Deletion Polymorphism of Glutathione S-Transferases M1 and T1 in The Tunisian Population

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RÉSUMÉ

Prérequis : Les glutathions S-transférases (GST) jouent un rôle vital dans la défense cellulaire contre les composés toxiques environnementaux. Ces enzymes présentent un polymorphisme génétique de délétion variable selon les ethnies.

But : Déterminer les fréquences de la délétion homozygote des gènes GSTM1 et GSTT1 dans la population Tunisienne.

Méthodes: Par la technique de PCR multiplex, nous avons évalué les fréquences de délétion homozygote des gènes GTSM1 et GSTT1 chez 145 individus Tunisien sains.

Résultats: Nous avons trouvé que 34,6% des individus avaient un génotype GSTM1 nul, 16,6% avaient un génotype GSTT1 nul et 4,82% avaient une délétion homozygote des deux gènes GSTM1 et GSTT1.

Conclusion: La fréquence de la délétion homozygote de gène GSTM1 chez les Tunisiens est plutôt dans l'intervalle de la population noire, et elle est plus basse que celle rapportée chez les Asiatiques, les Arabes et les Caucasiens. Cependant la fréquence de la délétion homozygote du gène GSTT1 est dans l'intervalle de plusieurs populations étudiées. La fréquence de la double délétion semble plus basse que celle décrite dans les différentes populations.

SUMMARY

Background : Glutathione S-transferases (GST) play a vital role in cellular defense against environmentally toxic compounds. These enzymes present a genetic deletion polymorphism, which varies with ethnicity.

Aim : To evaluate the frequencies of homozygous deletion of GSTM1 and GSTT1 genes in Tunisian population.

Methods: On the basis of multiplex PCR protocol, the frequency of the deleted genotypes of GSTM1 and GSTT1 genes was evaluated on 145 healthy Tunisian subjects.

Results: We found that 34.6% of the individuals had the GSTM1 null genotype, 16.6% had the GSTT1 null genotype and 4.82% had a double deletion of both GSTM1 and GSTT1.

Conclusion: The distribution of GSTM1 null in Tunisians is rather in the range of black populations and is lower than that reported in Asians, Arabs and Caucasians. However the frequency of GSTT1 null is in the range of several populations studied except Asians. The double deletion frequency seems lower than that described in different populations.

Mots-clés

Glutathion S-transférase T1, Glutathion S-transférase M1, Polymorphisme Génétique

Key-words

Glutathione S-transferase T1, Glutathione S-transferase M1, Genetic polymorphism

تعدّد أشكال القلوتاتيون س - ترانسفراز عند التونسيين

الباحثون : س .قارة - م . العباسي - ك .بن يامنة - م .عبد النبي - ف .قميرة.

الهدف من هذه الدراسة هو تحديد تواتر خبن الجينات 1ذ نئغ و 1ئ نئغ عند 145 شخصا تونسيا معافى و ذلك بواسطة تقنية .ؤضآ

توصّلنا إلى تحديد نسبة ٪ 34،6 سلبي بالنسبة للنمط الجيني 1ذ نئغ عند التونسيين و هو متواجد خاصة عند الزنوج .و نسبة ٪ 16،6 للنمط الجيني 1ئ نئغ سلبية ، و نسبة ٪4،82 يملكون خبنا على مستوى الجينيين 1ئ نئغ و 1ذ نئغ و هي نسب تتقارب مع الشعوب الأخرى.

الكلمات الأساسية : قلوتاتيون س - ترانسفراز -1ئ قلوتاتيون س - ترانسفراز -1ذ تعدّد الأشكال الجيني .

Glutathione S-transferases (GST) are a polymorphic super family of multifunctional enzymes, which exist as homodimers and heterodimers. These enzymes catalyze the conjugation of reduced gluthatione with various electrophilic compounds and are known to play an important role in the detoxification of several endogenous and exogenous toxic and carcinogenic substances (1). In humans, GST enzymes are divided into 5 subclasses: alpha (A), mu (M), pi (P), theta (T) and zeta (Z). Each class also includes several genes and isoenzymes. GSTM1 products catalyze the conjugation of glutathione to epoxide derivatives of polycyclic aromatic hydrocarbons (1). GSTT1 products are important in the detoxification of naturally occurring monohalomethanes, dichloromethanes and ethylene oxides (2).

Three different polymorphisms have been described at the GSTM1 locus on chromosome 1p13.3 (3). The most important polymorphism encodes for a partial gene deletion in GSTM1 (GSTM1 null genotype) resulting in complete absence of GSTM1 enzyme activity. The two other polymorphisms do not lead to functional differences (4). The GSTT1 gene has one polymorphism and has null allele (GSTT1 null genotype), that also results in no enzyme production in homozygote.

The frequencies of the deletion polymorphism in the GSTT1 and GSTM1 genes have been extensively studied in many ethnic groups, and the accumulated data show striking interethnic variation in the distribution of the deletion polymorphism (5, 6).

The aim of this study was to investigate the frequencies of homozygous deletion of GSTM1 and GSTT1 genes in Tunisian population.

PATIENTS AND METHODS

A- Subjects

One hundred forty five Tunisian healthy volunteers were included in this study. They were divided into 72 men and 73 women. The median age was 53.03 ± 8.64 years (40 - 70 years).

B- Genotyping

DNA was isolated from leukocytes in 10 ml of peripheral blood. The presence of the GSTM1 null and GSTT1 null deletion was screened by using a multiplex PCR procedure using primers described in table 1.

Table 1 : Primer sequences utilized for multiplex PCR

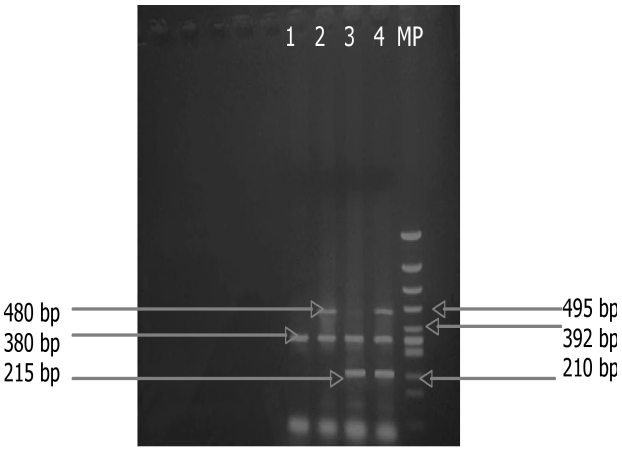
Genes	Primer Sequences	Product Length
GSTM1	5'-GAACTCCCTGAAAAGCTAAAGC- 3'	215 bp
	5'-GTTGGGCTCAAATATACGGTGG -3'	480 bp
GSTT1	5'-TTCCTTACTGGTCCTCACATCTC- 3'	380 bp
	5'-TCACCGGATCATGGCCAGCA- 3'	
Albumin	5'-GCCCTCTGCTAACAAGTCCTAC- 3'	
	5'-GCCCTAAAAAGAAAATCGCCAATC-3'	

The amplification of albumin gene provides a positive control for each reaction. The 50μl reaction mixture contained: 250 ng of genomic DNA, 22 pM for each primer, 200μM of each dNTP, 2U of Taq DNA polymerase. Cycling conditions consisted of 5'at 94°C for initial denaturation and 2'at 64°C for initial hybridization followed by 30 cycles of extension for 30''at 72°C, denaturating for 20''at 94°C, hybridization for 20''at 64°C and final extension at 72°C for 7'. Ten microliter of each PCR product were analyzed for efficient amplification on an agarose gel at 1%.

RESULTS

Frequencies of homozygous GSTM1 and GSTT1 deletions were respectively found in 34,5 % and 18,6% of individuals. The frequency of individuals lacking both genes was 4.82 % (fig 1, table 2.)

Figure 1 : PCR multiplex for genotyping GSTM1 and GSTT1



Lane 1: GSTM1 null and GSTT1 null; lane 2: GSTM1 null; lane 3: GSTT1 null; lane 4: corresponds to an individual who is positive for both GSTM1 and GSTT1

Table 2 : Frequencies of GSTM1 and GSTT1 null genotypes

Genotype	Number	Percentage
GSTM1 null	50	34,5
GSTT1 null	27	16,6
GSTM1 and GSTT1 null	7	4,82

There was no difference for the GSTM1 and GSTT1 null genotypes as a function of sex (table3).

Table 3 : Frequencies of GSTT1 and GSTM1 null genotypes as a function of sex.

Sex	Frequency (%)	
	GSTM1 null	GSTT1 null
Men	31,94	16,66
Women	36,98	20,54

DISCUSSION

Polymorphism in GST genes can affect the expression levels of the GST enzymes (7). Since GST's play a vital role in cellular defense against environmentally toxic compounds such as carcinogens, polymorphism of GST genes can increase susceptibility to diseases caused by such xenobiotics.
The distribution of GSTM1 and GSTT1 null genotypes varies among different ethnic groups (table 4).

Table 4 : Frequencies of GSTM1 and GSTT1 null genotypes in different ethnic groups.

Race	GSTM1 null	GSTT1 null	GSTM1 null / GSTT1 null	References
Caucasians	42 – 60 %	13 – 26 %	10,4%	8, 12
Asians	42 – 54 %	35 – 52 %	24,6%	8, 12
Africans	16 – 36 %	15 – 26 %	12,6%	8, 12
Middle East-Arabs	54,6%	25%		12
Egyptians	55,5%	29,5%	17,2%	13

Data is available for multiple populations but not for North-Africans. Several population based studies have reported a GSTM1 prevalence ranging from 16 to 60% (8). Asians and Caucasians have the highest frequencies although black populations including Africans, African-American and black populations of Brazil have the lowest ones (9, 10, 11). We found that 34.5% of Tunisians were homozygous for the GSTM1 deletion. Despite that Tunisians are Arabs; this frequency is lower than that described in Middle East Arabs (12) and Egyptians (13) whose frequencies are in the range of Caucasians. The distribution of GSTM1 null deletion in Tunisian is rather in the range of black populations, probably because of the geographic location of Tunisia. In fact, Tunisia is a North-African country located on the Mediterranean coast which has been affected by intercontinental transportation and South to North immigration.
The distribution of GSTT1 null frequency in the different populations is not as well documented as GSTM1's distribution. However, it seems that the highest frequencies are seen in Asian populations (14) although Caucasians, Arabs and Africans have equal distribution ranging from 13 to 26% (8)
We observed 16.6 % of Tunisians were homozygous for the GSTT1 deletion. This frequency is in the range of several populations studied except Asians in whom frequencies are higher, ranging from 35 to 52% (8).
Concerning the genotype combination analysis, 4.82% of Tunisians were homozygous for both the GSTM1 and GSTT1 deletion. This frequency is lower than that it was described in the different populations studied. Asians have the highest frequencies of genotypic combination, although Africans, Caucasians and Arabs are ranging from 10,4 to 17,2% (12).
Several studies have looked for a difference in distribution of GSTM1 and GSTT1 null genotype as function of sex, these studies showed that the frequencies of GSTT1 null genotype are not affected by sex (8). However, in African population the frequency of GSTM1 null was found significantly different between men and women (8). We found no difference for the GSTM1 and GSTT1 null genotype as function of sex as expected, because the genes are not located on the sex chromosome.

CONCLUSION

This study demonstrates that the frequencies of homozygous deletion of GSTM1, GSTT1 and of both genes are 34,6%, 16,6% and 4,82 % respectively in Tunisian population. There is no difference in genotype null frequency by sex for both GSTM1 and GSTT1.

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