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INNOVATIVE DIAGNOSTIC METHODS IN PATIENTS WITH CERVICAL INTRAEPITHELIAL NEOPLASIA OF THE CERVIX

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Abstract:
Background: *The primary causal factor in the development of cervical intraepithelial neoplasia (CIN) in women is considered to be infection with high-risk human papillomavirus (HPV). HPV infection and the integration of its genome into the chromosomal apparatus of cervical epithelial cells serve as an early key mechanism in the progression of cervical tumor lesions.*
Material and methods: *The study was conducted on 100 patients aged 30 to 59 years with cervical intraepithelial neoplasia. The control group consisted of 92 women without cervical pathology. The characteristics of the polymorphic genes MKI67 (rs10764749) were studied using standard allele-specific PCR. The cervix of healthy women and comparative controls was analyzed using the Hardy-Weinberg scale (HRW, p>0.05).*
Results: *Functional analysis of MKI67 (rs10764749) in patients with CIN 2 and healthy women of the main group revealed a statistically significant association between this genetic marker and the risk of CIN 2 development.*
Conclusion: *A statistically significant association was established between the risk of developing CIN 2 (HPV "-") and the unfavorable loci of the MKI67 (rs10764749) biomarker (T allele - χ^2=6.7; P=0.01; OR=2.3 and T/T genotype - χ^2=4.2; P=0.05; OR=4.2). An independent risk effect was demonstrated between the risk of developing CIN 2 (HPV "+") and the unfavorable loci of the MKI67 (rs10764749) genetic marker (T allele - χ^2=14.3; P=0.01; OR=3.1; C/T genotype - χ^2=5.8; P=0.03; OR=2.5; T/T genotype - χ^2=4.8; P=0.05; OR=4.5).*
Keywords: *cervical intraepithelial neoplasia, human papillomavirus, MKI67 (rs10764749).*

ИННОВАЦИОННЫЕ МЕТОДЫ ДИАГНОСТИКИ У ПАЦИЕНТОК С ЦЕРВИКАЛЬНЫМИ ИНТРАЭПИТЕЛИАЛЬНЫМИ НЕОПЛАЗИЯМИ ШЕЙКИ МАТКИ

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Резюме:
Введение: *У женщин основным этиологическим фактором развития цервикальной интраэпителиальной неоплазии (CIN) является инфицирование высокоонкогенными типами вируса папилломы человека (ВПЧ). Инфекция ВПЧ и интеграция его генома в хромосомный аппарат клеток шейки матки выступают исходным и ключевым механизмом в патогенезе опухолевых поражений шейки матки.*
Материалы и методы: *Исследование проведено у 86 больных в возрасте от 30 до 59 лет с цервикальными интраэпителиальными неоплазиями. Контрольную группу составили 92 женщины без патологии шейки матки. Характеристики полиморфных генов MKI67 (rs10764749) изучали с помощью стандартной аллель-специфической ПЦР. По шкале Харди-Вайнберга анализировали шейку матки здоровых женщин и сравнительного контроля (РХВ, p>0,05).*
Результаты: *Функциональный анализ MKI67 (rs10764749) у больных CIN 2 и здоровых женщин основной группы позволил установить статистически значимую связь между этим генетическим маркером и риском формирования CIN2.*
Заключение: *Установлена статистически значимая ассоциация между риском развития цервикальной интраэпителиальной неоплазии 2 степени (CIN 2) при отсутствии ВПЧ-инфекции (HPV "-") и наличием*

неблагоприятных локусов биомаркера MKI67 (rs10764749), а именно: Т-аллеля (χ^2=6,7; P=0,01; OR=2,3) и генотипа T/T (χ^2=4,2; P=0,05; OR=4,2). Продемонстрировано наличие независимого эффекта риска между развитием CIN 2 при наличии ВПЧ-инфекции (HPV "+") и неблагоприятными локусами генетического маркера MKI67 (rs10764749): Т-аллелем (χ^2=14,3; P=0,01; OR=3,1), генотипом C/T (χ^2=5,8; P=0,03; OR=2,5) и генотипом T/T (χ^2=4,8; P=0,05; OR=4,5).

Ключевые слова: *интраэпителиальная неоплазия шейки матки, вирус папилломы человека, MKI67 (rs10764749).*

BACHADON BO'YNI INTRAEPITELIAL NEOPLAZIYASI BILAN OG'RIGAN BEMORLARDA INNOVATSION DIAGNOSTIKA USULLARI

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Rezyume:

Kirish: *Ayollarda bachadon bo'yni intraepitelial neoplaziyasi (CIN) rivojlanishining asosiy sababchi omili yuqori xavfli odam papillomavirusi (OPV) infektsiyasi hisoblanadi. OPV infektsiyasi va uning genomini servikal epiteliy hujayralarining xromosoma apparatiga integratsiyalashuvi bachadon bo'yni o'smalari o'choqlari rivojlanishining dastlabki asosiy mexanizmi bo'lib xizmat qiladi.*

Material va metodlar: *Tadqiqot bachadon bo'yni intraepitelial neoplaziyasi bo'lgan 30 yoshdan 59 yoshgacha bo'lgan 100 nafar bemorlarda amalga oshirildi. Nazorat guruhi bachadon bo'yni patologiyasi bo'lmagan 92 ayoldan iborat edi. MKI67 (rs10764749) polimorf genlarining xususiyatlari standart allelga xos PCR yordamida o'rganildi. Xardi-Vaynberg balansiga muvofiq sog'lom ayollarning bachadon bo'yni va qiyosiy nazorati (RHV, p>0,05) amalga oshirilib tahlil qilindi.*

Natija: *Asosiy guruhdagi CIN 2 bilan kasallangan bemorlar va sog'lom ayollar orasida MKI67 (rs10764749) genetik belgisi funksional tahlili ushbu genetik marker va CIN 2 rivojlanish xavfi o'rtasida statistik jihatdan ahamiyatli bog'liqlik mavjudligini aniqlash imkonini berdi.*

Xulosa: MKI67 (rs10764749) biomarkeri (T alleli - χ^2=6.7; P=0.01; OR=2.3 va T/T genotipi - χ^2=4.2; P=0.05; OR=4.2) ning noqulay lokuslari bilan CIN 2 (HPV "-") rivojlanishi o'rtasida statistik jihatdan ahamiyatli bog'liqlik o'rnatildi. MKI67 (rs10764749) genetik markerining noqulay lokuslari (T alleli - χ^2=14.3; P=0.01; OR=3.1; C/T genotipi - χ^2=5.8; P=0.03; OR=2.5; T/T genotipi - χ^2=4.8; P=0.05; OR=4.5) bilan CIN 2 (HPV "+") rivojlanishi o'rtasida mustaqil xavf ta'siri ko'rsatildi.

Kalit so'zlar: *bachadon bo'yni intraepiyelial neoplaziyasi, inson papillomavirusi, MKI67 (rs10764749).*

RELEVANCE: The primary causal factor in the development of cervical intraepithelial neoplasia (CIN) in women is considered to be infection with high-risk human papillomavirus (HPV) [1]. HPV infection and the integration of its genome into the chromosomal apparatus of cervical epithelial cells serve as an early key mechanism in the progression of cervical tumor lesions [6,7].

It is reported that the pathogenic effect of HPV is mediated by the viral oncoproteins E6 and E7, which are responsible for the initial changes in epithelial cells. These oncoproteins contribute to the alteration of the activity of numerous genes involved in DNA repair processes, cell proliferation, growth factor activation, and angiogenesis [3].

MKI67 (rs10764749) is a genetic marker of cell proliferation, whose prognostic value has been demonstrated in the development of various neoplastic processes (including prostate carcinoma, brain tumors, and breast cancer) [4,8]. Furthermore, recent studies indicate that the MKI67 gene may serve as a potential marker for the progression of cervical intraepithelial lesions [2,5,9].

OBJECTIVE: To assess the significance of this genetic marker, we conducted a study to analyze its structure and functional characteristics in cervical intraepithelial neoplasia.

MATERIAL AND METHODS. A total of 86 Uzbek women aged 30 to 59 years were examined as the main study group, which was divided into two subgroups. The first subgroup included 46 (53.5%) patients who tested positive for high-risk HPV types, while the remaining 40 (46.5%) patients tested negative for HPV. The control group consisted of data from 92 practically healthy women with a negative HPV PCR test result and NILM findings from liquid-based cytology. Inclusion criteria for the study were based on the results of general clinical and gynecological examinations, including cytological analysis. Each patient underwent a comprehensive examination according to the protocol, which included the collection of clinical and anamnestic data, extended colposcopy, liquid-based cytology, quantitative polymerase chain reaction with real-

time detection (PCR-RT), pelvic ultrasound, histological examination of biopsy samples from pathological areas of the cervix, and analysis of the genotypic variant of the MKI67 (rs10764749) gene.

Collection of Biological Material for Molecular-Genetic Research.

For molecular-genetic studies, blood samples were collected from the cubital vein in dry plastic tubes with a volume of 4 ml, containing 0.5 ml of 0.5 M EDTA (pH=7.8) from patients with CIN 2 HPV (-) (n=40), CIN 2 HPV (+) (n=46), and the control group (n=92).

Polymerase Chain Reaction (PCR) for DNA Synthesis in Molecular-Genetic Analysis.

A sample of genomic DNA (50–200 ng) was added to a 25.0 µl reaction mixture containing 0.67 mM Tris-HCl (pH 8.8 at 25°C), 16.6 mM (NH4)2SO4, 1–6.7 mM MgCl2, 6.7 µM EDTA, 10 mM 2-mercaptoethanol, 170.0 µg BSA, a mixture of four main deoxynucleotide triphosphates (0.8 mM each), 0.2 U/µl of thermostable DNA polymerase ("SibEnzyme," Novosibirsk), and oligonucleotide primers at the final concentration. The specificity of amplification after PCR completion, as well as the quantity of the obtained amplicon, was verified using electrophoresis. If necessary, hydrolysis of the amplified DNA fragments was performed following the recommendations of the manufacturer ("SibEnzyme," Novosibirsk).

The frequency of allele and genotype variants (f) was calculated using the formulas: f=n/2N and f=n/N. The prognostic efficiency (AUC classifier) of the genetic markers studied was determined using the standard formula: AUC=(Se + Sp) /2. The correspondence of genotype frequencies to Hardy-Weinberg equilibrium was evaluated using the χ^2 test. Differences in allele and genotype frequencies were assessed using the χ^2 test and Fisher's exact test. The odds ratio and 95% confidence interval were calculated using the OpenEpi software package (ver. 9.3).

RESULTS.

The structure of the MKI67 (rs10764749) gene in the groups of women with CIN 2, depending on their negative or positive HPV status, was characterized by the observed frequency of the dominant **C allele** and the minor **T allele** at **72.5% and 66.3%**, as well as **27.5% and 33.7%**, respectively. The dominant **C/C genotype** was detected in **57.5% and 45.0%** of patients, while the heterozygous **C/T** and minor homozygous **T/T** genotypes were found in **30.0% and 41.3%**, as well as **12.5% and 13.0%** of cases, respectively, in the studied groups of women with CIN 2 HPV (-) and HPV (+).

When comparing the structural analysis results of the MKI67 (rs10764749) gene in the healthy control group, **a significantly higher frequency of the C allele (85.9%) and C/C genotype (75.0%)** was observed, whereas the unfavorable **T allele (14.1%)** and genotypes **C/T (21.7%)** and **T/T (3.3%)** were found at lower frequencies compared to patients with CIN 2 (see Table 1).

Table 1. Analysis of the MKI67 (rs10764749) Gene Structure in Groups of Women with CIN 2 and Healthy Controls

Alleles and Genotypes	Examined groups		
	CIN 2 HPV (-) Group (n=40), % (abs.)	CIN 2 HPV (+) Group (n=46), % (abs.)	Control Group (n=92), % (abs.)
C	72.5 (58)	66.3 (61)	85.9 (158)
T	27.5 (22)	33.7 (31)	14.1 (26)
C/C	57.5 (23)	45.7 (21)	75.0 (69)
C/T	30.0 (12)	41.3 (19)	21.7 (20)
T/T	12.5 (5)	13.0 (6)	3.3 (3)

This table provides a comparative analysis of the **MKI67 (rs10764749) gene structure** in three groups of examined women: those with **CIN 2 and HPV-negative status,** those with **CIN 2 and HPV-positive status**, and a **healthy control group.** The data highlight differences in allele and genotype distribution across these groups.

Table 2. Functional Analysis of the MKI67 (rs10764749) Gene in Groups of Patients with CIN 2 HPV (-) and Healthy Controls

Alleles and Genotypes	Frequencies of Alleles and Genotypes				χ^2	P	RR	95%CI	OR	95% CI
	CIN 2 HPV (-)		Control							
	n	%	n	%						
C	58	72.5	158	85.9	6.7	0.01	0.8	0.4 - 1.77	0.4	0.23- 0.82
T	22	27.5	26	14.1	6.7	0.01	1.2	0.69-2.02	2.3	1.22- 4.34

C/C	23	57.5	69	75.0	4.0	0.05	0.8	0.28-2.06	0.5	0.21- 0.98
C/T	12	30.0	20	21.7	1.0	0.40	1.4	0.47-4.03	1.5	0.67- 3.56
T/T	5	12.5	3	3.3	4.2	0.05	3.8	1.17-12.56	4.2	1.06- 16.92

Statistically significant differences in the frequencies of the minor T allele and T/T genotype of the MKI67 (rs10764749) gene between women with CIN 2 HPV (-) and healthy individuals confirm their association with an increased disease risk by 2.3 times (χ^2=6.7; P=0.01) and 4.2 times (χ^2=4.2; P=0.05), respectively.

In the groups of women with CIN 2 HPV (+) and healthy individuals, statistically significant differences were established for all allele and genotype variants. In particular, it was determined that the risk of disease was increased among carriers of the unfavorable A allele by 3.1 times (χ^2=14.3; P=0.01; OR=3.1; 95% CI: 1.72 - 5.54), the **C/T genotype by 2.5 times** (χ^2=5.8; P=0.03; OR=2.5; 95% CI: 1.19 - 5.4), and the **A/A genotype by 4.5 times** (χ^2=4.8; P=0.05; OR=4.5; 95% CI: 1.17 - 16.9).

It is also important to note that among women with CIN 2 HPV (+), there was a statistically significant reduction in the protective effect of the dominant C allele (χ^2=14.3; P=0.01; 95% CI: 0.18 - 0.58) and the C/C genotype (χ^2=11.6; P=0.01; 95% CI: 0.13 - 0.58) in relation to disease risk (see Table 3).

Table 3. Functional Analysis of the MKI67 (rs10764749) Gene in the Group of Patients with CIN 2 HPV (+) and Healthy Individuals

Alleles and Genotypes	Frequencies of Alleles and Genotypes				Alleles and Genotypes	P	RR	95%CI	OR	95% CI
	CIN 2 HPV (+)		Controls							
	n	%	n	%						
C	61	66.3	158	85.9	14.3	0.01	0.8	0.41-1.44	0.3	0.18- 0.58
T	31	33.7	26	14.1	14.3	0.01	1.3	0.73-2.31	3.1	1.72- 5.54
C/C	21	45.7	69	75.0	11.6	0.01	0.6	0.25-1.51	0.3	0.13- 0.58
C/T	19	41.3	20	21.7	5.8	0.03	1.9	0.78-4.64	2.5	1.19- 5.4
T/T	6	13.0	3	3.3	4.8	0.05	4.0	1.42-11.3	4.5	1.17- 16.9

Thus, the carriage of unfavorable alleles and genotypes of the MKI67 (rs10764749) gene is statistically significantly associated with an increased risk of CIN 2 in HPV (+) patients by 3.1 times (χ^2=14.3; P=0.01), 2.5 times (χ^2=5.8; P=0.03), and 4.5 times (χ^2=4.8; P=0.05).

Comparing the distribution differences of the MKI67 (rs10764749) gene in groups of women with CIN 2 HPV (-) and CIN 2 HPV (+), no statistically significant values were found in the frequency distribution of alleles (C allele - χ^2=0.8; P=0.4; OR=1.3; 95% CI: 0.7 - 2.57 and T allele - χ^2=0.8; P=0.4; OR=0.7; 95% CI: 0.39-1.43) and genotypes (C/C genotype - χ^2=1.2; P=0.3; OR=1.6; 95% CI: 0.69-3.78; C/T genotype - χ^2=1.2; P=0.3; OR=0.6; 95% CI: 0.25-1.49; T/T genotype - χ^2<3.84; P=0.95; OR=1.0; 95% CI: 0.27-3.39**) (Table 4).

Table 4.

Functional Analysis of the MKI67 (rs10764749) Gene in the Group of Patients with CIN 2 HPV (-) and HPV (+)

Alleles and Genotypes	Frequencies of Alleles and Genotypes				χ^2	P	RR	95%CI	OR	95% CI
	CIN 2 HPV (-)		CIN 2 HPV (+)							
	n	%	n	%						
C	58	72.5	61	66.3	0.8	0.40	1.1	0.53-2.25	1.3	0.7 - 2.57
T	22	27.5	31	33.7	0.8	0.40	0.9	0.52-1.6	0.7	0.39-1.43
C/C	23	57.5	21	45.7	1.2	0.30	1.3	0.51-3.12	1.6	0.69-3.78
C/T	12	30.0	19	41.3	1.2	0.30	0.7	0.27-1.99	0.6	0.25- 1.49

T/T	5	12.5	6	13.0	0.0	0.95	1.0	0.25-3.71	1.0	0.27- 3.39

Thus, the results of analyzing the structural and functional characteristics of the MKI67 (rs10764749) gene, conducted in groups of women with CIN 2 and healthy individuals, indicate a statistically significant role of this genetic marker in increasing the risk of cervical intraepithelial neoplasia.

The studies conducted in the main group of women with CIN 2, compared to healthy individuals, established a significant increase in the risk of CIN 2 among carriers of the unfavorable T allele by 2.5 times (χ^2=13.1; P=0.01), the C/T genotype by 1.9 times (χ^2=4.1; P=0.05), and the T/T genotype by 4.0 times (χ^2=5.1; P=0.03) in relation to the MKI67 (rs10764749) gene.

CONCLUSION. Thus, the study of the structural and functional characteristics of the genetic markers MKI67 (rs10764749) in comparison with healthy individuals yielded the following results:

- A statistically significant association was established between the risk of developing CIN 2 (HPV "-") and the unfavorable loci of the MKI67 (rs10764749) biomarker (T allele - χ^2=6.7; P=0.01; OR=2.3 and T/T genotype - χ^2=4.2; P=0.05; OR=4.2).
- An independent risk effect was demonstrated between the risk of developing CIN 2 (HPV "+") and the unfavorable loci of the MKI67 (rs10764749) genetic marker (T allele - χ^2=14.3; P=0.01; OR=3.1; C/T genotype - χ^2=5.8; P=0.03; OR=2.5; T/T genotype - χ^2=4.8; P=0.05; OR=4.5).

References:

1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA A Cancer J. Clin. 2018, 68, 394–424.
2. Diéguez N. C. et al. Detection of mRNA of CDKN2A, MKi67 and TOP2A in liquid-based cytology as biomarker of high-grade lesions of uterine cervix. – 2019.
3. Cheng, J.; Lu, X.; Wang, J.; Zhang, H.; Duan, P.; Li, C. Interactome analysis of gene expression profiles of cervical cancer reveals dysregulated mitotic gene clusters. Am. J. Transl. Res. 2017, 9, 3048–3059
4. Mrouj K. et al. Ki-67 regulates global gene expression and promotes sequential stages of carcinogenesis //Proceedings of the national academy of sciences. – 2021. – Т. 118. – №. 10. – С. e2026507118.
5. Øvestad I. T. et al. High-grade cervical intraepithelial neoplasia (CIN) associates with increased proliferation and attenuated immune signaling //International journal of molecular sciences. – 2021.
6. Wu, K.; Yi, Y.; Liu, F.; Wu, W.; Chen, Y.; Zhang, W. Identification of key pathways and genes in the progression of cervical cancer using bioinformatics analysis. Oncol. Lett. 2018, 16, 1003–1009.
7. Wang, C.; Davis, J.S. At the center of cervical carcinogenesis: Synergism between high-risk HPV and the hyperactivated YAP1. Mol. Cell. Oncol. 2019, 1–2.
8. Yu L. et al. Application of p16/Ki-67 dual-staining cytology in cervical cancers //Journal of Cancer. – 2019. – Т. 10. – №. 12. – С. 2654.
9. Sattarova, Kamola A., et al. "Clinical and Biological Importance of Micro RNA in the Formation of Women Reproductive Losses." Indian Journal of Forensic Medicine & Toxicology 14.4 (2020): 7355.
10. Абдубакиева, Ф. Б., К. А. Саттарова, and Г. Н. Бекбаулиева. "Социально-медицинские аспекты репродуктивного здоровья и контрацептивного поведения пациенток с внематочной беременностью." Журнал теоретической и клинической медицины 2 (2017): 122-123.
11. Сычева Е.Г., Назарова Н.М., Бурменская О.В., Прилепская В.Н., Трофимов Д.Ю., Сухих Г.Т. Персистенция вируса папилломы человека высокого онкогенного риска и другие молекулярно-генетические предикторы развития цервикальных интраэпителиальных неоплазий. Акушерство и гинекология. 2018; 12: 104-10.,
– Т. 23. – №. 1. – С. 373.
12. Babadjanova Gulzhakhon Sattarovna, Abdurazakova Mukhayo Dilshodovna, Razzakova Nilufar Saidakhmatovna, FEATURES OF PREGNANCY IN WOMEN WITH DISEASES OF THE BILIAR SYSTEM. Journal of Biomedicine and Practice 2020, vol. 6, issue 5, pp.48-53