



## “SPECTRUM OF CERVICAL PRECANCEROUS AND CANCEROUS LESION IN LIQUID BASED CYTOLOGY SMEARS AND ITS CORRELATION WITH HPV TYPING”

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### ABSTRACT

#### Background:

Cervical cancer remains a leading cause of morbidity and mortality among women worldwide, particularly in developing countries. Liquid-based cytology (LBC) has emerged as a more sensitive and reliable method compared to conventional Pap smears for detecting cervical epithelial abnormalities. Human papillomavirus (HPV), especially high-risk genotypes, plays a crucial etiological role in the development of cervical intraepithelial neoplasia and invasive carcinoma.

#### Objectives:

This study aims to evaluate the spectrum of precancerous and cancerous lesions in LBC smears and to correlate the cytological findings with high-risk HPV typing.

**Materials and Methods:** A total of 100 cervical samples were collected from women attending the gynecology outpatient department. LBC smears were prepared and evaluated using the Bethesda System 2014 for reporting cervical cytology. Concurrently, HPV DNA testing was performed using PCR-based methods to detect high-risk HPV genotypes. Cytological findings were then correlated with HPV typing results.

## **Results:**

Among the analyzed cases, the distribution of cytological findings ranged from ASCUS (Atypical Squamous Cell Of Undetermined Significance) to invasive carcinoma. A significant correlation was observed between high-grade lesions and positivity for high-risk HPV types, particularly HPV 16 and 18. The sensitivity of LBC in detecting high-grade lesions was enhanced when combined with HPV testing.

## **Conclusion:**

The study highlights a high prevalence of LSIL cases in the Vindhya region, with HPV-16 as the most common genotype. It recommends targeted screening, especially for LSIL cases, and strongly advocates HPV vaccination in younger females.

**Keywords:** Cervical cancer, Liquid-based cytology, HPV typing, Cervical intraepithelial neoplasia, High-risk HPV, Cytological screening, Bethesda system.

## **INTRODUCTION :**

Cervical cancer is a health concern globally and remains one of the leading causes of cancer-related deaths among women worldwide, despite significant advancements in screening and prevention methods<sup>1</sup>. There were 1,27,600 new cases and 79,900 deaths reported in India with a 5-year prevalence of 3,39,589 related to cervical cancer in 2022<sup>2</sup>. As early detection of cervical lesions can help us prevent development of cervical cancers, the screening methods play a pivotal role.

The integration of molecular and cytological diagnostic techniques has greatly enhanced the understanding of the spectrum of these lesions, facilitating early detection and improved management outcomes.

The identification of high-risk HPV types, such as HPV-16 and HPV-18, in cervical specimens has been associated with a higher likelihood of progression to invasive cancer.

## **AIM & OBJECTIVE: -**

### **AIMS: -**

To investigate the correlation between cervical precancerous and cancerous lesions in liquid based cytology smears with high risk HPV genotype (16/18) distribution using polymerase chain reaction (PCR) methodology, in tertiary care center of Vindhya region.

### **Objective: -**

1. To determine the spectrum of cervical precancerous and cancerous lesion in pap smear and its correlation with HPV typing.
2. To analyze the correlation between high-risk human papillomavirus (HPV) infection and precancerous lesions and cervical cancer.

## **MATERIAL & METHOD:**

The present study was conducted in the department of pathology at Shyam Shah Medical College, Rewa, M.P. after obtaining ethical clearance from Institutional Ethics Committee. It was cross sectional and analytical study conducted for a period of 12 months for 100 samples. Women attending the gynecology outpatient department, screening clinics, or referred for abnormal cervical findings were enrolled in the study. Socioeconomic status was graded using revised B.G. Prasad scale.

## **INCLUSION CRITERIA FOR SAMPLING:**

- Patient age 18-80 Years.
- High Risk patients.

### **INCLUSION CRITERIA FOR DNA EXTRACTION :**

Pap smear reported as Epithelial cell abnormality (ECA) according to Bethesda system for reporting cervical cytology 2014.

### **EXCLUSION CRITERIA :**

1. Pregnant and lactating
2. Previous cervical conization
3. Hysterectomy
4. Comorbid with immune system disorder
5. Patient reported as unsatisfactory & Negative for intraepithelial lesion and Malignancy[NILM]
6. Patient who don't agree with consent form

### **Sample Collection:**

Patients fulfilling the inclusion criteria were included in the study. The patients were explained about the study in detail and consent was taken. Cervical specimens were collected from patients attending out patient department of Obstetrics and Gynaecology after acquiring consent. A sterile cytobrush was used to obtain cells from the transformation zone of the cervix. The collected sample was immediately placed into a vial containing liquid-based preservative media of the EziPREP™. The specimens were transported to the laboratory for processing within a maximum of 24 hours.

### **Statistical Analysis**

The data were analyzed using Microsoft Excel and analysed using SPSS(versionX.X), descriptive statistics, such as mean, standard deviation, and frequency distribution, were used to summarize demographic and clinical characteristics of the study population. The association between HPV status and cytological findings was analyzed using chi-square tests or Fisher's exact test. A p-value of <0.05 was considered statistically significant.

### **RESULTS**

In total 100 patients the majority of patients (60%) fall within the 40–59 age group, suggesting that middle-aged women are most affected by cervical abnormalities in this study. A significant 67% of patients had their first coitus by age 20, with 24% initiating at or before 17, indicating early sexual debut— a known risk factor for HPV infection and cervical abnormalities. The highest proportion (43%) was in the 18–20 age group, followed by 33% in the 21–24 group, emphasizing the need for early HPV vaccination and education to reduce cervical health risks.

Over half of the patients (51%) had a parity index of P4 or higher, indicating high multiparity— a factor associated with increased cervical trauma and risk of cervical lesions. The P2–P3 group accounted for 40%, while only 9% were in the P0–P1 range, suggesting lower screening or risk in low-parity women. High parity also likely contributed to the high rate (45%) of UV prolapse, as repeated deliveries may intensify cervical stress and HPV-related damage.

UV prolapse was the most common presenting complaint (45%), closely linked to high parity (P4–P7 in 51%), highlighting the impact of repeated childbirth on pelvic and cervical health. AUB was the next most frequent (25%), possibly indicating underlying cervical or endometrial pathology. Less common symptoms included PID (9%), white discharge (7%), postmenopausal bleeding (6%), abdominal pain (6%), and post-coital bleeding (2%), which may suggest infection, inflammation, or advanced disease. This range of complaints underscores the need for thorough gynecological evaluation to detect cervical abnormalities, often associated with HPV or other risk factors.

**Table 1 :Region Wise Distribution of cases:**

| District  | Number of Cases | Percentage |
|-----------|-----------------|------------|
| Rewa      | 45              | 45%        |
| Satna     | 15              | 15%        |
| Maihar    | 09              | 09%        |
| Sidhi     | 09              | 09%        |
| Panna     | 08              | 08%        |
| Katni     | 05              | 05%        |
| Shahdol   | 06              | 06%        |
| Singrauli | 03              | 03%        |

The majority of cases originated from Rewa district (45%), followed by Satna (15%). Other districts like Maihar, Sidhi (9% each), and Panna (8%) contributed moderately, while Katni, Shahdol, and Singrauli reported fewer cases (5–3%). This distribution suggests a higher cervical disease burden or better screening access in Rewa, highlighting regional disparities in case detection. ASCUS was the most common cytological finding(46%), followed by LSIL(33%), HSIL(17%) and SCC(4%), indicating a significant burden of precancerous lesions in the population.

HPV DNA was positive in 44% of cases, confirming its key role in cervical lesions. The 56% negative rate indicates other contributing factors, highlighting the importance of HPV testing for early risk detection.

**Table 2:Distribution as per Socioeconomic Status(SES):**

| Socioeconomic status | Number of Cases | Percentage |
|----------------------|-----------------|------------|
| Class 2              | 06              | 06%        |
| Class 3              | 27              | 27%        |
| Class 4              | 43              | 43%        |
| Class 5              | 24              | 24%        |

The largest proportion of patients (43%) belongs to socioeconomic Class 4, indicating a middle-lower economic status, while Class 3 (27%) and 5 (24%) also represent significant portions, with only 06% in Class 2 (higher status). This distribution suggests that women from lower to middle socioeconomic backgrounds are more likely to seek care, possibly due to limited access to preventive screening in higher-income groups or greater disease burden in resource-limited settings. The predominance of lower class may correlate with reduced healthcare access, delayed diagnosis, and higher prevalence of risk factors like early marriage or multiparity.

**Table 3: Spectrum of Cervical Cytology (LBC Findings):**

| LBC Findings | Number of Cases | Percentage |
|--------------|-----------------|------------|
| ASC-US       | 46              | 46%        |
| LSIL         | 33              | 33%        |
| HSIL         | 17              | 17%        |
| SCC          | 04              | 04%        |

ASCUS was the most common cytological finding (46%), followed by LSIL (33%), HSIL (17%), and SCC (4%), indicating a significant burden of precancerous lesions in the population.

**Table 4: Shows overall percentage of HPV positive cases:**

| LBC Findings | Total Cases | HPV Positive | Overall HPV positive cases(%) |
|--------------|-------------|--------------|-------------------------------|
| ASC-US       | 46          | 08           | 18.18%                        |
| LSIL         | 33          | 24           | 54.54%                        |
| HSIL         | 17          | 11           | 25.00%                        |
| SCC          | 04          | 01           | 02.27%                        |

HPV positivity increases with increasing lesion severity, which is maximum in LSIL(54.54%) followed by HSIL(25.00%). showing a strong correlation with precancerous lesions. ASC-US showed a positive percentage of 18.18% and SCC was positive for 02.27% cases.

**Table 5: Genotype of HPV DNA Wise Distribution:**

| HPV Genotype        | Number of cases | Percentage of HPV Positive Cases out of total HPV positive cases |
|---------------------|-----------------|------------------------------------------------------------------|
| HPV-16              | 39              | 88.64% (39/44)                                                   |
| HPV-18              | 03              | 06.82% (03/44)                                                   |
| Both HPV16 & HPV-18 | 02              | 04.54% (02/44)                                                   |

The total cases positive for HPV were 44 cases out of which HPV 16 overwhelmingly dominates the genotype distribution (88.64%), reflecting its strong association with cervical precancerous and cancerous lesions in this study.

HPV 18 (6.82%) and dual infections (4.54%) are rare, suggesting that HPV 16 is the primary high-risk type driving disease in the Vindhya region. This high prevalence of HPV 16 supports the prioritization of HPV 16/18 vaccination and targeted screening strategies to mitigate its impact on cervical health.

## DISCUSSION:

The present study “SPECTRUM OF CERVICAL PRECANCEROUS AND CANCEROUS LESION IN LIQUID BASED CYTOLOGY SMEARS AND IT’S CORRELATION WITH HPV TYPING” was carried out in 100 samples.

### 1. Age Group and Epithelial Abnormalities:

In the present study, the most common age group showing epithelial cell abnormalities was 40–59 years, consistent with **Sharma et al. (2021)**<sup>3</sup> and **Biradar et al. (2019)**<sup>4</sup>, who reported peak prevalence in the 41–60 age range. This age group likely reflects increased vulnerability to persistent HPV infections and long-term viral effects.

### 2. Parity Distribution:

Higher parity was strongly associated with epithelial abnormalities in the present study, with 51% in the P4–P7 range. This aligns with **Subhadarshini et al. (2025)**<sup>5</sup>, who reported 82.3% multiparous cases, and **Agrawal et al. (2023)**<sup>6</sup>, with 62.51%. **Jaya Mishra et al. (2023)**<sup>7</sup> found 58% of cases in women with parity >3, while **Vedvathi et al. (2019)**<sup>8</sup> noted a higher percentage in P1–P2 (64.5%) but still reported 32.5% in P3 and above. The consistent findings highlight parity as a significant risk factor.

### 3. Presenting Complaints:

Uterovaginal (UV) prolapse was the leading complaint in the present study (45%), followed by abnormal uterine bleeding (25%). In contrast, **Subhadarshini et al. (2025)**<sup>5</sup> observed irregular menses (36.1%) and white discharge (24.8%) as common symptoms. **Agrawal et al. (2023)**<sup>6</sup> reported white discharge (38.75%) as the top symptom, while **Vedvathi et al. (2019)**<sup>8</sup> focused more on clinical signs like cervical erosion (18.5%) and hypertrophied cervix (10%).

### 4. Regional Distribution:

Rewa district contributed 45% of cases in the present study, compared to 25% in the MP registry (2020), suggesting a referral concentration to tertiary centers. Other districts like Satna (15%) and Sidhi (9%) had lower representation. This disparity highlights the need for decentralized screening strategies.

### 5. LBC Findings:

ASCUS was the most common abnormality in the present study (46%), followed by LSIL (33%) and HSIL (17%). These trends were similar to **Jaya Mishra et al. (2023)**<sup>7</sup>, who reported 58.1%

ASCUS, 19.1% LSIL, and 11% HSIL. **Patel et al. (2023)<sup>9</sup>** and **Gupta et al. (2019)<sup>10</sup>** also reported ASCUS as the leading category, supporting the role of LBC in early detection.

#### **6. LBC Findings vs. HPV Positivity:**

In the present study, HPV positivity was 18.18% in ASCUS, 54.54% in LSIL, 25% in HSIL, and 2.27% in carcinoma cases. These are notably lower than those reported by **Jaya Mishra et al. (2023)<sup>7</sup>**, who found 45% positivity in ASCUS and 86.67% in HSIL. **Pankaj S. et al. (2018)<sup>9</sup>** reported 50% HPV positivity in both LSIL and HSIL. The lower rates in high-grade lesions here may reflect testing sensitivity or viral integration effects.

#### **7. HPV Genotype Distribution:**

HPV-16 was the dominant genotype in the present study (88.64%), surpassing rates reported by **Kothari et al. (2024)<sup>12</sup>** at 68.65%, and **Shipra Gupta et al. (2022)<sup>11</sup>** at 60.4%. HPV-18 was found in 6.82% of cases, aligning with Gupta (6.25%) but much lower than Kothari (24.62%). Co-infection (HPV-16+18) was observed in 4.54%, compared to 10% in Flieder et al. (2023).

#### **8. Average Age of HPV Positive Cases:**

The mean age of HPV-positive women in the present study was 49.7 years—higher than **Jaya Mishra et al. (2023)<sup>7</sup>** at 44.3 years and **Depuydt et al. (2003)<sup>13</sup>** at 34.7 years. This may indicate delayed detection or prolonged persistence in older women, stressing the need for continued screening beyond reproductive age.

### **CONCLUSION:**

The study highlights a high prevalence of LSIL cases in the Vindhya region, with HPV-16 as the most common genotype. It recommends targeted screening, especially for LSIL cases, and strongly advocates HPV vaccination in younger females. The findings support the use of combined LBC and HPV co-testing, particularly in high-risk groups identified by age, high parity, early sexual debut, and low socioeconomic status.

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#### **Author's Contribution:**

**VM-** Definition of intellectual content, Literature survey, prepared first draft of manuscript, implementation of study protocol, data collection, data analysis, manuscript preparation and submission of article; **SKS-** Concept, design, clinical protocol, manuscript preparation, Design of study, statistical Analysis and Interpretation; **LT-** Coordination and Manuscript revision

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