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Research Article

CYTOLOGICAL, HPV GENOME, AND HISTOPATHOLOGICAL CORRELATION OF CERVICAL SMEARS IN FEMALES 30 YEARS AND ABOVE

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ABSTRACT

Objectives: The objectives of the study were to evaluate the patterns of cervical smear cytology in relation to human papillomavirus (HPV) and to correlate with histopathological diagnosis in females of 30 years and above.

Methods: The 2-year prospective study was done in the Department of Pathology in 100 patients who attended the Gynaecology OPD of Govt. Medical College, Patiala. The cervical brushings obtained were subjected to cytological examination by liquid-based cytology through SurePath method and for HPV evaluation by BD Onclarity HPV assay. The cytological findings were further correlated with histopathological examination.

Results: Majority of the females were in the age group of 30–45 years (62%). There was high incidence of dysplasia and malignancy in those who were of parity 3, 4, and above. The most common presenting symptom was discharge per vaginum (DPV) seen in 28% of cases. Maximum cases on cytology were diagnosed as NILM (including inflammatory pathology, 49%) followed by LSIL as 14% and malignant as 13%. Overall HPV genome was detected in 54% of the samples, the cases diagnosed as dysplasia and malignancy showed 68.42% and 100% presence of HPV, respectively. This was found to be statistically significant ($p<0.05$).

Conclusion: Pap smear along with HPV evaluation is an effective screening method for the detection of pre-invasive lesions and cancers of cervix that are potentially curable.

Keywords: Pap smear, Human papillomavirus, Liquid-based cytology.

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INTRODUCTION

Cervical cancer is the second most common cancer in women worldwide, and it is the principal cancer of women in most developing countries. Screening asymptomatic women with regular Papanicolaou (PAP) smears allow early diagnosis of treatable pre-invasive lesions of cervix [1]. Screening of cervical cancer underwent a major advancement after the introduction of liquid-based cytology (LBC) for Papanicolaou smears. Today, LBC accounts for over 90% of the Pap tests performed in the United States. This is largely a result of the improvement in overall clinical benefit and versatility of LBC as compared to conventional Pap test [2].

It has now been well established that human papillomavirus (HPV) is causally involved in pathogenesis of cervical cancer resulting in rich volume of scientific activity in this field. These activities range from the development of therapeutic vaccines designed to prevent infection with HPV to *in vitro* diagnostic tests for use as aids in cervical cancer screening and clinical patient management.

The Bethesda System 2014 classifies squamous cell abnormalities into four categories: (i) ASC (atypical squamous cells) with subcategorization into ASC-US (atypical squamous cells of undetermined significance) and ASC-H (atypical squamous cells – cannot rule out HSIL), (ii) LSIL (low-grade squamous intraepithelial lesions), (iii) HSIL (high-grade squamous intraepithelial lesions), and (iv) squamous cell carcinoma and glandular cell abnormalities in typical and atypical [3]. The ASC category was termed "atypical cells of undetermined significance (ASCUS)" in the previous version and is considered to be a category for reporting borderline or equivocal results. As the management of patients with ASCUS was unclear, the NCI sponsored clinical trial ASCUC/LSIL triage study (ALTS) to determine best management for these patients. The results have set in motion a new approach wherein screening may begin with HPV testing with cytology as a triage for HPV-positive samples as it is more cost effective and more sensitive for detecting high-grade lesions than repeat cytology [4].

Biopsy and histopathology are the gold standard to detect dysplastic changes along with microinvasive disease. It also shows characteristic pathologic features of HPV infection such as epithelial hyperplasia (acanthosis) and cytoplasmic vacuolization (koilocytosis) in terminally differentiated keratinocytes with atypical nuclei. HPV DNA can be demonstrated in biopsy tissues by *in situ* hybridization. *In situ* methods can be non-amplified, target amplification by PCR, or signal amplified [5].

The present study was undertaken to help in early detection of cervical cancer and other cervical abnormalities by studying changes in cervical cytology in females of 30 years and above in relation to human papillomavirus (HPV) and to correlate with changes on histopathological examination.

METHODS

The present study was conducted in the Department of Pathology, GMC, Patiala. It was a prospective study carried over a period of 2 years. The cervical brushings were prepared from women of 30 years and above who presented in the Department of Obstetrics and Gynecology, Rajindra Hospital, Patiala, after taking their informed consent. Samples were processed for cytological examination by LBC and for HPV DNA detection through polymerase chain reaction (PCR). Cervical biopsies were performed in all cases for cytohistopathological correlation.

Relevant history of illness was obtained from the patients and recorded on the pro forma.

Processing of the samples for LBC was done by SurePath (SP) method.

Sample collection for liquid-based cytology (LBC)

- Cervical brush was inserted into endocervical canal and rotated 5 times in clockwise direction. Then, detachable head of the device was dropped into BD SurePath vial and cap of the vial tightened. The BD SurePath vial contains ethanol-based preservative. The vial was then sent to the laboratory for processing.

Procedure of SP-LBC

Cell enrichment and preparing cell pellet

- After labeling each sample component (vial tube and glass slide), BD PrepMate rack was loaded by placing vials, centrifuge tubes and syringes on BD PrepMate rack. A 4 ml density reagent was dispensed to each empty centrifuge tube.
- The BD PrepMate rack was loaded into instrument tray and system was run. This transferred the sample from sample vials to centrifuge tubes automatically.
- Then, the tubes were centrifuged to produce a concentrated pellet of cells.

Centrifuge tubes and slides were placed on the BD PrepStain slide processor and the system was run which stained the slides automatically.

HPV DNA detection

In our department, the BD Onclarity HPV Assay was used. This is an amplified DNA test for the qualitative detection of high-risk types of human papillomavirus (HPV). The assay detects all high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and provides the capability for genotyping of six high-risk types (HPV 16, 18, 31, 45, 51, and 52).

Sample collection and transport

Cervical brush specimen collection

1. BD Onclarity HPV Cervical Brush was inserted into the endocervix and rotated ¼ to ½ turn in one direction.
2. The brush was placed into the bottom of the diluent tube.
3. The shaft was broken at the score line and cap was tightened on the tube.

The specimens were transported to the laboratory immediately and processed. However, these samples can be stored for 30 days after collection if kept at 2–30°C.

Processing procedure

Specimens undergo a pre-warm step in the BD Pre-warm Heater to homogenize the matrix, lyse cells, and release DNA capable of being extracted and amplified. After cooling, the specimens are loaded onto the BD Viper LT System which then performs all the steps involved in extraction and amplification of target DNA. This is done using amplification primers and fluorescently-labeled detector probes using real-time polymerase chain reaction (PCR). The presence or absence of HPV DNA is determined by the PCR cycle at which the signal crosses a pre-established threshold. In addition, the assay will extract, amplify, and detect a fragment of the human beta globin gene as an internal control.

The data collected was recorded in the pro forma and were subjected to statistical analysis.

RESULTS

Cervical brushings taken from 100 females were subjected to cytological examination through LBC and HPV DNA detection through PCR. Simultaneously biopsy was received in the department of pathology for cytohistopathological correlation. The data obtained were entered into Microsoft Excel worksheet. The data was analyzed and the observations and results was tabulated as under:

Majority of the females were in the age group of 30–45 years (62%). There was high incidence of dysplasia and malignancy in those who were parity 3, 4, and above. The most common presenting symptom was discharge per vaginum (DPV) seen in 28% of cases followed by pain lower abdomen in 15% of the cases.

Maximum cases on cytology were diagnosed as NILM (including inflammatory) 49% followed by LSIL as 14% and malignant as 13% (Table 1) and (Figs. 1-4).

On histopathological examination, 48% of cases were inflammatory and 27% malignant (Table 2).

Twenty-seven cases of malignancy were reported on histopathology, of these only 13 could be diagnosed on cytology with certainty (Table 3).
On statistical analysis,
F-statistic value=1.0989
p=0.3727

Hence, this was not found to be statistically significant ($p>0.05$).

Out of total of 100 cases, 54 cases were found to be HPV positive. All the cases of malignancy showed the presence of HPV while 68.42% of dysplastic lesions were positive for HPV (Table 4).

The Chi-square statistic is 12.1195. p =0.006985 was considered.

This is statistically significant ($p<0.05$).

Majority of cases (35.19%) showed HPV 16 followed by HPV18 (25.92%) (Table 5).

In our study, 10 cases of ASCUS were diagnosed on cytology. On HPV screening, six of these were positive for HPV. On further correlating with biopsy and HPE, two cases proved to be dysplastic and one case as malignant while six cases proved to be inflammatory and one as polyp (Table 6). The frequency of HPV infection was 60% in ASCUS.

Of the 14 cases diagnosed as LSIL on cytology, six were positive for HPV on screening. Of HPV-positive cases on biopsy and HPE, all proved to be dysplastic or malignant. One case showing dysplastic changes on biopsy was HPV negative (Table 7). The frequency of HPV infection was 42.85% in LSIL.

Of 12 cases of HSIL diagnosed on cytology, nine were positive on HPV screening. Of HPV-positive cases on biopsy and histopathology, eight

Table 1: Cytological diagnosis of 100 cervical brushings

Cytological diagnosis	Number of cases (*n*=100)	Percentage
NILM (including inflammatory)	49	49
ASC-US	10	10
LSIL	14	14
HSIL	12	12
Malignancy	13	13
Inadequate smear	2	2
Total	100	100

ASC-US: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion

Table 2: Histopathological diagnosis of 100 cervical biopsies

Histopathological diagnosis	Number of cases (*n*=100)	Percentage
Inflammatory	48	48
Polyp	6	6
Dysplasia (CIN)	19	19
Malignant	27	27
Total	100	100

Table 3: Correlation of cytological and histopathological diagnosis

Histopathological diagnosis	Number of cases	Cytological diagnosis					
		NILM (including inflammatory)	ASC-US	LSIL	HSIL	Malignant	Inadequate smear
Inflammatory	48	33	6	6	2	0	1
Polyp	6	3	1	1	1	0	0
Dysplasia (CIN)	19	8	2	5	4	0	0
Malignant	27	5	1	2	5	13	1
Total	100	49	10	14	12	13	2

ASC-US: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion

Table 4: Detection of human papillomavirus in patients with cervical lesions

Histopathological diagnosis	Total number of cases	Presence of HPV	Percentage
Inflammatory	48	13	27.08
Polyp	6	1	16.67
Dysplasia	19	13	68.42
Malignancy	27	27	100
Total	100	54	54

HPV: Human papillomavirus

Table 5: Genotype of human papillomavirus positive cases

HPV genotype	Number of cases	Percentage
P1	4	7.41
P2	3	5.56
P3	2	3.70
16	19	35.19
18	14	25.92
51	6	11.11
52	6	11.11
Total	54	100

HPV: Human papillomavirus

Table 6: Cytohistopathological and human papillomavirus correlation of 10 cases of atypical squamous cells of undetermined significance

Cytology	HPE	HPV	
		Present	Absent
ASC-US – 10 cases	Inflammatory - 6	3	3
	Polyp - 1	0	1
	Dysplasia - 2	2	0
	Malignant - 1	1	0

HPV: Human papillomavirus, ASC-US: Atypical squamous cells of undetermined significance

Table 7: Cytohistopathological and human papillomavirus correlation of 14 cases of low-grade squamous intraepithelial lesions

Cytology	HPE	HPV	
		Present	Absent
LSIL-14	Inflammatory-6	0	6
	Polyp-1	0	1
	Dysplasia-5	4	1
	Malignant-2	2	0

HPV: Human papillomavirus, LSIL: Low-grade squamous intraepithelial lesion

proved to be dysplastic and malignant. One case showing dysplastic changes on biopsy was HPV negative (Table 8). The frequency of HPV infection was 75% in HSIL.

Table 8: Cytohistopathological and human papillomavirus correlation of 12 cases of high-grade squamous intraepithelial lesions

Cytology	HPE	HPV	
		Present	Absent
HSIL-12	Inflammatory-2	0	2
	Polyp-1	1	0
	Dysplasia -4	3	1
	Malignant-5	5	0

HPV: Human papillomavirus, HSIL: High-grade squamous intraepithelial lesion

Of the 13 cases diagnosed as malignant on cytology, all were concordant on HPE and all were positive for HPV (Table 9). However, there were total 27 cases of malignancy diagnosed on HPE, of which only 13 could be diagnosed with certainty on cytology. The frequency of HPV infection was 100% in cytologically diagnosed malignant cases.

DISCUSSION

Majority of females (62%) belonged to the age group of 30–45 years. The age group of the present study is similar to the study conducted by Bal *et al*. [6], Dhakal *et al*. [7], and Kalyani *et al*. [8]. There was increased incidence of dysplasia and malignancy with increased parity of 3 and above which correlated with the studies by Alaknanda *et al*. [1].

The most common presenting symptom was discharge per vaginum (28%) and pain lower abdomen (15%), this correlates with the study by Sirasagi *et al*. [9], Bindroo *et al*. [10], and Atla *et al*. [11].

The maximum number of cases diagnosed on cytology were NILM (49%), similar to the studies by Bindroo *et al*. [10] and Saha *et al*. [12] (Table 10).

In the present study, 10 cases of ASCUS were diagnosed on cytology; histopathological concordance was seen in 3 cases (30%) only (diagnosed as dysplasia and malignancy), similar to the study by Sirasagi *et al*. [9], while the rest showed inflammatory pathology. This could be due to the fact many of these patients had reparative changes associated with cervical erosion and inflammation which might have been misdiagnosed as atypical squamous cells. Hormone replacement therapy and perimenopausal changes may also lead to overdiagnosis, especially in elderly females. Fine granularity of the chromatin is in favour of a reactive change which should be carefully studied. On further correlating with HPV DNA, it was seen that all three cases that were dysplastic and malignant on HPE were positive for HPV while additional three cases of inflammatory pathology were positive. It is important to follow up these HPV-positive patients with inflammatory pathology as chronic inflammation is associated with repeated tissue injury and development of mutations in vital tumor suppressor genes and is responsible for HPV-induced cancers [13,14].

Of the 14 cases diagnosed as LSIL on cytology, cytohistopathological concordance was seen in 7 cases (50%) while the rest showed inflammatory pathology. Reactive inflammatory changes could be the cause of overdiagnosis. In such cases, it is important to focus on nuclear contours which will be regular in case of inflammatory atypia. Any case with inflammatory change

must have repeat cytological examination after clearing infection to avoid false-positive or false-negative diagnosis. All these were also HPV negative. Of the seven cases diagnosed as dysplastic and malignant on HPE, six were positive for HPV while one case showing that dysplastic changes on biopsy were negative. In data from ASCUS/LSIL triage study (ALTS), it was seen that high-risk HPV types were detected in 85% of LSIL [3].

Of 12 cases of HSIL diagnosed on cytology, 9 (75%) correlated with histopathology (diagnosed as dysplasia and malignancy), while rest showed inflammatory pathology. Analysis of the biopsy material of these false-positive cases showed atrophic changes in cells along with inflammatory atypia and immature squamous metaplasia. One of the patients had a history of intrauterine device usage which produced changes that led to overdiagnosis. Repeat smears after application of estrogen cream along with a history of IUCD usage and radiation therapy should be taken to avoid such false-positive cases. HPV evaluation showed 8/9 (88.88%) HPV positivity in dysplastic and malignant lesions. Although HPV infection is important for the development of cervical dysplasia and cancer, not all females who have dysplasia may be HPV positive as certain uncharacterized factors may be important in causing cervical dysplasia and cancer. Highest cytohistological correlation was seen in HSIL lesions (75%) similar to the study by Patil *et al.* [15].

Of the 27 cases diagnosed as malignant on HPE, only 13 could be diagnosed with certainty on cytology, five were reported as HSIL, two as LSIL, and five as NILM. Those smears under reported as HSIL or LSIL could be due to sampling error. Hence, both cytology and histopathology samples should be from the same site or colposcopic-guided biopsies may be done to minimize such errors. Similar findings were seen in a study by Alta *et al*. [11], where 50% of HSIL cases proved to be of squamous cell carcinoma on HPE. Major area of concern in this category was five cases of malignancy missed on cytology and reported as NILM. The only way to reduce this false-negative rate is to repeat smears at regular intervals, especially the inflammatory smears. It is estimated that error rate can be reduced with three normal consecutive annual smears [16].

Table 9: Cytohistopathological and human papillomavirus correlation of 13 cases of malignancy

Cytology	HPE	HPV	
		Present	Absent
Malignant-13	Malignant-13	13	0

HPV: Human papillomavirus

Table 10: Comparative analysis of various cervical lesions

Author of study	NILM (%)	ASC-US (%)	LSIL (%)	HSIL (%)	Carcinoma (%)
Bindroo *et al*. [10]	59	16	15	7	2.4
Saha *et al*. [12]	51.16	2.33	18.6	20.93	6.98
Banik *et al*. [13]	91.8	0.18	6.36	1.18	0.35
Present study	49	10	14	12	13

ASC-US: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion

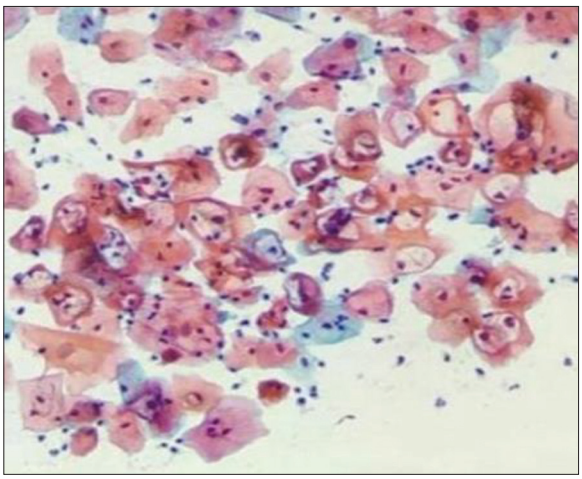

Fig. 1: Cytological smear showing koilocytic change suggestive of HPV infection. (Pap, ×400)

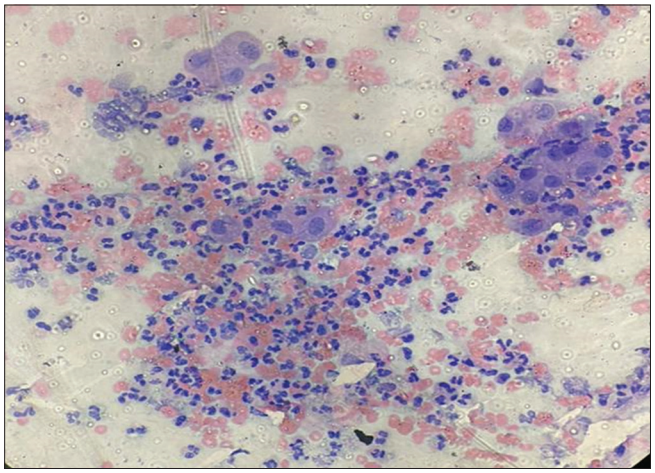

Fig. 2: Cytological smear showing features suggestive of ASCUS in a background of acute inflammatory cells (Pap, ×400)

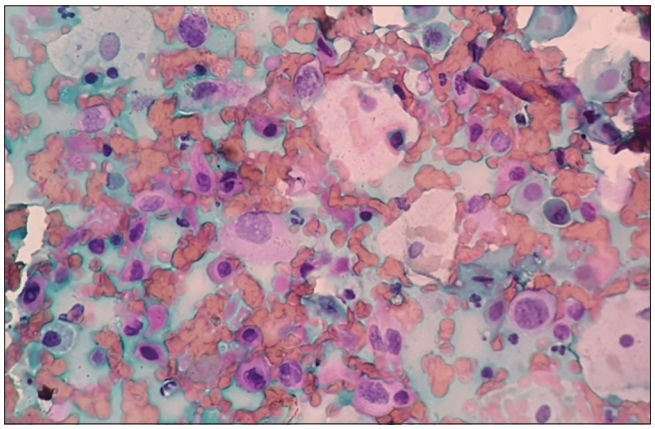

Fig. 3: Cytological smear showing squamous cell carcinoma, keratinizing (Pap, ×400)

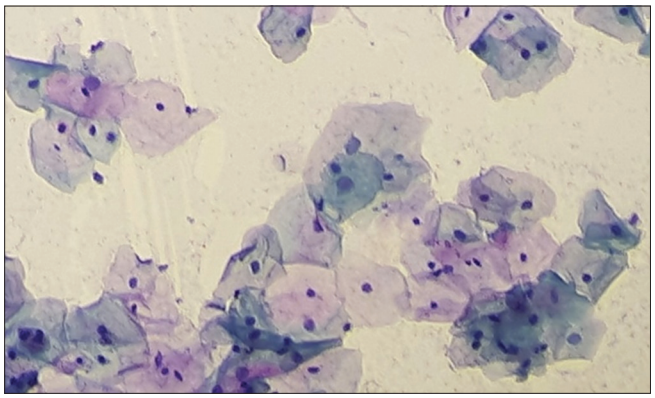

Fig. 4: Smear showing superficial squamous cells without any abnormality (NILM, Pap, ×400)

The frequency of HPV detection in our study was 60% in ASCUS, 42.85% in LSIL, 75% in HSIL, and 100% in malignancy. It was comparable to a study by Catteau *et al*. [17] done in Belgian population in which frequency of HPV was 65.76% in ASC (ASCUS +ASC-H), 86% in LSIL, and 98.4% in HSIL. The discrepancy of HPV positivity in LSIL in our study could be because of less number of cases and different geographical distribution of present study.

CONCLUSION

The present study highlighted the importance of screening, early diagnosis, and hence early management of cervical lesions which are important causes of morbidity and mortality in India. LBC has increased the detection of pre-invasive lesions and cancers that are potentially curable. HPV infection is highly prevalent and a major risk factor for the cervical abnormalities. Overall HPV positivity was 54%, for malignant lesions, it was 100%, while it was 65% for dysplastic lesions, therefore, its detection with screening tests will help in early diagnosis of cervical abnormalities.

AUTHOR CONTRIBUTIONS

Dr. Poonam Singal: Writing of manuscript and interpretation of data. Dr. Ninder Kumar: Analysis of data and proof reading of manuscript. Dr. Deepam Kundal: Collection of data and statistical analysis. Dr. Karanbir Singh: Collection of data. Dr. Ramesh Kumar and Dr. Manpreet Kaur: proof reading of manuscript.

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