

## MOLECULAR GENETIC CHARACTERISTICS OF PATIENTS WITH MULTIPLE MYELOMA FROM UKRAINE

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Was conducted comparative analysis of frequencies of polymorphous genes variants, involved in biotransformation of cytostatic medicine of patients with multiple myeloma. We determined frequencies of deletion polymorphism of genes *GSTT1*, *GSTM1*, frequencies of genotypes according to polymorphous variants *A313G*, *C3435T* of genes *GSTP1*, *MDR1* in 137 patients with multiple myeloma, which did not differ in patients from different regions of Ukraine. Genetic similarity was determined in patients with MM from European populations and patients from Ukraine according to studied genes. Received results show that genotype *CC* according to polymorphism *C3435T* of

gene *MDRI* is predictor of development of MM with unfavorable process at the expense of formation in most cases of Bence Jones type. Perspective of further researches means studying the role of genetic factors in mechanism of formation the phenotype of reaction on treatment with evaluation of risk of development refractory forms of multiple myeloma.

Key words: multiple myeloma, genes, polymorphism

**Introduction.** Nowadays appeared new possibilities in treatment patients with multiple myeloma (MM) due to implementation medicine of immunological direction besides standard schemes of treatment. Though, information about success achieved while implementing thalidomide, VELCADE etc, demonstrated also that not always effective response on treatment is achieved using this medicine as well as that which are included into general schemes, (2,3,4).

Information from literature shows dependence of effective implementation of medicine against genes polymorphism, which code ferments, involved into metabolism of pharmacological drugs. Speed of remaking drugs or their remaking into active compounds depends on presence and activity of ferments in patients, which shows individual differences, which are genetically determined (7,8,16,17).

Genes polymorphism of drugs biotransformation has also ethnic and population peculiarities, which indicates necessity of considering results of previous genetic researches in determining distribution of polymorphous variants of genes and their associations with risk of development refractory forms MM (16). In Ukraine such researches have not been conducted.

**The goal of this work** became research of occurrence of polymorphous variants of genes, which are connected to drugs metabolism in patients with MM from different regions of Ukraine.

## Materials and research methods

In order to achieve this goal we selected possible genes-candidates, whose polymorphism influences according to literature sources (5-17) metabolism of cytostatic drugs, which are used for treatment MM. 137 patients from different regions of Ukraine were examined, who in 2007-2011 for the first time were diagnosed with MM. Standard schemes of treatment of patients with MM were implemented. Patients were examined by molecular genetic method on deletion polymorphism of genes *GSTT1*, *GSTM1*, *A313G* polymorphism of genes *GSTP1*, *C3435T* polymorphism of genes *MDR1*. In order to conduct a research permission of ethical commission was acquired. After studying information letter all patients agreed to participate in this research.

Among patients with MM there were 60 (43,79%) male and 77 (56,21%) female. Average age of patients was 56 (27-80) years. In hematologic departments for determining diagnosis of patients standard methods of diagnosis verifying were implemented on the basis of clinical presentation, data of hemogramm and biochemical tests, immunochemical, morphological research of bone marrow (myelogram), histological (trepanobiopsy) of bone marrow and additional instrumental research methods.

Clinical presentation of examined patients was characterized by development of anemic syndrome, pains in bones of different intensity, more than 30% patients had hemorrhagic syndrome. 67,3% patients had pallor of skin integument, 40,4% - intoxication syndrome, 36,5% patients had clinical manifestation against lost of body weight. Signs of affect of nervous system in a form of peripheral pain polyneuropathy was determined not often – in 13,5 %, hepatomegaly – in 9,6% patients.

Patients, involved into the research, were examined by molecular genetic method with usage polymerase chain reaction (PCR) and polymerase chain reaction with determination polymorphism of length of restriction fragments (PCR-PLRF) for determining distribution of polymorphous variants of genes *GSTT1*, *GSTM1*, *GSTP1*, *MDR1*.

Statistical analysis was conducted with usage criteria  $\chi^2$  and Irwin Fisher with the help of Statistica 7 program.

## Results of the research and their discussion

Majority of patients involved into research were from Northern (29,92%) and Central (34,30%) parts of Ukraine. Patients from Southern Ukraine - 21,16%, Western Ukraine -13,13%. 1,45% patients were from Eastern Ukraine.

Analysis of clinical data showed that average age of patients and gender did not have evident differences in different regions of Ukraine. Among patients from Central and Western Ukraine percentage of female was higher amounting to 61,22% and 66,66%. Clinical presentation of the beginning and process of disease did not have differences depending on the region of living.

In general 137 patients with MM from Ukraine were diagnosed with deletion polymorphism of gene *GSTT1* in 20,43% patients, and deletion polymorphism of gene *GSTM1* – in 50,36% patients. On Diagrams 1 and 2 are presented results of research of deletion polymorphism of genes *GSTT1*, *GSTM1 glutathione S-transferase (GSTs)* in patients with MM from different regions of Ukraine. As it is seen from Diagram 1, frequencies of deletion polymorphism of gene *GSTT1* in patients with MM from different regions of Ukraine did not have evident differences, though there was a tendency to rise in frequency *GSTT1* of deletion polymorphism in patients from Central Ukraine as compared with other regions of Northern and Southern Ukraine (Fig. 1).

Among examined 137 patients with MM there were 2 female from Eastern Ukraine, who were not diagnosed with deletion polymorphism *GSTT1*, and deletion polymorphism *GSTM1* was diagnosed in one female patient. That is why we could not estimate frequency of examined polymorphous variants of genes in patients from Eastern Ukraine. Frequency of deletion polymorphism *GSTM1* had tendency to reduction in patients from Northern Ukraine as compared with patients from Central and Southern Ukraine, but not evident (Fig. 2).

Received frequencies of deletion polymorphism of genes *GSTT1*, *GSTMI* did not differ evidently from their occurrence in patients with MM, who were representatives of round-eyed. According to the data given in the research of Schlthuisen C. And others (14) it is shown that patients with MM from Netherlands frequency *GSTT1*"-" genotype amounted to 27%, *GSTMI*"-" genotype – 50% (7). In Russian-American research (8) it was determined that frequency *GSTT1*"-" genotype amounted 22%, *GSTMI*"-" genotype – 50% in patients with MM.

Among 137 patients with MM from Ukraine we determined genotype AA of gene *GSTP1* in 37,23%, AG - in 54,72%, GG - y 8,03%. In patients with MM from Southern Ukraine there was a presence of tendency to rise of frequency GG genotype of gene *GSTP1* as compared with other regions of Ukraine (Fig. 3). 2 female patients from Eastern Ukraine were diagnosed with genotype AA and AG of gene *GSTP1*.

Dasgupta and other indicated the following frequencies of genotypes of gene *GSTP1* in 222 patients with MM of British origin: AA – 44%, AG- 39%, GG – 17% (7). 204 patients from Netherlands (14) frequencies of genotypes of gene *GSTP1* did not have evident difference and amounted to: AA – 37%, AG - 48%, GG – 15%. 143 patients with MM from France (9) had not evident rise of frequency of genotype AA – 51,74%, probably at the expense of reduction of frequency of genotypes AG and GG among these patients, respectively 37,06%, 11,18%. Thus, we have not determined evident differences in frequencies of genotypes according to polymorphism A313G of gene *GSTP1* in patients with MM from Ukraine as compared with similar patients from European populations.

In the research of Dasgubta R.K. and co-authors it was shown that polymorphous variants of gene *GSTP1* modulate results of treatment patients with MM and influence on index of patients' survival (7). Possible functional effect of researched by us polymorphous variants of gene *GSTP1* associate with influence of polymorphous

variants of gene on enzymatic activity of ferment-isomere and his detoxification capacity (9), which is involved into metabolism of cytostatic medicine. During preliminary planning of research we paid attention that it is worth analyzing polymorphous variants of gene *MDR1*, since in literature sources there is information about associations of some polymorphous variants of this gene with results of treatment patients with MM, and theory shows that depending on genotype there are different functional variants of P- glycoprotein, involved into metabolism of medicine (6,9,11,13).

In general group of 137 patients from Ukraine frequency of genotype *CC* amounts to 25,54%, genotype *CT*- 46,71%, *TT* - 27,73%. Received by us frequencies did not evidently differ from similar in 115 patients with MM from Italy (6) who were diagnosed with the following frequency of genes: *CC* -28,69%, *CT* -47,82%, *TT* - 23,47% and 147 patients from France (10), who had the following frequency of these genotypes: *CC*- 36,73%, *CT*- 38,77%, *TT*-24,48%. Examined by us patients with MM from 4 regions of Ukraine did not have evident differences in frequencies of genotypes according to polymorphism *C3435T* of gene *MDR1* (Fig. 4), though patients from Western Ukraine had tendency to reduction frequency *TT* of genotype of gene *MDR1*. 2 female patients from Eastern Ukraine were diagnosed with genotype *TT* of gene *MDR1*.

Majority of researches show that population distinctions influence index of disease. Thus, among representatives of European populations disease happens with frequency from 2,6 for 100 000 population in England to 3,3 for 100 000 population in Sweden, and in Ukraine – 1,6 for 100 000 population (1). The biggest incidence of disease was determined in Negros in the USA – 8,25 for 100 000 population, and the smallest in China – 1,0 for 100 000 population (4,5). Determined differences, in our opinion, are connected with genetic heterogeneity of genes, associated with obvious development of disease. But there were no convincing data concerning role of genetic component in development MM. At the same time, majority of mentioned works shows

that genetic component is important constituent of reaction on treatment and it is necessary to study for individualizing usage of existing schemes and protocols.

In 66 examined patients, treated at Kyiv Centre for Bone Marrow Transplantation type of myeloma was determined by immunofixation method. 74,24% of these patients had G type, 13,63% - Bence-Jones type, 10,6% - A type, 1,51% - D type. Received frequencies of separate types of MM in examined patients correspond with given by other authors. For patients with Bence-Jones type prognosis concerning process of disease and reaction to treatment is the most unfavorable as compared with A and G types (2).

Difference in the process of disease in different types of myeloma associated with expression of genes of multiple medical resistance and genes involved in metabolism of medicine. That is why we analyzed researched genes depending on type of myeloma in examined patients (Fig. 5). Type D was determined in one patient, that is why this result was not included while examining distribution of polymorphous variants of genes *GSTT1*, *GSTM1*, *GSTP1*, *MDR1* depending on type of myeloma.

We conducted comparison of prevalence of examined genotypes of genes *GSTT1*, *GSTM1*, *GSTP1*, *MDR1* in patients with different types of myeloma. As shown on Fig. 5, patients with different types of myeloma had differences in prevalence of genotypes, which first of all concerned gene *MDR1*. In Table 1 we show results of distribution of polymorphous variants of gene *MDR1* in patients with different types of MM.

Table 1. Distribution of frequency of genotypes according to polymorphism *C3435T* of gene *MDR1* in patients with different types of MM.

Gene	Genotype	Type A (n=7)		Type G (n=49)		Type B-J (n=9)		Type D (n=1)	
		n	n	n	%	n	%	n	%
<i>MDR1 (C3435T)</i>	<i>CC</i>	1	14,28	14	28,57	5	55,55	0	0
	<i>CT</i>	2	28,57	22	44,89	3	33,33	1	100
	<i>TT</i>	4	57,14	13	26,53	1	11,11	0	0

In patients with MM with Bence Jones type was obviously raised frequency of genotype *CC* of gene *MDR1* ( $\chi^2=3,88$ ,  $p=0,0488$ ) and there was a tendency to reduction of frequency of genotype *TT* of gene *MDR1* ( $\chi^2=2,86$ ,  $p=0,0907$ ) as compared with their frequencies in patients with MM with type A. With type G frequency of genotype *CC* of gene *MDR1* was higher than in patients with A type, but less than in patients with Bence Jones type – determined differences were not evident. Similar tendency was determined for *TT* genotype of gene *MDR1*: in patients with G type was reduction of frequency as compared with patients with type A, but this frequency was higher than in patients with Bence Jones type.

According to known data, people with *TT* genotype according to polymorphism *C3435* of gene *MDR1* are characterized with reduction of level P- glycoprotein in cellule and raise of level of cytostatic medicine in cellule, which nowadays are not connected with toxic effect (9,16,14), and is considered as favourable predictor in formation phenotype of response on treatment (6). Achieved results concerning association of some types of myeloma with polymorphous variants of gene *MDR1* indicate its evident participation in mechanisms of formation some types of MM. According to the results of statistic analysis, people with genotype *CC* of gene *MDR1* obviously more often will experience formation of Bence Jones type disease.

## Conclusions

Conducted work allowed us to determine frequency of deletion polymorphism of genes *GSTT1*, *GSTM1*, frequency of genotypes according to polymorphous variants *A313G*, *C3435T* of genes *GSTP1*, *MDR1* in patients with multiple myeloma, who did not differ from patients of different regions of Ukraine. Genetic similarity was determined in patients with MM from European populations and patients from Ukraine according to studied genes. Received results show that genotype *CC* according to polymorphism *C3435T* of gene *MDR1* in patients obviously more often determine MM with Bence Jones type. Perspective of further researches means studying the role of genetic factors in mechanism of formation the phenotype of reaction on treatment with evaluation the risk of development refractory forms of MM.



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