DOI: https://doi.org/10.2298/SARH210620061T
UDC: 618.146-006.6:575

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Cytokine gene polymorphisms of TNF, IFN-γ, and IL-12 as potential predictors in the onset of cervical disease in HR HPV-positive women with behavioral risk cofactors

Dijana Tasić-Tomić[1], Vera Pravica[2,3], Lidija Tasić[1,2], Azra Lukač[4], Mirza Šačić[5], Maja Ćupić[2,3]

[1]University Clinic of Gynecology and Obstetrics Narodni Front, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[3]Institute of Microbiology and Immunology, Belgrade, Serbia;
[4]Community Health Center, Rožaje, Montenegro;
[5]State Hospital, Gynecology and Obstetrics Department, Novi Pazar, Serbia

SUMMARY

Introduction/Objective The aim of this study was to investigate the distribution of genotypes and alleles of proinflammatory cytokines TNF, IFN-γ, and IL-12 and their effect on the development of a cervical illness and also to determine their associated influence with cofactors in HR HPV-positive women in Serbia.

Methods We have investigated 24 women and based on the cytological findings they were classified into four groups: PAP II, ASCUS, LSIL, and HSIL. Analysis of TNF, IL-12, and IFN-γ polymorphisms was performed using the real-time PCR TaqMan method. Statistical analysis was performed using parametric and non-parametric tests and correlation and multiple regression analysis.

Results Significantly higher frequency of high production-related TNF AA genotype was observed in severe dysplasia. The correlation between TNF gene polymorphism and cervical findings were highly significant. There was a moderate, significant correlation between low production IFN-γ AA genotype and earlier cervical infections. There was a significant correlation between the IL-12 polymorphism of the low production IL-12 AA genotype and cervical lesions.

Conclusion Results of this study show that HSIL is associated with significantly higher frequency of high production TNF AA genotype. It is known that polymorphisms of certain cytokine genes encoding proteins involved in Th1 and Th2 cellular responses may be associated with better or worse prognosis of cervical disease in women with persistent HR HPV infection. Therefore, they may be considered as biomarkers that may have a predictive role in the development of cervical cancer.

Keywords: cervical cancer; cofactors; gene polymorphism; TNF; IFN-γ; IL-12

INTRODUCTION

Cervical cancer is the fourth most frequent cancer in women with an estimated 604,000 new cases in 2020. Of the estimated 342,000 deaths from cervical cancer in 2020, about 90% of these occur in low- and middle-income countries. This frequency varies by geographical areas and ranges 17.2–55 per 100,000 women [1, 2, 3]. This tumor is highly correlated with infection by highly oncogenic types of human papilloma virus (high-risk HPV, HR HPV), which are the most common sexually transmitted pathogens [4, 5]. Since Harald zur Hausen proved the presence of highly oncogenic HPV types 16 and 18 DNA in cervical cancer cells in the early 1980s, it was clear that HPV infection is the key factor in its emergence [5, 6, 7]. In a majority of cases (> 80%), a spontaneous regression of changes occurs and the virus is eliminated within two years of initial infection. However, in a minority of cases persistent infection is established, from which 25% of infected women develop cervical intraepithelial neoplasia in the first degree (CIN I), with further progression (CIN II/III). Cervical cancer would develop in 10% of all patients and in 1% of HR HPV-positive women over a number of years [6, 8].

Recently, more attention has been dedicated to the role of genetic predisposition in the development of cervical cancer associated with various predisposing environmental cofactors. Genome-wide association studies have discovered a vast number of genes whose individual alleles are associated with a predisposition to develop cervical cancer. There are still contradictory data from different research teams, related to different ethnic populations and geographical areas, on polymorphisms in genes encoding proteins involved in the functioning of the Th1 and Th2 cellular response and their role in the pathogenesis of emerging HR HPV cervical cancer [9, 10]. Single nucleotide polymorphisms (SNPs) in genes encoding relevant proinflammatory cytokines, such as tumor necrosis factor (TNF), interferon-gamma (IFN-γ), and interleukin-12 (IL-12), are becoming highly significant genetic markers for assessing the risk of developing cervical cancer associated with HR HPV [11, 12].

Received • Примљено: June 20, 2021
Revised • Ревизија: June 21, 2022
Accepted • Прихваћено: June 22, 2022
Online first: July 1, 2022

Correspondence to:
Dijana TASIĆ-TOMIĆ
Borska 47
11000 Belgrade, Serbia
dijanamicrogen@gmail.com

SNP at position -308 in the promoter region of the TNF gene (rs1800629) G/A, is highly correlated with CIN I associated with HR HPV. TNF plays an important role in various inflammatory diseases as one of the most important proinflammatory cytokines. It is considered that presence of A allele at the -308 TNF gene locus, which is a high secretory TNF phenotype, could have a strong influence on the development of HR HPV cervical cancer [13]. Studies show elevated TNF serum levels in women with CIN in comparison with healthy women. Also, AA genotype at the -308 locus of the TNF gene carries a higher risk for developing cervical tumors compared to GG and GA genotypes. A meta-analysis confirmed an increased risk of developing cervical cancer in the presence of the A allele, especially the AA genotype [14, 15].

IFN-γ is one of the most important Th1 immune modulators with antiviral and antitumor role. SNP at position +874 T/A (rs62559044) located in the first intron of the IFN-γ gene is associated with increased production of IFN-γ and effective defense against HPV infection, while its low level, which is present in cervical cancer, is associated with a poor prognosis [16, 17]. The DNA sequence containing the +874 T allele has a binding site for NF-kappa B-like transcription factor [18, 19]. Literature data shows that the presence of the T allele correlates with increased IFN-γ expression, thus reducing the cervical cancer risk, while a low secretory +874 IFN-γ AA genotype is associated with a high risk for developing HR HPV cervical tumors [7].

IL-12 is a heterodimer composed of two subunits – p35 (IL-12A) and p40 (IL-12B). Inheritance of functional SNP variants of this gene, which is associated with the destruction of cancer cells, leads to changes in expression, which also affects the function of other cytokines that are under its regulation. IL-12 stimulates the production of IFN-γ by signaling the molecular cascade, while inhibiting IL-4, which suppresses IFN-γ synthesis and stimulates the protective Th1 immune response. SNP at the +1188 A/C position in the 3'UTR region of the IL-12B gene (rs3212227) has been associated with a predisposition to cervical cancer in women with HPV HR. The AA genotype is also thought to be associated with the progression of HR HPV lesions in cervical cancer [20, 21].

The aim of this study was to investigate the distribution of genotypes and alleles of proinflammatory cytokines TNF, IFN-γ and IL-12 and their correlation with the grades of cervical illness. We also wanted to determine which genotypes possess a protective or favoring significance for the development of cervical cancer.

METHODS

From the patient registered with HR HPV positive cervical samples at the Institute of Microbiology and Immunology in Belgrade, Serbia, 24 patients were selected based on available colposcopic and cytological status determined during gynecological examination. Based on a Pap cytological findings and according to the Bethesda classification (2001), the patients were classified into four groups: women with normal cervical cytology or negative for intraepithelial lesion or malignancy (NILM) as control group; women whose cytology was defined as atypical cells of unknown origin (ASCUS); patients with low-grade squamous intraepithelial lesion (LSIL) or CIN I, corresponding to the slight changes in the cervical epithelium or koilocytosis (cells with perinuclear enlightenment indicative of HPV infection), and women with HSIL corresponding to moderate and/or severe cervical dysplasia (CIN II/III). The interview-based questionnaire was administered to all the participants involved in the study. It queried basic information about the patient, socio-demographic and behavioral data, patient's reproductive history, sexual habits, morbidity from other sexually transmitted diseases (STD), and other information of significance for HPV-related cervical disorders. To determine cytokine gene polymorphism, 5 ml of peripheral blood was taken from the patients using appropriate tubes (Becton Dickinson, New Jersey, USA) with anticoagulant ethylenediaminetetraacetic acid (EDTA), and has been transported within four hours to the Laboratory of Immunology of the Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, for further procedures.

Detection of TNF, IFN-γ, and IL-12B gene polymorphisms

DNA extraction

Genomic DNA was isolated from peripheral blood that had been sampled in tubes containing EDTA, using the Gene JET Whole Blood Genomic DNA Purification Mini Kit (Fermentas Thermo Fisher Scientific Inc., Vilnius, Lithuania) according to the manufacturer instructions.

SNP detection

Detection and analysis of the TNF –308 G/A (rs1800629) and IL12-B +1188 A/C (rs3212227) polymorphisms were performed using real-time PCR with commercial TaqMan probes (Applied Biosystems Inc., Foster City, CA, USA) and Maxima Probe qPCR Master Mix (Fermentas Thermo Fisher Scientific Inc.), according to the manufacturer instructions [22]. The IFN-γ +874 T/A (rs2430561) polymorphism was determined as previously described [23]. The thermal cycling conditions were 95°C for four minutes, followed by 40 cycles that were run for 15 seconds at 95°C, one minute at 55°C, and for 20 seconds at 68°C. Fluorescence readings were done at 68°C.

Written informed consent was obtained from all the women enrolled in the study. The protocol of the study was reviewed and approved by the Ethics Committee, Faculty of Medicine, University of Belgrade, decision number 29/XI-2.

Statistical analyses

Comparisons between genotype and allele frequencies in different populations were performed using the Pearson's χ^2 test, Fisher's exact test, or the Kruskal–Wallis test, followed by the Mann–Whitney U test, as appropriate. All

genotype frequencies were in Hardy–Weinberg equilibrium. Significance of differences was carried out at the probability level of $p < 0.05$.

RESULTS

Distribution of proinflammatory cytokine allele and genotype frequencies in cytological findings

The distribution of proinflammatory cytokine genotype frequencies in cytological findings is shown in Table 1. Statistical analysis of the significance of differences between the groups showed a significantly higher incidence of AA high secretory genotype of TNF cytokine (AA genotype, TNF gene, 75%, $p = 0.010$) only in cases of moderate and/or severe cervical dysplasia.

Table 1. Distribution of proinflammatory cytokine genotype frequencies in cytological findings

Genotype		Cytology results								p
		NILM		ASCUS		LSIL		HSIL		
		n	%	n	%	n	%	n	%	
TNF	GG	6	85.7	5	71.4	5	83.3	0	0	
	GA	1	14.3	2	28.6	0	0	1	25	0.010*
	AA•	0	0	0	0	1	16.7	3	75	
IFN-γ	**AA°**	1	14.3	4	57.1	3	50.0	0	0	
	AT	5	71.4	2	28.6	1	16.7	4	100	0.572
	TT	1	14.3	1	14.3	2	33.3	0	0	
IL-12	**AA°**	5	71.4	2	28.6	3	50	2	50	
	AC	2	28.6	5	71.4	2	33.3	2	50	0.513
	CC	0	0	0	0	1	16.7	0	0	

• – high production; ° – low production; LSIL – low-grade squamous intraepithelial lesion; HSIL – high-grade squamous intraepithelial lesions; ASCUS – atypical squamous cells of undetermined significance; NILM – negative for intraepithelial lesion or malignancy
*statistically significant – $p < 0.05$

In other groups of cytological findings (NILM, ASCUS, LSIL), allele distribution of the TNF gene did not differ significantly. The typical GG genotype dominance (72–86%) and an extremely rare occurrence of AA genotype was detected in all other groups.

In the analyzed sample, no significant difference was confirmed in the distribution of IFN-γ and IL-12 alleles in the control (NILM) and other groups ($p = 0.572$ and $p = 0.513$).

Low incidence of homozygous AA genotype of IFN-γ, which could, as hypothesized, pose an increased risk of cervical cancer, was slightly more common in ASCUS and LSIL groups (57.1% and 50%, respectively). The heterozygous AT genotype was predominant in the control group, but also in the group with HSIL findings (71.4% and 100%, respectively).

We got similar results for the distribution of IL-12 genotypes, such as that NILM (control group) had the highest frequency of homozygous AA genotype (71.4%), while the ASCUS group showed the same frequency of AC genotype of this gene (71.4%). High-risk low production AA genotype was detected in 50% of LSIL and HSIL cytological findings.

In order to achieve better overview of the distribution and influence of homozygous genotypes, we grouped them with respect to their heterozygous combinations and compared them according to the cytological findings in the control and other groups (Table 2).

Table 2. Distribution of proinflammatory cytokine genotype of high and low production according to the cytological findings in the control and other groups

Genotype		Control group and other results			
		NILM		ASCUS, LSIL, HSIL	
		n	%	n	%
TNF	GG + GA	7	35	0	0
	AA•	13	65	4	**100**
IFN-γ	**AA°**	10	62.5	7	**87.5**
	AT+TT	6	37.5	1	12.5
IL-12	**AA°**	10	**83.3**	7	58.3
	AC + CC	2	16.7	5	41.7

• – high production; ° – low production; LSIL – low-grade squamous intraepithelial lesion; HSIL – high-grade squamous intraepithelial lesions; ASCUS – atypical squamous cells of undetermined significance; NILM – negative for intraepithelial lesion or malignancy
*statistically significant – $p < 0.05$

Despite the high production TNF AA genotype being present in 65% in the control group of HR HPV-positive women without cytological changes, compared to all the other groups, statistical significance was not detected ($p = 0.160$).

The low production AA genotype of IFN-γ, which could potentially pose an increased risk of developing cervical cancer, was more prevalent in all other groups (87.5%) compared to the control group (65%) ($p = 0.204$).

The AA low secretory genotype of the IL-12 gene is mostly present in the control group (83.3%), compared to all others (58.3% – ASCUS, LSIL, HSIL), but statistically significant difference in this case was not proved ($p = 0.178$).

Previous analysis proved that, in the tested sample of proinflammatory cytokine gene polymorphisms, the mere presence of TNF AA genotype showed a statistically significant association with the progression of cervical dysplasia in HR HPV infection.

Correlation between cofactors and proinflammatory cytokines gene polymorphisms and their associated impact on the onset of cervical dysplasia

In the continuation of the statistical analysis, we applied the correlation and multiple regressions analyses to examine the correlations between polymorphisms and analyzed cofactors including environmental and behavioral cofactors such as high parity, oral contraceptives, tobacco smoking, infection with other STDs, early sexual intercourse, promiscuity, poor socio-economic conditions, dietary and nutritional factors, etc. Table 3 shows only the significant correlations of polymorphisms found in the studied group in relation to the tested cofactors.

TNF is related to cytological findings through a strong, significant and positive correlation coefficient. This means that a higher incidence of high production AA genotype occurs with less favorable cytological findings.

Table 3. Correlations of polymorphism with cytology results, previous infections, and cervical trauma

Cytology results, previous infections, and cervical trauma	p
TNF cytology results	
Spearman Correlation	0.592
Sig. (2-tailed)	0.002*
IFN-g	
Spearman Correlation	-0.472
Sig. (2-tailed)	0.020*
IL-12 cervical trauma	
Spearman Correlation	-0.444
Sig. (2-tailed)	0.030*

*statistically significant – $p < 0.05$

Table 4. High-grade squamous intraepithelial lesions prediction model based on proinflammatory cytokine polymorphisms

Prediction model HSIL	B	Std. error	t	p
(Constant)	0.229	0.172	1.333	0.198
TNF polymorphism	0.738	0.257	2.873	0.009*
IFN-g	0.007	0.201	0.034	0.973
IL-12 polymorphism	0.124	0.194	0.640	0.529

B – regression coefficient; HSIL – high-grade squamous intraepithelial lesions; *statistically significant – $p < 0.05$

Table 3 shows that there is a moderate but significant relationship between IFN-γ gene polymorphism and previously infections of the cervix. A negative sign of correlation (-0.472), indicates that low production of this cytokine is related to a greater number of previous cervical infections and vice versa.

The association between IL-12 production, represented by low production genotype, and the occurrence of cervical lesions is indicated by a negative and significant coefficient (-0.444.) This means that the high-risk low production AA genotype of the IL-12 gene is greatly correlated with the presence of cervical lesions.

The model for multiple regression analysis showed whether any of the polymorphisms examined may serve as potential genetic biomarker of susceptibility to cervical cancer in women with persistent HR HPV infection. In this regard, the occurrence of HSIL as a severe form of cervical dysplasia was taken as a dependent variable in relation to all the variables analyzed (Table 4). Through variance reduction, the step-by-step selection process identified only one significant predictor, namely the high production AA TNF genotype. This TNF gene polymorphism is significant in predicting the onset of HSIL in women with HR HPV infection.

DISCUSSION

In our previous study, a total of 541 women were processed and tested for the presence of HPV, out of which 105 were HPV-positive (19.4%) and 84 (15.5%) were HR HPV-positive [24]. The final investigated group included 84 women infected with HR HPV who were classified into four subgroups according to their cytological status of the cervix. The interview-based questionnaire which queried information of significance for HPV-related cervical disorders was administered to all the participants involved in the study. The cofactors found to be of significance in older age (46.7 ± 12.2 on average), body mass index > 25, lower educational level, long-term smoking (more than 20 years), previous genital infections and cervical interventions.

This study showed that, among the different groups of cytological findings, only the high production AA TNF genotype occurred with a significantly higher incidence in women with lesions of moderate to severe intensity. There was no significant difference between the other groups, both in genotypes determining TNF gene expression and in IFN-γ and IL-12 gene expressions. AA genotype prevailed in the lesions of moderate to severe intensity while in the other groups (PAP II, ASCUS, LSIL), the GG genotype was detected in 72–86% of findings. Correlation and multiple regression analysis showed that there was a highly significant, positive correlation coefficient between TNF and associated cytological findings. This means that the high production genotype of this cytokine was recorded in less favorable cytological findings.

We concluded that the presented sample of the analyzed cytokine gene polymorphisms provided indications of some of the presumed production trends that may have protective or better yet favoring significance for the occurrence of cervical cancer associated with HR HPV, but we were not able to prove the statistical significance in this sample size.

Previous analysis proved that, in the tested sample of proinflammatory cytokine gene polymorphisms, the mere presence of TNF AA genotype showed a statistically significant association with the progression of cervical dysplasia in HR HPV infection.

The association between SNP at position -308 and cervical cancer has been reported in many studies conducted among different races, where the presence of A allele has been associated with an increased risk of cervical cancer development [23].

In certain ethnic groups, SNP at position -308 has been shown to be associated with an increased risk of cervical cancer, but in some studies these results have not been confirmed [25].

Similar results were found in a study showing that A allele carriers were at higher risk for cervical cancer than individuals with both G alleles [26, 27].

In contrast, a study conducted in Africa showed that TNF gene polymorphism at position -308 had no effect on the development of cervical cancer [17].

Our results of the SNP gene study showed that there was no significant difference in allele distribution and IFN-γ production among patient groups with different cytological and colposcopic findings. Low IFN-γ production, determined by homozygous AA genotype, was found to be more common in the ASCUS and LSIL cervical findings, whereas the heterozygous AT genotype was more prevalent in the control and HSIL groups of patients.

Our study also established the existence of a significant relationship between IFN-γ gene polymorphism and earlier infections. A negative sign of correlation indicated that low

productions of this gene was related to a greater number of previous infections and vice versa.

The IFN-γ polymorphism at position +874 is associated with increased predisposition and progression of a number of diseases, including cervical cancer. The AA genotype (homozygous genotype) is responsible for low cytokine expression, since the DNA sequence containing T allele is a specific binding site for transcription factor NFκB, responsible for higher gene transcription and IFN-γ production. An *in vitro* study indicated that AA polymorphism is associated with a low AT polymorphism with a moderate and TT polymorphism with a high IFN-γ production [18]. These conclusions were also confirmed by a study by Zhou et al. [9], in which the IFN-γ AA genotype and the A allele were significantly more common in patients with CIN than healthy individuals.

The results of this study, as in the case of IFN-γ gene polymorphism, showed that there were no significant differences in IL-12 gene polymorphisms distribution and production among different groups of cytological findings. Low-production homozygous AA genotype had the highest incidence in the control group, whereas AC genotype was most common in the ASCUS group. The low-production AA genotype was detected in 50% of the LSIL and HSIL cytological findings.

This study showed that AA low-production genotype of the IL-12 is associated with the occurrence of cervical lesions by a strong, negative, and significant coefficient. This means that IL-12 low-production gene polymorphism correlates greatly with the presence of cervical lesions.

IL-12B polymorphism is associated with the development of diseases resulting from changes in IL-12 synthesis and secretion. A small number of studies have examined the correlation between IL-12B gene polymorphism and the development of cervical cancer. Studies conducted in Korea have shown that IL-12B with AC/CC genotypes (rs3212227) increases the risk of cervical cancer, but also that the difference is not statistically significant compared to women in whom this combination was not detected [28]. Similar results were reported in a study conducted in China. The IL-12B gene polymorphism (rs3212227) was not significantly correlated with an increased risk for the development of cervical pathological changes. A study conducted in China also suggested that IL12-B (rs3212227) AC/CC genotypes may, in some individuals, increase the risk of developing high-grade cervical lesions, especially if associated with a higher number of births [21].

By studying the IL-12 gene polymorphisms, we found that the AC genotype was not associated with the onset of cervical changes, whereas the AA genotype was significantly more common in patients with severe cervical dysplasia and cervical cancer. The AC genotype occurs more frequently in healthy individuals, indicating that the C allele has a protective role in the development of cervical lesions and their progression [20].

SNPs as genetic markers have received considerable attention from researchers in the past decade. Identifying SNPs in the gene's coding region that change the protein's amino acid sequence, which have been investigated in this study, in correlation with behavioral cofactors, can be helpful from a diagnostic point of view. According to the latest literature, statistical correlation also exists between polymorphisms present in the non-coding regions of the gene and enhanced risk of cervical cancer. But the effects of this polymorphisms residing in the non-coding regions (i.e., introns, promoters, 3′ and 5′ termini, etc.) have been investigated to a lesser extent as their exact mechanism of action is unknown and, therefore, demands additional attention [29].

CONCLUSION

It is known that polymorphisms of certain cytokine genes encoding proteins involved in Th1 and Th2 cellular responses may be associated with better or worse prognosis of cervical disease in women with persistent HR-HPV infection. Therefore, single nucleotide polymorphisms of TNF, IFN-γ and IL-12 genes may be considered as biomarkers that may have a predictive role in the development of cervical cancer, but further research would contribute to a better understanding of this subject. Also, combination of different gene polymorphism in one individual, a so-called gene profile, should be taken into consideration in future studies.

ACKNOWLEDGEMENT

The paper is part of Dijana Tasić-Tomić's doctoral thesis and presents some of the results that were published in the thesis; Dijana Tasić-Tomić. Cytokine genes polymorphisms of TNF-α, IFN-γ and IL-12 as potential predictors in the onset of cervical disease associated with HR-HPV infections [dissertation]. Serbia (RS): Faculty of Medicine University of Belgrade; 2019.

This study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Research Grant No. 175073.

Conflict of interest: None declared.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49. [DOI: 10.3322/caac.21660] [PMID: 33538338]
2. Mattiuzzi C, Lippi G. Cancer statistics: a comparison between World Health Organization (WHO) and Global Burden of Disease (GBD). Eur J Public Health. 2020;30(5):1026–7. [DOI: 10.1093/eurpub/ckz216] [PMID: 31764976]

3. Song B, Ding C, Chen W, Sun H, Zhang M, Chen W. Incidence and mortality of cervical cancer in China, 2013. Chin J Cancer Res. 2017;29(6):471–6. [DOI: 10.21147/j.issn.1000-9604.2017.06.01] [PMID: 29353969]
4. McBride AA. Oncogenic human papillomaviruses. Philos Trans R Soc Lond B Biol Sci. 2017;372(1732):20160273. [DOI: 10.1098/rstb.2016.0273] [PMID: 28893940]
5. Okunade KS. Human papillomavirus and cervical cancer. J Obstet Gynaecol. 2020;40(5):602–8. [DOI: 10.1080/01443615.2019.1634030] [PMID: 31500479]
6. Zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer. 2002;2(5):342–50. [DOI: 10.1038/nrc798] [PMID: 12044010]
7. Wang Z, Wang J, Fan J, Zhao W, Yang X, Wu L, et al. Risk factors for cervical intraepithelial neoplasia and cervical cancer in Chinese women: large study in Jiexiu, Shanxi Province, China. J Cancer. 2017;8(6):924–32. [DOI: 10.7150/jca.17416] [PMID: 28529603]
8. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. [DOI: 10.3322/caac.21492] Erratum in: CA Cancer J Clin. 2020 Jul;70(4):313. [PMID: 30207593]
9. Zhou C, Tuong ZK, Frazer IH. Papillomavirus Immune Evasion Strategies Target the Infected Cell and the Local Immune System. Front Oncol. 2019;9:682. [DOI: 10.3389/fonc.2019.00682] [PMID: 31428574]
10. Yuan Y, Cai X, Shen F, Ma F. HPV post-infection microenvironment and cervical cancer. Cancer Lett. 2021;497:243–54. [DOI: 10.1016/j.canlet.2020.10.034] [PMID: 33122098]
11. Ghebre RG, Grover S, Xu MJ, Chuang LT, Simonds H. Cervical cancer control in HIV-infected women: Past, present and future. Gynecol Oncol Rep. 2017;21:101–8. [DOI: 10.1016/j.gore.2017.07.009] [PMID: 28819634]
12. Stelzle D, Tanaka LF, Lee KK, Khalil AI, Baussano I, Shah ASV, et al. Estimates of the global burden of cervical cancer associated with HIV. Lancet Glob Health. 2020;9(2):E161–E169. [DOI: 10.1016/S2214-109X(20)30459-9] [PMID: 33212031]
13. Duvlis S, Dabeski D, Cvetkovski A, Mladenovska K, Plaseska-Karanfilska D. Association of TNF-a (rs361525 and rs1800629) with susceptibility to cervical intraepithelial lesion and cervical carcinoma in women from Republic of North Macedonia. Int J Immunogenet. 2020;47(6):522–8. [DOI: 10.1111/iji.12506] [PMID: 32662227]
14. De Moura EL, da Silva DM, dos Santos ACM, da Silva AF, Neto ABL, dos Santos IF, et al. TNF-α-308A>G and IL-10-819C>T polymorphisms as risk factors for cervical cancer: A case-control study, meta-analysis and trial sequential analysis. Meta Gene. 2021;29:100943. [DOI: 10.1016/j.mgene.2021.100943]
15. Yang J, Wang Y, Zhang S, Li Y, Li C, Liu W, et al. The Association of TNF-α Promoter Polymorphisms with Genetic Susceptibility to Cervical Cancer in a Chinese Han Population. Int J Gen Med. 2022;15:417–27. [DOI: 10.2147/IJGM.S350263] [PMID: 35046703]
16. Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-g production. Hum Immunol. 2000;61(9):863–6. [DOI: 10.1016/s0198-8859(00)00167-1] [PMID: 11053629]
17. Bao H, Sun X, Zhang Y, Pang B, Li H, Zhou L, et al. The artificial intelligence-assisted cytology diagnostic system in large-scale cervical cancer screening: A population-based cohort study of 0.7 million women. Cancer Med. 2020;9(18):6896–906. [DOI: 10.1002/cam4.3296] [PMID: 32697872]
18. Mehta AM, Mooij M, Branković I, Ouburg S, Morré SA, Jordanova ES. Cervical Carcinogenesis and Immune Response Gene Polymorphisms: A Review. J Immunol Res. 2017;2017:8913860. [DOI: 10.1155/2017/8913860] [PMID: 28280748]
19. Kiddane AT, Kang MJ, Ho TC, Getachew AT, Patil MP, Chun BS, et al. Anticancer and Apoptotic Activity in Cervical Adenocarcinoma HeLa Using Crude Extract of Ganoderma applanatum. Curr Issues Mol Biol. 2022;44(3):1012–26. [DOI: 10.3390/cimb44030067] [PMID: 35723290]
20. Chang SW, Xu GQ, Fan YL. Association of interleukin-12 gene polymorphisms with cancer susceptibility: a meta-analysis. Int J Clin Exp Med. 2015;8(4):5317–22. [PMID: 26131107]
21. Song J, Zhang Q, Wang R, Sun M, Jin S. Associations of IL-12, IL12R polymorphisms and serum IL-12 levels with high-risk human papillomavirus susceptibility in rural women from Luohe, Henan, China. Medicine (Baltimore). 2019;98(38):e16991. [DOI: 10.1097/MD.0000000000016991] [PMID: 31567936]
22. Popadic S, Savic E, Markovic M, Ramic Z, Medenica L, Pravica V, et al. TNF, IL12B, and IFNG Gene Polymorphisms in Serbian Patients with Psoriasis. Ann Dermatol. 2015;27(2):128–32. [DOI: 10.5021/ad.2015.27.2.128] [PMID: 25834350]
23. Popadic D, Savic E, Spuran Z, Markovic M, Mostarica Stojkovic M, Ramic Z, et al. Distinctive frequencies of +874T/A IFN-γ gene polymorphism in a healthy Serbian population. Clin Transl Sci. 2012;5(6):461–3. [DOI: 10.1111/cts.12000] [PMID: 23253667]
24. Tasic D, Lazarevic I, Knezevic A, Tasic L, Pikula A, Perisic Z, et al. The impact of environmental and behavioural cofactors on the development of cervical disorders in HR-HPV-infected women in Serbia. Epidemiol Infect. 2018;146(13):1714–23. [DOI: 10.1017/S0950268818001668] [PMID: 29923470]
25. Tian S, Yang X, Zhang L, Zhao J, Pei M, Yu Y, et al. Polymorphic variants conferring genetic risk to cervical lesions support GSTs as important associated loci. Medicine (Baltimore). 2019;98(41):e17487. [DOI: 10.1097/MD.0000000000017487] [PMID: 31593112]
26. Babapour N, Mehramiz M, Rastgar Moghadam A, Behboodi N, Yousefi Z, Maftouh M, et al. Association of TNF-308 G>A polymorphism located in tumor necrosis factor a with the risk of developing cervical cancer and results of pap smear. J Cell Biochem. 2019;120(4):5444–8. [DOI: 10.1002/jcb.27823] [PMID: 30387170]
27. Lei J, Ploner A, Elfström KM, Wang J, Roth A, Fang F, et al. HPV Vaccination and the Risk of Invasive Cervical Cancer. N Engl J Med. 2020;383(14):1340–8. [DOI: 10.1056/NEJMoa1917338] [PMID: 32997908]
28. Han SS, Cho EY, Lee TS, Kim JW, Park NH, Song YS, et al. Interleukin-12 p40 gene (IL12B) polymorphisms and the risk of cervical cancer in Korean women. Eur J Obstet Gynecol Reprod Biol. 2008;140(1):71–5. [DOI: 10.1016/j.ejogrb.2008.02.007] [PMID: 18367309]
29. Das AP, Saini S, Agarwal SM. A comprehensive meta-analysis of non-coding polymorphisms associated with precancerous lesions and cervical cancer. Genomics. 2022;114(3):110323. Online ahead of print. [DOI: 10.1016/j.ygeno.2022.110323] [PMID: 35227837]

Полиморфизми цитокинских гена *TNF*, *IFN*-гама и *IL*-12 као могући предиктори настанка цервикалне болести код жена позитивних на *HR HPV* са кофакторима ризичног понашања

Дијана Тасић-Томић[1], Вера Правица[2,3], Лидија Тасић[1,2], Азра Лукач[4], Мирза Шачић[5], Маја Ћупић[2,3]

[1]Гинеколошко-акушерска клиника „Народни фронт", Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија;
[3]Институт за микробиологију и имунологију, Београд, Србија;
[4]Дом здравља Рожаје, Рожаје, Црна Гора;
[5]Општа болница Нови Пазар, Нови Пазар, Србија

САЖЕТАК

Увод/Циљ Циљ ове студије је био да се испита дистрибуција генотипова и алела проинфламаторних цитокина *TNF*, *IFN*-гама и *IL*-12 и њихов утицај на настанак цервикалне болести, као и да се утврди њихов удружени утицај са кофакторима код *HR HPV* позитивних жена у Србији.

Методе Испитали смо 24 болеснице и на основу цитолошког налаза их поделили у четири групе: *PAP II*, *ASKUS*, *LSIL* и *HSIL*. Анализа полиморфизама *TNF*, *IFN*-гама и *IL*-12 извршена је методом *Real-time PCR TaqMan*. Статистичка анализа урађена је употребом параметарских и непараметарских тестова и корелационе и мултипле регресионе анализе.

Резултати Значајно већа учесталост високосекреторног генотипа *TNF AA* утврђена је у тежим облицима дисплазије. Позитивна корелација између високосекреторног полиморфизма *TNF* и цервикалних промена била је високо значајна. Утврђена је умерена, значајна корелација између нискосекреторног *IFN*-гама и ранијих цервикалних инфекција. Постоји значајна повезаност нискосекреторног генотипа *IL*-12 са раницама на грлићу материце.

Закључак Резултати ове студије показују да су *HSIL* промене у вези са значајно већом учесталошћу високосекреторног генотипа *TNF AA*. С обзиром на то да се полиморфизми одређених цитокинских гена који кодирају протеине укључене у *Th1* и *Th2* ћелијски одговор повезују са добром односно неповољном прогнозом цервикалне болести код жена са перзистентном *HR HPV* инфекцијом, могу се сматрати биомаркерима са предиктивном улогом у развоју цервикалног карцинома.

Кључне речи: цервикални карцином; кофактори; генски полиморфизми; *TNF*; *IFN*-гама; *IL*-12