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ORIGINAL RESEARCH

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Comparison of results of HPV infections and dysplastic changes of the uterine cervix in populations in Vojvodina (north region of Serbia) and Republic of North Macedonia

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Abstract

This study assesses human papilloma virus Human (HPV) infection prevalence among women in Vojvodina, Serbia and North Macedonia, considering cytological status and cervix-related pathological changes. The main goal of this study is to compare the HPV status (types of HPV, age of patients, histopathology diagnosis, *etc.*) in these two populations. The EUROArray HPV test, detecting 30 genotypes, was utilized on 1562 women (740 in Vojvodina and Serbia, 822 in North Macedonia) aged 20 to 80 with various cytological results. 164 of the 576 Serbian/Vojvodina samples that were categorised as negative of intraepithelial lesion or malignacy (NILM) had aberrant histology low grade squamous intraepithelial lesion/high grade squamous intraepithelial lesion (LSIL/HSIL cervical malignancy). Carcinogenic HPV genotypes were found in 252 (55%) samples, with 76.2% in the LSIL/HSIL/cervical cancer category. Top abnormal histopathology genotypes included HPV 16, 33, 31 and 56, while 18 and 39 were equally verified. Genotype 16 prevalence increased with higher histopathological grades: 18.8% in LSIL, 31.9% in HSIL, and 75% in cervical cancer samples. Among the North Macedonian samples, 221 had aberrant histopathology and 601 displayed NILM. Carcinogenic HPV genotypes were found in 43.4% of cases, with 70.1% in the LSIL/HSIL/cervical cancer category. Predominant genotypes associated with abnormal histopathology results included HPV 16, 31 and 35, with 18 and 52 equally verified. The most frequent genotype associated with higher-grade histopathological findings was genotype16, found in 20.1% of LSIL, 34.3% of HSIL, and 78% of cervical cancer cases. Multiple associated HPV genotypes were not correlated with histopathology. Older patients tended to exhibit higher-grade lesions when comparing histopathological diagnosis and age.

Keywords

Cervical cancer; HPV genotype; Prevalence

1. Introduction

The human papillomavirus (HPV) is the most common sexually transmitted virus worldwide and a leading cause of illness and death [1]. In 2020 there were 604,000 new cases and 342,000 deaths caused by cervical cancer, as the fourth most common cancer in women globally [2]. Almost all cervical cancers are caused by HPV and the whole process affects mental and physical health, as well as reproductive outcomes [3]. About 40 HPV genotypes among the more than 200 HPV genotypes that have been found so far infect the genital area, with 13–15 of the high-risk genotypes thought to be carcinogenic. 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68 are the high-risk HPVs [4, 5]. HPV infection is mostly multifocal, and it influences organs in lower reproductive tract. There is a possibility of developing cervical intraepithelial neoplasia (CIN) and cancer in several years in HPV positive patients. In the first two years, 90% of women under 30 naturally eliminate the virus, while the remaining 10% may get invasive cervical cancer, dysplasia or condyloma.

According to the data of the Institute of Public Health, cervical cancer is the sixth most common cancer among women in Republic North of Macedonia (RNM) in the last 10 years, with an incidence of 17.57/100,000 and an average of 51 deaths per year [6]. According to data from the Cancer Registry of the Vojvodina Public Health Institute, 298 newly diagnosed women were registered in Vojvodina in 2021, and 135 women died from cervical cancer [7].

It is anticipated that between 2018 and 2030, there would be a yearly increase in cervical cancer cases from 570,000 to

700,000, with 311,000 to 400,000 deaths [8]. To fulfil the WHO goal of eliminating cervical cancer by 2030, 70% of women must be screened with a high-performance test by the age of 35 and then again by the age of 45 [9].

The objective of this study was to investigate the prevalence and distribution of various HPV genotypes in a group of female patients exhibiting normal cytological findings. Additionally, the research aimed to assess the oncogenic potential of HPV infection genotypes in a specific group of female patients with histopathology confirmed dysplasia and cervical cancer. The study focused on the geographic regions of Vojvodina, located in the northern part of Serbia, and North Macedonia.

2. Material and methods

The research was conducted retrospectively at the Institute of Pathology and University Clinic of Gynecology and Obstetrics in Skopje, North Macedonia, the Oncology Institute of Vojvodina (OIV), and the Institute of Public Health of Vojvodina (IPHV). The study utilized data extracted from the medical records of patients who underwent treatment for cervical intraepithelial neoplasia or cervical cancer at the Department of Gynecology, Clinic for Surgical Oncology, Oncology Institute of Vojvodina in Sremska Kamenica, and the Department of Gynecology, Clinic for Surgical Oncology, Oncology Institute of Skopje, North Macedonia. The collected data was from the period from 2016 to 2021. Laboratory findings from the IPHV were included for a subset of patients with normal cytological results from the Papanicolau (PAP) smear.

The source material consisted of archival records from the OIV and IPHV, derived from medical documentation on histopathological material obtained during operations, cytological findings, identified HPV genotypes, and the age of the patients.

Data on the total number of instances in Republic North of Macedonia were gathered from patients whose attending physicians registered them in the state's official "My Appointment" database throughout the aforementioned time frame. These people looked for medical assistance or treatments pertaining to cervical cancer diagnosis and therapy as well as dysplatics alterations. Cases of dysplastic alterations and cervical cancer were identified using the diagnostic criteria based on the International Statistical Classification of Disease and Related Health Problems, Tenth Revision (ICD-10) code N87-C53. The Republic of North Macedonia's State Statistical Office provides official annual population numbers that cover the entire year up until December 31st. This is where the population data came from.

Inclusion criteria: —group of female patients with validated HPV typing on a cervical smear and a regular cytological PAP test; —female patients older than 18 years with verified histopathological cervical intraepithelial neoplasia or cervical cancer; —female patients with HPV typing on a cervical smear.

Non-inclusion criteria: —Patients who are female and have not had HPV typing done; they include pregnant women; patients with other malignant conditions or immunocompromised illnesses; patients with recurrent cervical dysplasia; and patients with histopathologically confirmed cervical intraepithelial neoplasia or cervical cancer [10].

The selection of participants (based on inclusion/exclusion criteria), the application of the protocol, the use of an HPV test, and staff competency were given particular consideration [11].

Through the analysis of documentation from the OIV and the IPHV, applying the specified sample selection criteria, a total of 164 patients with cervical pathological changes and 567 patients with regular cytological smears were included for further data processing. Conventional Papanikolau smear was used for collecting data that were used for the analyses.

Similarly, by analysing the documentation from the Department of Gynecology, Clinic for Surgical Oncology, Oncology Institute of Skopje, North Macedonia, and applying the criteria for sample selection, 221 patients with cervical pathological changes and 601 patients with regular cytological smears were included in further data processing. The cytology findings included in the analysis were also obtained through conventional Papanikolau smear.

By colposcopy guidance in patients with abnormal findings biopsies were performed and in some endocervical curettage.

In each EUR The genotyping of HPV DNA in cervical swab samples for the entire cohort was conducted using a qualitative amplification and hybridization test. Viral DNA extraction utilized the SaMag STD DNA Extraction Kit, with the SaMag-12 Automatic Nucleic Acids Extraction System (Sacace Biotechnologies, Como, Italy), following the manufacturer's instructions. OArray HPV test, 5 μL of the extracted DNA was used. Storage of processed samples was done at 2–8 °C for a maximum of five days or frozen at −80 °C for longer periods if amplification was not performed on the same day.

The EUROArray HPV test (EUROIMMUN, Luebeck, Germany) was employed for the HPV genotyping assay, detecting 30 HPV genotypes in a single reaction. This included 18 high-risk HPVs (HR HPV) genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) and 12 low-risk HPVs (LR HPVs) (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, 89). Amplification and hybridization steps allowed identification of both the target viral genetic material (E6/E7 genes) and the human HSP90 gene used as endogenous controls. The genotyping results were considered valid if all test controls met the manufacturer's instructions' satisfactory criteria.

The statistical software IBM SPSS Statistics (Version 23.0, Property of IBM Corp., registered in many jurisdictions worldwide) is used to process statistical data.

The statistical techniques listed below were used:

Percentages of structure are determined (%) in the series with attributive marks (NILM cytology (HPV negative, HPV positive, HR HPV positive, LR HPV positive, HR + LR HPV positive), HPV genotype (of NILM samples), Histopathology (LSIL, HSIL, Cervical cancer), and HPV genotype (Histopathology (LSIL, HSIL, Cervical cancer);

Fisher's Exact examine/Monte Carlo Sig. (2-sided)/(p) Pearson Chi-Square (p), and Pearson Chi-Square/Monte Carlo Sig. (2-sided) were used to examine the differences in the series with attributive features between Serbia and North Macedonia. A significance threshold of (p) < 0.05 was applied.

Both tabular and graphical forms of the data are displayed.

3. Results

The patients were categorized into two groups based on the country of enrolment: a Serbian group comprising 731 women aged 20 to 80 with varied cytological results and a North Macedonian group consisting of 822 women.

Vojvodina and North Macedonia with a normal cytological spread (negative for intraepithelial lesion or malignancy, NILM) are the subject of the results displayed in Table 1 and Fig. 1.

TABLE 1. Prevalence of HPV infection with normal cytological findings in Vojvodina and North Macedonia.

NILM cytology	HPV		Total
	Negative	Positive	
State			
Vovodina	325, 57.3%	242, 42.7%	567, 100.0%
North Macedonia	340, 56.6%	261, 43.4%	601, 100.0%
Total	665, 56.9%	503, 43.1%	1168, 100.0%

NILM: Negative for Intraepithelial Lesion or Malignancy; HPV: human papillomavirus.

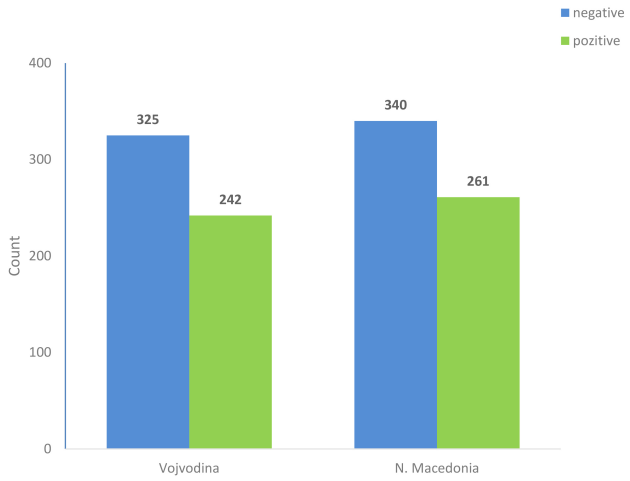

FIGURE 1. HPV infection with normal cytological findings in Vojvodina and North Macedonia.

Of the 567 patients in Vojvodina, 242 (42.7%) had an HPV infection that was confirmed, and 325 (57.3%) had an HPV negative result. Out of 601 patients in North Macedonia, 261 (43.4%) had HPV infection verified, while 340 (56.6%) had HIV negative results. There is no discernible difference between the distribution of HPV infection with normal cytological results in North Macedonia and Vojvodina (Pearson Chi-Square = 0.66 and $p > 0.05$ ($p = 0.797$)/2-sided).

Patients from Vojvodina and North Macedonia whose HPV infection was confirmed are included in the results displayed in Table 2 and Fig. 2.

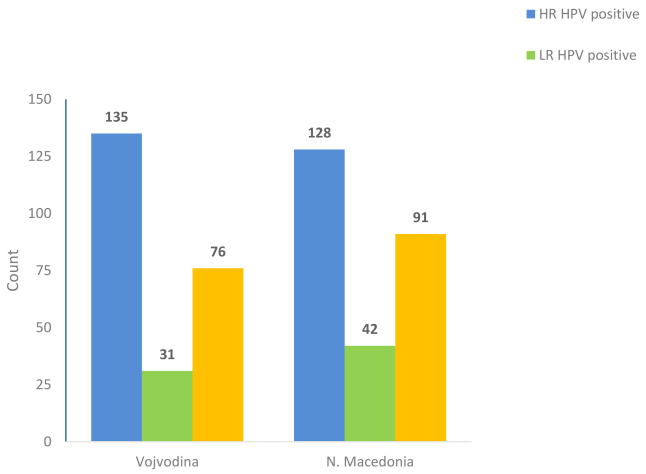

FIGURE 2. HPV infection with normal cytological findings in Vojvodina and North Macedonia/HPV positive.

In Vojvodina, of 242 patients, 135 (55.8%) had HR HPV infection, 31 (12.8%) had LR HPV infection, and 76 (31.4%) had a mixed group (HR + LR HPV) with proven HPV infection.

In North Macedonia, findings indicated that of 261 patients, 128 (49.0%) had HR HPV infection, 42 (16.1%) had LR HPV infection, and 91 (34.9%) had mixed HPV infection (HR + LR HPV).

The distribution of confirmed HPV infection in Vojvodina and North Macedonia is displayed with no significant difference for Pearson Chi-Square = 2.477 and $p > 0.05$ ($p = 0.287$)/Monte Carlo Sig. (2-sided)/0.275 − 0.299.

Table 3 and Fig. 3 present the HPV genotype prevalence in Vojvodina and North Macedonia. In Vojvodina, patients with negative cytology results were found to have HPV genotype 16 (26.2%), followed by genotypes 31 (15.9%), 51 (10.7%) and 18 (7.9%), with genotype 45 (0.8%) having the lowest

TABLE 2. Prevalence of HPV infection with normal cytological findings in Serbia and North Macedonia/HPV positive.

State	HPV positive			Total
	HR HPV positive	LR HPV Positive	HR + LR HPV positive	
Vojvodina	135, 55.8%	31, 12.8%	76, 31.4%	242, 100.0%
North Macedonia	128, 49.0%	42, 16.1%	91, 34.9%	261, 100.0%
Total	263, 52.3%	73, 14.5%	167, 33.2%	503, 100.0%

HR HPV: High-Risk HPV; LR HPV: Low-Risk HPV.

TABLE 3. Prevalence of HPV genotypes in female patients with NILM cytological findings/Carcinogens genotype.

HPV Genotypes	State		Total
	Vojvodina	North Macedonia	
16	66, 26.2%	70, 26.8%	136, 26.5%
31	40, 15.9%	46, 17.6%	86, 16.8%
51	27, 10.7%	30, 11.5%	57, 11.1%
18	20, 7.9%	18, 6.9%	38, 7.4%
56	17, 6.7%	13, 5.0%	30, 5.8%
39	17, 6.7%	10, 3.8%	27, 5.3%
59	14, 5.6%	16, 6.1%	30, 5.8%
52	14, 5.6%	13, 5.0%	27, 5.3%
33	14, 5.6%	13, 5.0%	27, 5.3%
58	13, 5.2%	13, 5.0%	26, 5.1%
35	8, 3.2%	16, 6.1%	24, 4.7%
45	2, 0.8%	3, 1.1%	5, 1.0%
Total	252, 100.0%	261, 100.0%	513, 100.0%

HPV: human papillomavirus.

TABLE 4. Histopathology characteristics of the examined female patients.

	State		Total
	Vojvodina	North Macedonia	
Histopathology			
LSIL	49, 29.9%	71, 32.1%	120, 31.2%
HSIL	90, 54.9%	118, 53.4%	208, 54.0%
Cervical Cancer	25, 15.2%	32, 14.5%	57, 14.8%
Total	164, 100.0%	221, 100.0%	385, 100.0%

LSIL: Low-grade Squamous Intraepithelial Lesion; HSIL: High-grade Squamous Intraepithelial Lesion.

representation.

HPV genotype 16 (26.8%) is the most prevalent among patients in North Macedonia with negative cytological results, followed by genotype 31 (17.6%), genotype 51 (11.5%), genotype 18 (6.9%), and the least common, genotype 45 (1.1%).

There is no significant difference in the distribution of HPV genotypes in Vojvodina and North Macedonia, as indicated by the Fisher's Exact Test values of 6.149 and $p > 0.05$ ($p = 0.878$)/Monte Carlo Sig. (2-sided)/0.868 − 0.884.

Table 4 and Fig. 4 display the results in patients who had histological verification. Of the 164 patients in Vojvodina, 25 (15.3%) had cervical cancer, 90 (54.9%) had high grade squamous intraepithelial lesions (HSIL), and 49 (29.9%) had low grade squamous intraepithelial lesions (LSIL). Among the 221 patients in North Macedonia, 32 (14.5%) had cervical cancer, 118 (53.4%) had high grade squamous intraepithelial lesions (HSIL), and 71 (32.1%) had low grade squamous intraepithelial lesions (LSIL). There is no significant difference in the distribution of patients with histopathological verification in Vojvodina and North Macedonia, as indicated by the following metrics: $p > 0.05$ ($p = 0.908$)/Monte Carlo Sig. (2-sided)/0.901 − 0.916.

Table 5 and Fig. 5 present the incidence of multiple infections and HPV genotypes in patients in Vojvodina and North Macedonia who had low-grade squamous intraepithelial lesions (LSIL). In Vojvodina, 40.6% of patients had multiple infections confirmed, 18.8% had HPV 16 confirmed, 9.4% had HPV 31, and 6.3% had HPV 39 and HPV 51 confirmed. In North Macedonia, 32.5% of patients had multiple infections confirmed; 20.0% had HPV 16, 10.0% had HPV 31 and 33, and 7.5% had HPV 51. For patients in Vojvodina and North Macedonia with low-grade squamous intraepithelial lesions (LSIL), the distribution of multiple infections and HPV genotypes is displayed for the following parameters: Fisher's Exact Test = 6.050 and $p > 0.05$ ($p = 0.989$)/Monte Carlo Sig. (2-sided)/0.986 − 0.992/no significant differences.

In Vojvodina and North Macedonia, Table 5.1 and Fig. 6 present the incidence of multiple infections and HPV genotypes in patients with high-grade squamous intraepithelial lesions (HSIL). In Vojvodina, it was found that 31.9% of patients had multiple infections, 31.9% had HPV 16 verified, 11.6% had HPV 33, 7.2% had HPV 56, and 5.8% had HPV 31. 23.9% of patients in North Macedonia had multiple infections confirmed, whereas 34.8% of patients had HPV 16, 10.9% had HPV 31, 6.5% had HPV 33, and 5.4% of female patients had HPV 56 verified.

The distribution of HPV genotypes and multiple infections in patients with high-grade squamous intraepithelial lesions

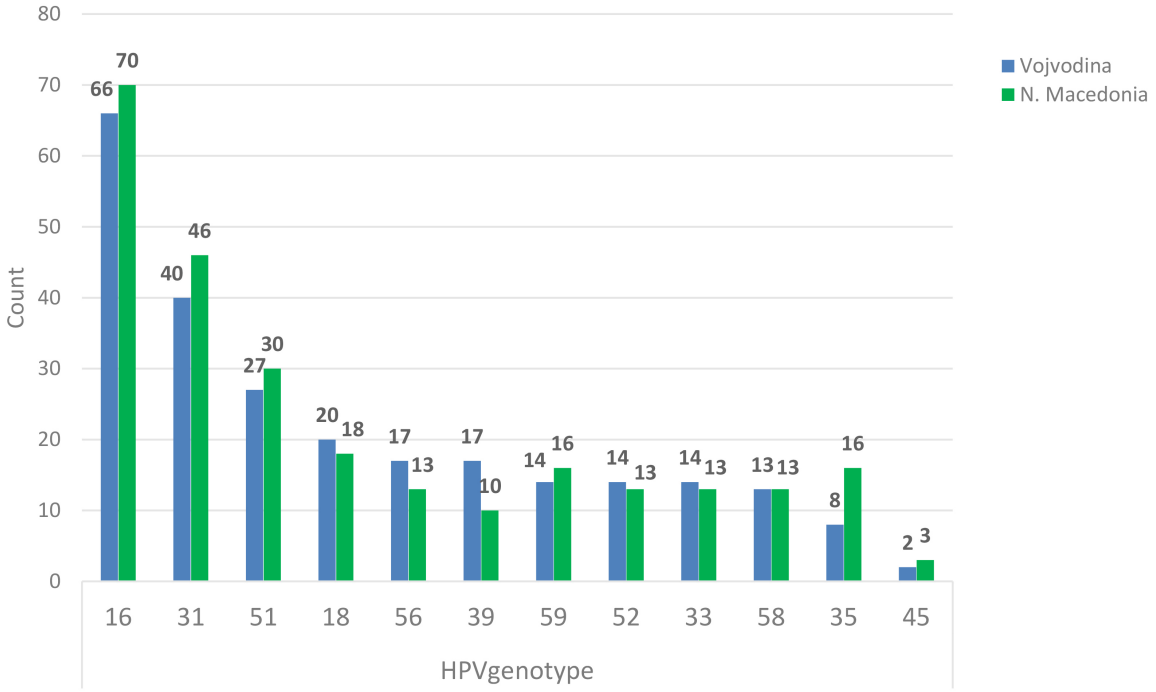

FIGURE 3. Prevalence of HPV genotypes in female patients with NILM cytological findings. HR HPV: High-Risk HPV; LR HPV: Low-Risk HPV.

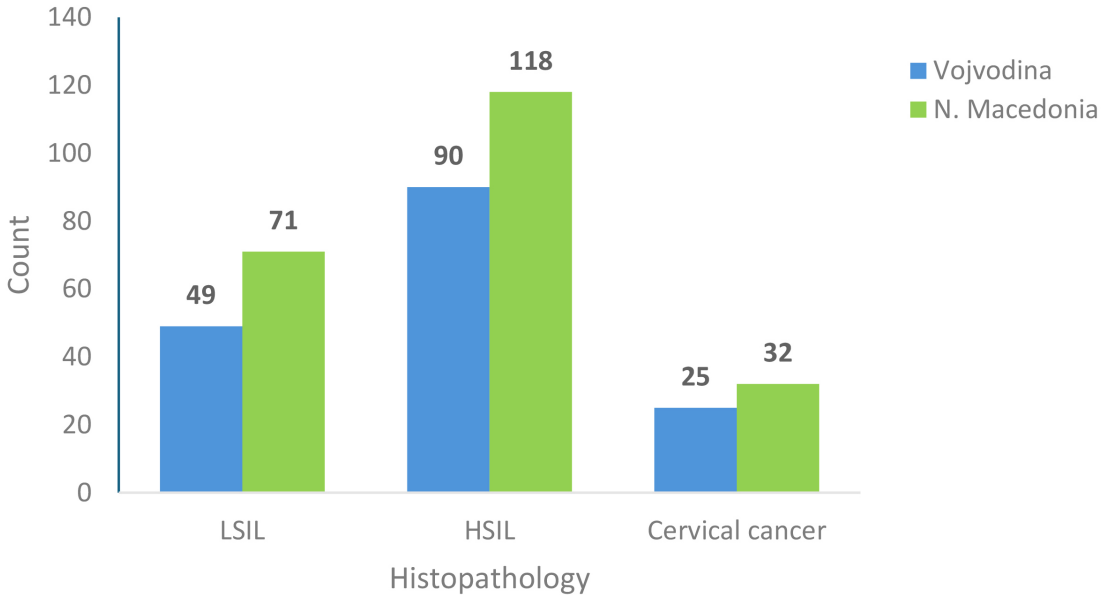

FIGURE 4. Prevalence of HPV infection obtained by cytological swabs in histopathology confirmed (LSIL/HSIL/Cervical cancer) of the cervix. LSIL: Low-grade Squamous Intraepithelial Lesion; HSIL: High-grade Squamous Intraepithelial Lesion.

(HSIL) in North Macedonia and Vojvodina is displayed, with no significant difference found for the Monte Carlo Sig. (2-sided)/0.938 − 0.950/and Fisher's Exact Test = 8.466 and $p >$ 0.05 ($p = 0.944$).

Table 5.2 and Fig. 7 illustrate the frequency of multiple infections and HPV genotypes in patients with cervical cancer in Vojvodina and North Macedonia. In Vojvodina, 8.3% of patients had multiple infections confirmed, 75.0% had HPV 16 verified, 8.3% had HPV 18, 4.2% had HPV 35, and 4.2% had HPV 45. 8.7% of patients in North Macedonia had multiple infections confirmed, 82.6% had HPV 16 verified, and 8.7% had HPV 18. There is no significant difference in the distribution of multiple infections and HPV genotypes in patients with cervical cancer in Vojvodina and North Macedonia, as indicated by the Fisher's Exact Test = 2.127 and $p > 0.05$ ($p =$ 1.000)/Monte Carlo Sig. (2-sided)/(1000) − 1000.

TABLE 5. Prevalence of multiple infections and HPV genotypes obtained by cytological swabs in histopathology confirmed (LSIL) of the cervix.

Histopathology LSIL	Stage		Total
	Vojvodina	North Macedonia	
HPV genotype			
Multiple infection	13, 40.6%	13, 32.5%	26, 36.1%
16	6, 18.8%	8, 20.0%	14, 19.4%
31	3, 9.4%	4, 10.0%	7, 9.7%
39	2, 6.3%	1, 2.5%	3, 4.2%
51	2, 6.3%	3, 7.5%	5, 6.9%
18	1, 3.1%	0, 0.0%	1, 1.4%
33	1, 3.1%	4, 10.0%	5, 6.9%
56	1, 3.1%	2, 5.0%	3, 4.2%
58	1, 3.1%	1, 2.5%	2, 2.8%
59	1, 3.1%	1, 2.5%	2, 2.8%
68	1, 3.1%	1, 2.5%	2, 2.8%
35	0, 0.0%	1, 2.5%	1, 1.4%
54	0, 0.0%	1, 2.5%	1, 1.4%
Total	32, 100.0%	40, 100.0%	72, 100.0%

LSIL: Low-grade Squamous Intraepithelial Lesion; HPV: human papillomavirus.

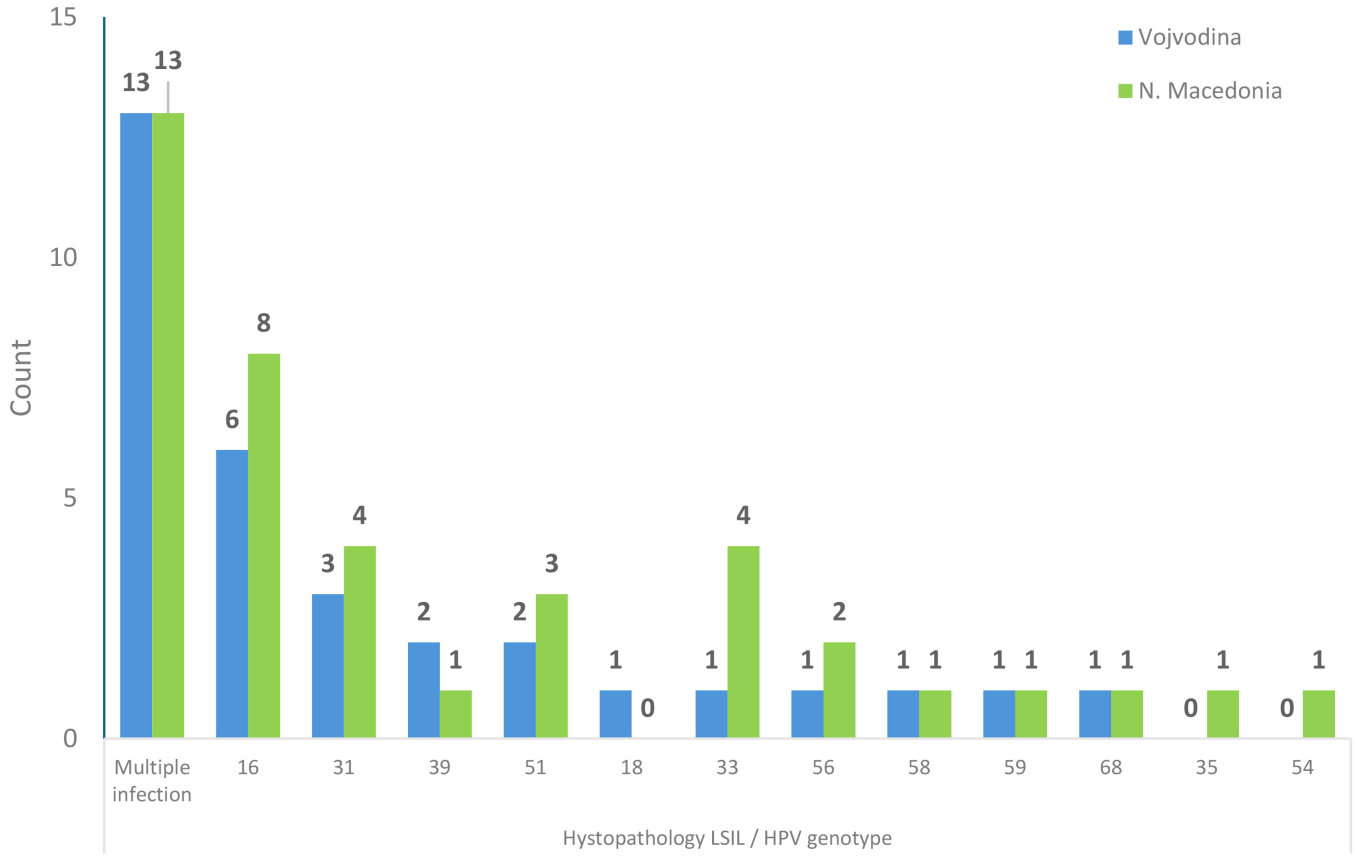

FIGURE 5. Histopathology LSIL/HPV genotype. LSIL: Low-grade Squamous Intraepithelial Lesion; HPV: human papillomavirus.

TABLE 5.1. Prevalence of multiple infections and HPV genotypes obtained by cytological swabs in histopathology confirmed (HSIL) of the cervix.

Histopathology HSIL	Stage		Total
	Vojvodina	North Macedonia	
HPV genotype			
Multiple infection	22, 31.9%	22, 23.9%	44, 27.3%
16	22, 31.9%	32, 34.8%	54, 33.5%
31	4, 5.8%	10, 10.9%	14, 8.7%
39	1, 1.4%	3, 3.3%	4, 2.5%
51	3, 4.3%	3, 3.3%	6, 3.7%
18	0, 0.0%	2, 2.2%	2, 1.2%
33	8, 11.6%	6, 6.5%	14, 8.7%
56	5, 7.2%	5, 5.4%	10, 6.2%
58	1, 1.4%	2, 2.2%	3, 1.9%
59	0, 0.0%	1, 1.1%	1, 0.6%
68	0, 0.0%	1, 1.1%	1, 0.6%
35	1, 1.4%	2, 2.2%	3, 1.9%
45	0, 0.0%	1, 1.1%	1, 0.6%
52	1, 1.4%	1, 1.1%	2, 1.2%
54	1, 1.4%	1, 1.1%	2, 1.2%
Total	69, 100.0%	92, 100.0%	161, 100.0%

HSIL: High-grade Squamous Intraepithelial Lesion; HPV: human papillomavirus.

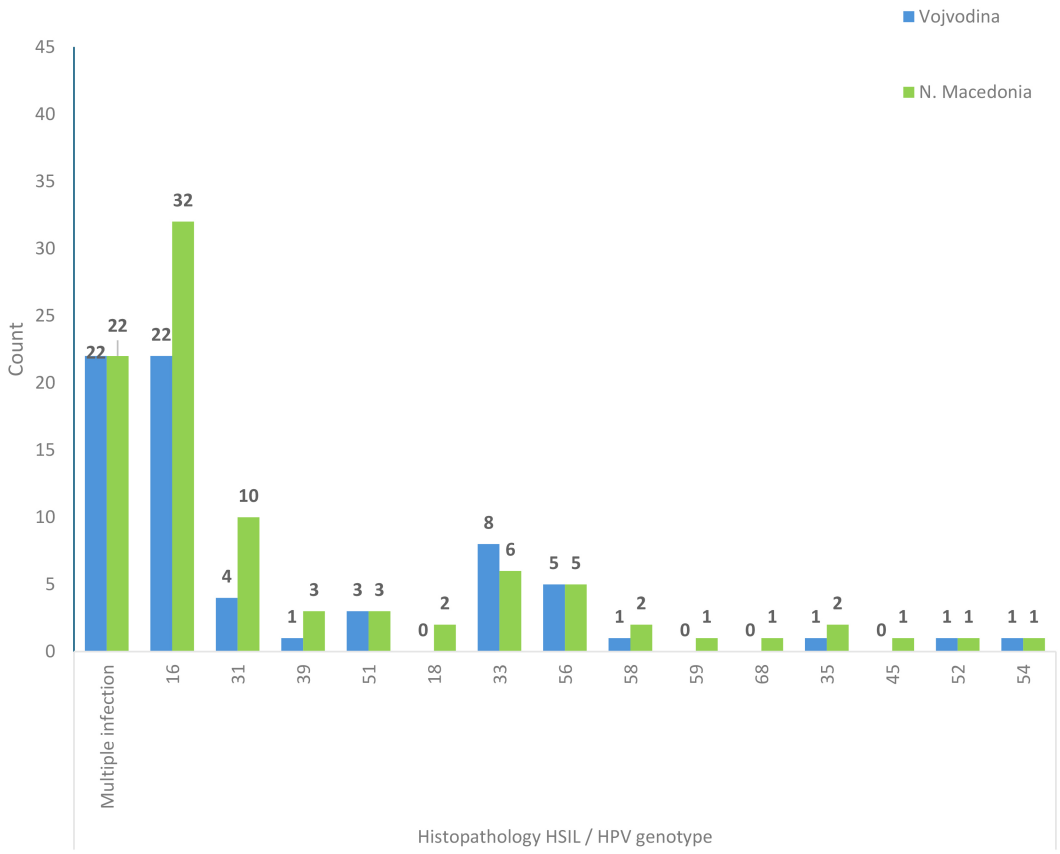

FIGURE 6. Histopathology HSIL/HPV genotype. HSIL: High-grade Squamous Intraepithelial Lesion; HPV: human papillomavirus.

TABLE 5.2. Prevalence of multiple infections and HPV genotypes obtained by cytological swabs in histopathology confirmed (Cervical cancer) of the cervix.

Histopathology Cervical cancer	Stage		Total
	Vojvodina	North Macedonia	
HPV Genotype			
Multiple Infection	2, 8.3%	2, 8.7%	4, 8.5%
16	18, 75.0%	19, 82.6%	37, 78.7%
18	2, 8.3%	2, 8.7%	4, 8.5%
35	1, 4.2%	0, 0.0%	1, 2.1%
45	1, 4.2%	0, 0.0%	1, 2.1%
Total	24, 100.0%	23, 100.0%	47, 100.0%

HPV: human papillomavirus.

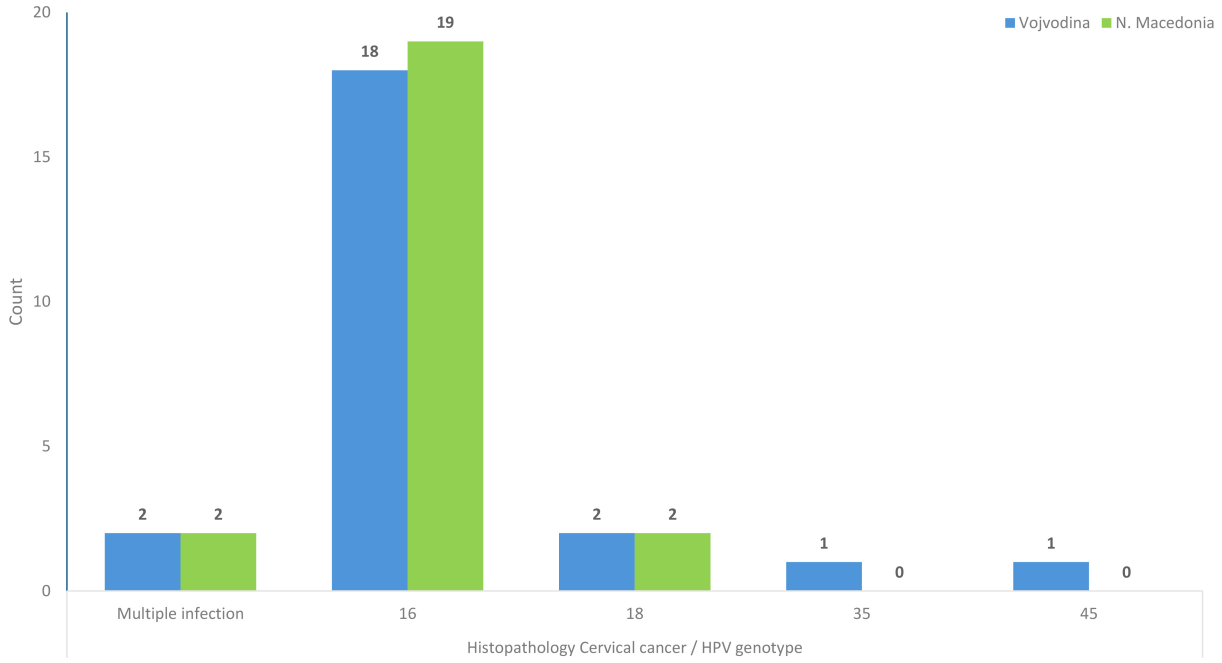

FIGURE 7. Histopathology cercical cancer/HPV genotype.

4. Discussion

To create more effective plans for the care and prevention of cervical cancer, it is beneficial to have a solid understanding of the prevalence and distribution of the HPV genotypes. Because of the various research contexts and demographics, as well as the various HPV genotypes that the employed HPV tests could detect, there was no absolute comparability in the overall HPV prevalence between different places in the studies that were accessible [12]. The results of our study demonstrated that HPV16, HPV31, HPV51 and HPV18 were the four most prevalent types in this study population, these results are consistent with earlier studies conducted in populations of Russians [13], Turks [14, 15], and Mongolians [16], where the most common kinds among women with abnormal cervical cytology were HPV16, HPV6, HPV45, HPV18, HPV53, HPV33 and HPV31. Understanding the varying incidence and genotype distribution of HPV in various countries and across various populations is crucial for optimising HPV vaccination regimens and maximising the effects of vaccination [17, 18].

The analysis of the oncogenic potential in the examined group with pathological changes in the cervix in Vojvodina and North Macedonia revealed that HPV genotype 16 predominates in high-grade squamous intraepithelial lesions (HSIL) and cancer, which is consistent with literature data from various regions. These results align with literature data. The most prevalent genotype of HPV16 is found worldwide in women with normal cytology, unvaccinated women with CIN, and women with cervical cancer [19]. In 70% of instances, HPV 16 and HPV 18 are implicated in cervical cancer [20]. For patients who test positive for HPV 16 or 18, urgent colposcopy has been implemented by numerous national cervical cancer screening programmes [21]. There is an increased incidence of CIN2 and CIN3 in the population infected with HPV 16 and 18 [22]. Our study's significant HPV 16 incidence is consistent with findings from earlier research on CIN2 women in affluent nations [23, 24].

Infections with multiple genotypes of viruses are more

prevalent in the younger population and milder lesions, 40.62% in LSIL *vs.* 8.33% in carcinoma in Vojvodina, as well as in North Macedonia (32.5% *vs.* 8.7%). However, the outcomes of some analyses were impressive. Brot *et al.* [25] pointed out that multiple HR-HPV infection is related with persistent low-grade squamous intraepithelial lesion (LSIL) and it is easier to recognize patients with a higher risk of progression to (HSIL) and cervical SCC (Squamous cell carcinoma). In contrast, Salazar *et al.* [26] suggested that multiple HPV infections can activate inter genotypic competition and immune response, and thus do not influence development of squamous intraepithelial lesion (SIL).

Interesting insights into the high frequency of other genotypes, such as HPV 31, HPV 33 and HPV 51, in various groups and lesions are also highlighted by our study. In Liaocheng City, Shandong province, the prevalence of HPV and the genotype distribution were mostly in line with the national HPV prevalence in China. The prevalence of HPV 53, HPV 39, HPV 59, HPV 66, HPV 51, HPV 56 and HPV 68 was high in this region in addition to HPV 16, HPV 52 and HPV 58. This has a significant guiding function in the optimisation and development of vaccine research [27].

Moreover, the study explores the HPV negativity in female patients with pathological changes on the cervix (LSIL/HSIL/cervical cancer). In Vojvodina, 21.78% of patients did not show HPV infection, and HPV negativity decreased with the severity of the lesion: 34.69% in LSIL, 23.33% in HSIL, and 4% in cervical cancer. Similarly, in North Macedonia, 22.17% of patients did not show HPV infection, and HPV negativity decreased with the severity of the lesion: 33.8% in LSIL, 19.5% in HSIL, and 6% in cervical cancer.

While 3 to 8% of instances of cervical cancer are HPV-negative, the majority of cases are linked to HPV. Cervical squamous cell carcinoma often has an HPV positive diagnosis; however, 15% to 38% of cervical adenocarcinomas have an HPV negative diagnosis. This can be the result of misdiagnoses of different neoplasms or misleading negative results. Consequently, increasing HPV detection in clinical settings helps to advance research on HPV-negative cervical malignancies as well as cervical cancer diagnosis. Cervical tumours that are HPV-negative are typically detected at an advanced stage, which leads to a less favourable prognosis. To develop more focused therapeutics for both HPV-negative and HPV-positive cervical cancers, further study is required to elucidate the carcinogenesis pathways and differential immune responses between these two types of malignancies [28].

It's important to consider the limitations and advancements in diagnostic techniques, as highlighted by the use of next-generation sequencing (NGS) in a study. In the scientific report for period from 1990 to 2010, 30,848 women were included in 243 studies, and it is reported that there was a gradual decrease in the number of HPV-negative cases [29]. Development of HPV testing and non-cervical cancer classification can be associated with the descending trend in HPV-negativity. In studies involving HPV testing, the true rate of HPV-negative invasive cervical cancer can be overestimated [30].

5. Conclusions

The results obtained in the cohort of female respondents in Vojvodina and North Macedonia are consistent with findings from other studies, confirming similarities of HPV infections and dysplastic changes in both regions. Notably, genotype 16 is most frequently associated with pathohistological changes on the cervix, and less frequent are 33, 31, 18 and 56. The analysis indicates that infection with multiple associated genotypes of HPV is not correlated with histopathology.

The observations on trends in HPV prevalence, especially high-risk genotypes, is necessary for shaping future strategies in primary and secondary prevention. Implementation of HPV testing as primary screening tool is a prerequisite for a good prevention program. The study contributes valuable insights for public health efforts aimed at reducing the burden of cervical cancer in these regions.

AVAILABILITY OF DATA AND MATERIALS

The complete data of this work will be available without any reservation.

AUTHOR CONTRIBUTIONS

VS—conceptualization, data curation, formal analysis, methodology, software, validation, visualization; writing review & editing. VS and AM—investigation. NN, BB, SM and BG—writing-original draft. GD and MM—administration. BB and AM—supervision. All authors read and approved the final work.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

In this study, all the materials that we used are in accordance with the Declaration of Helsinki and approved by Public Health Institution University Clinic for Gynecology and Obstetrics—Skopje. Informed consent was obtained from all individual participants included in the study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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