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Health effects and metabolic pathway of arsenic in children

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ABSTRACT

Our six years of field experience in Bangladesh showed that normally children under 11 years of age do not show arsenical skin manifestations. However, we have observed a few exceptions when (1) the arsenic content in water consumed by children is very high (≥1,000 µg/L) and (2) the arsenic content is not very high, but the children get poor nutrition. It appears that although 6.12% of 4,864 children showed arsenic skin lesions but hair and nail analysis of children (below 11 years) with or without arsenical skin lesions from affected villages of Bangladesh showed that 84% of the children had arsenic in hair more than the toxic level and 89% in nail above normal level. It appears that children living in arsenic affected villages have higher arsenic body burden but less dermatological symptoms. The value of arsenic excretion and arsenic intake per kg of body weight ratio for adults is 0.53 while that of the children is 0.69. This indicates total arsenic excretion is higher for children per kg of body weight than adults. The average values (n = 18) for speciation of arsenic in children urine are 15.60% In-As, 10.64% MMA, and 73.76% DMA. Overall percentage of MMA is higher, and DMA is lower in adults than children (average % MMA is 17.20 vs. 10.64 and % DMA is 64.62 vs. 73.76%, respectively). Therefore, the 2nd methylation step (MMA to DMA) could be more active in children than adults. The results indicate that the first reaction of the metabolism pathway is more active in adults than children, basically in adult's male. Also shows a significant (t-test positive) increase in the values of the DMA/MMA ratio in children compared to adults of the exposed group (8.15 vs. 4.11, respectively). Moreover, it is also observed that the second methylation step in the arsenic metabolic pathway is more active in children than adults. Because there is no significant difference of the values of MMA/iAs ratio, but the values of DMA/MMA ratio are significantly different between adults and children of the exposed group (0.92 vs. 0.73 for MMA/Asi and 4.11 vs 8.15 for DMA/MMA, respectively). Thus, from these results we may conclude that children retain arsenic shorter in their body comparing adults.

The distribution of the values of DMA/MMA ratios with different ages of exposed humans shows that the DMA/MMA ratio decreases with increasing age, i.e., the methylation process decreases with increasing age. This observation also supports that the 2nd methylation process of arsenic (MMA to DMA) in children is better than the adults.

In conclusions, i) it appears that children living in arsenic affected villages have higher arsenic body burden but less dermatological symptoms, ii) children require more water than adults in terms of ml, per kg body weight, iii) the average values for speciation of arsenic in children urine are 15.60% In-As; 10.64% MMA, and 73.76% DMA), iv) methylation capacity (2nd methylation step) in children is better than adults, v) methylation process of arsenic (MMA to DMA) decreases with increasing age, and vi) may be children excrete arsenic faster i.e., retain less arsenic in their cellular system than adults.

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Introduction

If we accept that the future of the world depends on its children, then all of us should try to protect them regardless of where they live. The exposure of children to environmental toxins and the resultant illness should be the concern of all. Arsenic toxicity is one example. Several studies over the years have shown that children are at higher risk of arsenic exposure[1-6]. Although children are showing less arsenical skin lesions than adults, they are more susceptible to arsenic toxicity[7]. Arsenic can damage the central nervous system; chronic encephalopathy symptoms include diminished recent memory and organic cognitive impairment[8]. Recent study shows that the percentage of children in the average IQ group decreased remarkably from 56.8 (n = 44) to 40.0 (n = 95) as the arsenic level increased in hair[9].

Methods and Materials

Reagents

All reagents were of analytical grade and Milli Q water was used throughout[10-14].

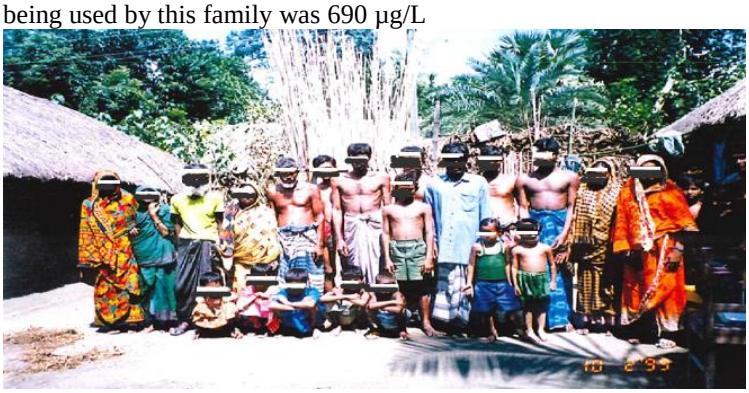
Analytical procedures

Sample collection, preservation, digestion, and analysis procedures

We collected and analyzed the groundwater and other biological samples for determination of total arsenic and arsenic metabolites from an arsenic exposed group and a controlled group of children in Bangladesh. The sampling, preservation, digestion, and analysis procedures of those samples were previously described[10,15,16].

Results and discussion

Our last 6 years of field experience in Bangladesh show that normally children under 11 years of age do not show arsenical skin manifestations. However, we have observed a few exceptions when (1) The arsenic content in water consumed by children is very high (1,000 µg/L) and (2) The arsenic content is not very high (around 500 µg/L) but the children get poor nutrition. For example, Photographs 1 & 2 show a group of children below 11 years in a poor family having arsenical skin lesions. It was also observed that 21 members out of 22 (including 6 children out of 7) had arsenical skin lesions in this poor family (Photograph 1). Table 1 shows the dermatological manifestation of these six children. The arsenic concentration in drinking water being used by this family was 690 µg/L



Photograph 1: A group of children below 11 years in a poor family having arsenical skin lesions (Vill: Bagoan, PS: Meherpur Sadar, Dist: Meherpur, Bangladesh)

Table 1: Dermatological manifestation of a group of children in a poor family in the village Bagon, under Meherpur Sadar police station, Meherpur, Bangladesh

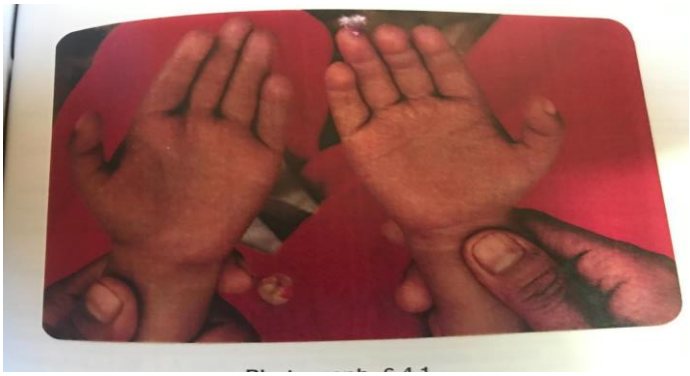
ID No.	Sex & age	Melanosis						Keratosis			
		Palm		Trunk		Leucomelanosis	Whole body	Palm		Sole	
		Spotted	Diffuse	Spotted	Diffuse			Spotted	Diffuse	Spotted	Diffuse
CPl	M, 9	-	+	++	++	-	+	+	+	+	-
CP2	F, 9	-	+	+	+	-	-	-	-	-	-
CP3	F, 8	-	+	+	+	-	-	-	-	-	-
CP4	M, 9	-	+	++	+	-	-	+	-	+	-

CPS	M, 11	-	+	+	+	-	-	-	-	+	-
CP6	F, 10	-	+	+	-	-	-	-	+	-	-

Mild (+), Moderate to severe (++)

Photograph 2: A group of children below 11 years in a poor family having arsenical skin lesions (Vill: Maharajpur Nimtala, PS: Nawabganj Sadar, Dist: Nawabganj, Bangladesh)

So far in Bangladesh the youngest child (age 18 months) arsenic patients with melanosis (+), keratosis (++) [spotted on palm and sole] is found in Payerpur village, Madaripur Sadar police station, Madaripur district, Bangladesh (Photograph 3). While discussing with his mother we came to know that from very childhood, the child used to drink very high quantity of water (2-3 liters per day).



Photograph 3: The youngest child (age 18 months) in the Vill: Payerpur, PS: Madaripur sadar, Dist: Madaripur, Bangladesh

So far, we had examined 4,864 children below 11 years drinking arsenic contaminated water from the affected villages of Bangladesh and out of that arsenical skin lesions registered from 298 children (6.12%) whereas in adults it is 24.47%. Normally arsenical, skin lesions observed in children are diffuse melanosis and spotted melanosis. Keratosis on the palm and sole are not common in children. A few exceptions are found when arsenic in drinking water is quite high (≥ 1,000 µg/L) and nutrition is also, poor. But we have not found children suffering from+++ stage of melanosis and keratosis (we used mild+, moderate to high++, severe+++). We have not also found any child patient during last 6 years in Bangladesh below 11 years with non-pitting oedema, gangrene, Bowens, dorsal, and cancer. Table 2 shows the dermatological manifestation of 24 child patients from 24 affected districts of Bangladesh (one child patient from each district) where we have identified child patients and Figure 1 shows a comparison of dermatological symptoms of the adults and children's patients in Bangladesh.

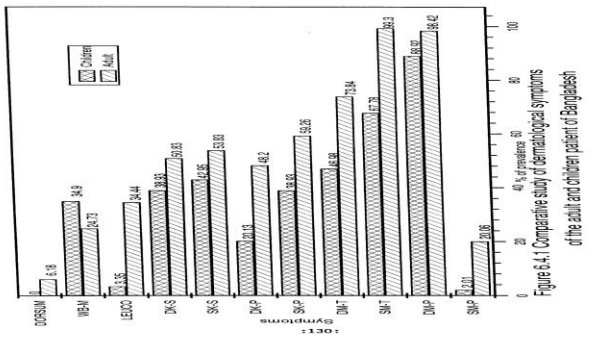


Figure 1: Comparative study of dermatological symptoms of the adults and children's patients in Bangladesh

Table 2: Dermatological features of 24 patients (≤11 years) from arsenic affected districts of Bangladesh (one child from each district)

ID No.	Districts	Sex & age	Melanosis						Keratosis			
			Palm		Trunk		Leucomelanosis	Whole body	Palm		Sole	
			Spotted	Diffuse	Spotted	Diffuse			Spotted	Diffuse	Spotted	Diffuse
CP I	Lakshmipur	F,9	-	+	+	+	-	-	-	-	+	-
CP2	Jessore	F,5	-	+	++	+	-	+	+	+	+	+
CP3	Pabna	F, 10	-	+	++	+	+	+	+	+	+	+
CP4	Faridpur	F, 11	-	+	+	+	-	-	-	-	-	-
CP S	Chandpur	F, 9	-	+	+	+	-	-	-	-	-	-
CP6	Narayanganj	M, 10	+	+	+	+	-	-	+	-	+	-
CP7	Nawabganj	M, 10	-	+	++	+	-	-	-	-	-	-
CP8	Rajshahi	M, 10	-	+	-	+	-	+	+	+	+	+
CP9	Rangpur	F, 10	+	+	+	+	+	+	+	+	+	+
CPl0	Meherpur	M, 10	-	+	+	+	-	-	+	-	+	+
CPI I	Jhsnaidaha	F,9	-	+	+	+	-	-	-	-	-	-
CP12	Camilla	F, 11	-	+	+	+	-	-	+	-	+	-
CP13	Manikganj	M,11	-	-	+	+	+	-	+	-	+	-
CP14	Bagerhat	F, 10	-	+	+	-	-	-	-	-	+	-
CP15	Bogra	M,10	-	+	+	+	-	-	+	-	-	-
CP1	Chuadanga	M,8	-	+	++	+	-	+	-	+	-	-

6												
CP17	Gopalganj	F,9	-	+	+	+	-	-	-	-	-	-
CPI8	Jamalpur	M,9	-	+	++	+	+	-	+	-	+	-
CP19	Khulna	F,9	-	+	+	+	-	+	+	+	+	+
CP20	Madaripur	F,11	-	+	++	+	-	+	+	-	+	-
CP21	Magura	F, 10	-	+	++	+	+	+	-	-	+	-
CP22	Narsingdi	F,10	-	+	+	+	-	+	+	-	+	-
CP23	Noakhali	M,10	-	+	+	+	-	-	+	-	+	-
CP24	Kustia	F, 11	++	++	+	+	-	++	+	++	++	++

We also analyzed hair, nail, and urine samples from children below 11 years from arsenic affected villages of Bangladesh. Table 3 shows a statistical comparison of arsenic in hair, nail, and urine of adults and children (both patient and non-patient). It appears that although 6.12% of 4,864 children showed arsenic skin lesions but hair and nail analysis of children (below 11 years) with or without arsenical skin lesions from affected villages of Bangladesh showed that 84% of the children had arsenic in hair more than the toxic level and 89% in nail above normal level (Table 3.). It appears that children living in arsenic affected villages have higher arsenic body burden but less dermatological symptoms. Table 3 also shows that the concentration (mean value) of arsenic in hair and nail of adults are higher than those of children. But for urinary arsenic, children are excreting more arsenic than that of adults.

Table 3: Status of biological samples collected from the adults and children of arsenic affected villages of Bangladesh (about 40-50% of samples are from people having arsenical skin lesions)

Parameters	Adults			Children		
	Arsenic in Hair* (µg/kg)	Arsenic in Nail** (µg/kg)	Arsenic in Urine*** (µg/L)	Arsenic in Hair* (µg/kg)	Arsenic in Nail** (µg/kg)	Arsenic in Urine*** (µg/L)
No. of valid observation	350	352	300	152	148	155
Mean	3400	9860	472	1850	5850	605
Median	2161	4512	225	1031	2516	310
Minimum	620	1700	37	570	1665	24
Maximum	9480	29600	2285	5930	17700	3085
Standard deviation	1900	5500	451	1150	3600	632
% of samples having arsenic above normal / toxic (hair) level	93	100	95	84	89	97

*Normal level of arsenic in hair ranges from 80-250 µg/kg;1000 µg/kg is the indication of toxicity[17]

**Normal level of arsenic in nail ranges from 430 - 1080 µg/kg[18]
***Normal excretion of arsenic in urine ranges from 5 - 40 µg/day[19]

We have further observed that children recover from diffuse melanosis (blackening of color) and light spotted melanosis (+) quickly if they use safe water and with better nutrition and with vitamins recovery is enhanced. Mild keratosis (+) also disappears but the children having moderate to high spotted melanosis (++) and spotted keratosis (++) even after drinking safe water and nutritious food, do not recover completely. In one of our follows up study in Harirampur village, Bagha police station in Rajshahi district of Bangladesh, we have found during August 1996, nine children (Photograph 4) had arsenical skin lesions and was drinking arsenic contaminated water from a tube well having arsenic 1,070 µg/L. The group started taking safe water <3 µg/L of arsenic from early1997 and we further went to the village in April 1999 for the follow up study. We had found diffuse melanosis fall the children disappear and those who had spotted melanosis (+) and keratosis (+) are no longer showing skin lesions but those who had ++ spotted melanosis and ++ spotted keratosis could not get rid of their skin lesions and spotted melanosis is replaced by Leucomelanosis and keratosis is less compared to what they had during August 1996. However, the children are still complaining about their weakness, breathing problems, and suffering from cough and cold. Finally, if it is accepted that children are at a higher risk due to arsenic exposure, the future of the next generation of Bangladesh living in arsenic affected villages may be grim as above 84% and 89% of the children's hair and nail contain arsenic above toxic (hair) or normal level (nail), respectively.

Photograph 4: A group of arsenic affected children in the Vill: Harirampur, PS: Bagha, Dist: Rajshahi, Bangladesh. Sum of urinary inorganic arsenic and its metabolites in children were measured by using FI-HG-AAS

Inorganic arsenic and its metabolites together were analyzed by the FI-HG-AAS method. In our FI-HG--AAS system arsenobetaine and arsenocholine do not form hydride. The results for children are given in Table 4. Exposed children's urine samples were collected from Datterhat village of Madaripur district in Bangladesh, where children use arsenic contaminated tube well water for drinking purposes. Controlled children's urine samples were collected from Medinipur district of West Bengal, India, where they are using arsenic safe water (<3 µg/L) for drinking purposes. The analytical result of control urine samples is given in Table 4.

Table 4: Concentration of arsenic in drinking water and corresponding urinary total arsenic of children in exposed and control groups collected from Datterhat village of Madaripur district in Bangladesh and Bautinagar block/PS in Medinipur district of West Bengal-India, respectively.

Group	**Sample No.**	**Age (Year)**	**Sex (M/F)**	**As in drinking Water (µg/L)**	**U-$As_{inorg+met}$ (µg/L)**
	EC1	10	F	300	370
	EC2	11	M	340	750

Exposed children (n=15)	EC3	11	F	460	1350
	EC4	6	M	460	370
	EC5	10	M	460	350
	EC6	8	M	620	1267
	EC7	6	M	620	2400
	EC8	7	M	540	1250
	EC9	10	F	540	320
	EC10	11	M	540	919
	EC11	11	M	118	164
	EC12	6	M	350	865
	EC13	9	F	390	1172
	EC14	5	M	390	340
	EC15	3	M	390	665
Control children (n=12)					
	CC1	8	M	<3	3
	CC2	6	M	<3	35
	CC3	9	M	<3	16
	CC4	11	F	<3	13
	CC5	6	F	<3	36
	CC6	7.5	F	<3	28
	CC7	10	F	<3	3
	CC8	10	M	<3	3
	CC9	10	M	<3	3
	CC10	5	F	<3	21
	CC11	10	M	<3	22
	CC12	4	M	<3	17

*EC= Exposed children and CC = Control children; ** We have no evidence that subjects drank water from the same source all the time.

Individual arsenic species in children urine samples were measured by using HPLC-ICP-MS

The samples measured for total arsenic by FI-HG-AAS were also analyzed by HPLC-ICP-MS for arsenic species. Results are given in Tables 5 and 6.

Table 5: Concentration (µg/L) and percentage of arsenic species in urine samples of children males (boys) living in arsenic contaminated area (Exposed group, n =13) and arsenic contamination free area (Control group, n = 4)

Sample ID	**Age (Year)**	**As in drinking water (µg/L)**	**Urine**					
			As^{III} (µg/L)	**As^{V} (µg/L)**	**$AS^{III}+As^{V}$ (µg/L)**	**MMA (µg/L)**	**DMA (µg/L)**	**Total $As_{inorg+met}$ (µg/L)**
Exposed Group (n=13)								
CM1	11	340	ND	90.5 (9.90%)	90.5 (9.90%)	106.5 (11.60%)	716.9 (78.44%)	913.9
CM2	6	460	13.5 (4.3%)	19.8 (6.35%)	33.3 (10.65%)	19.5 (6.25%)	259.7 (83.10%)	312.5
CM3	10	460	ND	52.7 (17.92%)	52.7 (17.92%)	29.6 (10.08%)	211.7 (72.00%)	294.0
CM4	7	540	ND	114.8 (10.86%)	114.8 (10.86%)	170.6 (16.15%)	771.3 (72.99%)	1056.7
CM5	11	540	ND	120.2 (11.03%)	120.2 (11.03%)	57.9 (5.32%)	911.4 (83.65%)	1089.5
CM6	7	460	ND	15.7 (12.14%)	15.7 (12.14%)	9.2 (7.12%)	104.4 (80.74%)	129.3
CM7	7	285	20.0 (13.26%)	3.6 (2.40%)	23.6 (15.66%)	11.5 (7.62%)	115.7 (76.72%)	150.8
CM8	10	285	22.5 (13.71%)	10.8 (6.58%)	33.3 (20.99%)	25.6 (15.61%)	105.2 (64.10%)	164.1
CM9	10	285	75.3 (7.52%)	19.7 (1.99%)	95.0 (9.52%)	62.7 (6.26%)	843.1 (84.23%)	1000.9
CM10	11	118	9.6 (6.22%)	18.6 (12.05%)	28.2 (18.27%)	16.0 (10.36%)	110.2 (71.37%)	154.4
CM11	6	350	1.0 (0.13%)	67.9 (8.79%)	68.9 (8.92%)	66.7 (8.64%)	636.4 (82.44%)	772.0
CM12	5	390	1.3 (0.35%)	93.6 (25.61%)	94.9 (25.96%)	48.9 (13.38%)	221.6 (60.66%)	365.4
CM13	3	390	53.9 (8.56%)	110.5 (17.55%)	164.4 (26.11%)	97.1 (15.42%)	368.2 (58.47%)	629.7
Control group (n=4)								
CM14	8	<3	0.50 (5.15%)	2.2 (22.68%)	2.7 (27.83%)	1.0 (10.32%)	6.0 (61.85%)	9.7

CM15	6	<3	4.6 (14.28%)	0.6 (1.86%)	5.2 (16.14%)	6.0 (18.65%)	21.0 (65.21%)	32.2
CM16	9	<3	ND	2.9 (20.87%)	2.9 (20.87%)	3.2 (23.02%)	7.8 (56.11%)	13.9
CM17	10	<3	5.9 (14.71%)	0.7 (1.75%)	6.6 (16.46%)	7.6 (18.95%)	25.9 (64.59%)	40.1

*CM= Children male (boys); ** We have no evidence that subjects drank water from same source all the time.

Table 6: Concentration (µg/L) and percentage of arsenic species in urine samples of children females

Sample ID	**Age (Year)**	**As in drinking water (µg/L)**	**Urine**					
			As^{III} (µg/L)	**As^{V} (µg/L)**	**$AS^{III}+As^{V}$ (µg/L)**	**MMA (µg/L)**	**DMA (µg/L)**	**Total $As_{inorg+met}$ (µg/L)**
Exposed Group (n=5)								
CF1	10	300	ND	52.8 (12.0%)	52.8 (12.0%)	82.8 (18.81%)	304.5 (69.19%)	440.1
CF2	10	540	ND	46.2 (12.37%)	46.2 (12.37%)	24.3 (7.06%)	277.5 (80.57%)	344.4
CF3	4	460	1.17 (0.11%)	129.03 (12.20%)	130.2 (12.31%)	119.8 (11.33%)	807.6 (76.36%)	1057.6
CF4	9	258	44.2 (17.31%)	14.8 (5.79%)	59.0 (23.10%)	33.1 (13.51%)	163.2 (63.39%)	255.3
CF5	9	390	66.8 (6.60%)	279.3 (27.59%)	346.1 (34.19%)	74.4 (7.35%)	591.8 (58.46%)	1012.3
Control group (n=5)								
CF6	11	<3	ND	3.8 (21.36%)	3.8 (21.36%)	3.6 (20.22%)	10.4 (58.42%)	17.8
CF7	6	<3	3.5 (10.35%)	4.0 (11.91%)	7.5 (22.26%)	6.1 (18.04%)	20.2 (59.7%)	33.8
CF8	10	<3	0.3 (3.09%)	0.7 (7.22%)	1.0 (10.31%)	1.1 (11.34%)	7.6 (78.35%)	9.7
CF9	7.5	<3	4.3 (17.26%)	4.1 (16.49%)	8.4 (33.74%)	4.0 (16.06%)	12.5 (50.20%)	24.9

CF10	5	<3	1.1 (6.14%)	1.2 (6.71%)	2.3 12.85%	2.1 (11.73%)	13.5 (75.42%)	17.9

(girls) living in arsenic contaminated area (Exposed group, n =5) and arsenic contamination free area

(Control group, n = 5)

*CF= Children female (girls); ** We have no evidence that subjects drank water from the same source all the time.

NIST SRM 2670 Freeze dried urine samples [(a) normal level (not certified but for information only) and (b) Elevated level (certified)] were analyzed by our FI-HG-AAS for inorganic arsenic and its metabolites. Table 7 shows the results. It appears that recovery of arsenic from normal level of urine is 70.66% and elevated level is 99.37%. This low result of arsenic for normal urine may be due to the percentage of arsenobetaine in NIST urine samples, which FI-HG-AAS did not measure. We used the concentration of arsenobetaine in NIST-SRM 2670 urine samples as available from literature[20-24] and made a correction. After correction the mentioned value of the arsenic NIST sample was well matched with our result (Table 7). Although NIST-SRM 2670 (elevated level) contains 15.6 ± 1 µg/L arsenobetaine[20], it is small compared to total arsenic, so found value is well in agreement with certified value. For example, Table 8 shows inorganic arsenic and its metabolites (µg/L) in a few numbers of same human urine samples measured by both FI-HG-AAS and HPLC-ICP-MS.

Table 7: Inorganic arsenic and its metabolites (U-Asinorg+met in µg/L) in NIST-SRM 2670 freeze dried urine samples were measured by FI-HG-AAS and HPLC-ICP-MS techniques.

Urine	Certified value	Found value		% of recovery by FI-HG-AAS	Arsenobetaine (HPLC-ICP-MS)
		FI-HG-AAS	HPLC-ICP-MS		
NIST-SRM 2670 (Normal level)	60[a]	42.4 ± 2.4	58.3 ± 2.2	70.66	15.4 ± 0.3
NIST-SRM 2670 (Elevated level)	480 ± 100	477 ± 30	504 ± 17.3	99.37	15.6 ± 1.0

[a]Not certified value, for information only.

Table 8: Inorganic arsenic and its metabolites (U-Asinorg+met in µg/L) in a few numbers of human urine samples from arsenic affected villages of Bangladesh were measured by FI-HG-AAS and HPLC-ICP-MS.

Urine samples	FI-HG-AAS	HPLC-ICP-MS
U1	260	275.5
U2	880	929.4
U3	580	560.2
U4	358	387.5
U5	1250	1056.7
U6	320	344.4
U7	577	601.5
U8	300	316.9
U9	650	707.2
U10	235	255.3
U11	967	1000.9
U12	774	847.3

Urinary arsenic concentration increases with increasing arsenic concentration in drinking water i.e., a linear correlation is expected. Linear regression shows a positive correlation between urinary arsenic (U-Asinorg+met) of children and arsenic concentration in their drinking water ($r = 0.598$, $p = 0.018$ ($n = 15$)). A good correlation was not observed, and the reason may be that subjects were drinking water from mixed sources; children normally drink water from many sources as they spend most of their daytime outside (in field, school etc.).

Table 9 shows that urinary average arsenic concentration in exposed adult males, adult females, and children are 29, 28, and 49 times higher than the corresponding control group. Table 9 indicates that average total urinary arsenic metabolites in children's urine is higher than adults considering consumption of water per day and concentration of arsenic in drinking water. I also observed the same trend in one of our studies in Datterhat (North) village in Madaripur district, Bangladesh (Table 10). To be sure whether this trend is followed or not and to avoid mixed sources, I analyzed urinary inorganic arsenic and its metabolites in urine from 4 families picking one adult and one child who were drinking from the same source during our survey and last few days before our survey. Table 11 shows the result that urinary arsenic metabolites in children is higher than adults in all four families. Del Razo et al. reported[25] that this is due to the fact that Children require more water than adults in terms of ml, per kg body weight.

To know whether arsenic excretion in children is higher than in adults due to higher methylation capacity (this work). I made a systematic study. From Datterhat (South) village we picked up 13 adult males and 15 children whose arsenic in drinking water we knew. We also knew their height, weight, and took the average intake water per day and volume of excretion of urine per day. From all these available data we calculated arsenic intake through drinking water per kg body weight for adults and children, and excretion of inorganic arsenic and its metabolites through urine. Tables 12 and 13 show that the value of arsenic excretion and arsenic intake per kg body weight ratio for adults is 0.53 while that of the children is 0.69. This indicates total arsenic excretion is higher for children per kg of body weight than adults. However, in this calculation we have not considered arsenic coming from food material, but we expect from our study that it follows the same trend.

Table 9: Average arsenic concentration and range of total arsenic (µg/L) in drinking water and in urine (µg/L) of exposed and control group of adults and children

Group	Source		Age (years)	Arsenic in drinking water (µg/L)	U-$As_{inorg+met}$
Exposed	Adult male (n=13)	Ave. Range	28.85 (14-70)	384 (285-540)	656 (183-2400)
	Adult female (n=16)	Ave. Range	26.86 (12-55)	377 (285-620)	511 (122-1554)
	Children (n=15)	Ave. Range	8.26 (3-11)	438 (118-620)	830 (164-2400)
Control	Adult male (n=13)	Ave. Range	32.33 (15-55)	3	22.22 (8-50)
	Adult female (n=16)	Ave. Range	31.64 (13-55)	3	18.09 (3-55)
	Children (n=15)	Ave. Range	8.04 (4-11)	3	16.67 (3-36)

Table 10: Average urinary arsenic metabolites (U-Asinorg + met) of exposed adults and children's groups of Datterhat village (North) in Madaripur district, Bangladesh.

Adults (n = 19)				Children (n = 9)			
Serial	Age (year) and Sex	U-$As_{inorg + met}$ ($\mu g\ l^{-1}$)	Average U-$As_{inorg + met}$ ($\mu g\ l^{-1}$)	Serial	Age (year) and Sex	U-$As_{inorg + met}$ ($\mu g\ l^{-1}$)	Average U-$As_{inorg + met}$ ($\mu g\ l^{-1}$)
1	14 (F)	76		1	10 (F)	675	
2	14 (F)	156		2	11 (M)	756	
3	14 (M)	145		3	6 (M)	372	
4	25 (M)	756		4	5 (M)	319	
5	28(M)	167		5	6 (M)	729	
6	18 (F)	97		6	4 (M)	319	
7	42 (M)	621		7	5 (F)	810	
8	35 (F)	648		8	11 (M)	156	
9	35 (M)	210		9	11 (M)	372	
10	20 (F)	281	307.05				500.88
11	35 (F)	162					
12	35 (F)	135					
13	12 (M)	113					
14	32 (F)	140					
15	30 (F)	729					
16	45 (F)	378					
17	30 (F)	210					
18	40 (F)	594					
19	25 (F)	216					

Table 11: Urinary arsenic concentration (U-Asinorg + met) of adult and child, exposed to same arsenic concentration in drinking water in the same family.

Family No.	Source	Age (years)	Arsenic concentration in drinking water ($\mu g\ l^{-1}$)	U $As_{inorg + met}$ ($\mu g\ l^{-1}$)
1	Adult	35	300	175.7
	Child	10		440.1
2	Adult	30	340	387.5
	Child	11		913.9
3	Adult	30	540	560.2
	Child	11		1089.5
4	Adult	25	460	494.7
	Child	4		1057.6

Table 12: Average arsenic consumed from drinking water and excreted through urine in per kg body weight of adults in Datterhat (South) village of Madaripur district in Bangladesh.

ADULT											
Serial No.	Age (years) / Sex (M/F)	Body weight (kg)	Water				Urine				B/A
			Volume of drinking water intake per day (liter)	Arsenic concentration in drinking water (μg l^{-1})	Arsenic consumed in μg kg^{-1} body weight	Average arsenic consumed in μg kg^{-1} body weight (A)	Volume of urine excreted per day (liter)	Arsenic concentration in urine (μg l^{-1})	Arsenic excreted in μg kg^{-1} body weight	Average arsenic excreted in μg kg^{-1} body weight (B)	
1	35, F	37	4	300	1200 ÷37 = 32.43	35.88	2	175.7	351.4 ÷ 37 = 9.49	19.03	0.53
2	30, F	51	4	340	1360÷51 = 26.66		2	275.5	551.0 ÷ 51 = 10.80		
3	18, M	54	4	340	1360÷54 = 25.18		2	387.5	775.0 ÷ 54 = 14.35		
4	30, F	41	4	540	2160÷41 = 52.68		2	560.2	1120.4÷41 = 27.32		
5	25, F	45	4	460	1840÷45 = 40.88		2	494.7	989.4 ÷ 45 = 21.98		
6	28, F	39	4	460	1840÷39 = 47.17		2	601.5	1203 ÷ 39 = 30.84		
7	25, F	43	4	460	1840÷ 43 = 42.79		2	354.6	709.2 ÷ 43 = 16.49		
8	23, F	50	4	460	1840÷50 = 36.80		2	316.9	633.8 ÷ 50 = 12.67		
9	40, M	44	4	460	1840÷ 44 = 41.81		2	707.2	707.2 ÷ 44 = 16.07		
10	17, M	55	4	460	1840÷ 55 = 53.45		2	319.6	639.2 ÷ 55 = 11.62		
11	35, M	42	4	285	1140÷ 42 = 27.14		2	847.3	1694.6÷42 = 40.34		
12	37, F	51	4	285	1140 ÷ 51 = 22.35		2	710.6	1421.2÷51 = 27.86		
13	14, M	31	4	285	140 ÷ 31 = 36.77		2	118.6	237.2 ÷ 31 = 7.65		

Table 13: Average arsenic consumed from contaminated drinking water and excreted through urine per kg of body weight of children at Datterhat (South) village of Madaripur district in Bangladesh.

CHILDREN											
Serial No.	Age (years)	Body weight (kg)	Water				Urine				B/A
			Volume of drinking water intake per day (liter)	Arsenic concentration in drinking water (μg l^{-1})	Arsenic consumed in μg kg^{-1} body weight	Average arsenic consumed in μg kg^{-1} body weigh (A)	Volume of urine excreted per day (liter)	Arsenic concentration in urine (μg l^{-1})	Arsenic excreted in μg kg^{-1} body weight	Average arsenic excreted in μg kg^{-1} body weight (B)	
1	10	23	2	300	600 ÷ 23 = 26.08	40.43	1	440.1	440.1÷ 23 = 19.13	28.04	0.69
2	11	26	2	340	680 ÷26 = 6.15		1	913.9	913.9 ÷ 26 = 35.15		
3	6	15	2	460	920 ÷ 15 = 61.31		1	312.5	312.5 ÷ 15 = 20.83		
4	10	24	2	460	920 ÷ 24 = 38.33		1	294.0	294.0 ÷ 24 = 12.25		
5	7	17	2	540	1080÷17 = 63.52		1	1056.7	1056.7÷17 = 62.15		
6	10	25	2	540	1080÷25 = 43.20		1	344.4	344.4÷25 = 13.77		
7	11	27	2	540	1080÷27 = 40.00		1	1089.5	1089.5÷27 = 40.35		
8	9	25	2	460	920 ÷25 = 36.8		1	183.0	183.0 ÷ 25 = 7.32		
9	11	34	2	460	920÷34 = 27.05		1	1350.0	1350 ÷ 34 = 39.70		
10	7	17	2	460	920÷17 = 54.11		1	129.3	129.3 ÷ 17 = 7.6		
11	4	12	2	460	920÷12 = 76.66		1	1057.6	1057.6÷12 = 88.13		
12	7	18	2	285	570÷ 18 = 31.66		1	105.8	105.8 ÷ 18 = 5.87		
13	9	18	2	285	570÷18 = 31.66		1	255.3	255.3 ÷ 18 = 14.18		
14	10	25	2	285	570÷25 = 22.80		1	164.1	164.1 ÷ 25 = 6.56		
15	10	21	2	285	570÷21 = 27.14		1	1000.9	1000.9÷21 = 47.66		

Speciation of arsenic in urine is generally considered to be more convenient for health risk assessment than measuring the total arsenic concentration. That is why I measured arsenic species in urine by using HPLC-ICP-MS for 24 adults (11 male, 13 females) and 18 children (11 male, 5 female). I measured As(III), As(V), MMA, and DMA only.

Figure 2 shows the chromatogram of a standard containing 100 ng of As/mL of each As(III), DMA, MMA, and As(V). Our results for As(III), DMA, MMA and As(V) for NIST-SRM-2670 are compared with results of other laboratories21-24 and also that reported from our laboratory earlier20. Table 14 shows the result. From the result it appears our system is capable to speciate inorganic arsenic and its metabolites in urine.

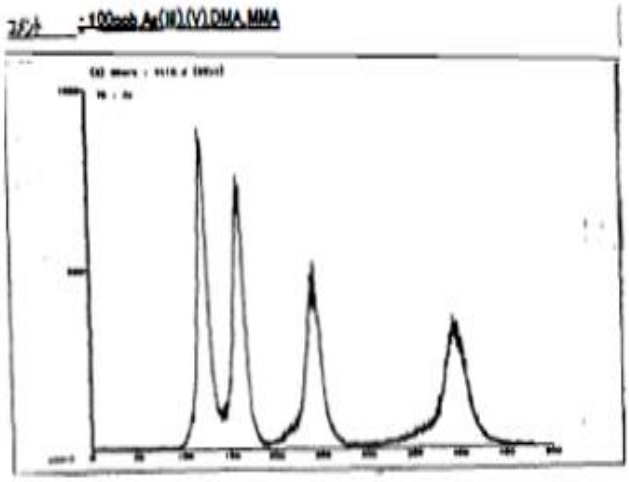


Figure 2: Chromatogram of standard containing 100 ng of As/mL of each As(III), DMA, MMA, and As(V) [retention time for As(III), DMA, MMA, and As(V) are 121 sec, 160 sec, 246 sec, and 397 sec, respectively.

Table 14: Arsenic species determined by HPLC- ICP - MS in NIST SRM 2670 toxic metals in freeze dried normal and elevated levels (μg As/L) urine by other workers.

Urine sample	Certified value	AB ($\mu g\ l^{-1}$)	DMA ($\mu g\ l^{-1}$)	As(III) ($\mu g\ l^{-1}$)	MMA ($\mu g\ l^{-1}$)	As(V) ($\mu g\ l^{-1}$)	Sum of all species ($\mu g\ l^{-1}$)
Normal level[19]	60[a]	15.4±0.3	30.8±0.6	ND	12.1±1.9	ND	58.3±2.2
Normal level[20]		ND	52.4±7.6	<2	12.8±2.0	<4	65.2
Normal level[20]		35.6±7.8	48.3±2.8	<4	18.4±4.4	<4	102.3
Normal level[21]		21.2±3.7	48.2±2.4	15.0±3.3	9.5±3	2.9±0.7	
Normal level[22]		ND	45.5±3.5	52.6±4.1	9.9±1.4	0.8±0.5	109±6
Normal level[24]		ND	49. ±5	ND	11.3	ND	60±7
Elevated level[19]	480±100	15.6±1.0	33.5±1.2	ND	13.7±0.6	442±18.6	504±17.8
Elevated level[20]		ND	52.3±10.2	<2	12.9±4.2	417±64	482.2
Elevated level[20]		32.0±8.4	48.9±2.0	<4	15.9±3.8	416±40	513
Elevated level[21]		24.7±0.7	51.6±3.4	13.1±4.5	10.9±2.1	386±50.9	
Elevated level[22]		ND	34.8±8.7	43.8±9.1	5.0±3.6	406±153	489±154
Elevated level[23]		15±3	49±3	ND	7±2	443±20	514±23

[a]Not certified value, for information only.

Inorganic arsenic in urine indicates recent exposure to inorganic arsenic[26,27]. Tables 5 and 6 indicate that during our survey villagers were drinking contaminated hand tube wells. Almost all children urine samples show 4 defined peaks [Figure 3 (child)]. Arsenobetaine peaks were not observed in our urine analysis of the exposed group in this village. Their food habit study indicates they do not eat seafood. Table 15 indicates the average concentration and range of total arsenic metabolites in urine and percentage of various species with respect to urinary inorganic arsenic and its metabolites. The results show that average percentage of In-As (AsIII + AsV), MMA, and DMA for exposed adult males are 15.52%, 17.49%, and 66.99%, respectively; for adult females 20.41%, 16.96%, and 62.63%, respectively; for children males (boys) 14.39%, 10.28%, and 75.33%, respectively and for children females (girls) 18.77%, 11.60%, and 69.63%, respectively. It appears from Table 15 that

for adult females the average inorganic arsenic is 4.9% higher than adult male and percentage of DMA is 4.36% higher in male than female. Again, for girl's average inorganic arsenic is 4.38% higher than boys and in case of DMA, it is 5.7% higher in boys than girls. Although the difference is not significant.

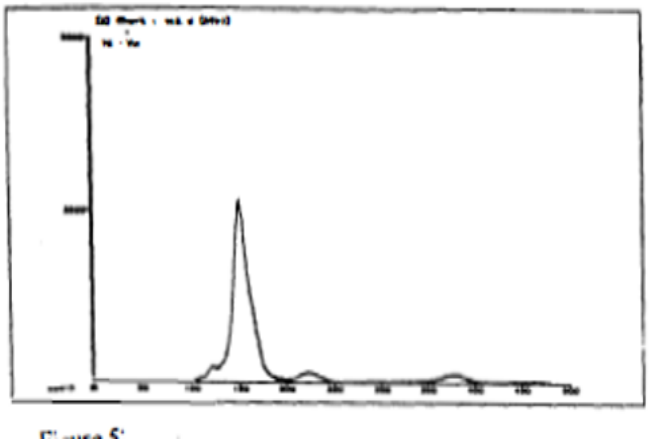


Figure 3: Chromatogram of a urine sample of an exposed child (male, 6 years); peak identities: As(III), DMA, MMA, and As(V). The experimental parameters are given in Table 5 (Sample ID# CM2).

Table 15: Average concentration (µg/L) and range of total arsenic metabolites (U-Asinorg+met) in urine (µg/L) and the percentages of inorganic arsenic As (In-As), MMA, and DMA in urine of adults and children in the arsenic contaminated area (exposed group) and arsenic concentration free area (control group).

Group	Sex	Age (Years)	U-$As_{inorg+met}$ ($\mu g\ l^{-1}$)	Urinary Arsenic Metabolites				
				Inorganic Arsenic			% MMA	% DMA
				% As (III)	% As (V)	% Inorganic As(III)+As(V)		
Exposed	Adult Males (n=11)	35.09 (14 -70)	556.58 (118.6-1325.3)	8.15 (0.10-22.85)	7.37 (2.47-16.51)	15.52 (11.77-23.84)	17.49 (10.30-29.51)	66.99 (50.35-79.38)
	Adult Females (n=13)	29.85 (13-55)	422.85 (89.9 - 929.4)	6.38 (0.34-21.87)	14.03 (0.94-24.74)	20.41 (11.83-26.81)	16.96 (11.00-21.73)	62.63 (51.80-76.33)
	Children Male (n=13)	8.00 (3-11)	541.01 (129.3-1089.5)	4.15 (0.13-13.71)	10.24 (1.99-25.61)	14.39 (8.92-26.11)	10.28 (5.32-16.15)	75.33 (58.47-84.23)
	Children Female (n=5)	8.40 (4-10)	621.94 (255.3-1057.6)	4.77 (0.11-17.31)	14.00 (5.79-27.59)	18.77 (12.0-34.19)	11.60 (7.06-18.81)	69.63 (58.46-80.58)
	Adults (n=24)	32.25 (13-70)	484.14 (89.9-1325.3)	7.19 (0.10-22.85)	10.99 (0.94-24.74)	18.17 (11.77-26.81)	17.20 (10.30-29.51)	64.62 (50.35-79.38)
	Children (n=18)	8.11 (3-11)	563.49 (129.3-1089.5)	4.32 (0.13-17.31)	11.28 (1.99-27.59)	15.60 (8.92-34.19)	10.64 (5.32-18.81)	73.76 (58.46-84.23)
Total = Adult + Children (n=42)		21.90 (3-70)	518.14 (89.9-1325.3)	5.96 (0.10-22.85)	11.11 (0.94-27.59)	17.07 (8.92-34.19)	14.39 (5.32-29.51)	68.54 (50.35-84.23)

Table 18 continued

Group	Sex	Age (Years)	U-$As_{inorg+met}$ ($\mu g\ l^{-1}$)	Urinary Arsenic Metabolites				
				Inorganic Arsenic			% MMA	% DMA
				% As (III)	% As (V)	% Inorganic As(III)+As(V)		
Control	Adult Males (n=11)	33.35 (17-55)	19.12 (8.60-34.20)	8.95 (3.49-21.65)	13.18 (11.63-8.36)	22.13 (10.67-43.38)	17.10 (7.76-26.29)	60.77 (48.86-81.33)
	Adult Females (n=7)	29.14 (13-50)	24.05 (2.8-36.3)	8.94 (10.38-20.48)	10.54 (3.53-30.78)	19.48 (0.71-30.95)	14.44 (8.13-21.79)	66.08 (47.43-78.57)
	Children Male (n=4)	8.25 (6-10)	23.97 (9.7-40.1)	8.53 (5.15-14.71)	11.79 (1.75-22.68)	20.32 (16.14-27.83)	17.72 (10.32-23.02)	61.96 (56.11-65.21)
	Children Female (n=5)	7.9 (5-11)	20.82 (9.7-33.8)	7.36 (3.09-17.26)	12.73 (6.71-21.36)	20.09 (10.31-33.74)	15.47 (11.34-20.22)	64.44 (50.2-78.35)
	Adults (n=18)	31.71 (13-55)	21.03 (2.8-36.3)	8.94 (3.49-21.65)	12.15 (3.53-38.36)	21.09 (0.71-43.38)	16.06 (7.76-26.29)	62.85 (47.43-81.33)
	Children (n=9)	8.05 (5-11)	22.22 (9.7-40.1)	7.88 (3.09-17.26)	12.31 (1.75-22.68)	20.19 (10.31-33.74)	16.47 (10.32-23.02)	63.34 (50.20-78.35)
	Total (Adults & children) [n=27]	23.82 (5-55)	21.42 (2.8-40.1)	8.58 (3.09-21.65)	12.20 (1.75-38.36)	20.78 (0.71-43.38)	16.20 (7.76-26.29)	63.02 (47.43-81.33)

When we compared arsenic species between adults and children, it has been observed inorganic arsenic in average is 2.57% and MMA average 6.56% lower for children than adult while DMA is 9.14% (average) higher in children than adults (average 18.17% vs. 15.60% In As, 17.20% vs. 10.64% MMA, and 64.62% vs. 73.67% DMA for adult's vs children, respectively) (Table 15). From the Table 15, the average values (n = 18) for speciation of arsenic in children urine are 15.60% In-As; 10.64% MMA, and 73.76% DMA.

There are a few previous reports of the speciation of As metabolites in the urine of children. Kalman et al. (1990)[28] reported average values of 13% inorganic As, 16% MMA, and 71% DMA in children in the United States (n = 158) and Buchet et al. (1980)[29] reported average values are 12% In-As, 28% MMA, and 60% DMA in children in Belgium (n = 14). However, while comparing with our results with Kalman (1990) and Buchet (1980) it appears that our values are 2.6% and 3.6% iAs higher, 5.36% and 17.36% MMA lower, and 2.76% &13.76% DMA higher from the children of United States and Belgium, respectively. But including all (adults and children) the percentage range and average percentage values (n = 42) for the speciation of As in urine are 8.92% to 34.19% (17.07%) iAs, 5.32% to 29.51% (14.39%) MMA, and 50.35% to 84.23% (68.54%) DMA. These results are good agreement with other reports. It was reported that without consumption of seafood the typical distribution of arsenic species in human urine is 10-30% inorganic arsenic, 10-20% MMA, and 60- 80% DMA[30,31]. A few others also reported that the distribution of arsenic species in urine are 10- 15% iAs, 10-15% MMA, and 60-80% DMA[32-34]. Our study for control group the range and average percentage (n = 27) of the speciation of arsenic are 0.71% - 43.38% (20.78%) iAs, 7.76% - 26.29% (16.20%) MMA, and 47.43% - 81.33% (63.02%) DMA. Therefore, there is no significant difference for the average percentage (%) of arsenic species in urine between exposed and control group. The total average urinary arsenic metabolites (U-As inorg +met) are 518 µg/L (range 89.9 to 1325.3 µg/L) and 21 µg/L (range 2.8 to 40.1 µg/L) for exposed and control group, respectively (Table 15).

Figure 4 shows the arsenic species present in control children urine samples (Sample ID CF8 in Table 6). It indicates that the presence of As(III), DMA, MMA, & As(V), and also the possibility of an unknown arsenic specimen at the retention time is 108 sec. This unknown peak is common for all control samples.

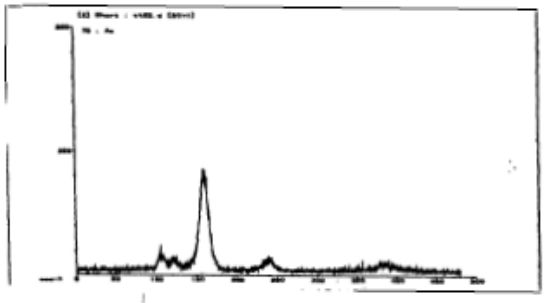


Figure 7:

Chromatogram of a control child urine sample (girl, 10 years); peak identities: As(III), DMA, MMA, As(V), and an unknown peak. The experimental parameters are given in Table 8 (Sample ID # CF8)

Figure 4: Chromatogram of a control child urine sample (girl, 10 years); peak identities: As(III), DMA, MMA, As(V), and an unknown peak. The experimental parameters are given in Table 6 (Sample ID # CF8)

Figures 5 and 6 show the distribution of %MMA and %DMA with the urinary arsenic metabolites (U- As inorg + met) of both exposed adults (n = 24) and children (n = 18), respectively. From these figures it appears that overall percentage of MMA is higher, and DMA is lower in adults than children (from Table 15 average % MMA is 17.20 versus 10.64 and % DMA is 64.62 versus 73.76%), respectively, where urinary arsenic metabolites concentration range 89.9 to 1325.3 µg/L for adults and for children this value is 129.3 to 1089.5 µg/L• Therefore, the 2nd methylation step (MMA to DMA) is more active in children than adults.

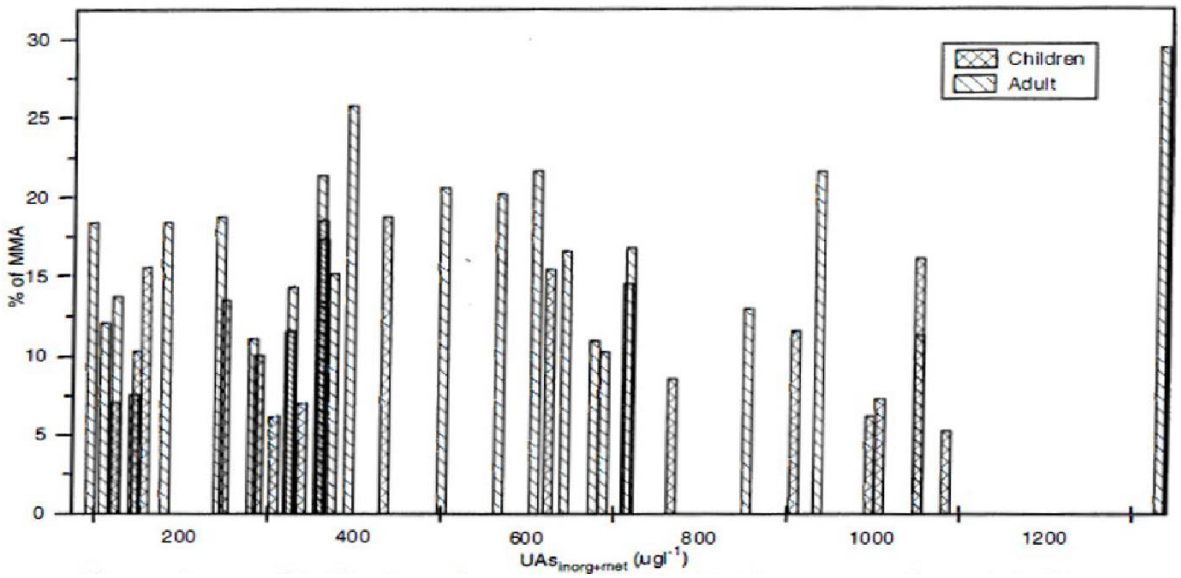


Figure 5. Distribution of percent MMA (%MMA) and urinary arsenic metabolites (UAsinorg+met) in exposed children and adults.

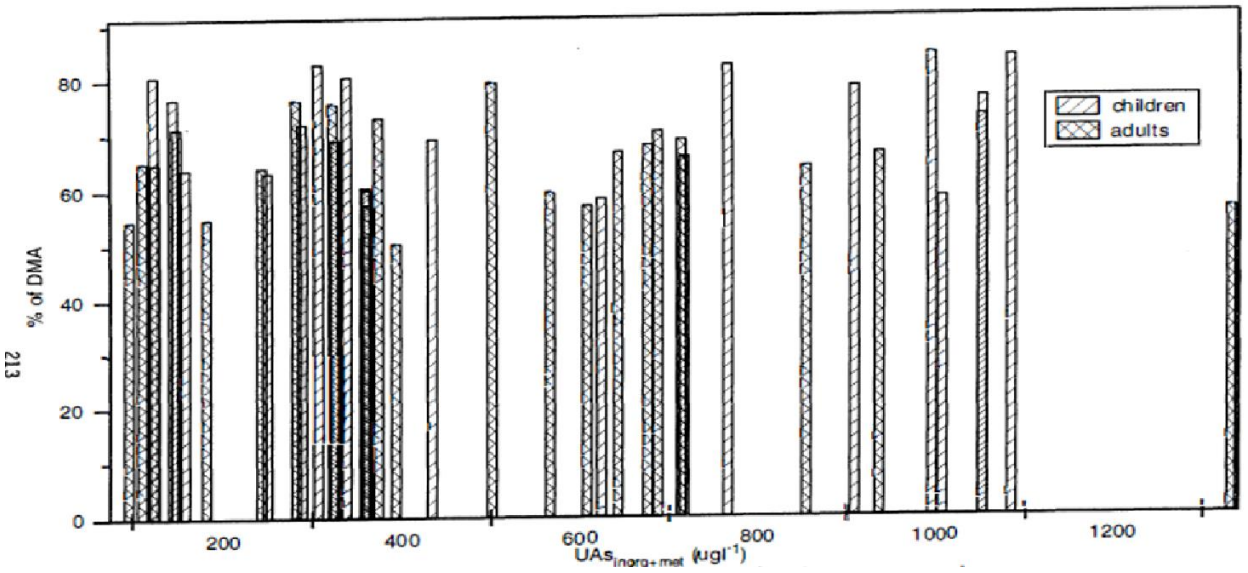


Figure 6. Distribution of percent DMA (%DMA) and urinary arsenic metabolites (UAsinorg+met) in exposed children and adults.

The efficiency of the methylation process is also assessed by the ratios between urinary concentrations of putative products and putative substrates of the As metabolism pathway. Higher values mean higher methylation capacity. Table 16 indicates the methylation capacity of different groups. Table shows, the values of the MMA/In-As ratio for adults' male and female 1.0 vs 0.87, for children male (boys) and female (girls) 0.72 vs 0.77, and for adults and children 0.92 vs 0.73, respectively. These results indicate that the first reaction of the metabolism pathway is more active in adults than children, basically in adult's male. Table 16 also shows a significant (t-test positive) increase in the values of the DMA/MMA ratio in children compared to adults of the exposed group (8.15 vs. 4.11, respectively). But there is no difference between the value of DMA/MMA ratio in adult males and females (average 4.22 vs. 4.03). Another interesting point is that DMA/MMA ratio in boys is greater than girls (8.62 vs 6.93). In a comparative study with control samples, there is no significant difference in the values of DMA/MMA and MMA/iAs ratios between exposed and control adults (4.11 vs. 4.98 and 0.92 vs. 0.98, respectively) and control adults and children (4.98 vs 4.22 and 0.98 vs 0.88, respectively). But there was a significant difference observed (positive t-test) in the values of DMA/MMA ratio between exposed and control children (8.15vs 4.22). Therefore, these results also indicate that methylation capacity in children is higher than adults, because the values of the DMA/MMA is significantly (positive t-test) higher in children than the adults (8.15 vs. 4.11) in the exposed group, especially for boys. Thus, from these results we may conclude that children retain less arsenic in their body than adults. Moreover, from Table 16 it is also observed that the second methylation step in the arsenic metabolic pathway is more active in children than adults. Because there is no significant difference of the values of MMA/iAs ratio, but the values of DMA/MMA ratio are significantly different between adults and children of the exposed group (0.92 vs. 0.73 for MMA/Asi and 4.11 vs 8.15 for DMA/MMA, respectively). This is like the findings of Yamauchi H., et al[35]. Yamauchi H., et al., reported that for low age persons (under 12 years old) the methylation capacity [2nd methylation (MMA to DMA)] was higher than the senior persons (over 13 years old).

Table 16: Relationship between the ratio of urinary average arsenic concentration of putative products and putative substrates of the arsenic metabolic pathway in adults and children of exposed (arsenic in drinking water, above 285 µg/L) and control (arsenic concentration in drinking water, below 3 µg/L) groups.

	N	MMM/Asinorg.	DMA/MMA
Exposed Group			
Adults (M)	11	1.0 (0.53-2.03)	4.22 (1.89-6.84)
Adults (F)	13	0.87 (0.53-1.83)	4.03 (2.42-6.87)
Children (M) (Boys)	13	0.72 (0.48-1.49)	8.62 (4.11-15.74)
Children (F) (Girls)	5	0.77 (0.21-1.57)	6.93 (3.68-11.42)
Adults (M+F)	24	0.92 (0.53-2.03)	4.11 (1.89-6.87)
Children (M+F)	18	0.73 (0.21-1.57)	8.15 (3.68-15.74)
Control Group			
Adults (M)	11	0.81 (0.18-1.55)	4.79 (1.98-10.17)
Adults (F)	7	1.24 (0.37-4.64)	5.29 (2.18-8.78)
Children (M) (Boys)	4	0.94 (0.37-1.15)	3.83 (2.43-6.00)
Children (F) (Girls)	5	0.83 (0.48-1.0)	4.53 (2.89-6.91)
Adults (M+F)	18	0.98 (0.18-4.64)	4.98 (2.43-6.91)
Children (M+F)	9	0.88 (0.37-1.15)	4.22 (2.43-6.91)

M = Male; F = Female

From Table 17, it appears that in the same family, children had lower percentage of In-As and MMA and higher percentage of DMA in most of the cases compared to adults. Similarly, the values of the DMA/MMA ratio are always significantly higher in child than adult in every family (3.86 vs. 2.98, 6.73 vs. 1.95, 15.74 vs. 2.94, and 6.74 vs. 3.85 for the family number 1,2,3, and 4, respectively). This observation also indicates that methylation process of arsenic in children is better than adults.

Table 17: Arsenic concentration (µg/L) and range of total arsenic metabolites (U-Asinorg+met) in urine (µg/L) and the percentage of inorganic As (In-As), MMA, and DMA in urine of adults and child from different exposed families.

Family No.	Source	Age (years)	Arsenic concentration in drinking water (µg l^{-1})	U-$As_{inorg+met}$	Total urinary arsenic metabolites			DMA / MMA
					Inorganic arsenic	MMA	DMA	
1	Adult	35	300	175.7	46.7 (26.57%)	32.3 (18.45%)	96.6 (54.98%)	2.98
	Child	10		440.1	52.8 (12.00%)	82.8 (18.81%)	309.4 (69.19%)	3.68
2	Adult	30	340	387.5	92.4 (23.84%)	100 (25.81%)	195.1 (50.35%)	1.95
	Child	11		913.9	90.5 (9.90%)	106.5 (11.66%)	716.9 (78.44%)	6.73
3	Adult	30	540	560.2	113.4 (20.24%)	113.4 (20.25%)	333.4 (59.51%)	2.94
	Child	11		1089.5	120.2 (11.03%)	57.9 (5.32%)	911.4 (83.65%)	15.74
4	Adult	25	460	494.7	ND	102.0 (20.62%)	392.7 (79.38%)	3.85
	Child	4		1057.6	130.2 (12.31%)	119.8 (11.33%)	807.6 (76.36%)	6.74

Figure 7 shows the distribution of the values of DMA/MMA ratio with different ages of exposed humans, and it appears that the DMA/MMA ratio decreases with increasing age, i.e., methylation process decreases with increasing age. This observation also supports the 2nd methylation process of arsenic (MMA to DMA) in children is better than adults.

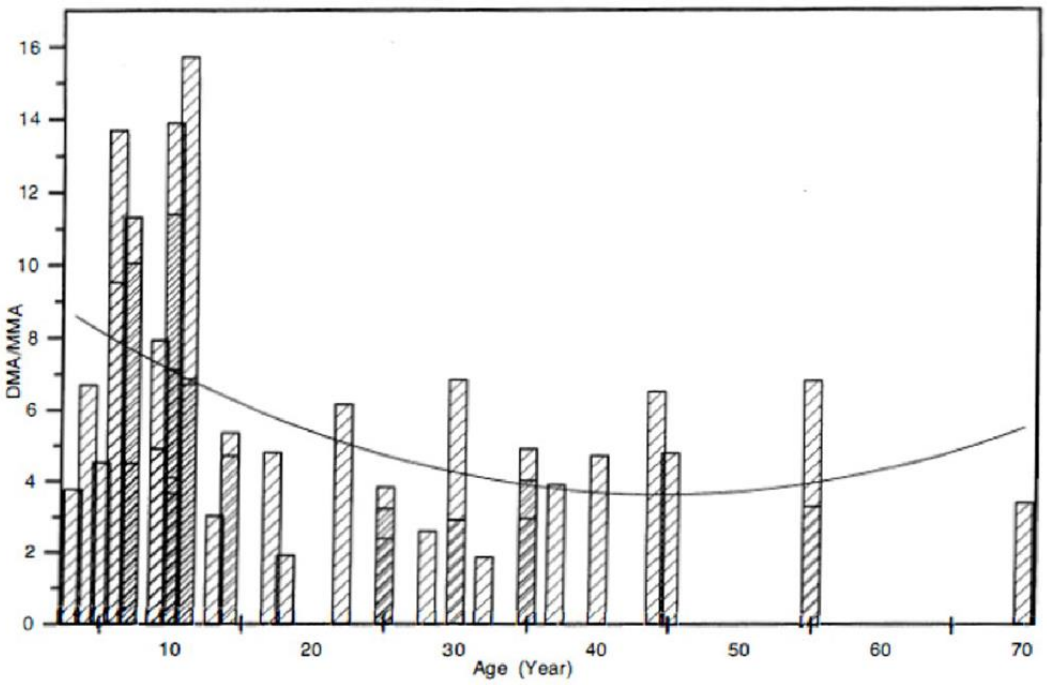


Figure 7: Distribution of DMA/MMA ratio with different ages of exposed human group.

Conclusions: The results of this study suggest concluding the following information (a) Normally children under 11 years of age do not show arsenical skin manifestations with a few exceptions when (1) The arsenic content in water consumed by children is very high (≥1,000 µg/L) and (2) the arsenic content is not very high but the children get poor nutrition, (b) Normally arsenical skin lesions observed in children are diffuse melanosis and spotted melanosis. Keratosis on the palm and sole are not common in children. We have not also found any child patient below 11 years with non-pitting oedema, gangrene, Bowens, dorsal, and cancer, (c) Arsenic toxicity depended on the amount of arsenic consumed. An example, the youngest child (age 18 months) arsenic patients with melanosis (+), keratosis (++) [spotted on palm and sole] is found and the child used to drink very high quantity of water (2-3 liters per day) from very childhood, (d) It appears that children living in arsenic affected villages have higher arsenic body burden/higher risk but less dermatological symptoms (84% of the children had arsenic in hair more than the toxic level and 89% in nail above normal level, but only 6.12% of 4,864 children showed arsenic skin lesions), (e) Arsenic in hair and nail of the adults are higher than those of children. But for urinary arsenic, children are excreting more arsenic than that of adults, (f) Children recover from diffuse melanosis (blackening of color) and light spotted melanosis (+) quickly if they use safe water and with better nutrition, and with vitamins recovery is enhanced, (g) Average total urinary arsenic metabolites in children's urine is higher than adults, that means children require more water than adults in terms of ml, per kg body weight, (h) the value of arsenic excretion and arsenic intake per kg body weight ratio for adult is 0.53 while that of the children is 0.69. This indicates total arsenic excretion is higher for children per kg body weight than adult, (i) The average values (n = 18) for speciation of arsenic in children urine are 15.60% In-As; 10.64% MMA, and 73.76% DMA), (j) it appears that overall percentage of MMA is higher, and DMA is lower in adults than children (average % MMA is 17.20 versus 10.64 and % DMA is 64.62 versus 73.76%), respectively). Therefore, may be the 2nd methylation step (MMA to DMA) is more active in children than adults, (k) Methylation capacity in children is higher than adults, because the values of the DMA/MMA are significantly (positive t-test) higher in children than the adults (8.15 vs. 4.11) in arsenic exposed group, and (l) The methylation capacity of arsenic decreases with increasing ages.

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