



Original Article

A study of cervical cancer screening using pap smear and correlation with high-risk genotypes and histopathology

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ABSTRACT

Background: Cervical cancer is a significant health concern in India, and early detection is crucial. This study aimed to evaluate the prevalence and genotype of human papillomavirus in abnormal pap smears and correlate findings with histopathology.

Methods and Materials: A one-year prospective study was done at Santosh Medical College, screening 3194 cases. HPV testing was performed on all patients, and biopsies were taken from those with abnormal epithelial lesions. The study includes sexually active women or women presenting with complaints such as abdominal pain, abnormal vaginal discharge or bleeding, or other vaginal sensations. It excludes unsatisfactory smears, unmarried females, pregnant women, lactating women, and diagnosed or treated cases of cervical carcinoma.

Results: The maximum number of cases reported as abnormal epithelial lesions occurred in the 5th decade of life. Out of 3194 cases, 37 cases were unsatisfactory, and 2890 (90.5%), 266 (8.32%), and 1 (0.03%) women were found to have normal, precancerous, and squamous cell carcinoma, respectively. Among 267 women, HPV was detected in 109 women, and the common HPV infection found in our study was HPV 16 (62.4%), followed by multiple HPV infections (21.2%). Most females reported as having chronic cervicitis on biopsy were positive for HPV 16.

Conclusions: This study suggests that pap and HPV co-testing from the initial visit can be cost-effective and reduce the need for follow-up appointments.

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INTRODUCTION

According to WHO, breast cancer, followed by cervical cancer and uterine cancer, are the most common cancers in women worldwide, as well as in India. Global data published by WHO in 2022 shows that around 6,04,000 new cases were reported and around 3,42,000 have died from cervical cancer worldwide in 2020.¹ The Pap test helps in the early diagnosis and treatment of patients. Worldwide, cervical cancer screening is a crucial public health strategy aimed at early detection and prevention of cervical cancer.²

A Pap smear is a sensitive tool for screening various cervical lesions. It has significantly reduced the incidence of cervical cancer since its introduction, but mortality due to cervical cancer is still a burden in this society.³ High-risk Human Papillomavirus (HR- HPV) testing identifies the presence of HPV types that are most commonly associated with cervical cancer. Persistent infection with these HR- HPV types, particularly HPV 16 and 18, is a well-established cause of cervical cancer.²

Combining Pap smear and HPV testing enhances the effectiveness of screening programs, enabling earlier and more accurate detection of potentially cancerous changes. Therefore, in this study, we aim to highlight the prevalence and genotypes of HR-HPV and correlate its association with positive cytology testing.

MATERIAL AND METHODS

A one-year prospective, observational study (1st October 2023 to 30th September 2024) was conducted at the Department of Pathology, Santosh Medical College, Ghaziabad, Uttar Pradesh, India. Institutional Ethical clearance- SU/R/2023/2489(90) was obtained.

Inclusion criteria: Sexually active women between 21-80 years of age who came with complaints of abdominal pain, bleeding per vagina, any discharge from the vagina, or something coming out of the vagina, etc., who gave consent for the study.

Exclusion criteria- Unsatisfactory smears, unmarried females, pregnant women, lactating women, diagnosed cases of cervical carcinoma, and treated cases of carcinoma cervix.

A detailed history was recorded by gynecologists, and pap smears were sent to the pathology laboratory for examination. Pap smears were taken by gynecology residents using modified Ayre's wooden spatula and cytobrush from the transition zone. Smears were prepared, properly labeled, and fixed in 95% ethyl alcohol immediately in Coplin Jar. This jar was then sent to the laboratory of the Pathology department for pap staining. Pap staining was done with a rapid pap stain kit, and the slides were mounted with DPX. These mounted slides were screened and reported by a pathologist according to the 2014 Bethesda system.

Cervical washings were taken in DNA LBC cervical sample transport medium along with a PAP smear. Collected samples were stored at 4°C.

DNA Extraction and HPV genotype detection- TRUPCR-HPV HR with 16/18 genotyping kit was used for the detection and genotyping of HPV DNA in samples. This kit uses fluorescent reporter dye probes specific for the detection of high-risk HPV genotypes, which are, 16,18,31,33,35,39,45,51,52,56,58,59,66, and 68. In this kit, three

independent reactions are running parallel in three tubes: 1- the first tube detects any of the HPV-HR genotypes 16,31,33,35,51,52,56,58,66; 2- the second tube detects any HPV-HR genotype 18,45,59 along with genotyping of HPV 18; 3-the third tube detects HPV-HR 39 or 68 along with genotyping of HPV 16.

Cervical biopsies were taken for patients reported with abnormal pap smears. The cervical biopsies were sent to the Histopathology Unit, Pathology Department in Santosh Medical College. There, the specimen grossing, tissue blocks, and slides were prepared. The slides were stained with Hematoxylin and Eosin (H&E) and reported by the pathologist.

The data was collected and analyzed using software version SPSS Statistics. Graphical representation of the variables and to assess the correlation, the data was analyzed using an Independent sample t-test.

RESULTS

A total of 3194 cases were studied during this period. Out of these cases, 37 cases (1.15%) were inadequate or unsatisfactory due to decreased cellularity, hence excluded. 3157 cases were included in this study, out of which 2890 cases (90.5%) were reported as negative for intraepithelial lesion or malignancy (NILM) and 267 cases (8.35%) showed epithelial cell abnormalities.

The maximum number of patients (mean age of 36.6 years and median of 35.98 years) were in the age group of 31-40 years (53.3%), followed by 21-30 years (23.5%). The most common presenting complaint in our study population was vaginal discharge (29.7%), followed by lower abdominal pain (17.7%), while 11.9% of women were asymptomatic. Most NILM cases were reported in the 4th decade, while most of the epithelial cell abnormalities were reported in the 5th decade. (Table 1)

Table 1: Age-wise distribution of various cervical lesions

Age group	21-30	31-40	41-50	51-60	61-70	>70	Total
NILM	741	1667	320	72	67	23	2890
ASCUS	01	12	149	35	09	02	208
ASC-H	00	00	00	01	00	00	01
LSIL	00	02	43	03	02	01	51
HSIL	00	00	01	03	01	01	06
SCC	00	00	00	00	01	00	01
AGC-NOS	00	00	00	00	00	00	00
Adenocarcinoma	00	00	00	00	00	00	00
Total	742	1681	514	113	80	27	3157

(NILM- Negative for Intraepithelial lesion or malignancy, ASCUS- Atypical squamous cell of undetermined significance, ASC-H- Atypical squamous cell- cannot exclude HSIL, LSIL- Low-grade squamous intraepithelial lesion, HSIL- High-grade intraepithelial lesion, SCC- Squamous cell carcinoma, AGC-NOS- Atypical Glandular cell, not otherwise specified.)

Among the NILM cases, 70% of smears were normal. Inflammation was the most common finding, i.e., around 466 cases (16.12%). Among infections, *Candida* infection was the most common, followed by bacterial vaginosis and *Trichomonas vaginalis*, i.e., 167 (5.7%), 69 (2.3%), and 24 (0.8%) cases, respectively. Around 85 smears (2.9%) showed cervical atrophy. Among cases showing abnormal cervical cytology, the most common case reported was atypical squamous cell of undetermined significance (ASCUS), about 6.5%, followed by low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL), i.e., 1.6% and 0.19% respectively. Single cases of atypical squamous cell-cannot exclude HSIL (ASC-H) and squamous cell carcinoma (SCC), 0.03% and 0.03% respectively, were reported.

These 267 abnormal pap smears were further evaluated for Human papillomavirus (HPV). Among them, HPV was detected in 109 cases (41%). Out of 109 HPV positive patients, the maximum was seen in the 5th decade of life, i.e., 67%, followed by the 6th decade (18.3%). The most common infection found in our study was HPV 16, about 62.4%,

then multiple HPV infections (multiple infection by low-risk HPV genotype- 31,33,35,51,52,56,58,66) (21.2%). In mixed HPV infection, more cases were seen of HPV 39,68 was detected in 7.3%. (Table 2)

Table 2: Age-wise distribution of HPV

Age	HPV 16	HPV 18	HPV 39,68	HPV 45,59	Multiple	Total
21-30	00	00	00	00	00	00
31-40	04	00	01	00	00	05
41-50	59	00	03	05	06	73
51-60	04	01	00	00	15	20
61-70	01	01	02	00	02	06
>70	00	03	02	00	00	05
Total	68	05	08	05	23	109

Out of 267 cases of abnormal epithelial lesions in pap smear, 93 cases (34.8%) underwent cervical biopsy. Our histopathological findings of all the samples are classified under chronic cervicitis, CIN I, CIN II, CIN III, SCC, and adenocarcinoma. (Table 3)

Table 3: Correlation of Pap smear findings with Histopathological findings

Pap smear findings	Histopathological findings						Total
	Chronic cervicitis	CIN I	CIN II	CIN III	SCC	Adeno carcinoma	
ASCUS	42	02	00	00	00	00	44
ASC-H	00	00	00	01	00	00	01
LSIL	05	35	01	00	00	00	41
HSIL	01	01	03	01	01	00	06
AGUS	00	00	00	00	00	00	00
SCC	00	00	00	00	01	00	01
ADENOCARCINOMA	00	00	00	00	00	00	00
Total	48	38	04	02	01	00	93

Of 208 cases of ASCUS, 44 patients underwent biopsy and were diagnosed as chronic cervicitis. 41 cases out of 51 cases of LSIL underwent cervical biopsy, but only 87.8% of cases were concordant. Similarly, ASC-H, HSIL, and SCC are 100%, 83.3%, and 100% concordant, respectively. The cytopathology and histopathology correlation observed in our study is 92.47%.

In correlation with HPV infection with histopathology, the most common histology found was chronic cervicitis, followed by CIN I and CIN II. (Table 4)

Table 4: Distribution of HPV genotype in pre-cancerous and cancerous lesions on histology with HPV detected

Histological type	HPV 16	HPV 18	HPV 39,68	HPV 45,59	Multiple*	Total	Percentage
Chronic Cervicitis	31	01	06	03	07	48	51.6
CIN I	30	00	01	00	07	38	40.8
CIN II	00	01	01	01	01	04	4.3
CIN III	00	01	00	01	00	02	2.1
SCC	00	01	00	00	00	01	1.2
Adenocarcinoma	00	00	00	00	00	00	00
Total	61	04	08	05	15	93	100

*Low risk HPV genotype- 31,33,35,51,52,56,58,66

DISCUSSION

Cervical carcinoma has a long premalignant latent phase that precedes the invasive stage and can be detected by cytological examination of cervical smears. Cervical cancer is the most widely screened cancer in both developed and developing countries. Papanicolaou (Pap) testing every 3-4 years has reduced cervical cancer incidence and mortality by up to 80% in developed countries in the last 5 decades.⁷

Our study includes women aged 21-80 years; most patients were in the 4th decade (53.3%), which is comparable to studies done by Verma et al, Ghosh et al, and Pahwa et al, as 56%, 50.2%, and 68.75% respectively.^{3,4,5}

In our study, the most common finding was NILM (90.5%), in which 16.8% were inflammatory, followed by 5.7% cases of Candida. Infectious cases are concordant with Misra et al (4.7%), Ghosh et al (6.1%), and Tailor et al (3.7%).^{6,4,7}

Prevalence of cervical epithelial abnormalities in our study was only 8.5% which is similar to the studies conducted by Sachan et al, Verma et al, Pratapan et al, and Gupta A et al, as 8.48%, 9%, 9%, 9.9% respectively.^{8,3,9,10}

Among 267 abnormal reported cases, only 109 were detected as HPV positive. 62.4% of cases were positive for HPV 16, followed by 21.1% of multiple HPV infections, which was in concordance with Gupta S et al, i.e., 60.4% and 20.8%, respectively.²

Out of 267 cases reported as epithelial cell abnormalities, only 93 women underwent cervical biopsies, i.e., 34.8% of cases. On comparison of HPV genotype with histopathology, all 93 cases that underwent cervical biopsies were evaluated. 48 cases (51.6%) were diagnosed as chronic cervicitis and were positive for HPV 16 genotype (31 cases), which was in concordance with Gupta S et al (60.4%).²

The cytopathology-histopathology correlation observed in our study was 92.47% (86/93 cases), which was comparable with Kumar et al (90.9%), Atla et al (83.3%), Patil et al (82.1%), and Joshi et al (80%).¹¹⁻¹⁴

A single female in her 4th decade was cytologically diagnosed with LSIL and was histologically reported as a moderate dysplastic (CIN II) lesion. Similarly, one woman in her 5th decade who was cytologically diagnosed with HSIL was diagnosed as chronic cervicitis histologically. These two cases were different from what is known in literature, giving it an importance of co-testing from the first visit, as both women were HPV negative.

A well-established literature says that a pap smear is a simple, non-invasive, cost-effective, and easily accessible procedure. It is a safe tool for the early detection of pre-malignant and malignant cervical lesions.⁷⁻⁹ Cervical cytology is a sensitive test to diagnosing cervical cell changes/ neoplasia,

and in conjunction with HPV-DNA and histopathology, its sensitivity increases and can detect changes in early stages.¹

The limitation of this study includes a single-institute study. Our histopathological sample was a little smaller as a few patients gave consent for biopsy.

CONCLUSION

This study concludes that we should do pap and HPV co-testing from the initial visit of patients, as it will decrease the follow-up and is cost-effective for patients. Also, not all high-risk HPV positive patients show high-grade lesions of the cervix if it is detected early. The patients who came to our hospital were from rural areas. Therefore, we strongly believe this small initiative and awareness can cause a great decrease in cervical cancer cases.

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