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Short Communication

Toenail Samples as an Indicator of Drinking Water Arsenic Exposure[1]

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Abstract

We conducted a pilot study to assess the utility of toenail arsenic concentrations as an indicator of ingestion of arsenic-containing water. We enrolled 21 individuals whose household drinking water supply was provided by a private well, including 10 individuals who lived in areas of New Hampshire where elevated water levels of arsenic had been reported previously. Participants were interviewed regarding use of their private (unregulated) wells for drinking and cooking, and each provided a sample of water and toenail clippings. All specimens were analyzed using instrumental neutron activation analysis with a sensitivity of approximately 0.001 parts per million (ppm). Trace concentrations of arsenic were detected in 15 of the 21 well water samples and in all toenail clipping samples. Among the 10 individuals who lived in areas with reportedly high arsenic levels in the water supply, the geometric mean toenail concentration was 0.39 ppm (SE, 0.12 ppm); among the other 11 persons, the geometric mean was 0.14 ppm (SE, 0.02 ppm; $P = 0.005$ for the difference between the two means). The overall Spearman correlation between toenail and well water arsenic was 0.67 ($P = 0.009$), and among those with detectable well water levels of arsenic, the Spearman correlation was 0.83 ($P = 0.0001$). Based on the regression analysis of those who had detectable water levels of arsenic, a 10-fold increase in well water concentrations of arsenic was reflected by about a 2-fold increase in toenail concentrations. These results indicate that concentrations of arsenic in toenails reflect use of arsenic-containing drinking water.

Introduction

Inorganic arsenic is present in a variety of ores and minerals and is fatal to humans in large doses (*e.g.*, about 2000 μg/kg/day; Ref. 1). An increased risk of cancers of the skin, lung, and other sites has been associated with exposure to high concentrations of arsenic in drinking water (2, 3), but virtually all studies conducted to date have relied on ecological measures (*e.g.*, classification based on water arsenic levels in a particular village or area rather than individual assessment; Ref. 4). Use of a biological marker of arsenic exposure would permit a more direct measure of disease risk associated with exposure, yet no marker has been validated for these purposes.

Arsenic can appear initially throughout the body, but it clears rapidly from most tissues, including the blood stream. After methylation in the liver, it is excreted in the urine (1); as a result, blood and urine concentrations reflect only relatively recent exposure. Because of its affinity for the sulfhydryl groups of keratin, arsenic will remain in scleroproteins (1). Therefore, the concentration of arsenic in hair and nail tissue can be used to track arsenic poisoning (5, 6). Toenail clippings are relatively easy to collect, and they have been used successfully to estimate intake of other trace elements (*e.g.*, selenium and zinc) in large epidemiological investigations (7); compared with fingernails, toenails provide a larger sample and represent exposures in the more distant past (because they take longer to grow out). As pilot work for a population-based case-control study of skin cancer, we assessed the suitability of toenail clipping samples as an indicator of ingestion of arsenic-containing water.

Materials and Methods

In May and June of 1991, we telephoned households in regions of New Hampshire where elevated arsenic levels had been detected previously in private water supplies by the New Hampshire Department of Environmental Services. We excluded households served by a public water source or by a private well serving 25 or more individuals (or 15 or more households); these sources were covered by Federal Safe Drinking Water Standards and thus should not contain appreciable levels of arsenic. We further restricted our sample to individuals who had lived in the current residence at least 1 year and who reported using their well water for drinking or cooking at least some of the time. For comparison, we selected a convenience sample of volunteers who resided in areas of New Hampshire or bordering regions of Vermont where elevated arsenic levels in well water had not been reported previously. The final study group included 10 individuals from areas with known arsenic and 11 from areas without reported arsenic in the water supply. No study participants shared the same water supply.

Using a structured interview, we ascertained participants' demographic characteristics, well types (soil, bedrock/artesian, and other), use of water treatment devices, and the proportion of time they used their household wells for drinking and cooking (<1/4, 1/4, 1/2, 3/4, or >3/4 of the time). A tap water sample was collected from each household in a 125-ml Nalgene polyethylene bottle. Toenail clipping samples were provided by the study participants in labeled paper envelopes.

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Table 1 Geometric mean toenail concentrations of arsenic by selected characteristics of study participants

Characteristic	No. of participants	Geometric mean concentration of arsenic (ppm)	SE	*P* value
Age (yr)				
<40	5	0.28	0.09	
40–59	11	0.22	0.05	
≥60	5	0.21	0.14	0.85
Sex				
Men	8	0.21	0.05	
Women	13	0.25	0.07	0.65
Type of well[a]				
Soil	4	0.18	0.07	
Bedrock/Artesian	16	0.23	0.05	0.83

[a] Type of well was unknown for one study participant.

Laboratory Methods. Drinking water samples were prepared for INAA[3] by transferring a total of 10 ml of water in 1-ml aliquots into high-purity quartz vials fashioned from quartz tubing with inside and outside diameters of 5 and 7 mm, respectively. Each 1-ml aliquot was quantified by mass and then dried using a centrifugal freeze-drier prior to the addition of the next aliquot. After the last aliquot had been added and dried, the quartz vial was heat-sealed under vacuum. Standards containing known amounts of arsenic and quality control samples were also prepared in quartz vials.

Toenail specimens were first scraped with a hard plastic edge to remove any attached debris and then cleaned by sonication, first in acetone to remove natural oils, and then by demineralized water to remove the acetone and loosen or dissolve surface contaminants. The nails were oven dried at approximately 50°C before being weighed and vacuum sealed into quartz vials of the type described above. Sample sizes ranged from 3 to 90 mg. The larger samples were subdivided into duplicate specimens.

Samples were analyzed for arsenic by INAA at the University of Missouri Research Reactor, using a standard comparison approach as described previously (8). Using this approach, samples are not subjected to chemical or thermal destruction, and thus mechanical or chemical (volatility) loss, separation yield, matrix modification, and external contamination are reduced substantially or eliminated entirely. Matrix-matched quality control samples, having known arsenic content, and analytical blanks were analyzed with the samples and standards. For this study, two standard reference materials were used that bear spectral resemblance to the samples [Orchard Leaves (NBS SRM 1573) and Bovine Liver (NBS SRM 1577), National Institute of Standards and Technology], as well as separate solution standards containing keratin and arsenic. In all cases, the arsenic concentrations measured in these quality control samples were in good agreement (within 1 SD) with the certified values or accepted values (*i.e.*, for keratin). The detection limit for arsenic measured by INAA is approximately 0.001 ppm.

Statistical Analysis. Water and nail concentrations were log transformed to provide more normally distributed data. Geometric means for individuals residing in reportedly high- and low-arsenic areas were compared using a *t* test. To estimate an overall Spearman correlation between the log-transformed measures of arsenic in toenails and arsenic in drinking water, a value of 10^{-5} ppm was added to the undetectable values of arsenic in drinking water. This value is in the range of the sensitivity of the equipment used to detect arsenic in a 10-ml water sample. Geometric means and SEs (9) were calculated for toenail arsenic concentrations for selected characteristics of the study participants. A regression analysis (10) was used to examine the relation between concentration of arsenic in toenails and detectable levels of arsenic in drinking water. Regression coefficients and 95% confidence intervals were transformed to the original scale to produce proportional changes and confidence intervals for geometric water and toenail arsenic concentrations. The statistical package SAS (11) was used, and all statistical tests were two sided.

Results

The study group included 8 men and 13 women, with a mean age of 47.6 years (SD, 16.1 years). Concentrations of arsenic in the well water specimens ranged from nondetectable to 0.137 ppm. Fifteen (71%) of the 21 specimens had detectable levels of arsenic. Three specimens had concentrations at or above the current Environmental Protection Agency standard of 0.05 ppm, and eight exceeded the 0.002 ppm proposed standard (12).

All 21 individuals had detectable arsenic in their toenail specimens. Toenail concentrations of arsenic ranged from 0.073 to 2.25 ppm (geometric mean, 0.23 ppm; SE, 0.05 ppm). The geometric mean toenail concentration was 0.39 ppm (SE, 0.12 ppm) for the 10 individuals who lived in areas with reportedly higher arsenic levels; among the other 11 individuals, the geometric mean concentration was 0.14 ppm (SE, 0.02 ppm; *t* test, $P = 0.005$). Geometric mean toenail concentrations did not differ appreciably by age, sex, or type of well (Table 1).

Toenail arsenic concentrations were highest among individuals whose well water samples contained higher levels of arsenic. The overall Spearman correlation between log-transformed values for toenail and well water arsenic was 0.67 ($P = 0.009$). Among the 15 persons with detectable well water levels of arsenic, the correlation was 0.83 ($P = 0.0001$; Fig. 1). When we restricted the analysis to those who reported using their well for drinking and cooking at least 75% of the time, the correlations were 0.76 ($P = 0.0004$) overall, and 0.92 ($P = 0.0001$) among those with arsenic-containing drinking water. On the basis of the regression analysis of those who had detectable water levels of arsenic, a 10-fold increase in well water concentrations of arsenic was reflected by about a 2-fold increase in toenail concentrations (Table 2).

[3] The abbreviations used are: INAA, instrumental neutron activation analysis; ppm, parts/million.

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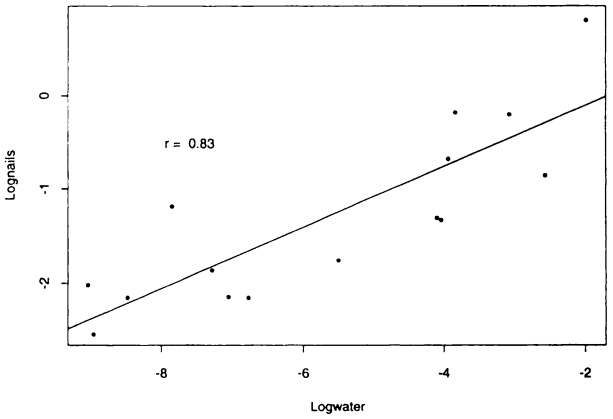

Fig. 1. Correlation between well water and toenail concentrations of arsenic in the 15 study participants who had detectable concentrations of arsenic in their drinking water.

Table 2 Proportional changes (and confidence intervals) in toenail concentrations of arsenic associated with a 10-fold increase in drinking water levels, among those with detectable arsenic in their drinking water

Model[a]	Number of subjects	Proportional change (95% confidence interval)[b]	*P* value
1	15	2.12 (1.63–2.75)	0.0001
2	12	2.16 (1.65–2.84)	0.0002

[a] Model 1 includes the total sample of subjects with detectable arsenic concentrations in their drinking water; model 2 is restricted to those who reported using their well for drinking or cooking at least 75% of the time.
[b] Proportional change and 95% confidence interval estimates were obtained from a regression analysis using the log-transformed values. Estimates shown were converted to the original scale.

Discussion

Our data indicate that drinking water was a primary contributor to toenail concentrations of arsenic among the individuals we studied. Under the current Environmental Protection Agency's Safe Drinking Water Act of 1974, public water supplies must be maintained at levels below 0.05 ppm; however, these regulations do not apply to private (*e.g.*, individual household) wells, which are used commonly in rural areas. Elevated levels of arsenic have been reported in several drinking water supplies in the United States (1), including those in New Hampshire. Although the source of arsenic in the drinking water is not known for the region we studied, natural mineral deposits as well as pesticide or waste-site contamination are possible contributors (1).

A limitation of our study was the absence of detailed information regarding diet, tobacco use, and water consumption on our study participants. Trace amounts of arsenic are found in a variety of foods, including grains, cereals, meat, fish, and poultry (13). Seafood contains the greatest concentrations of arsenic, mostly in the organic (methylated) form, which is essentially nontoxic, but elevated levels of inorganic arsenic have been reported in seafood as well (1). However, because concentrations of arsenic in foods are highly variable, it may be impossible to estimate an individual's intake of arsenic based on their dietary history. In a study of United States nurses,[4] fish intake was not associated with toenail arsenic concentrations. Concentrations of arsenic ranging from 0.01 to 5 ppm are also found in tobacco, presumably because of pesticide residuals (1). The correlations we observed suggest that the contribution of diet and tobacco use are minimal among individuals who consume arsenic-containing water. Also, although we lacked information on actual quantities of water consumed, this did not appear to limit our ability to detect an association between water levels and nail concentrations of arsenic.

To our knowledge, this is the first study to report a correlation between concentrations of arsenic in drinking water and nails. In a similarly designed study, Agahian *et al.* (14) found a strong correlation ($r = 0.89$) between arsenic concentrations in fingernails and air samples among workers in a gold mining operation. Another study examined nail levels of arsenic among residents of two Mexican towns, one with significant drinking water levels of arsenic (0.41 ppm), and the other with relatively low levels (0.005 ppm). Residents in the town with high arsenic content had significantly greater nail concentrations of arsenic than was found in the control town, but the correlation between the two measures was not assessed (15). In a highly exposed Alaskan population, individuals with elevated toenail concentrations (>8 ppm) were significantly more likely to consume well water containing 0.1 ppm or more of arsenic, but the overall correlation between well water and toenail concentrations was low ($r = 0.16$; Ref. 16). The authors hypothesized that nails may be a better integrated measure of past exposure, and thus did not necessarily reflect the household tested for arsenic. For this reason, we restricted our study sample to those who had lived in their current residence at least 1 year, the usual length of time it takes for a toenail to grow out (7). Our assay for arsenic determinations was also more sensitive than the atomic absorption methods used previously. The reliability of several trace elements as long-term measures of exposure was assessed by Garland *et al.* (17) by analyzing two toenail clipping samples taken 6 years apart from 127 United States nurses. The correlation for arsenic values between the two samples was among the highest of the 16 trace elements studied ($r = 0.54$).

In conclusion, quantification of arsenic concentrations in toenail clipping samples provides a measure of exposure on an individual level. The long-term effects of exposure to arsenic at the levels experienced by the general population of the United States are largely unknown, and studies using individual measures of arsenic exposure could provide more useful data than those involving ecological measures. Our study results suggest that arsenic determinations using INAA of toenail clipping samples constitute a useful biological marker of arsenic exposure, particularly among people whose drinking water contains arsenic.

[4] M. Garland, personal communication.

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