dependent variables were the hair and blood arsenic levels, and the associated factors were determined using univariate analysis. For the multivariable linear model, the logarithm of the dependent variables were calculated according to the Neperian base to convert the data to a normal distribution; the variables with a p-value of <0.10 in the univariate regression were included in the model. Values of p<0.05 were considered to be statistically significant.
significant.

**Results**

The study was carried out on 303 total people comprising 93 men (30.7%) and 210 women (69.3%). Of the participants, 13.9% were less than 35 years of age, and 23.8% were greater than 65 years of age with a mean age of 53.71±15.18 (min:20-max:81).

According to analyses of the water samples from the village fountain, the villages were divided into two groups as follows: Group I with an arsenic level of 10-20 µg/L (Kayaarasi-Kepez-Gumuskoy: mean water arsenic level: 10.15±0.05 µg/L) and Group II with an arsenic level of >20 µg/L (Igdekoy-Kirgil mean water arsenic level: 44.94±26.17 µg/L). There were no significant differences between these two groups in terms of age, gender, educational status, duration of residence or smoking status (p>0.05).

The villagers in Group II were found to use the village fountain for drinking water more frequently than those in Group I (p=0.028) (Table 1). Moreover, cancer-related deaths in the family (in the 1st degree relatives) and the presence of a chronic disease was higher in the participants living in Group II villages (p≤0.05). In total, 15.5% (n=47) had an arsenic exposure-related skin lesion; those living in Group II villages had a higher lesion prevalence than those living in Group I villages (Figure 1). The positive correlation between skin lesions and CAI was significant (spearman correlation r=0.198; p=0.001).

There were no significant differences between the two group of villages in terms of BMI or blood pressure (p>0.05). Hair and blood arsenic levels and CAI were found to be significantly higher in the participants living in Group II villages (p≤0.05) (Table 2).

The multivariate linear model of the variables associated with hair and blood arsenic levels found a positive association between body arsenic level and CAI in the participants of each group (Figure 2) (Table 3). Moreover, in the participants living in Group II villages, hair arsenic concentration was positively associated with age (p≤0.05) (Table 3).

![Figure 1. The Prevalence of Diseases in the Participants](image)

### Table 1. Sociodemographic and Several Clinical Characteristics of Participants Living in Group I or II Villages

<table>
<thead>
<tr>
<th></th>
<th>Group I villages (N=142)</th>
<th>Group II villages (N=161)</th>
<th>Total* N(%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47 (50.5)</td>
<td>46 (49.5)</td>
<td>93 (30.7)</td>
<td>0.394</td>
</tr>
<tr>
<td>Female</td>
<td>95 (45.2)</td>
<td>115 (54.8)</td>
<td>210 (69.3)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤35</td>
<td>20 (48.8)</td>
<td>21 (51.2)</td>
<td>41 (13.5)</td>
<td>0.684</td>
</tr>
<tr>
<td>35-55</td>
<td>53 (43.8)</td>
<td>68 (56.2)</td>
<td>121 (39.9)</td>
<td></td>
</tr>
<tr>
<td>≥55</td>
<td>69 (48.9)</td>
<td>72 (51.1)</td>
<td>141 (46.5)</td>
<td></td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>39 (44.8)</td>
<td>48 (55.2)</td>
<td>87 (28.7)</td>
<td>0.332</td>
</tr>
<tr>
<td>Primary</td>
<td>91 (46.2)</td>
<td>106 (53.8)</td>
<td>197 (65.0)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>12 (63.2)</td>
<td>7 (36.8)</td>
<td>19 (6.3)</td>
<td></td>
</tr>
<tr>
<td>Time of residing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 year</td>
<td>11 (42.3)</td>
<td>15 (57.7)</td>
<td>26 (8.6)</td>
<td>0.626</td>
</tr>
<tr>
<td>≥10 year</td>
<td>131 (47.3)</td>
<td>146 (52.7)</td>
<td>277 (91.4)</td>
<td></td>
</tr>
<tr>
<td>Drinking water sources</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>96 (51.9)</td>
<td>89 (48.1)</td>
<td>185 (61.1)</td>
<td>0.028</td>
</tr>
<tr>
<td>Village fountain</td>
<td>46 (39.0)</td>
<td>72 (61.0)</td>
<td>118 (38.9)</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>107 (45.3)</td>
<td>129 (54.7)</td>
<td>236 (77.9)</td>
<td>0.354</td>
</tr>
<tr>
<td>Yes</td>
<td>35 (55.2)</td>
<td>32 (47.8)</td>
<td>67 (22.1)</td>
<td></td>
</tr>
</tbody>
</table>

*Column percent

### Table 2. Comparison of Several Measurable Values between Participants from Group I and II

<table>
<thead>
<tr>
<th></th>
<th>Group I villages (mean±SH)</th>
<th>Group II villages (mean±SH)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>28.50±5.70</td>
<td>27.94±5.20</td>
<td>0.373</td>
</tr>
<tr>
<td>Sistol blood pressure (mm/Hg)</td>
<td>128.37±19.08</td>
<td>132.01±19.46</td>
<td>0.103</td>
</tr>
<tr>
<td>Diastol blood pressure (mm/Hg)</td>
<td>77.23±12.75</td>
<td>79.64±11.88</td>
<td>0.091</td>
</tr>
<tr>
<td>Hair As (µg/g)</td>
<td>0.10±0.46</td>
<td>0.19±0.62</td>
<td>0.036</td>
</tr>
<tr>
<td>Blood As (µg/L)</td>
<td>3.04±8.79</td>
<td>4.22±9.42</td>
<td>0.010</td>
</tr>
<tr>
<td>CAI</td>
<td>257.53±249.57</td>
<td>819.59±552.56</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The dermal effects due to arsenic exposure vary in various regions of the world. Water arsenic concentration has been found to be significantly associated with skin cancers in Argentina, Chile, US, China and Taiwan (Rodriguez-Lado et al., 2013). In countries with an arsenic concentration of <50 µg/L in drinking and tap water, the prevalence of arsenic-related skin lesions (hyperkeratosis/hyperpigmentation/melanosis) has been reported to be 21.6% in a cross-sectional study from Bangladesh (Ahsan et al., 2000) and 44% in Inner Mongolia, which found no significant correlation with the arsenic concentration in the water (Guo et al., 2006). In an Iranian study by Mosafari et al., the prevalence of skin lesions was high despite decreasing concentrations of arsenic in the water sources (Mosafari et al., 2008). However, many previous studies on this topic lack data about the effects of previous exposures, leading to inadequate data about exact exposure levels (Ahsan et al., 2000; Guo et al., 2006; Mosafari et al., 2008; Smith et al., 2009; Udensi et al., 2011). In addition, some studies have also reported an increased prevalence of skin lesions even with exposure to water containing 5-10 µg/L arsenic (Yoshida et al., 2004).

Of the participants, 15.5% had arsenic-induced skin lesions, and this prevalence was higher in the participants from Group II villages. Because a latent period is required for skin lesions to develop, the effects and duration of arsenic exposure may be higher in these villages. Moreover, increased CAI value with increasing lesion prevalence also supports this presumption. In addition, the skin lesions included in the prevalence analysis in the present study were limited to arsenic keratosis and pigmentation disorders. Skin lesions were evaluated during field observations, and those with suspicious lesions were invited to the hospital outpatient clinic. Because further examinations of these patients are ongoing, these data were not included in the study.

Because the sample, methodology and units used for the calculation of arsenic concentration differed between...
the countries, numerous studies have reported that the result may be variable from country to country (Yoshida et al., 2004; Guo et al., 2006; Hall et al., 2006; Moseferi et al., 2008; Smith et al., 2009; Udeni et al., 2011). Hair arsenic concentration has been suggested to be a good indicator of chronic exposure, reflect total body arsenic concentration, and be beneficial in evaluating ongoing exposure (Cohen et al., 2006; Hall et al., 2006; Marchiset-Ferlay et al., 2012). A study from the Ballia region of India reported that blood arsenic levels increased proportionally with the exposure duration and water arsenic concentration (Katiyar et al., 2014). Accordingly, Mazumder et al. have suggested that although low, prolonged water and food arsenic concentration is correlated with urinary arsenic concentration (Mazumder et al., 2014). In a field study in Pakistan, hair and blood arsenic levels were found to be 0.034-0.319 µg/g and <0.5-4.2 µg/L, respectively (Kazi et al., 2009). Several previous studies have suggested that after drinking water with an arsenic concentration of 5-410 µg/L, blood arsenic levels increase to 2-41 µg/L, and normal hair and blood arsenic levels are <1 µg/g and <2 µg/L in those with no arsenic exposure (Marchiset-Ferlay et al., 2012). In addition, body arsenic concentration is affected by several biological factors including gender and age as well as from behavioral factors such as excess use of sea products and smoking/alcohol use. Because of prolonged exposure as well as decreased metabolism and increased susceptibility, arsenic concentrations may be higher in advanced age groups than in younger groups (Calderon et al., 2013).

In parallel to the above-mentioned results, we also found near-normal results for mean hair and blood arsenic levels. However, the positive relationship found with CAI also supports the presumption that arsenic concentration in the body increases with the increasing exposure time and dose. However, there was no significant exposure in the participants in terms of nutritional or agricultural activities. Because the major source of exposure in our region is underground water, we focused on the water sources for the environmental exposure. Many of the villagers were found to be aware of the contaminated water and have reported that their local administration had searched for new water sources in the past 2-3 years. All the villagers reported that they are using the available water sources for cleaning, cooking and other purposes. However, only 39% were using these sources for drinking. In Group II villages, the percentage of people using these available water sources to drink and the level of blood arsenic level were higher, suggesting that exposure is still ongoing.

In addition, epidemiological studies have found that the duration of low-moderate concentration of arsenic exposure correlates with blood pressure and chronic diseases (Li et al., 2013; Zhang et al., 2013).

Interestingly, the prevalence of chronic disease and rate of cancer-related death in the family were higher in the Group II villages. To accurately evaluate the exposure, at least a 10 year follow-up period is necessary from the first exposure time. Moreover, medical records are lacking for retrospective follow-up studies. In light of all this information, researchers plan to carry out a future cohort study including a follow-up and further evaluation of the participants.

There are several limitations in the study. Firstly; the main limitation of our study is that it was a cross-sectional design, data are provided about only the current situation. Follow-up studies are needed to explain the causality. Secondly; as is in all field studies, the number of female participants was higher than that of males. Thirdly; inexact recalls of the participants about their drinking water history may lead to inaccuracies in calculating the CAI.

To our knowledge, this is the first study determining the arsenic concentration in the body samples of people living in the villages with arsenic-rich underground water in our region.

In conclusion, chronic exposure to arsenic in water has resulted in skin lesions in one of every 6 people living in these villages. These lesions and arsenic concentrations in the body samples were found to increase with the water arsenic level and exposure time.

We hope that sharing the results of the present study with local administrators will contribute to accelerate the rehabilitation of water sources. It is very important to provide health education programs related to environmental exposures and protection from toxic elements.

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