

Full Research Paper

GST polymorphic status modifying the arsenic induced clinical manifestations in people of the southern part of West Bengal, India

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Persons chronically exposed to environmental arsenic through their drinking water experience various arsenic induced clinical manifestations which includes keratosis and pigmentary changes in the skin. However the response varies widely among persons. To study whether Glutathione-S-Transferase (GST) gene polymorphism plays any role in this variation, a total of 78 study subjects were recruited from the villages of southern region of West Bengal, India. Concentration of arsenic in their urine and drinking water were determined by Atomic Absorption Spectrophotometry Hydride Generation (AAS) system. Extent of clinical manifestations in the form of pigmentation and keratosis were determined by evaluation of the severity and giving clinical symptom score according to their degree of severity. The individual's GST status was determined by multiplex PCR approach. The persons having skin manifestation given a score called clinical symptom score by which the degree of the clinical manifestation was determined. Results showed that genetic polymorphism of GSTM1 and T1 were significantly associated with urinary arsenic and clinical manifestation in higher exposure group. Persons having null genotype have significantly decreased urinary arsenic ($p < 0.01$) and increased clinical symptom ($p < 0.05$) score relative to persons with GSTM1 or GST T1 non-null genotype of same arsenic exposure group. The study signifies that GST status determines the extent of biotransformation of arsenic in the body which further determines the degree of arsenic induced clinical manifestations in exposed persons.

Key words: Arsenic, GST polymorphism, total urinary arsenic, clinical symptom score.

INTRODUCTION

Arsenicosis is associated with chronic arsenic exposure due to use of subsoil water in many places of eastern and north eastern India, including the basin of River Ganga in West Bengal.

The main arsenic species in subsoil water is arsenate. Inorganic pentavalent arsenate after entering into the body is readily absorbed from gastrointestinal tract and

reduced to arsenite. Arsenite is methylated mainly in the liver to monomethylarsonic acid (MMA) and dimethyl arsinic acid (DMA). The methylated metabolites of arsenic are less toxic, and being electrophilic, are readily excreted in urine. The concentration of arsenic in urine is considered a biological marker of arsenic exposure. Metabolism of arsenic occurs through repeated reduction and oxidative methylation of inorganic trivalent arsenic (AsIII) and pentavalent arsenic (AsV) to monomethyl arsonic acid (MMA)^V and dimethyl arsinic acid (DMA)^V. Oxidative addition of methyl groups to arsenic occurs by

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Table 1. Demographic distribution of the study population.

Age group	Sex	Group A	Group B	Group C
21 - 40	M = 9	9	7	3
	F = 3	3	3	1
41 - 60	M = 10	10	9	14
	F = 6	6	5	4
Smoker	M = all male	19	16	17
Non Smoker	F = all female	9	8	5
Total	N = 74	N = 28	N = 24	N = 22

the enzyme methyltransferase and the methyl group donor is S- adenosyl methionine (SAM) (Aposhian et al., 1997; Thompson, 1993; Vahter, 1999). SAM functions to methylate an assortment of acceptor molecules including Arsenic and DNA.

The biotransformation of inorganic arsenic to organic one is GSH dependent. As the serum level of GSH is further dependent upon the level of GST, it is hypothesized that genetic polymorphism of GST gene status may modulate the extent of arsenic metabolism. The absence of one or both M I allele or T I allele of GST gene (GST μ and θ gene respectively) has been associated with incomplete metabolism of arsenic which was studied in a population of Taiwan. The study showed that MI null allele is associated with increased inorganic arsenic and decreased DMA in urine (Chiou et al., 1997). As an extension of that study we have studied the role of GST polymorphism on total urinary arsenic and clinical severity in a population exposed to arsenic.

MATERIALS AND METHODS

Subjects

Study subjects were chosen from the Arsenic Clinic of Institute of Post Graduate Medical Education and Research, Kolkata, India, a tertiary referral center. Selection criteria was history of exposure to arsenic contaminated water (>50 $\mu\text{g/l}$) as a source of drinking water for more than 6 months, and presence of characteristic skin manifestation of chronic arsenic toxicity, viz, hyperpigmentation, hypopigmentation and keratosis. All the cases recruited were referred cases from south and north 24 Parganas, two major districts of South Bengal. History of arsenic exposure of each participant was obtained in detail including duration of intake of water from the source. Samples of water from the source and spot urine samples were collected from each participant. A control group with exposure level <50 $\mu\text{g/l}$ was recruited from the same area. Participants have been divided into three groups according to concentration of arsenic in their drinking water, A: <50 $\mu\text{g/l}$, B: 51 to 251 $\mu\text{g/l}$, C: 251 to 500 $\mu\text{g/l}$.

The average duration of exposure was about 10 years. The demographic distribution of the study population is described in Table 1. Criteria for diagnosis of arsenicosis were based on parameters described earlier (Guha Mazumder et al., 2001). Briefly these are as follows:

1. History of taking arsenic contaminated water (50 $\mu\text{g/l}$) for more than 6 months.
2. Presence of characteristic skin manifestation of chronic arsenic toxicity:

- a) Hyperpigmentation
- b) Hypopigmentation
- c) Keratosis (Guha Mazumder et al., 2001, 2003).

The skin lesions did not simulate with any other known skin disease.

Inclusion criteria for the study group are: Arsenicosis positive, that is, pigmentation and keratosis and degree of exposure is more than 6 months at the level of 50 $\mu\text{g/l}$.

An exclusion criteria for the same group is any type of skin disease without the history of arsenic exposure at for least 6 months at any phase of life at the level of at least 50 $\mu\text{g/l}$. The patient group has been described earlier in more detail (Chanda et al., 2006).

Most of the subjects were in 35 to 55 years of age group and engaged in small trading or farming. The average age of the study population is 45, 47 and 49 years respectively, for three different exposure groups. The males were mostly smokers and females were non-smokers and house wives. Age and gender matched controls were selected for the study.

Written informed consent was obtained from all participants before drawing their blood. The name of the institute where human studies were carried out is Institute of Post Graduate Medical Education and Research, Kolkata (IPGME & R), which is run by Government of West Bengal, a state government within the framework of Republic of India. Ethical principles followed by the institute are guided by rules as formulated by Indian Council of Medical Research and these are in agreement with Helsinki rules.

Clinical symptom score

Each proband was assigned a clinical symptom score which reflected severity of his/her skin manifestations. Both pigmentation and keratosis were graded 1, 2 or 3, depending on the level of symptoms. Sum of the two was clinical symptom score, so that a person can have maximum score of 6. The control subjects have no pigmentation and keratosis and therefore clinical symptom score of 0. Table 2 describes the scoring system according to degree of severity for arsenic induced clinical manifestations.

Determination of arsenic in drinking water and urine

Drinking water and spot urine samples obtained from each

Table 2. Dermatological criteria and graduation of chronic arsenic toxicity.

Pigmentation (Score)		
Mild (1)	Moderate (2)	Severe (3)
Defuse melanosis, mild spotty pigmentation, leucomelanosis.	Moderate spotty pigmentation	Blotchy pigmentation, pigmentation of under surface of tongue, buccal mucosa.
Keratosis (Score)		
Mild (1)	Moderate (2)	Severe (3)
Slight thickening, or minute papules (<2 cm) in palm and soles	Multiple raised keratosis papules (2 to 5 cm) in palm and soles with diffuse thickening.	Diffuse severe thickening, large discrete or confluent keratotic elevations (>5 cm), palm and soles (also dorsum of extremity and trunk).

participant was collected. The concentration of arsenic in drinking water and urine was determined by atomic absorption spectrophotometry hydride generation (AAS-HG, Specification: ContrAA 300) system purchased from Analyticjena, Germany. The unit for expression of the concentration of arsenic in water and urine is µg/l.

Genotyping of glutathione- S-transferase (GST) M1 and T1

The polymorphic deletion of M1 and T1 gene was genotyped from peripheral blood leukocyte DNA using the multiplex PCR approach described earlier (Mondol et al., 2005) using β globin gene as an internal control. The DNA was extracted from EDTA anti-coagulated whole blood using usual salting out procedure (Miller et al., 1988). Initially some PCR products amplified from genomic DNA of arsenic exposed people were purified and sequenced to confirm the deletion of one or both M I or T I allele of GST gene.

Statistics

As there is strong non normality in the data, non parametric tests were preferred and Kruskal-Wallis non parametric tests with exact p values were used for test of significance. Non parametric Median test is also used to test the significance of differences between median values of clinical symptom score and total urinary arsenic in different polymorphic status (Das and Das, 2008).

RESULTS AND DISCUSSION

Box plots and median values of clinical score and total urinary arsenic are provided in Figure 1. Box plots give some suggestion that the mean level of outcomes vary by GST polymorphic status in the B and C groups. Data was stratified by exposure group and test of significance was calculated by non parametric Kruskal-Wallis test for ANOVA. The p values for the significance of GST M1 null and T1 null genotypic status as a predictor were 0.027 for clinical score, 0.018 for total urinary arsenic. Particularly in group C the clinical symptom score is significantly high ($p < 0.01$ by Median test) in M-T+ genotype and M+T-genotype ($p < 0.05$) in comparison to M+T+ genotype of the same group. Similarly the M+T+ genotype of group C have a significantly high total urinary arsenic than M-T+

genotype and M+T- genotype ($p < 0.05$, by Median test) of the same group (Table 3).

Many workers had reported role of different Glutathione - S- Transferases in arsenic metabolism. Increase in GST concentration accompanies removal of arsenic from liver and kidney of arsenic exposed fish (Allen and Rana, 2004). Proteomic analysis of arsenic exposed rice seedling indicated increase in GST activity. Arsenic exposure in plants also increases GST activity (Ntebogeng et al., 2009). Toxicogenomic analysis on mice model revealed that arsenic exposure results in transcriptional activation and upregulation of GST gene which further signifies the importance of GST in arsenic metabolism (Liu et al., 2004).

The percentage of MMA in urine is affected in GSTM1 null genotype and both percentage of MMA and DMA are affected by GSTT1 null genotype after arsenic exposure in a population of Argentina. (Engström et al., 2007) It has been studied in Argentina on a population exposed to arsenic that women with GSTM1 null genotype is associated with higher percentage of MMA in urine (Steinmaus et al., 2007).

We hypothesized that increased urinary arsenic in the form of its final product DMA decreases the body burden and therefore decreases the risk for arsenic associated clinical symptoms. When the urine excretes less DMA and more MMA and inorganic arsenic it seems like that most of the ingested arsenic is not metabolized completely to be removed through urine in its final product form, DMA. It signifies that more the inorganic arsenic retained in the body more the occurrence of clinical symptoms associated with arsenic exposure.

Higher percentage of DMA(V), the final product of arsenic metabolism has been reported in urine of wild type exposed persons in Vietnam, compared to GST M1 null (Agusa et al., 2010). Arsenic methyltransferase polymorphism is also accountable in arsenic metabolism as it is revealed from a cross sectional study on Bangladesh and Argentina that this gene polymorphism associated with higher percentage of MMA in Bangladesh and higher percentage of DMA in Argentina (Engström et al., 2011). Total urinary arsenic and susceptibility to skin

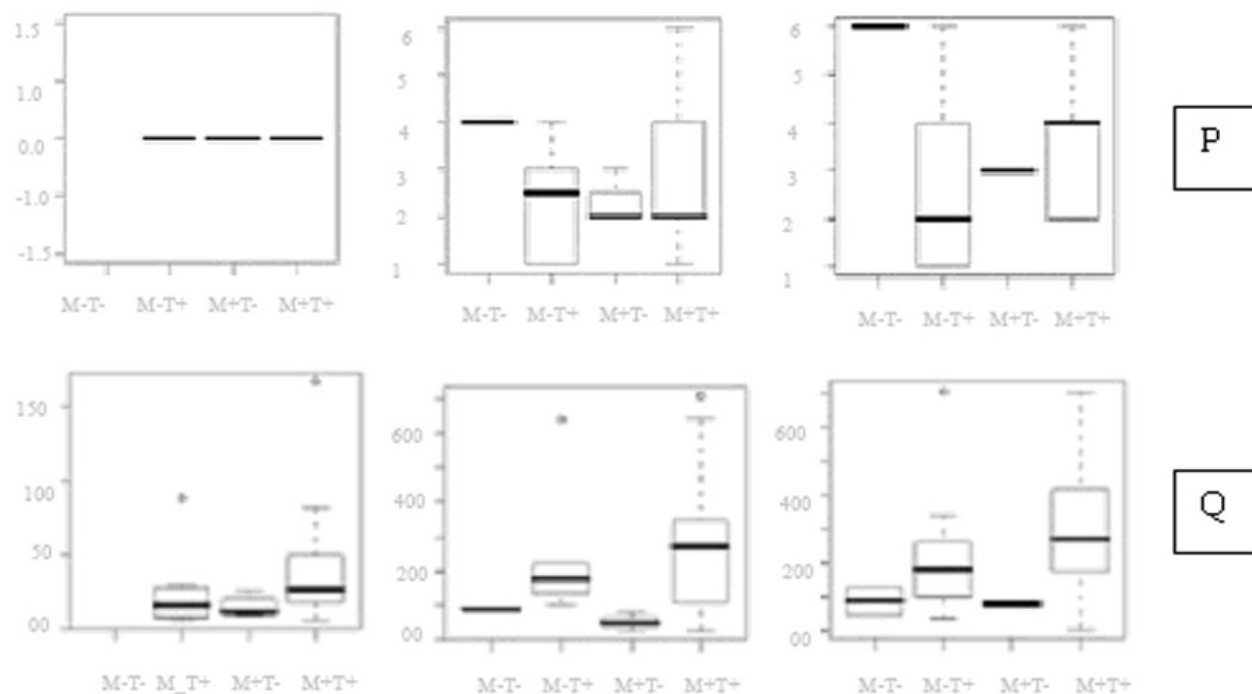


Figure 1. Box plots and median values of clinical score and total urinary arsenic for three different exposure groups (0 to 50, 50 to 250 and 250 to 500 $\mu\text{g/l}$ respectively) are provided in rows P and Q respectively.

Table 3. Representing the Median value of clinical symptom score and total urinary arsenic in different exposure group with different polymorphic variation.

	Group	M-T-	M-T+	M+T-	M+T+	χ^2	P
Median value of Clinical score	A (25)		0 (8)	0 (6)	0 (11)	8.303	0.027
	B (25)	4 (2)	3 (8)	2.0 (5)	2.0 (10)		
	C (28)	6 (2)	4 (10)	3 (1)	2 (15)		
	Overall	6	1	1	2		
Median value of Total urinary arsenic	A (25)		15.815 (8)	11.6 (5)	26 (11)	9.594	0.0188
	B (25)	89 (1)	177.6 (8)	48.7 (5)	272.8 (10)		
	C (28)	89 (2)	182.3 (7)	80 (4)	272.8 (9)		
	Overall	89	105.5	26	167.37		

lesions have been correlated with GST status in a large group of Chinese exposed to arsenic through indoor combustion of high arsenic coal. A significantly higher arsenic content in hair correlated with GST M1 null status (Lin et al., 2007).

Another case control study on Bangladesh population also failed to link skin lesions with GST M1 genotype, but found GST T1 null genotype beneficial, as the wild type shows relatively more skin lesions (McCarty et al., 2007). GST M1 null, on the other hand has been linked with incomplete metabolism of arsenic and consequent excretion of inorganic arsenic and MMA, instead of DMA, the end product of Arsenic metabolism in a study of

occupational exposure (Marcos et al., 2006). A study from Taiwan (Chiou et al., 1997) also showed incomplete methylation in GST M1 null persons, and reported increased methylation in GST T1 nulls.

From these different studies it is seen that lesser arsenic methylation and persistence of inorganic arsenic in body is detected in all the cases of GST M1 deficiency.

Our results indicate a decrease in total urinary arsenic and increase in clinical symptom score in GST deficient persons, within a particular exposure group. This can be explained in the light of persistence of inorganic arsenic in the body of GST deficient persons, which is known to alter arsenic metabolism (Chiou et al., 1997; Mc Karty et

al., 2007). Whether the increased retention of arsenic leads to decrease of arsenic excretion through urine in a particular exposure group is not evident from literature. Our data of decreased total urinary arsenic in GST deficient persons asserts so.

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