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Trace elements concentrations in biological samples and their correlation with urinary arsenic metabolites of chronic arsenic exposed people

Uttam K Chowdhury*

[a]*School of Environmental Studies (SOES), Jadavpur University, Kolkata 700032, India*

[b]*National Institute of Health Sciences (NIHS), Tokyo, Japan*

[c]*Department of Molecular and Cellular Biology, The University of Arizona, Life Sciences South, Tucson, Arizona, 85721-0106, USA*

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ABSTRACT

We collected and analyzed groundwater and other biological samples for the determination of arsenic and other trace elements from an arsenic exposed group and an unexposed group (control group) of people in Bangladesh. The results showed that arsenic in hair, nail, and urine increases with increasing arsenic in drinking water. Along with arsenic in hair and nail samples, I also analyzed Se, Zn, Cu, Hg, Pb, and Sb by ICP-MS after microwave digestion. In both cases (hair and nail), it appears that the mean concentration of Se and Zn are lower in the exposed group than in the control group. Again, the mean concentration of Hg, Pb, and Sb are higher in both hair and nail for the exposed group than the control group.

The regression analyses were carried out between arsenic and other metals and metalloids in hair and nail samples. The linear regression shows negative correlation between As & Se (r = - 0.84, p = 0.00005, n = 16) and As & Zn (r =- 0.78, p =0.0003. n = 16); some positive correlation between As & Pb (r = 0.58, p = 0.008, n = 19), As & Hg (r = 0.745. p 0.0002, n = 19) and As & Sb (r = 0.743, p = 0.0002, n = 19); no significant correlation between As & Cu (r = -0.04, p = 0.87, n = 17) for hair samples. For nail samples, a similar correlation was observed as in hair. The linear regression shows negative correlation between As & Se (r = - 0.53, p = 0.004, n = 27) and As & Zn (r = - 0.55, p = 0.0006, n = 35) and some positive correlation between As & Hg (r = 0.47, p = 0.004, n =35), As & Pb (r = 0.51, p = 0.003, n = 33), and As & Sb (r = 0.57, p = 0.0004, n = 33); no significant correlation between As & Cu (r = 0.15, p = 0.41, n =31) observed.

Also, urine and blood samples were collected from 191 subjects (98 females and 93 males) in the Lagunera area of Mexico. There were five groups that were based on total arsenic concentration (38-116 µg/L) in their drinking water. In urine, statistically significant correlations were not found between the concentrations (µg/g cre) of Se as well as Zn and % inorg As, % MMA, % DMA, as well as the ratios of % DMA to % MMA in urine for females. However, the relative concentrations of Se to As expressed as µg/g cre were positively and significantly correlated with % inorg As (r_s= +0.29, p<0.01) & % MMA (r_s= +0.25, p<0.05) and negatively correlated with % DMA (r_s= -0.34, p<0.001) & the ratios of % DMA to % MMA (r_s= -0.31, p<0.01) in urine for females. For males, the ratios of the concentrations (µg/g cre)) of Se or Zn to As were more positively correlated than the concentrations of Se or Zn were with % inorg As levels (r_s= +0.26, p<0.05 vs. r_s= +0.17 not significant and r_s= +0.22, p<0.05 vs. r_s= +0.086 not significant, respectively), but it was more negatively correlated with % DMA levels (r_s= -0.25, p<0.05 vs. r_s= -0.12 not significant and r_s= -0.19 not significant vs. r_s= -0.008 not significant, respectively) in urines.

In blood, the relative concentrations of Se or Zn to As than the corresponding element concentrations in blood were more significantly and positively correlated with urinary % inorg As (r_s= +0.36, p<0.001 vs. r_s= +0.11 not significant and r_s= +0.24, P<0.05 vs. r_s= -0.10 not significant, respectively), and negatively correlated with % DMA (r_s= -0.34, p<0.001 vs. r_s= -0.12 not significant and r_s= -0.24, p<0.05 vs. r_s=-0.005 not significant, respectively, and with the ratios of % DMA to % MMA (r_s= -0.32,

$p<0.01$ vs. $r_s=-0.063$ not significant and $r_s= -0.29$, $p<0.01$ vs. vs. $r_s=-0.049$ not significant, respectively) for females. The ratios of the concentrations of Se or Zn to As than the concentrations of Se or Zn in blood were also more positively and significantly correlated with urinary % MMA ($r_s= +0.27$, $p<0.01$ vs. $r_s= +0.033$ not significant and $r_s= +0.26$, $p<0.05$ vs. $r_s= +0.05$ not significant, respectively) for females. For males, better and significant correlations found between the ratios of the concentrations of Se or Zn to As in blood and the percentage of urinary arsenic metabolites than the correlations found between the corresponding element concentrations in blood and the percentage of urinary arsenic metabolites. The ratios of the concentrations of Se or Zn to As in blood were more positively correlated with urinary % inorg As ($r_s= +0.25$, $p<0.05$ vs $r_s= -0.043$ not significant and $r_s= +0.26$, $p<0.05$ vs. $r_s= +0.036$ not significant, respectively). The ratios of urinary % MMA to % inorg As were negatively correlated with the ratios of the concentrations of Se or Zn to As ($r_s= -0.20$ not significant and $r_s= -0.23$, $p<0.05$, respectively) in blood. But these correlations were not statistically significant with the concentrations of Se or Zn in blood. Also, the ratios of the concentrations of Se or Zn to As in blood were negatively correlated with the ratios of urinary % DMA to % MMA for males, but these correlations were not statistically significant.

In conclusions, the relative concentration of Se or Zn to As may be an important factor for arsenic methylation process. Also, the doses of Se and/or Zn may not be the same for everyone, and it could be dependent on the concentrations of arsenic in their drinking water.

∗Corresponding author. Tel.: +1-(520) 820-5861; e-mail: ukchowdh@email.arizona.edu

Background

There are many countries in the world where cases of arsenic contamination of groundwater are known. Out of these, Bangladesh calamity (Figs. 1 and 2) is the largest in the world.

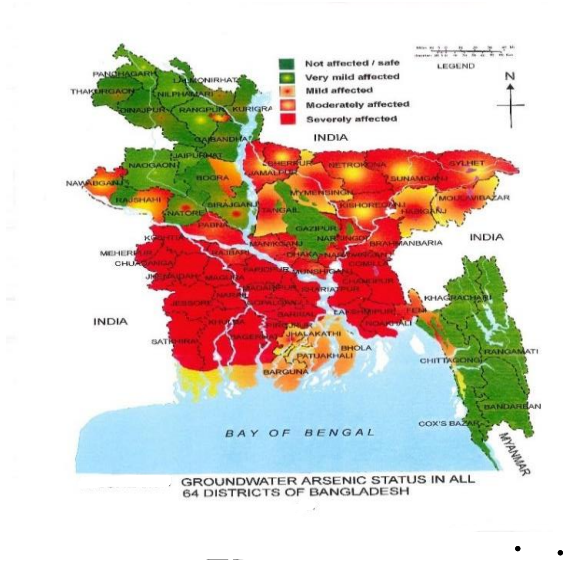

Figure 1: Groundwater arsenic status in all 64 districts

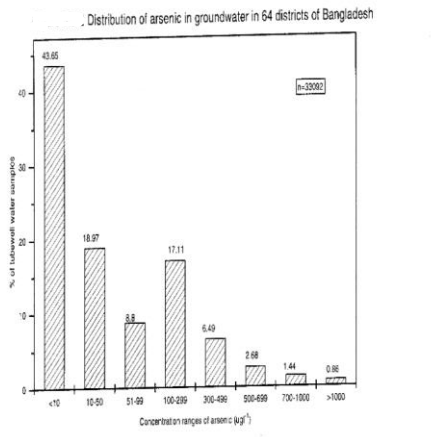

Figure 2: Distribution of arsenic in groundwater of Bangladesh in 64 districts of Bangladesh (n= 33,092)

Arsenic in urine, hair, nail, and blood are the biomarkers to measure the absorbed dose of inorganic arsenic. The stability of the various biomarkers to serve as indicators of acute or chronic exposure to inorganic arsenic and the various factors (e.g., dietary intake of arsenic compounds) that can influence the indicators - is important. Although absorbed arsenic is removed from the body mainly via the urine, a small amount of arsenic is removed via other routes (e.g., skin, nails, hair, sweat, and breast milk).

The concentration of total arsenic in urine has often been used as an indicator of recent exposure because urine is the main route of excretion of most arsenic species[1,2]. Analyses of blood, hair, and fingernail samples are also good indicators of

exposure. Because of the short half-life of As in blood, hematological estimation is useful in particular in the diagnosis of acute intoxication. Inorganic As is incorporated into hair and fingernails due to its affinity to the sulfhydryl groups in keratin. Following exposure to inorganic arsenic, the biological half time is about 4 days. It is slightly shorter following exposure to As(V) than to As(III)[3-6]. In a study of six humans, subjects who ingested radiolabeled ^{74}As-arsenate, 38% of the dose was excreted in the urine within 48 hours and 58% within 5 days[4]. The results indicate that the data were best fit to a three-compartment exponential function, with 65.9% excreted with a half time of 2.09 days, 30.4% with a half-time of 9.5 days, and 3.7% with a half-time of 38.4 days[5]. In three subjects, each of whom ingested 500 µg of arsenic in the form of arsenite in water, about 33% of the dose was excreted in the urine within 48 hours and 45% within 4 days[6]. The methylated metabolites MMA and DMA are excreted in the urine faster than the inorganic arsenic. In humans, about 78% of MMA and 75% of DMA were excreted in the urine within 4 days of ingestion of the dose[6]. In another study, two subjects ingested mineral water containing 200 µg As(V) and about 66% of the dose was excreted over 7 days[7]. Several studies on human subjects exposed to inorganic arsenic occupationally, experimentally, or environmentally have shown that, in general, U-As met consists of 10-30% inorganic arsenic (iAs), 10-20% MMA, and 60-80% DMA[8,9].

Arsenic concentrations are normally higher in hair and nail than in other parts of the body because of the high content of keratin (and the corresponding high content of cysteine). The -SH groups of keratins might bind trivalent inorganic arsenic (As^{III})[10-12]. The concentration of arsenic in the root of the hair is in equilibrium with the concentration in the blood. Hair might be considered an excretory pathway, and once incorporated in the hair, the arsenic is not biologically available. Arsenobetaine, the major organic arsenic compound in seafood, is not accumulated in hair[13]. That implies that arsenic in hair reflects exposure to inorganic arsenic only. But the speciation of arsenicals in water extracts of fingernails and hair at 90°C was carried out by HPLC-ICP-MS[14]. The results show that fingernails contained iAs^{III} (58.6%), iAs^{V} (21.5%), MMA^{V} (7.7%), DMA^{III} (9.2%), and DMA^{V} (3.0%); and hair contained iAs^{III} (60.9%), iAs^{V} (33.2%), MMA^{V} (2.2%), and DMA^{V} (3.6%)[14]. Fingernails contained DMA^{III}, but hair did not. The higher percentage of iAs^{III} both in fingernails and hair than that of iAs^{V} and other species suggest more affinity of iAs^{III} to keratin.

Segmental hair analysis (i.e., determination of the concentration along the length of the hair) might provide valuable information on the time of acute arsenic exposure[15-17]. Human hair grows at the rate of approximately 0.35 mm per day or 13 mm a month. Nail and hair have similar affinities for arsenic but data on nails is limited. However, the main disadvantage of using hair and nail as indicators of exposure to arsenic is that the arsenic concentrations might be influenced by external contamination via air, water, soaps, shampoos, and soil, and cannot be readily removed by washing. Therefore, external arsenic contamination must be completely excluded if hair and nail arsenic levels are to be used for assessment of poisoning. Thus, arsenic concentration in hair and nail can be used as markers / indicators of exposure rather than markers of absorbed dose[18].

Most of the absorbed inorganic and organic arsenic has a short half-time in blood, following a three- exponential clearance curve[19,20]. Most of the arsenic in blood is cleared with a half-time of about l hour. The half-times of the second and third phases are about 30 hr and 200 hr, respectively. Arsenic concentrations in blood are increased only for a very short time following absorption. If exposure is continuous and steady, as is often the case with exposure through drinking water, the blood arsenic concentration might reach a steady -state and then reflect the degree of exposure.

Blood is a difficult matrix for chemical analysis, so in general, only total arsenic concentrations are reported. Partial speciation of arsenic in blood has been reported in a few cases[21,22]. When using total arsenic in blood as an indicator of exposure to inorganic arsenic, the interference from organic arsenic compounds originating from seafood must be considered. Furthermore, because of the low concentrations, the analytical error might be significant unless the more-sensitive methods are used. In a population in northern Argentina with known exposure to inorganic arsenic and very little fish intake, the average blood arsenic concentration was 0.9 µg/L in children and 1.5 µg/L in adults[22-24].

In subjects exposed to arsenic via drinking water, blood arsenic concentrations are clearly increased and might reach several tens of micrograms per liter[23,25]. In people exposed to arsenic in drinking water (200 µg/L) in northern Argentina, arsenic concentration in blood was about 10 µg/L on average[22-24]. In studies carried out in California and Nevada, an arsenic concentration of 400 µg/L in the water corresponded to about 13 µg/L in the blood, and 100 µg/L in the water corresponded to 3-4 µg/L in the blood[25]. Obviously, compared with urine, blood is a much less sensitive biomarker of exposure to arsenic via drinking water.

Interaction of arsenic with selenium and zinc are very well known. Nutrition is the main source of selenium exposure of the general population. The content of this element in drinking water is low. Several studies demonstrated that low Se is an efficacious anticarcinogen whereas high Se can induce carcinogenesis, cytotoxicity and genotoxicity[26,27]. Some studies reported that As and Se can induce similar toxicity via different pathways[28].

Metabolic and toxicologic interactions between arsenic and selenium are multifaceted and complex. These interactions are of practical significance because large populations are simultaneously exposed to inorganic As in drinking water and varying levels of Se in the diet. Weanling female B6C 31-1 mice were maintained for 28 days on Torula yeast-based diets deficient (0.02 ppm. Se), sufficient (0.2 ppm Se) or excess (2.0 ppm Se) in Se; mice then received by oral dose either 0.5 or 5 mg (As)/kg as [^{73}As] sodium arsenate or 0.5 mg (As)/kg as [^{73}As] sodium arsenite[29]. Selenium deficient mice dosed with 5 mg (As)/kg As^{V} exhibited slower whole-body clearance from 8 hours-onward[29]. Total (urine and feces) cumulative excretion of As derived radioactivity was significantly lower in Se deficient As^{V} exposed mice at both dose levels compared to Se sufficient mice[29]. Significantly less As-derived radioactivity was also excreted in the feces of Se deficient mice exposed to 5 mg (As)/kg As^{V} compared to Se sufficient mice[29]. There was also a trend towards lower cumulative excretion of dimethylarsinic acid in urine of Se deficient compared to sufficient mice which was significant for mice exposed to As(III)[29]. Overall, studies indicate that Se deficiency is associated with altered As metabolism and deposition.

A new glutathione-arsenic selenium compound has been detected recently in rabbit bile[30]. Gaiter et al. (1999), injected intravenously with sodium selenite followed immediately with

intravenous sodium arsenite. Within 25 min a compound containing arsenic, and selenium was excreted in the bile. This newly compound, the seleno-bis (S-glutathionyl) arenium ion, explains a likely mechanism by which selenium prevents arsenic toxicity.

Inorganic arsenic increases the rate of formation of lipid peroxides and free radicals[31]. These compounds are associated with cancer as well as with cardiovascular diseases. Experiments have shown that arsenic exposure decreases the selenium content, which counteracts lipid peroxidation[32].

The clinical relevance of the interaction of arsenic and zinc is more tenuous. Injected parenterally, zinc protects mice against acute arsenic toxicity by way of an unknown mechanism[33], not related to the induction of metallothionein. Lin and Yang[34] measured unusually low zinc concentrations in blood and urine of Blackfoot-disease patients in Taiwan. Engel and Receveur[35] estimated the nutritional adequacy of the diet of the Taiwanese population in the Blackfoot-disease endemic area and believed that only zinc might be present in inadequate amounts. Therefore, important factors to consider in an evaluation diet to inhibit arsenic toxicity are methionine, cysteine, vitamin B_{12}, and folic acid as well as essential trace elements such as selenium and zinc.

A marginal zinc status could play a role in the severe vascular manifestations of chronic arsenic exposure with its atherosclerotic and thrombotic manifestations[36-38]. Low zinc could make endothelial cells more vulnerable to arsenic because zinc appears to be important in vascular endothelial barrier function[39,40] and membrane integrity in general[41]. Zinc has been reported to inhibit tumor necrosis factor-induced disruption of endothelial cell integrity[39] and tumor necrosis factor-mediated DNA fragmentation and cytolysis of murine cells[42].

Glutathione is involved in arsenic methylation[43] and may stimulate prostacyclin (an inhibitor of platelet aggregation and smooth muscle cell proliferation) in arsenic-exposed endothelial cells[44]. In zinc-deficient rats, blood glutathione levels and glutathione S-transferase activities were decreased[45,46] and were restored following zinc replacement[46].

Oxygen radicals may be involved in arsenite-induced damage for the addition of the radical- scavenging enzyme superoxide dismutase that decreased the frequency of arsenite-induced sister chromatid exchanges in human peripheral lymphocytes[47]. In rat liver cells, the activity of superoxide dismutase increased upon incubation with physiologic zinc levels[48].

In this study, I found that arsenic methylation decreased with increasing the relative concentration of Se or Zn to As in urine and blood of arsenic exposed people. There was also negative correlation with As and Se or Zn, but positive correlation with As and Hg, Pb or Sb in both hair and nail of chronic arsenic exposed people.

Methods and Materials

Reagents

All reagents were of analytical reagent grade and Milli Q water was used throughout[49-53].

Analytical procedures

Sample collection and preservation

We collected and analyzed the groundwater and other biological samples for determination of arsenic and other trace elements from an arsenic exposed group and a controlled group of people in Bangladesh. The sampling and preservation procedures are given below:

Water samples

Water samples were collected from both shallow and deep tube wells in pre-washed (with 1:1 $HN0_3$) polyethylene bottles after pumping off at least for 3-5 minutes. After collection concentrated nitric acid (1.0 ml per liter) was added as preservative[54]. Samples, which were not analyzed immediately, were kept in a refrigerator at 4°C.

Hair samples

The hair samples were collected from the people with arsenical skin lesions and without skin lesions from the arsenic affected areas of Bangladesh. A few samples were collected from the control zone of Bangladesh and West Bengal-India where arsenic in groundwater is below 3 µg/L. Samples were collected whenever possible close to the scalp by using stainless-steel scissors. At first the hair samples were washed by distilled water, followed by deionized water and finally with acetone (E. Merck, India Limited) as recommended by International Atomic Energy Agency[55]. After that it was dried in a hot oven at a temperature of 50-60°C and properly stored in polyethylene bottles or white and fresh paper packets with the proper level for the next step.

Nail samples

The nail samples (nails from hand and feet combined) were collected from the people of arsenic affected areas and few samples were also collected from the control zone. After collection the samples were washed by distilled water until free from dust, followed by deionized water and finally with acetone[55]. It was then dried in a hot oven at a temperature of 50-60°C and then stored in polyethylene bottles or white and fresh paper packets with proper level.

Skin-scale samples

Samples were collected from severely affected people with hyperkeratosis on the palm and sole. The affected skin becomes soft in warm water to make it easy to cut using stainless steel scissors / ceramic cutters. After collection the samples were washed and dried in the same way adopted for the hair/nail samples and properly stored with proper level[56,57].

Urine samples

Spot urine samples were collected from the people of arsenic affected areas in pre-washed polyethylene bottles during sampling when first void samples were not available, and few samples were collected from the control zone. The samples were not subjected to any chemical treatment. Immediately after collection, the samples were stored in a cool box with ice and later, after being brought back to the laboratory, kept at- 20°C until analyses were carried out.

Sample treatment for analysis

Decomposition of many biological substances by HNO_3, alone under pressure in Teflon vessels at temperature up to l60°C has been found to be incomplete. But for decomposition of hair, nail, and organs less violet, decomposition procedures e.g., mixture of nitric and with sulfuric acid[58] or nitric acid with hydrogen peroxide[59] or nitric acid alone[60] are adequate. In this study, HNO_3-H_2O_2 were used for the decomposition of hair, nail, and skin-scale for some specific samples. In our earlier publication[61], Teflon bomb acid digestion had been discussed for the determination of arsenic in hair, nail, and skin-scale samples. But for routine analysis of large numbers of samples,

this procedure is not suitable. Hot plate acid digestion procedure is very simple, and a large number of samples can be digested at a time by this technique which I had adopted throughout this study. To check the validity of this method, a known amount of arsenic was spiked in hair samples during digestion. Percentage recovery (98± 8%) was evaluated by spiking fifteen hair samples with known amounts of arsenic(V). Standard hair sample (NCSDC 73347) was analyzed by this procedure and found in good agreement with certified value.

Acid digestion of hair, nail, and skin-scales samples

Hair samples

0.02 to 0.07 g of hair sample was taken in a 25 ml Borosil glass beaker and added 5 ml concentrated nitric acid (E. Merck. India). Then closed the lid and heated on a hot plate with a temperature of 90- 100°C for a few minutes. Heating was discontinued and kept overnight. Next morning the lid was opened and l ml concentrated HNO_3 added again, and the sample evaporated about l00°C in an exhaust chamber. Nitric acid was added, if necessary, till the color of the solution turned into pale- yellow. On reaching a final volume of about l ml, heating was discontinued. The pale-yellow liquid was diluted and filtered through a Millipore membrane (0.45µm) filtering apparatus, then adjusted to a fixed volume[61].

Nail samples

0.02 to 0.07 gm of nail sample was taken in a 25 ml Borosil glass beaker and added 5 ml concentrated nitric acid. Then closed the lid and heated on a hot plate with a temperature of 90-100°C for a few minutes. Heating was discontinued and kept overnight. Next morning the lid was opened and 1 ml concentrated HNO_3 added again, and the sample evaporated at about 100°C in an exhaust chamber. Heating was continued with time-to-time addition of a known volume of concentrated HNO_3 until the color of the solution turned to almost colorless. Next steps taken were like those of hair samples[61].

Skin-scale samples

0.05-0.2 gm of sample was taken in a Borosil glass beaker and next steps taken were like those of nail digestion[61].

Microwave digestion of hair and nail samples.

Samples for digestion were weighed in the Teflon vessel (advanced composite vessel, HP 500) and added 2: l (V/V) of nitric acid and hydrogen peroxide (Waka Pure Chemicals Ind. Ltd., Japan). The vessels were closed using the lid provided. For safety of the vessel, rupture membrane was inserted in the lid. Vessels were set in the turntable of the microwave digestion machine (Model: MDS 2100, USA) and the below settings were programmed **(Table 1).**

Table 1: Optimum parameters for sample digestion by microwave system

Stages	1	2	3	4	5
Power (watt)	80	80	80	0	0
PSI	70	120	170	20	20
Time (min)	20	20	20	20	20
TAP (min)	5	5	5	5	0000

Hair, nail, and skin-scale samples were analyzed by FI-HG-AAS

Digested samples were analyzed by Fl-HG-AAS method against arsenate as the standard

Table 2: Optimum-Parameters for arsenic determination by flow infection (Fl) system

Parameters	**PerkinElmer (Model 3100)**	**Varian (Model Spectra AA-20)**
Lamp Current	400mA (EDL power supply)	10mA (hollow cathode)
Wavelength	193.7 nm	193.7 nm
Slit	0.7 nm	0.5 nm
HCl flow rate	1.25 ml/min	1 ml/min
HCl concentration	5M	5M
$NaBH_4$ flow rate	2 ml/min	1.5 ml/min
$NaBH_4$ concentration	1.25% (W/V) in 0.5% (W/V) NaOH solution	1.25% (W/V) in 0.5% (W/V) NaOH solution
Carrier gas	Nitrogen	Nitrogen
Carrier gas flow rate	130 ml/min	50 ml/min
Flame	Air-acetylene	Air-acetylene

Table 3: Analytical performance of FI-HG-AAS for the determination of arsenic

Parameter	Perkin-Elmer	Varian
Sensitivity (Au/ng)	3.8 X 10^{-2}	l.9x10^{-2}
Detection limit	0.1 ng/ml	0.16 ng/ml
Quantitation limit	0.3 ng/ml	0.47 ng/ml
Precision (CV%)	1.97	3.0
Sample frequency	100/h	70/h

Table 4: Analysis of Standard Reference Materials (SRM) for arsenic by FI-HG-AAS

Samples	Certified Value	Found Value
CRM (BND 301) NPL water	990 ±200 (µg/L)	960 ± 40 (µg/L)
SRM (QCS) Metals in Water	17.6 ± 2.21 (µg/L)	16 ± 3.5 (µg/L)
GBW 07601(Hair)	0.28 (µg/gm)	0.278 (µg/gm)
NIST, SRM 2670 (Urine)		
Elevated level	480±100 (µg/L)	477 ± 30 (µg/L)
Normal level	60[a] (µg/L)	42.4 ± 2.4 (µg/L)
NIST, SRM 1577b (Bovine liver)	0.047 ±0.006 (µg/gm)	0.043 ± 0.003 (µg/gm)
NIST, SRM 1572 (Citrus Leaves)	3. 1 ± 0.3 (µg/gm)	3.5 ± 0.5 (µg/gm)
CRM 278 (Muscle Tissue)	5.9 ± 0.2 (µg/gm)	6.1 ± 0.3 (µg/gm)
NIES-2 (Pond Sediment)	12.2 ± 2 (µg/gm)	9.85 ± 0.5 (µg/gm)
Chinese River Sediment 81-101 (of 1981)	56.0 ± 10.0 (µg/gm)	53.79 ± 2.0 (µg/gm)

[a]Not certified value, for information only

Table 2 shows the optimum parameters for arsenic determination with both Perkin-Elmer and Varian instruments. Low acid concentrations showed lower sensitivity probably due to incomplete reaction. The optimum HCl concentration was 5M and 1.25% $NaBH_4$ produced the maximum sensitivity in 5M HCI.

Several transition metals can interfere with the determination of arsenic during hydride generation[62] for the batch system. However, the flow injection hydride generation (FI-HG) system showed better tolerance toward hydride forming elements than the batch system[63,65]. This might be due to shorter reaction time and smaller sample volume. In such a short reaction period most of the interfering transition metal ions could not be reduced to metal and thus, could not absorb or decompose the hydride[64]. Sensitivity, detection limit and precision were determined for the proposed method. The results are summarized in **Table 3.** The analytical characteristics were evaluated in accordance with IUPAC recommendation[66]. To check the accuracy of the techniques I analyzed various types of SRM samples. The results are given in **Table 4** and the results show good agreement with the certified values.

ICP-MS Analysis

ICP-MS is an element selective detector. Twenty microliters (20 µL) of the microwave acid digested sample were injected into a carrier stream of Milli Q water with a sample loop. The Chromatographic areas were measured, and the concentrations of elements were calculated against the individual element standard curve. The experimental conditions of ICP-MS are given in **Table 5.**

To check the accuracy of the techniques I analyzed various types of NIST, SRM samples. The results are given in **Table 6** and **7,** and it shows good agreement with the certified values.

Table 5: Instrumental conditions for ICP-MS

Mobile phase	Milli Q water
Flow rate	1 ml/min
Infection volume	20 µL
Radio frequency (RF)	1300 W
RF refracting power	Below 5 W
Flow of plasma gas	15 L/ min

Flow of Carrier gas	1.2 L/min
Measuring time	2 min

Table 6: Analytical results of Standard Reference Material (SRM) by FI-HG-AAS

Sample	**Certified value (**As, µg/gm**)**	**Found value** (As, µg/gm**)**
NIST, SRM 1572 (Citrus Leaves)	3.1 ± 0.3	3.5 ± 0.5
NIES -2 (Pond Sediment)	12.2± 2	9.85 ±0.5
Chines River Sediment 81-101 (of 1981)	56.0± 10.0	53.79 ± 2.0
NIST, SRM 2709 (San Joaquin Soil)	17.7 ± 0.8	16.87 ± 0.34
NIST, SRM® 1568a (Rice Flour)	0.29 ± 0.03	0.28 ± 0.04
NIST, SRM 15708 (Spinach Leaves)	0.068 ± 0,012	0.062± 0.014
NIST, SRM 1573a (Tomato Leaves)	0.112 ± 0.004	0.100

Table 7: Analytical results of NIST Standard Reference Material (SRM) by ICP-MS

Samples	**Certified Value (µg/gm)**								**Found Value (µg/gm)**							
	As	Se	Mn	Cu	Hg	Pb	Ni	Zn	As	Se	Mn	Cu	Hg	Pb	Ni	Zn
NIST2709 (San Joaquin Soil)	17.7 ± 0.8	1.57 ± 0.08	538 ± 17	34.6 ± 0.7	1.4± 0.08	18.9 ±0.5	88±5	106±3	16.58 ±1.79	1.87 ± 0.07	495± 11.32	29.35 ±1.39	1.28 ± 0.06	21.8 ± 1.9	72.17 ±3.53	102.35 ±4.26
NIST 1568a (Rice Flour)	0.29± 0.03	0.38 ± 0.04	20.0 ± 1.6	2.4 ± 0.3	0.0058± 0.0005	<0.01	-	19.4 ± 0.5	0.29± 0.06	0.41 ± 0.08	17.8± 1.8	1.9± 0.7	<0.005	<0.003	<0.01	18.07 ±0.26
NIST 1515 (Apple leaves)	0.038 ± 0.007	0.05 ± 0.009	54± 3	5.64 ± 0.24	0.044 ± 0.004	0.47± 0.024	0.91 ± 0.12	12.50 ±0.30	0.039 ± 0.007	0.052 ± 0.01	55.16 ±0.22	5.28± 0.26	0.43 ± 0.001	0.47 ± 0.028	1.16± 0.04	12.98 ±0.05
NIST 1570a (Spinach Leaves)	0.068 ± 0.012	0.117± 0.009	75.9 ± 1.9	12.20 ±0.60	0.03± 0.003	0.20	2.14± 0.10	82±3	0.062 ± 0.005	0.127 ± 0.019	67.96 ±3.14	10.29 ±0.22	0.034± 0.006	0.16 ± O.01	2.27± 0.09	74.85 ±4
NIST 1573a (Tomato leaves)	0.112 ± 0.004	0.054± 0.003	246 ± 8	4.70 ± 0.14	0.034 ± 0.004	-	1.59± 0.07	30.9± 0.70	0.100 ± 0.01	0.058 ± 0.006	182±9	4.273 ±0.26	0.034± 0.008	<0.003	1.47 ± 0.11	26.86 ±2.86

Urine and Blood Collection

Urine and blood samples were collected from 191 subjects (98 females and 93 males), aged 18-77 years in the Lagunera area of Mexico. There were five groups, based on total arsenic concentration (38-116 µg/L) in their drinking water. The collection, processing, and analysis procedures of those samples were previously described[67].

Arsenics species analysis in urine

An HPLC-ICP-MS (High Performance Liquid Chromatography- Inductively Coupled Plasma-Mass Spectrometry) speciation method was used for the measurement of arsenic species (As^{V}, As^{III}, MMA^{V}, and DMA^{V}) including arsenobetaine (AsB)[67].

Trace elements analysis in urine

After acid digestion, we analyzed trace elements (As, Se, Zn, Co, Cu, Mn, Ni, Cd, Pb, and Hg) in urine samples collected from the subjects[67].

Trace elements analysis in whole blood

Whole blood samples were analyzed for total As, Se, Zn, Co, Cu, Mn, Ni, Cd, Pb, and Hg concentrations using Perkin Elmer Elan DRCe ICP-MS[67].

Results and Discussion

Total arsenic in biological samples (hair, nail, skin-scales, and urine) of the villagers in Bangladesh where we have identified arsenic patients

Literature survey shows that arsenic concentration in the body tissue and fluids are increasing with increase of arsenic concentration in the drinking water[68]. Since urine, hair and nail are available, these are used as the universal biomarker. Urinary arsenic has been considered as the most reliable indicator of recent exposure to inorganic arsenic and is used as the main biomarker of exposure[68-70]. In case of ingestion of inorganic arsenic, experimental studies show that around 60-75% of the dose is excreted in the urine within a few days[71-74]. In my study, I measured total arsenic ($Urinary_{inorg+metabolites}$), and inorganic arsenic and its metabolites in urine. Arsenic in hair and nails play an important role in evaluating arsenic poisoning from oral ingestion of arsenic. Although, determined arsenic concentration in normal hair and nail is not usually considered very reliable for biologic monitoring due to external contamination[75,76]. But the concentration of arsenic in hair and nail is usually quite high in arsenic affected villages in Bangladesh and external contamination is not a major problem. Also, arsenic contamination from dust is not a major problem too because villagers have higher arsenic body burden due to arsenic coming from highly arsenic contaminated drinking water.

Statistical presentation of arsenic in hair, nail, urine (inorganic+methylated arsenic), and skin scale samples from the villagers of the arsenic affected villages of Bangladesh where we have found arsenic patients and are presented in **Table 8.** About 40-50% of these samples were from people having arsenical skin lesions and rest of the samples from non-patients but they were living in the arsenic affected villages. The analytical report shows 95.11%, 83.15%, and 93.77% of the samples we had analyzed have arsenic in urine, hair, and nail above normal/ toxic level (hair), respectively. During our dermatological survey in the affected villages, we have observed that out of 5 people drinking the same arsenic contaminated water, 2 may not show arsenical skin lesions, but their hair, nail, and urine contain high levels of arsenic like other members. Thus, many of the villagers may not have arsenical skin lesions, but they are sub-clinically affected. However, we do not expect such elevated level of arsenic in biological samples from all villagers. The probable reason for such elevated levels of arsenic in hair, nail, and urine is that we have collected these samples from those villagers where arsenic patients exist and many tube wells are highly contaminated. The picture may be different in areas where groundwater is not much contaminated. The overall results from 50 districts show that 37% of the tube wells are safe to drink, according to the WHO recommended value (10 µg /L). Therefore, about 37% of the people should not show an elevated level of arsenic in the biological samples. A study was carried out by our group in 1998[77] to understand why body burden is higher among those using safe water for drinking and cooking, while living in arsenic affected villages[78]. In this study, safe water from a source having less than 3 µg/L arsenic was supplied for 2 years to 5 affected families to study the loss of arsenic through urine, hair, and nail. The study finally showed that despite having safe water for drinking and cooking, the study group could not avoid an intake of arsenic from contaminated food, food materials contaminated by washing, and the occasional drinking of arsenic contaminated water[78]. Arsenic in groundwater and in hair, nail, urine of the controlled population is presented in **Table 9.**

Table 8: Status of biological samples collected from the people of arsenic affected villages in Bangladesh where we had identified arsenic patients

Parameters	Arsenic in hair (µg/kg)	Arsenic in nail (µg/L)	Arsenic in urine (µg/L)	Arsenic in skin scales (µg/kg)
No. of observation	4386	4321	1084	705
Mean	3390	8570	280	5730
Median	2340	6400	116	4800
Minimum	280	260	24	600
Maximum	28060	79490	3086	53390
Standard deviation	3330	7630	410	9790
% of samples having arsenic above normal/toxic(hair) level	83.15	93.77	95.11	-

Normal level of arsenic in hair ranges from 80 - 250 µg/kg; 1000 µg/kg is the indication of toxicity[79]

Normal level of arsenic in nail ranges from 430 - 1080 µg/kg[80]

Normal excretion of arsenic in urine ranges from 5 - 40 µg/day[81]

There is no normal value for skin scale in literature

Table 9: Parametric presentation of arsenic in hair, nail, and urine of control population of Patia police station of Chittagong district, Bangladesh where arsenic in groundwater was below 3 µg/L.

Parameters	Arsenic in hair (µg/kg)	Arsenic in nail (µg/kg)	Arsenic in urine (µg/L)
No. of observation	62	62	62
Mean	410	830	31
Minimum	210	90	6
Maximum	850	1580	94
Standard deviation	180	680	20

Normal level of arsenic in hair ranges from 80 - 250 µg/kg; 1000 µg/Kg is the indication of toxicity[79]

Normal level of arsenic in nail ranges from 430 - 1080 µg/kg[80]

Normal excretion of arsenic in urine ranges from 5 - 40 µg/day[81]

From our overall study in Bangladesh, we have observed that arsenic in hair, nail, and urine increases with increasing arsenic in drinking water. **Figures 3-5** show our findings. It appears that correlations are not strongly positive (for hair samples r = 0.251, p = 0.01, n = 739; for nail samples r = 0.220, p = 0.01, n = 691; and for urine r = 0.547, p = 0.01, n = 910). Probable reason is that people are not drinking from the same source all the time. Our field data indicates that most of the villagers' drink water from more than one tube well.

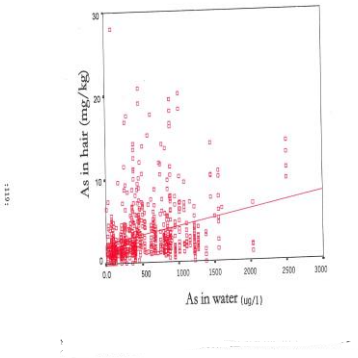

Figure 3: Correlation between arsenic in water and hair

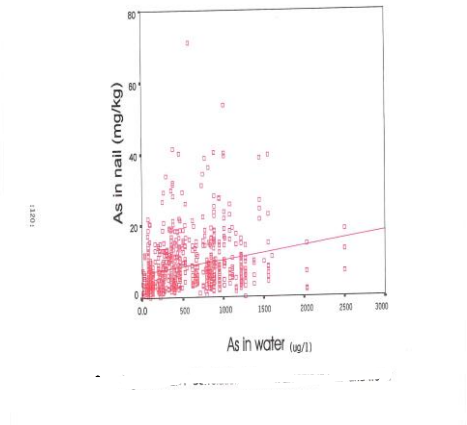

Figure 4: Correlation between arsenic in water and nail

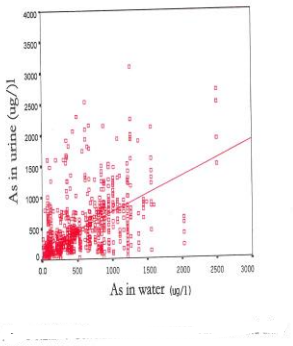

Figure 5: Correlation between arsenic in water and urine

Total arsenic, and other metals and metalloids in some biological samples [hair and nail] measured by using ICP-MS after microwave digestion

Along with arsenic in hair and nail samples, I also analyzed Se, Zn, Cu, Hg, Pb, and Sb by ICP-MS after microwave digestion. Our analysis of Standard human hair (NCS, CRM DC 73347) sample by the same procedure is in well agreement **(Table 10).** Statistical presentation of As, Se, Zn, Cu, Hg, Pb, and Sb in hair and nail samples of exposed and control population are shown in **Tables 11** and **12,** respectively. It appears in both cases (hair and nail) the mean concentration of Se and Zn are lower in exposed group than control group [for Se 167 vs. 295 µg/kg (hair) and 88 vs. 194 µg/kg (nail); and for Zn 92,013 vs. 123,255 µg/kg (hair) and 72,465 vs. 91,089 µg/kg (nail), where arsenic concentration (mean value) in hair 2,749 vs. 187 µg/kg and in nail 6,864 vs. 462 µg/kg for exposed and control group, respectively]. Again the mean concentration of Hg, Pb, and Sb are higher in both hair and nail for exposed group than control group [for Hg 789 vs. 476 µg/kg and 646 vs. 506 µg/kg; for Pb 5,531 vs. 4,587 µg/kg and 5,010 vs. 4,272; and for Sb 283 vs. 150 and 1049 vs. 225 µg/kg for exposed and control group, respectively, where arsenic concentration (mean value) in hair 2,749 vs. 187 µg /kg and in nail 6,864 vs. 462 µg/kg for exposed and control group, respectively]. But the mean concentration of Cu in both hair and nail is higher [6,778 vs. 5,677 µg/kg (hair) and 5,420 vs. 4,730 µg/kg (nail)] in control group than exposed group, respectively.

Table 10: Analysis of Standard Human Hair sample (NCS, CRM DC 73347) for arsenic by ICP-MS after microwave digestion

Sample	Elements	Certified value (µg/gm)	Found value (µg/gm)
	As	0.28 ± 0.04	0.28 ± 0.01
	Se	0.60 ± 0.03	0.709 ± 0.13

Human hair **(**CRM 73347)	Zn	190±5.00	182.84 ± 5.6
	Cu	10.60 ± 0.70	12.10 ± 0.57
	Hg	0.36 ± 0.05	0.35 ± 0.01
	Pb	8.8 ± 0.90	10.37±0.13
	Sb	0.095 ± 0.012	0.109 ± 0.005

Table 11: Statistical presentation of As, Se, Zn, Cu, Hg, Pb, and Sb in hair of chronic exposed and control population of Madaripur Sadar police station of Madaripur district, Bangladesh and Bhupatinagar police station of Medinipur district, West Bengal-India, respectively

Parameters	Exposed group (from Madaripur, Bangladesh) where arsenic in drinking water > 50 µg/L							Control group (from Medinipur, West Bengal-India) where arsenic in drinking water < 3 µg/L						
	As	Se	Zn	Cu	Hg	Pb	Sb	As	Se	Zn	Cu	Hg	Pb	Sb
	[µg/kg]							[µg/kg]						
No. of observation	19	16	16	17	19	19	18	30	13	28	17	19	17	18
Mean	2749	167	92013	5677	789	5531	283	187	295	123255	6778	476	4587	150
Median	2666	149	90306	5812	711	3444	224	178	216	122274	5794	440	3739	123
Minimum	1009	50	62270	3448	139	1148	69	53	139	60195	3467	242	1375	50
Maximum	6675	285	139088	10235	1766	16945	665	520	890	210650	12413	755	10787	322
Standard deviation	1650	71	22996	1886	397	5085	170	109	217	39058	2642	144	3004	76

Table 12: Statistical presentation of As, Se, Zn, Cu, Hg, Pb, and Sb in nail of chronic exposed and control population of Madaripur Sadar police station of Madaripur district, Bangladesh and Bhupatinagar police station of Medinipur district, West Bengal-India, respectively

Parameter	Exposed group (from Madaripur, Bangladesh) where arsenic in drinking water > 50 µg/L							Control group (from Medinipur, West Bengal-India) where arsenic in drinking water < 3 µg/L						
	As	Se	Zn	Cu	Hg	Pb	Sb	As	Se	Zn	Cu	Hg	Pb	Sb
	[µg/kg]							[µg/kg]						
No. of observation	40	27	35	31	35	31	39	29	11	26	17	18	18	19
Mean	6864	88	72465	4730	646	5010	1049	462	194	91089	5420	506	4272	225
Median	536	50	64878	3914	494	3864	850	420	150	80000	4941	452	2791	198
Minimum	1011	50	38057	1506	137	1239	97	189	103	63081	1575	233	455	77
Maximum	21174	202	124300	8971	1984	18190	4104	960	430	162000	12905	1062	11852	449
Standard deviation	4615	56	24170	2093	436	3491	834	198	93	25990	2885	247	3680	99

The regression analyses were carried out between arsenic and other metals and metalloids in hair and nail samples. The linear regression shows negative correlation between As & Se ($r = - 0.84$, $p = 0.00005$, $n = 16$) and As & Zn ($r = - 0.78$, $p = 0.0003$. $n = 16$); somewhat positive correlation between As & Pb ($r = 0.58$, $p = 0.008$, $n = 19$), As & Hg ($r = 0.745$. p 0.0002, $n = 19$) and As & Sb ($r = 0.743$, $p = 0.0002$, $n = 19$); no significant correlation between As & Cu ($r = -0.04$, $p = 0.87$, $n = 17$) for hair samples. For nail samples a similar correlation observed as in hair. The linear regression shows negative correlate between As & Se ($r = - 0.53$, $p = 0.004$, $n = 27$) and As & Zn ($r = - 0.55$, $p = 0.0006$, $n = 35$) and somewhat positive correlation between As & Hg ($r = 0.47$, $p = 0.004$, $n = 35$), As & Pb ($r = 0.51$, $p = 0.003$, $n = 33$), and As & Sb ($r = 0.57$, $p = 0.0004$, $n = 33$); no significant correlation between As & Cu ($r = 0.15$, $p = 0.41$, $n = 31$) observed. Filon, J., et al (2020)[82] found

statistically significant mean positive correlations between Pb and As in the hair of children with autism spectrum disorders (ASD). Epidemiologic studies suggest synergistic effects from binary combinations of Pb-As[83].

Toxicological and metabolic interactions of selenium (Se) with arsenic (As) have been reported in many experimental studies. However, for human populations, possible interactions between As and Se, and their toxicological significance have not been established. Miyazaki, K., et al (2003)[84] have examined the relationship between Se and As in spot urine samples collected from the inhabitants of two rural communities of northeast Bangladesh and negative correlation between UAs and USe was found in both females (($r = –0.25$, $p < 0.01$) and males ($r = –0.16$, $p < 0.05$)[84]. They explain this inverse correlation might be due to inverse intakes of these two elements, i.e., high As containing tube well water may contain low Se and vice versa[84].

Segmental hair analysis report

Arsenic concentration is normally higher in hair and nail than in other parts of the body because of the high content of keratin, the -SH group of which might bind trivalent inorganic arsenic[85-87]. Therefore. Hair and nail might be considered an excretory pathway of arsenic from the body. Arsenobetaine, the major organic arsenic compound in seafood, is not accumulated in hair[88].

That implies that arsenic in hair and nail reflects exposure to inorganic arsenic only. Experimental studies in which radiolabeled DMA was administered to mice and rats showed very low incorporation of DMA in skin and hair compared with that of inorganic arsenic [89]. However, others reported presence of DMA in hair and nail[90, 91].

Segmented hair analysis (i.e., determination of the concentration along the length of the hair) might provide valuable information on the time of acute arsenic exposure[92-94]. For example, Smith, H. (1964)[92] reported a case in which a single fatal ingestion of 800 mg of arsenic trioxide gave rise to a concentration of only 860 µg/kg for the whole length of hair 30 cm long. Further, analysis showed that the concentration of arsenic in the first millimeter, including the root, was 90,000 µg/kg. In another fatal case[95] of arsenic poisoning, centimeter segmental analysis revealed that the concentrations of arsenic varied between 28,000 and 226,000 µg /kg.

For my experiment I had collected long hair samples from four chronic female patients having arsenical skin lesions. Before washing I cut the samples in different segments from root to top (each segment about 4 cm length) and analyzed them by ICP-MS after microwave digestion. The analytical results are shown in **Table 13. Table 13** shows the distribution of arsenic in different segments of hair sample (from root to top). It appears that arsenic concentration in hair is decreasing trend with increased length from root to top. However, the decrease is not sharp as observed for acute toxicity[92].

Table 13: Distribution of arsenic concentration (µg/kg) in hair segment samples (each segment 4 cm length) collected from villagers who were drinking arsenic contaminated water

Sample ID#	Age and Sex	Patient	Arsenic concentration (µg/kg)							Whole sample arsenic (µg/ kg)
			Segment No.							
			1st (root)	2nd	3rd	4th	5th	6th	7th (top)	
Pl	40, F	Yes	4647	2746	2508	2076	1842	1757	-	-
P2	40, F	Yes	-	14004	11379	10908	-	-	-	-
P3	16, F	Yes	10934	3014	2271	2191	2084	2025	1225	4536
P4	30, F	Yes	2469	2449	2128	1429	1305	1503	-	1659

Trace elements concentrations in urine and whole blood of arsenic exposed people from subjects in the Lagunera area of Mexico[67]

The concentrations of As, Se, and Zn in urine and blood are reported in **Table 14**. The elements concentrations in urine expressed as ug/g cre were higher for females (F) compared to males (M). But the concentrations of As and Zn were lower, but Se was almost same in blood for females compared to males.

Table 14. As, Se, and Zn concentrations in urine and whole blood for females (F) and males (M). Values are the mean ± SE (F, n=98 and M, n=93).

	As	Se	Zn
In urine (µg/g cre)			
F	121.43±6.4	94.30±6.0	395.56±31.2
M	108.52±6.1	70.09±3.8	358.25±25.0

In blood (µg/L)			
F	9.48±0.2	234.95±3.2	5452.65±60.8
M	11.18±0.4	232.77±3.0	5764.80±77.7

Influence of the relative concentrations of Se or Zn to arsenic in urine on the percentage of urinary arsenic metabolites[67]

There were better correlations between (a) the ratio of other element (Se or Zn) to arsenic (As) in urine (µg/g cre) and the percentage of urinary arsenic metabolites than (b) the correlations found between the corresponding element concentrations expressed as µg/g cre in urine and percentage of urinary arsenic metabolites (**Table 15**).

Statistically significant correlations were not found between the concentrations (µg/g cre) of Se as well as Zn and % inorg As, % MMA, % DMA, as well as the ratios of % DMA to % MMA in urine for females. But the ratios of the concentrations of Se to As expressed as µg/g cre were positively and significantly correlated with % inorg As (r_s= +0.29, p<0.01) as well as % MMA (r_s= +0.25, p<0.05), and negatively correlated with % DMA (r_s= -0.34, p<0.001) as well as the ratios of % DMA to % MMA (r_s= -0.31, p<0.01) in urine for females (**Table 15**).

For males, the ratios of the concentrations (µg/g cre)) of Se or Zn to As than the concentrations of Se or Zn were more strongly and positively correlated with % inorg As levels (r_s= +0.26, p<0.05 vs. r_s= +0.17 not significant and r_s= +0.22, p<0.05 vs. r_s= +0.086 not significant, respectively), but more strongly and negatively correlated with % DMA levels (r_s= -0.25, p<0.05 vs. r_s= -0.12 not significant and r_s= -0.19 not significant vs. r_s= -0.008 not significant, respectively) in urines (**Table 15**).

Table 15. Comparison of spearman correlation coefficients between (a) the ratio of the concentrations of Se or Zn to As or (b) the concentration of element (µg/g creatinine) in urines and the percentage (%) of urinary As metabolites for females and males

	(a) Ratio of Se to As	(b) Se	(a) Ratio of Zn to As	(b) Zn
Females:				
% Inorg As	0.290[b]	0.160	0.084	-0.080
% MMA	0.250[a]	-0.004	-0.027	-0.181
% DMA	-0.337[c]	-0.168	-0.109	0.070
% MMA/% inorg As	-0.026	-0.081	-0.124	-0.094
% DMA/% MMA	-0.312[b]	-0.038	-0.014	0.197
Males:				
% Inorg As	0.264[a]	0.174	0.223[a]	0.086
% MMA	0.100	0.090	-0.028	-0.092
% DMA	-0.254[a]	-0.117	-0.189	-0.008
% MMA/% inorg As	-0.163	-0.080	-0.242[a]	-0.115
% DMA/% MMA	-0.145	-0.094	-0.034	0.081

[a]p<0.05, [b]p<0.01, [c]p<0.001

Influence of the relative concentrations of Se or Zn to arsenic in blood on the percentage of urinary arsenic metabolites[67]

The ratios of the concentrations of Se or Zn to As than the corresponding element concentrations in blood were more significantly and positively correlated with urinary % inorg As (r_s= +0.36, p<0.001 vs. r_s= +0.11 not significant; r_s= +0.24, P<0.05 vs. r_s= -0.10 not significant, respectively), and negatively correlated with % DMA (r_s= -0.34, p<0.001 vs. r_s= -0.12 not significant; r_s= -0.24, p<0.05 vs. r_s=-0.005 not significant, respectively, and with the ratios of urinary % DMA to % MMA (r_s= -0.32, p<0.01 vs. r_s=-0.063 not significant; r_s= -0.29, p<0.01 vs. vs. r_s=-0.049 not significant, respectively) for females (**Table 16**).

Table 16. Comparison of spearman correlation coefficients between (a) the ratio of the concentrations of Se or Zn to As or (b) the concentration of element (µg/L) in bloods and the percentage (%) of urinary As metabolites for females and males

	(a) Ratio of Se to As	(b) Se	(a) Ratio of Zn to As	(b) Zn
Females:				
% Inorg As	0.361[c]	0.111	0.237[a]	-0.102
% MMA	0.267[b]	0.033	0.257[a]	0.050
% DMA	-0.335[c]	-0.124	-0.237[a]	-0.005
% MMA/% inorg As	-0.150	-0.007	-0.047	0.159
% DMA/% MMA	-0.316[b]	-0.063	-0.290[b]	0.049
Males:				
% Inorg As	0.250[a]	-0.043	0.259[a]	0.036
% MMA	0.093	-0.018	0.060	-0.053
% DMA	-0.296[b]	0.017	-0.313[b]	-0.050
% MMA/% inorg As	-0.197	0.057	-0.229[a]	-0.065
% DMA/% MMA	-0.190	0.007	-0.159	-0.013

[a]p<0.05, [b]p<0.01, [c]p<0.00

The ratios of the concentrations of Se or Zn to As than the concentrations of Se or Zn in blood were also more positively and significantly correlated with urinary % MMA (r_s= +0.27, p<0.01 vs. r_s= +0.033 not significant and r_s= +0.26, p<0.05 vs. r_s= +0.05 not significant, respectively) for females.

For males, better and more significant correlations found between the ratios of the concentrations of Se or Zn to As in blood and the percentage of urinary arsenic metabolites than the correlations found between the corresponding element concentrations in blood and the percentage of urinary arsenic metabolites (**Table 16**). The ratios of the concentrations of Se or Zn to As in blood were more positively correlated with % inorg As in blood (r_s= +0.25, p<0.05 vs r_s= -0.043 not significant; r_s= +0.26, p<0.05 vs. r_s= +0.036 not significant, respectively). The ratios of urinary % MMA to % inorg As in blood were negatively correlated with the ratios of the concentrations of Se or Zn to As (r_s= -0.20 not significant and r_s= -0.23, p<0.05, respectively) in blood. But these correlations were not statistically significant with the concentrations of Se or Zn in blood. The ratios of the concentrations of Se or Zn to As in blood were negatively correlated with the ratios of urinary % DMA to % MMA for males, but these correlations were not statistically significant.

Many observational studies of human populations have evaluated the associations between the Se status and proportions of As metabolites, suggesting a protective effect of Se on increased As methylation efficiency. However, conflicting results have also been reported. For example, in children, Skröder Löveborn et al. (2016)[96] have found a positive correlation between the erythrocyte concentration of Se and the percentage of inorganic As and MMA^V, and a negative relationship with the percentage of DMA^V in urine (suggesting Se-mediated inhibition of As methylation). This can be explained by the fact that, especially in excessive doses in contrast to low doses, and in excessive doses, Se can suppress As methylation by competing for metabolic substrates such as GSH or SAM[97].

Another important aspect is that Se, especially when overdosed, can enhance the deleterious effects of inorganic As by inhibition of As methylation, as described by Sun et al. (2014)[98]. Since selenite and arsenite share similar metabolic pathways, which require the presence of GSH and SAM (a methyl donor), a

higher concentration of selenite can compete with arsenite for the availability of GSH and SAM, and consequently, inhibit the formation of methylated As metabolites. This in turn increases the cellular retention of more toxic inorganic and monomethylated As forms[99]. In addition, the inhibition of As methylation in the presence of Se is often linked with the function of AS3MT. Studies demonstrated that selenite inhibited the methylation of arsenite by recombinant rat AS3MT in a competitive manner, probably through direct interaction between selenite and the enzyme[100]. Further studies have shown that selenite inhibited the activity of recombinant human AS3MT, and the mechanism of inhibition probably involved the interaction of selenite with cysteines in the structural residues of the enzyme[101]. Overall, these findings demonstrate that excessive selenite can adversely affect the metabolism of inorganic As through decreasing the GSH and SAM levels and suppressing the activity of AS3MT.

In both females and males, there were significant positive correlations between the percentage of U_{iAs} ($\%U_{iAs}$) and the relative concentrations of Se to As (ratios of Se to As), but negative correlations between the percentage of DMA (%DMA) and the relative concentrations of Se to As (ratios of Se to As) in both urine and blood samples of chronic arsenic exposed people in the Lagunera area of Mexico. This result suggests that As methylation decreases with increasing relative concentrations of Se to As.

Arsenic was negatively correlated with selenium in both hairs and nails of chronic arsenic exposed people in Bangladesh where we had identified arsenic patients. It could be the mechanism that As replaced Se from tissues and increased cellular Se with increasing As concentration in drinking water. Researchers have reported that As can replace iodine (I), selenium (Se), and phosphorus (P)[102,103].

Both As(III) and Se interact with -SH groups in tissues. It could be more As interacted with -SH groups when increasing As concentration in drinking water and replacing more Se from -SH groups. Due to insufficient -SH groups more Se be interacted with SAM with increasing relative concentrations of Se to As. Inorganic Se biotransformation process is similar to As and As competes with Se for methyl donors (SAM)[104], and decreasing As methylation due to depleting SAM pools. Kenyon, E.M. and Hughes, M.F. (1997)[105] have been reported that excess Se decreased As methylation in mice is by more effectively competing for SAM or depleting SAM pools, but more likely depleting SAM pools. In addition, the formation of the highly toxic methylated trivalent arsenicals decreases with decreasing As methylation and Se also reacts with arsenite to form $[(GS)_2AsSe]$[106].

Human Se deficiency in arsenic-endemic regions is associated with arsenic-induced disease, but there are inconsistent results between in *vitro* and *in vivo* studies[107]. Rats dosed with arsenite and selenite are protected from As toxicity through increased biliary elimination of $[(GS)_2AsSe]^-$, but *in vitro* work with primary rat hepatocytes have suggested that selenite increases arsenite cellular retention, decreases As methylation, and increases As toxicity[108-111].

It was reported that Se (IV) as well as mercuric chloride ($HgCl_2$) inhibited As (III) methyltransferase and MMA (III) methyltransferase in rabbit liver cytosol. Mercuric chloride was found to be a more potent inhibitor of MMA (III) methyltransferase than As (III) methyltransferase[112,113]. These results suggested that Se and Hg decreased arsenic methylation. The inhibitory effects of Se and Hg were concentration dependent[112,113].

The toxicity of one metal or metalloid can be dramatically modulated by the interaction with other toxic and essential elements[114]. Arsenic and Hg are toxic elements, and Se is required to maintain good health[115]. But Se is also toxic at high levels[116]. Recent reports point out the increased risk of squamous cell carcinoma and non-melanoma skin cancer in those treated with 200 µg/day of selenium (Nutritional Prevention of Cancer Trial in the United States)[117]. However, it is well known that As and Se as well as Se and Hg act as antagonists[118]. It was also reported that inorg-As (III) influenced the interaction between selenite and methyl mercury[119]. A possible molecular link between As, Se, and Hg has been proposed by Korbas et al. (2008)[120].

These results also suggest that more than one methylase may be involved in the inorg-arsenic biotransformation pathway because the ratios of the concentrations of the elements (Se or Zn) to As were positively correlated with % MMA, but negatively correlated with % DMA. Similarly, to our findings, other researchers have also suggested that more than one methylase is involved in the oxidative methylation of inorg As[121-124].

Conclusion

It would be concluded that a) the relative concentration of Se or Zn to As may be an important factor for arsenic methylation, b) a proper dose of Se and/or Zn should be maintained, c) the dose may not be the same for everyone, and it could be dependent on the concentration of arsenic in their drinking water, and d) further research is needed with combination of inorg-arsenic, Se, and Hg at different levels in different animal models.

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I want to dedicate this paper to the memory of the people who died or were affected and suffered from arsenic toxicity in Bangladesh, India, and all over the world.

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