RESEARCH ARTICLE

GSTM1 and GSTT1 Allele Frequencies among Various Indian and non-Indian Ethnic Groups

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Abstract

Background: Glutathione-S-transferase (GST) is an important phase II xenobiotic compound metabolizing enzyme family, involved in tolerance to a particular drug or susceptibility to a diseasec. This study focused the GSTM1 and T1 null allele frequency in the Gujarat population with a comparison across other Inter- and Intra-Indian ethnic groups to predict variation in the possible susceptible status. Methods: DNA was isolated by a salting out method and GSTM1 and T1 homozygous null genotypes were detected by multiplex polymerase chain reaction in 504 unrelated individuals. The genotype distribution of null alleles was compared with Indian and non Indian ethnics reported earlier in the literature using Fisher's test. Results: The frequencies of the homozygous null genotypes of GSTM1 and GSTT1 were 20% (95%CI 16.7-23.9) and 35.5% (95%CI 31.4-39.9) respectively. GSTM1 null frequency did not deviate from most other Indian ethnic groups but differed from the majority of those of non Indian ethnicity studied. The frequency of homozygous null type of GSTT1 was significantly higher and deviated from all Indian groups and a few of non Indian ethnicity. Conclusions: Gujarat ethnicity, possibly the most susceptible for GSTT1 dependent drug disposition and diseases regarding effects of pollution. Further, the results have implications for GSTT1 dependent drugs used for treatment, a serious problem which needs to be solved by physicians and clinical researchers.

Keywords: Ethnic variation - GST null genotypes - homozygosity - disease susceptibility - xenobiotics

Asian Pacific J Cancer Prev, **13 (12)**, 6263-6267

Introduction

Drug/xenobiotic compound metabolizing enzymes (XMEs) are essential for various disease-free state of an individual. They are capable of converting the active carcinogens/toxic compounds to inactive/non toxic compounds and vice versa (Lacko et al., 2009). The combination of Phase I and Phase II biotransforming enzymes along with the receptors associated to them act inevitably accurate to maintain an individual's safety from environmental pollutants and other xenobiotics. One of the Phase II enzyme system, Glutathione-S-Transferase (GST) constitutes a family of multifunctional enzymes that has the ability to conjugate electrophiles and detoxify the agents of environmental concern including pesticides, therapeutic drugs, dietary components and a wide range of epoxides (Reszka, 2006).

Knowledge of GST has lead to hypotheses about the role of the GSTM1 and GSTT1 genes in cancer etiology (Rebbeck, 1997). High activity of Phase I enzymes or high exposure to environmental agents and null activity of Phase II enzymes increases the individual risk association to various disease or cancer, as the enzymes majorly inactivate the toxic and/or active pro-carcinogenic intermediates of Phase I enzymes (Hirvonen, 1999). The null allele genotype with deletion mutation in GST has complete loss of gene function. The homozygous null types were associated with increased susceptibility to various genetic and metabolic disorders (Habdous et al., 2004; Anantharaman et al., 2007; Nosheen et al., 2010; Amer et al., 2011; Masood et al., 2011; Lordelo et al., 2012; Jiang et al., 2012). The variations in frequency distributions of the alleles are ethnic dependent and are even responsible for the efficacy and toxicity with various drugs (Kurose et al., 2012).

Growing literature on genetic polymorphism of GSTM1 and T1 null alleles with different ethnic groups or population affiliation were observed recently as they differ significantly from each other. Even though few studies have been reported in Indian ethnics for GST polymorphism (Roy et al., 1998; Buch et al., 2001; Mishra et al., 2004; Naveen et al., 2004; Vetriselvi et al., 2006; Konwar et al., 2010) all were not able to show the exiting deviation among the intra ethnics of Indians significantly and none of them have the reports from Gujarat, which is the highly polluted state of India (Kathuria, 2007; Singh and Kohli, 2012). Studies from other non Indian ethnics also claims the existing inter ethnic differences

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among the world population and Indian ethnics (Oke et al.,1998; Rossini et al., 2002; Hamdy et al., 2003; Magno et al., 2009; Ebeshi et al., 2011; Kurose et al., 2012). In this study, we investigated the hypothesised intra-Indian ethnic's and inter-Indian ethnic's differences among different populations with reference to our population based subjects from Gujarat for GSTM1 and GSTT1 null type allele frequency. The data acquainted from the study could be useful for understanding the significant deviation among XMEs in different populations and also useful for predicting the possible GSTM1 and T1 dependent drug tolerance, toxicity and/or susceptibility to various cancers among Gujaratians.

Materials and Methods

Subjects

The study was approved by the institutional ethical committee of Sh.NP Cancer Institute, Rajkot Cancer Society; India. Two mL of blood samples were collected from 504 healthy unrelated (2 male: 1 female) volunteers of Gujarat origin, after signing the informed consent to participate in the study. The age of subjects ranged from 40-80 with the mean age of 60 years.

DNA isolation and Genotyping

Genomic DNA from whole blood was isolated by salting out method of Lahiri and Nurnberger (1991). Multiplex polymerase chain reaction by Kui et al. (2006) method was performed for identification of GSTM1 and T1 null types with albumin gene as internal control. Primers used were GSTM1-F: 5' GAA CTC CCT GAA AAG CTA AAG C 3', GSTM1-R: 5' GTT GGG CTC AAA TAT ACG GTG G 3', GSTT1-F: 5' TTC CTT ACT GGT CCT CAC ATC TC 3', GSTT1-R: 5' TCA CCG GAT CAT GGC CAG CA 3', Albumin F: 5' GCC CTC TGC TAA CAA GTC CTA 3' and Albumin R: 5' GCC CTA AAA AGA AAA TCG CCA ATC 3'. The amplified 215 bp, 480 bp, and 350 bp were analyzed for GSTM1, GSTT1, and albumin presence respectively. Homozygous null types were identified by the absence of respective bands and the efficiency of the reaction was confirmed by the presence of the albumin band in all the samples. 25 μl reaction was used to amplify the template DNA with 10 μl of 1:10 diluted DNA sample; 200 μM of all dNTPs; Forward and reverse primers were used in 5 pM for GSTM1, T1 and albumin; 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$ and 2.5U of Taq DNA polymerase. (All molecular biology grade chemicals used were from Merkgenei private Limited, Mumbai - India).

Different ethnics used in the study

The GST null allele frequency comparative analysis included the previous reports from Indian ethnics (Table 2 and 4) and other non Indian ethnics (Table 3 and 5). Among the intra Indian ethnics, reports from South India (Naveen et al., 2004; Vetriselvi et al., 2006), Lucknow (Konwar et al., 2010), North India (Mishra et al., 2004), Western central India (Buch et al., 2001), Orissa (Roy et al., 1998) and other stratified report of Tamilnadu-Pondicherry, Andhra Pradesh, Kerala and Karnataka (Naveen et al., 2004) were comparatively studied with reference to Gujaratians. Combined review report of GST null allele frequency from ethnics of Eastern Asians and Europeans countries (Kurose et al., 2012) and reports from various investigators of Egyptian (Hamdy et al., 2003), Brazilian (Rossini et al., 2002; Magno et al., 2009) Nigerian (Ebeshi et al., 2011) and Turkish (Oke et al., 1998) were used for comparative study as the inter Indian ethnic groups. Asian and European countries were grouped under population affiliation of i) Eastern Asians countries - Japan, Korea, China, Taiwan and Mongolia ii) South Eastern Asia countries - Vietnam, Thailand, Philippines, Indonesia, Malaysia and Singapore iii) Northern European countries - Norway, Sweden, Denmark, Finland and UK iv) Southern European countries - Italy, Spain, Portugal, Slovenia and Greece v) Western European countries - Netherland, Germany and France vi) Eastern European countries - Checz, Bulgaria, Poland, Slovakia and Russia and other ethnics as vii) Caucasian includes Caucasian American and Caucasian Canadians and viii) African includes African Americans and South African Xhosa (Kurose et al., 2012).

Statistics

Null Allele frequency of GSTM1 and GSTT1 was calculated using Statistical Package for Social Studies (SPSS 16) software for Windows. Two sided Fisher's exact test was done with statistical significance set at $p<0.05$ to compare frequency variation of null alleles among other inter-Indian and intra-Indian ethnics with reference to ethnics of Gujarat.

Results

Among the 504 samples investigated, GSTM1 and T1 null genotype was found to be 20% and 35.5% respectively as in Table 1. The Comparative analysis of the GSTM1 null allele frequency of Gujaratians, according to the two sided Fisher's test as shown in Table 2 was unexpectedly not significantly different to the previous reports from India for various Indian ethnic's frequency of 23-33% (Roy et al., 1998; Buch et al., 2001; Mishra et al., 2004; Naveen et al., 2004; Vetriselvi et al., 2006; Konwar et al., 2010), but as in Table 3, it was significantly different from 21.1-56.2% frequency of majority of non Indian ethnic's reported by various investigators away from India (Rossini et al., 2002; Hamdy et al., 2003; Magno et al., 2009;

Table 1. GSTM1 and GSTT1 Null Type Frequency in Gujarat Population

Genotype	Number	Frequency (%)	95%CI
GSTM1	504		
+/+	185	36.7	32.5-41.1
+/-	218	43.3	38.9-47.7
-/-	101	20.0	16.7-23.9
GSTT1	504		
+/+	149	29.6	25.7-33.8
+/-	176	34.9	30.8-39.3
-/-	179	35.5	31.4-39.9

*Homozygous wild type = +/+; Heterozygous = +/-; Homozygous null type = -/-; CI - Confidence Interval

Table 2. Intra Ethnic Differences at GSTM1 Null Type Frequency among Indians

Location	Sample No.	GSTM1 Null %	P value	Literature
Gujarat	504	20	Reference	Present Study
Karnataka	110	36.4	0.01183*	Naveen et al., 2004
Lucknow	200	36.5	0.01773*	Konwar et al., 2010
Andhra Pradesh	115	33	0.05395	Naveen et al., 2004
Kerala	122	31.9	0.07561	Naveen et al., 2004
North India	370	33	0.1125	Mishra et al., 2004
South India	772	27.72	0.2463	Naveen et al., 2004, Vetriselvi et al., 2006
Western Central India	883	26.6	0.317	Buch et al., 2001
Tamilnadu-Pondicherry	170	23.5	0.6089	Naveen et al., 2004
Orissa	72	23.8	0.6089	Roy et al., 1998

*Significance at P<0.05

Table 3. Inter Ethnic Difference in GSTM1 Null Type Frequency

Location	Sample No.	GSTM1 Null %	P value	Literature
Gujarat	504	20	Reference	Present Study
South Eastern Asia	1666	56.2	0.0000002343***	Kurose et al., 2012
Egyptian	200	55.5	0.0000002343***	Hamdy et al., 2003
Northern Europe	3686	53.3	0.000001955**	Kurose et al., 2012
Caucasian	2714	52.9	0.000001955**	Kurose et al., 2012
Western Europe	6486	51.5	0.000003825**	Kurose et al., 2012
Eastern Asia	8931	52.1	0.000003825**	Kurose et al., 2012
Eastern Europe	1184	51.1	0.000007352**	Kurose et al., 2012
Southern Europe	3770	50.9	0.000007352**	Kurose et al., 2012
Brazilian	714	39.7	0.003192*	Rossini et al., 2002, Magno et al., 2009
Nigeria	300	30	0.1412	Ebeshi et al., 2011
Africans	594	26.6	0.317	Kurose et al., 2012
South African Xhosa	128	21.1	1	Kurose et al., 2012

*Significance at P<0.001, **Significance at P<0.00001, ***Significance at P<0.000001

Table 4. Intra Ethnic Differences at GSTT1 Null Type Frequency among Indians

Location	Sample No.	GSTT1 Null %	P value	Literature
Gujarat	504	35.5	Reference	Present Study
Tamilnadu-Pondicherry	170	13.0	0.0002481***	Naveen et al., 2004
Western Central India	883	13.0	0.0002481***	Buch et al., 2001
Lucknow	200	14.0	0.0005253***	Konwar et al., 2010
Kerala	122	15.6	0.002018**	Naveen et al., 2004
South India	772	17.09	0.003691**	Naveen et al., 2004, Vetriselvi et al., 2006
North India	370	18.4	0.006471**	Mishra et al., 2004
Karnataka	110	19.1	0.01091*	Naveen et al., 2004
Andhra Pradesh	115	18.8	0.01091*	Naveen et al., 2004

*Significance at P<0.05, **Significance at P<0.01, ***Significance at P<0.001

Table 5. Inter Ethnic Difference in GSTT1 Null Type Frequency

Location	Sample No.	GSTT1 Null %	P value	Literature
Gujarat	504	35.5	Reference	Present Study
Northern Europe	2291	16.5	0.003691**	Kurose et al., 2012
Western Europe	5562	18.3	0.006471**	Kurose et al., 2012
Eastern Europe	1169	18.8	0.01091*	Kurose et al., 2012
Southern Europe	2660	19.5	0.01773*	Kurose et al., 2012
Caucasian	1223	19.7	0.01773*	Kurose et al., 2012
Turkish	240	20	0.01773*	Oke et al., 1998
Africans	594	23.1	0.06231	Kurose et al., 2012
Eastern Asia	7875	47.6	0.1148	Kurose et al., 2012
Brazilian	794	26.7	0.2232	Rossini et al., 2002, Magno et al., 2009
Egyptian	200	29.5	0.4522	Hamdy et al., 2003
South African Xhosa	128	40.6	0.5612	Kurose et al., 2012
South-Eastern Asia	890	35.1	1	Kurose et al., 2012
Nigeria	300	37	1	Ebeshi et al., 2011

*Significance at P<0.05, **Significance at P<0.01

Ebeshi et al., 2011; Kurose et al., 2012). Table 4 shows the GSTT1 null frequency (35.5%) was significantly different from 13-19.1% of various Indian ethnic's (Roy et al., 1998; Buch et al., 2001; Mishra et al., 2004; Naveen et al., 2004; Vetriselvi et al., 2006; Konwar et al., 2010) and Table 5 shows it was unexpectedly similar with most of non Indian ethnic's frequency range of 26-48% in Nigerian, Asian, African, Egyptian and Brazilian reports by various investigators (Oke et al.,1998; Rossini et al., 2002; Hamdy et al., 2003; Magno et al., 2009; Ebeshi et al., 2011; Kurose et al., 2012).

Discussion

XMEs were vital to consider for any drug before its practice in an individual. The null/defective allelic frequency estimate for a drug to get metabolized in a given population is the deciding factor for the effectiveness of the drug. Clinical trials for lethal/effective curable dose of a drug among the subjects were critical in approval of the drug (Deisseroth et al., 2012; Poste et al., 2012). In addition the difference in the ethnicity of the subjects used for clinical trials and the population used for treatment were crucial for the main effect and side effect of the drug designed (Andres et al., 2012; Kappert et al., 2012). On the other hand, XMEs defective or null type alleles were reported as the risk factors associated with various cancers and diseases (Habdous et al., 2004; Anantharaman et al., 2007; Nosheen et al., 2010; Amer et al., 2011; Masood et al., 2011; Lordelo et al., 2012; Jiang et al., 2012). In this population based basic study, one of the phase II XMEs, GST has been investigated in the suspected population of highly polluted Gujarat for the first time. GSTM1 null allele frequency of Gujarat (20%) was significantly similar to most of known reports by various other authors from ethnics of Orissa, South India, North India and Western Central India. But was significantly different and low to (36.4%) Karnataka and (36.5%) Lucknow population reported earlier by Naveen et al. 2004 and Konwar et al. 2010 respectively among Indian ethnics at $p<0.5$ level. This observation of intra ethnic difference shows the diversity of GSTM1 null type among some Indian population if not all. Therefore any drug that requires GSTM1-XMEs could be used among majority of Indian ethnics, as Intra ethnic difference for GSTM1 among Indians was less. At the same time the existence of Inter ethnics difference among Indians and non Indians ethnics for GSTM1 null allele have to be considered significantly for GSTM1-XMEs dependent drug's uptake or dosage, as the difference was more than two and half fold high in South Eastern Asian and Egyptian at P<0.000001 level (Table 3). The existence of significant GSTM1 null allele frequency's similarity among majority ethnics of Indians was unexpectedly deviating from other reports of various authors (Roy et al., 1998; Buch et al., 2001; Mishra et al., 2004; Naveen et al., 2004; Vetriselvi et al., 2006;

Konwar et al., 2010; Kurose et al., 2012) while, non-Indians ethnics significant deviation was in agreement to the previous reports from various regions (Rossini et al., 2002; Hamdy et al., 2003; Magno et al., 2009; Ebeshi et al., 2011; Kurose et al., 2012).

GSTT1 null frequency of Gujarat (35.5%) was significantly higher than all other reported Indian populations (Roy et al., 1998; Buch et al., 2001; Mishra et al., 2004; Naveen et al., 2004; Vetriselvi et al., 2006; Konwar et al., 2010) of 13-19.1% and also significantly different to Western Central India, Tamilnadu-Pondicherry and Lucknow at P<0.001 (Table 4). This significant deviation in null allele frequency for GSTT1 was similar to the expectations of the existence of intra ethnic variation by other investigators from India (Roy et al., 1998; Buch et al., 2001; Mishra et al., 2004; Naveen et al., 2004; Vetriselvi et al., 2006; Konwar et al., 2010). Among the world populations, (Table 5) GSTT1 null allele frequency of European, Caucasian and Turkish populations were low to Gujaratians and significantly different at P<0.05 level was as per the expectations of various investigators (Oke et al., 1998; Kurose et al., 2012). This observation of significant intra and inter ethnic difference among majority of Indian and non Indian ethnics for GSTT1 and GSTM1 null allele frequency has to be considered critically for the GST- XMEs dependent drug's uptake or dosage of inter-individuality response was in agreement to Kunak et al. (2012). The existing intra ethnic differences among various Indian ethnics were in accordance to various investigators' for GSTM1 but with unexpected significant similarity for GSTT1 (Roy et al., 1998; Buch et al., 2001; Mishra et al., 2004; Naveen et al., 2004; Vetriselvi et al., 2006; Konwar et al., 2010). Gujarat was known for its highly polluted environment and the null allele frequency of GSTT1 observed was to be high among the populations. This combined interaction of high pollution and high GSTT1 null frequency among the population, insists the probable susceptible status of Gujaratians for various diseases or disorders and ethnic-variable drug responses. Therefore the population under risk has to be considered for ethnic specific drugs treatments, as recommended earlier by Laing et al. (2011) with precaution to the environmental and genetic risks involved in them.

In conclusion, the distribution pattern of GSTM1 null alleles in Gujarat was not significantly different to other Indian ethnics but differ from most of non Indian ethnics. GSTT1 null allele's frequency of Gujarat population varies among intra ethnics of India and non Indian ethnics of Europe. The data acquainted from this population study adds basic information about the defective null alleles of phase II XMEs such as GSTT1 and GSTM1 in Gujaratians. This information of Gujaratians GSTM1 and GSTT1 null allele's comparisons with other populations suggests the existence of significant deviation in frequency of inter-Indian ethnics and intra-Indian ethnics respectively. The study also reports for the first time the presence of possible GSTT1 dependent drug tolerance/toxicity, variability in drug response among the Gujaratians in India. And the population might be at high risk for susceptibility to the diseases and various cancers associated with null alleles of GSTT1, as the environmental pollution rate was also alarming in the region. On the other hand, the physicians' too have difficulties in treating the ethnics with GSTT1 dependent drugs for various diseases. Further studies in clinical molecular biology are required for eliminating the alarming susceptible status of Gujaratians and their cure without GSTT1 dependent drugs for various cancers or diseases, accustomed to gene-environment interactions and ethnicity.

Acknowledgements

The authors wish to thank Bharathidasan University and Shree M. and N. Virani Science College for providing the facilities. The authors gratefully acknowledged the co-operation received from all the subjects who participated in this study and Dr. V. K. Gupta, Medical Director, Sh.NP Cancer Institute, Rajkot Cancer Society-India, for his support during the study. Conflict of Interest Statement: None declared.

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