ROLE of MOLECULAR-GENETIC FACTORS FOR PROGNOSIS of CHEMOTHERAPY TOXICITY in PATIENTS with BREAST CANCER

Syvak L.A., Lyalkin S.A., Svergun N.M., Gubareva G.O., Maidanevich N.M., Klimanov M.Y., Askolskiy A.V., Kasap N.V.

National cancer institute, Kiev

Summary: Individual differences in activity of GSTP1 and MTHFR, mediated by gene polymorphism, may be associated with the risk of development of malignant diseases, their natural histories, chemotherapy resistance and toxicity. The analysis of genotype of genes of GSTP1 and MTHFR was performed in 130 patients with breast cancer. It was proved that toxicity of chemotherapy in breast cancer patients is genetically predisposed - a presence of mutant alleles genes of GSPT1 and MTHFR is the risk factor of development of gastrointestinal and cardiac toxicity.

Key words: breast cancer, chemotherapy, polymorphism of genes.

The frequency of side effects caused by chemotherapy (CT) in patients with malignant tumors (sometimes life-threatening) remains quite high. Individualized approach to the selection of chemotherapy for a particular patient, aimed on the improving of the efficacy and reducing of the toxicity remains a keystone. Prognostic factors of chemotherapy toxicity are not fully understood. The literature data suggest that individual differences in efficacy and toxicity of CT may be genetically determined [4-6]. Recently, more researches are devoted to the role of inherited individual metabolic potential.

Throughout the world the potential role of genetic polymorphisms of genes GSTs is widely investigated. They catalyze the reaction of glutathione with a variety of aliphatic, aromatic, and heterocyclic epoxy radicals of exogenous harmful substances. Subfamily π-GSTs includes one enzyme - glutathione-S-transferase P1

(GSTP1), which is involved in detoxification processes of wide variety of compounds, including mutagens and carcinogens of environment and is involved in regulating cell proliferation and apoptosis. Some cytotoxic agents such as anthracyclines, platinum agents, alkylating agents, steroids are also substrates of GSTP1. The gene encoding GSTP1 is polymorphic. Polymorphism of GSTP1 gene by one nucleotide in the 105 th codon (exon 5) is the result of nucleotide substitution of adenine (A) to guanine (G), resulting in amino acid substitution of isoleucine for valine (Ile → Val).

It is known that different genotypes are characterized by polymorphic activity [5]. According to different authors, individual differences in enzymatic activity of GSTP1, mediated gene polymorphism may be associated with the risk of cancer, its progression, the development of resistance to chemotherapy and toxicity [5-7].

Methylen tetra-hydrofolatreductase (MTHFR) is a key enzyme in the metabolism of folate and methionine and an important factor for the synthesis and methylation of DNA. The enzyme is also involved in the metabolism of cytotoxic drugs (methotrexate, 5-FU). Reduced activity of the enzyme leads to increased levels of homocysteine in the blood (hyperhomocysteinemia), activation of oncogenesis and is sensitive to factors that can damage DNA (as a consequence of lack of methionine). To date, there are about two dozen mutations in this gene that violate the function of the enzyme. The most studied is a mutation in which a nucleotide cytosine (C) at position 677 is replaced by thymidine (T), which leads to substitution of amino acid residue alanine to valine residue (position 223) in the folate binding site. In individuals with the presence of mutations note thermolability MTHFR enzyme and reduce its activity to about 35% on average (with genotype C / T) and 70% (with genotype T / T). Some researchers state that it is possible for genotyping MTHFR polymorphism S677T will highlight different pharmacogenetic effects of CT genotypes in different patients in the future will personalize cancer therapy [3,7].

Therefore, the aim of our study was to evaluate the molecular and genetic prognostic factors of chemotherapy toxicity in patients with breast cancer.

MATERIALS and METHODS

The study included 130 patients with malignant tumors of the breast (BC), aged 27 to 75 (mean age 49,8 ± 9,8) years.

To study the gene GSTP1 genotype and MTHFR, genomic DNA was isolated from peripheral blood by adsorption of nucleic acids on «silica» membrane through speakers "QIAamp DNA Blood Mini Kit" ("QIAGEN", USA), according to the recommendations of the manufacturer. Amplification of polymorphic regions of GSTP1 and MTHFR gene was performed by allele-specific polymerase chain reaction detection results in real time on the instrument 7300/7500 Real-Time PCR Systems ("Applied Biosystems", USA). Prior to reaction amplification of DNA concentration obtained was adjusted to 2-8 ng / ml. Measuring the concentration of DNA was performed by spectrophotometry on a spectrophotometer NanoDrop1000 («Thermo Scientific», USA). To investigate single nucleotide polymorphisms of genes we used TaqMan-MGB-probe type. Sequences of primers and TaqMan probes were selected using the program Primer Express ® Software v. 3.0 ("Applied Biosystems", USA) and synthesized by "Applied Biosystems" (USA).

BC patients receive from 4 to 6 cycles of chemotherapy using FAC regimen (Cyclophosphamide - 500 mg/m2 intravenously on day 1, doxorubicin - 50 mg/m2 intravenously on day 1, fluorouracil - 500 mg / m2 intravenously at 1 and 8 days of the cycle). Totally 732 cycles of chemotherapy were performed.

Toxicity was assessed before chemotherapy, 7 and 14 days after each cycle. All chemotherapy side effects were evaluated by CTC (Common Toxicity Criteria) NCI. Statistical analysis of the data was performed using the statistical package of applied programs «Statistica v.6». To determine of the most important clinical and laboratory

factors of toxicity prediction was performed the method of variation statistics using Student's criterion.

RESULTS and DISCUSSION

Analysis of the distribution of patients by stage (TNM) showed that most of them (97) had stage II (stage IIa (T2N0M0) - 42 women, stage IIb (T2N1M0) - 55 patients), stage IIIA (T3N1M0, T3N2M0, T4N1M0) – 19, stage IIIb (T4N1M0) - 8, IV stage - 6 patients.

Distant metastases were reported in 6 patients, including 2 patients with bone lesions, the other two had lung and mediastinal lymph node metastases and the rest 2 - liver, bones and lung lesions.

The morphology of tumor in 65 patients was adenocarcinoma. Lobular infiltrative ductal carcinoma was observed in 23 patients, solid carcinoma - in 36, infiltrative low differentiated cancer – in 8, 4 had mixed adeno-skirrous

Immunohistochemical study and determination of the degree of differentiation was performed in 117 patients, including 39 patients diagnosed with Grade III (G3), the majority (78 patients) had G2. Estrogen receptors (ER) and progesterone (PR) were positive in 97 patients. Thirty three patients were ER and PR negative. HER-2/neu positivity was found in 68 patients ("+" - at 52, "+ +" - at 9 and "+ + +" - 7 cases).

All 130 patients had several unfavorable prognostic factors. They are presented in table. 1.

Table 1 - Risk factors in the prognosis of breast cancer patients (n = 130).

Prognostic factors	Number of cases, (%)
negative estrogen and progesterone receptor	33 (25,4)
Positive lymph nodes	87 (66,9)
tumor size ≥ 2 cm	130 (100)
the degree of malignancy G 2-3	117 (90)
age of the patients up to 35 years	16 (12,3)
overexpression of HER2/neu	68 (52,3)

19 patients had concomitant pathology of the gastrointestinal tract (chronic cholecystitis, gastritis and pancreatitis).

In all 130 patients GSTP1 gene polymorphism and MTHFR were analyzed. In addition was analyzed the frequency distribution of polymorphic variants of GSTP1 gene in the group of healthy people (89 people). The study revealed: homozygous genotype for GSTP1 wild-type allele - Ile / Ile - in 5 BC patients (17.86%) and in 42 of control group (47.19%), GSTP1 genotype homozygous for mutant type allele - Val / Val - in 5 patients (17.86%) and 10 in the control group (11.24%), heterozygous genotype GSTP1 - Ile / Val - in 18 patients (64.28%) and 37 individuals in the control group (41.57%) (see fig. 1).

In the group of healthy people the frequency of mutant type allele (Val) was 0.32 and was comparable with the frequencies described previously for other members of the Caucasian (0,28-0,36), lower than in African Americans (0,42-0, 45), but higher than in Africans and Asians (0,14-0,27).

At the same time, in patients with type BC mutant allele frequency was 0.50, significantly higher than the corresponding figure in the group of healthy subjects. It should be noted that the frequency of allele Val-GSTP1 gene in patients with BC

young age (45 years) did not differ from the control group and the index was 0.35, whereas in patients aged over 45 years was equal to 0.55. This may indicate a connection between the type of inheritance of the mutant allele (Val) gene GSTP1 and risk of BC in older women (see fig. 2).

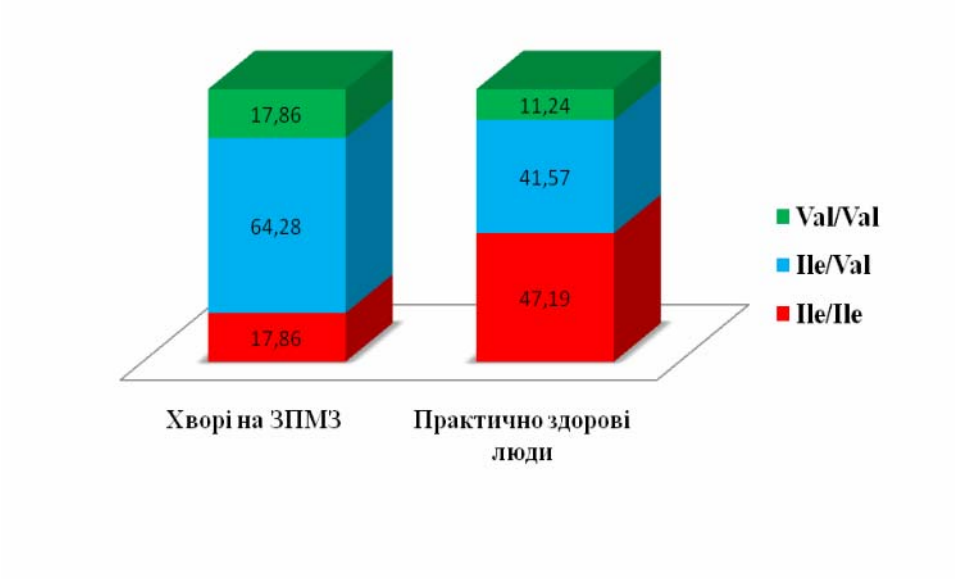

Fig. 1. Frequency distribution of polymorphic variants in the GSTP1 gene healthy individuals and in patients with malignant breast cancer (%). Хворі на ЗПМЗ – breast cancer patients; Практично здорові люди — healthy subjects

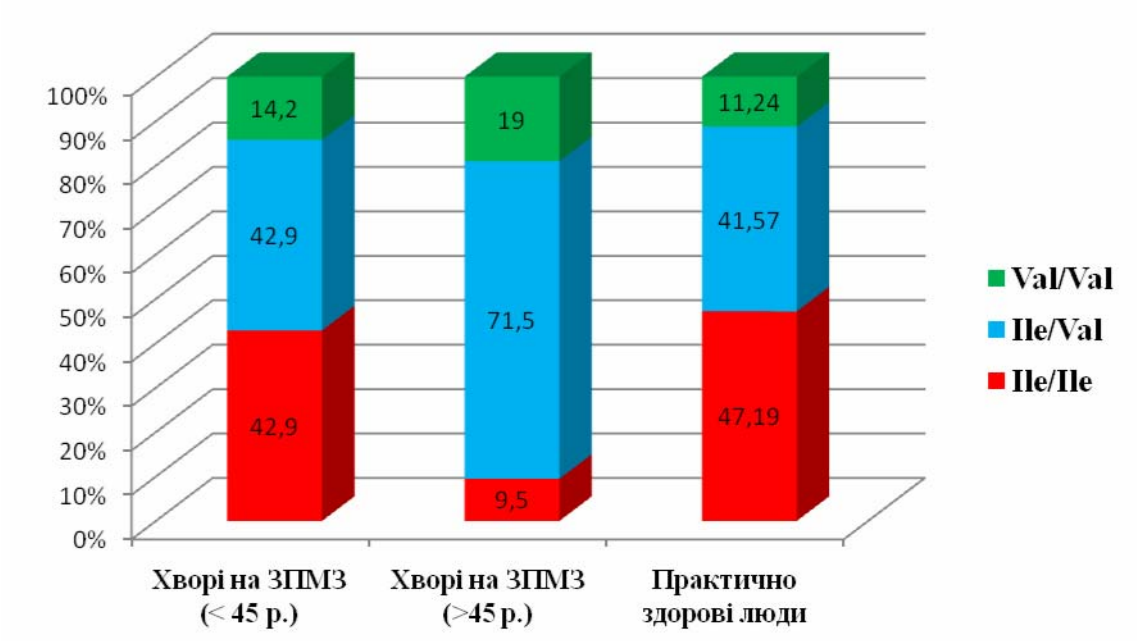

Fig. 2. Frequency distribution of polymorphic variants in the GSTP1 gene healthy individuals and in patients with malignant breast cancer of all ages (%).Хворі на ЗПМЗ – breast cancer patients; Практично здорові люди — healthy subjects

The study GSTP1 genotype homozygous for the wild-type allele - A / A - was found in 63 patients BC (46.3%) and 42 in the control group (47.19%), GSTP1 genotype homozygous for the mutant allele type - G / G - was found in 19 patients (14%) and 10 in the control group (11.24%), heterozygous genotype GSTP1 - A / G - was found in 54 patients (39.7%) and 37 in the control group (41.57%).

The distribution of genotypes of GSTP1 gene in the control group of healthy people is not statistically different from the estimated distribution law of Hardy-Weinberg equilibrium ($\chi 2 = 0,12$; $p = 0,73$). In the group of healthy frequency of mutant type allele G was 0.32 and was comparable to the frequencies described previously for other members of the Caucasian (0,28-0,36) and lower than this in the African (0,42-0,45) but higher than in Africans and Asians (0,14-0,27).

The distribution of genotypes of GSTP1 gene in patients with BC also responsible genetic law of Hardy-Weinberg equilibrium ($\chi 2 = 0,75$; $p = 0,39$). The frequency of the mutant allele G gene did not differ from the rate in the group of healthy people and was 0.338.

Overall GSTP1 gene inheritance model presented in Table 2 and 3.

Table 2

Multiplication model of GSTP1 gene inheritance in patients with malignant breast tumors and in healthy subjects.

Allele gene GSTP1	Patients with breast cancer	Virtually healthy people	χ^2	*P*	*OR*[2]	
	n = 136	n = 98			Consequence	95 % CI
Allele A	0,662	0,680	0,16	0,69	0,92	0,62 – 1,38
Allele G	0,338	0,320			1,08	0,73 – 1,62

Remarks:
1 χ2 - test, the number of degrees of freedom df = 1;
2 OR2 (odds ratio) - ratio of odds.

Table 3

General model GSTP1 gene inheritance in patients with malignant breast tumors and in healthy subjects

Gene genotypes GSTP1	Patients with breast cancer	Virtually healthy subjects	χ^2	*P*	*OR*[2]	
	n =136	n =98			Consequence	95 % CI
Genotypes A/A	0,463	0,472	0,37	0,83	0,97	0,57 – 1,65
Genotypes A/G	0,397	0,416			0,93	0,54 – 1,59
Genotypes G/G	0,14	0,112			1,28	0,57 – 2,90

Remarks:
1 χ2 - test, the number of degrees of freedom df = 2;
2 OR (odds ratio) - ratio of odds.

Analysis of the frequency distribution of polymorphic variants of GSTP1 gene showed no statistically significant differences between the group of patients with BC and a group of healthy people. The results indicate that there is no correlation between GSTP1 gene polymorphism and risk of BC.

In patients with BC also found association of polymorphic variants of the GSTP1 gene demographic and clinical characteristics of patients (age, disease stage, ER, PR and HER2 status).

At the same time, the distribution of polymorphic variants of MTHFR gene in patients with BC significantly different from the distribution in the group of healthy people. Thus, in the group of healthy men received a distribution of genotypes of the gene MTHFR: C / C - 61,5% (24 people), C / T - 28,2% (11 people), T / T - 10,3% (4 people), which was not statistically different from the estimated distribution law of Hardy-Weinberg equilibrium ($\chi 2 = 1,1$; $p = 0,29$). In the group of healthy frequency of mutant type allele T was 0.244 and was comparable to the frequencies described previously for other members of the Caucasion race.

In patients with BC genotype C / C was detected in 56 (41.2%) patients with genotype C / T - in 60 (44.1%) patients with genotype T / T - in 20 (14.7%) patients. The distribution of genotypes of MTHFR gene in patients with BC also responsible genetic law of Hardy-Weinberg equilibrium ($\chi 2 = 0,21$; $p = 0,65$). The frequency of the mutant gene allele T was 0.368, which is significantly higher than the corresponding figure in the group of healthy subjects.

Multiplication and dominant models of inheritance of MTHFR gene are presented in table. 4 and 4.

Table 4

Multiplication model MTHFR gene inheritance in patients with malignant breast tumor and healthy people

Allele gene MTHFR	Patients with breast cancer	Virtually healthy people	χ^2	*P*	*OR*[2]	
	n = 136	n = 39			Consequence	95 % CI
Allele C	0,632	0,756	4,16	0,04	0,55	0,31 – 0,98
Allele T	0,368	0,244			1,81	1,02 – 3,20

Remarks:
1 χ2 - test, the number of degrees of freedom df = 1;
2 OR2 (odds ratio) - ratio of odds.

Table 5

Dominant model in the MTHFR gene inheritance in patients with malignant breast tumor and healthy people

Gene genotypes MTHFR	Patients with breast cancer	Virtually healthy people	χ^2	*P*	*OR*[2]	
	n=136	n=39			Consequence	95% CI
Genotypes C/C	0,412	0,615	5,06	0,02	0,44	0,21 – 0,91
Genotypes C/T+T/T	0,588	0,385			2,29	1,10 – 4,74

Remarks:
1 χ2 - test, the number of degrees of freedom df = 1;
2 OR2 (odds ratio) - ratio of odds.

As shown in table 3 and 4 BC risk is twice as high in women with genotype C / T or T / T than in women with genotype C / C. Accordingly, the risk of BC women - a

wild allele carriers of the gene (genotype C / C) 56% lower than in homo-and heterozygous carriers of the mutant allele of the gene.

It is worth noting that the risk of BC was significantly higher in older women and in women of menopause. BC risk in women aged over 50 years was 3 times higher in genotype C / T or T / T (OR = 2,95; 95% CI = 1,32-6,59, $\chi 2$ = 7,22; p = 0.007) than in those with genotype C / C, and in women during menopause - almost 3.5 times in genotype C / T or T / T (OR = 3,43; 95% CI = 1,39 - 8,47; p = 0,007), than in genotype C / C.

In order to compare the cytogenetic data from clinical and laboratory manifestations of chemotherapy toxicity we have analyzed the results of treatment of 39 patients with BC who received 4-6 cycles of chemotherapy. To determine the individual (genetically determined, innate) susceptibility to chemotherapy toxicity we have studied GSTP1 gene polymorphism and MTHFR in patients with BC as potential individual genetic risk factors for chemotherapy toxicity. Comparison of genetic data with parameters chemotherapy toxicity revealed the association of gene polymorphisms of MTHFR and GSTP1 A313G S677T in toxicity of FAC chemotherapy in patients with BC.

Thus, patients with BC - carriers of mutant allele of the gene GSTP1 (allele G) had a significantly higher risk of gastrointestinal toxicity (OR = 3,13; 95% CI = 1,22-7,99; p = 0 , 02) compared with patients with BC - only native wild allele of the gene (allele A). Among patients with genotype G / G 75% had gastrointestinal complications of chemotherapy, whereas among patients with genotype A / G - 50% with genotype A / A - only 26.7%. The risk of gastrointestinal toxicity was significantly higher in the succession homozygous mutant allele of the gene GSTP1 (genotype G / G). Thus, in patients with homozygous BC - G allele carriers of the gene GSTP1 risk of gastrointestinal toxicity is almost 5 times higher (OR = 4,75;

95%, CI = 0,82-27,5; p = 0,02), than in patients with homozygous wild genotype gene GSTP1 (genotype A / A). The data presented in fig. 3.

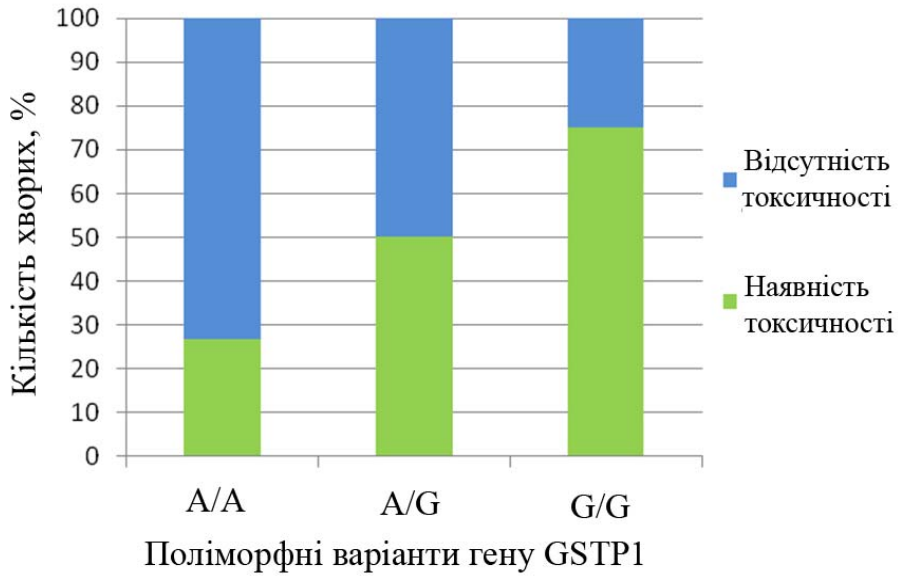

Fig. 3 - Gastrointestinal toxicity of chemotherapy in patients malignant breast tumor based on genotype gene GSTP1.

Remarks: 1. allele A: OR = 0,32; 95% CI = 0,19-0,82; 2. allele G: OR = 3,13; 95% CI = 1,22-7,99; p = 0,02; 3. genotype G / G: OR = 4,75; 95% CI = 0,82-27,5; p = 0,02. Кількість хворих – number of patients; Відсутність токсичності – no toxicity; Наявність токсичності – toxicity; Поліморфні варіанти гену GSTP1 – gene GSTP1 polymorphism

In patients with breast cancer homozygous mutant genotype of the gene GSTP1 (genotype G / G) also observed an increased frequency of complications of chemotherapy of the cardiovascular system compared with homozygous carriers of the wild allele of the gene (genotype A / A) and patients with heterozygous genotype (genotype A / G). For example, among patients with genotype G / G in 50% of

patients observed cardiovascular complications of chemotherapy of varying severity, whereas among patients with genotype A / G or A / A - only 25% (see fig. 4).

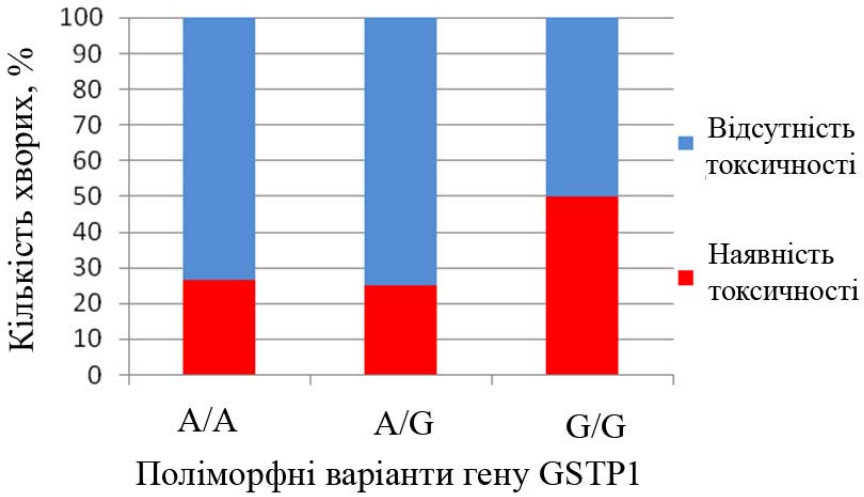

Fig. 4. Cardiovascular complications of chemotherapy in patients with malignant breast tumors depending on the genotype gene GSTP1. Кількість хворих – number of patients; Відсутність токсичності – no toxicity; Наявність токсичності – toxicity; Поліморфні варіанти гену GSTP1 – gene GSTP1 polymorphism

The incidence of gastrointestinal toxicity was also slightly higher in patients - carriers of the mutant allele of the gene MTHFR (allele T). Thus, 83.3% of patients with BC with genotype T / T was observed complication of chemotherapy on the gastrointestinal tract of varying severity, whereas among patients with genotype C / T - in 68.4%, and C / C - in 57 1% (see fig. 5).

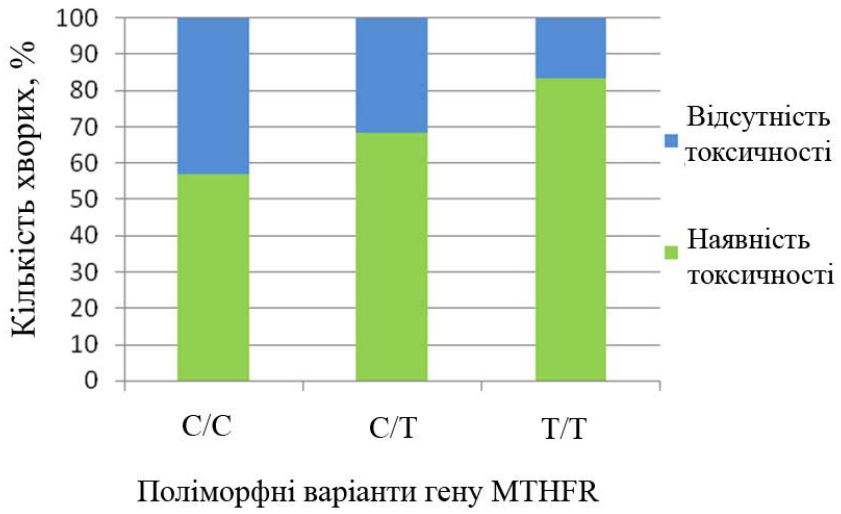

Fig. 5. Gastrointestinal toxicity of chemotherapy in patients with malignant breast tumors depending on the genotype gene MTHFR. Кількість хворих – number of patients; Відсутність токсичності – no toxicity; Наявність токсичності – toxicity

1. The risk of breast cancer is 3.5 times higher in women during menopause, which are carriers of the mutant allele of the gene than in female carriers of a wild-type allele of the gene.

2. The toxicity of chemotherapy in patients with BC is genetically determined (the presence of mutant alleles of GSTP1 and MTHFR genes are risk factors for gastrointestinal and cardiovascular toxicity).

3. |In patients with BC- carriers of at least one mutant allele G gene GSTP1 risk of gastrointestinal toxicity of chemotherapy is 3 times higher than in patients with homozygous wild genotype gene GSTP1 (genotype A / A).

4. For homozygous carriers of the T allele of the gene MTHFR (genotype T / T) the risk of gastrointestinal toxicity of chemotherapy almost 5 times higher than in patients with homozygous wild genotype gene GSTP1 (genotype A / A).

REFERENCES

1. Shapiro C.L., Recht A. Side effects adjuvant therapy of breast cancer glands / / N. Engl. J. Med. - 2001. - Vol. 334. - P. 1997-2008.

2. Kazyulyn A.N., Kozlov S.V., Queen I.A., Kucheryaviy Y.A. The frequency of lesions of the gastrointestinal tract when conducting cancer chemotherapy breast cancer glands / / Journal of Russian gastroenterology, hepatolohyy, Coloproctology. - 2007. - № 5. - P. 173-176.

3. Herrstedt1 J., Roila F. et. al. Guideline update for MASCC and ESMO in the prevention of chemotherapy and radiotherapy-induced nausea and vomiting: results of the Perugia consensus conference / / Ann. Oncol. - 2010. - Vol. 21 (5). - R. 232-243.

4. Hayes J.D. Glutathione transferases / J.D. Hayes, J.U. Flanagan, I.R. Jowsey / / Annu Rev Pharmacol Toxicol. - 2005. - № 45. - R. 51-88.

5. McIlwain C.C. Glutathione S-transferase polymorphisms: cancer incidence and therapy / CC McIlwain, D.M. Townsend, K.D. / / Oncogene. - 2006. - № 25. - P.1639-1648.

6. Fernandez-Peralta A.M., Daimiel L., Nejda N. et al. Association of polymorphisms MTHFR C677T and A1298C with risk of colorectal cancer, genetic and epigenetic characteristic of tumors, and response to chemotherapy / / Int. J. Colorectal Dis. - 2010. - Vol. 25 (2). - R. 141-51.

7. Toffoli G., Russo A., Innocenti F. et al. Effect of methylenetetrahydrofolate reductase 677C ~> T polymorphism on toxicity and homocysteine plasma level after chronic methotrexate treatment of ovarian cancer patients / / Int. J. Cancer. - 2003. - Vol. 103 (3). - R. 294-299.