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Prevalence of Human Papillomavirus (HPV) Genotypes in Women from North Macedonia

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Abstract

Cervical cancer remains one of the most prevalent malignancies among women both globally and within North Macedonia. Persistent infection with high-risk oncogenic genotypes of Human Papillomavirus (HPV) is a critical etiological factor in its development. This study aimed to determine the genotype distribution and prevalence of HPV among women in North Macedonia and to explore associated infection risk factors.
Between June 2023 and July 2024, a total of 300 cervical samples were collected from women for HPV analysis. Molecular detection was performed using conventional PCR and real-time qPCR methods. Two commercial kits were utilized: the AmpliSens® HPV HCR Screen-FEP kit was applied to 50 samples for the detection of high-risk HPV (HR-HPV) genotypes only, while the remaining 250 samples were tested using the NeoPlex™ HPV29 Detection kit, which identifies a broader spectrum of genotypes, including HR- HPV, probable high-risk (pHR-HPV), and low-risk (LR-HPV) types. DNA extraction and purification were carried out using the AmpliSens® DNA-sorb-AM kit, following the manufacturer's protocols. Statistical analysis was conducted using binary logistic regression, with a 95% confidence level, to assess associations between HPV positivity and the independent variables: age group, infection complexity (single vs. multiple genotypes), and genotype risk classification.
The overall HPV prevalence was 18.33%. The 36–66 age group exhibited a higher infection rate (67.27%) compared to the 26–36 age group (32.73%). Genotypes HPV 16 and HPV 31 emerged as the most frequently detected and are known for their strong oncogenic potential. HR-HPV types constituted 94.5% of the positive cases, while LR-HPV types were present in 21.8% of infections.
The predominance of high-risk HPV types and the age-related increase in infection rates underscore the need for targeted vaccination strategies, routine cervical screening, and long-term monitoring to reduce the burden of cervical pre-cancerous conditions.

Key Words: Cervical cancer, Human Papillomavirus (HPV), Genotyping, Age groups, Oncogenic risk.

Introduction

Cervical cancer continues to pose a significant public health challenge worldwide, particularly in low- and middle-income countries, where access to screening and vaccination is limited [1]. Globally, it ranks as the fourth most commonly diagnosed cancer among women, with an estimated 604,127 new cases and 341,831 related deaths reported in 2020 [2]. In North Macedonia, cervical cancer is among the six most frequent malignancies affecting women, with an incidence rate of 9.2 per 100,000 and a mortality rate of 2.9 per 100,000 annually [3].

Persistent infection with high-risk genotypes of Human Papillomavirus (HPV) is the principal etiological factor in cervical carcinogenesis [4]. HPV is a small, non-enveloped, double-stranded DNA virus from the *Papillomaviridae* family, with a genome of approximately 8,000 base pairs [5], [6], organized into early (E), late (L), and regulatory regions [7]. Of particular importance are the viral oncoproteins E6 and E7, which disrupt the function of tumor suppressor proteins p53 and pRb, respectively, thereby facilitating malignant transformation [8], [9].
To date, over 200 HPV genotypes have been identified, more than 40 of which are known to infect the genital tract [10]. These are categories based on oncogenic potential into high-risk (HR), probable high-risk (pHR), and low-risk (LR) types. The six most prevalent HR-HPV types worldwide are HPV-16, -18, -45, -52, -31, and -58 [11], [12], [13]. However, the prevalence and distribution of HPV genotypes vary significantly by region [4]. Among the HR types, HPV-16 and HPV-18 alone are responsible for around 70% of all cervical cancer cases [14], [15], while other genotypes such as HPV-31, -33, -45, -52, and -58 are also recognized as major contributors to cervical carcinogenesis [16]. In certain regions including East Asia, parts of Europe, Latin America, and Africa, HPV-58, -52, -31, and -45 have been reported as especially common [11], [13], [17], [18].

HPV genotypes are further classified into lineages and sublineages, which can influence infection persistence, viral load, and the likelihood of progression to high-grade lesions or invasive cancer [19], [20], [21]. Additionally, host genetic factors such as HLA haplotypes have been shown to modulate susceptibility to cancer progression in infections with specific HPV variants [22]. For example, a study by Mejia et al. (2016) in Ecuadorian women found that the European lineage of HPV-16 was the most frequently detected, and also among the least carcinogenic [23].

Data from North Macedonia indicate a substantial prevalence and genetic diversity of HPV. A retrospective study involving 7,411 women reported an overall HPV prevalence of 35.77%, with the highest rates observed in women under 30 years old [24]. The most frequently detected types were HPV-16 (23.39%), HPV-31 (10.68%), and HPV-53 (10.6%), followed by HPV-18 (6.19%) [24]. A more recent study (2019–2023) analyzing 300 women aged 18–65 found a bimodal age distribution of HPV infections, with peaks in the 18–34 and 55–64 age groups [25]. Multiple-type HPV infections were more common in women with high-grade cervical lesions (54.76%) compared to those with low- grade lesions (41.18%) or normal cytology (31.96%) [25].
Interestingly, HPV-66—a genotype typically classified as probably high-risk—has been frequently observed in women with abnormal cytology in North Macedonia [26]. Furthermore, viral load studies have indicated that high levels of HR-HPV DNA correlate with increased severity of cervical pathology, although some types, such as HPV-18, -45, -56, and -59, exhibited an inverse association [25].

Given the growing burden of HPV-associated disease and observed genotype variation, continued molecular surveillance and population-level genotyping are essential to inform vaccination strategies and improve screening effectiveness [27], [28]. Although prophylactic vaccines targeting HPV-16 and -18 have demonstrated high efficacy, their impact may vary depending on the regional distribution of HPV genotypes [29], [30].

In response, the World Health Organization (WHO) has launched a global initiative to eliminate cervical cancer as a public health problem by 2030 through widespread HPV vaccination, regular screening, and effective treatment [31]. Local data on HPV prevalence and genotype distribution are crucial to support these global efforts and adapt them to regional needs [32].

The aim of this study is to determine the prevalence of HPV infections in a group of women in North Macedonia and to characterize the distribution of HPV genotypes using PCR-based methods with melting curve analysis. The study further investigates differences in genotype risk levels (HR, pHR, LR), the presence of single versus multiple infections, and the potential relationship between genotype complexity and the risk of developing precancerous cervical lesions.

Materials and Methods

This study aimed to detect and characterize Human Papillomavirus (HPV) infections through molecular methods in a population of women in North Macedonia. A total of 300 cervical epithelial cell samples were analyzed, collected from women attending routine gynecological examinations across different regions of the country. Participants were stratified into two age groups: 26–36 years and 36–66 years.

Sample collection and storage: Cervical samples were obtained using a sterile cytobrush and transferred into tubes containing 1.5 mL of 0.9% sodium chloride solution. All samples were immediately stored and transported under refrigerated conditions to ensure the preservation of nucleic acid integrity.

DNA extraction: Genomic DNA was isolated using the AmpliSens® DNA-sorb-AM kit, following the standard operating procedure outlined in protocol K1-11-100-CE and based on silica-membrane technology as described by Knebelsberger and Stöger (2012). The procedure included cell lysis using guanidine thiocyanate, selective DNA binding to silica particles, ethanol-based washing, and elution in a buffer solution. The final DNA eluates (up to 150 µL) were stored at 2–8°C for short-term or at –16°C for long-term preservation.

HPV genotyping and amplification: Two commercially available molecular kits were employed for HPV detection:

- AmpliSens® HPV HCR Screen-FEP kit was used for 50 samples, targeting exclusively high-risk HPV (HR-HPV) genotypes via endpoint PCR.
- NeoPlex™ HPV29 Detection kit was applied to the remaining 250 samples, enabling simultaneous identification of HR-HPV, probable high-risk (pHR-HPV), and low-risk (LR-HPV) genotypes through real-time PCR (qPCR).

Each reaction mixture was prepared in accordance with the manufacturers' instructions. For the NeoPlex™ kit, the master mix included genotype-specific primers, DNA polymerase, dNTPs, $MgCl_2$, buffer solution, and nuclease-free water. DNA samples were added under sterile conditions to minimize contamination.

Amplification was conducted using the Bio-Rad CFX96 Touch™ Real-Time PCR Detection System. The PCR/qPCR protocol consisted of three main phases: DNA isolation, reaction mix preparation, and thermal cycling. Genotype identification was based on melting curve analysis, using characteristic melting temperatures (Tm) for each HPV type. The analytical sensitivity of the assays ranged between 97.5% and 100%, depending on the genotype.

Data processing and statistical analysis: Descriptive and inferential statistical analyses were performed using SPSS (v.26) and STATA (v.14) software. HPV prevalence and genotype distribution were evaluated across age groups. Binary logistic regression was used to explore associations between infection status and clinical variables, with a significance level set at $p < 0.05$ and 95% confidence intervals (CI) applied for interpretation.

All analyses were carried out at the certified histopathology laboratory Indus Medika, located in Skopje, under standardized biosafety and quality control protocols.

Results

The study was conducted between June 2023 and July 2024, during which a total of 300 cervical epithelial cell samples were analyzed for the presence of HPV infection. Among these, 245 samples (81.67%) tested negative, while 55 samples (18.33%) were positive for at least one HPV genotype. Out of the 29 genotypes targeted through real-time PCR analysis, 23 distinct genotypes were identified among the positive cases, indicating a broad genotypic distribution within the studied population.

Table 1. Genotypes count (frequency) divided by age group and genotype number divided by complexity presence

				Age		**Frequency of single and multiple genotypes**			
				26-36	36-66	26-36		36-66	
				Count	Count	S	M	S	M
Genotypes			Negative	61	184				
	HPV 16		Positive	0	6	0	0	6	0
	HPV 16, 56, 66		Positive	1	0	0	1	0	0
	HPV 16, 31, 53		Positive	0	1	0	0	0	1
	HPV 16, 31, 54		Positive	0	1	0	0	0	1
	HPV 16, 56, 40, 44		Positive	0	1	0	0	0	1
	HPV 18		Positive	1	3	1	0	3	0
	HPV 18, 58, 73, 61		Positive	0	1	0	0	0	1
	HPV 31		Positive	3	3	3	0	3	0
	HPV 31, 42, 70, 6		Positive	0	1	0	0	0	1
	HPV 31, 53		Positive	0	1	0	0	0	1
	HPV 33		Positive	1	1	1	0	1	0
	HPV 39		Pozitive	2	0	2	0	0	0
	HPV 39, 51, 61		Positive	0	1	0	0	0	1

	HPV 42		Pozitive	1	1	1	0	1	0
	HPV 45		Positive	1	2	1	0	2	0
	HPV 51		Positive	3	2	3	0	2	0
	HPV 52		Positive	2	3	2	0	3	0
	HPV 53		Positive	0	1	0	0	1	0
	HPV 54		Positive	0	1	0	0	1	0
	HPV 56		Positive	1	0	1	0	0	0
	HPV 59		Positive	0	1	0	0	1	0
	HPV 66		Positive	2	2	2	0	2	0
	HPV 67		Positive	0	1	0	0	1	0
	HPV 68		positive	0	2	0	0	2	0
	HPV 70		Positive	0	1	0	0	1	0
Total				18	37	17	1	27	7
Prevalence				32,7%	67,27%	30,91%	1,82%	49,09%	12,73%

As shown in Table 1, the prevalence of HPV infection varied significantly across age groups. In women aged 26–36 years, HPV was detected in 32.73% of cases (18 out of 61), whereas in the 36–66-year group, the prevalence increased markedly to 67.27% (37 out of 184).

When analyzing the complexity of infection—i.e., whether individuals carried a single genotype or multiple—the younger group showed that 30.91% of positive cases were single-genotype infections, with only 1.82% involving multiple genotypes. In contrast, among older participants, 49.09% of infections were attributed to a single genotype, while 12.73% represented co-infections with multiple HPV types.
This trend highlights a greater infection complexity with increasing age, suggesting cumulative exposure to risk factors over time. The likelihood of harboring multiple HPV types may be linked to a combination of factors, including persistent infection, viral reactivation, reinfection, and limited cross-immunity between genotypes.

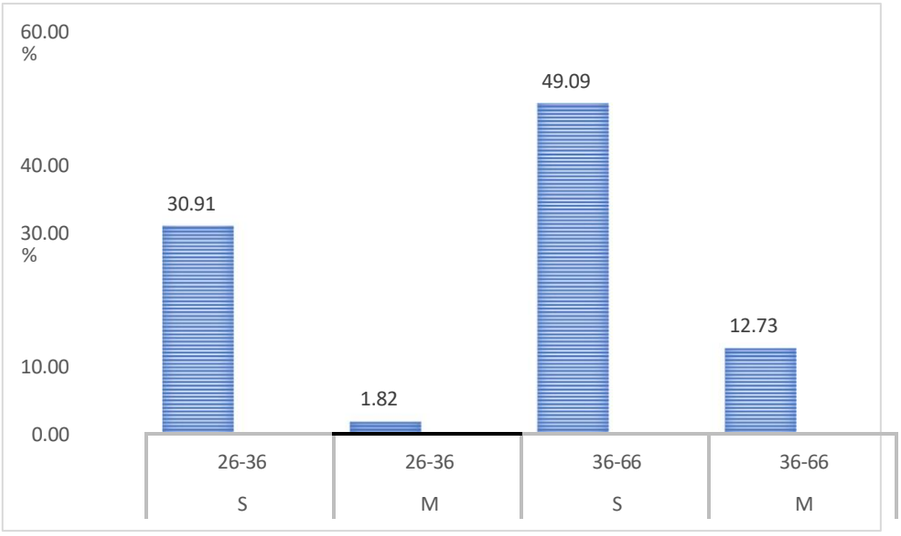
Such co-infections complicate both the clinical evaluation and management of HPV-related disease, especially when high-risk (HR) genotypes are involved. Notably, genotypes such as HPV 16, 18, and 31—which possess stable viral genomes—are frequently identified in tandem with low-risk genotypes, raising concerns about synergistic effects on disease progression.

Understanding the dynamics of multiple infections is crucial for refining screening protocols and improving patient stratification, particularly for individuals at elevated risk of precancerous transformation and cervical malignancy.

Among the genotypes identified in this cohort, a substantial proportion were classified as high-risk, including HPV 16, 18, 31, 33, 45, 51, 52, 56, and 58—all of which have been strongly implicated in the pathogenesis of cervical and other anogenital cancers. HPV 16 and 31 emerged as the most prevalent genotypes in this study, accounting for 10 positive cases, particularly in the older age group (36–66 years). This aligns with global findings identifying these types as dominant in the epidemiology of HPV infections in women.

While the World Health Organization (2022) notes that initial HPV infections are most commonly detected in younger women, HPV 16 and 18 continue to be the primary oncogenic drivers of cervical cancer globally. The persistence of infection, rather than the mere presence of the virus, plays a critical role in determining disease outcomes. Importantly, over 90% of infections with HPV 16, 18, and 31 are self-limited and resolve within 6 to 18 months, particularly in immunocompetent individuals without concurrent infections or underlying pathology.

One genotype of emerging concern is HPV 39, which, although less prevalent in this sample, has demonstrated significant oncogenic potential and may play an underrecognized role in genital carcinogenesis. By contrast, low-risk (LR) genotypes, such as HPV 6, 42, 44, and 70, were detected infrequently, consistent with their typically non-oncogenic behavior and self-limiting clinical course.



Graphic 1. Prevalence of genotypes as single and multiple for 2 age groups

Chart 1. Ilustrates the prevalence of single and multiple HPV infections across age groups, clearly showing that the 36–66 age group has both a higher overall infection rate and a greater proportion of multiple genotype infections. This finding underscores the critical need for early screening and consistent monitoring within this demographic.

Table 2 offers a detailed breakdown of the HPV genotype distribution among the analyzed samples, categorized by their associated risk levels: high-risk (HR-HPV), probable high- risk (pHR-HPV), and low-risk (LR-HPV) genotypes.

Table 2. Prevalence of the genotypes, prevalence of the genotypes grouped by risk grade

Risk grade	Genotype	Count	Prevalence	Prevalence of cases by Risk
High	HRHPV16	10	18.2%	
High	HRHPV18	5	9.1%	
High	HRHPV31	10	18.2%	
High	HRHPV33	2	3.6%	
High	HRHPV39	3	5.5%	

High	HRHPV45	3	5.5%	
High	HRHPV51	6	10.9%	94,5%
High	HRHPV52	5	9.1%	
High	HRHPV56	3	5.5%	
High	HRHPV58	1	1.8%	
High	HRHPV59	1	1.8%	
High	HRHPV68	2	3.6%	
High	HRHPV73	1	1.8%	
PHigh[1]	pHRHPV53	3	5.5%	
PHigh	pHRHPV66	5	9.1%	16.36%
PHigh	pHRHPV67	1	1.8%	
Low	LRHPV6	1	1.8%	
Low	LRHPV40	1	1.8%	
Low	LRHPV42	3	5.5%	
Low	LRHPV44	1	1.8%	21,8%
Low	LRHPV54	2	3.6%	
Low	LRHPV61	2	3.6%	
Low	LRHPV70	2	3.6%	

[1] PHigh indicate Probably high or potentially dangerous

According to the data presented in Table 2, high-risk HPV genotypes (HR-HPV) constitute 94.5% of all positive cases, highlighting their predominance within the studied population. The most frequently detected genotypes were HPV16 and HPV31, each with a prevalence of 18.2%. These genotypes are strongly linked to cervical cancer development and rank among the most oncogenic HPV types. HPV51 followed with a prevalence of 10.9%, while HPV18, HPV52, and HPV66 each accounted for 9.1%.

The substantial presence of these high-risk genotypes emphasizes the critical importance of regular screening and active clinical monitoring, particularly for HPV16 and HPV18, which are targeted by current HPV vaccines.

Probable high-risk genotypes (pHR-HPV), including HPV53, HPV66, and HPV67, represented 16.36% of positive cases. Notably, HPV66, which has an ambiguous risk classification, showed a prevalence of 9.1%, whereas HPV53 and HPV67 were less frequent at 5.5% and 1.8%, respectively. Although these genotypes are not definitively classified as high-risk, their notable presence—especially in co-infections—warrants clinical attention.

Low-risk HPV genotypes (LR-HPV) comprised 21.8% of positive cases. HPV42 was the most common among these (5.5%), followed by HPV54, HPV61, and HPV70, each with a prevalence of 3.6%. While low-risk genotypes generally do not lead to malignancies and are associated with benign conditions such as genital warts, their detection remains relevant for comprehensive genital tract health monitoring.

This analysis confirms the dominance of HPV16 and HPV31, underscoring their significant oncogenic potential.

The genotype distribution observed aligns with global epidemiological patterns, where HPV16, HPV18, and HPV31 are prevalent in cervical cancer cases. The notable occurrence of multiple HR-HPV genotypes within this population suggests an elevated risk for the development of CIN2+ lesions and reinforces the necessity of integrating genotype-specific testing into national screening protocols.
Furthermore, these findings provide essential insights for vaccination strategies, given that the most prevalent genotypes identified correspond to those covered by existing vaccines.
For clearer visualization, Chart 2 presents the prevalence of all genotypes stratified by risk category, complementing the data summarized in Table 2.

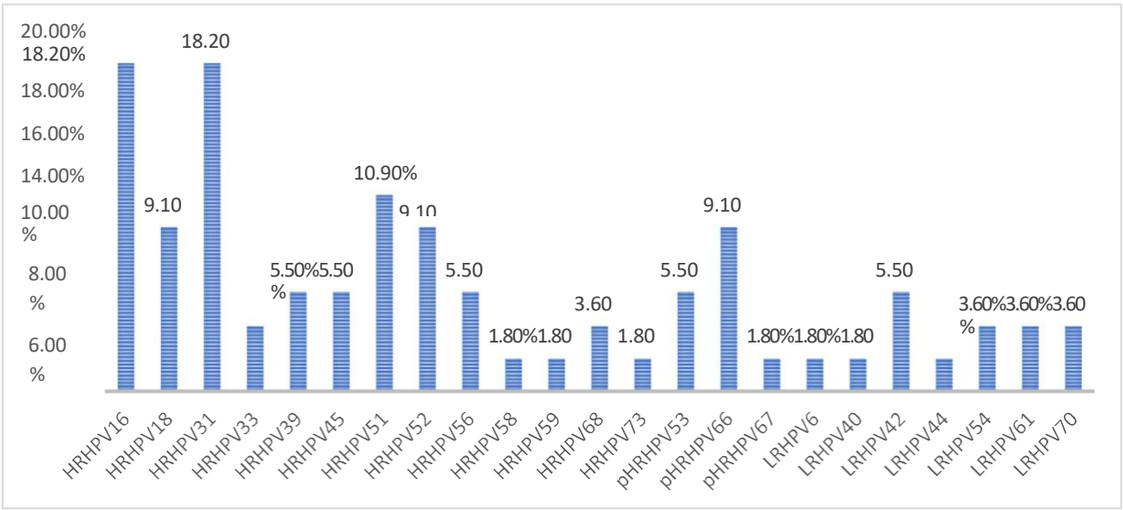


Chart 2. Prevalence of genotypes

The distribution of HPV genotypes across risk categories, as illustrated in Chart 2, offers a comprehensive overview of their prevalence within the studied cohort. The high-risk (HR-HPV) group encompasses 13 genotypes, including the well-established oncogenic types HPV 16, 18, and 31, which exhibit the highest prevalence rates. Additional HR genotypes such as HPV 33, 39, 45, 51, 52, 56, 58, 59, 68, and 73 were also identified, albeit at lower frequencies. These findings underscore that the majority of HPV infections in this study are attributable to high-risk variants, corroborating existing evidence linking these genotypes to an elevated risk of cervical cancer and other HPV-associated malignancies.

The probable high-risk (pHR-HPV) category consists of genotypes HPV 53, 66, and 67, which carry a moderate oncogenic potential. Although their prevalence is comparatively lower, this group warrants clinical attention due to its possible involvement in cervical dysplasia and, in rare instances, malignant transformation. Notably, genotype HPV 66 requires ongoing surveillance to clarify its long-term clinical implications.

Low-risk (LR-HPV) genotypes identified include HPV 6, 40, 42, 44, 54, 61, and 70, typically associated with benign conditions such as genital warts and infrequently linked to cancer. Despite their lower oncogenicity, the relatively high prevalence of LR genotypes observed highlights the necessity to differentiate between high-risk and low-risk infections during routine screening and clinical management, given their potential to cause other health issues like low-grade cervical lesions.

Chart 3 visually depicts these distributions through bars, where the X-axis represents the risk categories (HR-HPV, pHR-HPV, and LR-HPV), and the Y-axis indicates the percentage prevalence of positive cases within each group.

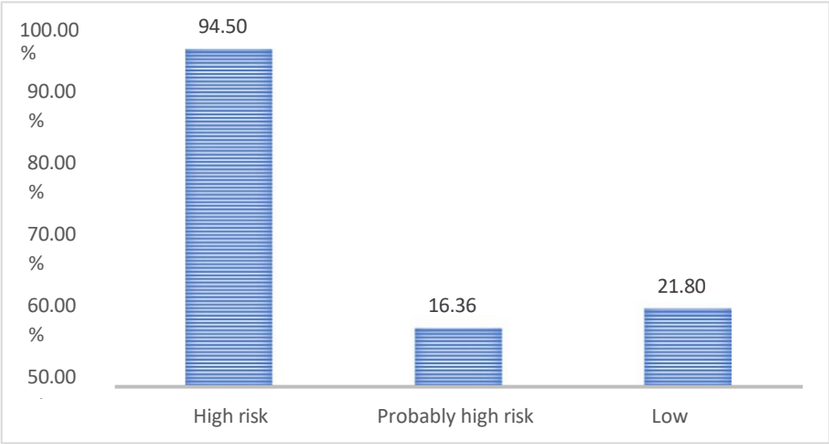


Chart 3. Prevalence of genotypes by risk

The data summarized in Chart 3 show the prevalence of HPV across risk categories: high-risk HPV (HR-HPV) at 94.5%, probable high-risk HPV (pHR-HPV) at 16.36%, and low-risk HPV (LR-HPV) at 21.8%. The total percentages exceed 100% due to multiple infections occurring in some patients, involving more than one HPV genotype.

Analysis of Table 1 and Table 2: An ordinal logistic regression model was applied to estimate the likelihood of HPV infection based on the following predictors:

- Age group (26–36 coded as 1, 36–66 coded as 0),
- Infection complexity (single genotype coded as 0, multiple genotypes as 1),
- Genotype risk level (low risk coded as 0, high risk coded as 1).

The model is expressed as:

P (HPV positive) = $\beta_0 + \beta_1$ (age group) + β_2 (multiple infection) + β_3 (high-risk genotype) + e_i

Key regression results:

Variable	Coefficient (β)	p-value	Odds Ratio (OR)
Age group 26–36	0.954*	0.049	2.6×
Multiple genotype infection	1.802**	0.012	6.1×
High-risk genotypes	2.112**	0.002	8.3×

(*Significant at α = 0.05, **Significant at α = 0.01)

Interpretation:

- Women aged 26–36 have 2.6 times higher odds of testing positive for HPV.
- Infections involving multiple HPV genotypes increase the risk by approximately sixfold.

- Presence of high-risk HPV genotypes (e.g., HPV 16, 18, 31) is associated with an 8.3 times greater likelihood of infection.

Model fit indicators:

Indicator	Value
Chi-square	24.68
Degrees of freedom (df)	3
p-value	0.000
Nagelkerke R^2	0.278
McFadden R^2	0.209

The model is statistically significant and demonstrates strong predictive capability, with pseudo R^2 values exceeding 0.20.

Conclusions:

- The principal factors associated with HPV positivity are younger age, infections involving multiple genotypes, and the presence of high-risk HPV types.
- This predictive model may be valuable in identifying women at elevated risk, enabling improved prevention and clinical monitoring.
- Incorporating further clinical parameters, such as cervical intraepithelial neoplasia (CIN) status, colposcopy results, and co-infection profiles, could enhance the model's accuracy and utility.

Discussion

This study offers a comprehensive analysis of the prevalence and distribution of Human Papillomavirus (HPV) genotypes within a representative sample from North Macedonia. The overall HPV prevalence of 18.33% falls within expected ranges when compared to global literature, which reports prevalence rates varying from approximately 11% in high-income countries to over 5% in low-income settings [11], [31].

A key finding is the predominance of high-risk oncogenic HPV genotypes (HR-HPV), with HPV-16 and HPV-31 emerging as the most frequently detected types. This observation is consistent with regional and international studies identifying HPV-16 as the genotype most strongly associated with cervical cancer [16], [15], [13].

Clinically significant is the notably higher prevalence of HPV infection (67.27%) observed in women aged 36–66 years. This contrasts with earlier regional reports such as Andonovska (2014), which reported higher prevalence among women under 30 years of age [24]. Our findings align more closely with recent data by Krstevska-Kelepurovska et al. (2024), which describe a bimodal distribution of HPV prevalence, peaking in younger women and again in those aged 55–64 years [25]. This pattern likely reflects reactivation of latent infections, immune senescence, and cumulative risk exposure over time [19].

Another important aspect is the frequent occurrence of multiple HPV genotype infections in this population, which are linked to an increased risk of precancerous and cancerous lesions through synergistic interactions of the viral oncoproteins E6 and E7 [9], [6]. This finding echoes the results of Krstevska-Kelepurovska et al. (2024), who reported multiple infections in 54.76% of cases with high-grade cervical lesions [25].

Additionally, the detection of probable high-risk (pHR-HPV) and low-risk (LR-HPV) genotypes, such as HPV-66, HPV-42 and HPV-54, is important for clinical management.

For example, Duvlis et al. (2001) reported a lower percentage of HPV-66 in abnormal cytology in Macedonia, suggesting that being classified as moderate-risk may report a greater role in carcinogenesis than previously thought [26].

From a vaccination perspective, these findings underscore the need for tailored immunization strategies. Current vaccines—including bivalent, quadrivalent, and nonavalent formulations—target HPV16 and HPV18, which together account for approximately 70% of cervical cancer cases globally [31], [11]. However, given the considerable prevalence of genotypes such as HPV-31, HPV-51, and HPV-53 in our study cohort, national vaccination programs should integrate local epidemiological data to optimize preventive impact [30], [33].

Beyond prevention, these data emphasize the importance of incorporating HPV genotyping into routine cervical screening programs. Early identification of high-risk HPV infections is critical for timely clinical management and reducing cervical cancer incidence at both national and regional levels.

Finally, these results support global initiatives aiming to eliminate cervical cancer, reinforcing the WHO's "90-70-90" strategy: achieving 90% vaccination coverage, 70% screening uptake, and 90% treatment of detected cases [32].

Conclusions

HPV infection is prevalent in the studied population, with a predominance of high-risk genotypes (HR-HPV), including HPV 16 and HPV 31, which are associated with the development of cervical cancer.

Infection complexity increases with age, as older women (36–66 years) show higher prevalence rates and more frequent multiple HPV genotype infections.

Moderate-risk (pHR-HPV) and low-risk (LR-HPV) genotypes are also detected, typically associated with benign conditions, but warrant careful monitoring to maintain genital health.

These findings reinforce the critical role of regular HPV screening and vaccination, especially targeting dominant genotypes such as HPV 16 and HPV 31, which are covered by existing vaccines.

A personalized, risk-based approach is essential for effective monitoring and management of HPV infections, with intensified follow-up recommended for individuals harboring multiple or high-risk HPV types.

This study supports enhanced awareness of early and sustained HPV screening and provides valuable epidemiological data to guide vaccination policies and cervical cancer prevention strategies in the region.

References:

1. World 1. World Health Organization (WHO). (2022). Human papillomavirus (HPV) and cervical cancer. Available from: https://www.who.int/news-room/fact-sheets/detail/human-papillomavirus-and-cervical-cancer

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 71(3):209–49. https://doi.org/10.3322/caac.21660.

3. Institute of Public Health of North Macedonia. (2025). Cervical cancer statistics 2013–2022. Portalb.mk.

4. International Agency for Research on Cancer (IARC). (2012). Human papillomaviruses: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 100B. Lyon, France: WHO.

5. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM. (2010). Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology, 401(1):70–79.

6. Doorbar J. (2006). Molecular biology of human papillomavirus infection and cervical cancer. Clin Sci, 110(5):525–41.

7. Tommasino M. (2014). The human papillomavirus family and its role in carcinogenesis. Semin Cancer Biol, 26:13–21.

8. Munger K, Phelps WC, Bubb V, Howley PM, Schlegel R. (1989). The E6 and E7 genes of HPV-16 and HPV-18 are sufficient for transformation of human keratinocytes. J Virol, 63(10):4417–21.

9. Münger K, Baldwin A, Edwards KM, et al. (2004). Mechanisms of human papillomavirus–induced oncogenesis. J Virol, 78(21):11451–60.

10. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. (2004). Classification of papillomaviruses. Virology, 324(1):17–27.

11. Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S. (2010). Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis, 202(12):1789–99. https://doi.org/10.1086/657321.

12. Ciapponi A, Bardach A, Glujovsky D, Gibbons L, Picconi MA. (2011). Type-specific HPV prevalence in cervical cancer and high-grade lesions in Latin America and the Caribbean: systematic review and meta-analysis. Plos One, 6(10):e25493. https://doi.org/10.1371/journal.pone.0025493.

13. Guan P, Howell-Jones R, Li N, Bruni L, de Sanjosé S, Franceschi S, et al. (2012). Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. Int J Cancer, 131(10):2349–59. https://doi.org/10.1002/ijc.27485.

14. Schiffman M, Castle PE. (2005). The promise of global cervical cancer prevention. N Engl J Med, 353(20):2101–4.

15. Bosch FX, de Sanjosé S. (2003). Chapter 1: Human papillomavirus and cervical cancer—burden and assessment of causality. J Natl Cancer Inst Monogr, (31):3–13.

16. Muñoz N, Bosch FX, de Sanjosé S, et al. (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med, 348(6):518–27.

17. Ogembo RK, Gona PN, Seymour AJ, Park HS-M, Bain PA, Maranda L, et al. (2015). Prevalence of human papillomavirus genotypes among African women with normal cervical cytology and neoplasia: a systematic review and meta-analysis.Plos One, 10(4):e0122488. https://doi.org/10.1371/journal.pone.0122488.

18. Chan PKS, Ho WCS, Chan MCW, Wong MCS, Yeung ACM, Chor JSY, et al. (2014). Meta-analysis on prevalence and attribution of human papillomavirus types 52 and 58 in cervical neoplasia worldwide. Plos One, 9(9):e107573. https://doi.org/10.1371/journal.pone.0107573.

19. Schiffman M, Rodriguez AC, Chen Z, Wacholder S, Herrero R, Hildesheim A, et al. (2010). A population-based prospective study of carcinogenic human papillomavirus variant lineages, viral persistence, and cervical neoplasia. Cancer Res, 70(8):3159–69. https://doi.org/10.1158/0008-5472.can-09-4179.

20. Burk RD, Harari A, Chen Z. (2013). Human papillomavirus genome variants. Virology, 445(1–2):232–43. https://doi.org/10.1016/j.virol.2013.07.018.

21. Chen Z, Ho WCS, Boon SS, Law PTY, Chan MCW, DeSalle R, et al. (2017). Ancient evolution and dispersion of human papillomavirus 58 variants. J Virol, 91(21):e01285. https://doi.org/10.1128/jvi.01285-17.

22. Zehbe I, Tachezy R, Mytilineos J, Voglino G, Mikyskova I, Delius H, et al. Human papillomavirus 16 E6 polymorphisms in cervical lesions from different European populations and their correlation with human leukocyte antigen class II haplotypes. Int J Cancer. 2001;94(5):711–6. https://doi.org/10.1002/ijc.1520.

23. Mejía L, Muñoz D, Trueba G, Tinoco L, Zapata S. (2016). Prevalence of human papillomavirus types in cervical cancerous and precancerous lesions of Ecuadorian women: HPV types on cervical cancer in Ecuadorian women. J Med Virol, 88(1):144–
52. https://doi.org/10.1002/jmv.24310.

24. Andonovska J. (2014). Genotypic distribution and prevalence of the HPV infection in Macedonia. 16th ICID Abstracts / Int J Infect Dis, 21S:1–460.

25. Krstevska-Kelepurovska E, Kaftandzieva A, Nikolovska J, et al. (2024). Human papillomavirus infections, viral load and age in women with cervical cancer. Acad Med J, 4(1).

26. Duvlis S, Plaseska-Karanfilska D, Basheska NT, et al. (2001). A variant of human papillomavirus type 66 is common among HPV-infected women in Macedonia. Balkan J Med Genet, 4(3–4):53–57.

27. Bruni L, Albero G, Serrano B, et al. (2021). Human papillomavirus and related diseases in Europe. ICO/IARC HPV Information Centre.

28. Clifford GM, Franceschi S, Diaz M, et al. (2006). HPV type-distribution in women with and without cervical neoplastic diseases. Vaccine, 24(Suppl 3):S26–S34.

29. Markowitz LE, Drolet M, Perez N, Jit M, Brisson M. (2022). Human papillomavirus vaccine effectiveness by number of doses. Lancet Oncol, 23(9):1167–78.

30. Drolet M, Bénard É, Pérez N, et al. (2019). Population-level impact and herd effects following the introduction of HPV vaccination programs: updated systematic review. Lancet Public Health, 4(11):e599–e617.

31. World Health Organization (WHO). (2020). Global strategy to accelerate the elimination of cervical cancer as a public health problem. Geneva: WHO.

32. World Health Organization (WHO). (2021). Guidelines for screening and treatment of precancerous lesions for cervical cancer prevention. 2nd ed. Geneva: WHO.

33. Arbyn, M., Weiderpass, E., Bruni, L., de Sanjosé, S., & Ferlay, J. (2020). Estimates of cancer incidence and mortality in women in 2020: A global analysis by country. The Lancet Oncology, 21(11), 1671-1684. https://doi.org/10.1016/S1470-2045(20)30353-4.