THE ROLE OF MOLECULAR GENETIC MARKERS IN THE CLINICAL COURSE OF CERVICAL INTRAEPITHELIAL NEOPLASIA

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Abstract
When studying the clinical course of cervical intraepithelial neoplasia in 226 women with molecular genetic analysis, it was revealed that the correlation of alleles and genotypes of genes of oncosuppressor proteins, enzymes of estrogen metabolism and matrix metalloproteinases, confirmed by indicators of relative risk, were the basis for establishing the severity of the clinical course and the risk of developing this multifactorial pathology, by studying anamnestic, somatic data, complaints and the presence of urogenital infections in women with neoplasia of the cervical epithelium.

INTRODUCTION

The prevalence of cervical intraepithelial neoplasia (CIN) and its association with many risk factors suggests that certain alleles and genotypes of the genes for estrogen metabolism enzymes, matrix metalloproteinases, and oncosuppressor proteins are associated with the risk of development and severity of CIN in women [3,4].

The studied genetic polymorphisms play a fundamental role in the occurrence of neoplasia of the cervical epithelium, determining the possible oncological transformation, the level of oncogenic estrogens, and the severity of the destruction of the extracellular matrix. The synthesis of products regulated by the studied genes is inducible and depends on both genetic polymorphism and genetic combinations, the interaction of determines which is the level of specific response and the nature of the manifestation of neoplastic disorders [1,2,5,6].

In this regard, it is important to study gene interactions associated with specific phenotypic manifestations of cervical intraepithelial neoplasia.

The aim of the study was to evaluate the combination of polymorphic loci of oncosuppressor protein genes - TP53, estrogen metabolism enzymes CYP1A2, SULT1A1 and matrix metalloproteinases - MMP-1 in the clinical course of cervical intraepithelial neoplasia.

MATERIALS AND METHODS

We examined 226 patients who underwent outpatient examination and treatment at the Women's Health Center of the Tashkent Medical Academy with a confirmed diagnosis of CIN of varying severity. The age of the observed patients ranged from 18 to 45 years (mean age 36.9±1.1 years). By nationality, all the surveyed were Uzbeks, born and living in the city of Tashkent. The diagnosis of CIN was established on the basis of cytological and colposcopic findings. The control group consisted of 165 healthy women comparable with the study group in terms of age and ethnicity. All participants received voluntary informed consent to participate in the study. The material for molecular genetic analysis was blood samples from the cubital vein. For the analysis of gene polymorphisms, the allel-specific polymerase chain reaction (PCR) method with electrophoretic detection was used.

RESULTS AND DISCUSSIONS

The results of a molecular genetic study showed that the symptom of dysuria in patients with CIN was associated with a significant increase in the frequency of the genotype 1G2G 1799750 MMT-1 to 6.19% in CIN versus 1.21% in the control group (χ^2 = 6.003; 95% CI 1.201 – 24.015); itching and burning - with a combination of genotypes 1G / 2G 1799750 MMT-1 + G 638A SULT1A1 - 9.30% versus 3.03% (χ^2 = 3.278; 95% DI 1.201 - 8.884); abundant mucopurulent discharge - with 1G / 2G 1799750 MMT-1 + TP53 rs 17884159 + G 638A SULT1A1 + Arg72Pro TP53 - 10.18% vs. 3.63% (χ^2 = 3.002; 95% DI 1.194 - 7.550); pain during menstruation is associated with a combination of genotypes 1799750 MMT-1 + G 638A SULT1A1 - 11.06% vs. 2.42% (χ^2 = 5.006; 95% CI 1.707 - 14.678); in patients with menstrual irregularities, a significant increase in the frequency of occurrence of the combination of G 638A SULT1A1 and CYP1A2 C-734A genotypes was found to be 8.41% versus 1.21% 2.42% (χ^2 = 7.484; 95% CI 1.718 - 32.582); intrauterine contraception was associated with the CYP1A2 C-734A + TP53 rs 17884159 genotype combination; and taking oral contraceptives - with a combination of gen-

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otypes of estrogen metabolism enzymes genes - G 638A SULT1A1 and CYP1A2 C-734A, the registration frequency of which was 12.39% versus 4.85% (χ^2 = 2.775; 95% DI 1.231 - 6.252); at the same time, in patients with a burdened obstetric history, the combination G 638A SULT1A1 + 1799750 MMT-1 was significantly more common - in 8.41% versus 1.82% (χ^2 = 4.957; 9%); carriage of the TP53 rs 17884159 and + Arg72Pro TP53 genotypes was associated with early (less than 15 years of age) sexual debut (χ^2 = 13.368; P ≤ 0.001; OR = 5.882; 95% CI 2.015–16.993); smoking more than 10 cigarettes per day correlates with the carriage of the TP53 rs 17884159 and + Arg72Pro TP53 genotypes (χ^2 = 6.013; P ≤ 0.015; OR = 2.394; 95% CI 1.172 - 4.891); the presence of oncological pathology of the first line of kinship correlated with the combination of the genotypes TP53 rs 17884159 and + Arg72Pro TP53 + G 638A SULT1A1 and CYP1A2 C -734A (χ^2 = 6.408; P ≤ 0.02; OR = 3.149; 95% CI 1.257 - 47.886); and the presence of cervical cancer in a family history with a high degree of statistical significance is associated with all studied genotypes TP53 rs 17884159 and + Arg72Pro TP53 + G 638A SULT1A1 and CYP1A2 C-734A + with 1G/2G 1799750 MMT-1 (χ^2 = 8.517; P ≤ 0.004; OR = 5.243; 95% DI 1.531 - 17.952) (Table 1.1).

Table 1.1

Molecular genetic polymorphisms and their combinations associated with complaints of patients with cervical intraepithelial neoplasia

Complaints and anamnestic data	Polymorphism and combination of genes	Frequency in %		OR	95% DI
		Patients with CIN n-226	Control n-165		
Dysuria	1G2G 1799750 MMT-1	14/6,2	2/1,2	5,4	1,2-24,0
		x^2=6,03; P<0,015			
Itching, burning	1G/2G 1799750 MMT-1 + G 638A SULT 1A1	21/9,3	5/3,03	3,3	1,2-8,9
		x^2=5,82; P<0,016			
Profuse mucopurulent discharge	1G/2G 1799750 MMT-1 + TP53 rs 17884159 + G 638A SULT 1A1 + Arg72Pro TP53	23/10,2	6/3,6	3,0	1,2-7,6
		x^2=5,94; P<0,015			
Pain during menstruation	1G/2G 1799750 MMT-1 + G 638A SULT 1A1	25/11,1	4/2,4	5,0	1,7-14,7
		x^2=10,28; P<0,002			
Menstrual dysfunction	G 638A SULT 1A1 + C-734A CYP 1A2	19/8,4	2/1,2	7,5	1,7-32,3
		x^2=9,71; P<0,002			
intrauterine contraception	CYP 1A2 C-734A + TP53 rs 17884159	27/11,9	7/4,2	3,1	1,3-7,2
		x^2=7,13; P<0,008			
Taking oral contraceptives	G 638A SULT 1A1 + CYP 1A2 C-734A	28/12,4	8/4,9	2,8	1,2-6,3
		x^2=6,48; P<0,011			
Aggravated obstetric anamnesis - more than 3 births and abortions	1G/2G 1799750 MMT-1 + G 638A SULT 1A1	19/8,4	3/1,8	4,9	1,4-17,0
		x^2=7,79; P<0,006			
Age of sexual debut <15 years; >3 sexual partners	TP53 rs 17884159 + Arg72Pro TP53	29/12,8	4/2,4	5,9	2,0-16,9
		x^2=13,36; P<0,001			
Smoking more than 10 cigarettes a day	TP53 rs 17884159 + Arg72Pro TP53	33/14,6	11/6,7	2,4	1,2-4,9
		x^2=6,01; P<0,015			
Oncological pathology in relatives of the 1st line of kinship	TP53 rs 17884159 + Arg72Pro TP53 + G 638A SULT 1A1 + CYP 1A2 C-734A	24/10,6	6/3,6	3,1	1,3-7,9
		x^2=6,40; P<0,012			
Family history of cervical cancer	TP53 rs 17884159 + Arg72Pro TP53 + G 638A SULT 1A1 + CYP 1A2 C-734A + 1G/2G 1799750 MMT-1	20/8,9	3/1,8	5,2	1,5-17,9
		x^2=8,51; P<0,004			

An analysis of the prevalence of the studied genotypes and their combinations showed that in patients with CIN more than 3 deliveries and a history of postpartum complications are statistically significantly associated with the combination of genotypes G 638A SULT1A1 and CYP1A2 C-734A + 1G/2G 1799750 MMT-1 – 7, 53% vs 2.42% (χ^2 = 4.877; P ≤ 0.028; OR = 3.233; 95% DI 1.067 - 9.797); more than 3 abortions and post-abortion complications are statistically significantly associated with the combination of genotypes 1G/2G 1799750 MMT-1 + TP53 rs 17884159 - 14.60% versus 4.24% (χ^2 = 11.145; P ≤ 0.001; OR = 1.378; 95 % DI 0.537 – 3.532); frequent miscarriages and their complications are associated with a combination of genotypes of estrogen metabolism enzymes and metalloproteinases - G 638A SULT1A1 and CYP1A2 C-734A + with 1G / 2G 1799750 MMT-1 - 7.53% - 6.19% versus 1.82% (χ^2 = 4.392; P ≤ 0.037; OR = 3.566; 95% DI 1.008 - 12.618).

It should be noted that such a complication as ectopic pregnancy in CIN was statistically significantly associated with the genotype of the intracellular matrix and estrogen metabolism gene - 1G / 2G 1799750 MMT-1 + G 638A SULT1A1 and CYP1A2 C-734A, amounting to 6.64% versus 1.82 % (χ^2 = 5.043; P ≤ 0.025; OR = 3.839; 95% DI 1.093 - 13.485).

At the same time, in more than 17.26% of patients with CIN versus 6.67% in the control group, a burdened reproductive history was statistical significantly associated with a combination of all studied genotypes G638A SULT1A1 + CYP1A2 C-734A + TP53 rs 17884159 and + Arg72Pro TP53 + 1G/ 2G 1799750 MMT-1 (χ^2 = 9.590; P ≤ 0.002; OR = 2.920; 95% DI 1.447 - 5.893).

The same combinations of genotypes, but with a lower frequency, were found in patients with a history of a combination of 2 complications - 10.62% vs. 4.85% (χ^2 = 4.227; P ≤ 0.040; OR = 2.332; 95% CI 1.020 - 5.331) and with 3 complications, respectively, 6.64% versus 1.82% (χ^2 = 5.043; P ≤ 0.025; OR = 3.839; 95% DI 1.093 - 13.485).

Thus, a detailed analysis of genetic polymorphisms will make it possible to identify combinations of genotypes associated with reproductive history and capable of influencing the development of CIN.

Known pathogenetic role of microorganisms of the vaginal biotope in the occurrence, development and malignancy of the pathology of the cervix. Qualitative assessment of the microflora of the genital tract by PCR showed that the detection of Chlamydia trachomatis, Mycoplasma genitalis and Ureaplasma urealyticum in patients with CIN was significantly associated with a combination of polymorphic variants of estro-

gen metabolism genes and tumor markers: G638A SULT1A1 + CYP1A2 C-734A + Arg72Pro TP53. The frequency of registration this combination of genotypes in patients with CIN when registering Chlamydia trachomatis was 5.75% versus 1.21% in the control group (χ^2 = 5.427; P ≤ 0.020; OR = 4.974; 95% CI 1.107 - 22.352); with the isolation of Mycoplasma genitalis - 4.87% versus 1.21% (χ^2 = 4.043; P ≤ 0.045; OR = 4.974; 95% DI 1.107 - 22.352); when Ureaplasma urealyticum was detected in 6.19% versus 1.82% (χ^2 = 4.392; P ≤ 0.037; OR = 3.566; 95% CI 1.008 - 12.618).

Facultative anaerobic and opportunistic microorganisms, which are part of the resident microflora of the vaginal biotope, can exhibit pathogenic properties under risk factors, contributing to hormonal imbalance and stimulating neoplastic transformation, which ultimately can contribute to malignancy.

In all studied patients with CIN, a statistically significant association was found between the combination of oncosuppressor protein genotypes and the estrogen metabolism gene Arg72Pro TP53 + TP53 rs 17884159 + G638A SULT1A1 with viral infections: Herpes symp.virus I, Herpes symp.virus II and Cytomegalovirus. So, with Herpes symp.virus I, this combination is recorded in 14.16% of patients with CIN versus 7.27% of the examined control group (χ^2 = 4.529; P ≤ 0.034; OR = 2.103; 95% CI 1.048 - 4.220); with Herpes symp.virus II and Cytomegalovirus, respectively, in 11.95% versus 4.85% (χ^2 = 5.813; P ≤ 0.034; OR = 2.663; 95% CI 1.177 - 6.023) and 15.28% versus 6.67% (χ^2 = 4.529; P ≤ 0.015; OR = 2.394; 95% DI 1.172 - 4.892).

The course of CIN was associated with HPV infection and a statistically significant increase in the frequency of registration of the combination of genotypes Arg72Pro TP53 + TP53 rs 17884159 + G638A SULT 1A1 + CYP 1A2 C-734A. Thus, the overall frequency of registration of this combination in Human papilloma virus infection was 91.15% in patients with CIN versus 29.70% in the control group (χ^2 = 158.775; P ≤ 0.001; OR = 24.384; 95% CI 13.822 - 43.016); with Human papilloma virus infection of 16/18 types, respectively, 79.65% vs. 22.42% (χ^2 = 12.644; P ≤ 0.001; OR = 13.537; 95% DI 8.305 - 22.065) and with Human papilloma virus infection of 16/18 types 19 .47% vs. 9.09% (χ^2 = 8.017; P ≤ 0.001; OR = 2.366; 95% DI 1.267 - 4.416).

Thus, the study of the effect of virus associations on the functional state and features of the proliferation of the stratified squamous epithelium of the cervix in CIN and the initial stages of cervical cancer remains an urgent problem. Considering that preventive medicine is a modern and new area of research, the solution of these issues is of great clinical importance for determining the risk factors for the development and course of precancer and cervical cancer, as well as for developing principles for individual prevention of these forms of pathology in the presence of genital infections.

Thus, the result of the study was the identification of a combination of genotypes that predispose to the development and severity of the clinical course of cervical intraepithelial neoplasia, which makes it possible to diagnose early, predict the development of the disease and oncotransformation, develop management tactics and personalized therapy based on genetic testing.

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