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Cytogenetic Effects of Cytostatics and Their Relationship With p53 Gene Polymorphism

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ABSTRACT
The karyological parameters of buccal epithelial cells were studied as markers of the effects of chemotherapy drugs (doxorubicin with cyclophosphamide (DC) and paclitaxel) in 68 patients with diagnosed breast cancer taking into account p53 gene polymorphism (rs17884159). It was shown that paclitaxel has a stronger mutagenic effect compared with the regimen of DC: the share of cells with micronuclei and protrusions and binuclear cells increases. Evaluation of the karyological parameters of buccal epithelial cells and the significance of the minor p53 gene T allele (26 + 4486C> T) for tumor development in patients with breast cancer shows that chemotherapy is accompanied by integral processes that enhance nuclear destruction processes and cause the genome instability. It is shown that the indicators of karyological analysis can be biomarkers of the mutagenic effect of therapeutic treatment using cytostatics.

Keywords: *karyological parameters, chemotherapy, cytostatics, p53 gene polymorphism, breast cancer*

1. INTRODUCTION

Despite possibilities of the early diagnosis and treatment of cancer, the number of patients is growing. Every year at least 12 million new cases of tumors are registered in the world. It is necessary to treat more than 80 tumors using chemotherapy drugs in independent or adjuvant modes. The search for optimization mechanisms for chemotherapeutic treatment, the best modes of its use and the most effective combinations is ongoing. However, it is difficult to predict sensitivity to chemotherapy. The main group of antitumor drugs are cytostatics, which have a strong mutagenic and carcinogenic effect, disrupt the tumor development and mechanisms of cell division, thereby initiating the development of new foci of the pathology. Given the focus of chemotherapy on the destruction of the genome and / or the mechanism of tumor cells division, studies are being conducted on the effectiveness of new generation cytostatics both in a mono mode and in a combination with other drugs [4].

To identify destructive changes in the nuclei of cells, a karyological analysis of buccal epithelial cells is used. The state of epithelial cells is an objective reflection of intensity of the mechanisms of destabilization; it is advisable to take into account changes in the differentiation of the epithelium as rapid tests for assessing the general state of health, severity of diseases, determining the biological age of a person [3], genetic risk groups in environmental pollution [5]. The functional activity of buccal epithelial cells is determined by their degree of maturity; differentiation, proliferation, as well as parameters that determine the function of mature cells are regulated by factors of local and central origin. Some karyological parameters, such as micronuclei and protrusions, are markers of the biological effect. They reflect carcinogenesis processes that disrupt the structure of the genetic material of cells and activate oncogenes [13–15].

The aim of this work is to study cytogenetic parameters of the micronuclear test as markers of the effects of chemotherapy drugs in patients with breast cancer taking into account the p53 gene polymorphism (rs17884159).

2. METHODS AND MATERIALS

The study involved 68 patients suffered from breast cancer and treated in the Republican Oncology Center (Grozny, Chechen Republic). The participation was voluntary. The control group was represented by 65 healthy women who had no significant pathologies.

A molecular genetic study of the polymorphism 26 + 4486C> T of the p53 gene (rs17884159) was carried out by PCR-RV using a CFX-96 amplifier (BioRad, USA) and a SNP-Screen reagent kit (Synthol CJSC, Moscow).

A karyological analysis of buccal epithelial cells (material sampling, preparation and analysis of drugs) was carried out in accordance with the methodological recommendations "Assessment of the cytological and

cytogenetic status of mucous membranes of the nasal cavity and mouth in humans" [10, 12]. In accordance with the classification by L.P. Sycheva (2007), the following indicators were taken into account: the frequency of cytogenetic disorders: the share of cells with micronuclei and protrusions; the frequency of cells with proliferation: the share of cells with two or more nuclei, dual nuclei; apoptotic cell frequencies: the share of cells with chromatin condensation, karyorexis, karyopiches, nucleus vacuolization, karyolysis. For each respondent, two smears of buccal cells of the oral cavity were prepared and an analysis of at least 1000 cells was carried out.

Statistical processing of the results was carried out in Statistica 6.0.

3. RESULTS

We studied the cytogenetic status of patients with breast cancer treated with cytostatics – doxorubicin with cyclophosphamide and / or paclitaxel. The results of the study are presented in tables 1–5.

Cytogenetic indicators of the karyological analysis show a significant difference in the control and experimental groups. In patients with breast cancer, the number of epithelial cells with micronuclei (2.0 ± 0.37) is 8.3 times higher than that in healthy women (0.23 ± 0.04) ($p < 0.001$), which is due to the mutagenic effects of chemotherapeutic drugs Table 1).

Table 1 The cytogenetic status of patients with breast cancer

№	Indicator	Share of cells (M±m, ‰)	
		BC (N=68)	**Control (N=65)**
Cytogenetic indicators, ‰			
1	Micronuclei	2.0±0.37	0.23±0.04
	protrusions	4.8±1.14	0.28±0.02
Indicators of nuclear destruction (apoptosis / necrosis), ‰			
	Vacuolization	184.59±30.75	17.3±2.11
	Karyolysis	33.47±7.02	42.51±2.10
	Karyorexis	3.06±0.74	7.26±0.54
	Karyopiches	9.12±2.77	26.64±0.72
Proliferation rates, ‰			
	Binuclear cells	13.29±1.80	2.11±0.08
	Dual nuclei	3.59±0.47	1.40 ± 0.06

Protrusions are a significant indicator of the karyological analysis which reflects the mutagenic effect of various factors on the genome structure. They are membrane DNA-containing formations connected to the main core by a jumper [7, 12]. The results identified an increase in the share of cells with protrusions (4.8 ± 1.14) in patients with breast cancer by 17.1 times compared with the average values of this indicator in healthy women (0.28 ± 0.02) ($p < 0.001$) [10]. Given that protrusions are fragments of chromosomes and occur during DNA breaks, or may be separate chromosomes that lag behind in mitotic disorders, i.e. are a consequence of the carcinogenic and mutagenic effects of various factors [10], it can be assumed that a significant number of protrusions is the result of endofactors and chemotherapy cytostatics.

The group of indicators characterizing proliferative processes in cell populations is binuclear and dual cells which develop in the process of enhancing cell proliferation, aimed at the formation of cells that replace damaged cells. Binuclear cells develop mainly as a result of incomplete acytokinetic mitosis [1]. This indicator, as well as cytogenetic abnormalities, showed a significant 12.5-fold increase in the number of cells with two nuclei (13.3 ± 1.80) compared with the average in healthy women (1.06 ± 0.07) [10].

Indicators of proliferative activity are cells containing two or more nuclei that appear as a result of damage to the fission spindle [10]. The share of cells with this indicator is 3.60 ± 0.52. It is higher than in the control group by 1.7 times (2.11 ± 0.08) ($p > 0.05$) (Table 1). Disorders of cytotomy causing the dual nuclei can be indicators of the mutagenic nature of drugs.

The apoptosis is genetically controlled, therefore the changes in apoptosis intensity are attributed to genotoxic events (Byakhova 2008). The apoptosis removes functionally defective cells, thereby ensuring physiological tissue

regeneration [8, 12]. In patients with cervical cancer, there was an increase in cells with nuclear destruction, in particular, an increase in the share of cells with vacuolization of the nucleus to 25.0 ± 1.2 ‰, while in the control group these indicators were not revealed [6]. As can be seen Table 1, in the experimental group, there was a significant increase in the share of cells with vacuolization (184.6 ± 30.75), whereas such cells are absent in healthy women [6, 11].

Other indicators of nuclear destruction (karyolysis, kraiopyknosis, karyorexis) show a decrease in the frequency of apoptotic cells when treating with drugs. A low frequency of cells with destructive changes was revealed: karyolysis (33.47 ± 7.02), karyopicnosis (3.06 ± 0.74) and karyorexis (9.12 ± 2.77), the share of cells with these indicators was 1.5–2.2 times lower than in healthy women (42.51 ± 2.10; 7.26 ± 0.54 and 26.64 ± 0.72, respectively).

A decrease in the ability to apoptosis is characteristic of cells with a violated DNA structure which can cause the development of malignant tumors [2]. In addition, there is a decrease in apoptosis in individuals undergoing chemotherapy with a significant increase in the share of cells with cytogenetic and proliferative indicators.

A comparative analysis of the frequency of karyological parameters and the type of drugs used showed that the share of cells with nuclear disorders in patients using different chemotherapy treatments (docetaxel in combination with cyclophosphamide (DC) and paclitaxel), showed different levels of their genotoxic or a mutagenic effect on the nucleus structure (Table 2). The mutagenic effect of the DC complex is manifested as an increase in the share of cells with vacuolization and karyopicosis, however, the frequency of the remaining indicators is lower than with paclitaxel. Differences do not reach a statistical significance (p> 0.061).

Paclitaxel has a stronger mutagenic effect compared to the "DC" mode: the share of cells with micronuclei is 1.6 times, and with protrusions 3.4 times higher (2.1 ± 0.49 and 6.2 ± 1.56, respectively). It also increases the frequency of binuclear cells (14.45 ± 2.43). When treating with paclitaxel, an increase in the share of cells with karyolysis was observed. Apoptosis reduces the frequency of cells with cytogenetic disorders.

Table 2 Frequency of cells with karyological parameters in patients using different types of chemotherapeutic drugs

№	Indicator	Share of cells (M±m, ‰)	
		docetaxel + cyclophosphamide, **n=29**	paclitaxel, **n=39**
Cytogenetic indicators, ‰			
1	Micronuclei	1.8±0.73	2.1±0.49
2	protrusions	1.8±0.92	6.2±1.56
Indicators of nuclear destruction (apoptosis / necrosis), ‰			
3	Vacuolization	253.8±65.89	167.0±32.99
4	Karyolysis	26.2±6.44	43.9±7.75
5	Karyorexis	3.6±1.29	3.00±0.99
6	Karyopiches	11.8±5.55	8.5±3.56
Proliferation rates, ‰			
7	Binuclear cells	10.6±2.99	14.45±2.43
8	Dual nuclei	3.4±0.40	3.3±0.63

Thus, the study of violations of the nucleus structure, the mechanisms of apoptosis and cell division, indicate the effects of both endogenous factors and chemotherapeutic drugs. Moreover, a more significant mutagenic effect is observed when treating patients paclitaxel.

The p53 gene is a key gene that ensures stability of the genome and violation of its structure. It affects the cells. The gene is localized on chromosome 17 (17p13.1). There are 19 polymorphisms of this gene; three of them are located in the 3rd and 6th introns and the 4th exon reflecting the influence of carcinogens. We analyzed rs17884159 (NM_001126112.2: c.-26 + 4486C> T) whose frequency is 0–0,2 according to the database https://www.ncbi.nlm.nih.gov.

The results of genotyping are presented in Table 3.

Table 3 Frequency of distribution of genotypes and alleles of the polymorphic variant of p53 gene

Gene p53 26+4486C>T	Genotypes						Allele	
	C/C		C/C		T/T		C	T
	abs.	abs.	abs.	%	abs.	%	%	%
BC	32	32	32	47.05	4	5.8	66.67	33.33
Control	41	41	22	40.27	2	2.7	80.00	20.0

These studies showed that a third of genotypes (33.33 %) possess a minor allele of this gene. Accordingly, the frequency of the wild-type allele is 66.67 %. In the control group, there were 20.0 % of mutant alleles and 80.00 % of the wild type alleles.

A study of the distribution of genotypes showed that the frequency of the homozygous genotype for the wild-type allele reaches 56.94 %. A homozygous genotype with a minor allele was observed only in two cases and amounted to 2.7 %. The frequency of the heterozygous genotype reached 40.27 % (Table 3)

In the group of patients with breast cancer, the genotypes were distributed as follows: homozygotes for the dominant C allele and heterozygotes were identified with the same frequency of 47.05 %, the homozygous genotype for the minor T allele was 5.8 %.

A comparative analysis of the genotype frequency revealed an increased frequency of heterozygous carriers (1.2 times) and the homozygous genotype for the minor allele (2.15 times) compared with the control group (Table 4). However, the differences are not statistically significant.

Table 4 Analysis of the conjugation of polymorphism of the p53 rs17884159 gene with the development of breast cancer

Genotypes	BC	Control	p	OR	95%CI
CC	47.05	56.94	0.276	0.52	0.23–1.19
CT	47.05	40.27	0.276	1.74	0.86–3.51
TT	5.8	2.7	0.601	2.03	0.28–14.75

An analysis of the conjugation of the polymorphic variant of the p53 rs17884159 gene with malignant transformation of breast cells showed the significance of the minor T allele for the tumor development. However, the differences did not reach the statistical significance (Table 4) due to the small sample size.

In order to determine the role of the polymorphic locus of the p53 rs17884159 gene in nuclear degradation processes, an associative analysis of the conjugation of indicators of the karyological analysis of epithelial cells with the genotypes of the polymorphic variant of the p53 gene was performed (Table 5). In homozygotes for the wild-type T T / T allele of the p53 gene, the average frequency of cells with vacuolated nuclei was 215.00 ± 44.73. Chromatin condensation and lysed nuclei were detected with a uniform frequency of 17.87 ± 4.97 and 18.12 ± 4.04, respectively. Cells with more than one nucleus were observed with a frequency of 13.37 ± 3.14, respectively. The frequency of protrusions, incisions, and rexis in wild-type homozygotes varied from 3.00 to 3.5. The smallest values were observed in the frequency of cells with micronuclei: 1.75 ± 0.49. In heterozygous carriers of the mutant C/T allele, an abnormally high degree of vacuolization of 173.25 ± 45.01 was observed. The frequency of cells with lysis of the nuclei was 40.75 ± 10.36, the share of cells with the condensed nucleus was larger: 43.25 ± 10.68. Cells with two or more nuclei and cells with pyknotic nuclei were equally distributed: 13.12 ± 2.41 and 12.50 ± 5.25, respectively. Cells with protrusions showed the greatest value (6.12 ± 2.16). The frequency of rexis and incisions varied from 3.37 ± 1.45 to 3.50 ± 0.68. The most rare disorders of the nucleus in heterozygotes and wild-type homozygotes were micronuclei (2.25 ± 0.64).

The study showed that the share of cells with nuclear destruction is much larger than that with cytogenetic disorders (Table 5).

A comparative analysis of the level of nuclear damage depending on the genotypes of the respondents showed significant differences in heterozygotes and homozygotes for the following indicators: micronuclei, protrusion, pycnosis condensation and lysis. The frequency of these indicators is significantly higher in heterozygous carriers, compared with homozygotes for the wild-type allele ($p<0.05$). By the frequency of violations of the type of karyorexis and incision, differences between carriers of different genotypes were not observed. The frequency of vacuolization in heterozygotes was lower compared with homozygotes: 173.25 ± 45.01 versus 215.00 ± 44.73. The analysis shows that the polymorphic variant (rs17884159) of the P53 gene affects genomic instability, enhancing nuclear destruction in cells.

Table 5 The distribution of cytogenetic parameters in accordance with the genotypes of the polymorphic variant of the rs17884159 of the p53 gene

Cytogenetic indicators	Genotypes p53 (rs17884159)		
	C/C	C/T	T/T
Micronuclei	1.5±0.71	2.25±0.64	1.75±0.49
protrusions	3.5±0.71	6.12±2.16	3.50±1.05
Binuclear cells	13±1.41	13.12±2.41	13.37±3.14
notches	5.5±2.12	3.50±0.68	3.25±0.61
Karyorexis	0.5±0.71	3.37±1.45	3.00±0.65
Karyopiches	4±1.41	12.50±5.25	6.50±2.53
condensation	56.5±6.36	43.25±5.68	17.87±4.97
Vacuolization	29.5±3.54	173.25±45.01	215.0±44.73
Karyolysis	105±9.89	40.75±6.36	18.12±4.04

4. CONCLUSION

Evaluation of karyological parameters of buccal epithelial cells of the oral cavity and p53 gene polymorphism (26 + 4486C> T) shows that treatment of oncopathology is accompanied by integral processes causing the genomic instability. A significant excess in the frequency of cytogenetically altered cells is a reflection of the genotoxic effects of drugs.

Thus, indicators of a karyological analysis may be biomarkers of the effect of therapeutic treatment with cytostatics in breast cancer.

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