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Evaluation of arsenic levels in rice grains available in market and household cooked rice in five selected regions of Chattogram division

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Abstract

Rice has been implicated as a major dietary source of arsenic exposure. Under this study, total arsenic levels of 100 Bangladeshi rice grain samples collected from wholesale and retail markets in Chattogram Division were measured by Hydride Generation Atomic Absorption Spectrometry (HG-AAS). Twenty-five foreign rice types were used as controls. The mean arsenic concentration in the samples was 0.18 µg/gm, falling within the "global normal range" of 0.08 - 0.20 µg/gm. None of the Bangladeshi samples exceeded the recommended maximum limit of 1.0 µg/gm. We estimated that rice contributed 37.29% of the maximum tolerable daily intake (MTDI) of arsenic in local consumers. Cooking rice samples containing arsenic below detection limit with water sample containing 216 µg/L arsenic resulted in marked elevation of arsenic content in the cooked samples (0.79 to 1.08 µg/gm). The present findings suggest that arsenic exposure through rice consumption should not be neglected while devising mitigation policies.

Key words: Rice, arsenic, hydride generation atomic absorption spectrometry (HG-AAS), maximum tolerable daily intake (MTDI)

Introduction

Arsenic (As) is a well-studied toxic metalloid and is referred to as the "King of Poisons"[1]. In humans, arsenic exposure has been implicated in the development of skin lesions, cancers, cardiovascular diseases (CVDs), peripheral vascular disease and many other diseases[2]. Humans are exposed to arsenic mainly through drinking water, food and air[3]. An estimated 35 to 77 million Bangladeshis have been chronically exposed to arsenic through drinking water[4]. The permissible range of arsenic level in drinking water in Bangladesh has been set to 50 µg/L, which is 5 times higher than the WHO standard of 10 µg/L. In a study, 35% of all tube wells in the country were found to contain > 50 µg/L arsenic[5]. In areas where the environment is not polluted with arsenic, crops usually do not accumulate arsenic to levels that would be toxic to humans. On the other hand, the situation becomes threatening in areas where soil contains high arsenic levels. In those types of areas, arsenic uptake by plant tissues is elevated significantly, especially in edible crops and vegetables[6]. In Bangladesh, arsenic contamination of groundwater has become a matter of further concern due to its use for irrigation purposes, leading to arsenic contamination of food chain. Lin *et al.* reported that translocation of arsenic through root to rice grain can adversely affect human health[6]. Crops are grown with local arsenic contaminated groundwater in Bangladesh which is adding significant amount of this metalloid in rice and in the topsoil[7]. When the irrigation water had arsenic levels of more than 10 µg/L, arsenic concentrations up to 83 µg/gm were found in the soil[8]. Abedin *et al*. reported that arsenic levels in cultivated rice grains increased significantly with increasing arsenic levels in the water used for irrigation[9]. These reports clearly suggest that arsenic contamination of food chain poses additional threat to human health besides arsenic exposure through drinking water.

The provisional maximum tolerable daily intake (MTDI) of inorganic arsenic suggested by the World Health Organization (WHO) is 2.1 µg/kg body weight[10]. The dominant arsenic species in rice has been reported to be inorganic[10]. Chowdhury *et al.* showed that inorganic arsenic constituted 95% of the total arsenic in rice samples from Kolsur village- a highly arsenic-affected area in West Bengal, India[11]. In a study conducted in Bangladesh, the average total arsenic level in rice grains was reported to be 0.358 µg/gm in 46 samples[12]. In another study, concentrations of arsenic in rice in a village of Jessore district ranged from 0.160 to

0.580 µg/gm[13]. In a market-based study, the mean arsenic concentration of 15 rice samples of Bangladeshi origin bought from wholesale market was reported to be 0.130 µg/gm[14]. Research has also showed that cooking rice with arsenic contaminated water can result in an additional increase in its arsenic concentration[15].

Arsenic content assessment of rice grain and cooked rice has been done to some extent in mostly the Northern and central sides of the country, targeting specific severely arsenic affected areas. But such research is yet to be done in the Chattogram region where some prominent wholesale market of rice and other crops are located. Rice is supplied from all over the country in this densely populated region of the country. Besides, many areas inside and adjacent to Chattogram have been identified as severely arsenic affected, where, even if not a prominent rice producing area, rice is cooked with arsenic contaminated water[1, 16]. Under this study we investigated a total arsenic levels of rice grains commonly available in the market as well as arsenic content of household cooked rice in some selected regions of Chattogram Division and to estimate their contribution to arsenic load in the local consumers.

Materials and Methods

Sample collection, processing and storage

Rice grain

A total of 100 rice grain samples were collected from Chattogram City, Hathazari, Mirsarai, Noakhali, and Cox's Bazar. Since it was mainly a market-based study, we collected rice grain as samples from markets randomly located in different areas irrespective of their arsenic contamination status. The typical way in which local consumers would buy rice and the level of popularity of various rice types commonly available in the markets were reflected in our sample collection strategy. As controls, 25 imported and packaged rice grains were collected from prominent supermarkets located in Chattogram city of which 15 were of Indian and 10 were of Pakistani origin (Table 1). Structured data collection sheets were used to keep record of relevant information regarding the rice grains such as size, processing method etc. The rice grains were heated at 60°C in an oven to ensure complete dryness. They were then powdered by grinding and stored it in airtight plastic containers for avoiding cross-contamination. The grinder was thoroughly cleaned after powdering each sample and control rice.

Table 1 Sample collection areas and sources of controls

	Area of collection	Number of rice grains
Sources of rice grains samples	Chattogram City	40
	Hathazari	15
	Mirsarai	15
	Noakhali	15
	Cox's Bazar	15
		Total = 100

	Country of origin	Number of rice grains
Control	India	15
	Pakistan	10
	Total	25

Cooked rice and cooking water

For assessing arsenic absorption by cooked rice, we collected 4 uncooked rice samples from 4 different households located in the following reported arsenic-contaminated areas in Chittagong City namely South Bakalia, East Sholoshahar, North Halishahar, and in Mirsarai[1,16,17,18]. We also collected water samples from those households for arsenic concentration measurement and for cooking rice. Since one household reported that they used water collected from 2 different sources for cooking purposes, we collected those 2 water samples to represent that household. Thus, we had 5 water samples collected from 4 households. The water samples were collected in sterilized plastic bottles with liquid-tight stopper, acidified with 70% HNO_3 and stored at -20 °C.

To avoid differences in rice cooking methods used by various households, we cooked a portion of the 4 uncooked rice samples collected from the households in our laboratory using the 5 different sources of water samples that were collected from the same households. Thus, each rice sample was cooked 5 times with different water sources using the common water and rice ratio of 2:1 for approximately 30 minutes in a gas cooker[19]. The excess water was drained out after each rice sample was fully cooked. All the cooked rice samples were then oven-dried, ground to fine powder and stored as described in the previous section.

Rice consumption survey

We also conducted a survey regarding the amount of rice consumed daily by the members living in the participating households. A total of 16 people were asked about their daily rice consumption quantities. These data were later used for assessing the health impact of arsenic consumed through rice.

Arsenic concentration measurement

Arsenic concentration was measured by Atomic Absorption Spectrometry equipped with Hydride Generation (HG-AAS)[20]. Analytical grade reagents were used throughout the study. All the solutions were prepared using deionized-distilled water.

Preparation of rice samples

The rice grains were first washed thoroughly with tap water to remove debris, rinsed twice afterwards with deionized water, and oven-dried at 60 °C for 24 hours. The grains were then ground to a fine powder using a grinder. For digestion of each sample, 0.25 gm of the rice powder was weighed into a 50 mL glass beaker and concentrated acids were added in the following order: 4.0 mL HNO_3, 1.5 mL H_2SO_4, and 1.0 mL $HClO_4$. The beaker was then placed in a fume hood and was allowed to stand for 15–30 minutes before being gently heated on a hotplate. The temperature was increased gradually and maintained at 100–110 °C until the solution became clear (typically 60–120 minutes). After cooling down the solution to room temperature, 5 mL of deionized water was added to it and the digest was filtered through Whatman No. 42 filter paper into a 25 mL volumetric flask and made up to the mark with deionized water.

Preparation of water samples

Each water sample was stabilized by adding concentrated HNO_3 at the time of collection to restrict precipitation and stored at -20 °C. Prior to analysis by HG-AAS, 1 mL of 5M HCl, and 1 mL of 20% KI were added to 10 mL of each water sample in a test tube which was then heated to 80 °C for 30 minutes.

Measurement of arsenic

An iCE 3300 AAS fitted with VP100 continuous flow vapour generation system (Thermo Scientific) was used for the HG-AAS analysis. The spectrometer was operated at 193.7 nm wavelength and the lamp current was set at 75%. Other parameters were also set accordingly. Absorbance values were recorded following background correction. For quantization, the peak heights were used by the dedicated software. Limit of Detection (LOD) for arsenic in rice grains and water samples were 0.25 µg/gm and 5 µg/L, respectively.

Statistical analysis

Statistical analysis required for the study was performed by using Microsoft Excel. T-test was used to compare the arsenic content of sample and control rice grains, and standard errors (SE) of the means were calculated. For classifying rice grains according to the size, the following scale used by International Rice Research Institute (IRRI) was used: Short grain: <5.50 mm; Medium grain: 5.51 to 6.60 mm; and Long grain: >6.61 mm. The rice grains for which arsenic level was below the detection limit (<0.25 µg/gm), a value of 50% LOD (0.13 µg/gm) was used in accordance with previous similar studies[21,22].

A strategy combining market-based study and household survey was used to assess the potential health risk of arsenic exposure through rice by estimating its contribution to arsenic load in the local consumers. A standard 60 kg body weight was considered for estimating the contribution of rice to the maximum tolerable daily intake (MTDI) of arsenic [10]. The following percentages reported in previous literature were used in various calculations-

1. Average inorganic arsenic level (out of total arsenic concentration) in Bangladeshi rice grain: 80%[14]
2. Bioavailability of ingested inorganic arsenic through consumption of cooked rice: 90%[23]

Results and Discussion

Characteristics of the rice grains

Market survey is a widely employed strategy to monitor the safety of food supplies globally[24,25]. In our study, the market rice samples were collected from wholesale, retail and reputed supermarkets to reflect a wide range of commercially available rice of Bangladeshi origin. We compared those Bangladeshi rice grains to control rice samples from two globally important rice producing countries (India and Pakistan) to find any trend of differences in their arsenic concentrations.

Regarding the processing method, most of the samples (n = 62) were parboiled and the rest (n = 38) were sunned. Taking into account the grain size, medium sized grains were predominant, followed by short and long sized grains (Table 2). Globally, consumer preferences for rice types are heterogenous and medium sized rice is the most popular choice in Bangladesh[26]. While collecting sample rice grains from various markets, we also found medium sized rice to be predominant - the trend also being reflected in our samples. The packaged foreign rice that were bought as controls from supermarkets were all long grain, parboiled basmati rice, since packaged rice that are imported in Bangladesh are almost entirely of those types.

Table 2 Characteristics of the collected samples

Criteria	Characteristics	Number of samples (n = 100)
Processing method	Sunned	38
	Parboiled	62
Grain size	Short	34
	Medium	59
	Long	7

Arsenic concentration in market rice

The mean arsenic concentration in the samples was found to be 0.18 µg/gm, whereas in the controls, the mean arsenic concentration was 0.23 µg/gm. No significant difference (P=0.28) was observed between samples and controls regarding arsenic concentration (Figure 1). In our study, the mean arsenic concentration in the samples i.e., Bangladeshi rice grains was within the “global normal range” or background range of 0.08-0.20 µg/gm as specified by previous study[27]. Though the average arsenic concentration of the controls was slightly above this global normal range, it might possibly be attributed to one control with an extreme value (1.20 µg/gm). This particular control (foreign origin) is the only rice grain in our study to exceed the recommended maximum limit of 1.0 µg/gm[28,29]. None of the Bangladeshi sample rice grains exceeded this recommended limit of 1.0 µg/gm and the maximum concentration of arsenic in the samples (0.56 µg/gm) was well below this limit, the findings being consistent with previous reports[29,30].

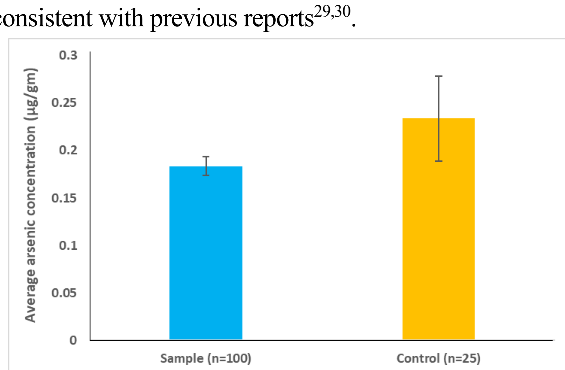

Figure 1 Average arsenic concentration in samples and controls (Error bars represent the standard error of the mean)

Arsenic concentration of rice grains has been shown to be strongly influenced by the origin and variety of the rice[31]. However, available evidence for the effect of grain size on arsenic content is still insufficient and inconclusive[32,33]. Since all the controls (imported rice) were long and parboiled grains, we calculated whether grain size and processing method had any impact on the arsenic content of the samples. We found that short grains had the highest average arsenic concentration (0.20 µg/gm), which is seemingly contradictory with a previous meta-analysis[32] but is supported by a study conducted in rural West Bengal[33]. Both medium and long grains had very similar arsenic content (0.18 µg/gm) (Figure 2). On the other hand, parboiled rice had higher average arsenic (0.19 µg/gm) compared to sunned grains (0.17 µg/gm) (Figure 3), which may be attributed to the use of arsenic contaminated water during parboiling. However, no significant differences were observed (P>0.05) among the samples with regard to arsenic content based on size and processing method.

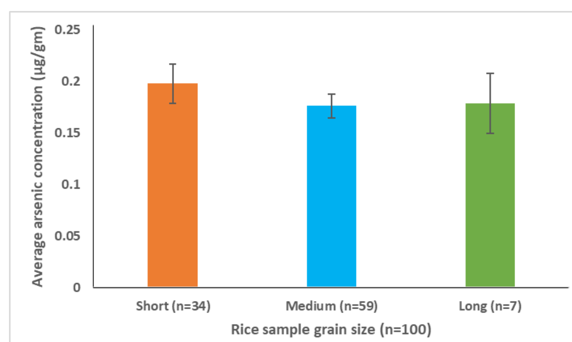

Figure 2 Average arsenic concentration in samples based on size (Error bars represent the standard error of the mean)

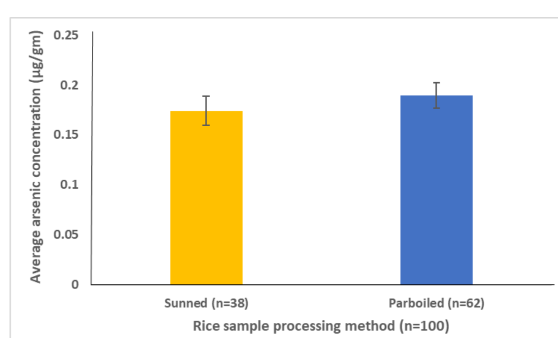

Figure 3 Average arsenic concentration in samples based on processing method
(Error bars represent the standard error of the mean)

Arsenic concentration in household water samples

With a goal of assessing arsenic absorption by rice grains during cooking, arsenic concentrations of 5 water samples from 4 households of reported arsenic-contaminated areas were measured. Four of the samples had arsenic levels above 10 µg/L (WHO limit) and 1 sample had arsenic level below detection limit (BDL). The water sample with the highest arsenic level had 216 µg/L concentration (Table 3).

Table 3 Arsenic concentrations of water samples

Household No.	Water Sample No.	As Concentration (µg/L)
1.	1.	10.77
2.	2.	BDL
	3.	11.13
3.	4.	18.20
4.	5.	216.0

BDL- Below Detection Limit (<5 µg/L)

Arsenic concentration in uncooked and cooked rice samples collected from households

Four uncooked rice grain samples that were collected from 4 different households were cooked using the 5 water samples collected from those same households. All 4 uncooked rice grain samples had arsenic concentration below the detection limit (<25 µg/gm).

In cooked rice, total arsenic comes from two sources- (i) the uncooked rice that is being used for cooking, and (ii) the arsenic absorbed from water during cooking. With typical preparation procedures, rice absorbs almost 100% of its mass in water while cooking[34]. After cooking each sample 5 different times with the 5 water samples, we found that when the rice samples were cooked using water containing 10.77, 11.13 and 18.20 µg/L of arsenic, the cooked samples did not have detectable arsenic content in them. But when cooked using the water sample containing 216 µg/L arsenic, every sample not only showed detectable arsenic concentrations but also in quite high levels (range: 0.79 to 1.08 µg/gm). The results are shown in Table 4.

Table 4 Arsenic concentrations in uncooked and cooked rice samples

Household Rice Sample No.	Arsenic concentration before Cooking (µg/gm)	Arsenic concentration after Cooking	
		Cooking Water arsenic concentration (µg/L)	Cooked Rice arsenic concentration (µg/gm)
1.	BDL	10.77	BDL
		BDL	BDL
		11.13	BDL
		18.20	BDL
		216	**0.79**
2.	BDL	10.77	BDL
		BDL	BDL
		11.13	BDL
		18.20	BDL
		216	**0.87**
3.	BDL	10.77	BDL
		BDL	BDL
		11.13	BDL
		18.20	BDL
		216	**1.08**
4.	BDL	10.77	BDL
		BDL	BDL
		11.13	BDL
		18.20	BDL
		216	**0.83**

BDL- Below Detection Limit (<25 µg/gm)

Though we did not find water samples with intermediate arsenic concentrations (e.g., 50 µg/L, 100 µg/L etc.) to cook rice for comparison, still it is obvious from our results that if arsenic concentration is very high in the water used for cooking, it will substantially increase the arsenic content of the cooked rice. Interestingly, a previous study showed that cooking rice with low to moderately arsenic-contaminated water (up to 58 µg/L) resulted in a significant decrease in arsenic levels of the cooked rice[15]. In that same study, using highly arsenic-contaminated water for cooking (up to 105 µg/L) significantly increased the arsenic content of the cooked rice which is clearly consistent with our observation.

Average daily rice consumption

For estimating the contribution of arsenic ingested through rice to the overall arsenic load in the consumers, all members from the households in reported arsenic-contaminated areas from which uncooked rice and cooking water samples were collected were also surveyed regarding the quantity of their daily rice consumption. Household no. 3 had the highest average daily rice intake (450 gm). Overall, the average daily rice consumption by the surveyed population was 362.50 gm (Table 5), which is slightly higher than the quantity (328.90 gm) reported by the Bangladesh Bureau of Statistics (BBS)[35].

Table 5 Average daily rice consumption by the surveyed population (n = 16)

Household No.	Number of Surveyed Members (n = 16)	Average Daily Rice Consumption (gm)	Overall Average (gm)
1.	4	300	362.50
2.	3	400	
3.	4	450	
4.	5	300	

Contribution of arsenic intake through rice to the MTDI

It has been reported that, even at background levels, arsenic contained in rice contributes to a considerable extent to dietary arsenic exposure in the regions of the world where rice is the staple diet[14]. From our study, considering the average arsenic concentration of rice grain samples (0.18 µg/gm) and daily average rice consumption (362.50 gm), the daily average total arsenic intake from rice was- 0.18×362.50 = 65.25 µg. A previous study in Bangladesh reported an average daily dietary intake of 56.40 µg total arsenic from rice[29]. Another study conducted in West Bengal, India reported a daily average dietary intake of 161.15 µg and 174.84 µg total arsenic through rice consumption in two different blocks[36].

Inorganic As species have been reported to be more toxic than the organic ones[37]. The provisional maximum tolerable daily intake (MTDI) of arsenic specified by WHO (2.1 µg/kg body weight) is based upon inorganic arsenic concentration[10]. To assess the health impact of arsenic ingested through rice, we first converted the concentration of total arsenic into inorganic As and then into bioavailable inorganic arsenic[14,23]. Considering 80% average inorganic arsenic content of Bangladeshi rice grains[14] and our calculated daily average ingestion of 65.25 µg total arsenic by the surveyed people, the daily average inorganic arsenic intake from rice was- 65.25×0.80 = 52.20 µg.

The bioavailability of inorganic arsenic in rice is 90% when cooked[23]. So, of the 52.20 µg inorganic arsenic consumed daily through rice was equivalent to- 52.20×0.90 = 46.98 µg bioavailable inorganic As.

Contribution of rice consumption to maximum tolerable daily intake (MTDI) of arsenic assumes a standard body weight of 60 kg[10]. The WHO's provisional MTDI for arsenic is 2.1 µg/kg body weight[10]. For a person weighing 60 kg, the maximum daily tolerable amount of arsenic intake would be- 60×2.1 = 126 µg. So, if a person weighing 60 kg intakes 46.98 µg (bioavailable) inorganic arsenic daily through rice consumption as per our calculation, then the average rice grain total arsenic level of 0.18 µg/gm found in our study would ultimately be equal to- (46.98/126)×100 = 37.29% of the MTDI.

This is a considerable percentage keeping in mind the fact that arsenic contaminated drinking water, not food, is the principal source of arsenic exposure in

Bangladesh[38]. Let us consider a scenario where a 60 kg person drinks 2 L of water daily which has an arsenic concentration at the Bangladeshi guideline of 50 µg/L. Regarding the bioavailability of arsenic in drinking water after ingestion, arsenic in drinking water is rapidly and almost completely absorbed from the gastrointestinal tract[39]. So, in that scenario, the contribution of arsenic in drinking water to the MTDI would be 79.37%. If we add the contribution of arsenic contaminated rice estimated in our study (37.29%), the combined contribution of drinking water and rice would be 116.66%, exceeding the MTDI. We also have to consider other sources of exposure such as vegetables, cigarettes, occupational environment etc. which can further add to this amount[40].

Conclusion

There have been repeated concerns from scientists across the world regarding elevated levels of arsenic in food, especially in rice grains. We conducted a market-based study where we tried to assess the arsenic content of rice grains of Bangladeshi origin and to compare it with that of rice imported from two prominent rice producing countries. We found the average arsenic content of Bangladeshi rice samples (0.18 µg/gm) to fall within the reported range of global 'normal' or background arsenic level in rice. Moreover, no Bangladeshi sample exceeded the recommended maximum limit of 1.0 µg/gm. Our data suggest that, arsenic exposure through rice is not an immediate threat - not individually at least. But, considering our estimated 37% contribution of rice to the maximum tolerable daily intake of arsenic even at this "normal" level, it is clear that consuming arsenic-contaminated rice along with heavily contaminated drinking water for a long period of time might have serious adverse effects on human health. We also found that using water with very high arsenic levels for cooking rice can substantially increase the arsenic content of the cooked rice. Our results reinforce the idea that intervention strategies must take all possible sources of arsenic exposure into account rather than solely focusing on arsenic-contaminated drinking water. Further studies are required where water containing varied levels of arsenic would be used to cook rice for precisely determining how cooking with contaminated water affects the arsenic content of rice. We hope that our study will raise public awareness regarding the possible sources and harmful effects of arsenic exposure. Policies should be developed that will promote sustainable agricultural practices and will help to take necessary actions for minimizing the transfer of arsenic to soil and crops.

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