Evaluation of low to Moderate Arsenic Exposure, Metabolism and Skin Lesions in a Turkish Rural Population

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Abstract

Background: There is no human data regarding exposure, metabolism and potential health effects of arsenic (As) contamination in drinking water in the Central Anatolian region, Turkey.

Methods: Residents in the 10 villages with drinking water total As (T-As) level >50 µg/L and 10-50 µg/L were selected as an exposed group (n=420) and, <10 µg/L as a control group (n=185). Time-weighted average-As (TWA-As) intakes were calculated from T-As analysis of drinking water samples (DWS). Other metals in DWS, concentrations of T-As in urine and hair samples, urinary As species (S-As) (As (III), As (V), MMA-V and DMA-V), and some micronutrients in serum samples of residents were analyzed. Primary and secondary methylation indexes (PMI, SMI; respectively) were assessed from S-As concentrations. . Skin lesion presence was examined.

Results: TWA-As intake was 75.15 ppb in exposed group. Urinary and hair T-As and urinary S-As concentrations were significantly higher in exposed group *(p<0.05).* PMI and SMI values are revealed that methylation capacities of the residents were efficient and there was no saturation in As metabolism. No significant increase was observed in the frequency of skin lesions (hyperpigmentation, hypopigmentation, keratosis) of exposed group *(p>0.05).* Only frequency of keratosis either at hand or foot was higher in individuals with hair As concentration >1 µg/g *(p<0.05).*

Conclusion: Current study findings of chronic As exposure through drinking water of individuals living in the study area, could contribute to overall health risk assessment of regulatory agencies' meta-analysis efforts on low-to moderate As exposure (<100 µg/L).

1. Introduction

Major factors leading water pollution are known to be microbial factors, chemicals and radionuclides. Arsenic is one of the major chemical contaminants. Arsenic is a naturally occurring element which has widespread distribution worldwide. It exists in the form of inorganic arsenic species (i As), namely arsenite (As III) and arsenate (As V), in drinking water. The most significant environmental exposure results from contamination of drinking water in geographic regions with abundant inorganic arsenic species. The inorganic arsenic species are classified within group 1 (human carcinogen) in the International Agency For Research on Cancer classification, which is most hazardous contaminant in drinking water regarding long-term health risks (IARC 2004; IARC 2012; Vahter 2002).

It is thought that As contamination in drinking water is global threat affecting 137 million people in more than 70 countries since it is estimated that 57 million people exposes to As levels > 50 µg/L and 137 million to As levels > 5–10 µg/L (Ravenscroft August 2007). In epidemiological studies from Asian and Latin American countries such as Taiwan, Bangladesh, India and Argentina, it was established that there is an association between long-term exposure to water contaminated by high doses of inorganic arsenic species (150–1000 µg/L) and visceral organ cancers (lung, bladder, kidney, liver, colon and prostate cancers), skin lesions (hypo- or hyper-pigmentation and hyperkeratosis) and development of skin cancer other than melanoma. In addition, a dose-response relationship was shown in some of these studies (Ferreccio et al.

2013; IARC 2004; NRC 2001). It can be also suggested that long-term exposure to high doses of inorganic arsenic species has negative influences on diseases other than cancer including vascular disorders, neuropathy, chronic cough, diabetes mellitus, development and immune system (Naujokas et al. 2013; NRC 2001). Abnormal skin characteristics are known to be early finding seen in arsenic toxicity. Unlike visceral organ cancers related to arsenic, premalignant skin lesion generally presents within a period ranging from 6 months to 10 years after exposure, comprising most important findings in arsenic toxicity. However, arsenic-induced skin lesions occur in only 15–20% of population (Centeno et al. 2002; Tseng et al. 1968).

In humans, oral route is the most important way of As intake, while exposure via dermal route is minimal. After being ingested, inorganic As is metabolized to methylarsonic acid (MMA) and dimethylarsinic acid (DMA) via methylation. The methylation occurs via alternating reduction of As (V) to As (III) and addition of a methyl group. Because As (III) is more toxic than As (V), this initial step in the biotransformation of arsenate may be regarded as a bioactivation. However, majority of As (III) formed is distributed to the tissues where it is methylated to MMA and DMA (Vahter 2002). As MMA and DMA are readily excreted in urine, evaluation for efficiency of arsenic methylation is mainly based on the relative amounts of the different metabolites in the urine (Vahter 1999). Irrespective of the type and extent of exposure, the average relative distribution of arsenic metabolites in the urine of various population groups seems to be fairly constant, i.e. 10–30% inorganic As, 10–20% MMA, and 60–80% DMA (Vahter 2002). Thus, speciation of As in biological samples from people chronically exposed to As through drinking contaminated water would help elucidating to what extent the methylated metabolites contribute to the observed toxicity (Vega et al. 2001).

There is an individual variation regarding arsenic metabolites in urine, which is interpreted as arsenic methylation activity. Urinary MMA/iAs and DMA/MMA ratios can be used as a marker for activity of primary and secondary methylation. In a few study in humans, it was proposed that there is an association between primary and secondary methylation rates and higher cancer risk (e.g. skin, bladder) and formation of skin lesions (Chen et al. 2003; Steinmaus et al. 2006; Valenzuela et al. 2005; Wei et al. 2017; Yu et al. 2000; Zhang et al. 2014). It has been established that genetic polymorphism is major factor affecting the metabolism of inorganic arsenic species while it is also affected from level of exposure, age and gender by 30% (Lindberg et al. 2007; Nermell et al. 2008).

In several studies, it has been demonstrated that arsenic, a major carcinogenic contaminant, is a natural source for pollution of drinking water and tap water as well as geothermal water in Western and Central Anatolian regions in Turkey (Atabey and Unal 2008; Bundschuh et al. 2013; Dogan and Dogan 2007; Gunduz et al. 2010; Simsek 2013; Yılmaz and Yılmaz 2006). In most recent geological studies from Central Anatolian region, it was found that levels of arsenic varied from 11 to 500 µg/L in drinking water at residential areas of Nevşehir Province (Atabey 2009; Atabey 2013; Atabey and Unal 2008).

In Turkey, the preliminary data regarding adverse health impact in humans due to contamination of ground water with arsenic came from Emet district of Kütahya Province. In this region, the findings for effects contamination with iAs species on humans were limited and based on ecological data in general (Col et al. 1999; Dağıstanlı 1995; Dogan et al. 2005; Yildiz et al. 2008). In a study on mortality statistics from Simav

district of Kütahya Province, it was found that lung, prostate, colon and gastric cancer rates were higher among individuals living in villages with high arsenic levels (> 10 µg/L) when compared to those living in villages with low arsenic levels (< 10 µg/L) in drinking water (Gunduz et al. 2014).

However, there is no data regarding short- and long-term arsenic exposure, arsenic metabolism and potential effects on health resulting from arsenic contamination in drinking water in Nevşehir Province. The main objectives of the present study are: (1) to conduct a study in the population living in several villages of Nevşehir Province, Turkey having different arsenic levels in drinking water (> 50µg/L, 10−50 µg/L and < 10µg/L), (2) to determine recent and long-term iAs exposure by measuring total As levels in urine and hair, (3) to determine As metabolism by measuring As species (As (III), As (V), MMA-V and DMA-V) in urine samples and to evaluate methylation capacity as primary methylation index-PMI; and secondary methylation index; SMI, (4) to determine barium (Ba), cadmium (Cd) chromium (Cr), nickel (Ni), antimony (Sb), selenium (Se) and zinc (Zn) and vanadium (V) concentrations other than total As concentrations in water samples used (drinking and cooking) by villagers, (5) to determine some micronutrient levels (serum folate and vitamin B12) of subjects exposed to iAs, (6) to determine the prevalence of skin lesions.

2. Materials And Methods

2.1 Study setting

The study was conducted in a region at Nevşehir Province of Turkey. The Nevşehir Province has 8 districts and 153 villages and a total population of 298,389 based on 2018 data retrieved in 19.09.2019 (https://biruni.tuik.gov.tr). Nevşehir Province was build over Western hillside of a wide plateau formed accumulation of ash and lava from Mount Erciyes, Mount Hasan and Mount Melendiz localized within southeastern volcanic region of middle Anatolia. The streambed of river Kızılırmak divides land of Nevşehir Province into 2 regions. There are mostly volcanic rock areas at south to Kızılırmak and those surrounding Nevşehir. In this region, volcanic history and natural hydrogeological conditions can lead elevated arsenic concentrations (up to 500 µg As/L) in groundwater (Atabey 2013).

To select study area, pilot analysis was conducted by measurement of total arsenic levels (µg/L) in drinking water samples from tap water and village fountain water in 19 villages and 2 municipality areas within Nevşehir Province. Based on pilot analysis, overall 10 villages were selected: Dadaği, Küçükayhan, Gülpınar and Emmiler villages with total As level > 50 µg/L and Kızılkaya and Yassıca villages with total As level > 10−50 µg/L as exposed group and Eskiyaylacık, Civelek, Alkan and Altıpınar villages with total As level < 10 µg/L as control group (Fig. 1). Figure 1 shows geographic localizations of study area in geological map of Nevşehir Province. There is no industrial activity in residential areas included to the study or nearby these areas.

2.2 Study population and biological sampling

Informed consent was obtained from all individual participants included in the study.

This study was performed in line with the principles of the Declaration of Helsinki. The study was conducted between March, 2011 and April, 2012. The individuals who were residing in the study area for at least one year were included to the study. The individuals previously resided in another region of Nevşehir Province known to have high mesothelioma incidence were excluded. The individuals with cancer or hepatitis were also excluded.

All participants gave written informed consent.

All participants were asked to complete a survey including items on age, gender, education level, smoking, alcohol consumption (yes/no), nutritional habits, general health status, medication and supplement intake, water sources used for cooking and drinking, residential duration in the area, occupation and degree of relationship. Diabetes mellitus and hypertension were defined as self-reported use of insulin/oral hypoglycemic agents or antihypertensive medication, respectively. In the selected study areas, tap water, village fountain water and well water are being used for drinking and cooking. The individuals who are smokers for at least one year were considered as current smokers while those quit smoking for more than one year were considered as ex-smokers (Burgaz et al. 2011). In the study region, no pesticide containing arsenic was used within prior 10 years. The subjects were asked not to consume seafood within a week prior to urine sample collection. Thus, the interference between organic As compounds with seafood origin and total As concentrations was minimized. Urine, blood and hair samples were simultaneously drawn from individuals exposed to arsenic (n = 420) and controls (n = 185) early at morning. Skin examination was performed following biological sampling.

The study was approved by Ethics Committee of Gazi University, Medicine School (approval#340-15.06.2009).

2.3 Measurement of As exposure

2.3.1 Determination of total arsenic concentration in samples of drinking water

In each village included to the study, tap water, village fountain water as well as private well water samples (100 mL) were collected in polypropylene containers previously washed with a mixture of nitric acid-water (1:9,v/v) at time of biological sampling.

Total arsenic (AsIII + AsV) concentrations were measured using hydride generation–atomic absorption spectroscopy (HG-AAS) (Unicam 939) (Dedina and Tsalev 1995). All water samples were prepared in three parallel solutions and measured at least three times using HG-AAS. Results were expressed as µg/L ±standard deviation. Limit of detection (LOD) and limit of quantification (LOQ) for arsenic determination by HG-AAS was 0.3 µg/L and 1 µg/L, respectively. The accuracy for total arsenic in water samples was tested using Standard Reference Material TMDA61 (National Research Institute, Canada). Observed values (31.8 ± 2.2 µg/L As) were in good agreement with the certified values (33.6 ± 5.2 µg/L As) (p > 0.05).

2.3.2. Derivation of time-weighted average (TWA) As intake

Given the different sources of As exposure including tap water, village fountain water and private well water samples in the study areas, we also calculated a time-weighted (TWA) As intake as a function of arsenic

concentrations in water sources and of drinking durations of water ($TWA\text{-}As\ in\ \mu g/L = \sum C_i x T_i / \sum T_i$ where C_i and T_i denote water As concentration and drinking duration of i th water sources) (Ahsan et al. 2007). The duration of water use for each individual is based on data retrieved from questionnaires.

2.3.3 Urinary collection and determination of total arsenic and arsenic species (As III, As V, MMA, DMA)

In order to prevent interference from organic arsenicals, individuals were asked to avoid consuming sea food for one week prior to collecting the urine samples. The early morning spot urine samples (50–100 mL) were collected in polypropylene containers previously washed with a mixture of nitric acid-water (1:9, v/v). As soon as the samples were collected, specific gravity (SG) values were measured using a refractometer and recorded. It has been suggested that urinary creatinine levels are affected by body mass index, age, gender and seasonal factors. In addition, it is also suggested that there is a significant correlation between urinary As concentrations and creatinine levels, proposing that one could not definitely show true elevation in As exposure (Nermell et al. 2008). Thus, in our study, concentrations of As compounds in urine samples were adjusted according to specific gravity values.

The specific gravity-adjusted urinary As concentration was calculated using the formula: Urine As concentration x (1.012-1) / (measured SG-1) (Cone et al. 2009; Nermell et al. 2008). The samples were stored at – 20°C until assays. Arsenic speciation analysis was performed within 6 months of the sample collection.

Total arsenic levels in urine samples were measured with hydride generation–atomic fluorescence spectrometry (HG-AFS, PS Analytical Excalibur Millennium System, PS Analytical, UK). The method for total arsenic determination was validated using standard reference material "SRM NIST 2669 arsenic species in frozen human urine" (Level I, Low-Level and Level II, High-Level). The SRM's were digested and analyzed for total arsenic by using HG-AFS. The certificate values for low and high level SRM were 22.2 ± 4.8 µg/l and 50.7 ± 6.3 µg/l, respectively, while the values found were 22.3 ± 0.4 µg/l and 50.7 ± 0.6 µg/l (n = 5). The results obtained were statistically not different from the certified values at 95% confidence level ($p > 0.05$).

The quantification of arsenic speciation [inorganic Arsenic (iAs) including iAs (III) and iAs (V), MMA-V and DMA-V] was carried out by high performance liquid chromatography- hydride generation–atomic fluorescence spectrometry (HPLC-HG-AFS) (Gomez-Rubio et al. 2012). In our study, the amounts of MMA (III) and DMA (III) metabolites, excreted by urine and considered as toxic, were not studied since detection of trivalent arsenic species depends on storage conditions of samples and urinary concentration. These species are highly susceptible; thus assays should have to be performed immediately after urine sampling. We were unable to perform such a rapid assay in our study design. Due to such challenges, trivalent arsenic species are not used as a eligible marker for As methylation (Del Razo et al. 2011).

The chromatographic separation of arsenic species was performed using a Hewlett Packard HPLC system, 1050 model (Agilent Technologies, USA) equipped with an isocratic pump, a 100 µL injection loop, a Hamilton PRP-X100 anion-exchange column (250 mm x 4.1 mm, 10 µm) and a Hewlett Packard Hypersil guard column (20 mm x 2.1 mm, 5 µm). The signal output of the atomic fluorescence spectrometer (AFS) system was recorded with a computer having a chromatographic software (Agilent ChemStation for LC

systems software, Rev. B. 03.01) interfaced to the AFS instrument via an analogue-to-digital converter "Agilent Interface 35900 E". Separations were performed in an isocratic mode. The mobile phase, consisting of 8.0 mM KH_2PO_4/K_2HPO_4 at pH 5.80, was used at a flow rate of 1.0 mL min^{-1} and an injection volume of 100 µL. An AFS instrument (PS Analytical Ltd. Millennium Excalibur, UK) was used to detect the separated species. A boosted hollow cathode lamp (Photron Super Lamp) was used as the excitation source at 193.7 nm. Lamp current, argon and nitrogen as the carrier and drying gases, respectively, were used at the flow rates recommended by the manufacturer. The separated arsenic species were derivatized into their volatile hydrides by a post-column hydride generation with 1.5 M HCl and 1.4 % w.w^{-1} NaBH$_4$ (prepared in 0.4 % w.w^{-1} NaOH) at a flow rate of 4.0 mL min^{-1}.

Results were expressed as µg/L ±standard deviation. LOD values with HPLC-AFS were found to be 0.3µg/L for As (III) and DMA, 0.7µg/L for MMA and As (V). LOQ were found as 1.0 µg/L for As(III) and DMA, 2.3 µg/L for MMA and As(V). Accuracy of HPLC – HG – AFS was assessed by analyzing the standard reference material "SRM NIST 2669 arsenic species in frozen human urine" (level II, High-Level). The certified values for As (III), As(V), DMA and MMA were 5.03 ± 0.3, 6.16 ± 0.49, 25.3 ± 0.7 and 7.18 ± 0.56 µg l^{-1} while the results obtained were 5.12 ± 0.45, 6.64 ± 0.49, 26.5 ± 1.0 and 7.21 ± 1.04 µg l^{-1}, respectively. The results obtained were statistically not different from the certified values at 95% confidence level (p > 0.05).

Urinary As proportions and methylation indices were calculated using the following formulas; Σ As species = iAs + MMA + DMA; %i As =[iAs/Σ As species] x100; %MMA= [MMA/Σ As species] x100; %DMA= [DMA/Σ As species] x100, primary methylation index(PMI) = MMA-V: iAs (AsIII + AsV); and secondary methylation index (SMI) = DMA-V:MMA-V (Del Razo et al. 2011; Hsueh et al. 1997; Steinmaus et al. 2010).

2.3.4. Determination of total As in hair samples

The hairs near to scalp (300–500 mg) were collected from the area behind the ears (post auricular region). The samples were kept in polyethylene cups at 4 °C until analysis. All samples were washed for 15 min with deionized water while magnetically stirred, then washed with a mixture of acetone-deionized water (1:1) (Basu et al. 2002) in order to minimize external As contamination.

For the total arsenic determination, each urine sample was digested in parallel, and two parallel solutions were prepared from each digest and measured as described by Rahman et al. (Rahman et al. 2000).

Results were expressed as µg/g ± standard deviation. LOD and LOQ values for the total arsenic determination in hair sample were 0.01 and 0.03 µg/g, respectively. The accuracy for total arsenic in hair samples was tested using Standard Reference Material NCS DC73347a Human hair, Beijing, China). Observed values (0.28 ± 0.01 µg/g As) were in good agreement with the certified values (0.28 ± 0.05 µg/g As) (p > 0.05).

2.4. Determination of other metals in drinking water samples

V, Cr, Ni, Zn, Se, Cd, Sb and Ba concentrations in drinking water samples collected from the selected study area were also analyzed with ICP-MS (Inductive Coupled Plasma-Mass Spectrometry-Thermo X Series) equipped with flow injection assembly. 500 µL injection volume was used in the analyses. Interference free

isotopes $^{51}V^+$, $^{52}Cr^+$, $^{60}Ni^+$, $^{66}Zn^+$, $^{82}Se^+$, $^{111}Cd^+$ and $^{121}Sb^+$ and $^{137}Ba^+$ were selected for the determination of these elements with external calibration method. LOD, LOQ (as µg/L) and % RSD values, respectively were 0.28;0.92; 2.16 for ^{51}V, 0.31;1.04;4.66 for ^{52}Cr,1.66;5.52;0.47 for ^{60}Ni,0.42;1.39;4.96 for ^{66}Zn, 0.82;2.73;8.66 for ^{82}Se, 0.05;0.18;0.07 for ^{111}Cd, 0.11;0.36;1.15 for ^{121}Sb and 0.02;0.07;1.40 for ^{137}Ba.

2.5. Analysis of serum folate and vitamin B12 levels

Serum total folate and vitamin B12 concentrations were analyzed by using enzyme linked chemiluminescent immunosorbent assay (ECLIA) kit while following the manufacturer's protocol. The manual offered the normal reference value of serum folate and vitamin B12, respectively: 3.1–17.5 ng/ml and 197–866 pg/ml. The inter-assay coefficients of variation (CVs) for folate and Vitamin B12 were 5.82 % and 2.99 %, respectively.

3. Skin Examination

Skin examination was performed by a dermatologist blinded to arsenic values in tap water at the area where examinations were performed. Skin examination included pigmentation type (hypopigmentation-leukomelanosis, hyperpigmentation-melanosis) and presence of keratosis (yes/no) in palm/dorsum of hands, sol/dorsum of feet, trunk, back and upper and lower extremities. In skin examination, clinical conditions mimicking keratosis or melanosis were excluded (Fatmi et al. 2009). In addition, premalignant/malignant lesions (actinic keratosis, Bowen disease etc.) were also assessed. The results of dermatological examination were summarized as hyperpigmentation (yes/no), hypopigmentation (yes/no), keratosis at hand or foot (yes/no), keratosis at both hand and foot (yes/no) and presence of hyperpigmentation plus keratosis (yes/no).

4. Statistical Analysis

The normal distribution of continuous variables was assessed using Kolmogorov Smirnov test. Levene test was used for the evaluation of homogeneity of variances. The categorical variables are presented as number of cases and percentages while descriptive statistics for continuous variables are presented as mean ± SD or median (min-max), where applicable. The Student's t test was used to compare differences in mean values between groups in case of normal distribution, while Mann Whitney U test was used to compare data with skewed distribution. When the number of independent groups was more than two, the continuous variables were analyzed by Kruskal Wallis test. When the p-values from the Kruskal Wallis test statistics were statistically significant, Conover's multiple comparison test was used to determine the group accounting from difference. Categorical data were analyzed by Pearson's Chi-square, Fisher's exact or Likelihood Ratio test, where appropriate. Degrees of association between continuous variables were evaluated by Spearman's Rank Correlation analyses.

Multiple linear regression (MLR) analyses were performed to determine the best predictors which effect on PMI and SMI measurements. Any variable found to have p value < 0.10 in univariate analysis was accepted as a candidate for the multivariable model along with all variables of known clinical importance. Coefficients of regression (B) and 95% confidence intervals (CIs) for each independent variable were also

calculated. Logarithmic transformation was used for both PMI and SMI in multiple linear regression analyses due to not normal distribution.

Self-reported data on body weight and height and drinking water consumption (as a glass of water per day) were found to be unreliable, thus, they were not included in the statistical analysis.

We excluded participants with urinary As (III), As (V), MMA and DMA concentrations below detection limit (LOD) for methylation efficiency (Steinmaus et al. 2010). Measured values of urinary As (III), As (V), MMA and DMA concentrations < LOD were replaced with half the LOD for other all statistical analysis (Del Razo et al. 2011; Gomez-Rubio et al. 2012; Gribble et al. 2013).

Data analysis was performed by using IBM SPSS Statistics version 17.0 software (IBM Corporation, Armonk, NY, USA). A p value less than 0.05 was considered statistically significant.

5. Results

Table 1 presents major characteristics of individuals living in selected 10 villages as study area based on criteria given in the Sect. 2.1. In our study population, overall 605 individuals were analyzed including 185 controls. In the study, exposed individuals and controls were compatible regarding mean age, age groups of ≤ 50 years and > 50 years, alcohol consumption status, smoking status and smoking frequency. When subgroups were assessed according to age, the number of individuals aged 51–60 years and aged 71–84 years was higher in the exposed group when compared to control data (*p = 0.028* and *p = 0.011*, respectively).

Table 1
General characteristics of study population

Parameters	Control group (n = 185)	Exposed group (n = 420)	p value
Gender [n (%)]			0.605
Male	83 (44.9)	198 (47.1)	
Female	102 (55.1)	222 (52.9)	
Age [year; mean ± sd]	47.0 ± 13.8	49.1 ± 15.5	0.106
Age, years [n (%)]			**0.033**
19–30 years	26 (14.1)	55 (13.1)	
31–40 years	30 (16.2)	84 (20.0)	
41–50 years	49 (26,5)	95 (22.6)	
51–60 years	49 (26.5)	78 (18.6)[a]	
61–70 years	22 (11.9)	60 (14.3)	
71–84 years	9 (4.9)[b]	48 (11.4)[b]	
Age groups [n (%)]			0.812
≤ 50 years	105 (56.8)	234 (55.7)	
> 50 years	80 (43.2)	186 (44.3)	
Education [n (%)]			**< 0.001**
0	19 (10.3)[c]	95 (22.6)[c]	
1–5 years	138 (74.6)	285 (67.9)	
> 5 years	28 (15.1)[d]	40 (9.5)[d]	
Residence time [(year; mean(range)]	35(1–79)	40 (2–84)	**0.010**

n: number of subjects, sd: standard deviation, [a]: *p = 0.028*, [b]:*p = 0.011*, [c]:*p < 0.001*, [d]:*p = 0.044*, [e]:*median(min.- max)*

Parameters	Control group (n = 185)	Exposed group (n = 420)	p value
Residence time [n (%)]			**0.008**
≤ 30 years	84 (45.4)	143 (34.0)	
> 30 years	101 (54.6)	277 (66.0)	
Smoking status [n (%)]			0.994
Nonsmoker	113 (61.1)	258 (61.4)	
Former smoker	21 (11.4)	48 (11.4)	
Current smoker	51 (27.6)	114 (27.1)	
The number of cigarettes per day [n (%)]			0.294
< 20	21 (41.2)	57 (50.0)	
≥ 20	30 (58.8)	57 (50.0)	
Alcohol consumption [n (%)]	27 (14.6)	42 (10.0)	0.101
Diabetes Mellitus Type II [n (%)]	7 (3.8)	38 (9.0)	**0.023**
Hypertension [n (%)]	30 (16.2)	81 (19.3)	0.369

n: number of subjects, sd: standard deviation, [a]: $p = 0.028$, [b]:$p = 0.011$, [c]:$p < 0.001$, [d]:$p = 0.044$, [e]:median(min.- max)

When education level was assessed, it was seen that number of individuals with no formal education was significantly higher in the exposed group while number of those with secondary school graduation was significantly higher in the control group ($p < 0.001$ and $p = 0.044$, respectively). The residential duration of individuals at study region was significantly longer in exposed group when compared to controls ($p = 0.01$). In addition, residential duration of individuals at study region was less than 30 years in 45.4% of controls and 34% of exposed group. On the other hand, the frequency of individuals residing in the study region more than 30 years was significantly higher in exposed group than control group (66% vs. 54.6%; $p = 0.008$). Diabetes Mellitus Type II was more common in the exposed group ($p = 0.023$) while exposed group and controls were not different regarding history of hypertension. As suggested by residential duration in Table 1, it is possible to propose that mobility of individuals living in the study region is highly restricted and they can represent the study region.

5. 1 Measurement Of As Exposure

As the locations of villages were represented in Fig. 1. Median (min.-max.) time-weighted average As intake (TWA-As-µg/l-year) levels in drinking water were 0.50 (0.40-31.53) for control group and 75.15 (10.42–300.60) for exposed group. The TWA-As level was significantly higher for exposed group than that of control group ($p < 0.001$) which indicated the TWA-As level as appropriate for evaluation of cumulative As exposure for control and exposed groups.

Descriptive statistics regarding levels of total urine As and As species including As (III), As (V), MMA and DMA of the study population were presented in Table 2. As seen in Table 2, total As exposure was significantly higher (by 6-folds) in exposed group when compared to controls ($p < 0.001$). It was found that total urine As level and As (III), As (V), MMA and DMA levels were significantly higher in individuals living at areas with As concentration ranged $10-50$ µg/L and > 50 in drinking water when compared to those living in areas with As concentration < 10 µg/L in drinking water and that the statistical significance was increased with tap water As concentration ($p < 0.05$, $p < 0.001$, respectively, data not shown).

Table 2
Descriptive statistics of total As ve As species -As(III), As(V), MMA, DMA-(µg/L) in urine samples

Groups	n	Mean (SD)	Median (Min.-Max.)	p value
Total As				<0.001
Control	181	17.82 (26.59)	10.89 (0.94−198.60)	
Exposed	413[c]	110.74 (85.04)	85.91 (3.45−875.30)	
As(III)				< 0.001
Control	150	0.13 (0.12)	0.10 (0.03−1.12)	
Exposed	415	3.85 (6.21)	0.24 (0.02-37.00)	
As(V)				< 0.001
Control	150	0.40 (0.18)	0.35 (0.12−1.84)	
Exposed	415	2.11 (11.58)	0.37 (0.03-215.38)	
MMA				< 0.001
Control	150	0.52 (0.41)	0.35 (0.09−2.35)	
Exposed	415	10.00 (16.47)	3.41 (0.13-133.79)	
DMA				< 0.001
Control	150	1.19 (1.22)	1.14 (0.03−5.97)	
Exposed	415	50.36 (65.87)	25.76 (0.05-708.48)	
Total As species				< 0.001
Control	150	2.25 (1.49)	2.08 (0.27−7.93)	
Exposed	415	66.79(80.06)	39.06 (0.42-853.72)	

[a] Unsufficient urine sampling from four and five subjects in control and exposed group, respectively. [b]As-species analysis were not carried out in thirty-one control subjects. [c] Two missing total As values due to technical problems.

Table 3 presents descriptive statistics for total As levels in hair samples according to exposure, age, gender and smoking status in the study population. Total hair As levels were significantly higher (by 6-folds) in the exposed group when compared to controls ($p < 0.001$).

Table 3

Descriptive statistics of total As levels (μg/g) in hair samples by exposure, age, gender and smoking status

Hair total As	n	Mean (SD)	Median (Min.-Max.)	p value
Groups				
Control	178	0.17 (0.14)	0.14 (0.01−0.98)	**< 0.001**
Exposed	419	1.08 (0.96)	0.79 (0.12−6.59)	
Drinking water As				
<10 μg/L [a,b]	178	0.17 (0.14)	0.14 (0.01−0.98)	**< 0.001**
10−50 μg/L [a,c]	155	0.56 (0.30)	0.51 (0.12−1.91)	
> 50 μg/L [b,c]	264	1.39 (1.07)	1.06 (0.20−6.59)	
Age-years				
≤ 50	336	0.70 (0.84)	0.45 (0.02−6.48)	**< 0.001**
> 50	261	0.95 (0.97)	0.63 (0.01−6.59)	
Gender				
Male	273	0.77 (0.92)	0.52 (0.04−6.59)	0.843
Female	324	0.84 (0.90)	0.56 (0.01−4.84)	
Smoking status				
Nonsmoker	367	0.89 (0.99)	0.57 (0.01−6.59)	0.139
Former smoker	69	0.75 (0.82)	0.52 (0.07−4.30)	
Current smoker	161	0.66 (0.70)	0.47 (0.04−5.58)	

*Unsufficient hair sampling from five and one control and exposed subjects, respectively. [a,b,c] p < 0.001

When assessed according to drinking water As categories, it was found that total hair As level was significantly increased by elevating drinking water As level ($p < 0.001$). Although total hair As level was significantly higher in individuals aged > 50 years compared to those aged ≤ 50 years ($p < 0.001$), no significant difference was shown in hair As levels according to gender or smoking status (yes/no, ex-smoker) ($p > 0.05$). A significant, positive correlation was detected between total As level in hair samples and TWA-As level (r = 0.752; $p < 0.001$). When toxic cut-off level was considered as 1 μg/g (Arnold 1990) total As level in hair samples was higher than cut-off level in 8.3% and 91.7% of individuals living in the areas with drinking water As concentration of 10−50 μg/L and > 50 μg/L in drinking water, respectively ($p < 0.001$).

5.1.1. Measurement of other metals in drinking water samples

Table 4 presents concentrations (with min-max values) of metals other than arsenic measured in drinking water samples (tap water, village fountain water and well water) obtained from study areas during biological sampling. It was found that Cr, Ni, Sb, Se and Zn concentrations were at or below the limits proposed by national and international organizations. Ni and Sb values were above limits in only two private wells of two villages with As concentration > 50 µg/L in drinking water (Dadağı and Küçükayhan). Vanadium concentration in drinking water was lower in villages with As concentration < 10 µg/L while it was higher in villages with As concentration > 10 µg/L in drinking water. There is no national or international limit value for vanadium levels in drinking water.

Table 4

Summary of trace element concentrations (µg/L) (minimum-maximum) other than arsenic in drinking water samples from the study area

Study area	V	Cr	Ba	Ni	Zn	Se	Cd	Sb
< 10 µg As/L	1.38–5.67	ND-3.47	7.3–68.5	ND	N.D.-6.80	ND	ND	ND-2.70
10–50 µg As /L	31.56–67.60	ND	127.5-138.6	ND	ND-5.89	ND	ND	0.52–0.61
> 50 µg As /L	6.51–44.87	ND-30.8	94.8-163.1	ND-22.5	ND-201	ND-4.8	N.D.	ND-75.31
National Drinking Water Guideline [a] and/or WHO [b] alues	–	50 µg/L	700 µg/L	20 µg/L	< 3000 µg /L	10 µg/L	5 µg/L	5 µg/L

ND. Undetectable (< LOD); [a] National official newspaper (No.28580, March 7,2013); [b] WH0,2003

Fluoride (F) concentrations in drinking water samples obtained from villages with As concentration of 10–50 µg/L and > 50 µg/L were measured by using fluoride electrode, resulting the F concentrations between 0.19 and 0.46 mg/L, respectively. The measured concentrations were below the limit value (1.5 mg/L) for F proposed by National Drinking Water Guideline. No fluoride measurement was performed in drinking water samples obtained from areas designated as controls.

5.2 Arsenic methylation capacity and urinary methylation indices

Tables 5 and 6 present descriptive statistics for methylation capacity and indices of 339 individuals with urine AS (III), As (V), MMA and DMA concentrations > LOD.

Table 5
Descriptive statistics of urinary As proportions in the study population

Groups	n	Mean (SD)	Median (Min.-Max.)	*p value*
iAs (%)				<0.001
Total	339	10.95 (8.09)	9.13 (0.26−52.52)	
Control	48	13.72 (4.01)	14.15 (5.91−22.25)	
Exposed	291	10.50 (8.50)	8.51 (0.26−52.52)	
MMA (%)				*0.054*
Total	339	25.13 (9.97)	25.88 (1.09−50.14)	
Control	48	27.74 (4.69)	27.71 (14.54−41.90)	
Exposed	291	24.70 (10.53)	25.40 (1.09−50.14)	
DMA (%)				< 0.001
Total	339	63.91 (12.58)	62.28 (29.09−96.46)	
Control	48	58.54 (6.50)	57.76 (46.88−73.98)	
Exposed	291	64.80 (13.11)	63.44 (29.09−96.46)	

[a] It is excluded participants with urinary As (III), As (V), MMA and DMA concentrations < LOD.

Table 6

Descriptive statistics of methylation indices (PMI and SMI) by exposure, age, gender and smoking status

		PMI			SMI		
	n	Mean (SD)	Median (Min.-Max.)	p value	Mean (SD)	Median (Min.-Max.)	p value
Groups							
Control	48	2.22 (0.82)	2.08 (1.13–4.61)	0.016	2.21 (0.65)	2.12 (1.17–5.09)	0.021
Exposed	291	4.70 (6.65)	2.60 (0.13–80.44)		4.48 (7.60)	2.45 (0.85–88.78)	
Drinking water As							
< 10 µg/L	48	2.22 (0.82)	2.08 (1.13–4.61)[a]	< 0.001	2.21 (0.65)	2.12 (1.17–5.09)[a]	< 0.001
10–50 µg/L	114	5.15 (5.14)	3.13 (0.16–26.38)[b]		2.34 (1.98)	2.13 (0.85–22.14)[b]	
> 50 µg/L	177	4.40 (7.46)	2.37 (0.13–80.44)[a,b]		5.85 (9.37)	2.83 (0.88–88.78)[a,b]	
Age-year							
≤ 50	202	3.61 (3.50)	2.47 (0.16–26.38)	*0.442*	3.37 (5.57)	2.17 (0.85–70.32)	< 0.001
> 50	137	5.44 (8.73)	2.48 (0.13–80.44)		5.32 (8.76)	2.73 (1.13–88.78)	
Gender							
Male	179	3.64 (3.93)	2.40 (0.13–26.38)	*0.210*	3.68 (6.11)	2.24 (0.85–70.32)	*0.095*
Female	160	5.13 (8.00)	2.60 (0.16–80.44)		4.69 (8.03)	2.50 (0.88–88.78)	
Smoking status							
Nonsmoker	198	5.16 (7.56)	2.59 (0.16–80.44)	*0.051*	4.38 (7.36)	2.34 (0.85–88.78)[c]	0.014
Former smoker	47	2.72 (2.56)	2.04 (0.13–14.49)		5.55 (10.75)	2.73 (1.24–70.32)[c,d]	
Current smoker	94	3.44 (3.63)	2.45 (0.31–26.28)		2.98 (2.89)	2.14 (1.01–19.27)[d]	

[a]p<0.001,[b]p=0.002,[c]p=0.048,[d]p=0.03

The percentage of As was significantly lower and the percentage of DMA was significantly higher in exposed group when compared to controls ($p < 0.001$; Table 5). No significant difference was detected in percentage of MMA. As shown in Table 6, PMI and SMI values were significantly increased in exposed group ($p = 0.016$, $p = 0.021$, respectively) and in drinking water As categories ($p < 0.001$). Age, gender and smoking status had no effect on PMI value. However, age > 50 and being in former smoker group were significantly increased SMI value ($p < 0.001$ and $p < 0.05$, respectively; Table 6).

5.3. Measurement of serum folate and vitamin B12 levels

Table 7 presents descriptive statistics for categories (low, normal or high) of serum folate (ng/mL) and vitamin B12 in subjects according to As levels in drinking water.

Table 7
Descriptive statistics of serum folate (ng/ml) and vitamin B12 level (pg/ml) categories in study subjects by arsenic exposure

Parameter	Control	Exposed	*p-value*
Serum folate [Mean (SD)]	8.94 (3.19)	8.59 (3.05)	*0.226*
Serum folate [n(%)]*			*0.471*
Low	—	2 (0.5)	
Normal	182 (98.4)	404 (98.1)	
High	3 (1.6)	6 (1.5)	
Serum Vitamin B12 [Mean (SD)]	320.82 (151.70)	287.41(175.14)	< 0.001
Serum Vitamin B12 [n(%)]**			< 0.001
Low	28 (15.1)[a]	136 (33.0)[a]	
Normal	154 (83.2)[a]	270 (65.5)[a]	
High	3 (1.6)	6 (1.5)	

Serum folate	n	Mean (SD)	Median (min.-max)	p-value
Drinking water As				*0.190*
< 10 µg/L	185	8.94 (3.19)	8.60 (3.70−20.00)	
> 10−50 µg/L	155	8.36 (3.10)	8.10 (2.50−19.20)	
> 50 µg/L	257	8.73 (3.01)	8.40 (3.60−19.00)	

Serum Vitamin B12	n	Mean (SD)	Median (min.-max.)	p-value
Drinking water As				< 0.001
< 10 µg/L	185	320.82 (151.70)	294[b,c] (105.00-1248.00)	
> 10−50 µg/L	155	270.68 (141.25)	236[b] (106.00-1074.00)	
> 50 µg/L	257	297.50 (192.29)	252[c] (105.00-1673.00)	

n = number of subjects, [a] p < 0.001, [b,c]p<0.001, * reference range : 3.1−17.5 ng/ml, ** reference range: 197−866 pg/m

When mean folate levels in low, normal and high categories were assessed, no significant difference was found among individuals exposed to As and controls (*p > 0.05*). In addition, serum folate levels showed no significant difference according to drinking water As categories (*p > 0.05*; Table 7). It was found that mean serum vitamin B12 level was significantly lower in individuals exposed to As when compared to controls (*p < 0.001*). When mean B12 vitamin in low and normal categories were assessed, the frequencies of individuals with normal B12 levels were lower in control group and and those with low B12 levels were

significantly higher in exposed group ($p < 0.001$; Table 7). It was observed that serum vitamin B12 levels were decreased according to drinking water As categories ($p < 0.001$; Table 7).

5.4. Skin lesion findings

It was observed that frequencies(%) of hyperpigmentation, hypopigmentation, keratosis at either hand or foot, keratosis at both hand and foot and keratosis plus hyperpigmentation were similar between exposed group (6.7, 2.9, 11.2, 6.5 and 11.2, respectively) and controls(7.2, 2.2, 8.7, 4.9 and 8.7, respectively) ($p > 0.05$). The frequency of skin findings observed did not differ according to As categories of drinking water ($p > 0.05$). When frequency of skin finding was assessed according to TWA-As levels, the median TWA-As value with no skin finding observed was 34.05μg/L while median TWA-As value where skin findings were observed ranged from 34.05 to78.07 μg/L. No significant difference was detected between TWA-As values in which skin findings were observed or not ($p > 0.05$).

Pathological examination was performed by a dermatologist in three individuals living in villages with As concentration > 50 μg/L (Dadağı and Emmiler) and one individual living in village with As concentration of 10–50 μg/L (Kızılkaya) in drinking water, revealing no malignant lesion. The lichenoid dermatitis was found in two individuals and hyper-keratotic skin with non-specific morphological findings was detected in remaining two individuals.

When frequency of skin findings was assessed according to toxic cut-off level for hair samples, it was found that only frequency of keratosis either at hand or foot in individuals with hair As concentration > 1 μg/g (15.8%) was higher than that of As concentration ≤1 μg/g (8.7%) ($p < 0.05$).

Table 8 presents analysis of skin findings according to methylation indices (PMI and SMI). PMI values were found to be significantly lower in individuals with hyperpigmentation ($p < 0.05$) while SMI values were significantly higher in individuals with keratosis at both hand and foot ($p < 0.001$). It was shown that SMI value was significantly higher in individuals with skin findings compared to those without any skin findings ($p = 0.003$; Table 8).

Table 8
PMI and SMI values by skin lesions in the study population

Skin lesion	n	PMI Mean (SD)	PMI Median (Min.-Max.)	PMI p-value	SMI Mean (SD)	SMI Median (Min.-Max.)	SMI p-value
Hyperpigmentation				0.045			*0.775*
No	303	4.37(6.28)	2.51(0.13–80.44)		4.05(7.29)	2.33 (0.85–88.78)	
Yes	24	3.32(4.70)	1.77(0.31–18.81)		5.32(5.85)	2.14(1.01–21.54)	
Hypopigmentation				*0.895*			*0.315*
No	326	4.37(6.30)	2.49(0.13–80.44)		3.91(5.45)	2.33 (0.85–70.32)	
Yes	9	4.49(4.64)	2.37(0.44–12.69)		14.11(28.31)	4.58 (1.09–88.78)	
Keratosis (Hand/Feet)				*0.261*			*0.196*
No	281	4.42(6.62)	2.42(0.13–80.44)		3.69 (6.27)	2.29 (0.85–88.78)	
Yes	29	4.88(4.43)	2.83(0.36–14.88)		5.48(5.97)	2.39(1.17–29.80)	
Keratosis (Hand + Feet)		4.40(6.31)	2.48(0.13–80.44)	*0.586*	3.84(6.20)	2.31(0.85–88.78)	< 0.001
No	315	2.76(1.71)	2.37(0.28–7.28)		9.43(15.42)	4.58 (1.33–70.32)	
Yes	19						
Hyperpigmentation + Keratosis		4.29(6.18) 7.61(8.91)		*0.266*			*0.052*
No	327		2.45(0.13–80.44)		4.14(7.19)	2.32 (0.85–88.78)	
Yes	8		3.67(0.44–26.25)		5.68(3.75)	5.11 (1.32–12.33)	

	PMI			SMI	
Presence of skin lesion			*0.662*		0.003
No	250	4.42(6.68)	2.48(0.13–80.44)	3.15 (3.26)	2.30 (0.85–30.98)
Yes	85	4.23(4.86)	2.51(0.28–26.25)	7.22(12.57)	2.73 (1.01–88.78)

5.5. Factors related to PMI and SMI

Regression analysis results [B (95%CIs)] indicated that As exposure living in villages with As level of 10–50 µg/L in drinking water [0.4310 (0.1194–0.7425)] and being former smoker [-0.4231 (-0.7158–0.1305)] significantly affected the PMI (p = *0.007, 0.005; respectively).* The other factors selected for the analysis (living in villages with As level of > 50 µg As/L in drinking water, current smoking, folic acid, vitamin B12, hyperpigmentation) were not affected the PMI levels (p > 0.05).

Regression analysis data [B(95%CIs)] showed that As level > 50 µg /L in drinking water [0.5297 (0.3243–0.7352)], age [0.0127 (0.0077–0.0178)], folic acid level [0.0409 (0.0167–0.0652)] (*p < 0.001*), presence of keratosis (hand plus feet) [0.4553 (0.1609–0.7497)] (*p = 0.003*), former smoking [0.2602 (0.0332–0.4872)] (*p = 0.025*) and female gender [0.1967 (0.0128–0.3806)] (*p = 0.036*) were positively correlated with SMI. The other factors selected for the analysis (living in villages with As level of 10–50 µg/L in drinking water, current smoking, vitamin B12, diabetes, hypertension, hyperpigmentation + keratosis) were not affected the SMI levels (p > 0.05).

6. Discussion

The paper presents quantitative information concerning the recent and long-term As exposure, As metabolism and efficiency and skin lesions in the population living in several villages of Nevşehir Province, Turkey having different arsenic levels in drinking water (> 50µg/L, 10–50 µg/L and < 10µg/L).

The major route for As exposure is food and/or drinking water in individuals other than those with occupational As exposure. Although food is a negligible source of pollution in areas with As concentration > 50 µg/L in drinking water, it has become increasingly important to investigate effects of As taken by diet and smoking on human health while As level is decreasing in drinking water (Marchiset-Ferlay et al. 2012).

Recent epidemiological studies aid to identify health risks due to arsenic exposure. In recent years, contradictory results have been reported about cancer risk in lower doses of inorganic arsenic (< 100 µg/L) in many studies (Baastrup et al. 2008; Chen et al. 2010; Dauphine et al. 2013; Snow et al. 2004). Scarcity in validated animal models which may present carcinogenic effect induced by inorganic arsenic has impeditive effect on understanding mechanism of action of inorganic arsenic. However, lack of scientific data, regarding dose-response curve form (sublinear, linear, supralinear) at low doses of arsenic, adversely influences legal arrangements about permissible limits of arsenic. Thus, measurements with accurate

biomarkers for both exposure and effect in human tissues have become extremely important to stratify health risks, particularly in low exposure (Abernathy et al. 1996; Bates et al. 1995; Kitchin 2001; Mead 2005; Mo et al. 2006).

Furthermore, in the recent guidance report of US Environmental Protection Agency (EPA), it is suggested to carry out meta-analysis studies which take into consideration of health risks (pulmonary disease, bladder cancer, ischemic heart diseases and skin lesions, etc.), individual arsenic exposure (preferably measured by biomarkers) associated with low to moderate arsenic exposure (< 100 μg/L) via drinking water. In addition, it was recommended that 3 or more peer-reviewed manuscripts with 3 or more exposure levels investigated, should be included to meta-analysis on dose-response relationships (NRC 2013). Accordingly, our study findings would be contributing significantly to this guidance report by assessing individuals living in a study region with low-to-moderate As exposure.

In our study, As levels in drinking water sources are measured and at the same time, time-weighted As (TWA-As) levels in drinking water were calculated to determine individual As exposure of residents to eliminate to the variations in water sources and duration of use of drinking waters in the study region. It is known that TWA-As calculation is preferred approach for stratifying exposure groups in epidemiological studies which do not use biological markers for individual As exposure (Ahsan et al. 2007; Chen et al. 2011).

In our study, 3 study regions, based on total As level in drinking water as < 10 μg/L (control), 10–50 μg/L and > 50 μg/L, are also confirmed by TWA-As values of individuals living in these regions. Also, it can be concluded that it would be appropriate to use TWA-As values in analyses for cumulative As exposure. Another important suggestion of the study is, the mobility of individuals living in the study region is highly restricted according to the duration of residency data and they can represent the study region.

6.1. Assessment of recent and long-term arsenic exposure

Although total urinary As has been used to determine iAs exposure in many studies, it is important to distinguish iAs and its metabolites from organic forms. In particular, some seafood contains organic As in form of arsenobetaine (less toxic), which is rapidly excreted via urine after recent fish consumption; thus, misleadingly showing high iAs. The speciation methods can distinguish iAs, MMA and DMA from remaining As compounds and it is thought that urinary As metabolites are better predictors for As toxicity when compared to total As (ATSDR 2007; Huang et al. 2009). Herewith, to estimate absorbed iAs doses in the individuals living in study region, besides total urinary As measurements As species (AsIII, AsV, DMA and MMA) levels are also used which is allowing better quantitative estimation for recently absorbed As (Huang et al. 2009; WHO 2001).

In our study, total urinary As level was found to be higher than total urinary level of As species. The potential causes of this finding include lack of hydride formation by organic As species other than seafood consumed, inability to detect such species in samples where no degradation could be achieved, and partial analyte loss during filtering of urine samples before chromatographic separation in speciation studies (Chen et al. 2002; Heitland and Koster 2008). Total As and As species data are pointing out individuals living in the Nevşehir Province are still exposing to As in dose-dependent manner.

As level in hair sample is widely used to determine long-term exposure in epidemiological studies and many studies have shown a close correlation between As concentration in drinking water and hair As levels (Agusa et al. 2014; Phan et al. 2011; Uchino et al. 2006).

In our study, hair As levels revealed that individuals living in Nevşehir Province had cumulative As exposure pointing out drinking water as the important factor. Individuals living in study areas with As concentration > 50 µg/L in drinking water, hair As concentrations were found to be compatible to those with occupational As exposure in Turkey (Akbal et al. 2014). On the other hand, it was found that there are differences in hair As levels among populations living in different regions around world with similar As levels in drinking water (Agusa et al. 2014; Ghosh et al. 2006; Mosaferi et al. 2005; Normandin et al. 2014; Phan et al. 2011; Uchino et al. 2006) to our study. This variation is thought to be due to other factors affecting hair As level. Several endogenous (gender, ethnicity, genetic polymorphism), exogenous factors (smoking, dietary habits, residence), general health status or medication and presence of absence of other metals such as zinc and selenium in hair can affect As levels in hair (Arnold 1990; ATSDR 2007; Chakraborti et al. 2003; Cone et al. 2009; Marchiset-Ferlay et al. 2012; Nermell et al. 2008).

Normal As level in hair sample ≤ 1 mg/kg, which is proposed as a toxicity marker (such as skin lesions) (Arnold 1990), can increase up to 100-folds and remains to be increased up to 6–12 months (ATSDR 2007). Accordingly, it may be predicted that 8.3% and 91.7% of individuals in the exposed groups of our study are at risk for toxic effects and can be monitored.

6.2. Levels of metals other than arsenic in drinking water

It is suggested that other environmental contaminants in drinking water may play role in arsenic toxicity (Farias et al. 2003; Francisca and Carro Perez 2009; McCarty et al. 2004). Thus, it is thought that it is important to know levels of metals other than As in drinking water in epidemiological studies and it may be helpful for toxicological risk assessment related to low dose, chronic As exposure (Gebel 2000).

No further analysis was performed regarding modulation of As-induced toxic effect of elements (V, Cr, Ba, Ni, Zn, Se, Cd, and Sb) analyzed other than As in water samples, since levels of important toxic elements including Cr, Cd, Ni and Sb are not exceeded limit values in almost all water samples. No limit value has been established for vanadium by national or international organizations. Vanadium concentrations were found be higher in the areas with As concentration > 10 µg/L. Although V levels in drinking water range from 0.2 to > 100 µg/L across geographic regions, it is known that in general it is around 1–6 µg/L (WHO 2000). In our study, V concentrations ranged from 1.38 to 67.60 µg/L in water samples. There is a significant gap of knowledge for reliable toxicological classification and risk assessment of V compounds (Assem and Levy 2009; Rodriguez-Mercado et al. 2011). In an epidemiological study conducted at some regions where well water containing < 1-747 µg/L As and < 1 µg/L Sb in Bangladesh, it was shown that Sb had no significant interaction with effects of As (McCarty et al. 2004).

It was shown that drinking water contains Ba element may have influence on pathogenesis of diseases caused by arsenic, suggesting that limit value for Ba (700 µg/L) should be reduced in drinking water. Researchers demonstrated that Ba alone at doses of 343–687 µg/L promoted transformation of

keratinocyte, fibroblast and melanocyte while lower doses of Ba (137 µg/L) caused inhibition of As-induced apoptosis in the co-presence of As (224.8 µg/L) (Kato et al. 2013; Yajima et al. 2012). In our study, it can be suggested that Ba concentration in drinking water (97.8-163.1 µg/L; Table 4) may contribute to efforts to understand pathogenesis of diseases caused by As in the future.

On the other hand, fluoride is also an important geogenic, inorganic contaminant that may be commonly present in the environment, which can be detected in underground water. However, information about the effects of F exposure on As metabolism and further As toxicity are limited. Although detailed mechanism has not yet been understood, previous experimental data indicate synergistic, antagonist and independent effects of F and As (Mondal and Chattopadhyay 2020). However, a recent epidemiological study by (Jimenez-Cordova et al. 2019) suggested that high F exposure level (≥ 1.5 mg/L in water) is only significantly related to an increase in mono-methylated arsenic levels. In our case, F levels in all water samples were below WHO maximum permissible limit in drinking water; 1.5 mg/L. Thus, we have assumed that F levels might not modify As metabolism.

6.3. Skin lesions

Skin lesions are the first symptoms of toxicity in chronic As exposure (Centeno et al. 2002; NRC 2001; Tseng et al. 1968). In several studies, it was shown that the severity and frequency of As-induced skin lesions such as hyperkeratosis at palm and sole and/or hyper-pigmentation and hypopigmentation (typically both may develop concurrently) at areas where sunlight exposure is limited, depend on As concentration in water and duration of use. In regions such as Bangladesh, India, China and Pakistan where arsenic exposure is high, skin lesion incidence varies from 2.08–20.6% (Fatmi et al. 2013; Rahman et al. 2006). In studies on individuals living at regions with arsenic contamination, it was reported that toxic effects other than cancer-including skin lesions and other effects occur when As level is above 280–350 µg/L (NRC 2001).

On the other hand, (Karagas et al. 2015) published as systematic review including 25 studies on skin lesions (15 studies) and skin cancer (10 studies) in individuals having individual As exposure data with As levels ≤ 100 µg/L in drinking water. In this systematic review, it is reported that majority of studies on skin lesions were from Southern Asia while others from China and As-induced skin lesion development was dose-dependent. On the other hand, it was shown that risk for As-related skin lesions and skin cancer can vary based on several factors including nutritional factors (e.g. folate and selenium status, dietary diversity), lifestyle (e.g. smoking and body mass index) and genetic polymorphism of As metabolism (Karagas et al. 2015).

In individuals living in the villages at study region, TWA-As exposure ranged from 15.0 to 134.35 µg/L. In the residents with higher TWA-As exposure, incidences of hyperpigmentation, hypopigmentation, keratosis at hand or foot, keratosis at hand and foot and keratosis plus hyperpigmentation were similar to those living in the areas without As contamination. The incidence of keratosis at hand and foot was significant higher in individuals with hair As level > 1 µg/L (marker of toxicity) when compared to those with As level < 1 µg/L (15.8% vs.8.7%). This may be an incidental finding; however, it will important to evaluate other risk factors modifying skin lesion formation in this risk group.

In Turkey, there is limited number of comprehensive studies on As exposure and related skin lesions in individuals living in the areas with As contamination in drinking water. In this issue, preliminary findings were noted in individuals living in İğdeköy and Dulkadir villages at Emet district of Kütahya Province, who consumed drinking water with high As levels (Dağıstanlı 1995). Author reported that hyperkeratosis and hyperpigmentation were present in 9 of 30 individuals from İğdeköy village where As concentration was 140–1700 μg/L in drinking water. Chronic As exposure was shown using hair As levels in these individuals. However, there is no data about follow-up of these individuals after confinement of these problematic drinking water sources. (Dogan et al. 2005) found As-related palmo-plantar keratosis frequency as 17.5% and 1.8% in 97 individuals from İğdeköy and 56 individuals from Dulkadir villages at district of Kütahya Province where As concentration was 0.3–0.9 mg/L, respectively. However, there was no data regarding chronic As exposure of individuals. In a study on 303 individuals living in several villages of Kütahya Province, (Arikan et al. 2015) reported that As-related skin lesion frequency was significantly higher in individuals living in villages with mean drinking water As level of 10 ppb when compared to those living in villages with drinking water As level of 445 ppb. The chronic As exposure was assessed using hair As level. Although hair As level was significantly higher in individuals living at areas with high As level when compared to those living at areas with low As level in drinking water, it should be noted that hair As levels in both groups were within normal range. Thus, these results were not comparable with hair As levels in the groups (exposed group and controls) in our study. Moreover, it is difficult to compare with results from other studies conducted at Emet district of Kütahya Province since there are differences in study design, sample size and study population.

6.4. Methylation efficiency

The percent arsenic metabolites in urine (iAs, MMA and DMA) show efficiency of arsenic metabolism. The metabolite ratios (MMA: iAs and DMA: MMA) are widely used markers for estimation of individual methylation capacity. Similar indices are used in the epidemiological studies from several countries (Del Razo et al. 2011; Huang et al. 2009; McCarty et al. 2007; Normandin et al. 2014; Xu et al. 2008).

For calculation of metabolite proportions, (Steinmaus et al. 2010) suggest that a little mistake in laboratory measurements can derive relatively large errors at low metabolite concentrations; thus, for more appropriate calculations for methylation efficiency, in our study we excluded participants with urinary As (III), As (V), MMA and DMA concentrations below detection limit (LOD).

In our study, urinary iAs% and DMA% were within range seen in other populations (Heck et al. 2007; Hopenhayn-Rich et al. 1996; Hsu et al. 2017; Huang et al. 2008; Lindberg et al. 2008; Yu et al. 2000). However, it was seen that MMA% values in both control and exposed groups were slightly higher (27.71% and 25.40%, respectively) (Table 5) than other studies. Similarly, (Chiou et al. 1997) and (Hsueh et al. 1997) found urine MMA levels as 18.5–31.5% independent from As dose. PMI and SMI values showed that individuals in our study had efficient methylation capacity and that there is no saturation in As metabolism.

The epidemiological studies on individuals with environmental iAs exposure showed associations between skin lesions, skin cancer, bladder cancer, lung cancer, peripheral vascular disease and hypertension incidences and increased PMI (MMA: iAs) or decreased SMI (DMA: MMMA) values (Ahsan et al. 2007; Chen

et al. 2003; Chung et al. 2013; Hsu et al. 2017; Hsueh et al. 1997; Huang et al. 2008; Lindberg et al. 2008; McCarty et al. 2007; Pu et al. 2007; Tseng et al. 2005). In some studies, it was shown that there was a negative correlation between iAs exposure and DMA: MMA ratio due to saturation or inhibition of iAs methylation pathway at high levels of As exposure (TWA-As: 158 μg/L) (Ahsan et al. 2007), while low DMA: MMA or high %MMA was associated with increased susceptibility to iAs exposure-related diseases including skin lesions (Chung et al. 2013; Hsu et al. 2017; Lindberg et al. 2008).

Low PMI values in individuals with hyperpigmentation and high SMI levels in individuals with keratosis at hand and foot or skin lesions in our study suggest that low-to moderate As levels in drinking water on the risk of pre- malign skin lesions (as PMI and SMI) may modifiable by other factors, e.g. nutritional and lifestyle factors and genetic polymorphisms in genes related to arsenic metabolism.

In many studies, it was shown that inorganic arsenic metabolism is affected by several factors including genetics, demographic characteristics (e.g. age, gender), environmental factors (As dose in drinking water, exposure duration and termination of exposure), lifestyle- and diet-related factors, dietary habits and general health status. Although effects of above-mentioned factors could not be shown consistently, they should be considered as factors which must be taken into consideration (Gribble et al. 2013; Lindberg et al. 2008; Lindberg et al. 2007; Tseng 2009). In our study, one limitation is lack of body mass index data (BMI) which has been proposed as a factor that may affect As metabolism.

Reactions involved in arsenic methylation occur via single carbon metabolism. Methylation is mediated by enzymes which use S-adenosyl methionine (SAM) as substrate. Thus, it is proposed that several factors (e.g. folic acid, cysteine, methionine, niacin, choline, S-adenosyl methionin, vitamin B12) affecting single carbon metabolism can alter As metabolism and that it may have influence on human health (Gamble et al. 2005; Heck et al. 2007; Huang et al. 2009). It is known that folate is an important co-factor involved in transportation and use of methyl groups during single carbon metabolism. In addition, it contributes to DNA biosynthesis process including gene expression, transcription, chromatin structure, genomic repair and stability regulation as single carbon transporter. The role of folate deficiency has become increasingly important in many diseases such as atherosclerotic disorders, cardiovascular diseases, neurological, neuropsychiatric and congenital disorders, and cancer (Choi and Mason 2000; Stanger 2002).

In descriptive epidemiological studies in regions where As-related disorders are seen, it was seen that diet is weak without diversity. In case-control studies from China, Taiwan, Western Bengal and India, diet content is poor from fruits, vegetables and food of animal origin (Heck et al. 2007). It was found that blood folate levels can be more reliable parameter than survey-based folate estimations (Huang et al. 2008). Thus, we measured serum folate levels to estimate dietary folate intake. It can be suggested that dietary folate intake was not different between controls and exposed group (Table 7). The higher proportion of individuals with low vitamin B12 levels in exposed group may be due to other factors (age, medication). (Chen et al. 2007) suggest that higher dietary selenium intake may reduce the risk of arsenic-related skin lesions. However, blood selenium levels are lacking in our study.

7. Conclusion

This paper is part of our bigger project aimed at elucidating the effect of low-to moderate As exposure on skin lesions and genotoxicity parameters (e.g. Micronuclei) in target and surrogate tissues as well as individual susceptibilities (e.g. GSTM1, GSTT1, GSTP1, GST01-1 and AS3MT genetic polymorphisms).

In our study, all environmental and biological measurements demonstrated that individuals living in the study area were chronically exposed to low-to-moderate As due to geological contamination in drinking water. No significant increase was observed in the frequency of skin lesions in individuals exposed to As in our study area. Given the contradictory results about health risks (e.g. lung, skin and bladder cancer and skin lesions) to low-to-moderate As exposure (Baastrup et al. 2008; Chen et al. 2010; Dauphine et al. 2013; Snow et al. 2004), it is important to initiate long-term follow-up studies about effects on health in this region which would be considered as risky for As contamination.

DECLARATIONS

Declarations

Acknowledgment

Conflicts of interest/Competing interests

I have read the manuscript and I hereby affirm that the content of this manuscript or a major portion thereof has not been published in a refereed journal, and it is not being submitted fully or partially for publication

elsewhere. The manuscript has been read and approved by all authors.

The authors have no conflicts of interest to declare that are relevant to the content of this article

Availability of data and material

 All data analysed during this study are included in this published article

Code availability

Data analysis was performed by using IBM SPSS Statistics version 17.0 software (IBM Corporation, Armonk NY,USA) by a statistician (Salih Ergöçen). This issue has been indicated in Statistical Analysis and Acknowledgement sections.

Authors' contributions

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Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki.This study was approved by Gazi University Faculty of Medicine Local Ethics Committee (15.06.2009, No.340).

This issue has been indicated indicated in Sections 2.2 and Acknowledgment in the text.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

This issue has been indicated indicated in Section 2.2 in the text.

Consent for publication

The participant has consented to the submission of the case report to the journal.

References

Abernathy CO, Chappell WR, Meek ME, Gibb H, Guo HR (1996) Is ingested inorganic arsenic a "Threshold" Carcinogen? Fund Appl Toxicol 29:168-175

Agusa T, Trang PT, Lan VM, Anh DH, Tanabe S, Viet PH, Berg M (2014) Human exposure to arsenic from drinking water in Vietnam Sci Total Environ 488-489:562-569 https://doi.org/10.1016/j.scitotenv.2013.10.039

Ahsan H et al. (2007) Arsenic metabolism, genetic susceptibility, and risk of premalignant skin lesions in Bangladesh Cancer Epidemiol Biomarkers Prev 16:1270-1278 https://doi.org/10.1158/1055-9965.EPI-06-0676

Akbal A, Yilmaz H, Tutkun E (2014) Arsenic exposure associated with decreased bone mineralization in male Aging Male 17:256-258 https://doi.org/10.3109/13685538.2013.819326

Arikan I, Namdar ND, Kahraman C, Dagci M, Ece E (2015) 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 Asian Pacific Journal of Cancer Prevention 16:3183-3188

Arnold HL, Odam, R.B., James, W.D. (1990) Disease of the skin. In: Clinical Dermatology. Philadelphia: W.B. Saunders

Assem FL, Levy LS (2009) A review of current toxicological concerns on vanadium pentoxide and other vanadium compounds: gaps in knowledge and directions for future research J Toxicol Environ Health B Crit Rev 12:289-306 https://doi.org/10.1080/10937400903094166

Atabey E (2009) Arsenik ve Etkileri. Maden Tetkik ve Arama Genel Müdürlüğü, Yerbilimleri ve Kültür Serisi:3. MTA yayınları, Ankara

Atabey E (2013) Nevşehir İli Tıbbi Jeolojik Unsurları ve Halk Sağlığı. Nevşehir Belediyesi yayını, Ankara

Atabey E, Unal H (2008) Batı Anadoludaki jeolojik unsurlar ve halk sağlığı projesi 2006-2007 yılı Tıbbi Jeoloji ve Etüt Raporu. Maden Tetkik ve Arama Genel Müdürlüğü, Ankara

Agency for Toxic Substances and Disease Registry (ATSDR) (2007) Toxicological profile for Arsenic, Atlanta, GA: U.S. Department of Health and Human Services Public Health Service

Baastrup R et al. (2008) Arsenic in drinking-water and risk for cancer in Denmark Environ Health Perspect 116:231-237 https://doi.org/10.1289/ehp.10623

Basu A et al. (2002) Enhanced frequency of micronuclei in individuals exposed to arsenic through drinking water in West Bengal, India Mutat Res-Gen Tox En 516:29-40 https://doi.org/Pii S1383-5718(02)00014-1

Bates MN, Smith AH, Cantor KP (1995) Case-control study of bladder cancer and arsenic in drinking water Am J Epidemiol 141:523-530 https://doi.org/10.1093/oxfordjournals.aje.a117467

Bundschuh J et al. (2013) Naturally occurring arsenic in terrestrial geothermal systems of western Anatolia, Turkey: potential role in contamination of freshwater resources J Hazard Mater 262:951-959 https://doi.org/10.1016/j.jhazmat.2013.01.039

Burgaz S, Coskun E, Demircigil GC, Kocabas NA, Cetindag F, Sunter O, Edinsel H (2011) Micronucleus frequencies in lymphocytes and buccal epithelial cells from patients having head and neck cancer and their first-degree relatives Mutagenesis 26:351-356 https://doi.org/10.1093/mutage/geq101

Centeno JA et al. (2002) Pathology related to chronic arsenic exposure Environ Health Perspect 110 Suppl 5:883-886 https://doi.org/10.1289/ehp.02110s5883

Chakraborti D et al. (2003) Arsenic groundwater contamination in Middle Ganga Plain, Bihar, India: a future danger? Environ Health Perspect 111:1194-1201 https://doi.org/10.1289/ehp.5966

Chen CL, Chiou HY, Hsu LI, Hsueh YM, Wu MM, Wang YH, Chen CJ (2010) Arsenic in drinking water and risk of urinary tract cancer: a follow-up study from northeastern Taiwan Cancer Epidemiol Biomarkers Prev 19:101-110 https://doi.org/10.1158/1055-9965.EPI-09-0333

Chen Y et al. (2011) Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study BMJ 342:d2431 https://doi.org/10.1136/bmj.d2431

Chen Y, Hall M, Graziano JH, Slavkovich V, van Geen A, Parvez F, Ahsan H (2007) A prospective study of blood selenium levels and the risk of arsenic-related premalignant skin lesions Cancer Epidemiol Biomarkers Prev 16:207-213 https://doi.org/10.1158/1055-9965.EPI-06-0581

Chen YC, Amarasiriwardena CJ, Hsueh YM, Christiani DC (2002) Stability of arsenic species and insoluble arsenic in human urine Cancer Epidemiol Biomarkers Prev 11:1427-1433

Chen YC et al. (2003) Arsenic methylation and skin cancer risk in southwestern Taiwan J Occup Environ Med 45:241-248 https://doi.org/10.1097/01.jom.0000058336.05741.e8

Chiou HY et al. (1997) Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan Mutat Res-Rev Mutat 386:197-207 https://doi.org/Doi 10.1016/S1383-5742(97)00005-7

Choi SW, Mason JB (2000) Folate and carcinogenesis: an integrated scheme J Nutr 130:129-132 https://doi.org/10.1093/jn/130.2.129

Chung CJ, Huang YL, Huang YK, Wu MM, Chen SY, Hsueh YM, Chen CJ (2013) Urinary arsenic profiles and the risks of cancer mortality: a population-based 20-year follow-up study in arseniasis-endemic areas in Taiwan Environ Res 122:25-30 https://doi.org/10.1016/j.envres.2012.11.007

Col M, Col C, Soran A, Sayli BS, Ozturk S (1999) Arsenic-related Bowen's disease, palmar keratosis, and skin cancer Environ Health Perspect 107:687-689 https://doi.org/10.1289/ehp.107-1566498

Cone EJ, Caplan YH, Moser F, Robert T, Shelby MK, Black DL (2009) Normalization of urinary drug concentrations with specific gravity and creatinine J Anal Toxicol 33:1-7 https://doi.org/10.1093/jat/33.1.1

Dağıstanlı S (1995) Evaluation of the genotoxic damage of the peripheral blood lymphocyte of individuals exposed to arsenic by well water (Ph.D Thesis) Gazi University Faculty of Pharmacy, Ankara

Dauphine DC, Smith AH, Yuan Y, Balmes JR, Bates MN, Steinmaus C (2013) Case-control study of arsenic in drinking water and lung cancer in California and Nevada Int J Environ Res Public Health 10:3310-3324 https://doi.org/10.3390/ijerph10083310

Dedina J, Tsalev DL (1995) Hydride Generation Atomic Absorption Spectrometry. Wiley, Chichester, U.K

Del Razo LM et al. (2011) Exposure to arsenic in drinking water is associated with increased prevalence of diabetes: a cross-sectional study in the Zimapan and Lagunera regions in Mexico Environmental Health 10:73

Dogan M, Dogan AU (2007) Arsenic mineralization, source, distribution, and abundance in the Kutahya region of the western Anatolia, Turkey Environ Geochem Health 29:119-129 https://doi.org/10.1007/s10653-006-9071-z

Dogan M, Dogan AU, Celebi C, Baris YI (2005) Geogenic arsenic and a survey of skin lesions in the Emet region of Kutahya, Turkey Indoor and Built Environment 14:533-536 https://doi.org/10.1177/1420326x05060121

Farias SS, Casa VA, Vazquez C, Ferpozzi L, Pucci GN, Cohen IM (2003) Natural contamination with arsenic and other trace elements in ground waters of Argentine Pampean Plain Sci Total Environ 309:187-199 https://doi.org/10.1016/S0048-9697(03)00056-1

Fatmi Z, Abbasi IN, Ahmed M, Kazi A, Kayama F (2013) Burden of skin lesions of arsenicosis at higher exposure through groundwater of taluka Gambat district Khairpur, Pakistan: a cross-sectional survey Environ Geochem Hlth 35:341-346 https://doi.org/10.1007/s10653-012-9498-3

Fatmi Z et al. (2009) Health burden of skin lesions at low arsenic exposure through groundwater in Pakistan. Is river the source? Environmental research 109:575-581

Ferreccio C et al. (2013) Case-control study of arsenic in drinking water and kidney cancer in uniquely exposed Northern Chile Am J Epidemiol 178:813-818 https://doi.org/10.1093/aje/kwt059

Francisca FM, Carro Perez ME (2009) Assessment of natural arsenic in groundwater in Cordoba Province, Argentina Environ Geochem Health 31:673-682 https://doi.org/10.1007/s10653-008-9245-y

Gamble MV et al. (2005) Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh Environ Health Perspect 113:1683-1688 https://doi.org/10.1289/ehp.8084

Gebel T (2000) Confounding variables in the environmental toxicology of arsenic Toxicology 144:155-162 https://doi.org/10.1016/s0300-483x(99)00202-4

Ghosh P et al. (2006) Cytogenetic damage and genetic variants in the individuals susceptible to arsenic-induced cancer through drinking water Int J Cancer 118:2470-2478 https://doi.org/10.1002/ijc.21640

Gomez-Rubio P et al. (2012) Indigenous American Ancestry Is Associated with Arsenic Methylation Efficiency in an Admixed Population of Northwest Mexico J Toxicol Env Heal A 75:36-49 https://doi.org/10.1080/15287394.2011.615107

Gribble MO et al. (2013) Body composition and arsenic metabolism: a cross-sectional analysis in the Strong Heart Study Environ Health 12:107 https://doi.org/10.1186/1476-069X-12-107

Gunduz O et al. (2014) Statistical analysis of death causes (2005-2010) in villages with high arsenic levels in drinking water supplies of simav plain, Turkey Archives of environmental and occupational health 70:35-46

Gunduz O, Simsek C, Hasozbek A (2010) Arsenic Pollution in the Groundwater of Simav Plain, Turkey: Its Impact on Water Quality and Human Health Water Air and Soil Pollution 205:43-62 https://doi.org/10.1007/s11270-009-0055-3

Heck JE et al. (2007) Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh Am J Clin Nutr 85:1367-1374 https://doi.org/10.1093/ajcn/85.5.1367

Heitland P, Koster HD (2008) Fast determination of arsenic species and total arsenic in urine by HPLC-ICP-MS: concentration ranges for unexposed german inhabitants and clinical case studies J Anal Toxicol 32:308-314 https://doi.org/10.1093/jat/32.4.308

Hopenhayn-Rich C, Biggs ML, Smith AH, Kalman DA, Moore LE (1996) Methylation study of a population environmentally exposed to arsenic in drinking water Environ Health Perspect 104:620-628 https://doi.org/10.1289/ehp.96104620

Hsu KH, Tsui KH, Hsu LI, Chiou HY, Chen CJ (2017) Dose-Response Relationship between Inorganic Arsenic Exposure and Lung Cancer among Arseniasis Residents with Low Methylation Capacity Cancer Epidem Biomar 26:756-761 https://doi.org/10.1158/1055-9965.Epi-16-0281

Hsueh YM et al. (1997) Serum beta-carotene level, arsenic methylation capability, and incidence of skin cancer Cancer Epidemiol Biomarkers Prev 6:589-596

Huang YK, Huang YL, Hsueh YM, Wang JT, Yang MH, Chen CJ (2009) Changes in urinary arsenic methylation profiles in a 15-year interval after cessation of arsenic ingestion in southwest Taiwan Environ Health Perspect 117:1860-1866 https://doi.org/10.1289/ehp.0900560

Huang YK, Pu YS, Chung CJ, Shiue HS, Yang MH, Chen CJ, Hsueh YM (2008) Plasma folate level, urinary arsenic methylation profiles, and urothelial carcinoma susceptibility Food Chem Toxicol 46:929-938 https://doi.org/10.1016/j.fct.2007.10.017

International Agency for Research on Cancer (IARC) (2004) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Drinking-Water Disinfectants and Contaminants, including Arsenic.vol. 84. pp. 1–512

International Agency for Research on Cancer (IARC) (2012) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Arsenic, Metals, Fibres,and Dusts. pp. 41-85

Jimenez-Cordova MI, Sanchez-Pena LC, Barrera-Hernandez A, Gonzalez-Horta C, Barbier OC, Del Razo LM (2019) Fluoride exposure is associated with altered metabolism of arsenic in an adult Mexican population Science of the Total Environment 684:621-628 https://doi.org/10.1016/j.scitotenv.2019.05.356

Karagas MR, Gossai A, Pierce B, Ahsan H (2015) Drinking Water Arsenic Contamination, Skin Lesions, and Malignancies: A Systematic Review of the Global Evidence Curr Environ Health Rep 2:52-68 https://doi.org/10.1007/s40572-014-0040-x

Kato M et al. (2013) Comparison of Barium and Arsenic Concentrations in Well Drinking Water and in Human Body Samples and a Novel Remediation System for These Elements in Well Drinking Water Plos One 8:e66681 https://doi.org/ARTN e6668110.1371/journal.pone.0066681

Kitchin KT (2001) Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites Toxicol Appl Pharmacol 172:249-261 https://doi.org/10.1006/taap.2001.9157

Lindberg AL, Ekstrom EC, Nermell B, Rahman M, Lonnerdal B, Persson LA, Vahter M (2008) Gender and age differences in the metabolism of inorganic arsenic in a highly exposed population in Bangladesh Environ Res 106:110-120 https://doi.org/10.1016/j.envres.2007.08.011

Lindberg AL et al. (2007) Metabolism of low-dose inorganic arsenic in a central European population: influence of sex and genetic polymorphisms Environ Health Perspect 115:1081-1086 https://doi.org/10.1289/ehp.10026

Marchiset-Ferlay N, Savanovitch C, Sauvant-Rochat MP (2012) What is the best biomarker to assess arsenic exposure via drinking water? Environ Int 39:150-171 https://doi.org/10.1016/j.envint.2011.07.015

McCarty KM et al. (2007) Arsenic methylation, GSTT1, GSTM1, GSTP1 polymorphisms, and skin lesions Environ Health Perspect 115:341-345 https://doi.org/10.1289/ehp.9152

McCarty KM, Senn DB, Kile ML, Quamruzzaman Q, Rahman M, Mahiuddin G, Christiani DC (2004) Antimony: An unlikely confounder in the relationship between well water arsenic and health outcomes in Bangladesh Environ Health Persp 112:809-811 https://doi.org/DOI 10.1289/ehp.6800

Mead MN (2005) Arsenic: in search of an antidote to a global poison Environ Health Perspect 113:A378-386 https://doi.org/10.1289/ehp.113-a378

Mo J, Xia Y, Wade TJ, Schmitt M, Le XC, Dang R, Mumford JL (2006) Chronic arsenic exposure and oxidative stress: OGG1 expression and arsenic exposure, nail selenium, and skin hyperkeratosis in Inner Mongolia Environ Health Perspect 114:835-841 https://doi.org/10.1289/ehp.8723

Mondal P, Chattopadhyay A (2020) Environmental exposure of arsenic and fluoride and their combined toxicity: A recent update J Appl Toxicol 40:552-566

Mosaferi M, YOUNESIAN M, Mesdaghinia A, NASERI S, Mahvi A, Nadim H (2005) Correlation between arsenic concentration in drinking water and human hair

Naujokas MF, Anderson B, Ahsan H, Aposhian HV, Graziano JH, Thompson C, Suk WA (2013) The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem Environ Health Perspect 121:295-302 https://doi.org/10.1289/ehp.1205875

Nermell B, Lindberg AL, Rahman M, Berglund M, Persson LA, El Arifeen S, Vahter M (2008) Urinary arsenic concentration adjustment factors and malnutrition Environ Res 106:212-218 https://doi.org/10.1016/j.envres.2007.08.005

Normandin L et al. (2014) Biomarkers of arsenic exposure and effects in a Canadian rural population exposed through groundwater consumption J Expo Sci Environ Epidemiol 24:127-134

https://doi.org/10.1038/jes.2013.80

National Research Council (NRC) (2001) Arsenic in Drinking Water. National Research Council Washington DC

National Research Council (NRC) (2013) Critical aspects of the EPA's IRIS Assessment of inorganic arsenic: Interim report. Committee on Inorganic Arsenic, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies. Washington, DC: The National Academies Press

Phan K, Sthiannopkao S, Kim KW (2011) Surveillance on chronic arsenic exposure in the Mekong River basin of Cambodia using different biomarkers Int J Hyg Environ Health 215:51-58 https://doi.org/10.1016/j.ijheh.2011.07.002

Pu YS et al. (2007) Urinary arsenic profile affects the risk of urothelial carcinoma even at low arsenic exposure Toxicol Appl Pharmacol 218:99-106 https://doi.org/10.1016/j.taap.2006.09.021

Rahman L, Corns WT, Bryce DW, Stockwell PB (2000) Determination of mercury, selenium, bismuth, arsenic and antimony in human hair by microwave digestion atomic fluorescence spectrometry Talanta 52:833-843 https://doi.org/10.1016/s0039-9140(00)00436-7

Rahman M et al. (2006) Arsenic exposure and age-and sex-specific risk for skin lesions: a population-based case–referent study in Bangladesh Environ Health Persp 114:1847-1852

Ravenscroft P Special session: Arsenic in the natural environment; the geography of a global problem. In: Annual Conference of the Royal Geographical Society, London, August 2007.

Rodriguez-Mercado JJ, Mateos-Nava RA, Altamirano-Lozano MA (2011) DNA damage induction in human cells exposed to vanadium oxides in vitro Toxicol In Vitro 25:1996-2002 https://doi.org/10.1016/j.tiv.2011.07.009

Simsek C (2013) Assessment of naturally occurring arsenic contamination in the groundwater of Sarkisla Plain (Sivas/Turkey) Environmental Earth Sciences 68:691-702 https://doi.org/10.1007/s12665-012-1771-3

Snow ET, Sykora P, Durham TR, Klein CB (2004) Arsenic mode of action at biologically plausible low doses: What are the implications for low dose cancer risk? Toxicology and Applied Pharmacology 197:170-170

Stanger O (2002) Physiology of folic acid in health and disease Curr Drug Metab 3:211-223 https://doi.org/10.2174/1389200024605163

Steinmaus C et al. (2006) Arsenic methylation and bladder cancer risk in case-control studies in Argentina and the United States J Occup Environ Med 48:478-488 https://doi.org/10.1097/01.jom.0000200982.28276.70

Steinmaus C et al. (2010) Individual differences in arsenic metabolism and lung cancer in a case-control study in Cordoba, Argentina Toxicol Appl Pharmacol 247:138-145

https://doi.org/10.1016/j.taap.2010.06.006

Tseng CH (2009) A review on environmental factors regulating arsenic methylation in humans Toxicol Appl Pharmacol 235:338-350 https://doi.org/10.1016/j.taap.2008.12.016

Tseng CH, Huang YK, Huang YL, Chung CJ, Yang MH, Chen CJ, Hsueh YM (2005) Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in blackfoot disease-hyperendemic villages in Taiwan Toxicol Appl Pharmacol 206:299-308 https://doi.org/10.1016/j.taap.2004.11.022

Tseng WP, Chu HM, How SW, Fong JM, Lin CS, Yeh S (1968) Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan J Natl Cancer Inst 40:453-463

Uchino T, Roychowdhury T, Ando M, Tokunaga H (2006) Intake of arsenic from water, food composites and excretion through urine, hair from a studied population in West Bengal, India Food Chem Toxicol 44:455-461 https://doi.org/10.1016/j.fct.2005.08.018

Vahter M (1999) Variation in human metabolism of arsenic. In: Arsenic Exposure and Health Effects III. Elsevier, pp 267-279

Vahter M (2002) Mechanisms of arsenic biotransformation Toxicology 181-182:211-217 https://doi.org/10.1016/s0300-483x(02)00285-8

Valenzuela OL, Borja-Aburto VH, Garcia-Vargas GG, Cruz-Gonzalez MB, Garcia-Montalvo EA, Calderon-Aranda ES, Del Razo LM (2005) Urinary trivalent methylated arsenic species in a population chronically exposed to inorganic arsenic Environ Health Perspect 113:250-254 https://doi.org/10.1289/ehp.7519

Vega L, Styblo M, Patterson R, Cullen W, Wang C, Germolec D (2001) Differential effects of trivalent and pentavalent arsenicals on cell proliferation and cytokine secretion in normal human epidermal keratinocytes Toxicol Appl Pharmacol 172:225-232 https://doi.org/10.1006/taap.2001.9152

Wei B et al. (2017) Arsenic methylation and skin lesions in migrant and native adult women with chronic exposure to arsenic from drinking groundwater Environ Geochem Health 39:89-98 https://doi.org/10.1007/s10653-016-9809-1

World Health Organisation (WHO) (2000) Regional Office for Europe, Air Quality Guidelines Chapter 6.12: Vanadium, Copenhagen, Denmark

World Health Organisation (WHO) (2001) Arsenic and Arsenic Compounds. Environmental Health Criteria 224. Geneva

Xu Y et al. (2008) Association of oxidative stress with arsenic methylation in chronic arsenic-exposed children and adults Toxicol Appl Pharmacol 232:142-149 https://doi.org/10.1016/j.taap.2008.06.010

Yajima I et al. (2012) Barium inhibits arsenic-mediated apoptotic cell death in human squamous cell carcinoma cells Arch Toxicol 86:961-973 https://doi.org/10.1007/s00204-012-0848-9

Yildiz A et al. (2008) Effect of chronic arsenic exposure from drinking waters on the QT interval and transmural dispersion of repolarization J Int Med Res 36:471-478 https://doi.org/10.1177/147323000803600311

Yılmaz A, Yılmaz H (2006) Characteristic features and structural evolution of a post collisional basin: The Sivas Basin, Central Anatolia, Turkey Journal of Asian Earth Sciences 27:164-176

Yu RC, Hsu KH, Chen CJ, Froines JR (2000) Arsenic methylation capacity and skin cancer Cancer Epidemiol Biomarkers Prev 9:1259-1262

Zhang Q, Li YF, Liu J, Wang D, Zheng QM, Sun GF (2014) Differences of Urinary Arsenic Metabolites and Methylation Capacity between Individuals with and without Skin Lesions in Inner Mongolia, Northern China International Journal of Environmental Research and Public Health 11:7319-7332 https://doi.org/10.3390/ijerph110707319