



Obstetrics & Gynaecology

AN ANALYSIS OF ABNORMAL PAP SMEAR FOLLOW UP THROUGH COLPOSCOPY AND HPV DNA TESTING

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ABSTRACT

Introduction: Cervical cancer is a major health burden in India despite being preventable. Cytology alone may miss pre-malignant lesions due to limited sensitivity. HPV DNA testing and colposcopy enhance detection of high-risk cases. This study evaluates their role in the follow-up of abnormal Pap smears. **Materials and Methods:** This prospective study was conducted at Alluri Sitarama Raju Medical College, Eluru, from January 2023 to January 2025. One hundred women aged 30–60 years with Pap smear results of ASCUS, LSIL, or HSIL were enrolled. All participants underwent clinical examination, HPV DNA testing via real-time PCR for high-risk genotypes, and colposcopic evaluation using 3% acetic acid and Lugol's iodine. Colposcopic findings were interpreted using the modified Reid's Index, and suspicious areas were biopsied for histopathological confirmation. **Results:** The study included 100 women aged 30–60 years with abnormal Pap smears (ASCUS, LSIL, HSIL). LSIL was the most common cytologic abnormality (42%), followed by ASCUS (32%) and HSIL (26%). Colposcopy revealed abnormal findings in 52% of cases, with significantly more abnormalities in HSIL (69.2%) than ASCUS (34.4%) or LSIL (54.8%) ($p = 0.027$). The Reid's Colposcopic Index (RCI) scores mostly suggested low-grade lesions, without significant correlation to Pap smear grades. p16 immunohistochemistry was positive in 59% of cases, significantly higher in younger women ($p = 0.034$). No significant association was found between Pap smear grade and p16 positivity ($p = 0.156$). Combined colposcopy and p16 results showed no significant correlation with Pap smear categories ($p = 0.152$). **Conclusion:** Cervical cancer screening is vital, but resource limitations challenge the use of HPV DNA testing in our setting. Colposcopy adds value, especially when combined with Pap smears, though its role in triaging ASCUS is limited. An integrated approach using affordable methods can improve early detection and outcome.

KEYWORDS :**INTRODUCTION**

Cervical cancer remains the second most common cancer among Indian women and a leading cause of cancer-related deaths, particularly in low- and middle-income countries. According to GLOBOCAN 2020, India accounted for 18.3% (123,907) of new cervical cancer cases worldwide. Despite a 39.7% decline in incidence from 1990 to 2016, the disease continues to pose a significant public health burden due to lack of structured screening programs. While cytology-based screening has helped reduce incidence in high-income countries, its limited specificity and low sensitivity in detecting high-grade lesions hinder its effectiveness when used alone. Various screening methods such as Pap smear, visual inspection with acetic acid (VIA), HPV DNA testing, colposcopy, and their combinations have been employed, with newer modalities like E6/E7 mRNA testing and p16^{INK4a} immunostaining offering improved accuracy.

High-risk HPV is detected in over 95% of high-grade squamous intraepithelial lesions (HSIL), making HPV testing a valuable tool for identifying both current disease and future risk. Colposcopy remains essential for evaluating abnormal cytology and suspected invasive disease. Biomarkers such as p16^{INK4a} help differentiate true precancerous lesions, especially in HPV-associated cellular transformation. Incorporating HPV testing into national screening programs, alongside efforts to increase HPV vaccination, offers a promising strategy to reduce cervical cancer burden, especially in resource-limited settings.

AIMS AND OBJECTIVES :**Aim:**

To determine positivity of human papilloma virus by p16 testing & Colposcopy in abnormal pap smears.

Objectives:

- To determine positivity of human papilloma virus by p16 testing in abnormal pap smears ASCUS, LSIL, HSIL
- To know various colposcopic findings in abnormal pap smears

ASCUS, LSIL, and HSIL.

MATERIALS AND METHODS :

This prospective study was conducted at Alluri Sitarama Raju Medical College, Eluru, from January 2023 to January 2025. One hundred women aged 30–60 years with Pap smear results of ASCUS, LSIL, or HSIL were enrolled. Exclusion criteria included pregnancy, STDs, immunocompromised status, prior HPV vaccination or cervical surgery, and refusal to consent.

All participants underwent clinical examination, HPV DNA testing via real-time PCR for high-risk genotypes, and colposcopic evaluation using 3% acetic acid and Lugol's iodine. Colposcopic findings were interpreted using the modified Reid's Index, and suspicious areas were biopsied for histopathological confirmation.

Inclusion Criteria :

All patients who give informed consent to be part of the study, All women between 30-60 years of age, Women with pap test results with ASCUS or LSIL or HSIL.

Exclusion Criteria :

All patients who do not give informed consent to be part of the study, Sexually transmitted disease, Pregnancy and puerperium, Individuals with history of genital warts, History of prior HPV vaccination, Age <30 years and >60 years, Individuals who are immunocompromised, Past history of cervical neoplasia, Patients with frank cervical growth, Past history of cervical conization or hysterectomy.

RESULTS AND ANALYSIS :

A total of 100 women aged 30–60 years with abnormal Pap smear results (ASCUS, LSIL, HSIL) were included.

Table: 1 Comparison Between Pap Smear With Colposcopy By Pearson's Chi-square Test

Pap smear		Colposcopy		Total	p-value
		Abnormal	Normal		
ASCUS	Count	11	21	32	0.027 *
	%	34.4%	65.6%	100.0%	
HSIL	Count	18	8	26	100.0%
	%	69.2%	30.8%	100.0%	
LSIL	Count	23	19	42	100.0%
	%	54.8%	45.2%	100.0%	
Total	Count	52	48	100	100.0%
	%	52.0%	48.0%	100.0%	

* Statistical Significance at $p < 0.05$ level

Colposcopic examination revealed abnormal findings in 52% of cases. The breakdown showed:

- ASCUS: 34.4% had abnormal colposcopy
- LSIL: 54.8% had abnormal colposcopy
- HSIL: 69.2% had abnormal colposcopy

The association between Pap smear grade and colposcopic abnormality was statistically significant ($\chi^2 = 7.204, p = 0.027$).

Table: 2 Comparison Between RCI With Pap Smear By Pearson's Chi-square Test

RCI		Pap smear			Total	p-value
		ASCUS	HSIL	LSIL		
0 - 2	Count	10	9	13	32	0.237 #
	%	31.3%	28.1%	40.6%	100.0%	
3 - 4	Count	1	6	6	13	100.0%
	%	7.7%	46.2%	46.2%	100.0%	
≥ 5	Count	0	3	4	7	100.0%
	%	0.0%	42.9%	57.1%	100.0%	
Total	Count	11	18	23	52	100.0%
	%	21.2%	34.6%	44.2%	100.0%	

No Statistical Significance at $p > 0.05$ level

Among 52 patients with abnormal colposcopy, the majority (61%) had RCI scores between 0–2, suggesting low-grade CIN. There was no statistically significant correlation between Pap smear category and RCI grade ($p = 0.237$), although higher RCI scores (≥ 5) were more common among HSIL and LSIL cases.

Table3 : Comparison Between Pap Smear With Colposcopy & IHC By Pearson's Chi-square Test

Pap smear		Colposcopy & IHC				Total	p-value
		C Normal & IHC +ve	C Normal & IHC -ve	C Abnormal & IHC +ve	C Abnormal & IHC -ve		
ASCUS	Count	11	11	4	6	32	0.152 #
	%	34.4%	34.4%	12.5%	18.8%	100.0%	
HSIL	Count	6	4	9	7	26	100.0%
	%	23.1%	15.4%	34.6%	26.9%	100.0%	
LSIL	Count	14	7	15	6	42	100.0%
	%	33.3%	16.7%	35.7%	14.3%	100.0%	
Total	Count	31	22	28	19	100	100.0%
	%	31.0%	22.0%	28.0%	19.0%	100.0%	

No Statistical Significance at $p > 0.05$ level

When evaluating the combined findings of colposcopy and p16 IHC:

- 35.7% of LSIL cases had both abnormal colposcopy and positive p16
- 34.6% of HSIL cases showed abnormal colposcopy and positive p16
- In ASCUS, most cases had normal colposcopy, with equal distribution of IHC positive and negative results

No statistically significant association was observed between Pap smear and combined colposcopy-IHC findings ($p = 0.152$).

DISCUSSION:

According to the World Health Organization (WHO), cervical cancer remains a significant public health issue. It is the fourth most common cancer among women globally but ranks as the second most common cancer in developing countries, particularly affecting middle-aged women aged 15 to 44 years. Cervical cancer is also ranked among the top three cancers affecting women under the age of 45^[64].

The implementation of high-quality screening methods with adequate

coverage and follow-up can significantly reduce the incidence of cervical cancer.

However, cytology-based screening has had limited success in developing countries like India due to constraints such as the shortage of trained personnel, lack of laboratory infrastructure, high costs, and inadequate follow-up systems. Hence, alternative screening methods with higher sensitivity and specificity, such as **colposcopy**, are necessary^[65].

Human papillomavirus (HPV) is the most common sexually transmitted infection, with more than 170 identified genotypes. Two major species, alpha-9 (HPV types 16, 31, 33, 35, 52, 58, and 67) and alpha-7 (HPV types 18, 39, 45, 59, 68, and 70), account for over 80% of all cervical cancer cases. While early research suggested that HPV16 positivity was associated with a poor prognosis, some studies later reported HPV18 to be a stronger predictor of poor outcomes.

Cervical cancer progresses gradually from cellular atypia to various grades of dysplasia or Cervical Intraepithelial Neoplasia (CIN), before becoming invasive. Factors such as the cervix's accessibility, high epithelial turnover, ease of exfoliation of cervical cells, and a long preinvasive phase make it amenable to early detection through screening.

Colposcopy is a clinically validated and accurate method for assessing an unhealthy cervix. It excels in distinguishing high-grade from low-grade cervical lesions and is considered the gold standard when combined with directed biopsy. During colposcopy, the squamo-columnar junction and transformation zone are visualized, and application of acetic acid helps delineate abnormal areas, which can be biopsied for histopathological confirmation. Routine Pap smears, though useful, can have false-negative rates ranging from 1.8% to 20%. Colposcopy allows visualization of lesions not seen with the naked eye and aids in biopsy site selection, conservative CIN treatment, and follow-up.

The **p16(INK4a)** protein, a cyclin-dependent kinase inhibitor, inhibits cell cycle progression by inactivating kinases involved in RB phosphorylation. High-risk HPV oncogenes (E6 and E7) disrupt the RB-p16 pathway, resulting in p16 overexpression. p16 overexpression is common in precancerous and cancerous cervical lesions, making it a useful biomarker for HPV-related pathogenesis and a reliable diagnostic marker for cervical neoplasia.

Colposcopy Results

Out of 100 cases with abnormal Pap results, four had unsatisfactory colposcopy. Among the satisfactory ones, 52% revealed abnormal findings. Notably, 65.6% of ASCUS cases showed normal colposcopy, indicating colposcopy's limited sensitivity in low-grade cytology.

In contrast, 69.2% of HSIL and 54.8% of LSIL cases had abnormal colposcopic findings. Our abnormal colposcopy rate was 82%, . Vascular abnormalities were seen in 3% of our cases.

REID'S COLPOSCOPIC INDEX (RCI)

In our study, 61% of abnormal colposcopy cases were categorized as RCI 0–2 (suggestive of CIN I). Most ASCUS and LSIL cases fell into this group. However, high-grade cytology did not always correspond to high RCI scores, likely due to interobserver variability.

CONCLUSION:

Screening for cervical cancer remains an important health and economic concern. Use of HPV tests in co-testing has been implemented in recent screening guidelines. However, co-testing in our country is challenging because HPV DNA tests are too expensive. Hence, wherever feasible and affordable HPV DNA test and Pap smear can be combined for better sensitivity. Colposcopy performed well in the overall detection of abnormal pap cytology, though its capability for accurate categorisation of degree of abnormality by Reid's colposcopic index was poor. Colposcopy is less useful to triage ASCUS pap cytology as there was no colposcopic abnormality detected in majority of ASCUS group. When comparing HPV DNA testing and colposcopy as a triage for abnormal cytology, colposcopy was more useful as it was helpful in immediate detection of cervical CIN changes and directed biopsy. The major limitation of our study is that the final diagnosis was not established by the histology of cervical Biopsy specimens in majority of the cases. This was not possible as study also included cancer outreach screening program. HPV testing

and colposcopy can have an important role in primary screening for cervical cancer as an adjunct tool to augment existing Pap cytology programs.

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