

POLYMORPHISM OF GLUTATHIONE S TRANSFERASES GENES (GSTM1 AND GSTT1) IN ARAB POPULATION RESIDING IN UNITED ARAB EMIRATES

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ABSTRACT

The cluster of glutathione S-transferase (GST) genes play an important role in phase II biotransformation of environmental compounds, pollutants, drugs and other xenobiotics. The prevalence of GST polymorphism is dependent on ethnicity. GST gene polymorphisms have been shown to be associated with predisposition to disease and disease outcomes. The frequencies of GSTM1 and GSTT1 polymorphisms in 110 unrelated healthy Arab volunteer subjects residing in UAE was investigated. Blood was collected and DNA extracted by using the Wizard genomic DNA purification kit. Demographic information was also recorded. GSTM1 and GSTT1 gene polymorphisms were analyzed by a PCR-Multiplex procedure. We found that 55.45% of the individuals had the GSTM1 null genotype, whereas 29% had the GSTT1 null genotype whereas 20.9% of the individuals had double null genotype ie both GSTT1 and GSTM1 absent. The frequencies of GST polymorphisms in the Arab population residing in UAE were found to be different from those observed in other population. This exhibits the impact of environmental factors and ethnicity and thus provides the basis for future clinical studies.

INTRODUCTION

Genetic polymorphism stimulates diversity within a population. It frequently continues over several generations because no sole form has complete advantage or disadvantage over the others concerning natural selection. Variability created by genetic polymorphism can affect genes which encode the metabolism, disposition, transportation or targeting of the drugs and this has the potential to amend the individual's response (Marin, F. 2009). Pharmacogenetics is the study of how inheritance of genetic variations influences an individual's response to drugs (Relling MV, et al 2011). It lists out the variations in human genome so that the new or existing therapeutic agents can be used with maximal efficacy and minimal toxicity (Pfost, 2000). Pharmacogenetics approaches, in which patient specific factors, such as the presence of defective phase II alleles that may impact on drug pharmacokinetics, could be used to direct drug dosage and provide opportunities to tailor therapy to individuals (Townsend D, et al 2003). The major reasons of patient's heterogeneity is primarily due to polymorphisms in genes which are involved in the metabolism of xenobiotics (Ginsberg G, et al 2009). Evaluation of polymorphisms of these genes in different populations may elucidate the variation in response to cancer therapy and to other toxic chemicals.

Glutathione S-transferases (GSTs) belong to a family of multigene and multifunctional Phase II detoxification enzymes, that catalyze the conjugation of glutathione to a

varied range of chemical metabolites, xenobiotic and oxidative stress. Glutathione S-transferases plays a vital role in cellular defense from environmental and oxidative stress, and in cellular resistance to drugs (McIlwain CC, 2006). Glutathione S-transferases are existing in many tissues, and add to the protection from broad range of compounds including carcinogens (Ginsberg G et al, 2009; Bolt HM et al, 2006)

Human GSTs are allocated into cytosolic, mitochondrial and membrane-bound microsomal families. The cytosolic family is further divided into diverse classes: Alpha, Mu, Omega, Pi, Sigma, Theta and Zeta. GSTM1 gene (Mu class) is located on chromosome 1p13 and GSTT1 gene (Theta class) is located on chromosome 22q11. These genes showed polymorphisms which result from gene deletion, and therefore associated with absent enzymatic activity in individuals carrying both deletions (i.e. null genotype) (XuS, et al 1998; Pemble S et al 1994). The GSTM1 and GSTT1 null genotype is associated with differential susceptibility to various forms of cancer (Strange RC, et al 1999) resistance to chemotherapy treatment, drug response (Hayes JD, et al 1995), and cancer susceptibility (Lear J, et al 1996; Fryer AA et al 2000).

GSTM1 and GSTT1 null genotypes are associated with increased risk of hepatocellular carcinoma in Chinese population (Yu et al 2011). Children with acute myeloid leukemia who are homozygous for deletions in GSTT1 gene are three times as likely to die of toxicity as those

patients who have at least one copy of GSTT1 gene following intensively timed anti leukemic therapy (Davies et al 2001).

Distribution of GSTM1 and GSTT1 null genotypes vary in different populations. About half of the European populations have homozygous deletions for GSTM1 null allele, and thus fail to express the enzyme (Giensberg et al 2009). The prevalence of homozygous deletions of GSTM1 gene are higher in Caucasians and Asians than in African Americans (Bailey LR et al, 1998), whereas homozygous deletions of GSTT1 gene are greater in Asians and Africans than in Caucasians (Strange RC et al, 1999). The aim of our study was to analyse the frequency of Glutathione S Transferases genes (GSTM1 and GSTT1) in Arab population since polymorphism in these low penetration genes may in turn predispose these populations to certain adverse drug reactions or disease occurrence. Cataloguing inter-ethnic differences in the distribution of genotype of drug metabolic genes provide valuable information for profiling the pharmacogenetics of a population.

MATERIALS AND METHODS

The study group consisted of healthy Arab female students from Dubai Pharmacy College. A total of 110 students were recruited for the study. Volunteers with history of any disease were not included in this study.

They signed an informed consent form describing information regarding nature and implications of the study. The information obtained and study was approved by ethical committee of the college. The study was conducted in Department of Molecular Biology in Dubai Pharmacy College. For the present study blood samples from healthy Arab Nationals studying in Dubai Pharmacy College were obtained. The blood samples were collected during the duration from September 2015 - December 2019.

The subjects were healthy females who did not have family history of any disease. All the subjects were not fasting at the time of blood collection.

Genomic DNA was isolated from 1 mL whole blood collected in EDTA anticoagulated tubes using the Wizard genomic DNA purification kit (Promega, Madison, USA). GSTM1 and GSTT1 genetic polymorphism were evaluated using multiplex polymerase chain reaction technique. The PCR primers were synthesized by Alpha DNA, Montreal Quebec H4C3N9(Abdel-Rahman SZ et al 1996; Salem AH, et al 2011).

Primers for GSTM1

Forward:

5'-GAA CTC CCT GAAA AGCTAAAG-3'

Reverse:

5'GTT GGG CTC AAA TAT ACG GT GG-3'

Primers for GSTT1

Forward:

5'-TTC CTT ACT GGT CCT CAC ATC TC-3'

Reverse:

5'-TCA CCG GACAT GGC CAG CA-3'

The B globin locus was used as an internal control to avoid false negative results.

Primers for B globin:

Forward:

5'-CAA CTT CAT CCA CGT TCA CC-3'

Reverse:

5'-GAA GAG CCA AGG ACA GGT AC-3'

PCR reaction was carried out in a total volume of 50µl containing

- 1) 25µl of master mix containing 2.5mmol/L of MgCl₂, 0.2mmol/L of each deoxyribonucleotide triphosphate.
- 2) (dNTP's, dATP's, dCTP's, dGTP's, dTTP's) and 1 unit of Taq Polymerase
- 3) 30P moles of each primers.
- 4) 5µl of Template / Genomic DNA
- 5) 8µl of nuclease free water.

Implication was performed by initial denaturation at 94°C for 5 minutes followed by 30 cycles at 94°C for 1 minute, 64°C for 1 minute and 72°C for 1 minute and a final extension of 72°C for 7 minutes.

The amplified products were identified by electrophoresis in a 2% agarose gel and stained with 0.5 mg/ml ethidium bromide. The product lengths were 210bp, 480bp and 260bp for GSTM1, GSTT1 and B globin respectively.

Absence of PCR product for GSTM1 or GSTT1 in the presence of B globin band was indicative of a null genotype for GSTM1 or GSTT1.

Individuals with one or two copies of the relevant gene were classified as a positive genotype and individuals with homozygous deletions as a null genotype.

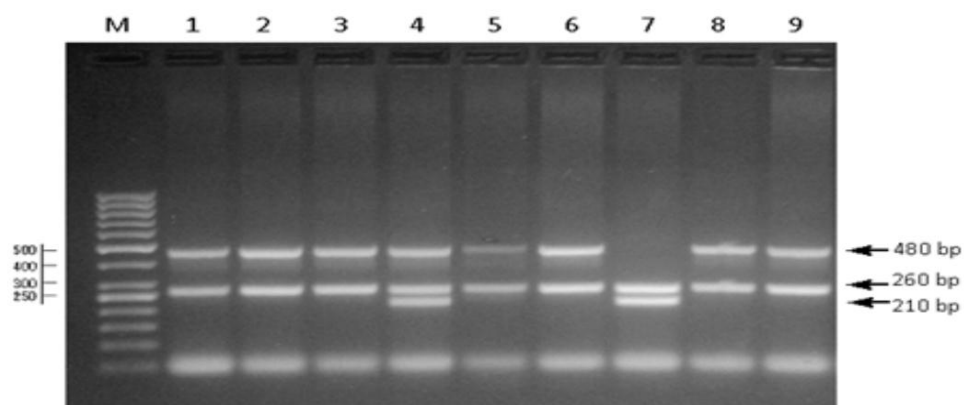


Figure 1. PCR products evaluated on 2% agarose gel. The presence or absence (null) of GSTM1 and GSTT1 was identified by the presence or absence of a band at 480 bp (corresponding to GSTT1) and a band at 210 bp (corresponding to GSTM1). β -Globin is considered an internal control (260 bp). 50 bp ladder Lane 1; GSTM1 and GSTT1 wild type Lane 4; GSTM1 null Lanes 1-3, 5, 6, 8, 9; GSTT1 null genotype Lane 7.

RESULTS

Table 1: Variable drug metabolic genes in arab population.

GENOTYPE (GSTM1)	PERCENTAGE
NULL (-)	55.45%
PRESENT (+)	44.55%
GENOTYPE (GSTT1)	PERCENTAGE
NULL (-)	29%
PRESENT (+)	71%

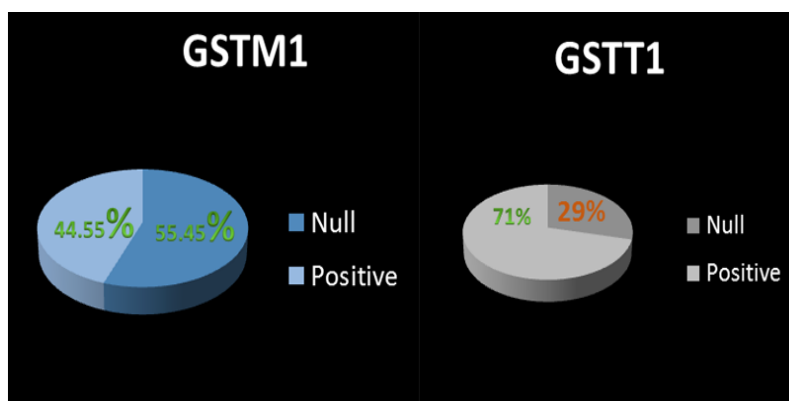


Figure 1: Variable drug metabolic genes in arab population.

Table 2: Different genotype combinations in arab population.

GENOTYPE	PERCENTAGE
GSTT1 (Null)/GSTM1 (Null) = Double Null	20.9%
GSTT1 (Positive)/GSTM1 (Positive) = Both Present	40.9%
Either Null	38.2%

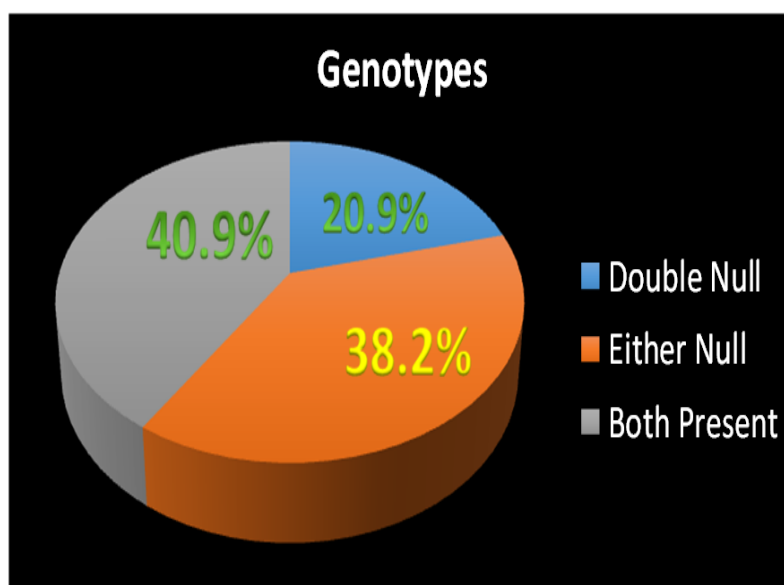


Figure 2: Different genotype combinations in arab population.

Table 3: Distribution of polymorphic genes in different populations.

GENOTYPE	ARABS	CAUCASIANS	OTHER ASIANS	AFRICANS
GSTT1 Null	29%	19.7%	47%	26%
GSTM1 Null	55.45%	53.1%	52.9%	26.7%
Double Null	20.9%	10.4%	24.6%	12.6%

Piacentini Set.al. 2011; Chen CL et.al., 1996; Naveen AT et.al., 2004;

We observed that 29% of the Arab population was homozygous for the GSTT1 deletion. This frequency is similar to that reported in other studies that analyzed the GSTT1 polymorphism in Africans. (Piacentini Set al., 2011)

We observed 55.45% of these Arabs were homozygous for the GSTM1 deletion. This frequency is similar to that reported in the other studies that analyzed GSTM1 polymorphism in Caucasians and Asians. (Chen CL et al., 1996; Naveen AT et al., 2004)

The frequency of the double nulls observed in the present study (20.9%) is higher than that observed in Caucasian population and Africans. (Chen CL et al., 1996)

Several studies have reported a relationship between combination of the GST genotype and the risk of various diseases such as chronic lymphocytic leukemia, thyroid cancer and breast cancer and some of them suggested possible synergistic effect between GST genotypes. Although our study included only females subjects, given that the genotype frequencies are not affected by sex in general (Garte S et al., 2001). Our data can represent the population genotype frequencies. Indeed our data did not show any significant differences when compared with other studies that included male subjects (Garte S et al., 2001).

DISCUSSION

Polymorphism in GST genes can affect the expression levels of GST enzymes. Since GST enzymes play a vital role in cellular defense against environmentally toxic compounds such as carcinogens polymorphisms at GST gene can increase susceptibility to disease caused by such xenobiotics.

The distribution of these metabolic genes is available for multiple population but not for Arabs.

Although this study has limitations due to the nature of the subject's enrolled (preponderance of female blood donors) previous studies have indicated no significant influence of age and gender on the distribution of these polymorphism in a given population.

As seen in table 4 the population genetic structure of the Arabs is unique and the characteristics resemble more that to Caucasians for some genes, Africans and Asians for others.

In fact Arabs are usually identified as Caucasians, modern Arabic populations especially in Egypt, Palestine, Jordan, and Lebanon are the result of long blending with different human races. (Des Kalustian et al., 1980) But we can expect different allele frequencies from the Caucasians because Arabs and other Asians and Africans intermarried during wars, mass migrations,

trade and religious practices (pilgrimage). On account of outbreeding Arabic population are more susceptible to genetic disorders. When it comes to consanguinity outbreeding introduced different deleterious recessive alleles common among other populations besides those alleles already common among Arabs.

Variations in these genes have also been demonstrated to influence drug efficacy and toxicity. In addition this also modifies individuals susceptibility to cancer (Liu D et al, 2013, Zhao Y et al, 2014, Zhang H et al, 2014).

GSTs participate in the metabolism of alkylating agents, anthracyclins and steroids. Variations in GSTT1 and GSTM1 can significantly influence treatment outcome (Davies SM et al., 2001).

CONCLUSION

From the finding of this study, it is concluded that the genotype data for polymorphic variants of GST genes provide further evidence for ethnic variations in metabolism and disposition. The study was focused to uncover the frequency distribution pattern in *GST M1* and *T1 null* genotypes among geographically Assorted Human Populations It is believed that this data will help other genetics studies on GSTM1 and GSTT1 polymorphism in association with disease risks and drug effects in Arabs.

FUTURE IMPLICATIONS

Our findings presented here are preliminary because of the small number of subjects and that the study requires confirmation in a separate larger cohort. Therefore, extensive population-specific studies are needed.

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