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Human Papillomavirus (HPV) Proportion and Genotype Distribution among Reproductive age Women of a Hilly District of Nepal

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ABSTRACT

Background: Human papillomavirus (HPV) is a leading cause of cervical cancer, particularly in low- and middle-income countries (LMICs) with limited access to preventive services. This study was aimed to assess high-risk HPV prevalence and genotype distribution among reproductive-age women in Palpa district of Nepal and compare them with demographic and clinical data.

Methods: A small, community based, non-probability convenience sampling approach was used for data collection in which cervical swabs were collected from 2,331 women aged 20 to 45 years from Rampur and Rambha municipalities of Palpa district, who attended the health camps organized from 7th February 2025 to 7th October 2025, after obtaining ethical approval from the Nepal Health Research Council (NHRC), Nepal. Out of 2,331 cervical swab samples, 2,024 swab samples were only processed due to pre-analytical errors for HPV DNA detection using the Nucleic Acid Extraction Kit and genotypes identified through RT-PCR using the Human Papillomavirus Nucleic Acid Amplification Test Kit.

Results: HPV positivity among participants were found to be 3.5%. Other HPV genotype (non 16/18) were most frequently observed among HPV positive participant 35(49.3%), followed by HPV 16 genotype (29.5%) and HPV 18 (16.9%). The women between 35–40 years age group had the highest proportion of HPV positive cases (36 cases). The logistic regression between HPV infection and the presence of cervical lesions yielded an odds ratio of 2.999, indicating that women with cervical lesions were nearly three times more likely to have HPV infection than those without lesions.

Conclusions: The study showed low HPV positive proportion rate, with non-16/18 genotypes predominating. Women aged 35–40 years had the highest HPV positivity, and HPV infection was found to be more common among women with cervical lesions.

Keywords: Cervical swabs; genotypes; High risk- HPV; real time PCR.

INTRODUCTION

Cervical cancer remains one of the most preventable yet deadly forms of cancer among women worldwide. Persistent infection with high-risk human papillomavirus (HR-HPV) types is recognized as the primary cause of cervical cancer.[1] HPV is a double-stranded DNA virus comprising over 200 genotypes, of which approximately 14 are considered high-risk due to their oncogenic potential. These HR-HPV types, particularly genotypes 16 and 18, are responsible for over 70% of cervical cancer cases globally.[2] Cervical cancer ranks as the fourth most common cancer affecting women worldwide. In 2020 alone, there were an estimated 604,000 new cases and 342,000 deaths, with approximately 90% of these fatalities occurring in low- and middle-income countries (LMICs), where access to preventive healthcare is often limited.[3]Community-based studies in Nepal have revealed notable regional variations in the prevalence of high-risk human papillomavirus (HR-HPV). For instance, the Kavre District reported an overall HPV prevalence of 14.4%, with 7.9% identified as HR-HPV.[4] Studies conducted in districts such as Kavre and Jumla have demonstrated the value of RT-PCR in identifying the distribution of high-risk HPV genotypes and their association with cervical lesions.[4,5]Similarly, the far-western region

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recorded a 9.6% HR-HPV prevalence, while a study in eastern Nepal found an 8.9% HR-HPV rate among Nepali and Bhutanese women.[6] In central urban areas of Nepal, the prevalence was slightly lower, with 8.6% overall HPV and 6.1% HR-HPV reported.[7]Moreover, genotype-specific data from the Information Centre on HPV and Cancer (ICO), Nepal highlight the predominance of HPV types 16 and 18 in cervical disease, with these types detected in 30.2% of low-grade squamous intraepithelial lesions (LSIL), 63.4% of high-grade squamous intraepithelial lesions (HSIL), and 80.3% of cervical cancer cases.[8]

METHODS

This study was conducted at Rampur municipality and Rambha rural municipality of Palpa district among women between the age group of 20 to 45 years who attended the health camps organized from 7th February 2025 to 7th October 2025. Collected cervical swab samples were tested at Province Public Health Laboratory (PPHL), Lumbini Province located in Rupandehi district and molecular lab of Central Department of Biotechnology, TU, Kirtipur, Nepal.

Ethical approval for the study was obtained from the Nepal Health Research Council (ethical approval no. 55-2025), ensuring adherence to ethical guidelines for human subject research. Prior to sample collection, informed written consent was obtained from all participants. Non-probability convenience sampling technique was used for data collection. Health camp attended participants who gave consent were enrolled in the study, and relevant socio-demographic and clinical information were obtained through interview using a structured questionnaire. Females of age group 18-45 years, willing to participate, not pregnant, no recent history of cervical treatment or abnormal bleeding were included in the study. But females with a history of cervical surgery, hysterectomy, or any known medical condition affecting immune response were excluded in the study.

Cervical swab specimens were collected from health camp participants residing in Rampur Municipality and Rambha rural municipality of Palpa district, Nepal. Commercially available self-swab collection kit (Mole Bioscience) was used for sample collection. After providing proper instruction to participants for cervical swab collections, they collected their cervical swab sample by themselves. Collected Cervical swabs were preserved in a leakproof Viral Transport Medium (VTM) to maintain nucleic acid integrity for molecular analysis. A total of 2,331 participants cervical swab samples were collected. Out of 2,331 cervical swab samples, 2,024 swab samples were only processed for HPV DNA detection and remaining 307 samples were rejected due to improper labeling of sample, sample leakage and poor storage condition of the samples. Collected Samples were properly labelled with unique identification code indicating the participants study Id, date of collection and site (Rampur or Rambha) but participants name were not mentioned to maintain confidentiality of participants. The collected swabs were kept at the temperature of 2-8°C throughout the field works and transported safely to PPHL and Central department of biotechnology in batches under cold chain conditions within 24 to 48 hours of collection. After receiving the samples, the respective laboratories stored those samples at -20 to -80°C for further molecular analysis. Genomic DNA was extracted from the cervical swab specimens using the Nucleic Acid Extraction Kit (Catalog No. P08802T2096, Mole 96M), manufactured by Jiangsu Mole Bioscience Co., Ltd., China following the manufacturer's protocol. The extracted DNA was subsequently subjected to genotyping using real-time polymerase chain reaction (RT-PCR).[9,10] The RT-PCR assays were performed using the DLAB PCR Thermal Cycler and Rotor-Gene platform (Qiagen). For amplification and detection, Human Papillomavirus Nucleic Acid Amplification Test Kit (Fluorescence probe based RT-PCR Assay; Jiangsu Mole Bioscience Co., Ltd., Jiangsu Province, China) was used [10]. The assay specifically targeted the L1 gene region of the HPV genome, enabling the simultaneous detection and genotyping of multiple high-risk HPV types.[11]

Collected data were entered and analyzed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were computed to summarize demographic and clinical characteristics. Associations between HPV status and cervical lesions were analyzed using logistic regression.

RESULTS

A total of 2,331 participant's cervical swab samples were collected. Out of 2,331 cervical swab samples, 2,024 swab samples were only processed for HPV DNA detection and remaining 307 samples were rejected due to improper labeling of sample, sample leakage and poor storage condition of the samples. Out of the 2,024 samples processed for HPV screening, 71 (3.5%) cases tested positive for human papillomavirus (HPV), including high risk HPV types (16 & 18), and low risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, & 68) and 1953 (96.5%) samples were tested negative.

Table 1. Distribution of HPV Genotypes. (n = 71)

Genotypes	Positive cases	Percent (%)
Other HPV	35	49.3
HPV-16	21	29.58
HPV-18	12	16.9
All Genotypes	1	1.41
HPV-16 and others	1	1.41
HPV-18 and others	1	1.41
Total	71	100

**Other HPV genotypes includes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.*

Among 71 HPV-positive cases, the highest proportion of 35 (49.3%) cases were infected with other HPV genotypes (non-16 and 18) indicating a substantial proportion of non-16/18 genotypes in the study population. HPV 16 genotype was the second most common genotype, detected in 21 (29.58%) cases, followed by HPV 18 in 12 (16.9%) cases. HPV infections involving multiple HPV genotypes were rare. Only one case (1.41%) was found to be concurrently positive for all three categories: HPV 16, HPV 18, and other HPV genotypes. Similarly, one case each (1.41%) was positive for both HPV 16 and other types, and HPV 18 and other types, respectively as shown in table 1.

Table 2. HPV test status of participants with Ethnicity.

	HPV test status			
Ethnicity	Negative	Positive (%)	Total	Chi Square P-value
Brahmin	708	18(2.48)	726	0.547
Chhetri	237	10(4.0)	247	
Dalit	276	12(4.17)	288	
Magar	488	19(3.75)	507	
Newar	117	6(4.88)	123	
Others (Thakuri/Gurung/Rai/Tharu)	127	6(4.51)	133	
Total	**1,953**	**71**	**2,024**	

The table 2 illustrates the distribution of HPV (Human Papillomavirus) testing status across various ethnic groups among 2,024 participants. Out of the total, 1,953 (96.5%) tested negative, while 71 (3.5%) were positive for HPV. The Brahmin group had the highest representation (726 individuals) and recorded the lowest positive rate (2.48%) of HPV infection. Conversely, the Newari group, though smaller in number (123 individuals), showed the highest positive rate (4.88%) of HPV infection. However, the p-value shows no association between ethnicity and HPV test status (p-value=0.547, Pearson chi-square value= 4.015 and df= 5).

Table 3. Distribution of HPV genotypes across different age group.

	HPV status		
Age group	Negative (%)	Positive (%)	Total
20-25	21 (100)	0	21
25-30	120 (96.77)	4 (3.23)	124
30-35	553 (95.84)	24 (4.16)	577
35-40	950 (96.35)	36 (3.65)	986
40-45	309 (97.78)	7 (2.22)	316
Total	**1953 (96.49)**	**71 (3.51)**	**2024**

The table 3 illustrates the distribution of HPV genotypes by age group, with a low HPV-positive proportion rate of 3.51% among 2,024 participants. The highest HPV proportion rate (4.16%) was observed among 30–35 age group, while the 20–25 age group had no positive cases. Across all age categories, the majority tested negative, suggesting a low HPV- positive proportion in the study area with no clear trend based on age.

Table 4. Risk level of HPV infections with symptoms, STI history & cervical lesions.

		HPV risk level		
Symptoms		**HPV Other (%)**	**HPV-16/18(%)**	**Total**
1	Abnormal Vaginal Bleeding	5 (50)	5 (50)	10
2	None	10 (52.63)	9 (47.37)	19
3	Pain during intercourse	1 (14.29)	6 (85.71)	7
4	Pelvic pain	12 (70.59)	5 (29.41)	17
5	Unusual Discharge	7 (38.89)	11 (61.11)	18
	Total	35 (49.3)	36 (50.7)	71 (100%)
STI history				
1	No	33 (52.38)	30 (47.62)	63
2	Yes	2 (25)	6 (75)	8
	Total	35 (49.3)	36 (50.7)	71 (100%)
Cervical Lesions				
1	Absent	30 (55.56)	24 (44.44)	54
2	Present	5 (29.41)	12 (70.59)	17
	Total	35 (49.3)	36 (50.7)	71 (100%)

Out of 71 HPV-positive individuals, the distribution was nearly equal, with 50.7% having HPV-16/18 and 49.3% infected with other types. Symptoms like abnormal vaginal bleeding and the absence of symptoms showed an almost even split between both risk groups. However, some symptoms showed a relation with specific HPV types. Pain during intercourse was mostly linked to HPV-16/18 (85.71%), while pelvic pain was more frequently associated with other HPV strains (70.59%). Additionally, unusual discharge was more commonly seen in those with HPV-16/18 (61.11%) as shown in table 4 that indicates certain symptoms may be more possibly connected to particular HPV risk categories. Due to small numbers and possible confounding, we did not explicitly test for differences by symptoms, thus they might not accurately represent underlying risk differences.

The table 4 also illustrates the association between a history of sexually transmitted infections (STIs) and HPV (Human Papillomavirus) infection. Among 71 HPV positive cases 63 individuals had no any history of STI with 33(52.38%) cases HPV other types infected and 30 (47.62%) cases high-risk HPV-16/18 genotypes infected. In contrast 8 individuals had the history of STI among them 6(75%) cases were infected with HPV-16/18, and only 2(25%) cases had other HPV type infection. These findings suggest that individuals with a history of STIs may have a higher likelihood of acquiring high-risk HPV strains.

Out of the 71 positive cases the cervical lesions were present in 17 and absent in 54 cases. The cases with cervical lesions had 12(70.9%) individuals infected with HPV-16/18 and 5(29.41%) individuals infected with HPV other types. While the cases without lesions were 24(44.44) HPV-16/18 infected and 30(55.56) HPV other types infected as shown in table 4. This shows a possible connection between HPV-16/18 and the emergence of cervical lesions, suggesting that high-risk HPV strains might be more important in the development of lesions.

Table 5. Risk of cervical lesions in HPV positive cases (n=71) across different Age groups.

	HPV risk level		
Age group (yrs)	**HPV other (%)**	**HPV-16/18 (%)**	**Total**
25-35	15 (53.57)	13 (46.43)	28
35-45	20 (46.51)	23 (53.49)	43
Total	**35 (49.3)**	**36 (50.7)**	**71 (100%)**

The distribution of HPV positive cases according to age group showed 23 cases of HPV-16/18 genotypes in age group 35-45 years and only 13 cases of HPV-16/18 genotypes in age group 25-35 years. Similarly, 20 cases of HPV other types were seen in age group 35-45 years and 15 cases in the 25-35 age groups as shown in table 5. This indicates the high-risk HPV-16/18 genotypes infections were more common in the older age group (35-45 age group).

Table 6. Logistic regression between HPV status and cervical lesions

	Odds ratio	p-value
Intercept		0.4152
Cervical lesions Present	2.999	0.0665

Note: Cervical lesions were found in 143 Negative HPV cases and 17 in positive HPV cases.

The odds ratio and associated p-value evaluating the association among the existence of cervical lesions and the result under study are shown in the table 6. With an odds ratio of 2.999, those who were observed with cervical lesions were nearly three times more likely to have HPV compared to those who do not have cervical lesions.

DISCUSSION

HPV types 16 and 18, which infect roughly 2% of women at any given time, are responsible for 80.3% of instances of cervical cancer, the first most frequent malignancy among women between 15 and 44 years of age in Nepal.[12] The American Cancer Society (ACS) released updated cervical cancer screening guidelines in 2020 that endorse a shift in practice to primary human papillomavirus (HPV) screening in people with a cervix, beginning at ages of 25-65 years.[13] Our study showed a low HPV proportion rate of 3.51%, with the highest proportion rate (4.16%) in women aged 30-35 years, however, no any cases were identified in 20-25 years age group, consistent with global and Nepalese studies showing peak HPV prevalence in mid-adulthood. The absence of cases in younger women may relate to smaller sample sizes or lower sexual activity.[14]

Several studies have indicated a potential association between sexually transmitted infections (STIs), persistent HPV infection, and the progression of cervical neoplasia.[15] In a study of associations between sexually transmitted infections and high-risk human papillomavirus infection found that positive rate of high-risk HPV infection in total STIs positive group was 1.47 times higher than that of total STIs negative group which is similar to our study.[16]

In our study, the distribution of the 71 HPV-positive cases was almost equal, with 50.7% having HR-HPV-16/18 and 49.3% having other types. Certain symptoms, however, were more possibly associated with particular HPV strains. Pelvic pain was more commonly connected with other HPV strains (70.59%), whereas pain during sexual activity was primarily linked to HPV-16/18 (85.71%). Furthermore, individuals with HPV-16/18 were more likely to experience atypical discharge (61.11%). This implied that some symptoms might be more closely associated with specific HPV risk groups which is comparable with a study performed in Nepal.[1] High-risk HPV-16/18 was possibly linked to symptoms like pain during intercourse (85.71%) and cervical lesions (70.59%), which is similar with the various study findings that highlight these types as predominant in cervical neoplasia.[17,18] However, We did not formally test for differences by symptoms due to small numbers and potential confounding thus they may not reflect true underlying risk differences. HPV-16/18 prevalence increases with age (35-45), aligning with persistence patterns reported in a study performed in Nepal.[8] These findings underscore the need for comprehensive, genotype-specific screening and follow-up in Nepalese women to reduce the risk of cervical cancer.

CONCLUSION

The study found that the test group had a low overall HPV positivity rate of 3.5%, with non-16/18 HPV genotypes being the most prevalent, followed by HPV 16 and HPV 18. The largest percentage of HPV-positive infections were seen in women between the ages of 35 and 40. HPV infection was almost three times more in women with cervical lesions than in those without cervical lesions. These results highlight the significance of early cervical lesion diagnosis and routine HPV screening for successful cervical cancer prevention.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this study.

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