MARKERS OF LOW LEVEL ARSENIC EXPOSURE FOR EVALUATING HUMAN CANCER RISKS IN A US POPULATION*

MARGARET R. KARAGAS[1], CHRIS X. LE[2], STEVEN MORRIS[3], JOEL BLUM[4], XIUFEN LU[2], VICKY SPATE[3], MARK CAREY[1], VIRGINIA STANNARD[1], BJOERN KLAUE[4] and TOR D. TOSTESON[1]

[1] Section of Biostatistics and Epidemiology
Department of Community and Family Medicine
Dartmouth Medical School
Lebanon, NH, USA.

[2] Department of Public Health Sciences
University of Alberta
Edmonton, Alberta, Canada

[3] Missouri University Research Reactor Center
Columbia, MO, USA

[4] Department of Geological Sciences
University of Michigan
Ann Arbor, MI, USA

Abstract. Epidemiologic studies conducted in the US have not previously detected an association between regional drinking water arsenic concentrations and corresponding cancer occurrence or mortality rates. To improve our estimation of cancer risk and arsenic exposure in the USA, we have investigated the reliability of several exposure markers. In the current study, we specifically evaluated the long-term reproducibility of tap water and toenail concentrations of arsenic, and the relation between water, toenail, and urinary measurement. Subjects included 99 controls in our case-control study on whom we requested a household tap water sample and toenail clipping three to five years apart. Additionally, participants were asked to provide a first morning void sample at the second interview. Tap water arsenic concentrations ranged from undetectable (<0.01 μg/L) to 66.6 μg/L. We found a significant correlation between both replicate water and toenail samples (intraclass correlation coefficient = 0.85, 95% confidence interval = 0.79–0.89 for water, and intraclass correlation coefficient = 0.60, 95% confidence interval = 0.48–0.70 for toenails). The inter-method correlations for water, urinary and toenail arsenic were all statistically significant ($r = 0.35$, $p = 0.0024$ for urine vs water; $r = 0.33$, $p = 0.0016$ for toenail vs water and $r = 0.36$, $p=0.0012$ for urine vs toenails). Thus, we found both toenail and water measurements of arsenic reproducible over a three- to five-year period. Our data suggest that biologic markers may provide reliable estimates of internal dose of low level arsenic exposure that can be used to assess cancer risk.

Key words:
Heavy metals, Toxic metals, Measurement, Toenails, Water, Urine, Epidemiology

INTRODUCTION

Arsenic is an established human carcinogen based on studies of populations ingesting high levels of arsenic in their drinking water [1,2]. But, it is unclear whether effects occur at the low levels experienced in the USA. Studies of cancer endpoints require long-term measures of exposure since the latency period for clinically evident disease may be several years if not decades. Also, because cancer is a rare event, studies, such as ours [3] often use a case-control design. A major advantage to using biologic or environmental markers (versus patient recall) is that

they are not subject to differential misclassification, resulting from interviewer or recall biases inherent to case-control studies. Blood levels of arsenic generally do not reflect long term exposure since arsenic is cleared from the blood within a few hours. Urinary arsenic is a potential biologic marker of arsenic exposure, but only may be a short-term measure since arsenic is cleared from the body via the kidneys within a few days [4]. Moreover, it is uncertain how well urinary arsenic reflects intake in populations exposed to low levels of arsenic in their drinking water, and whether the disease process itself (i.e., bladder cancer) affects concentrations. Arsenic accumulates in hair and nail tissue due to its affinity for sulfhydryl groups in keratin [5,6]. We previously reported a significant correlation between drinking water and toenail concentrations among those whose drinking water contained 1 μg/L to 180 μg/L of arsenic in the New Hampshire population [7].

To examine the long-term reproducibility of drinking water and toenail measures of arsenic, we re-tested a sample of participants from our original study. We further tested the inter-method correlations of drinking water, toenail and urinary measures.

METHODS

We selected controls from our non-melanoma skin cancer case-control study on whom we analyzed a toenail and water sample three to five years ago [3]. We first sent a letter to these subjects, summarizing the trace element results from the original study and that we might re-contact them for the biologic marker sub-study. The letter was followed by a telephone call from an interviewer who arranged a time to meet those who agreed to take part. Prior to the interview, we sent the instructions [8] and materials to collect toenail clippings. Based on the findings of Calderon and colleagues, we asked participants to collect a "first morning" urine sample in the morning of the interview, to place it in an insulated thermos and to refrigerate it until the interviewer arrived. We provided participants with gloves and instructions for sample collection. The interviewer collected a tap water sample using 125 ml HDPE bottles (I-CHEM) following a previously described protocol [3] and asked subjects whether they moved or changed water supplies since they were originally tested.

Laboratory methods

Toenail clipping samples were analyzed for arsenic and other trace elements by the instrumental neutron activation analysis (INAA) at the University of Missouri Research Reactor, using a standard comparison approach as described previously [9,10]. Samples of sufficient weight were split and run in replicate. If the coefficient of variability (CV, mean divided by the standard deviation) exceeded 15%, the batch was recounted to ensure that the mass and signal were accurately measured and recorded. The limit of detection for arsenic measured by INAA is approximately 0.001 μg/g.

Samples of drinking water were analyzed for arsenic concentration using a Finnigan MAT Corp. ELEMENT high resolution inductively coupled mass spectrometer (HR-ICP-MS) equipped with a precision glass blowing corp. VS-1 membrane gas liquid separator to allow hydride generation (HG) for enhanced sensitivity [11]. All sample preparations and analyses were carried out in a trace-metal clean HEPA-filtered-air environment. Analytical blanks and potential instrumental drifts were carefully monitored, and instrument standardization and reproducibility were performed with Certified Standard Reference Materials. The limit of detection, using HG-HR-ICP-MS is 0.01 μg/L, two orders of magnitude lower than conventional methods.

Analyses of arsenic speciation in urine samples were carried out by using ion pair chromatographic separation with hydride generation atomic fluorescence detection (HPLC-HGAFD) [12]. A HPLC system consisted of a Gilson (Middleton, WI) HPLC pump (Model 307), a Rheodyne 6-port sample injector (Model 7725i) with a 20-μL sample loop, and a reversed phase C18 column (ODS-3, 150 • 4.6 mm) with 3 μm particle size packing materials (Phenomenex, Torrance, CA). The column was mounted inside a column heater (Model CH-30, Eppendorf) which was controlled by a temperature controller (Model TC-50, Eppendorf). Column temperature was maintained at 50°C. A mobile phase solution (pH 5.9) contained 5 mM tetrabutylammonium hydroxide, 3 mM malonic acid, and 5% methanol, and its flow rate was 1.2 mL/min. A hydride generation atomic fluorescence detector (HGAFD)

(Model Excalibur 10.003, P.S. Analytical, Kent, UK) was used for the detection of arsenic. The combination of HPLC and HGAFD has been described previously [13]. An aliquot (20 uL) of a urine sample was filtered through a 0.45 μm membrane prior to be subjected to HPLC-HGAFD analysis. The limit of detection for each fraction was 0.5 to 1.0 μg/L.

Statistical Analysis

We linked current data on toenail, water and urine arsenic with data obtained from the original interview (including demographic data, toenail and water analysis). To assess the reproducibility of the water and toenail samples over a three- to five-year period, we used normal random effect models to estimate the intra class correlation between each of the repeated measures along with appropriate confidence intervals. The three water samples with unde- tectable levels were set to 0.005 μg/L, one-half of the usual detection limit, and the two subjects who moved were excluded. We also computed the correlation between measurements, water, urine and toenail concentrations in our population measured at the second interview. In this analysis, we excluded individuals with undetectable uri- nary or tap water arsenic. We summed over urinary frac- tions to obtain total urine concentration. We carefully examined scatter plots for linearity and normality and chose a natural log transformation on this basis.

RESULTS

We enlisted 99 controls previously interviewed in the bio- logic marker reliability study. The mean interval between the original and second interview was 3.8 years (SD = 0.35 years). Forty-four percent were women and 56% were

Table 1. Values of toenail, water and urine levels of arsenic in samples collected during the original and second interviews

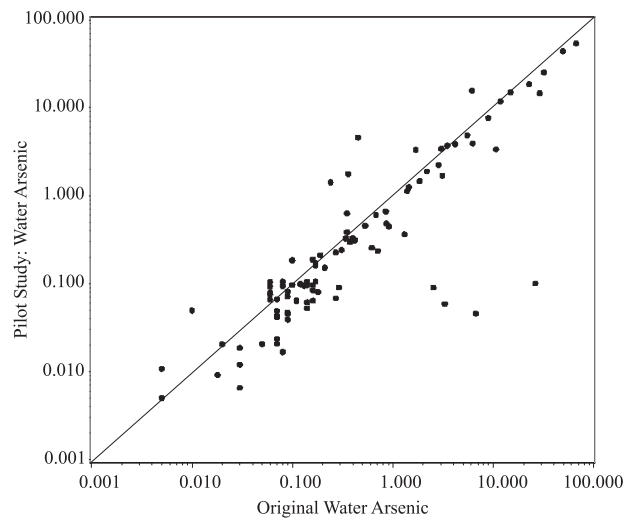|	Original Interview		Second Interview	
	n	Mean (SD)	n	Mean (SD)
Toenail μg/g	99	0.13 (0.10)	96	0.11 (0.08)
Water μg/L	99	3.51 (9.86)	96	2.72 (8.06)
Urine μg/L	–	–	77	9.89 (14.50)

Fig. 1. Water arsenic concentrations at the first and second interviews, Intraclass correlation 0.85 (95% CI: 0.79–0.89).

men with overall mean age of 66.9 years (SD = 10.8). Of these subjects, 96 provided a toenail clipping sample, 92 a urine sample and 98 a water sample.

Concentrations of toenail, water and total urinary arsenic are shown in Table 1. We found detectable levels of arsenic in all toenail, and all but three water samples. Concentration in water ranged from undetectable (<0.01 μg/L) to 54.1 μg/L and in toenails varied from 0.02 to 0.53 μg/g. Seventy-seven out of 91 subjects (84.6%) had at least one detectable fraction of urinary arsenic.
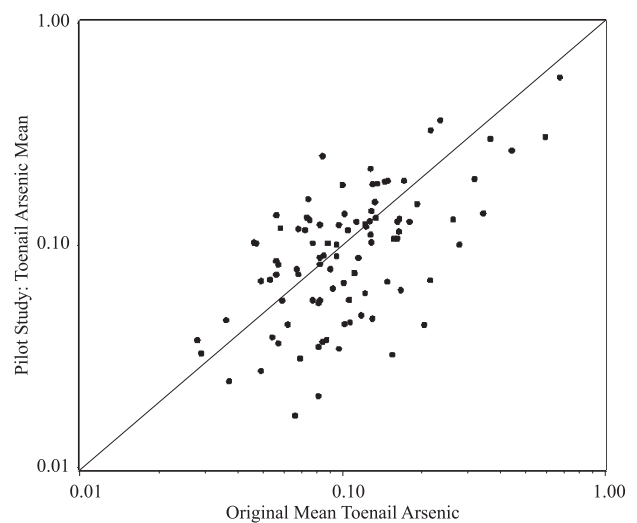

Fig. 2. Toenail arsenic concentrations at the first and second inter- views. Intraclass correlation 0.60 (95% CI: 0.48–0.70).

Table 2. Correlations between tap water, urine and toenail arsenic concentrations from the second interview

	Water Arsenic< 1 μg/L	Water Arsenic> μg/L	Overall
	correlation (p-value)	correlation (p-value)	correlation (p-value)
Urine vs Water	0.02 (p = 0.90)	0.46 (p = 0.029)	0.35 (p = 0.0024)
Toenail vs Water	-0.19 (p = 0.13)	0.64 (p = 0.006)	0.33 (p = 0.0016)
Urine vs Toenail	0.25 (p = 0.071)	0.42 (p = 0.044)	0.36 (p = 0.0012)

We found a strong correlation between the original and subsequent tap water concentrations (intraclass correlation coefficient = 0.85, 95% CI: 0.79 - 0.89) (Fig. 1). Likewise, we found a significant correlation between the replicate toenail measurements (intraclass correlation coefficient = 0.60, 95% CI: 0.48 - 0.70) (Fig. 2).

In our data, both toenail and urine concentrations correlated with water concentrations, particularly among those with water arsenic greater than or equal to 1 μg/L (r = 0.46, p = 0.029 for urine vs water; r= 0.64, p = 0.006 for toenail vs water) (Table 2). Toenail and urine concentrations also correlated with each other (r = 0.36, p = 0.0012 for urine vs toenails) (Table 2).

DISCUSSION

To our knowledge, our study is among the first to evaluate the reliability of various measurements of arsenic exposure in population exposed to low, however potentially carcinogenic, levels of arsenic.

In regions with relatively high drinking water concentrations, urinary arsenic generally correlates with water levels [8,14,15]. In a study of two Chilean towns, inorganic arsenic and the methylated metabolites were strongly correlated with drinking water concentrations in the two towns combined, but correlations within each town were lower [14]. In the town with elevated drinking water arsenic concentrations (up to 670 μg/L), the overall correlation between water and urine levels was 0.26 (p < 0.003, and in the other town, with lower levels (15 μg/L), it was 0.25 (p < 0.009). An important limitation of our study was that a number of subjects had undetectable levels of urinary arsenic (levels below 0.5 or 1.0 μg/L). We also were unable to examine the reproducibility of urine concentra-

tions over time because we only collected urine samples at the second interview. Nonetheless, our results are consistent with the Chilean study, and suggest that other sources of exposure (e.g., diet) may contribute to urinary arsenic levels in populations with low drinking water levels.

Both hair and nails have been used forensically to track arsenic poisoning over a period of several months [5,6]. Airborne, water and food exposure to arsenic each have been related to nail concentrations [1,2]. Nails are generally considered less susceptible to external contamination than hair. They are easy to collect and can be analyzed for an array of trace elements (e.g., selenium, mercury, zinc). In a study of female nurses tested 6 years apart, toenail arsenic concentrations were most highly correlated among the trace elements studied (r = 0.54), suggesting that these measurements reflect exposures over even longer periods [16]. Our data confirm the long-term reproducibility of toenail measures in populations exposed to low levels of arsenic through the drinking water supply.

Exposure assessment based on drinking water samples is an alternative approach to the use of a biomarker, particularly if there is minimal exposure from other sources (i.e., diet or occupational activities). But, the extent to which water concentrations vary over time is not well-characterized. Cebrian and colleagues collected 18 to 20 water samples between 1975 in two Mexican towns, one with a mean concentration of 411 μg/L and another with a mean concentration of 5 μg/L [17]. In the high-exposure town, values varied considerably from 160 μg/L to 590 μg/L (SD = 0.114 μg/L). In the low exposure town, values appeared more stable (SD = 0.007 μg/L). Recently, a study conducted in Maryland, USA found significant variation in water samples taken every two months for one year [18]. Thus, fluctuations in water concentrations may depend on

the water supply and its geochemical characteristics. In our study of New Hampshire drinking water supplies, levels of arsenic remained surprisingly consistent. Moreover, in our previous analysis of 217 households, we found no seasonal pattern in water arsenic levels [7].

In summary, we found that both drinking water and toenail measurements of arsenic remained fairly consistent over a three- to five-year period. In our population, toenail, water and urinary arsenic concentrations were each significantly correlated. But, our results indicated that factors other than drinking water may be contributing to urine and toenail concentrations. Use of biologic markers may more accurately reflect total dose of exposure in populations exposed to low, but potentially carcinogenic levels of drinking water arsenic.

REFERENCES

1. Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Arsenic.* Atlanta: US Department of Health and Human Services; 1993.

2. Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Arsenic*. Atlanta: US Department of Health and Human Services; 1999.

3. Karagas MR, Tosteson TD, Blum J, Morris JS, Baron JA, Klaue B. *Design of an epidemiologic study of drinking water Arsenic exposure and skin and bladder cancer risk in a US population.* Environ Health Perspect 1998; 106: 1047–50.

4. Malachowski ME. *An update on arsenic*. Clin Lab Med 1990; 10: 459–72.

5. Henke G, Nucci A, Queiroz L. *Detection of repeated arsenical poisoning by neutron activation analysis of foot nail segments*. Arch Toxicol 1982; 50: 125–31.

6. Schroeder H, Balassa J. *Abnormal trace metals in man: arsenic.* J Chron Dis 1966; 19: 85–106.

7. Karagas MR, Tosteson TD, Blum J, Klaue B, Weiss JE, Stannard V, et al. *Measurement of low levels of arsenic exposure: a comparison of water and toenail concentrations.* Amer J Epidemiol 2000; 152 [in press].

8. Calderon RL, Hudgens E, Le XC, Schreinemachers D, Thomas D. *Excretion of arsenic in urine as a function of exposure to arsenic in drinking water.* Environ Health Perspect 1999; 107: 663–7.

9. Cheng TP, Morris JS, Koirtyohann SR, Spate VL, Basket CK. S*tudy of the correlation of trace elements in carpenter's toenails*. J Radioanal Nucl Chem 1995; 195: 31–42.

10. Nichols TA, Morris JS, Mason MM, Spate V, Baskett CK, Cheng TP, et al. T*he study of human nails as an intake monitor for arsenic using neutron activation analysis.* J Radioanal Nuclear Chem 1998; 236: 51–6.

11. Klaue B, Blum JD. *Trace analysis of Arsenic in drinking water by inductively coupled plasma mass spectrometry: High Resolution versus Hydride Generation*. Anal Chem 1999; 71: 1408–14.

12. Le XC, Lu X, Ma M, Cullen WR, Aposhian V, Zheng B. S*peciation of key arsenic metabolic intermediates in human urine*. Anal Chem 2000; 72: 5172–77.

13. Le XC, Ma M. *Short-column liquid chromatography with hydride generation atomic fluorescence detection for the speciation of arsenic.* Anal Chem 1998; 70: 1926–35.

14. Biggs ML, Kalman DA, Moore LE, Hopenhayn-Rich C, Smith MT, Smith AH. *Relationship of urinary arsenic to intake estimates and a biomarker of effect, bladder cell micronuclei.* Mutat Res 1997; 386: 185–95.

15. Chiou H, Hsueh Y, Hsieh L, Hsu L, Hsu Y, Hsieh F, et al. A*rsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic exposed residents in Taiwan*. Mutat Res 1997; 386: 197–207.

16. Garland M, Morris JS, Rosner BA, Stampfer MJ, Spate VL, Baskett CJ, et al. *Toenail trace element levels as biomarkers: reproducibility over a 6-year period* [published erratum appears in Cancer Epidemiol Biomarkers Prev 1994 Sep;3 (6): 523]. Cancer Epidemiol Biomarkers Prev 1993; 2: 493–7.

17. Cebrian ME, Albores A, Aguilar M, Blakely E. *Chronic arsenic poisoning in the north of Mexico.* Human Toxicol 1983; 2: 121–33.

18. Ryan PB, Huet N, MacIntosh DL. *Longitudinal investigation of exposure to arsenic, cadmium, and lead in drinking water*. Environ Health Perspect 2000; 108: 731–5.