Analysis of the GSTM1 gene polymorphism in patients with tuberculosis with regard to the version of MBT resistance

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Abstract. An analysis of the occurrence of alleles and genotypes of GSTM1 gene in patients with pulmonary tuberculosis regarding the MBT resistance version allowed to establish that under the conditions of pulmonary tuberculosis infection GSTM1 gene deletion mutation can be found in one out of five (21.87% of cases), and the occurrence due to the MBT resistance version is: with NDTB - 17.39%, with MDR-TB 35.0% and - PRTB 20.0% respectively. According to the nature of the distribution of allelic gene GSTM1 a favorable functional 1 allele prevails (73.29%) in the normal inbreeding among patients and deficiency of heterozygosity among healthy people, which generally forms a normal population distribution for the European race.

Keywords: *tuberculosis, deletion polymorphism, GSTM1, resistance, MBT.*

Introduction. Some of the genes, the expression of which plays a key role in the resistance of cells to the effects of free radicals by lipid peroxidation and oxidative modification of proteins, preventing breakage of DNA, biosynthesis of prostaglandins, transportation and metabolism of bilirubin, hormones are genes which code the synthesis of glutathione-S -transferase (GST) [1, 3]. GST are enzymes of the second phase of detoxification systems which protect the body against endogenous oxidative stress and exogenous toxins, catalyzing conjugation of sulfhydryl groups of reduced glutathione and rendering harmless various electrophilic compounds, including products of lipid and DNA oxidation [2, 4].

Objective. To identify GSTM1 gene polymorphism in patients with tuberculosis regarding the MBT resistance version.

Material and methods. The study involved 100 patients with newly diagnosed pulmonary TB who had been hospitalized in Chernivtsi Regional TB Dispensary. The control group consisted of 50 healthy individuals. Genomic DNA was isolated from the whole venous blood. GSTM1 polymorphic areas were isolated by means of multicomplex polymerase chain reaction, according to the protocol for instantaneous analysis of polymorphism by M. Arana et all (1996). Deletion of gene corresponds to the lack of appropriate strips in the electropherogram. We used the program STATISTICA, version 10.0.228.8 (StatSoft, Inc.) for statistical analysis of the findings. The difference in the distribution of occurrence of genotypes and their combinations between groups were calculated using $\chi 2$ criteria . Differences were regarded as significant at significance level $p<0,05$. The association of genotypes with susceptibility to tuberculosis was judged by the size of the odds ratio (odds ratio, OR).

Results and discussion. Despite the fact that the activity of the enzyme glutathione-S-transferase of class M is encoded by five GST genes of class M (M1-M5), the dominant cause of genetically caused dysregulation of antioxidant activity is deletion (null) polymorphism of the gene GSTM1. Due to the above, we have analyzed the occurrence of alleles and genotypes of GSTM1 gene in patients with pulmonary tuberculosis due to MBTresistance version.

Results and discussion. Lack of 0-genotype was found in 214 (73,29%) cases out of 292 isolated alleles (n=107), while the "mutant" deletion (0-allele) was observed by 2.74 times less frequently – in 78 (26,71%) cases (n=39) (χ^2=63,34, p<0,001) (Chart. 1).

Chart 1. Distribution of deletion polymorphism of the gene glutathione-S-transferase of class M1 (GSTM1)

Study groups	Experimental group, n=96	Control group, n=50	χ^2 p	Total, n=146 (%)
No 0-genotype, n (%)	75 (78,13)	32 (64,0)	χ^2=3,35 p=0,067	107 (73,29)
0-genotype, n (%)	21 (21,87)	18 (36,0)	χ^2=3,67 p=0,052	39 (26,71)
χ^2 p	χ^2=60,75 p<0,001	χ^2=7,84 p=0,005	-	χ^2=63,34 p<0,001

The relative occurrence of 0-genotype and its absence among TB patients and healthy individuals did not differ significantly (p>0,05). Thus, in both groups the functional allele of gene GSTM1 was found much more frequently: by 3.57 times in the experimental group (χ^2=60,75 p<0,001) and by1.78 times in the control group (χ^2=7,84 g=0,005) (chart 3.1). The resulting distribution in observation groups reflected the general one in the surveyed population , which was also dominated by those with wild 1 allele by 2.74 times over those with non-functional 0-genotype (p<0,001).

Race and population analysis of gene GSTM1 null polymorphism showed that the frequency of homozygous null genotype gene appointed above among the examined tuberculosis patients was lower than in European population (PD=0,42-0,60 vs PD=0,22, p<0,05) and Asian races (PD=0,42-0,54, p<0,05), it did not differ significantly from the corresponding figure of the equatorial race (PD=0,16-0,36, p<0,05). Occurence of null genotype in the control group of the examined patients did not differ significantly from the rate for Caucasians (p>0,05). In addition, the occurence of

GSTM1 0/0-genotype in our experimental (PD=0,22) and control groups (PD=0,36) corresponded to averages in Ukrainian (south-eastern and central Ukraine) and some Eastern European populations (PD=0,15-30).

Allelic distribution according to the polymorphic variant of gene GSTM1 among TB patients and healthy individuals in general corresponds to the expected population equilibrium Hardy-Weinberg (Chart 2). In quantitative terms, an allele without genotype-0 is dominant (P1=54,0%), while the relative occurence of alleles did not differ significantly. We found statistically significant heterozygote deficiency in the control group (F=0,28, p=0,033), which does not generally cover the entire sample (F=0,24, p>0,05) and shows a normal population distribution.

Chart 2. Analysis of heterozygosity of null polymorphism of the gene glutathione-S-transferase of class M1 (GSTM1)

Groups	Genotypes, alleles, n (%)		P_D	P_1	H_O	H_E	F	χ^2	P
	DD	1_{allele}							
Experimental group, n=96	21 (21,87)	75 (78,13)	0,41	0,59	0,38	0,48	0,20	2,33	>0,05
Control group, n=50	18 (36,0)	32 (64,0)	0,54	0,46	0,36	0,50	0,28	4,56	0,033
Total, n=146	39 (26,71)	107 (73,29)	0,46	0,54	0,38	0,50	0,24	3,27	>0,05

Notes: 1. P_1 – relative occurrence of 1 allele; P_D – relative occurrence of deletion allele D. 2. H_0 – real heterozygosity; H_E – expected heterozygosity; F – inbreeding factor. 3. χ^2p – criterion of correctness of "null" hypothesis between real and expected heterozygosity.

The occurence of 00-gene GSTM1 genotype in patients with pulmonary tuberculosis depending on the type is shown in chart 3. We found significantly more frequent presence of a functional allele than its absence, in patients with newly diagnosed pulmonary tuberculosis (NDTB) by 4.75 times (p<0,001) and in those with poly-resistant pulmonary tuberculosis (PRTB) by 4 times (p<0,001), respectively. There was no substantial difference in frequency in patients with multi-drug resistant tuberculosis (MDR-TB) (p=0,056). It should be noted that among the carriers of non-functional allele in the experimental group there were more patients with NDTB than those with MDR-TB and PRTB by 2,92 (χ^2=18,57, p<0,001) and 1,58 (χ^2=5,39, p=0,02) times. At the same time there were more patients with PRTB and without mutated GSTM1 gene than those with MDR-TB: 32,0% vs 17,33% (χ^2=4,34, p=0,037), respectively. There were no significant differences between the occurrence of certain types of pulmonary tuberculosis (NDTB, MDR-TB, PRTB) among homozygous carriers of the gene GSTM1 of the deletion genotype (chart 3).

Chart 3. Occurrence of the gene GSTM1null genotype in patients with TB due to its type

Study groups		No 0-genotype, n=75 (%)	0-genotype, n=21 (%)	LOD [95% CI]	χ^2 p
Newly diagnosed tuberculosis, n=46 (%)		38 (82,61)	8 (17,39)	22,56 [7,67-66,3]	χ^2=39,13 p<0,001
Multidrug resistant tuberculosis, n=20 (%)		13 (75,0)	7 (35,0)	3,45 [0,94-12,6]	χ^2=3,60 p=0,056
Poly-resistant tuberculosis, n=30 (%)		24 (80,0)	6 (20,0)	16,0 [4,51-56,7]	χ^2=21,60 p<0,001
χ^2p	NDTB-MDR-TB	χ^2=18,57 p<0,001	χ^2<1,0 p>0,05	–	–
	NDTB-PRTB	χ^2=5,39 p=0,02	χ^2<1,0 p>0,05		
	MDR-TB-PRTB	χ^2=4,34 p=0,037	χ^2<1,0 p>0,05		
Control, n=50 (%)		32 (64,0)	18 (36,0)	3,16 [1,40-7,15]	χ^2=7,84 p=0,005

Note. LOD – logarithm of the odds ratio score; CI – confidence interval; p – differences in probability; NDTB – newly diagnosed tuberculosis; MDR-TB – multidrug resistant tuberculosis; PRTB poly-resistant pulmonary tuberculosis.

An analysis of heterozygosity of null polymorphism of the gene GSTM1 heterozygous gene GSTM1, taking into account diagnosed MBT resistance variation (chart 4), showed normal allelic distribution, which corresponded to the scale of population equilibrium by Hardy-Weinberg (p> 0,05). In quantitative terms, the dominant allele in the experimental group regardless of the type of tuberculosis is functional variant 1 (75,0-82,61% vs 17,39-35,0%). **Conclusions**. 1. Among the patients with pulmonary tuberculosis one out of five persons (21,87% of cases) was diagnosed with deletion mutation of GSTM1 gene; and the occurrence due to MBT resistance variation is: in NDTB – 17,39%, in MDR-TB – 35,0% and in PRTB – 20,0% respectively.

Chart 4. Analysis of heterozygosity of null polymorphism of the gene glutathione-S-transferase of class M1 (GSTM1) due to the MBT resistance variation

Groups	Genotypes, alleles, n (%)		P_D	P_1	H_O	H_E	F	χ^2	P
	DD	1_{allele}							
NDTB, n=46 (%)	8 (17,39)	38 (82,61)	0,39	0,61	0,43	0,48	0,09	1,32	>0,05
MDR-TB, n=20 (%)	7 (35,0)	13 (75,0)	0,53	0,48	0,35	0,50	0,30	2,36	>0,05
PRTB, n=30 (%)	6 (20,0)	24 (80,0)	0,37	0,63	0,33	0,46	0,28	2,23	>0,05
Total, n=96	21 (21,87)	75 (78,13)	0,41	0,59	0,38	0,48	0,20	2,33	>0,05

Notes: 1. – NDTB – newly diagnosed tuberculosis; MDR-TB – multidrug resistant tuberculosis; PRTB poly-resistant pulmonary tuberculosis. 2. P_1 – relative occurrence of I allele; P_D – relative occurrence of deletion allele D. 3. H_0 – real heterozygosity; H_E – expected heterozygosity; F –inbreeding factor. 4. χ^2p – criterion of the correctness of null hypothesis between real and expected heterozygosity.

2. According to the nature of allele distribution of GSTM1 gene the favorable functional 1 allele prevails (73,29%) in case of normal inbreeding in patients (F=0,20, p>0,05) and lack of heterozygosity in healthy individuals (F=0,28 p=0,033), which, in general forms a normal population distribution [OR=14,06, p=0,005].

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Анализ полиморфизма гена GSTM1 у больных туберкулезом в зависимости от варианта резистентности МБТ

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Аннотация. Проведенный анализ частоты аллелей и генотипов гена GSTM1 у больных туберкулезом легких с учетом варианта резистентности МБТ позволил установить, что за условий наличия туберкулезной инфекции легких делеционная мутация гена GSTM1 оказывается у каждого пятого (21,87% случаев), соответственно частота по варианту резистентности МБТ составляет: при ВДТБ - 17,39%, при МРТБ - 35,0% и ПРТБ - 20,0% соответственно. По характеру аллельного распределения гена GSTM1 преобладает благоприятный функциональный 1 аллель (73,29%) при нормальном инбридинге среди больных и дефиците гетерозиготности у здоровых, что в целом формирует нормальное популяционное распределение для европейской расы.

Ключевые слова: *туберкулез, делеционний полиморфизм, GSTM1, резистентность, МБТ.*