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Frequency of single and combined genotypes of *GSTM1*, *GSTT1* and *GSTP1* in Mexican individuals: a pilot study.

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Abstract

The Glutathione S-transferase is supergene family polymorphic involved in Phase II metabolism. Participate together in the detoxification of xenobiotics. There are few reports where these set of polymorphism of GSTs genes of the same individual are studied. These polymorphisms can be used as biomarkers for predicting disease risk or response to treatment.
Objective: Determine the genotype set of each individual harbouring polymorphisms *GSTT1*, *GSTM1* and *GSTP1* in healthy volunteers of Mexican origin.
Methods: We studied 160 samples of healthy volunteers. DNA was extracted from peripheral blood. *GSTT1* and *GSTM1* polymorphisms were identified by multiplex end point PCR. The identification of *GSTP1b* (exon 5) and *GSTP1c* (exon 6) was performed separately by PCR-RFLP.
Results: The frequency for the *GSTM1* null was 47%; *GSTT1* null 65%; *GSTP1* exon 5 for heterozygotes (P1a/P1b) 51.9%, 35.6% in homozygotes (P1b/P1b) and exon 6 heterozygous 25.6% (P1a/P1c). The most common genotypes set were, wild M1; null T1; a/b P1b; a/a P1c and wild M1; null T1; b/b P1b; a/a P1c, both with a frequency of 12.5%. The null M1; null T1; a/b P1b; a/a P1c frequency was 11.9%. The highest risk genotypes were 3.8% (null M1; null T1; a/b P1b, a/c P1c) and 2.5% (null M1; null T1; b/b P1b; a/c P1c).
Conclusion: The frequencies show genotypes that can be considered high risk, indicating increased susceptibility to xenobiotics. These results are the first report of *GSTM1*, *GSTT1* and *GSTP1* combined genotype in Mexican population.

Keywords: Glutathione S-transferase, Polymorphisms, Mexican population.

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Introduction

About 99.9% of the DNA sequence of the individuals is shared with each other. However, in the remaining 0.1% is where genetic variations are found. These types of variations are known as genetic polymorphisms and they represent different DNA sequences [1,2].

The enzymes of the Glutathione S-Transferase (GST) family consist of several genes that code for a group of isoenzymes involved in Phase II metabolism. The cell can be protected from oxidative damage through the conjugation of glutathione with electrophilic substrates, which generate more soluble compounds [3-5]. The most studied GST subclasses in mammals are Mu (μ), Pi (π) and Theta (θ) [5,6]. The *GSTM1* (μ) gene, located on chromosome 1p13.3, consists of eight exons with a length of 4.2 kb and four allelic variants: A (wild), B, C and 0 or null. *GSTM1* null is the most frequent polymorphism, which generally result in loss of enzymatic activity [5,7]. On the other hand, the *GSTT1* (θ) gene is located on chromosome 22p11.2. It consists of six exons and is flanked by two homologous regions HA3 and HA5. It has two allelic variants: wild *GSTT1a* and *GSTT1-0* or null. This polymorphism is due to a homologous recombination of the HA3 and HA5 regions resulting in a deletion of 5.4 kb [5,8]. The *GSTP1* gene (π) is located on chromosome 11q13, it consists of nine exons, with a length of 3.2 kb and it has three allelic variants: *GSTP1a*, *GSTP1b* and *GSTP1c*. The wild allele is *GSTP1a* and the polymorphic allele *GSTP1b* is located in exon 5 as a result of the mutation of ATC (Ile) to GTC (Val) at codon 104. The third allele, *GSTP1c*, also polymorphic, has the same mutation at codon 104. Additionally, it has a second mutation in exon 6, consisting of a base substitution of GCG (Ala) to GTG (Val) [2,9-11].

The GSTs are important because their expression increases significantly in mammalian tumor cells. They have been implicated in resistance to the treatment of different types of cancer drugs [2,3,5,12].

Regularly xenobiotics are metabolized by a group of enzymes that work simultaneously to remove them from the body. The altered enzyme activity by GST polymorphisms is attributed to the *GSTT1* and *GSTM1* gene deletions, meanwhile for the *GSTP1*, the SNPs can induce lower enzyme activity [13]. There are several reports in the literature on the frequencies of each of these genes in different populations and also the comparison between them. Nowadays, in literature there are few reports can be found where these set of polymorphism of GSTs genes of the same individual are studied. Most analysis focus on the frequencies of each gene [2,5,11,13-20]. However, the glutathione S-transferase family of enzymes participate together in the detoxification of xenobiotics. Therefore, studying several genes could give a better prognosis of possible susceptibilities in a population. Therefore, the aim of this study was to determine the genotype set of each individual harbouring polymorphisms *GSTT1*, *GSTM1* and *GSTP1* in healthy volunteers of Mexican origin.

Material and Methods

Studied group

We recruited participants from January 2014 to January 2015. The participation in this study was voluntary and by invitation. When individuals agreed to take part, they were reported if they had a chronic disease, if they were in treatment or if they were taking any medication. These criteria were used to include apparently healthy individuals. Those who agreed to participate signed a letter of informed consent.

Ethical considerations

The authors certify that all research was done under full compliance with all government policies and the Helsinki Declaration 2013. This study was approved by the Ethics and Research Committee of the Research Center in Medical Sciences at the Autonomous University of the State of Mexico.

DNA extraction

A 4 ml sample of peripheral blood from each participant was collected into a Vacutainer tube with heparin. DNA extraction was performed using a ZR Genomic DNA Kit II (Zymo Research, USA). The products were verified by horizontal electrophoresis in agarose (1%).

Genotyping of GSTT1 and GSTM1

GSTM1 and *GSTT1* polymorphisms were identified by multiplex PCR with *CYP1A1* gene as control, according to the modified method using specific primers [21]. The mixture had a final volume of 25 µl containing 5l of 5X PCR buffer (Promega), 4 µl of 25 mM $MgCl_2$ (Promega), 1 µl dNTPs, 10 µM (Fermentas), 6.7 µl of molecular biology grade H_2O, 0.3 µl of 5 U/µl Taq Polymerase (Promega), 2 µl of DNA template and 1 µl of each primer *CYP1A1-f*, *CYP1A1-r*, *GSTM1-f*, *GSTM1-r GSTT1-f*, *GSTT1-r*. All primers had a concentration of 30 pM/µl. The PCR conditions were 5 min at 94°C, 35 cycles of denaturation for 1 min at 94°C, alignment 1 min at 62°C, 1 min extension at 72°C, and a final elongation of 5 min at 72°C. PCR products were verified by horizontal electrophoresis using 1.5% agarose. The determination of the polymorphism of *GSTT1* was based on the presence of a 480 bp band, corresponding to wild *GSTTl*, while its absence denoted a null *GSTT1*. €Similarly, for *GSTM1* the presence of a 215 bp band indicated wild *GSTM1* while its absence implied null *GSTM1*. Finally, a 312 bp band, corresponding to *CYP1A1* [21].

Genotyping of *GSTP1*

Because of *GSTP1* polymorphisms are in different regions of the gene, exon 5 (*GSTP1*b) and exon 6 (*GSTP1*c), the amplification was performed separately. The PCR-RFLPs was carried out using specific primers for each polymorphism. The mixture had a final volume of 25 µl, containing 5 µl 5X PCR buffer (Promega), 4 µl of 25 mM $MgCl_2$ (Promega), 1 µl dNTPs 10 µM (Fermentas), 10.7 µl of molecular biology grade H_2O, 0.3 µl 5 U/µl Taq Polymerase (Promega), 2 µl of DNA template and 1 µl of each primer according to the gene to be amplified *GSTP1*b-*f*, *GSTP1b-r GSTP1c-f* or *GSTP1c-r*; all primers had a concentration of 30 pM/µl [2]. The same PCR conditions for M1 and T1 were used for *GSTP1c* genotyping. For *GSTP1b*, the alignment temperature was 59°C, everything else remained the same.

For exon 5, an amplicon of 176 bp was obtained; while for exon 6 an amplicon of 332 bp. The digestions were performed with enzymes BsmAI (Fermentas) to determine *GSTP1b* and *AciI* (Fermentas) to determine *GSTP1*c. The content of the digestion mixture was: 7 µl H_2O (molecular biology grade), 2 µl FastDigest Green buffer (Fermentas), 1 µl Fast-Digest Enzyme (Fermentas) and 10 µl PCR product DNA, for a total volume of 20 µl. Both digestion reactions were incubated at 37°C for 5 minutes. The digestion products were verified by horizontal electrophoresis in 2% of agarose. The identification of polymorphisms was based on the presence of DNA fragments of different sizes. For exon 5, 176 bp, 91 bp and 85 bp fragments correspond to heterozygote P1b, while 91 bp and 85 bp P1b fragments correspond to homozygote and a 176 bp fragment corresponds to wild P1a. Regarding exon 6 polymorphisms, the 332 bp fragment corresponds to homozygote P1c; three fragments, 332 bp, 174 bp and 158 bp correspond to heterozygote P1c and two fragments, 158 bp and 174 bp, to wild P1a [2].

Results

In this study, 160 healthy volunteers participated, 58 were women and 102 were men, representing respectively 36.3% and 63.8% of all cases. The average age was 39.57 years, in a range of 16-72 years.

For *GSTM1*, the number of individuals with gene deletion or null polymorphism was 76 (47.5%), while the wild-type gene was presented in 84 cases representing 52.5%. In the *GSTT1* gene, it was found that 104 cases have deletion and 56 had the wild type, 65% and 35% respectively. For *GSTP1b* gene, the exon 5 and 6 were analysed. In exon 5, it was found: 83 heterozygous (P1a/P1b) 51.9%, 57 homozygous (P1b/P1b) 35.6% and the wild-type gene (P1a/P1a) was identified in 20 individuals (12.5%). For exon 6, it was found that 41 cases had the mutation corresponding to 25.6% of heterozygous (P1a/P1c) and 119 individuals (74.4%) have the wild-type gene (P1a/P1a), no homozygous was found (Figure 1).

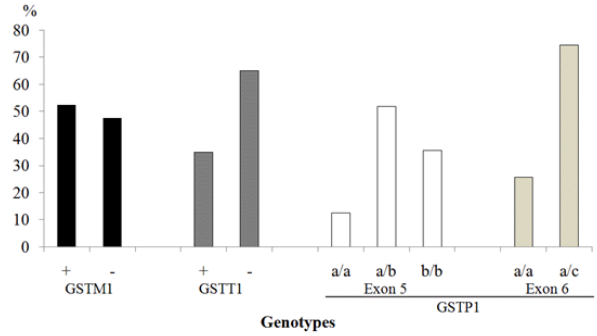

Figure 1. *Distribution of GST polymorphisms.*

To determine the genotype at each individual, the results of *GSTT1*, *GSTM1* and *GSTP1* were grouped. Later, they were identified and grouped individuals according to genotype.

We found that the most common genotype set was: wild *GSTM1*; null *GSTT1*; heterozygote (P1a/P1b) exon 5 and wild *GSTP1c* (P1a/P1a) exon 6 and wild *GSTM1*; null *GSTT1*; homozygote (P1b/P1b) exon 5 and wild *GSTP1*c (P1a/P1a) exon 6, and wild *GSTM1*; null *GSTT1*; homozygote (P1a/P1b) exon 5 and wild *GSTP1c* (P1a/P1a) exon 6, both with a frequency of 12.5%. The genotype with all the genes and the wild alleles: wild *GSTM1*; wild *GSTT1*; wild (P1a/P1a) exon 5 and wild *GSTP1*c (P1a/P1a) exon 6 was present only in the 2.5%. Also, the genotype that confers higher susceptibility to xenobiotics: null *GSTM1*; null *GSTT1*; homozygote (P1b/P1b) exon 5 and heterozygote (P1a/P1c) exon 6 had a frequency of 2.5%. The details of these results are presented in Table 1.

Table 1. *Frequency distribution of genotypes sets.*

Genotypes				
GSTM1	*GSTT1*	*GSTP1*	*GSTP1*	**%**
		exon 5	**exon 6**	
+	-	a/b	a/a	12.5
+	-	b/b	a/a	12.5
-	-	a/b	a/a	11.9
-	-	b/b	a/a	7.5
+	-	a/b	a/c	7.5
+	+	a/b	a/a	5.6
+	+	b/b	a/a	5.6
-	+	a/b	a/a	5
-	-	a/a	a/a	4.4
-	-	a/b	a/c	3.8
-	+	b/b	a/a	3.1
+	+	a/b	a/c	3.1
-	-	b/b	a/c	2.5
-	+	a/a	a/a	2.5
-	+	a/b	a/c	2.5
-	+	b/b	a/c	2.5
+	+	a/a	a/a	2.5
+	-	a/a	a/a	1.3
+	-	a/a	a/c	1.3
+	-	b/b	a/c	1.3
-	+	a/a	a/c	0.6
+	+	b/b	a/c	0.6

Discussion

It was found that the frequency for the *GSTM1* null polymorphism (47%) in this study is similar to that reported by Montero et al. in a Mexican population, with 42.6 % for the null polymorphism [22]. These results are also consistent with those reported in other populations of the planet. For example, Ye et al. reported a frequency of *GSTM1* null genotype of 50% in Caucasian and 51% in Asian and Salah et al. in Tunisian population with a 53.9% for this polymorphism [11,23].

However, the frequencies in this study were higher than the observed in other studies. For instance, Molina et al. and Perez et al. found a frequency of 30% and 37% in Mexican population [24,25]. Investigation in Chile by Quinones et al. and in Africa by Dandara et al. found similar frequencies of 24% and 33 % for GSTM null [19,26]. The present study identified a frequency of 65% for the *GSTT1* null polymorphism. Such result differs from findings in other studies. For instance, Perez et al. and Molina et al. reported in Mexican population a frequency of 12%, 15% and 19% [20,24,25]. In Caucasian population, Nelson et al. observed a frequency of 15% and in African population, Dandara et al. and Salah et al. observed a frequency of 25% and 28% [18,19,23].

With respect to the polymorphisms of *GSTP1* exon 5, the percentage was 51.9 for heterozygotes (P1a/P1b) and 35.6 in homozygotes (P1b/P1b). These frequencies are similar to the results in Mexican population by Molina et al., Perez et al. and Mejia et al. that report a frequencies for the heterozygote polymorphism P1a/P1b of 50%, 51% and 50% [2,24,25]. In another study in Tunez, Salah et al. the frequency of the heterozygote and homozygote polymorphisms was 54% and 21% respectively [23]. Dandara et al. reported for heterozygote

and homozygote polymorphisms of *GSTP1* frequencies of 25% and 7% [19]. Similar findings were reported by Ye et al. found a frequency of 10% for Europe, 14% in African Americans and 20% in Asia. However, it is not specified if the polymorphism is heterozygous (P1a/P1b) or homozygous (P1b/P1b) [11]. For polymorphism *GSTP1*c exon 6, in this work only the heterozygous (P1a/P1c) was identified with a frequency of 25.6%. In contrast, Ye et al. reported only 1% of prevalence. However, they do not specify whether the polymorphism is heterozygous or homozygous [11]. On the other hand, Mejia et al. did not identify any polymorphism in this gene region and reported 100% of the wild allele [2].

With regard to the genotype set of GST, in this study the more frequently genotypes detected were wild *GSTM1*; null *GSTT1*; *GSTP1* heterozygote exon 5 (P1a/P1b) and *GSTP1* wild exon 6 (P1a/P1a). However, in the literature there exist a few studies reporting the genotype set for the GST genes family. Salah et al. found in Tunez population wild *GSTM1*, wild *GSTT1*, wild (P1a/P1a) as the most frequent combined genotype [23].

The combined frequencies of genotypes are considered high risk when contain null polymorphisms and SNPs at a greater extent. In contrast, the risk is considered lower when the combined genotype has larger proportion of wild alleles. The present study identified the genotype of higher risk null *GSTM1*; null *GSTT1*; *GSTP1* homozygote exon 5 (P1b/P1b) and *GSTP1* heterozygote exon 6 (P1a/P1c) in 2.5%, and the genotype of lower risk wild *GSTM1*; wild *GSTT1*; *GSTP1* wild exon 5 (P1a/P1a) and *GSTP1*wild exon 6 (P1a/P1a) in 2.5%.

Knowing the combined genotype is important because the xenobiotics are metabolized by a set of isoenzymes coded by GST genes. The identification of a single gen and its polymorphisms is not enough to estimate the risk.

The variability of the frequencies can relate to the ancestry of the population and the miscegenation in the different regions of the world. The interbreeding of people of different races differs in Mexico. Caucasian genes predominate in the north of Mexico and genes from mixtures between Spanish people and Mesoamerican natives predominate in the south of Mexico. It is important to consider that there is a big genetic diversity worldwide, which may explain these differences [27].

The results suggested that the frequencies of these polymorphisms could be a handy tool to monitor susceptible groups or populations. Also, the study of the association of polymorphisms can be a useful predictive value of treatment response. In conclusion, these results represent the first combined genotype frequencies reported of *GSTM1*, *GSTT1* and *GSTP1* in Mexicans.

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