

Full Length Research Paper

# Glutathione–S-transferase polymorphic status modifies the arsenic induced clinical manifestation in Nadia District of West Bengal, India

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Subsoil water contamination with arsenic is a burning global health issue. People experiencing exposure to contaminated water by arsenic through out years can give rise to development of myriad clinical manifestations with a chief of arsenicosis which is the collective form of pigmentation and keratotic lesion of the skin. We surveyed over a population of above 500 people in West Bengal who are chronically exposed to arsenic at various doses through their drinking water through out years which revealed a discreet variation in the development of such symptoms. This discrimination may be due to the error in metabolism which comes from the polymorphic association of genes particularly involved in arsenic metabolism. To check our hypothesis we conducted a case control study over 78 study subjects including control and arsenic exposed people, with different level of exposure, chosen from the Nadia district of West Bengal, India. Our result revealed that GST polymorphism is closely associated with the degree of urinary excretion of arsenic in people with arsenic exposure. Persons with GSTMI and TI null genotype showed a significantly decreased level of total urinary arsenic than GSTMI and TI non null genotype of the same exposure group and also from the unexposed control group. The degree of keratosis and pigmentation also goes high in exposed people with GSTMI or TI null genotype. The overall skin manifestation is also significantly higher in persons with null genotype of GST in comparison to non null counterpart of the study population.

**Key words:** Pigmentation, keratosis, arsenic exposure, GST polymorphism, total urinary arsenic.

## 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 (NRC 1999, 2001). 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. Therefore, concentration of arsenic in urine is considered a biological marker of arsenic exposure (NRC 2001; Vahter 1999). Metabolism of arsenic occurs through repeated reduction and oxidative methylation of inorganic trivalent arsenic (AsIII) and pentavalent arsenic (AsV) to monomethyl arsonic acid (MMA)<sup>V</sup> and dimethyl arsinic acid (DMA)<sup>V</sup> (Vahter, 1999). Oxidative addition of methyl groups to arsenic occurs by the enzyme methyltransferase

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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 reduced glutathione (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 (Hayakaya et al., 2004). The absence of one or both M1 allele or T1 allele has been associated with incomplete metabolism of arsenic which was studied in a population of Taiwan. The study showed that M1 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 (The date of collection of the blood samples are March 2004 to April 2005). 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, including, hyperpigmentation, hypopigmentation and keratosis (Guha et al., 2003). 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 patient group has been described previously in more detail (Chanda et al., 2006).

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 (Guhamazumder et al., 2001, 2003).

## Determination of arsenic in drinking water and urine

Drinking water and urine samples obtained from each participant was collected. The concentration of arsenic in drinking water and urine was determined by atomic absorption spectrophotometry hydride generation (AAS-HG) system.

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

The polymorphic deletion of M1 and T1 gene was genotyped using the multiplex PCR approach described previously. The primer used in GST polymorphic status analysis for GSTM1 and T1 allele were P1 and P3 for GSTM1 and F46 and R 137 for GSTT1. The PCR was conducted in 20  $\mu\text{l}$  containing 20 ng genomic DNA, 10X PCR reaction buffer, 0.5  $\mu\text{l}$  of Taq Polymerase (Bangalore Genei). After initial denaturation at 94°C, the PCR was performed for 30 cycles of 30 s at 94°C, 1 min at 58°C and 1 min at 72°C. Internal control was served by a 861 bp fragment from  $\beta$ -globin gene using 619A and 619 B primer pair (Mondol et al., 2005). Initial cases were confirmed by sequencing.

## Statistics

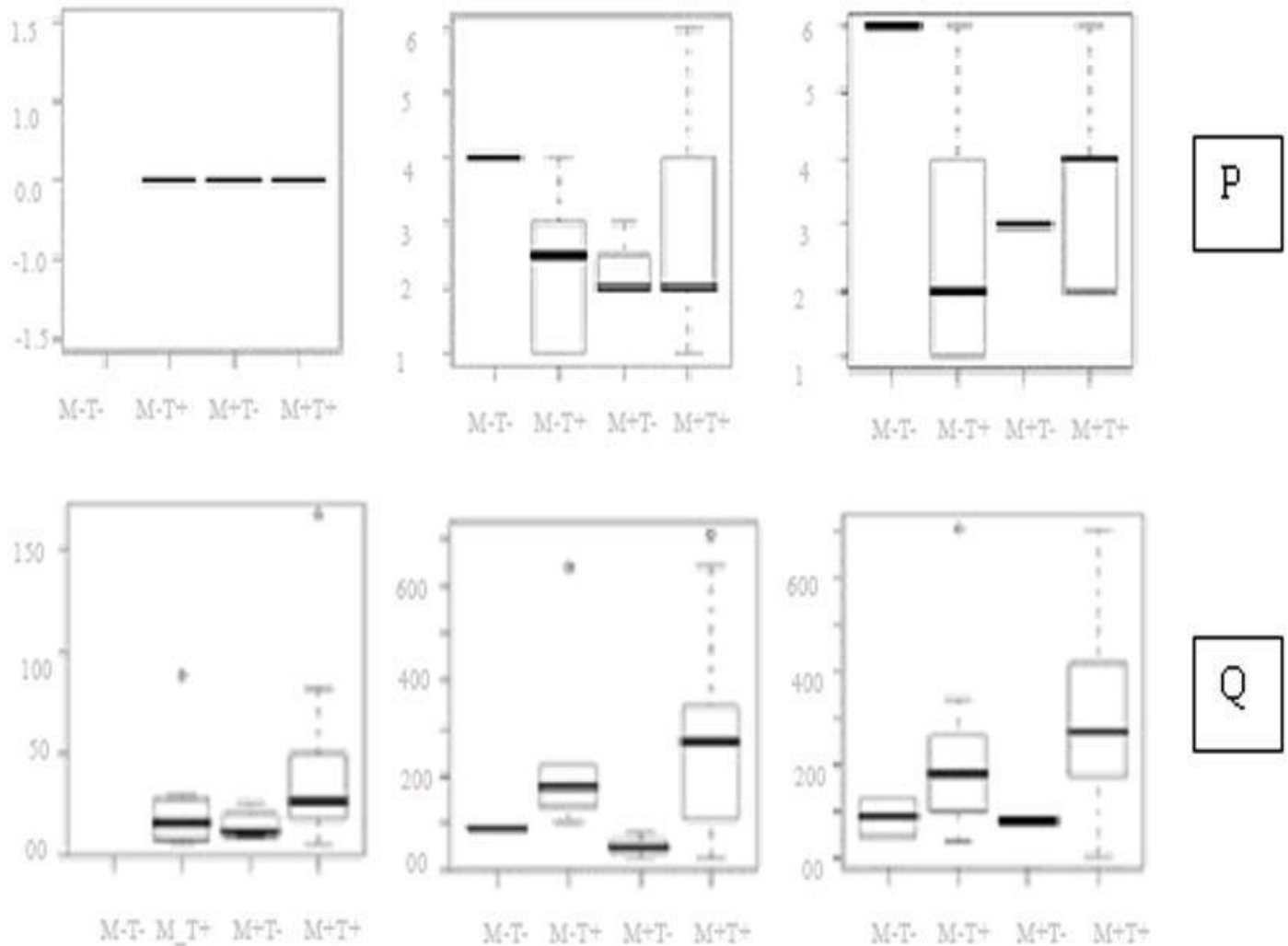
As there is strong non normality in the data, non parametric tests were preferred and Kruskal-Wallis non parametric tests with exact p values were used for test of significance. Non parametric median test is also used to test the significance of differences between median values of clinical symptom score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

## RESULTS

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. The p values for the significance of GST M1 null and T1 null 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.

## DISCUSSION

Many workers had reported role of different Glutathione-S-transferases in arsenic metabolism (Chiou et al., 1997; Georing et al., 1999; Hayakaya et al., 2004; Mc Karty et al., 2007). 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



**Figure 1.** Box plots and median values of clinical score and total urinary arsenic for three different exposure groups (0-50 µg/l and 50-250 µg/l and 250-500 µg/l respectively) are provided in rows P and Q respectively.

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 had 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 the more the inorganic arsenic retained in the body, the more the occurrence of clinical symptoms associated with arsenic exposure (Table 1).

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 is 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 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).

**Table 1.** 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+	$\chi^2$	P
<b>Clinical symptom score</b>						
A (25)		0 (8)	0 (6)	0 (11)		
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	8.303	0.027
<b>Total urinary arsenic</b>						
A (25)		15.815 (8)	11.6 (5)	26 (11)		
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	9.594	0.0188

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; McCarty 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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