4 Speciation of arsenic in rice and vegetables from arsenic exposed areas in Bangladesh

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Abstract

Foodstuffs from arsenic-exposed areas in Bangladesh may contain high concentration of total arsenic but their speciations are not known. About 600 samples of raw rice and vegetables were collected from two arsenic-exposed Upazillas. Speciation of arsenic was done using column chromatography and hydride generator atomic absorption spectrometer. The mean concentrations of inorganic arsenic, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in raw rice were 296.3, 222.5 and 363.4 μg/kg respectively. High concentrations of inorganic arsenic (range 270-377 μg/kg) were found in arum lati, arum leaf, arum root and snake gourd. Low concentrations of inorganic arsenic (range 0-62 μg/kg) were present in potato, pumpkin, amaranth leaf and kalmi leaf. Vegetables contain 27.9% inorganic arsenic, 21.5% MMA and 50.6% DMA. The average daily intake of arsenic by an adult was 1017.9 μg of which 54.3% was from the raw rice and vegetables. The remaining 45.7% of arsenic intake was from drinking water. Considering the total intake of inorganic arsenic, raw rice and vegetables contributed only 27.4%. This study suggests that foodstuffs contributed a higher concentration of arsenic intake than drinking water in two arsenic-exposed areas in Bangladesh.

Introduction

About half of the total populations (about 57 million), of Bangladesh are consuming high concentrations of arsenic through drinking water (Mudur, 2000). The World Health Organization's drinking water guideline value for arsenic is 10 μg/l. Arsenic in drinking water is present as arsenite and arsenate, which show acute toxicity.

Recent studies show that foodstuffs of Bangladesh also contain high concentrations of arsenic (Huq and Naidu, 2003; Das et al, 2004). Raw rice with arsenic levels of 1.8 mg/kg have been recorded in the arsenic-affected tube well areas of Bangladesh (Meharg and Rahman, 2003). With an arsenic concentration of 1.5 mg/l in water used for cooking, a raw rice arsenic level of 1.8 mg/kg contributes to about 35% of the dietary arsenic intake. It has been found that seafood contributed almost 90% of daily arsenic intake in USA (Gunderson, 1995), about 70% in Canada (Dabeka et al., 1993) and 60~70% in Japan (Tsuda et al., 1995). Bangladeshi people usually do not consume seafood, though rice and vegetables are consumed with small amounts of fresh water fish (Jahan and Hossain, 1998).

Arsenic in foodstuffs is present in both inorganic and organic forms. Ninety percent of the arsenic in seafood as foodstuffs is in organic form. Organic arsenic is less absorbable from the gut and shows less acute toxicity. In Bangladeshi, Indian and European rice, 80%, 81% and 64%, respectively, of the recovered arsenic is found to be inorganic (William, 2005). In contrast, DMA is the predominant species (42%) in the rice of USA. However, the percentage of inorganic and methylated arsenic in other foodstuffs of Bangladesh is not known. There is an emerging hypothesis that methylated forms of arsenic may play a larger role in arsenic toxicity (Styblo et al, 2000). The objective of the present study was to do speciation of typical foodstuffs and to identify the foodstuffs that have capability to metabolize arsenic.

Materials and Methods

Chemicals: Arsenic trioxide and sodium borohydride were obtained from Loba Chemie (India). DMA was purchased from Sigma Chemical Co. (USA). Monomethylarsonic acid (MMA) was purchased from Chem Service (USA). AG50 WX-8 resin was obtained from Bio-Rad Laboratories (USA). Concentrated hydrochloric acid, sulfuric acid, nitric acid and perchloric acid were purchased from E. Merck (Germany). Sodium borohydride solution prepared freshly on each day of arsenic estimation by dissolving borohydride powder in deionized water and stabilized with 0.1% w/v sodium hydroxide.

Collection of samples: About 600 samples of different foodstuffs were collected from two different Upazillas (smallest administrative unit): Bashail Upazilla of Tangail District and Shahrasti Upazilla of Chadpur District during June and July 2005. In each Upazilla, a house was randomly selected from which five Upazilla Health Assistants moved toward five different directions. Each Upazilla Health Assistant selected one house from the 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th}, 60^{th}, 70^{th}, 80^{th}, 90^{th}, and 100^{th} cluster of houses. We collected 8 samples of foodstuffs in addition to tube well water and soil from each house that the owner cultivated in his own field. Selection of items depended entirely on the individual house owner's choice. Each sample (ranging 100-500 g) was collected in a polyethylene bag and identified with a code number. All samples were then transported to the laboratory on the same day of collection and kept at -20°C until analysis for arsenic.

Eighty four samples of midstream tube well water were collected in plastic bottles containing 0.1% concentrated nitric acid as preservative. Similarly, 73 samples of surface soils (0-15 cm) were collected from the houses. After collection, samples (about 2-5 g) were air dried, ground and screened to pass through a 0.5 mm sieve and stored in polyethylene vials for all laboratory analysis.

Speciation of arsenic: Each sample was weighed and homogenized using an electric blender and the extraction of arsenic was done with a mixture of water-methanol (50:50, v/v) for 10 hours (D'Amato et al, 2004). It was then filtered and the filtrate passed through ion exchange chromatography on columns of AG50 WX-8 resin. Inorganic arsenic, MMA and DMA were eluted sequentially from the column by passing 0.5 M hydrochloric acid, deionized water and 4M ammonia (Tam et al., 1978). Finally, the amount of inorganic arsenic, MMA and DMA were estimated using a continuous flow hydride generator with atomic absorption spectrometer (HG-AAS; model 210 VGP, Buck Scientific Co., CT, USA).

Digestion of soil sample: Extractable arsenic of the soil was assessed following aqua-regia digestion of soils. Soil (500 mg) was digested with 10 ml of aqua-regia solution. The procedure was repeated. Following digestion, the extracts were diluted to 50 ml using aqua regia and the amount of total arsenic in the extract was estimated using continuous flow HG-AAS.

Estimation of arsenic(Wang et al., 1994): Each sample introduced into the HG-AAS by continuous flow of 10% hydrochloric acid, 3% sulfuric acid and 1% sodium borohydride into a gas-liquid separator. The arsine vapor produced by arsenic and the hydrogen gas (produced by sodium borohydride and acid) was carried by flowing argon gas into the quartz T-tube. The tube was heated in an air-acetylene flame, which served as an atomization cell.

Quality control: The limit of detection and limit of quantification were estimated using 3 and 10 standard deviation of low concentration of standard estimated for 6 times. The limit of quantification of total arsenic was 0.88 μg/l.

Results

The mean (± S.D.) concentration of arsenic in tube well water (n = 84) from two arsenic-exposed areas were 108.2 ± 122.4 μg/l. More than 50 μg/l of total arsenic (Bangladesh standard) was detected in 54.8% of the tube wells.

The highest concentration detected was 531.6 μg/l. The mean concentration of arsenic in surface soil (n = 73) was 637.3 ± 585.4 μg/kg with highest concentration 1905.4 μg/kg.

The mean concentrations of inorganic arsenic, MMA and DMA in raw rice were 296.3, 222.5 and 363.4 μg/kg respectively (Table 1). That is, 33.6% were in inorganic form. DMA was the predominate species (41.2%).

Frequently used non-leafy vegetables in cooking were green papaya (17.7%), lady's finger (16.5%), egg plant (16.5%), and amaranth (15.3%). These vegetables were usually cooked with small amounts of fresh water fish or dried). The concentrations of arsenic in non-leafy vegetables ranged from 619.4 to 962.9 μg/kg. The highest concentration was found in arum stem and the lowest concentration in amaranth stem. Pumpkin did not contain any detectable amount of inorganic arsenic. Non-leafy vegetables show 24.9% inorganic arsenic, 27.5% MMA and 47.6% DMA.

The arsenic concentrations in arum root and arum lati were more than 1 mg/kg. The percentage of inorganic arsenic, MMA and DMA were 28.7%, 16.8% and 54.5% respectively.

Among the leafy vegetables, indian spinach (33.4%), arum leaf (20.6%) and amaranth leaf (15.5%) are usually cultivated during the months of June and July. The highest concentration of inorganic arsenic was detected in arum leaf (369.3 μg/kg) whereas very low concentrations were found in amaranth leaf (39.1 μg/kg) and kalmi leaf (62.3 μg/kg). The percentage of inorganic arsenic, MMA and DMA were 30%, 20.4% and 49.6% respectively.

Only two samples of potatoes were found in the collected samples and the concentration of inorganic arsenic was below the limit of detection. The amount of MMA was higher than that of DMA. In addition, the concentration of total arsenic was only 448.5 μg/kg, the lowest value among the foodstuffs examined. However, more samples are necessary for speciation of arsenic.

Table 2 shows the average daily intake of inorganic and methylated arsenic calculated on the basis of average intake of foodstuffs according to the Bangladesh National Nutrition Survey 1995-96 (Jahan and Hossain, 1998). On average the daily intake of total arsenic was 1017.9 μg of which 62.7% were as inorganic arsenic, 13.7% as MMA and 23.6% as DMA. Of the total intake of arsenic, 54.3% were from the rice and vegetables. That is, half of total intake of arsenic was from foodstuffs. While considering the intake of

Table 1: Speciation of arsenic in rice and vegetables from two arsenic-exposed areas in Bangladesh

Foodstuffs	*Local name*	*Number of samples*	*Speciation of arsenic in foodstuffs (µg/kg)*			
			Inorganic	*MMA*	*DMA*	*Total*
Raw rice	Chaal	75	296.3	222.5	363.4	882.2
Non-leafy vegetables						
Amaranth stem	Data	39	166.0	144.4	309.0	619.4
Arum stem	Kachur data	40	229.7	323.1	410.1	962.9
Dhundal	Dhundal	9	214.7	241.7	182.2	638.6
Egg plant	Begoon	42	252.6	191.6	449.1	893.3
Lady's finger	Dherosh	42	211.6	155.9	318.0	685.5
Papaya (green)	Kacha pepe	45	177.9	220.5	282.5	680.9
Pumpkin	Kumra	2	0	331.4	433.5	764.9
Ridge gourd	Jhingha	11	141.2	89.3	486.0	716.5
Snake gourd	Chichinga	22	270.3	121.6	294.7	686.6
Roots and tubers						
Arum lati	Kachur lata	34	377.6	232.3	533.5	1143.4
Arum root	Maan kachu	16	274.4	149.8	706.7	1130.9
Leafy vegetables						
Amaranth leaf	Data shak	34	39.1	134.3	284.8	458.2
Arum leaf	Kachu shak	45	369.3	230.6	534.4	1134.3
Halancha leaf	Halancha leaf	22	196.0	130.2	348.1	674.3
Indian spinach	Pui shak	73	227.7	157.5	386.0	771.2
Jute leaf	Paat shak	10	212.1	98.4	321.8	632.3
Kalmi leaf	Kalmi leaf	3	62.3	0	49.2	111.5
Potato leaf	Alu shak	5	249.8	128.7	326.5	705.0
Pumpkin leaf	Kumra shak	26	225.3	193.4	365.9	784.6
Potato	Alu	2	0	270.7	177.8	448.5

Table 2: Average daily intake of inorganic and methylated forms of arsenic

Foodstuffs	*Amount of food* and drinking water/day***	*Speciation of arsenic (µg/day)*			
		Inorganic arsenic	MMA	DMA	Total
Raw rice	427 g	126.5	95.0	155.1	376.6
Water for cooking rice	1 liter***	108.2	-	-	108.2
Non-leafy vegetables	92 g	20.7	19.4	26.2	66.3
Roots and tubers	70 g	22.8	13.3	43.4	79.5
Leafy vegetables	21 g	3.2	5.4	11.1	19.7
Pulse	10 g	NA	NA	NA	NA
Potato	24 g	0	6.4	4.2	10.6
Fruits	13 g	NA	NA	NA	NA
Fish	32 g	NA	NA	NA	NA
Meat	6 g	NA	NA	NA	NA
Egg	3 g	NA	NA	NA	NA
Milk and milk products	13 g	NA	NA	NA	NA
Sea fish	1.2 g	NA	NA	NA	NA
Drinking water	3.3 liters	357.0	0	0	357.0
		638.4	139.5	240.0	1017.9
		62.7%	13.7%	23/6%	

* Jahan and Hossain, 1998; **Sinha et al, 2003; ***Bae et al, 2002; NA means data not available

inorganic arsenic raw rice and vegetables contributed only 27.4%. Of the total intake of arsenic per day, MMA and DMA were supplied totally from the rice and vegetables. So, vegetables contained 27.9% inorganic arsenic, 21.5% MMA and 50.6% DMA.

Discussion

At present, 61 out of 64 districts of Bangladesh are affected with high concentrations of arsenic (more than 50 μg/l, the Bangladesh standard level) in drinking water of shallow tube well origin. About half of the total populations are exposed to arsenic (Mudur, 2000). In our studied areas, more than 50% of the total tube wells are contaminated with arsenic. The mean (± S.D) concentration of arsenic in tube well water was 108.2 ± 122.4 μg/l whereas its concentration in soil was only 637.3 ± 585.4 μg/kg indicating that the surface soils are not contaminated. A study conducted by Huq et al., (2003) shows that arsenic concentrations in the soils of different districts of Bangladesh were less than 10 mg/kg. The surface (0-15 cm) soil contains more arsenic than the subsurface (16-30 cm) soil. It may be due to arsenic contaminated water that is carried out every year by flood.

Bangladesh is typically a rice growing country. About 80% of the total agricultural land (61% of the land area of the country) is under rice cultivation. Bangladeshi people mainly consume rice and vegetables (Jahan and Hossain, 1998). Fish, meat, eggs and milk products represent less than 15% of the total intake of foodstuffs. Rice comprises 73% of a Bangladeshi's calorific intake (del Ninno and Dorosh, 2001) whereas in USA it is only 5% of mean food intake (Tao and Bolger, 1999). The mean concentration of arsenic in raw rice was 882.2 μg/kg. This concentration is actually 10-30% higher than the raw rice in plant which is due to processing of raw rice immediately after cultivation by arsenic contaminated water (Misbahuddin, 2003). The mean concentrations of arsenic in the rice collected from different districts of Bangladesh were reviewed (Williams et al., 2005). The range of arsenic was 100 to 950 μg/kg. The mean concentration of arsenic in the rice of our study is within this range. Of the total amount of arsenic in rice, 33.6% were inorganic arsenic, 25.2% were MMA and the rest 41.2% were DMA. Williams et al, (2005) showed that the amount of inorganic arsenic in Bangladeshi rice is 80%, which is about 2-fold higher than that of USA rice (42%). Our data has similarity with the rice from USA but the reason for the dissimilarity with Williams result is unclear. The amount of

DMA, in our study, was highest as the similar result obtained by Scoof et al., (1991).

Among the vegetables, arum is common and widely cultivated in rural Bangladesh. All the parts of this vegetable (leaf, stem and root) are edible. It contains high concentrations of arsenic, though the levels of arsenic in different parts of this plant vary. The highest concentration is in the root and the lowest concentration in the stem. On the other hand, kalmi leaf and amaranth contain low concentrations of arsenic.

Roychowdhury et al., (2002) shows that Indian (West Bengal) people are consuming about 715 g of rice and 100 g of vegetables per day. Total daily intake of arsenic was 171-189 μg from foodstuffs, though the amount of inorganic, MMA and DMA were not studied. Their recent data (Uchino et al, 2006) show that rice and vegetables contributed about 94%, 73% and 42% of arsenic intake in mild (water arsenic concentration of 2.8 μg/l), moderate (30.7 μg/l) and high (118 μg/l) arsenic-affected families. Using the data of our National Nutritional Survey 1995-96 (Jahan and Hossain, 1998), the mean daily intake of arsenic by the Bangladesh people of arsenic exposed areas is 909.7 μg of which 552.7 μg (about 60.7%) from the rice and vegetables. High intake of arsenic through food is due to higher concentration of methylated arsenic in foodstuffs.

Potato and pumpkin had non-detectable level of inorganic arsenic whereas concentrations of inorganic arsenic in amaranth leaf and kalmi leaf are very low. More studies are required to confirm these data. High concentration of inorganic arsenic in arum lati and arum root may be due to direct contact of this part of the plant with the arsenic present in the soils. The wide differences in between arum leaf and amaranth leaf, though both are away from the roots, are not known.

Among the vegetables, arum (root, leaf, and lati) contained high concentrations of DMA. Low concentrations of DMA were detected in kalmi leaf, potato and dhundul. If the people of arsenic-exposed areas avoid cooking different parts of arum, then the average daily intake of arsenic through vegetables would be around 80 μg instead of 165.5 μg (Table 2). That is, total avoidance of arum consumption only reduces about 9.4% of the total intake of arsenic.

In conclusion, this study suggests that foodstuff of arsenic-exposed areas contributed higher concentrations of arsenic intake than the drinking water, though the intake as inorganic arsenic is less.

Acknowledgement

This work was supported by a grant from World Health Organization, Bangladesh (under ICP-budget). We thank Dr. Deoraj Caussy (epidemiologist, SEARO) and Dr. Andrew Trevett (WHO Advisor for Environmental Health) for their critical comments of the study.

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