

Role of Glutathione-S-Transferase Polymorphism on Arsenic Induced DNA Hypermethylation

Chanda Sarmishtha^{1,2*}, Dasgupta Uma², Guha Mazumder Debendranath³,
Chaudhuri Utpal² and Ganguli Bhaswati⁴

¹Department of Physiology, Presidency College, Kolkata - 700073, West Bengal, India;
csarmishtha@yahoo.com

²Department of Biophysics, Molecular Biology and Genetics, University of Calcutta,
Kolkata - 700073, West Bengal, India

³Institute of Post Graduate Medical Education and Research, Kolkata - 700020, West Bengal, India

⁴Department of Statistics, University of Calcutta, Kolkata - 700073, West Bengal, India

Abstract

People chronically exposed to environmental arsenic through their drinking water experience various arsenic induced clinical manifestations and there is modulation of methylation level in their genome. However the response varies widely among persons. The present manuscript deals with the evaluation of role of individual's GST status in altering the degree of DNA methylation after chronic arsenic exposure in human. To study whether Glutathione-S-Transferase (GST) gene polymorphism plays any role in this variation, a total of 92 study subjects were recruited from the villages of southern region of West Bengal, India. Concentration of arsenic in their urine and water, extent of clinical manifestation, GST status and p53 and p16 gene promoter methylation status were determined. Results showed that genetic polymorphism of GSTM1 and T1 were significantly associated ($p < 0.05$) with degree of methylation in p53 and p16 gene promoter region and urinary arsenic in higher exposure group. Persons having null genotype have significantly decreased urinary arsenic and increased DNA methylation level relative to persons with GSTM1 or GSTT1 nonnull genotype of same arsenic exposure group. In the present work we are therefore trying to show that individual's GST polymorphic status may alter the degree of DNA methylation after arsenic exposure.

Keywords: Arsenic, Clinical Symptom Score, DNA Hypermethylation, GST Polymorphism, Total Urinary Arsenic

Received: 03-09-2015 Revised: 02-10-2015 Accepted: 16-10-2015

1. Introduction

Arsenicosis is precipitated by chronic 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 Arsonic Acid (DMA). The methylated metabolites of arsenic are less toxic, and being electrophilic, are readily excreted in urine. The inorganic arsenic which is not metabolised and is not excreted, retained in the body and stored as inorganic arsenic in hair, nail of fingers and toes and also in the soft tissues of

the body. Concentration of arsenic in such body stores is reliable biomarker for arsenic exposure. This internal pool of arsenic may also alter some internal metabolic processes as inorganic arsenic can depresses the activities of various enzymes like methyltransferases, e.g., selenocysteinemethyltransferase and others. Therefore, concentration of arsenic that is retained in the body can modulate the DNA methylation by altering the activity or expression of DNA methyltransferase. The concentration of arsenic in urine is considered as a biological marker of arsenic exposure as it is excreted via urine. 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 arsonic acid (DMA)V. Oxidative addition of methyl groups to arsenic occurs

* Author for correspondence

by the enzyme methyltransferase and the methyl group donor is S-Adenosyl Methionine (SAM) (Aposhian, 1997; Thompson, 1993; Vahter, 1999). SAM functions to methylate an assortment of acceptor molecules including arsenic and DNA. Further, GSH is the enzyme involved in arsenic metabolism which can justify the role of GST in arsenic metabolism. Therefore, we hypothesize that GST status and arsenic burden of the body can contribute to modulate DNA methylation status of exposed persons.

Alteration of DNA methylation after chronic arsenic exposure has been demonstrated in tissue culture system, experimental animals and humans (Mass and Wang, 1997; Zhao *et al.*, 1997; Zhong and Mass, 2001; Liu *et al.*, 2004; Chanda *et al.*, 2006). As an extension of that work, the role of GST M1 and GST T1 polymorphism on DNA methylation level, clinical symptoms score, total urinary arsenic level has been studied.

2. Materials and Methods

Sample selection and source of sample:

Study samples were chosen from the Arsenic Clinic of Institute of Post Graduate Medical Education and Research, Kolkata, India, is a tertiary referral centre. 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: $51\text{--}251\mu\text{g/l}$; C: $251\text{--}500\mu\text{g/l}$ for degree of methylation study. The average duration of exposure was about 10 years.

The patient group has been described earlier 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 clinical studies were carried out is Institute of Post Graduate Medical Education and Research, Kolkata (IPGME & R), which is run by Govt.

of West Bengal, a state government within the framework of Republic of India. The basic research was carried out in department of Biophysics, Molecular Biology and Genetics, University of Calcutta. 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.

2.1 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 (Chanda *et al.*, 2006).

2.2 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 according to manufacturer's protocol (Atallah and Kalman, 1991).

2.3 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 by Mondal *et al.*, (2005). Initial cases were confirmed by sequencing.

2.4 Methylation Status Analysis of Promoter Region of p53 and p16 Genes

The p53 and p16 tumor suppressor gene methylation status was analyzed in each subject by the method described earlier (Chanda *et al.*, 2006).

2.5 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. Degree of correlation was analyzed for dose response analysis between arsenic accumulation and concentration

of drinking water arsenic and for accumulation of arsenic in hair and p53 promoter methylation by non parametric Spearman's rank-difference correlation coefficient (rho).

3. Results

Epigenetic alterations (alteration in DNA methylation in p53 and p16 gene promoter region) have been studied in people of West Bengal chronically exposed to environmental arsenic through their drinking water. It has been shown that the degree of clinical symptom score, total urinary arsenic and gene promoter methylation is modulated by the individual's GST gene polymorphic status in exposed persons. Clinical symptom score and total urinary arsenic (eliminated arsenic species through urine) also have shown GST polymorphic status dependent variations even in the same exposure groups. Persons with null genotype of GSTM1 and T1 have a high degree of arsenical skin lesions (assigned as clinical symptom score) in contrast to persons of non null genotype of same exposure groups (Table 1) though not at significant level. Persons with null genotype for GSTM1 and T1 have statistically significant low total urinary arsenic in comparison to similarly exposed persons of non null genotype for the same gene (Table 2).

Person, having GST null genotype for GSTM1 and T1, exposed to similar level of arsenic through their drinking water shows a greater degree of DNA methylation status in comparison to non null genotype of similar exposure

group (Table 3 and 4). All these findings are the reflection of association of GST polymorphic status with arsenical skin lesions and arsenic induced changes in DNA methylation.

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 status as a predictor were 0.05721 for Clinical Score, 0.018 for Total urinary arsenic, 0.0007 for p53 methylation status and 0.0017 for p16 methylation status tabulated in Table 1, 2, 3 and 4 respectively.

Table 1. Median value of clinical score and comparison between different arsenic exposure groups and subgroups

Group	M-T-	M-T+	M+T-	M+T+	χ ²	P
A (23)		0 (8)	0 (4)	0 (11)		
B (24)	4 (2)	2.5 (7)	2.0 (5)	2.0 (10)		
C (28)	6 (2)	2 (10)	3 (1)	4 (15)		
Overall	6	1	1	2	7.13	.05721

Median value of Clinical score

Table 2. Median value of Total Urinary arsenic comparison between different arsenic exposure groups and subgroups

Group	M-T-	M-T+	M+T-	M+T+	χ ²	p
A (23)		15.815 (8)	11.6 (4)	26 (11)		
B (24)	89 (1)	177.6 (7)	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

Median value of Total Urinary arsenic

Table 3. Median value of p53 methylation status comparison between different arsenic exposure groups and subgroups

Group	M-T-	M-T+	M+T-	M+T+	χ ²	p
A (23)		0.31 (8)	0.22 (4)	0.19 (11)		
B (21)	2.69 (1)	2.36 (7)	2.02 (4)	0.90 (9)		
C (28)	2.99 (2)	3.145 (10)	1.84 (1)	1.06 (15)		
Overall	2.69	1.93	1.025	0.66	14.7239	.0007

Median value of p53 methylation status

Table 4. Median value of p16 methylation status comparison between different arsenic exposure groups and subgroups

Group	M-T-	M-T+	M+T-	M+T+	χ ²	p
A (23)		0.24 (4)	0.078 (8)	0.189 (11)		
B (15)		2.57 (6)		0.995 (9)		
C (28)	2.22 (1)	4.225 (4)	3.106 (8)	1.217 (15)		
Overall	.7355	3.45	1.592	0.6185	13.8112	.00307

Median value of p16 methylation status
 NB: M+T+ denotes GSTM1T1 wild type form. M-T+ denotes GSTM-T+ form where both of μ allele or at least 1 of the allele is deleted. M+T- denotes GSTM+T- form where 1 or both of the θ allele is deleted. M-T- denotes GSTM-T- form where 1 or both of the μ and θ allele are deleted.

4. Discussion

Many workers had reported role of different Glutathione-S-Transferases (GSTs) 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).

After arsenic exposure, GST M1 null individuals in Argentina excrete relatively more MMA through urine. The result showed similar pattern in other areas also (Engström *et al.*, 2007; Engström *et al.*, 2011; Steinmaus *et al.*, 2007). 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)¹⁶. 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. However skin lesions did not seem significantly correlated with GST status, similar to our data (Lin *et al.*, 2007). Another case control study on Bangladeshi 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 KM *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 arsenic 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 (Steinmaus *et al.*, 2007; Agusa *et al.*, 2010; Kile *et al.*, 2005). Our results indicate a decrease in total urinary arsenic and increase in DNA methylation in GST deficient persons, in moderate to high 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 up regulate DNA methyltransferase expression (Zhong and Mass, 2001; Dumond and Sing, 2007). As reported in previous works that arsenic induces p53 and p16 gene promoter hypermethylation and genomic hypermethylation in exposed persons and also in arsenic

induced cancer patients (Chanda *et al.*, 2006; Majumder *et al.*, 2010). It is also reported that chronic arsenic exposure to human also induces non-tumor suppressor gene methylation (Human GDP- Mannose 4,6 dehydrase gene methylation) (Dumond and Sing, 2007). Whether the increased retention of arsenic leads to a decrease in 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. To best of our knowledge this is the first report indicating the association between GST polymorphism and p16 and p53 gene promoter methylation in arsenic exposed population. The degree of DNA methylation in normal unexposed persons (median value of DNA methylation in unexposed group) has been taken as a normal basal degree of DNA methylation (Chanda *et al.*, 2006). Results of clinical score are not significant, though there is increase of clinical score in cases which are deficient in one or other gene. Clinical symptom score is an indicative here to demonstrate the severity of arsenic induced skin manifestations which is higher in high exposure group and also in null genotype where the urinary arsenic is low in comparison to non null genotype of the same exposure group. In case of chronic/high level of exposure there is an increase in the clinical severity reflected as high degree of keratosis and pigmentation which may develop Bowen's disease or cancer. In the present study population case of cancers are not included. This study is reflecting the association between GST polymorphic status and p53 and p16 gene promoter hypermethylation in persons chronically exposed to graded degree of arsenic. However, reports available indicating association of GSTMI and TI polymorphism with p16 and p14 gene CpG island hypermethylation in patients (not related to arsenic exposure) with ulcerative colitis without neoplastic lesion (Tahara *et al.*, 2011) and in a separate study, with CpG island hypermethylation in promoter region in a patient population of oesophageal carcinoma from China (Liu *et al.*, 2010).

5. Conclusion

To sum up we can conclude that GST null genotype is a determinant for degree of DNA methylation in arsenic exposed people of West Bengal, India. Person with GST M1 null retain more arsenic and excrete less arsenic from the body.

Conflict of Interest: The authors declare that there are no conflicts of interest.

6. References

1. Aposhian HV. Enzymatic methylation of arsenic species and other new approaches to arsenic. *Ann Rev of Pharm and Tox.* 1997; 37:397–419.
2. Thompson DJ. A chemical hypothesis for arsenic methylation in mammals. *Chemico-Biologic Inter.* 1993; 88:89–114.
3. Vahter M. Variation in human metabolism of arsenic. *Arsenic Exposure and Health Effects.* 2nd ed. 1999; 267–75.
4. Mass MJ, Wang L. Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: A model for a mechanism of carcinogenesis. *Mutat Res.* 1997; 386:263–77.
5. Zhao CQ, Young MR, Diwan BA, Coogan TP, Waalkes MP. Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression. *Proc Natl Acad Sci.* 1997; 94:10907–12.
6. Zhong CX, Mass MJ. Both hypomethylation and hypermethylation of DNA associated with arsenite exposure in cultures of human cells identified by methylation-sensitive arbitrarily-primed PCR. *Toxicol Lett.* 2001; 122:223–34.
7. Liu J, Xie Y, Ward JM, Diwan BA, Waalkes MP. Toxicogenomics analysis of aberrant gene expression in liver tumors and nontumorous livers of adult mice exposed in utero to inorganic arsenic. *Toxicol Sci.* 2004; 77:249–57.
8. Chanda S, Dasgupta UB, Mazumder DG, Gupta M, Chaudhuri U, Lahiri S, et al. DNA Hypermethylation of Promoter of Gene p53 and p16 in arsenic-exposed People with and without Malignancy. *Toxicol Sci.* 2006; 89:431–7.
9. Atallah R, Kalman DA. Online photooxidation for the determination of organic arsenic compounds by AAS with continuous arsine generation. *Talanta.* 1991; 38:167–73.
10. Mondal BC, Paria N, Majumder S, Chandra S, Mukhopadhyay A, Chaudhuri U, et al. Glutathione-S-transferase M1 and T1 null genotype frequency in chronic myeloid leukemia. *European Journal of Cancer Prevention.* 2005; 14:281–4.
11. Allen T, Rana SV. Effect of arsenic (AsIII) on glutathione-dependent enzymes in liver and kidney of the freshwater fish *Channa punctatus*. *Biol Trace Elem Res.* 2004; 100:39–48.
12. Ntebogeng S, Flores-Tavizón E, Castillo-Michel H, Peralta-Videa JR, Gardea-Torresdey JL. Arsenic tolerance in mesquite (*Prosopis* sp.): Low molecular weight thiols synthesis and glutathione activity in response to arsenic. *Plant Physiol and Biochem.* 2009; 47:822–6.
13. Engström KS, Broberg K, Concha G, Nermell B, Warholm M, Vahter M. Genetic polymorphisms influencing arsenic metabolism: Evidence from argentina. *Environ Health Perspect.* 2007; 115:599–605.
14. Engström KS, Vahter M, Mlakar SJ, Concha G, Nermell B, Raqib R, et al. Polymorphisms in arsenic(+III oxidation state) methyltransferase (AS3MT) predict gene expression of AS3MT as well as arsenic metabolism. *Environ Health Perspect.* 2011; 119:182–8.
15. Steinmaus C, Moore LE, Shipp M, Kalman D, Rey OA, Biggs ML, et al. Genetic polymorphisms in MTHFR 677 and 1298, GSTM1 and T1, and metabolism of arsenic. *J Tox Environ Health, Part A.* 2007; 70:159–70.
16. Agusa T, Iwata H, Fujihara J, Kunito T, Takeshita H, Minh TB, et al. Genetic polymorphisms in glutathione S-transferase (GST) superfamily and arsenic metabolism in residents of the Red River Delta Vietnam. *Toxicol Appl Pharmacol.* 2010; 242:352–62.
17. Lin GF, Du H, Chen JG, Lu HC, Kai JX, Zhou YS, et al. Glutathione S-transferases M1 and T1 polymorphisms and arsenic content in hair and urine in two ethnic clans exposed to indoor combustion of high arsenic coal in Southwest Guizhou, China. *Arch Toxicol.* 2007; 81:545–51.
18. McCarty KM, Chen YC, Quamruzzaman Q, Rahman M, Mahiuddin G, Hsueh YM, et al. Arsenic Methylation, GSTT1, GSTM1, GSTP1 Polymorphisms, and Skin Lesions. *Environ Health Perspect.* 2007; 115:341–5.
19. Marcos R, Martínez V, Hernández A, Creus A, Sekaran C, Tokunaga H, et al. Metabolic profile in workers occupationally exposed to arsenic: Role of GST polymorphisms. *J Occup Environ Med.* 2006; 48:334–41.
20. Chiou HY, Hsueh YM, Hsieh LL, Hsu LI, Hsu YH, Hsieh FI, et al. Arsenic methylation capacity, body retention and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. *Mut Res.* 1997; 386:197–207.
21. Kile ML, Houseman AE, Rodrigues ETJ, Quamruzzaman Q, Rahman M, Mahiuddin G, et al. Toenail arsenic concentrations, GSTT1 gene polymorphisms, and arsenic exposure from drinking water. *Cancer Epidemiol Biomarkers Prev.* 2005; 14:2419–26.
22. Dumond JW, Sing KP. Gene expression changes and induction of cell proliferation by chronic exposure to arsenic of mouse testicular leydig cells. *J Tox and Environ Health A.* 2007; 70:1150–4.
23. Majumder S, Chanda S, Ganguli B, Mazumder DN, Lahiri S, Dasgupta UB, et al. Arsenic exposure induces genomic hypermethylation. *Environ Toxicol.* 2010; 25(3):315–8.
24. Chanda S, Dasgupta UB, Mazumder DNG, Saha J, Gupta B. Human GMDS gene fragment hypermethylation in chronic high level of arsenic exposure with and without arsenic induced cancer. *Springer Plus.* 2013; 2:557.
25. Tahara T, Shibata T, Nakamura M, Okubo M, Yamashita H, Yoshioka D, et al. Association between polymorphism in XRCC1 and GST genes and CpG island methylation status in colonic mucosa in ulcerative colitis. *Virchows Arch.* 2011; 458:205–11.
26. Liu R, Yin L, Pu Y, Li Y, Liang G, Zhang J, et al. Functional alteration in Glutathione S-Transferase Family associated with enhanced occurrence of esophageal carcinoma in China. *J Toxicol Environ Health A.* 2010; 73:471–82.