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# Association of Arsenic Induced Bladder Cancer with GST O1and GSTO2 Polymorphism with High Cumulative Arsenic Exposure in Residents of West Bengal

**Sarmishtha Chanda[1,2], Tushar Chakraborty[1] and Debedranath Guhamazumder [3]**

*[1]Department of Physiology, Presidency College, 86/1, College Street, Kolkata, West Bengal, India*
*[2]Department of Gene Regulation, CSIR, Indian Institute of Chemical Biology, Raja S.C. Mullick Road, Kolkata , West Bengal, India*
*[3]DNGM Research Foundation, New Alipur Block B, Kolkata, West Bengal, India*

**Abstract**

**Background:** *Arsenic contamination through food chain causes skin manifestations and cancer. However, the response varies widely among persons despite receiving similar cumulative exposure. These differentiations in manifestation may be due to polymorphic distribution of arsenic metabolizing genes among exposed people.*

**Method:** *Polymorphism of GST omega gene and their frequency distribution ratio may modify skin manifestations and development of arsenic induced cancer in persons receiving arsenic through food chain. Polymorphic variations of GST omega genes have been studied on 133 study subjects. Subjects were recruited from one of major arsenic affected district, Nadia of West Bengal, India, having high arsenic content in their food. Exposed subjects were categorized into three groups, i.e, with arsenical skin lesions and with out arsenical skin lesions and arsenic induced urothelial cancer. Concentration of arsenic in their urine, hair, drinking water, food, extent of clinical manifestations, GST status was determined.*

**Result:** *Results showed that genetic polymorphism of GSTO1 and O2 are significantly associated ($p<0.05$) with arsenic induced clinical symptoms and urothelial cancer. Frequency of GSTO1 and O2 mutant variety and wild type variety was studied and tested in relation to Hardy Weinberg Equation. GSTO1 and GSTO2 wild type are significantly associated with arsenic induced skin manifestations and arsenic induced urothelial cancer.*

**Keywords:** *Arsenic, genetic polymorphism , metabolism, skin lesion, food chain.*

**Introduction:** Humans are exposed to environmental arsenic mostly through drinking water and food. The subsoil water of many countries of the world including India has been contaminated with arsenic. In India, the basin of river Ganga in West Bengal is highly contaminated with arsenic. The allowable limit for arsenic in drinking water set by the US

Protection Agency is 10 µg/l. Though intensive programs had been taken by Government of West Bengal to block the arsenic affected tube wells and supplying arsenic free safe water to affected villages, no restriction had been made to use this arsenic contaminated shallow wells for irrigation purpose. Due to irrigation with arsenic contaminated water, vegetables are also depositing inorganic arsenic in it and therefore transmitted to people consuming it. Urine examination had revealed evidence of current exposure. It indicates that they are still receiving environmental arsenic through any alternative source, may be from food. The subsoil water which contains inorganic arsenic is also used in irrigation purpose and therefore may be transmitted to plants and enter in to the food chain. Evidences coming from Bangladesh, India, Combodia, Vietnam and other countries revealed that food grains can accumulate arsenic when harvested in arsenic contaminated soil and ground water (Zhao et al. 2010, Ma et al 2008, Abedin et al 2002). Outcomes of recent research indicate that rice is the more potent accumulator of arsenic (Abedin et al 2002, Ahmed et al. 2011, Zhang et al. 2005, Su et al. 2010). Paddy field flooded with arsenic contaminated ground water produces paddy containing significant amount of inorganic arsenic in it (Dwivedi et al. 2010). Live stock in this manner exposed to environmental arsenic is another alternative route of exposure to human (Bera et al. 2010, Eisler et al. 2004). Guha Mazumder and his group reported in a separate study that participants coming from arsenic endemic area of West Bengal have

exposure of arsenic through food and the main component of dietary arsenic exposure is rice (Biswas et al. 2014). In the present report we are going to explain the association of polymorphic variation of GST omega genes with arsenic induced skin manifestations and arsenic induced bladder cancer or more precisely speaking urothelial cancer in persons with arsenic exposure through food. Subjects are selected from arsenic endemic area and collection of samples and subject recruitment has been done during 2009-2010 and 2010-2011.

We observed in our previous studies that people receiving similar level of exposure with a similar duration reflecting differential degree clinical manifestations. This is due to different degree of arsenic metabolism in different people which is influenced by genetic polymorphism of arsenic metabolizing genes. Glutathione S Transferase omega (GSTO) and arsenic (III) methyltransferase (AS3MT) are involved in classic arsenic metabolism pathway in human and other animals. GSTO1 and GSTO2 are involved directly in the reduction of MMA(V) to MMA(III), which is the rate limiting step of arsenic methylation in human (Hsu et al 2011). The association of arsenic induced clinical manifestation and GSTO1 and GSTO2 polymorphism has been studied in different arsenic exposed population of the World (Esgtrom et al. 2007, Paiva et al. 2008, Lindberg et al. 2007). The polymorphism of GSTO1 and GSTO2 could be a significant modifier of arsenic methylation capacity of individuals exposed to arsenic and thereby involved in arsenic induced skin manifestation and arsenic induced urothelial carcinoma (Ahsan et al.2007, Wang et al.2009). We are therefore trying to find out the relationship between polymorphism of GSTO1 and GSTO2 genes with their frequency distribution in study population and induction of arsenic induced skin manifestations after food chain contamination by arsenic. However, this is the first report of association of GSTO1 and GSTO2 gene polymorphism with presence or absence of skin lesions and urothelial cancer in persons chronically exposed to arsenic through their food chain in West Bengal, India.

**2. Material and Methods:**

We have recruited a total 111 study subjects. The subjects were selected from Nadia district, a highly arsenic affected district of West Bengal. A total number of 76 arsenic exposed subjects have been chosen from this area. They were exposed to similar level of environmental arsenic through food. The total numbers of arsenic exposed subjects (76 subjects) are divided in three groups on the basis of having arsenical skin manifestations and arsenic induced urothelial cancer. Exposed subjects were selected on the basis of their urine, food and hair arsenic concentration with presence or absence of skin manifestations which are checked by group of specialized clinicians (Guha Mazumder et al. 2001, Chanda et al. 2006). In group I there were 33 subjects having definitive arsenical skin manifestations and in group II (n=33), these 33 subjects did't have any arsenical skin manifestations, although both the groups have received similar level of arsenic exposure through food. Group III is arsenic induced urothelial cancer (n= 15). Group IV is arsenic unexposed control (n= 19). Group I is designated as arsenic induced skin manifestation positive, and group II is arsenic induced skin manifestation negative. Age, sex and socio economic status matched control subjects were selected from arsenic unexposed blocks of a district of West Bengal, North 24 Parganas, and designated as group IV. Participants of this group neither have any history of current or past arsenic exposure nor arsenical skin lesions. The current and past drinking water arsenic concentration of this group was <10 µg/L. Current drinking and cooking water samples, morning void urine samples and 24 hrs food samples and hair samples had been collected in sterilized polypropylene container from all of the study subjects to detect the concentration of arsenic in their drinking water, urine, hair and food. Control subjects had a food and urine arsenic level below detection limit.

The mean concentration of arsenic in raw rice in arsenic exposed population was 315µg/kg and 298µg/kg for group I and group II respectively and the range of exposure of arsenic through food were 118-672 µg/kg in group I and 212-478 µg/kg in group II as measured in our laboratory from 24 hours food samples collected from the participants. The method of collection of food samples are described in detail in our previous paper.[15] Blood was also collected from every participant in EDTA vial. History of arsenic exposure from each participant was obtained in detail including duration of water intake, food sampling and food arsenic concentration. Demographic data, social characteristics and occupational data were collected for each recruit (Deb et al. 2012, Biswas et al. 2013).

All male participants were involved in sedentary to moderate works and smokers. Mostly they were small traders or farmers or involved in office jobs in small concerns. Women were mostly housewives or involved in sedentary works and non smokers. Written informed consent was obtained from all participants before drawing their blood. 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. The name of the institute where human studies were carried out is Indian Institute of Chemical Biology (IICB) which is run by Govt. of India, a CSIR research Unit.

**2.1 Field Study:**

A village level sampling frame was created within two blocks, Chakdah and Haringhata of Nadia district, having at least one tube well contaminated with >10 µg/l of arsenic.

**2.2 Collection of Blood samples:**

EDTA anticoagulated blood samples were collected from each participants and kept at –20°C at DNGM research foundation, (DNGMRF), Kolkata. All the blood samples were transported in icebox from DNGMRF to the Department of Physiology, Gene Regulation laboratory, IICB, Kolkata for further storage at -70°C and subsequent analysis after DNA extraction.

**2.3 Clinical Symptom Score:**

Each study subject had been assigned a clinical symptom score according to their skin manifestations, i.e presence of pigmentation and keratosis present in the exposed area of the body. Although in group II subjects absence of skin manifestation causes assignment of clinical score as 0. 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. This scoring system is applicable in group I and group II subjects while group II and IV subjects had a score 0 (Guha Mazumder 2008).

**2.4 Determination of Arsenic in drinking water, hair and urine:**

The concentration of arsenic in drinking water, hair, 24 hours food sample and urine was determined by atomic absorption spectrophotometry hydride generation (FI- AAS-HG) system according to manufacturer's instruction**.** Hair and urine arsenic concentration was measured according to Das et al (Das et al. 1995) using FI-HG-AAS (PerkinElmer A Analyst 200). The limit of detection determined at the 90% confidence level was 3µg/L.

**2.5 Genotyping of Glutathione- S-Transferase Omega**

**PCR RFLP for GST O A 140 D:**

In humans, five classes of GST genes exist: α (*GSTA*), µ (*GSTM*), π (*GSTP*), θ (*GSTT*), and ζ (*GSTZ*) with one or more genes in each class. The enzymes have different, but sometimes overlapping, substrate affinity (Buchard et al. 2007,). GST O1 A140 D genotype was determined by PCR RFLP. The primers for PCR were 5' AAA GTT GTT TCT TAA ACG TGC C-3' and 5' –AAG TGA CTT GGA AAG TGG GAA-3' (Hsu et al.2011). The reaction was incubated at 95º C for 10 min and subjected to 30 cycles of 94º C for 60 s, 55ºC for 60 s, and 72º C for 60 s and a final extension step at 72ºC for 10 min. The PCR product was digested for 12 hrs with one unit of Cac 8 I (New England, Biolabs) and then separated on 2.5% agarose gel for 2hrs. The genotypes were determined as follows: homozygous wild type C/C or AA: 243, 145 and 67 bp; heterozygous C/A or AD: 388, 243, 145, and 67 bp; and homozygous varientA/A or DD :388 and 67bp. [20]

**PRC-RFLP for GST O 2 N 142 D polymorphism:**

The PCR was performed using the primers 5' ACT GAG AAC CGG AAC CAC AG 3' and 5' GTA CCT CTT CCA GGT TG-3' (Hsu et al 2011). The reaction was incubated at 95ºC for 10 min and subjected to 30 cycles of 95ºC for 60 s, 60ºC for 60s, and 72ºC for 60 s and a final extension step at 72ºC for 12 min. The PCR product was digested by MboI (New England, Biolabs), and the products were resolved on 2.5% agarose gel. The genotypes were determined as followed: homozygous wild type A/A or NN: 280 bp, heterozygous A/G or ND: 280, 231 and 49 bp; homozygous variants G/G or DD: 231 and 49bp (Hsu et al. 2011).

**2.6. Statistical Analysis:**

Assuming non-normal distribution of data in various groups of genetic polymorphisms and arsenic exposure with and without skin manifestations we have done nonparametric statistics to find out the association between genetic polymorphism and arsenic induced skin manifestations (Das and Das 2008). We have analyzed the data by nonparametric Median test to find out the association between GSTO1 and GSTO2 polymorphism with arsenic induced skin manifestation and arsenic induced urothelial cancer.

**3. Result:**

Demographic distribution and frequency of occurrence of different polymorphic variations has been tabulated in table 1 and 2 respectively.

**Table 1:**

## Demographic distribution of study population in three groups

| Group name | Age (yrs) | Sex | Average Ht, Wt | Smoking habit | Occupation | Average duration of exposure | average Food arsenic | average Urine arsenic | average Water Arsenic |
|---|---|---|---|---|---|---|---|---|---|
| Group I Past water arsenic leve 0- 50µg/l N=30 | i). 20-35 N=08 ii). 35-50 N= 15 iii). 51-65 N= 07 | i).M= 7 , F= 1 ii).M=11, F=4 iii).M=6, F=1 | M= Ht=159cmWt= 63kg F= Ht= 150 cm, Wt=51 kg | M= all smoker F= all non smoker | 16 farmers, rest M are small traders F=housewives | 7 yrs. | 0- 7.9 µM/l | 0-9 µg/l | 6.6 µM/l |
| Group II 50- 250µg/l N=16 Urothelial carcinoma | i). 20-35 N=01 ii). 35-50 N= 11 iii).51-65 N=04 | i).M=1, F=0 ii).M=8 F=3 iii).M=3, F=1 | M=Ht= 159cm Wt= 63 kg F=Ht= 150 cm, Wt=51 kg | M= all smoker F= non smoker | M=8 farmers, 4 small traders F= 4, house wives | 6 yrs | 329µg/kg | 87µg/l | 129µM/l |
| Group III 50- 250µg/l N=30 | i). 20-35 N=5 ii). 35-50 N= 15 iii).51-65 N= 10 | i).M=5, F=0 ii).M=11 F=4 iii).M=10, F=0 | M=Ht=164cm Wt= 65 kg F=Ht= 152 cm, Wt=52 kg | M= all smoker F= non smoker | M= 20 farmers, 5 small traders 1 service holder F=house wives | 0 yrs. | 128 µg/kg | 119µg/kg | 97.8µM/l |

In group I the occurrence of GSTOI AA genotype is 63%, AD is 30 % and DD is 7 %. In group II that is arsenic induced urothelial cancer the occurrence of AA is 86%, AD is 12% and DD is 2%. In group III category, i.e, similar level of arsenic exposure without arsenic induced cancer the occurrence of AA is 81%, Ad is 14% and DD is 5%. Hardey-Weinberg equation shows a significant deviation of AA genotype in arsenic induced cancer and non cancer patients with high skin manifestation when compared to normal unexposed persons having AA genotype ($p<0.05$). When GSTO2 N142D polymorphism was studied, it is seen that the frequency distribution NN wild type is 45%, ND is 45% and DD is 10% in group I participants. In group II arsenic induced urothelial cancer patient the frequency of NN genotype is 48%, ND is 50% and DD is 2%. According to Hardey Weinberg equation the differences between wild genotype (NN) in different arsenic exposure groups with normal unexposed group is not significant. The frequency distribution pattern is not significantly different in ND and DD genotype between normal unexposed control and exposed persons with and without malignancy.

**Table 2:**

## Distribution of frequency of occurrence of different polymorphic variation in all three groups

| Group Name | GST O1 A140D (% of occurence) | GST O2 N142D (% of occurence) | GST O1 A140D | GST O2 N142D | P value |
|---|---|---|---|---|---|
| Group I (control) N=30 | AA (63%)<br>AD (30%)<br>DD ( 07%) | NN ( 45% )<br>ND ( 45%)<br>DD (10%) | AA (N=19)<br>AD (N=9) 95% CI<br>DD (N=2) | NN<br>ND<br>DD | |
| Group II (urothelial cancer) N=16 | AA (86%)<br>AD (12%)<br>DD ( 2%) | NN (48%)<br>ND ( 50%)<br>DD (2%) | AA ( N= 14)<br>AD (N= 2)<br>DD (N= 0) | NN (N= 7)<br>ND (N=8)<br>DD (N= 1) | AA Vs AD 0.0048<br><br>A Vs D allele 00.0144 |
| Group III (arsenicosis + UC-) N=30 | AA (81%)<br>AD (14%)<br>DD ( 5%) | NN (48%)<br>ND (47%)<br>DD (5%) | AA ( N= 24)<br>AD ( N= 4 )<br>DD ( N= 2) | NN (N= 14)<br>ND (N= 14)<br>DD (N= 2) | GSTO2 N142 D is not significantly differ from normal. |

The median value of clinical symptom score was significantly higher ($p<0.01$, $p<0.05$) in GSTO1 wild genotype (AA) in arsenic induced urothelial cancer and arsenic exposed persons without cancer respectively (table 3) in comparison to AD and DD genotype1 of same group. While although a difference in skin manifestation has been notified in NN genotype in comparison to ND genotype of arsenic induced cancer patient and non cancer participant, it is not significant within same exposure group.

Total urinary arsenic is significantly lower in wild type AA population of GSTO1 in comparison to mutant AD in group II ($p<0.01$) and group III ($p< 0.001$) (table 3). In AD and DD genotype the total urinary arsenic is significantly higher in comparison to AA genotype of the same exposure level. MDA level was studied for each participant to ensure the oxidative damage induced by chronic arsenic exposure. It is seen from our study that MDA level is significantly higher in Group II and III participant in comparison to group I participants. Moreover, the MDA concentration in urothelial cell lysate indicates a significantly ($p<0.05$) higher concentration in AA genotype in comparison to AD and DD genotype of GSTO1 polymorphism. Similarly, in GSTO2 genotype, the total urinary arsenic is significantly higher in NN wild type participants of group II only. The group III participants do not show any significant differences in total urinary arsenic at different polymorphic subgroups. But regarding MDA, the wild type NN genotype shows significantly higher MDA level in comparison to ND and DD genotype in group II and group III participants.

No significant difference occurs in respect to skin score in these three genotypes of group I.

**Table 3:**

**Comparative chart for GSTO1 and GSTO2 polymorphic variation with T.U.As, Skinscore and p53 methylation**

| Group Name | GSTOI Polymorphism | | | | GSTO2 Polymorphism | | |
|---|---|---|---|---|---|---|---|
| | T.U. AS (µg/l) | Skin Score | MDA level (nmol/mg prot) | | T.U. AS (µg/l) | Skin Score | MDA level (nmol/mg prot) |
| Group I | | | | | | | |
| AA | 3.3 | --- | 10.31 | NN | 2.41 | ---- | 9.26 |
| AD | 4.1 | ---- | 9.28 | ND | 3.01 | ---- | 7.33 |
| DD | 1.9 | ---- | 9.37 | DD | 1.07 | ------ | 9.24 |
| Group II | | | | | | | |
| AA | 79 | +++ + | 22.46 | NN | 88 (P<0.05) | +++ | 20.947 ♣ |
| AD | 144 | ++ | 18.79 | ND | 123 | +++ | 17.56 |
| DD | ----- | ++ | ------- | DD | 137 | ++ | ---- |
| Group III | | | | | | | |
| AA | 88 | ++++ | 16.14 | NN | 87.7 | +++ | 18.891 ♣ |
| AD | 123 | +++ | 17.88 | ND | 96 | ++ | 13.671 |
| DD | 137 | +++ | 11.54 | DD | 114 | +++ | 11.474 |

**Footnote: ♣ indicate P<0.05.**

**4. Discussion:**

GST enzymes are highly polymorphic. Some of these polymorphisms affect enzyme expression and/or activity. Functional alteration due to genetic polymorphisms of these enzymes thought to be linked with genotoxic effects as well as overall clinical manifestations of xenobiotics (Djukic et al. 2013, Chung et al.2011, Whitbread et al. 2005) The GST omega class belongs to the GST enzyme super family which has a cysteine amino acid in its active site. Two actively transcribed GST genes (GSTO1 and GSTO2) are located on the long arm of chromosome 10. Both of the GSTO1 and GSTO2 are polymorphically distributed among in our population. GSTO1 and 2 and Arsenic methyl transferase (AsMT or Cyt 19) are involved in arsenic methylation in variety of animals including human. The GSTOs can catalyze the reduction of MMA(V) to MMA(III), which is the rate limiting step for arsenic biotransformation in human (Hsu et al 2011). In addition to MMA(V) / DMA(V) reductase activity GSTOs also exhibit high thioltransferase activity as well as dehydroascorbate reductase activity. The enzyme therefore can participate in intracellularthiolhomeostatic reactions and ascorbate recycling.

Result of one Taiwanian study revealed that GSTO2 wild type (DD) is associated with excretion of increased inorganic arsenic, while, a study conducted on Mexican Population showed that GSTO1 homovarient (DD) is associated with increased inorganic arsenic either as AsIII or As V (Hsu et al 2011) . Researchers showed that GSTO1 and GSTO2 are actively involved in disease activity of certain types of cancers. In human papilloma virus induced cervical cancer, it has been tested that GSTO1 wild type is responsible for poor prognosis and the frequency distribution of GSTO1 wild type (AA) is significantly greater in cervical cancer (Zamani et al. 2018).While in the same study carried out on Iranian population it has been reflected that GSTO2 wild type (NN) is not significantly involved in the disease activity or prognosis (Zamani et al.2018). In a separate Taiwanian study conducted over 764 subjects, role of GSTM1, GSTT1, GSTO1 and GSTO2 on the occurrence of arsenic induced urothelial carcinoma was performed (Hsu et al 2011). The result revealed that there was no correlation between GSTO2 or GSTO1 polymorphism and occurrence of non arsenic exposed urothelial carcinoma. But when considered with cumulative arsenic exposure and arsenic exposed urothelial carcinoma, a significant correlation was observed between the polymorphism and urothelial cancer.

Our study also shows significant association between arsenic exposure and urothelial cancer in homozygous wild variant of GSTO1 (AA) and homozygous wild variant of GSTO2 (NN) genotype where, we can see a higher percentage of total urinary arsenic and arsenic induced skin manifestation. For both of the gene, GSTO1 and GSTO2, homozygous variant and heterozygous variant have significantly higher percentage of total urinary arsenic reflecting , a good prognostic value of those genotype in chronic arsenic exposure. Lower incidence of arsenic induced urothelial cancer is also correlated with higher total urinary arsenic in those genotype among our study subjects.

In two separate studies it was further demonstrated that GSTO2 N142D homozygous mutant genotype was associated with higher percentage of urinary inorganic arsenic (Chung et al. 2009). Studies displayed that GSTO1 E155 del was associated markedly changed parcentage of inorganic arsenic when compared to wild homotype (Marnell et al. 2003, Agusa et al.2010). But surprisingly, the association of GSTO1 and GSTO2 in arsenic metabolism and different arsenic induced cancer shows inconsistent results due to small sample size, ethnicity, and differential nutritional status.

Despite its conclusive results regarding association of GSTOs polymorphism and arsenic induced urothelial carcinoma our present study has several limitations. Firstly, the small sample size with a limited number of urothelial cancer cases. Based on such a small nuber of participant we have got a very few number of cases in each of the genotypic variation under GSTO1 and GSTO2 polymorphism. A second limitation is that we do not study the urinary arsenic profiling, i.e. the speciation of arsenic metabolism in urinary arsenic by which we can specifically point out the role of GSTO1 and GSTO2 in arsenic metabolism. Lack of speciation of arsenic from urinary arsenic limits the power of the work to definitely demonstrate the role of GSTOs in arsenic metabolism and potentiation of arsenic induced urothelial cancer. Finally, the estimates of exposure status from food source (24 hours recall process for food items and their amount) decreases the power of actual estimate of the type and amount of foods taken by each participants. Taking together these factors, the statistical analysis on which the data was estimated for significance study and correlation study, the interaction between genes, environment and disease become underestimated.

In conclusion, our present findings have shown an association of the wild type homozygous polymorphism of the GSTO1 and GSTO2 , both individually, with arsenic induced skin manifestations and arsenic induced urothelial cancer in chronic arsenic exposed population. Apparently, this constitutes a fairly high risk group of arsenicosis. Occurrence of GSTO1 AA wild type homozygous polymorphic variety is high in Group II and III which constitute a high risk group of arsenicosis and arsenic induced urothelial cancer in group II. However, the data does not show any correlation of polymorphism with the age, sex and occupational status of the exposed population. In our future studies we will look into the combined etiological roles of smoking, nutritional and dietary status vis-à-vis the methylation profile of arsenic within arsenic exposed population to identify other risk factors.

**Competing interest** The authors declare that they have no competing interests.

**Author's Contribution** SC standardize and performed wet lab experiments, analyze the data and wrote the manuscript.TC design the experiments, and furnish the manuscript, DNGM guide clinical assessments and patients selection.

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