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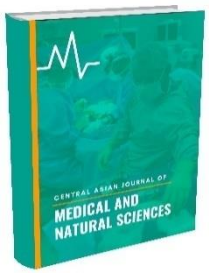

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The role of the genetic polymorphism of the gene - oncosupressor TP53 rs 17884159 in women with cervical intraepithelial neoplasia

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ABSTRACT: Analysis of the frequency of distribution of alleles and genotypes of polymorphism of the TP53 rs 17884159 gene depending on the severity of the disease at the time of the study showed negative associations, consisting in a significant increase in the prevalence of carriage of the heterozygous C/T genotype associated with the severity of CIN: carriage of the heterozygous C/T genotype increases the risk of pathology in LSIL 1.947 times; HSIL CIN II - 3.957 times and HSIL CIN III - 2.250 times. In our studies, the carriage of unfavorable C/T and T/T genotypes is associated with an increase in the severity of CIN.

Key words: cervical intraepithelial neoplasia (CIN), human papillomavirus (HPV) viruses, oncosupressor TP53 rs 17884159, heterozygous C/T genotype, C/T and T/T genotypes.

Relevance of the work. Cervical intraepithelial neoplasia (CIN) - a disease characterized by the neoplastic transformation of the cervical epithelium with a possible outcome in cervical cancer [2,3,6]. Human papillomavirus (HPV) viruses of high oncogenic risk types 16 and 18 are recognized as the etiological factor of CIN [5,7,14].

In the implementation of the neoplastic transformation of the cervical epithelium, the leading role is assigned to the integration of the human papillomavirus into the host cell genome. Uncontrolled HPV multiplication is accompanied by the release of oncogenic proteins of early (E2, E4, E5, E6, E7) and late (L1, L2) HPV genes. Overexpression of the viral oncogenes E6 and E7 initiates the activation of pathological proliferation, a decrease in apoptosis, an increase in cell division, neoangiogenesis, and invasion, i.e. oncological transformation. At the same time, there is a violation of genomic integrity [8,14,17,19,20].

The molecular basis of multifactorial diseases is numerous genetic disorders, including chromosomal aberrations, mutations, and epigenetic changes in key genes that control cell division and DNA repair, which include tumor suppressor genes [4,6].

One of the most well-studied suppressor genes is the TP53 gene (rs 17884159), which regulates the processes of cell cycle control, apoptosis, DNA repair, and angiogenesis [1,3].

The TP53 gene and its product p53 play a key role in protection against cancer by maintaining genome stability and genetic homogeneity of cells in the whole organism. Its activity is manifested in response to a deviation from the norm, with the normal differentiation of the cells does not need the protein p53 [2,4,16]. In the aspect of the pathogenesis of the development of pathology, it is important that damage to the function of the TP53 gene (mutations), its cooperation with viral and cellular oncogenes lead to cell transformation. [10,21,22].

The association of the expression of mutant p53 with oncological pathology of the female reproductive sphere was established [9,11,12,13,15,18].

The study of the features of changes in the individual genetic variability of the TP53 gene will make a significant contribution to the study of genetically determined mechanisms of CIN development.

This work aims to investigate the associations of allelic and genotypic variants of TP53 gene polymorphism (rs 17884159) in cervical intraepithelial neoplasia and their relationship with the severity of the clinical course of the disease.

Materials and methods. We examined 226 patients undergoing outpatient treatment at the Women's Health Center of the Tashkent Medical Academy for cervical intraepithelial neoplasia (CIN). All surveyed were born and permanently resided in Tashkent and were of childbearing age in the range from 18 to 45 years (the average age was 36.9±1.1 years), belonged to the Uzbek nationality. The diagnosis of CIN was established based on clinical data and materials from colposcopic and cytological studies. The control group consisted of 165 healthy women matched with the study group in terms of age and ethnicity.

Isolation of genomic DNA from whole blood was performed using the Ampli Prime Ribot-prep reagent kit (Next Bio LLC, Russia). Detection of DNA samples for rs 17884159 of the TP53 gene was carried out by allele-specific PCR on an Applied Biosystems 2720 thermocycler, using sets of NPF Litekh, according to the manufacturer's instructions.

Results and Discussions. Statistical analysis of the differences in the distribution of alleles and genotypes of the TP53 rs 17884159 gene between the group of patients with cervical intraepithelial neoplasia and the control group of women without pathology of the reproductive system made it possible to establish that "cases" and "control" are in Hardy - Weinberg equilibrium, which allows data analysis and comparison research results.

The analysis of allelic variants combinations TP53 gene polymorphism rs 17884159 showed that in a cohort of patients with CIN observed statistically significant reductions in the frequency of the T allele, which amounted to 11.95%, compared with 5.15% in healthy women (control group) (χ^2 = 10,680; P≤ 0.002; OR = 2.480; DI 95% - 1.420 - 4.395). Thus, carriage of the T allele increases the risk of developing CIN by more than 2.289 times. At the same time, as shown by the research results, allele C has a protective effect, reducing the risk of developing CIN by 0.400 times (χ^2 = 10.680; P≤ 0.002; OR = 0.400; DI 95% - 0.228 = 0.704).

Analysis of the frequency distribution of the genotypes of the p53 rs17884159 gene polymorphism demonstrated negative associations consisting of an increased frequency of the

heterozygous C/T gene variant by 2.06 times compared with the control group and the detection of a homozygous T/T genotype. Thus, the frequency of carriage of the heterozygous C/T genotype in patients with cervical intraepithelial neoplasia is 21.24% versus 10.30% in the control group ($\chi^2 = 8.229$; $P \leq 0.005$; OR = 2.250; DI 95% 1.238 - 4.089) and genotype T / T, respectively, 1.33% versus absence in the control group ($\chi^2 = 2.207$; P = 0.138).

Expected and observed frequencies of genotypes of C/T polymorphism of the TP53 gene (rs 17884159) according to the Hardy-Weinberg distribution

The main group of the patient with CIN n = 226					
Alleles	**Allele frequency**				
C	0,88				Df
T	0,12				1
Genotypes	**Genotype frequency**		χ^2	P	Df
	Observed	Expected			
C/C	0,77	0,78	0,029	0,866 P>0,05	
C/T	0,22	0,21	0,030	0,864 P>0,05	
T/T	0,01	0,01	0,00	1,000 P>0,05	
Bcero	1,0	1,0	0,030	0,986 P>0,05	2
Control group n = 165					
Alleles	**Allele frequency**				
C	0,95				df
T	0,05				1
Genotypes	**Genotype frequency**		χ^2	P	df
	Observed	Expected			
C/C	0,90	0,90	0,00	1,0 P>0,05	
C/T	0,10	0,10	0,00	1,0 P>0,05	
T/T	1,0	1,0	0,00	1,0 P>0,05	
Total			0,00	1,0 P>0,05	2

The TP53 gene is a key gene for the stability of the genome, all changes in its structure lead to disruption of cell functioning, 3 out of 19 polymorphic variants of the PT53 gene are involved in carcinogenesis. The polymorphic variant rs17884159 is localized in the region of the intron NM_001126112.2: c.-26 + 4486 with a frequency from 0 to 0.02, the C>T substitution is associated with carcinogenesis [11].

In this work, we analyzed the rs17884159 polymorphism of the TP53 gene in patients with different severity of the disease.

The results of the analysis established changes in the frequency of the polymorphic allele T, which consisted of a statistically significant increase in the frequency of carriage of the T allele associated with the severity of the disease.

Thus, with a relatively favorable course in patients with clinically and colposcopically verified LSIL, the frequency of the T allele was 9.14% and significantly exceeded the values of the control group - 5.15% (χ^2 = 4.129; P≤0.043; OR = 1.852; CI 95% 1.014 - 3.382); with an increase in the severity of the clinical course of HSIL CIN II, the frequency of carriage of the T allele increased to 21.875 (χ^2 = 20.681; P≤0.001; OR = 5.155; CI 95% 2.392 - 11.110); and in patients at risk of malignant malignancy with HSIL CIN III, the frequency of T allele carriage was 37.50% (χ^2 = 25.733; P≤0.001; OR = 11.047; CI 95% 3.591 -33.983).

Analysis of the dynamics of the distribution of the genotype frequencies of the TP53 rs 17884159 gene polymorphism revealed a statistically significant increase in the frequency of the heterozygous C / T genotype and the homozygous T / T genotype associated with an increase in the severity of CIN and the level of its oncological transformation.

Thus, in patients with LSIL, the frequency of carriage of the C / T genotype was 18.28% versus 10.30% in the control group (χ^2 = 4.480; P≤0.035; OR = 1.947; CI 95% 1.043 - 3.637); in patients with HSIL CIN II, the frequency of the C / T genotype increased to 31.25% (χ^2 = 9.943; P≤0.002; OR = 3.957; CI 95% 1.608 - 9.737); with a high risk of malignant transformation in patients with HSIL CIN III, the frequency of the T / C genotype is 50.00% (χ^2 = 8.229; P≤0.005; OR = 2.250; CI 95% 1.238 - 4.089).

An association of the frequency of the T / T genotype of the p53 rs17884159 gene with the severity of the clinical course of cervical neoplasia and the risk of transformation of cells of the cervical epithelium was established: in the control sample of patients, the T / T genotype was absent; in LSIL, the T / T genotype was absent; with LSIL, the frequency of carriage of the C / T genotype is minimal - 0.54% (χ^2 = 0.890; P ≥ 0.890); HSIL CIN II during and HSIL CIN III corresponding carrier frequency genotype T / T are equal to 3,13% (χ^2 = 7,540; R≤0,007) and 12,59% (χ^2 = 20,745; R≤0,001).

Discussion. Carriage of alleles and genotypes of TP53 rs17884159 gene polymorphism was studied in patients with cervical intraepithelial neoplasia. In the group of patients with CIN established predominance carrier T allele (11.95% versus 5.15%) and reduction carrier allele C (88.05% vs. 94.85%). Analysis of the distribution of genotypes of the p53 rs17884159 gene polymorphism in patients with cervical intraepithelial neoplasia revealed an increase in the carriage of the homozygous C / T genotype (21.24% versus 10.30%) and the presence of the homozygous T / T genotype in 1.33%, which was absent in the control. Thus, homozygous - C / C gene TP53 and more favorably exerts a protective effect on the occurrence of CIN.

Analysis of the frequency of distribution of alleles and genotypes of polymorphism of the TP53 rs 17884159 gene depending on the severity of the disease at the time of the study showed negative associations, consisting in a significant increase in the prevalence of carriage of the heterozygous C / T genotype associated with the severity of CIN: carriage of the heterozygous C / T genotype increases the risk of pathology in LSIL 1.947 times; HSIL CIN II - 3.957 times and HSIL CIN III - 2.250 times.

In our studies, the carriage of unfavorable C/T and T/T genotypes is associated with an increase in the severity of CIN. Since the presence of the T allele is also associated with the severity of the pathology, it is clear that genotypes with the T allele are a significant risk factor for the development of C/T and T/T genotypes.

The C>T substitution in the PT53 gene leads to an increase in the carriage of the C/T and T/T genotypes, decreases the expression of the p53 tumor suppressor protein, which is realized in apoptosis disorders, stimulation of neoplastic transformation of the cervical epithelium and the progression of CIN.

Conclusion. According to the results of our studies, the carriage of the T allele, the heterozygous C/T genotype, and the homozygous T/T genotype of the TP53 rs 17884159 gene variants are associated with the presence of neoplastic changes in the cervical epithelium, the risk of their development and the severity of the clinical course, which indicates the contribution of these disorders to neoplastic transformation and the progression of pathology. In the pathogenesis of the development of CIN, an important role is played by impaired control over the regulation of apoptosis caused by the consequences of mutations in the gene TP53 rs 17884159, which leads to uncontrolled proliferation of the cervical epithelium and its neoplastic transformation. Assessment of the activity of apoptotic processes and their relationship with the regulator genes of the p53 molecule can become the basis for the diagnosis and prognosis of neoplastic changes in the cervical epithelium.

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