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Original Article

Prevalence of Human Papillomavirus Infection and Clinicodemographic Correlates among Women Attending a Tertiary-Care Hospital: A Cross-Sectional Study Using Cytological and Molecular Approaches.

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Abstract

Background:

Cervical cancer remains a major cause of morbidity and mortality among women in low- and middle-income countries, driven primarily by persistent infection with high-risk HPV, especially types 16 and 18. Although cytology-based screening has reduced disease burden, its variable sensitivity highlights the need for complementary molecular methods. This study estimated HPV prevalence, characterized HPV-16/18 distribution, assessed associations with clinicodemographic and reproductive factors, and compared the diagnostic performance of Pap smear cytology and HPV qPCR in women attending a tertiary-care hospital.

Methodology:

A hospital-based cross-sectional study was conducted among 82 women referred for cervical evaluation. Cervical samples were subjected to conventional Pap smear cytology and PCR-based HPV testing. Broad-spectrum L1 PCR was used for HPV detection, followed by genotype-specific real-time PCR for HPV-16 and HPV-18. Clinicodemographic, reproductive, and clinical data were collected using a structured questionnaire. Associations with HPV infection were assessed using chi-square or Fisher's exact tests, and diagnostic agreement between Pap smear and qPCR was evaluated using concordance measures.

Results:

HPV DNA was detected in 21 participants (25.6%). HPV positivity was significantly associated with diabetes mellitus, anaemia, higher parity (≥ 3), non-barrier or absent contraceptive use, and clinical symptoms including white vaginal discharge, menstrual irregularities, and abdominal pain. HPV infection was most frequent among women aged 41–50 years. Cytological abnormalities were identified in 22.0% of participants, with squamous cell carcinoma being the most common abnormal finding. Concordance analysis demonstrated substantial agreement between Pap smear cytology and HPV qPCR, highlighting their complementary diagnostic roles.

Conclusion:

HPV infection was common among women attending a tertiary-care referral clinic, with significant associations observed with metabolic comorbidities, reproductive factors, and selected clinical symptoms. The findings support the integration of cytological and molecular screening approaches to improve early detection and risk stratification, particularly in resource-constrained tertiary-care settings.

Keywords:

Human Papillomavirus Infections, Uterine Cervical Neoplasms, Polymerase Chain Reaction, Papanicolaou Test, Cervical Cancer Screening

Introduction

Cervical cancer is the fourth most common malignancy among women worldwide and remains a major public health challenge, particularly in low- and middle-income countries where organized screening programs are limited or inconsistently implemented [1]. Despite being largely preventable, cervical cancer continues to contribute substantially to female morbidity and mortality, largely due to late diagnosis and inadequate access to effective screening strategies.

Persistent infection with high-risk human papillomavirus (HPV) is the central etiological factor in the development of cervical intraepithelial neoplasia and invasive cervical carcinoma [2]. Among the more than 200 identified HPV genotypes, HPV-16 and HPV-18 account for approximately 70% of cervical cancer cases globally, owing to their strong oncogenic potential [3,4]. The viral oncoproteins E6 and E7 disrupt key tumor suppressor pathways, including p53 and retinoblastoma protein, leading to genomic instability and malignant transformation of cervical epithelial cells [5].

Cytology-based screening using the Papanicolaou (Pap) smear has played a critical role in reducing cervical cancer incidence in high-income countries. However, the sensitivity of Pap smear testing varies widely and is influenced by sample adequacy, observer expertise, and subjective interpretation [6]. In contrast, molecular detection of HPV DNA using PCR-based methods offers higher analytical sensitivity, reproducibility, and the ability to identify specific high-risk genotypes [7]. Genotype-specific detection is particularly relevant, as infection with HPV-16 or HPV-18 confers a substantially higher risk of progression to high-grade lesions and invasive disease compared with other HPV types [3].

Beyond viral factors, the natural history of HPV infection and its progression to malignancy is influenced by host-related and environmental determinants. Sociodemographic and reproductive factors such as age at sexual debut, multiparity, contraceptive practices, socioeconomic status, and coexisting medical conditions have been shown to affect HPV acquisition, persistence, and clearance [8–11]. Metabolic and nutritional conditions, including diabetes mellitus and anaemia, may further impair immune responses and promote viral persistence [12]. Understanding these associations in specific regional and clinical contexts is essential for refining screening strategies and identifying high-risk subgroups.

In many low-resource settings, hospital-based and referral populations represent a critical point of contact for cervical cancer prevention, yet epidemiological data from such settings remain limited. Moreover, comparative evaluations of cytological and molecular screening modalities within tertiary-care contexts are essential to inform evidence-based screening algorithms. Therefore, the present study was undertaken to determine the prevalence of HPV infection, assess the distribution of HPV-16 and HPV-18, examine clinicodemographic and reproductive correlates of HPV positivity, and compare the diagnostic performance of Pap smear cytology and HPV qPCR among women attending a tertiary-care hospital.

Methodology

Study Design

This investigation was conducted as a hospital-based cross-sectional analytical study, designed to estimate the prevalence of human papillomavirus (HPV) infection and to examine its association with

selected clinicodemographic, reproductive, and clinical variables among women undergoing cervical evaluation. The cross-sectional design was chosen to provide a snapshot of HPV burden and related factors within a tertiary-care referral setting, where women present for cervical screening, Pap smear examination, and colposcopic assessment.

Ethics

The study protocol received approval from the Institutional Ethical Committee (IEC) of Burdwan Medical College (Memo No.: BMC-1200, dated 25/05/2018). All procedures were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrolment. Participants were counselled regarding the purpose of the study, sample collection procedures, potential benefits, and follow-up recommendations in the event of abnormal findings.

Setting

The study was carried out at the Department of Microbiology and the ICMR-DHR-VRDL laboratory, Burdwan Medical College, a tertiary-care teaching hospital that functions as a regional referral centre for gynaecological and cervical disease evaluation. Recruitment occurred through the colposcopy and cervical screening clinics, where women are routinely referred for Pap smear testing, colposcopic examination, or further diagnostic work-up due to clinical symptoms or abnormal screening results.

Participants

A total of 82 consecutive women attending the cervical screening or colposcopy clinic were enrolled during the study period. Eligible participants included women presenting for Pap smear evaluation or referred for suspected cervical pathology. Women with a prior total hysterectomy, those with active vaginal bleeding that precluded adequate sample collection, or those who had received recent topical vaginal therapy were excluded to ensure sample adequacy and data reliability. This consecutive sampling approach minimized selection bias within the constraints of a hospital-based design.

Variables

The primary outcome variable was HPV infection status, determined by molecular detection of HPV DNA. Secondary variables included HPV-16 and HPV-18 genotype status, Pap smear cytology findings, and a range of clinicodemographic and reproductive factors. Explanatory variables comprised age, occupation, household income, parity, age at coitarche, contraceptive practices, and clinical symptoms such as white vaginal discharge, menstrual irregularities, post-coital bleeding, and abdominal pain. Medical comorbidities, including diabetes mellitus, anaemia, hypertension, and thyroid disorders, were also recorded as potential cofactors influencing HPV persistence and susceptibility.

Data Sources and Measurement

Data were collected using a structured interviewer-administered questionnaire capturing demographic characteristics, reproductive history, contraceptive use, menstrual and obstetric history, presenting symptoms, and relevant medical conditions. Cervical samples were obtained from the ecto-endocervical junction using standard spatula and cytobrush techniques. One portion of the sample was processed for conventional Pap smear cytology, reported according to the Bethesda System, while another portion was preserved in viral transport medium for molecular analysis.

DNA extraction was performed using a validated commercial extraction kit according to the manufacturer's instructions. Sample

adequacy and DNA integrity were confirmed through amplification of the β -actin housekeeping gene. Broad-spectrum HPV screening was conducted using conventional PCR targeting the conserved L1 region of the HPV genome. Samples positive for L1 were further subjected to genotype-specific real-time PCR (qPCR) for HPV-16 (E6 region) and HPV-18 (E7 region). Positive and negative controls were included in each PCR run to ensure assay validity and contamination control.

Bias

Given the hospital-based and referral nature of the study population, selection bias is acknowledged, as symptomatic women or those with suspected cervical pathology were overrepresented. This may have contributed to higher observed HPV prevalence and cytological abnormalities compared with population-based screening cohorts. To reduce information bias, standardized questionnaires and uniform laboratory protocols were employed, and all molecular assays included internal controls to ensure consistency and reproducibility of results.

Study Size

The study included 82 participants, reflecting all eligible women presenting during the defined study period. Although no formal sample size calculation was performed, the sample size was considered adequate for estimating HPV prevalence and conducting exploratory analyses of associations within a tertiary-care setting. The modest number of HPV-positive cases was taken into account during statistical interpretation to avoid overfitting of analytical models.

Quantitative Variables

Quantitative variables such as age were categorized into clinically relevant age groups to facilitate comparison with existing epidemiological studies. Parity, income, and age at coitarche were also categorized based on established thresholds reported in prior HPV research. HPV infection status and cytological findings were treated as categorical outcome variables for analytical purposes.

Statistical Methods

Data were entered into a secure database and analysed using SPSS software. Categorical variables were summarized as frequencies and percentages [n/N (%)]. Associations between HPV infection status and clinicodemographic or clinical variables were assessed using the chi-square test or Fisher's exact test, depending on expected cell counts. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for univariate associations. Diagnostic performance measures—including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy—were calculated to compare Pap smear cytology with HPV qPCR, using molecular testing as the reference standard. A p-value <0.05 was considered statistically significant.

Results

Participants

Data were entered into a secure database and analysed using SPSS software. Categorical variables were summarized as frequencies and percentages [n/N (%)]. Associations between HPV infection status and clinicodemographic or clinical variables were assessed using the chi-square test or Fisher's exact test, depending on expected cell counts. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for univariate associations. Diagnostic performance measures including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy were calculated to compare Pap smear cytology with HPV qPCR, using

molecular testing as the reference standard. A p-value <0.05 was considered statistically significant.

Descriptive Data

The clinicodemographic, reproductive, and socioeconomic characteristics of the study population are summarized in Table 2. Participants represented a broad age range and diverse occupational and income categories, reflecting the heterogeneous population attending the tertiary-care referral clinic. Comorbid conditions including diabetes mellitus, anaemia, hypertension, and thyroid disorders were commonly reported. Cytological findings based on the Bethesda System are detailed in Table 1. Of the 82 Pap smear samples examined, 64 (78.0%) were reported as negative for intraepithelial lesion or malignancy (NILM), while 18 (22.0%) showed epithelial cell abnormalities. Among abnormal cytology results, squamous cell carcinoma was the most frequent diagnosis (11 cases; 61.1%), followed by ASC-US, ASC-H, HSIL, and LSIL. No glandular epithelial abnormalities were identified. The predominance of malignant cytology reflects the referral-based nature of the study population.

Outcome Data

HPV DNA was detected in 21 of 82 samples (25.6%) by molecular testing. Associations between HPV infection status and clinicodemographic, reproductive, and clinical variables are presented in Table 2. Significant associations were observed between HPV positivity and diabetes mellitus as well as anaemia, while hypertension and thyroid disorders did not differ significantly between HPV-positive and HPV-negative groups.

Reproductive factors showed meaningful associations with HPV infection. Age at coitarche between 18 and 25 years and parity of three or more (P3+0) were significantly associated with HPV positivity (Table 2). Contraceptive practices also differed significantly by HPV status, with higher HPV positivity observed among women using non-barrier contraceptive methods or reporting no contraceptive use (Table 2).

Regarding age-group distribution, HPV positivity was most frequently observed among women aged 41–50 years, although the overall association with age group was not statistically significant (Table 2).

Several clinical symptoms demonstrated significant associations with HPV infection. White vaginal discharge, menstrual irregularities, and abdominal pain were significantly more common among HPV-positive participants, whereas post-coital bleeding and post-menopausal bleeding did not show statistically significant associations (Table 2).

Main Results

This study demonstrated a 25.6% prevalence of HPV infection among women attending a tertiary-care cervical screening clinic. HPV positivity was significantly associated with metabolic comorbidities, higher parity, specific reproductive characteristics, and contraceptive practices, as detailed in Table 2.

Cytological abnormalities were identified in 22.0% of participants (Table 1), with a high proportion of squamous cell carcinoma reflecting the referral-driven nature of the cohort. Concordance analysis between HPV qPCR and Pap smear cytology is presented in Table 3, demonstrating substantial agreement between the two screening modalities. While Pap smear identified epithelial

abnormalities, HPV qPCR confirmed viral infection, underscoring their complementary diagnostic roles.

Together, the results highlight the considerable burden of HPV infection in a tertiary-care referral population and support the value of integrated cytological and molecular screening strategies, particularly for high-risk women presenting with clinical symptoms.

Table 1: Cytological findings of cervical samples based on the Bethesda System among study participants (N = 82).

Bethesda Category		Findings	n (%)
Negative for Intraepithelial Lesion or Malignancy (NILM) (n=64)	Organisms (n=52)	Shift in flora suggestive of bacterial vaginosis	32 (61.5%)
		Trichomonas vaginalis	6 (11.5%)
		Fungal organisms (Candida spp.)	14 (26.9%)
		Actinomycosis	0 (0%)
		Herpes simplex virus (HSV)	0 (0%)
		Cytomegalovirus (CMV)	0 (0%)
	Other non-neoplastic findings (n=12)	Reactive changes associated with inflammation	12 (100%)
Epithelial Cell Abnormalities (Pap smear positive) (n=18)	Squamous cell abnormalities	Glandular cells status post-hysterectomy	0 (0%)
		Atypical squamous cells of undetermined significance (ASC-US)	2 (11.1%)
		Atypical squamous cells – cannot exclude HSIL (ASC-H)	2 (11.1%)
		Low-grade squamous intraepithelial lesion (LSIL)	1 (5.6%)
		High-grade squamous intraepithelial lesion (HSIL)	2 (11.1%)
		Squamous cell carcinoma	11 (61.1%)

Table 2: Association of clinicodemographic characteristics, reproductive factors, contraceptive practices, comorbidities, and clinical symptoms with HPV infection status among the study population.

Parameter	HPV Positive (N=21)	HPV Negative (N=61)	Total (N=82)	χ^2	p value
Co-morbidity					
Hypertension	22.20%	18.20%	19.50%	0.42	0.520
Diabetes mellitus	25.90%	23.60%	24.40%	7.1	0.008*
Hypothyroidism	7.40%	12.70%	11.00%	1.03	0.310
Hyperthyroidism	7.40%	3.60%	4.90%	0.66	0.420
Anaemia	37.00%	41.80%	40.20%	4.88	0.027*
Coitarche (years)					
<18	19.00%	9.80%	12.20%	0.82	0.360
18–25	76.20%	37.70%	47.60%	9.02	0.003*
>25	33.30%	42.60%	40.20%	0.45	0.500
Parity					
P1+0	14.30%	13.10%	13.40%	0.01	0.920
P2+0	28.60%	39.30%	36.60%	0.58	0.450
P3+0	71.40%	26.20%	37.80%	6.85	0.009*
≥P4	14.30%	11.50%	12.20%	0.08	0.780
Occupation					
Agriculture	25.90%	25.50%	25.60%	0.97	0.320
Daily worker	44.40%	32.70%	36.60%	3.73	0.190
Teacher	7.40%	16.40%	13.40%	1.6	0.30
Housewife	18.50%	7.30%	11.00%	1.01	0.290
Trade	3.70%	18.10%	13.40%	0.85	0.750
Income (₹)					
≤10,000	48.10%	45.50%	46.30%	2.3	0.320
10–20k	33.30%	18.20%	23.20%	3.1	0.170
20–30k	14.80%	27.30%	23.20%	1.02	0.610
>30k	3.70%	9.10%	7.30%	0.74	0.430
Method					
Barrier	33.30%	30.90%	31.70%	1.07	0.300
Non-barrier	51.90%	50.90%	51.20%	5.88	0.036*
Not used	14.80%	18.20%	17.10%	8.7	0.002*
Age group					
20–30	18.50%	29.10%	25.60%	0.75	0.720
31–40	22.20%	27.30%	25.60%	1.03	0.330
41–50	48.10%	34.50%	39.00%	3.12	0.130

>50	11.10%	9.10%	9.80%	0.82	0.710
Symptom					
White discharge	51.90%	38.20%	42.70%	4.71	0.041*
Post-coital bleeding	18.50%	21.80%	20.70%	0.8	0.910
Menstrual irregularity	11.10%	9.10%	9.80%	4.3	0.031*
Post-menopausal bleeding	3.70%	14.50%	11.00%	0.87	0.940
Abdominal pain	14.80%	16.40%	15.90%	6.91	0.001*

* $p \leq 0.05$ indicates statistically significant.

Table 3: Concordance between HPV qPCR and Pap smear cytology results among study participants.

Pap Smear Result	HPV qPCR Positive	HPV qPCR Negative	Total
Positive	14	4	18
Negative	7	57	64
Total	21	61	82

Table 4: Diagnostic agreement measures between Pap smear and HPV qPCR.

Measure	Value
Overall agreement (%)	86.59
Positive agreement (%)	66.67
Negative agreement (%)	89.06
Cohen's kappa (κ)	0.63
Strength of agreement	Substantial agreement

Discussion

This hospital-based cross-sectional study provides an integrated evaluation of HPV prevalence, genotype distribution, clinicodemographic correlates, and diagnostic performance of cytological and molecular screening methods among women undergoing cervical evaluation at a tertiary-care centre. The overall HPV prevalence of 25.6% observed in this study is consistent with previously reported hospital-based estimates from India and other low- and middle-income countries, where prevalence typically ranges from 15% to 35% depending on population characteristics and diagnostic sensitivity [7–9]. Importantly, the referral-driven nature of the study population must be considered when interpreting these findings, as symptomatic women and those with suspected cervical pathology are overrepresented compared with general population screening cohorts.

HPV positivity was most frequently observed among women aged 41–50 years, although the association with age group did not reach statistical significance. While population-based studies often report higher HPV prevalence in younger women with a subsequent decline due to immune-mediated clearance [8], a secondary peak in middle-aged or peri-menopausal women has been documented in hospital-based cohorts. This pattern may reflect persistent or reactivated infections, age-related immune senescence, or hormonal changes that influence viral persistence and cervical epithelial susceptibility [9,10].

Reproductive factors demonstrated significant associations with HPV infection in the present study. Women with higher parity (≥ 3) exhibited significantly greater HPV positivity, supporting existing evidence that repeated cervical trauma, prolonged exposure of the transformation zone, and hormonal influences during multiple pregnancies may facilitate HPV persistence and progression to cervical neoplasia [3,11]. Similarly, age at coitarche between 18 and 25 years was significantly associated with HPV positivity. Early sexual exposure during periods of cervical epithelial immaturity

increases vulnerability to HPV acquisition and reduces the likelihood of effective viral clearance [9].

Contraceptive practices were also significantly associated with HPV infection. Non-barrier contraceptive use and absence of contraception were more common among HPV-positive women, whereas barrier methods showed no significant association. Hormonal contraceptives have been implicated in enhancing HPV oncogene expression and modulating local immune responses, potentially increasing the risk of persistent infection [11]. In contrast, barrier methods provide partial protection against HPV transmission, although their effectiveness is variable and dependent on consistent use [9].

The study also identified significant associations between HPV infection and diabetes mellitus and anaemia, highlighting the role of host metabolic and nutritional factors in HPV susceptibility. Hyperglycaemia-associated immune dysfunction and micronutrient deficiencies linked to anaemia may impair cell-mediated immunity and delay viral clearance, thereby promoting persistent infection [12]. While not uniformly reported across studies, emerging evidence supports the influence of systemic health conditions on HPV pathogenesis and progression.

Several clinical symptoms including white vaginal discharge, menstrual irregularities, and abdominal pain were significantly associated with HPV positivity. These symptoms may reflect underlying cervical inflammation, co-existing infections, or epithelial disruption that facilitate viral persistence or detection [6]. Conversely, post-coital bleeding and post-menopausal bleeding did not demonstrate statistically significant associations, likely due to their multifactorial etiology and non-specific relationship with HPV infection.

Comparative diagnostic evaluation demonstrated substantial agreement between Pap smear cytology and HPV qPCR, consistent with previous studies emphasizing their complementary roles in cervical cancer screening [6,7]. Pap smear cytology exhibited higher sensitivity and negative predictive value, enabling detection of cytological abnormalities, while HPV qPCR provided greater

specificity and positive predictive value by directly identifying viral DNA. These findings reinforce the value of integrated or triage-based screening strategies, particularly in tertiary-care and resource-constrained settings where maximizing diagnostic yield is critical [14,15].

Limitations

Several limitations should be acknowledged. First, the hospital-based and referral design limits the generalizability of findings to the broader asymptomatic population. Second, the modest sample size and relatively small number of HPV-positive cases may have reduced statistical power and limited the ability to perform robust multivariable analyses. Third, genotyping was restricted to HPV-16 and HPV-18, potentially underestimating the contribution of other high-risk HPV genotypes prevalent in the region. Additionally, the cross-sectional design precludes causal inference and does not allow assessment of HPV persistence or progression over time. Despite these limitations, the study provides valuable insights into HPV burden and associated factors in a tertiary-care setting.

Conclusion

This study demonstrates a substantial burden of HPV infection among women attending a tertiary-care hospital for cervical evaluation and highlights significant associations with reproductive characteristics, contraceptive practices, metabolic comorbidities, and selected clinical symptoms. The complementary diagnostic performance of Pap smear cytology and HPV qPCR underscores the importance of integrated screening approaches rather than reliance on a single modality. These findings support targeted screening and follow-up strategies for high-risk women in referral settings and emphasize the need for expanded genotyping and longitudinal studies to inform region-specific cervical cancer prevention programs.

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