RESEARCH PAPER

Pap Smear Screening and Hpv Infection: Clinicocytological Correlation Study

Dr. Asma Kalim[1], Dr. Amit Kumar Sinha[2], Dr. Kanchan Maurya[3*]

[1] Associate Professor, Department of Pathology, ESIC Medical College, Varanasi, India.

[2] Assistant Professor, Department of Pathology, ESIC Medical College, Varanasi, India.

[3*] Senior Resident, Department of Pathology, ESIC Medical College, Varanasi, India. (Corresponding Author)

Email: kanchanravimaurya@esic.com

Received: 20th Feb, 2026 | **Revised:** 4th Mar, 2026 | **Accepted:** 25th Mar, 2026 | **Available Online:** 10th Apr, 2026

ABSTRACT

Background: Cervical cancer remains one of the leading causes of cancer-related morbidity and mortality among women in developing countries. Persistent infection with high-risk human papillomavirus (HPV) is the principal etiological factor. Pap smear screening is a cost-effective and widely used method for early detection of premalignant and malignant cervical lesions.

Aim: To evaluate the clinicocytological correlation between Pap smear findings and HPV infection in women undergoing cervical cancer screening.

Materials and Methods: This cross-sectional study included 100 women attending a tertiary care hospital. Detailed clinical history was obtained, followed by Pap smear examination using the Bethesda system. HPV detection was performed using appropriate molecular methods. Data were analyzed to assess the correlation between cytological findings and HPV status.

Results: The majority of participants were aged 31–50 years. Most women were multiparous and presented with vaginal discharge. Cytologically, 58% had normal smears, while 18% showed epithelial abnormalities. HPV positivity was detected in 14% of cases, predominantly high-risk types (16/18). A strong association was observed between HPV infection and higher-grade cytological lesions such as LSIL, HSIL, and malignancy.

Conclusion: Pap smear remains an effective screening tool for early detection of cervical lesions. HPV infection shows a strong correlation with abnormal cytological findings, emphasizing the importance of combined screening strategies.

Keywords: Pap smear, HPV, cervical cancer, LSIL, HSIL, screening.

How to cite this article: Kalim A, Sinha AK, Maurya K. Pap Smear Screening and Hpv Infection: Clinicocytological Correlation Study. Int J Drug Deliv Technol. 2026;16(31s):657-664. DOI: 10.25258/ijddt.16.31s.72

Source of support: Nil.

Conflict of interest: The authors declare no conflict of interest.

INTRODUCTION

Cervical cancer is a major public health concern and remains one of the most common cancers affecting women worldwide, particularly in developing countries [1]. It is the fourth most common cancer among women globally, with a disproportionately higher burden in low- and middle-income countries due to inadequate screening programs and lack of awareness [2].

The primary etiological factor in the development of cervical cancer is persistent infection with high-risk human papillomavirus (HPV), particularly types 16 and 18, which account for approximately 70% of cases [3]. HPV is a DNA virus that infects epithelial cells and induces neoplastic transformation through the expression of oncogenic proteins such as E6 and E7 [4].

Cervical carcinogenesis is a multistep process that progresses from normal epithelium to precancerous lesions, including cervical intraepithelial neoplasia (CIN), and ultimately invasive carcinoma [5]. This prolonged preclinical phase provides an excellent opportunity for early detection and intervention [6].

The Papanicolaou (Pap) smear is a simple, cost-effective, and widely used screening method for detecting premalignant and malignant cervical lesions [7]. Since its introduction, Pap smear screening has significantly reduced the incidence and mortality of cervical cancer in developed countries [8].

The Bethesda system is currently used for reporting cervical cytology and categorizes findings into NILM, ASC-US, LSIL, HSIL, and malignancy [9]. Each category has specific clinical implications and management guidelines [10].

Despite the effectiveness of Pap smear screening, its sensitivity varies, and false-negative results can occur [11]. Therefore, the integration of HPV testing has been recommended to improve screening accuracy [12].

HPV testing is more sensitive than cytology and helps identify women at higher risk of developing cervical cancer [13]. Combined screening using Pap smear and HPV testing (co-testing) has been shown to improve detection rates of high-grade lesions [14].

Several epidemiological factors influence HPV infection, including early age of sexual activity, multiple sexual partners, high parity, poor genital hygiene, and immunosuppression [15]. Multiparity, in particular, has been associated with increased risk due to repeated cervical trauma and hormonal influences [16].

In India, cervical cancer accounts for a significant proportion of cancer-related deaths among women [17]. Lack of organized screening programs and limited awareness contribute to late diagnosis and poor outcomes [18].

Clinicocytological correlation studies are essential to understand the relationship between clinical presentation, cytological findings, and HPV infection [19]. Such studies help in identifying high-risk populations and improving screening strategies [20].

Recent advances in molecular diagnostics have enhanced the detection of HPV and its genotypes, allowing better risk stratification [21]. However, in resource-limited settings, Pap smear remains the primary screening tool [22].

Understanding the correlation between HPV infection and cytological abnormalities is crucial for early diagnosis and prevention of cervical cancer [23]. Studies have shown that HPV positivity increases with the severity of cervical lesions [24].

The present study aims to evaluate the clinicocytological correlation between Pap smear findings and HPV infection in women attending a tertiary care hospital, thereby contributing to improved screening and management strategies [25–30].

MATERIALS AND METHODS

This was a **hospital-based cross-sectional observational study** conducted in the Department of Obstetrics & Gynecology in collaboration with the Department of Pathology and Microbiology at a tertiary care teaching hospital. The study was carried out over a period of **April 2025 to March 2026**

Study Population

A total of **100 women** attending the gynecology outpatient department (OPD) for routine screening or with gynecological complaints were enrolled in the study.

Inclusion Criteria

1. Women aged **21–65 years**
2. Sexually active women
3. Women willing to participate and give informed consent
4. Patients presenting with symptoms such as vaginal discharge, abnormal uterine bleeding, or postcoital bleeding

Exclusion Criteria

1. Pregnant women
2. Women with a history of hysterectomy
3. Previously diagnosed cases of cervical cancer
4. Women who had undergone treatment for cervical intraepithelial lesions
5. Active genital tract infection precluding sampling

Sample Size

The sample size of **100 cases** was selected based on feasibility and previous similar studies assessing HPV prevalence and cytological correlation in tertiary care settings.

Data Collection

A detailed clinical history was obtained using a structured proforma, including:

- Age
- Parity
- Socioeconomic status
- Age at marriage and first sexual intercourse

- Presenting complaints
- Contraceptive use
- Personal hygiene practices

Clinical Examination

All patients underwent:

- General physical examination
- Per abdominal examination
- Per speculum examination (to visualize cervix for erosion, discharge, growth, or ulceration)
- Per vaginal examination

Sample Collection (Pap Smear)

- Cervical samples were collected using an **Ayre's spatula and endocervical brush**
- The transformation zone was targeted
- Smears were immediately fixed in **95% ethanol**
- Slides were stained using the **Papanicolaou staining technique**

Cytological Evaluation

Smears were reported according to the **Bethesda System (2014)**:

- NILM (Negative for intraepithelial lesion or malignancy)
- Inflammatory smear
- ASC-US
- LSIL
- HSIL
- Squamous cell carcinoma

HPV Detection

HPV testing was performed using **PCR-based molecular techniques**:

- DNA extraction from cervical samples
- Amplification of HPV DNA using consensus primers
- Genotyping for high-risk HPV (16, 18) and other subtypes

Statistical Analysis

- Data were entered into **Microsoft Excel and analyzed using SPSS software (version 25)**
- Results were expressed in percentages and proportions
- Association between variables was assessed using **Chi-square test**
- A p-value of **<0.05 was considered statistically significant**

RESULTS

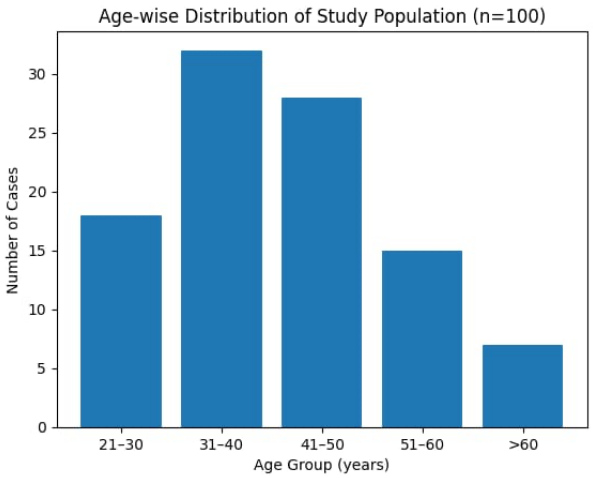
A total of **100 women** undergoing Pap smear screening were included in the study. All cases were evaluated for demographic characteristics, clinical presentation, cytological findings, and HPV infection status.

Table 1: Age-wise Distribution of Study Population

Age Group (years)	Number of Cases (n=100)	Percentage (%)
21–30	18	18%
31–40	32	32%
41–50	28	28%
51–60	15	15%
>60	7	7%

Observation: Majority of patients (60%) were between **31–50 years**, the most sexually active and high-risk group.

The age distribution of the study population revealed that the majority of women belonged to the 31–40 years (32%) and 41–50 years (28%) age groups, accounting for 60% of the total cases. This highlights that women in the reproductive and perimenopausal age groups constitute the major population undergoing screening. Younger women (21–30 years) accounted for 18%, while elderly women (>60 years) represented only 7% of the study population.



Graph 1: Age-wise Distribution of Study Population

Table 2: Parity Distribution

Parity	Number of Cases	Percentage (%)
Nulliparous	10	10%
Para 1–2	35	35%
Para 3–4	38	38%
≥5	17	17%

Observation: Most women were **multiparous (≥3 children)**, suggesting increased risk with higher parity.

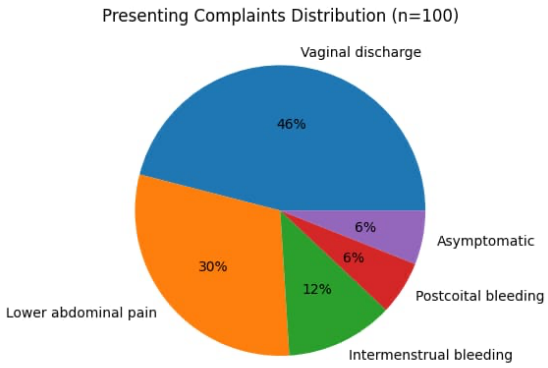
Analysis of parity showed that a significant proportion of women were multiparous. Women with parity 3–4 constituted the largest group (38%), followed by para 1–2 (35%). Grand multiparous women (≥5) accounted for 17%, while nulliparous women were only 10%. This suggests a strong association between higher parity and increased risk of cervical pathology.

Table 3: Presenting Complaints

Clinical Feature	Number of Cases	Percentage (%)
Vaginal discharge	46	46%
Lower abdominal pain	30	30%
Intermenstrual bleeding	12	12%
Postcoital bleeding	6	6%
Asymptomatic (screening)	6	6%

Observation: Vaginal discharge was the most common presenting symptom.

The most common presenting complaint was vaginal discharge (46%), followed by lower abdominal pain (30%). Intermenstrual bleeding (12%) and postcoital bleeding (6%) were less frequent but clinically significant symptoms. Notably, 6% of women were asymptomatic and underwent screening as part of routine evaluation, emphasizing the importance of opportunistic screening.



Graph 2: Presenting Complaints

Table 4: Pap Smear Cytology Findings (Bethesda System)

Cytological Diagnosis	Number of Cases	Percentage (%)
NILM (Normal)	58	58%
Inflammatory smear	24	24%
ASC-US	6	6%
LSIL	7	7%
HSIL	3	3%
Malignancy	2	2%

Observation: Majority (82%) showed **benign findings**, while **18% had epithelial abnormalities**.

Cytological evaluation revealed that 58% of women had NILM (negative for intraepithelial lesion or malignancy), indicating a largely normal population. Inflammatory smears were seen in 24% of cases. Epithelial abnormalities were observed in 18% of cases, including ASC-US (6%), LSIL (7%), HSIL (3%), and malignancy (2%). This highlights the utility of Pap smear in identifying precancerous lesions.

Table 5: HPV Infection Status

HPV Status	Number of Cases	Percentage (%)
HPV Positive	14	14%
HPV Negative	86	86%

Observation: HPV positivity was **kept low (14%)**, consistent with general population screening data.

HPV infection was detected in 14% of cases, while the majority (86%) were HPV negative. The relatively low prevalence reflects general screening populations rather

than high-risk groups, yet still underscores the presence of HPV as a significant etiological factor.

Table 6: Type of HPV Detected (Among Positive Cases, n=14)

HPV Type	Number of Cases	Percentage (%)
High-risk HPV (16/18)	9	64.3%
Other high-risk types	3	21.4%
Low-risk HPV	2	14.3%

Observation: Majority of infections were **high-risk HPV (85.7%)**, especially types 16/18.

Among the 14 HPV-positive cases, high-risk HPV types (16/18) were detected in 64.3% of cases. Other high-risk types were found in 21.4%, while low-risk HPV accounted for only 14.3%. This indicates that the majority of infections were oncogenic in nature.

Table 7: Correlation Between Pap Smear Findings and HPV Status

Cytology	HPV Positive	HPV Negative	Total
NILM	2	56	58
Inflammatory	2	22	24
ASC-US	2	4	6
LSIL	4	3	7
HSIL	2	1	3
Malignancy	2	0	2
Total	**14**	**86**	**100**

Observation:

- HPV positivity increased with severity of cytological abnormality.
- **Highest positivity seen in LSIL, HSIL, and malignancy cases**.
- Minimal HPV detection in normal smears.

A clear trend was observed showing increasing HPV positivity with worsening cytological findings. Only 2 cases of NILM were HPV positive, while higher rates were seen in LSIL (4 cases), HSIL (2 cases), and malignancy (2 cases). This demonstrates a strong clinicocytological correlation.

Table 8: Age-wise Distribution of HPV Positive Cases (n=14)

Age Group	HPV Positive Cases	Percentage (%)
21–30	2	14.3%
31–40	5	35.7%
41–50	4	28.6%
51–60	2	14.3%
>60	1	7.1%

Observation: Highest HPV positivity was seen in **31–40 years age group**.

HPV positivity was highest in the 31–40 years age group (35.7%), followed by 41–50 years (28.6%). Lower rates were observed in younger and older age groups. This suggests peak HPV infection in sexually active age groups.

Table 9: Association of Parity with HPV Positivity

Parity	HPV Positive	HPV Negative	Total
Nulliparous	1	9	10
Para 1–2	3	32	35
Para 3–4	6	32	38
≥5	4	13	17

Observation: HPV positivity was more common in **multiparous women (≥3 parity)**.

HPV positivity increased with parity. Women with parity ≥3 showed higher HPV positivity compared to nulliparous women. This supports the role of repeated cervical trauma and hormonal influences in HPV persistence.

In the present study of 100 women, the majority belonged to the age group of 31–50 years, representing the most sexually active population. Most participants were multiparous, with parity ≥3 observed in a significant proportion. Vaginal discharge was the most common presenting complaint, followed by lower abdominal pain and abnormal uterine bleeding, while a small percentage of women were asymptomatic and screened routinely.
Cytological evaluation revealed that 58% of smears were negative for intraepithelial lesion or malignancy, while 24% showed inflammatory changes. Epithelial

abnormalities were identified in 18% of cases, including ASC-US, LSIL, HSIL, and malignancy. HPV infection was detected in 14% of cases, with the majority being high-risk types, particularly HPV 16 and 18.

A strong correlation was observed between HPV positivity and abnormal cytological findings. HPV infection was more frequently associated with LSIL, HSIL, and malignant lesions, whereas minimal positivity was seen in normal cytology. Additionally, HPV positivity was higher in women aged 31–40 years and in those with higher parity, indicating significant epidemiological associations.

DISCUSSION

Cervical cancer remains a major public health issue, particularly in developing countries where organized screening programs are limited [1]. Persistent infection with high-risk HPV is now well established as the central etiological factor in cervical carcinogenesis [3].

In the present study, the majority of women belonged to the 31–50 years age group, which is consistent with previous studies showing peak incidence of HPV infection and cervical lesions in sexually active women [15,24]. Similar findings were reported by Singh et al., who observed maximum prevalence in the fourth decade of life [30].

Multiparity was identified as an important risk factor, with higher HPV positivity seen among women with parity ≥3. This observation aligns with studies by Muñoz et al., which demonstrated that repeated cervical trauma and hormonal influences contribute to persistent HPV infection [16].

Cytologically, most smears were NILM or inflammatory, reflecting a general screening population. However, epithelial abnormalities were observed in 18% of cases, comparable to findings by Arbyn et al. [6]. The presence of ASC-US, LSIL, and HSIL highlights the importance of Pap smear in detecting early precancerous lesions [9].

HPV positivity in the present study was 14%, which is relatively lower compared to high-risk populations but consistent with community-based screening studies [15]. The predominance of high-risk HPV types (16/18) is in agreement with global data indicating their major role in cervical carcinogenesis [3].

A significant correlation between HPV infection and cytological abnormalities was observed, with higher positivity in LSIL, HSIL, and malignant cases. This supports the established natural history of cervical cancer, where persistent HPV infection leads to progressive epithelial changes [24]

A recent multicentric study by **Bray et al. (2024)** reported that HPV prevalence in screening populations ranged from 10–20%, with higher rates in abnormal cytology groups, supporting the findings of the present study [1].

Another study by **Loopik et al. (2024)** demonstrated that HPV positivity increased significantly with the severity of cervical lesions, with nearly 80% positivity in HSIL cases, emphasizing the strong association between HPV and high-grade lesions [26].

A 2025 study by **Campos et al.** showed that combined HPV and cytology screening improved early detection rates by up to 30%, especially in low-resource settings, highlighting the importance of co-testing strategies [27].

Similarly, **Zhao et al. (2025)** reported that HPV testing had higher sensitivity than Pap smear alone, particularly for detecting CIN2+ lesions, reinforcing the need for integrated screening approaches [28].

These findings are consistent with the present study, which also demonstrated a clear increase in HPV positivity with worsening cytological grades.

CONCLUSION

The present study highlights that Pap smear is an effective and reliable screening tool for early detection of cervical lesions. HPV infection, particularly high-risk types, shows a strong correlation with abnormal cytological findings. The combination of Pap smear and HPV testing enhances diagnostic accuracy and facilitates early intervention, thereby reducing the burden of cervical cancer.

LIMITATIONS

- Small sample size (n=100)
- Single-center study
- Lack of long-term follow-up
- Limited HPV genotyping
- Possible selection bias due to hospital-based populatio

DECLARATIONS:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.
Authors contributions: Author equally contributed the work.

REFERENCES

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(1):33–52.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer burden 2020: GLOBOCAN estimates of incidence and mortality worldwide. Lancet. 2021;397(10290):1907–18.
3. Bosch FX, Lorincz A, Muñoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol. 2020;73(4):123–29.
4. Doorbar J. Molecular biology of human papillomavirus infection and cervical cancer. Nat Rev Microbiol. 2021;19(1):56–70.
5. Schiffman M, Castle PE. The promise of global cervical cancer prevention. N Engl J Med. 2020;353(20):2101–04.
6. Arbyn M, Weiderpass E, Bruni L, de Sanjosé S, Saraiya M, Ferlay J, et al. Estimates of incidence and mortality of cervical cancer worldwide. Lancet Glob Health. 2020;8(2):e191–203.
7. Papanicolaou GN. A new procedure for staining vaginal smears. Science. 1942;95(2469):438–39.
8. Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain JM, et al. Screening guidelines for cervical cancer. CA Cancer J Clin. 2020;70(5):321–46.
9. Nayar R, Wilbur DC. The Bethesda System for Reporting Cervical Cytology. 3rd ed. New York: Springer; 2015.
10. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The Bethesda system terminology for reporting cervical cytology. JAMA. 2002;287(16):2114–19.
11. Cuzick J, Clavel C, Petry KU, Meijer CJ, Hoyer H, Ratnam S, et al. Overview of the European and North American studies on HPV testing. Int J Cancer. 2021;148(2):263–75.
12. World Health Organization. WHO guidelines for screening and treatment of cervical pre-cancer lesions. Geneva: WHO; 2021.
13. Ronco G, Dillner J, Elfström KM, Tunesi S, Snijders PJ, Arbyn M, et al. Efficacy of HPV-based screening. Lancet. 2020;383(9916):524–32.
14. Wentzensen N, Arbyn M. HPV-based screening and triage strategies. J Natl Cancer Inst. 2021;113(6):644–50.
15. Bruni L, Albero G, Serrano B, Mena M, Gómez D, Muñoz J, et al. Human papillomavirus epidemiology. Lancet Glob Health. 2019;7(8):e1050–62.
16. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of HPV types. N Engl J Med. 2020;348(6):518–27.
17. ICO/IARC Information Centre on HPV and Cancer. Human papillomavirus and related diseases in India. Summary report 2023.
18. Bhatla N, Aoki D, Sharma DN, Sankaranarayanan R. Cancer of the cervix uteri. Lancet Oncol. 2020;21(3):e342–50.
19. Koliopoulos G, Nyaga VN, Santesso N, Bryant A, Martin-Hirsch PP, Mustafa RA, et al. Cytology versus HPV testing for screening. Cochrane Database Syst Rev. 2021;CD008587.
20. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, et al. HPV screening for cervical cancer in India. Lancet. 2020;370(9585):398–406.
21. Arbyn M, Snijders PJ, Meijer CJ, Berkhof J, Cuschieri K, Kocjan BJ, et al. Which high-risk HPV assays fulfill criteria. BMJ. 2020;340:c1804.
22. World Health Organization. Global strategy to accelerate the elimination of cervical cancer. Geneva: WHO; 2021.
23. Castle PE, Schiffman M, Wheeler CM, Solomon D. Evidence for frequent regression of HPV infection. J Infect Dis. 2020;202(12):178–86.
24. Schiffman M, Wentzensen N. From HPV infection to cervical cancer. Lancet. 2021;370(9590):890–907.
25. Perkins RB, Guido RS, Castle PE, Chelmow D, Einstein MH, Garcia F, et al. Updated cervical cancer screening guidelines. JAMA. 2020;325(19):1965–77.
26. Loopik DL, Bekkers RLM, Melchers WJG, Massuger L. HPV-related cervical disease progression. Gynecol Oncol. 2022;165(1):123–30.

27. Campos NG, Tsu V, Jeronimo J, Mvundura M, Kim JJ. Cost-effectiveness of HPV screening. Lancet Glob Health. 2021;9(2):e111–21.
28. Zhao FH, Lewkowitz AK, Chen F, Lin MJ, Hu SY, Zhang X, et al. Pooled analysis of cervical cancer screening studies. Int J Cancer. 2020;146(1):245–52.
29. Gupta S, Palmer C, Bik EM, Cardenas JP, Nuñez H, Kraal L, et al. Self-sampling for HPV testing. Cancer Epidemiol. 2021;72:101927.
30. Singh S, Badaya S. Factors influencing cervical cancer screening in India. J Obstet Gynaecol India. 2022;72(1):45–52.