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Original Research Article

Cytomorphological study of pap smear and its efficacy to detect various cervical lesions

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ABSTRACT

Background: Cervical lesions require screening for early diagnosis to differentiate between non-neoplastic and neoplastic lesions of cervix. The present research aimed to evaluate the prevalence of various cervical lesions and detect the efficacy as a screening test of pap smear for early diagnosis of cervical lesions.
Materials and Methods: The current retrospective study was conducted in the Department of Pathology, SSMC, Rewa, M.P. Total 658 cervical pap smears were retrieved, re-examined and re-classified according to the revised Bethesda system for reporting of cervical cytology, 2014. Out of 658 pap smears, 234 biopsies were available, which were re-examined histopathologically and correlation was done with their corresponding cytological findings.
Results: Out of 658 pap smears, negative for intraepithelial lesion or malignancy (NILM) was found in 74.47% smears, while the prevalence of epithelial cell abnormalities (ECA) was 24.32%. Amongst ECA, most common (11.86%) cervical lesion was low-grade squamous intraepithelial lesion (LSIL). Diagnostic sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy for the diagnosis of pre-invasive and invasive cervical lesions was found 70.54%, 81.9%, 82.72%, 69.35% and 75.6% respectively.
Conclusion: The current study showed that pap smear cytology is able to detect various cervical lesions with a diagnostic accuracy of 75.6%. So, women belonging to reproductive or postmenopausal age-group should be routinely screened for cervical lesions by non-invasive pap smear method. Conclusively, early diagnosis of cervical lesions is possible, thus reducing the incidence of invasive cervical carcinoma.

1. Introduction

Cervical cancer is one of the common cause of death amongst all cancers in the world.[1] It is the 4^{th} leading cause of cancer death in the women worldwide.[2] Most common etiology of cervical cancer is HPV infection.[3] Since it has a protracted pre-invasive state, invasive cervical cancer is considered preventable if detected at an early stage.[4]

Cervical cytology screening programmes are available for the early detection of epithelial cell abnormalities (ECA). Cervical cancer incidence and mortality have decreased since the introduction of cervical screening programmes.[5] Papanicolaou (pap) test was developed by George Papanicolaou which is used as a screening tool for the cervical lesions.[6] Evaluation of cervical lesions based on the 2014, revised Bethesda System (for cervical cytology) can provide the cytological evidence of ECA quickly and safely.[7] Cervical ECA in the pap smears show a wide range of intraepithelial lesions (IEL) that can

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range from mild dysplasia to invasive malignancy.[4,7] Cyto-histological correlation (CHC) is a special method used to determine whether the results are concordant or discordant and to evaluate errors in cytologic screening.[8] Literature reviews have reported that CHC discrepancy accounts for approximately 8–28% of all cytology-biopsy cases as a result of interpretive, screening, and sampling errors.[9,10]

The present study aimed to evaluate the frequencies of various cervical lesions according to the 2014 revised Bethesda system for cervical cytology, to detect the efficacy of pap smear as a screening test for early diagnosis of cervical lesions, and to determine the cytohistological correlation according to the CHC protocol guidelines of the ASC (2017).

2. Materials and Methods

The present retrospective descriptive study was conducted during the period of March 2018 to March 2021 in the Department of Pathology, Shyam Shah Medical College, Rewa, Madhya Pradesh, India. After taking ethical clearance from the Institutional Ethical Committee, data regarding cytological diagnosis of different lesions were collected from the departmental cytological record registers. Total 658 pap smears were retrieved, re-examined and re-classified according to the "revised 2014, Bethesda system for cervical cytology",[11] as shown in Table 1.

Departmental histological records were checked for cervical biopsies/hysterectomy. Out of 658 pap smears, only 234 biopsies were available in the department, which were retrieved and re-processed for histopathological examination (HPE). Histopathological reporting of these biopsies was done and noted. All biopsies were matched with their corresponding pap smears on the basis of patients' unique ID of the hospital.

The cytological findings of unsatisfactory cervical lesions and negative for intra-epithelial lesion or malignancy (NILM) were considered as negative pap smear result, whereas atypical squamous cells of underdetermined significance (ASC-US), atypical squamous cells- cannot rule out HSIL (ASC-H), low-grade squamous intra-epithelial lesion (LSIL), high-gradesquamous intra-epithelial lesion (HSIL) and squamous cell carcinoma (SCC) were classified as positive pap smear results. Histopathological findings of inflammatory cervical lesions were classified under negative results whereas; LSIL, HSIL and SCC were considered as positive results. According to the CHC protocol, a two-step difference between the cervical cytology result and the corresponding biopsy was considered a major discrepancy, while a one-step difference was considered a minor discrepancy. Pairs with an exact agreement were designated as an agreement.

2.1. Statistical analysis

Analysis of data was done by using Statistical Package for Social Sciences (SPSS) ver. 22 (Chicago), IL. Frequency counts (percentage) were used to express categorical data. Histopathological findings of all biopsies were compared with their corresponding pap smears findings.We regarded histopathological findings as the benchmark for diagnosing cervical lesions in our research. Cases with positive cervical lesions on both cytology and histopathology were classified as true positives (TP), whereas cases with negative cervical lesions on both cytology and histopathology were classified as true negatives (TN). The cases with negativecytological findings but positive histopathological findings, were classified as false negative (FN). The cases with positive cytological findings but negative histopathological findings, were classified as false positive (FP). The sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and overall accuracy of pap smear cytology for the diagnosis of different cervical lesions was calculated.

3. Results

3.1. Pap smear findings

Out of 658 cases, 1.21% (8/658) of the cases were unsatisfactory for evaluation and 98.79% (650/658) smears were satisfactory, which were further classified into NILM and epithelial cell abnormalities (ECA)/ intra-epithelial lesion (IEL). 74.47% (490/658) of the smears were reported as NILM, whereas 24.32% (160/658) smears were positive for IEL/ECA.

NILM was further classified into inflammatory, atrophic and reactive cervical lesions. 58.03% smears (382/658) were diagnosed as inflammatory/non-specific infectious cervical lesions (Figure 1), whereas, atrophic and reactive cervical lesions were seen in 9.44% (62/658) and 7% (46/658) smears respectively. ECA were also classified into ASC-US, ASC-H, LSIL, HSIL and SCC. The most common ECA was LSIL, seen in 11.86% (78/658) smears, followed by ASC-US in 6.23% (41/658) smears. HSIL and SCC was seen in 3.79% (25/658) and 1.68% (11/658) smears respectively (Figure 2). The least common ECA was ASC-H seen in 0.76% (5/658) smears (Table 2). Other glandular neoplasms or adenocarcinoma were not seen in our study.

3.2. Cytohistopathological correlation

Out of 658 cases, HPE was done in 234 cases which were compared with their corresponding cytological findings. Cytologically diagnosed 2 unsatisfactory cases and 84 NILM cases were diagnosed as chronic cervicitis in HPE. As unsatisfactory, NILM and chronic cervicitis are non-neoplastic cervical lesions, so total 86 cases were considered as TN (Tables 3 and 4).

11 ASC-US cases, 1 ASC-H case and 7 LSIL cases were misdiagnosed by cytological examination, which were correctly diagnosed by HPE as chronic cervicitis. These 19 cases were considered as FP in our study (Tables 3 and 4).

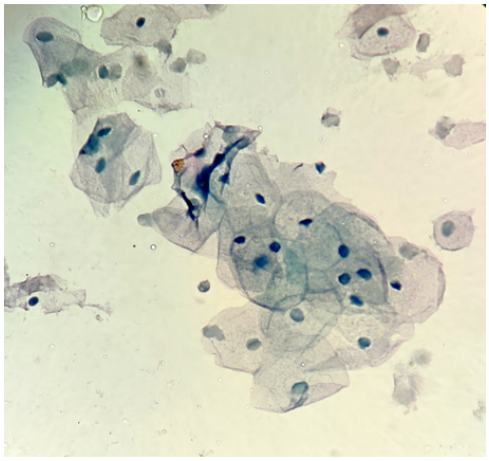

Figure 1: 27 year old female, pap smear showing benign looking squamous epithelial cells interpreted as Negative for intra-epithelial lesion or malignancy (NILM). (Pap stain; 40X)

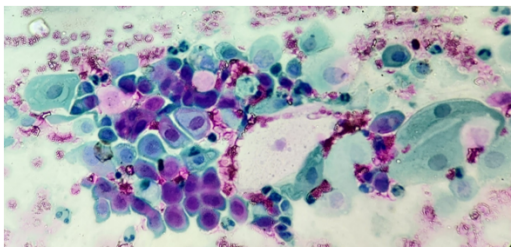

Figure 2: Pap smear and histopathological findings of a 62-year-old female, presenting with cervical growth, Pap smear showing dysplastic squamous epithelial cells, interpreted as high-grade squamous intra-epithelial lesion (HSIL). (40X; Pap stain).

Cytologically diagnosed 15 ASC-US cases, 1 ASC-H case and 48 LSIL cases were diagnosed as LSIL on HPE, while cytologically diagnosed 2 ASC-US cases, 2 LSIL cases and 17 HSIL cases were diagnosed as HSIL on HPE. 6 cases were diagnosed as SCC both cytologically and histopathologically. As ASC-US, ASC-H, LSIL, HSIL and SCC are neoplastic cervical lesions, so these 91 cases were taken as TP (Tables 3 and 4).

1 unsatisfactory case and 28 NILM cases were misdiagnosed by cytological examination, which were correctly diagnosed as LSIL on HPE. Cytologically diagnosed 9 NILM cases were also correctly diagnosed as HSIL on HPE, so these 38 cases were considered as FN (Tables 3 and 4).

Cyto-histological agreement was observed in 75.6% (177/234) of the cases, whereas discrepancies were seen in 24.4% (57/234) of the cases. Major under-call discrepancies were seen in 3.84% of the cases (reported as NILM in cytology, with HSIL in biopsy). However, 20.6% of the cases showed minor discrepancies between the cytological and histological diagnoses (Table 3). These discrepancies were due to sampling errors, problems due to processing and staining or interpretation errors.

Diagnostic sensitivity and specificity of pap smear was 70.54% and 81.9% respectively for the diagnosis of neoplastic cervical lesions, whereas the PPV, NPV and diagnostic accuracy was 82.72%, 69.35% and 75.6% respectively (Tables 3 and 4).

4. Discussion

The progress of any cancer can be reduced by early diagnosis and effective treatment. Carcinoma of the cervix is a well-recognized disease in the women of pre-and post-menopausal age-group which can be diagnosed at an early stage by a screening programme.[12]

On screening examination, we found 1.21% smears as unsatisfactory which may due to inadequate squamous component or obscuring inflammation and hemorrhage. Percentage of unsatisfactory smears reported by other previous researchers Lahari NA et al[17] (10.4%), Rana R et al[14] (7%), Goel NM[16] (6%) and Bamanikar SA et al[13] (5.99%) was higher in comparison to our study (Table 5).

NILM corresponds to cervical lesions with no cellular evidence of neoplasia.[19] Pap smear findings of NILM was the most common cervical lesion reported in our study with 74.47% of the smears. This finding is in line with the studies done by previous researchers[13,17] as shown in Table 5. Bamanikar SA et al[13] found infection as the most common etiology in NILM which was similar to our study.

ECA are characterised by squamous dysplasia ranging from mild to severe. Various studies have shown the prevalence of ECA in India ranging from 1.32% to 11.95%.[20,21] Nair GG et al[12] and Lahari NA et al[17] reported LSIL as the most common cervical lesion amongst ECA. This finding is in accordance with our study, but in contrast to above finding, Singh M et al,[15] Rana R et al,[14] Bamanikar SA et al[13] and Goel NM[16] found ASC-US as the most common ECA (Table 5).

CHC showed discrepancy in 24.4% of the total cases which correlates with the data of previous studies where CHC ranged between 8-28%.[9,10] Previously, various studies had been done to assess the diagnostic efficacy of pap smears for the diagnosis of various cervical lesions.[22–24] In our study, sensitivity and specificity of pap smears for the diagnosis of neoplastic cervical lesions was 70.54% and 81.9% respectively, while PPV and NPV were 82.72% and 69.35% respectively (Table 4). These findings are in agreement with those reported by previous researchers[4,13,16,18] (Tables 4 and 6). The diagnostic accuracy of pap smear for detecting neoplastic cervical lesions in our study was 75.6%, while Verma I et

Table 1: The revised Bethesda system for reporting of cervical cytology,[11] 2014

	Interpretations of cervical lesions
Specimen Type	• Conventional pap smears • Liquid based cytological preparations
Specimen Adequacy	• Unsatisfactory • Satisfactory
General categorisation	• NILM (Negative for intraepithelial lesion or malignancy) • Squamous cell: 1. ASC-US (Atypical squamous cells of undetermined significance) 2. ASC-H (Atypical squamous cells cannot rule out HSIL) 3. LSIL (Low-grade squamous intraepithelial lesion) 4. HSIL (High-grade squamous intraepithelial lesion) 5. SCC (Squamous cell carcinoma) • Glandular cells: 1. Atypical: Endocervical, endometrial, glandular 2. Adenocarcinoma in situ 3. Adenocarcinoma: Endocervical, endometrial, glandular

Table 2: Findings of pap smears according to "revised Bethesda system for reporting of cervical cytology, 2014"

Pap smear report	**No of patients**	**Percentage**
Specimen Adequacy (n=658)		
Unsatisfactory	8	1.21
Satisfactory	650	98.79
Interpretation/Results		
1. **NILM*** (490/658=74.47%)		
• Non-specific infections/Inflammatory	382	58.03
• Atrophic	62	9.44
• Reactive	46	7
2. **ECA/IEL** (160/658=24.32%)**		
• ASC-US$	41	6.23
• ASC-H$$	5	0.76
• LSIL!	78	11.86
• HSIL&	25	3.79
• SCC#	11	1.68
Total	658	100

*-NILM: negative for intraepithelial lesion or malignancy
$-ASC-US: Atypical squamous cell of undetermined significance
$$- ASC-H: Atypical squamous cell, cannot rule out HSIL
!- LSIL: Low-grade squamous intra-epithelial lesion
&- HSIL: High-grade squamous intra-epithelial lesion
#- SCC: Squamous cell carcinoma

Table 3: Correlation between histopathology and cytopathology

		Pap Smear report							**Total**
		Unsatifactory	**NILM***	**ASC-US** $	**ASC-H** $$	**LSIL** !	**HSIL** &	**SCC** #	
Histopathological findings	Chronic Cervicitis	2	84	11	1	7	-	-	105 (44.88%)
	LSIL	1	28	15	1	48	-	-	93 (39.74%)
	HSIL	-	9	2	-	2	17	-	30 (12.82%)
	SCC	-	-	-	-	-	-	6	6 (2.56%)
	Total	3 (1.28%)	121 (51.7%)	28 (11.96%)	2 (0.85%)	57 (24.35%)	17 (7.26%)	6 (2.6%)	234 (100%)

*-NILM: negative for intraepithelial lesion or malignancy
$-ASC-US: Atypical squamous cell of undetermined significance
$$- ASC-H: Atypical squamous cell, cannot rule out HSIL
!- LSIL: Low-grade squamous intra-epithelial lesion
&- HSIL: High-grade squamous intra-epithelial lesion
#- SCC: Squamous cell carcinoma

Table 4: Efficacy of pap smear cytology for the diagnosis of cervical lesions

		Histopathology findings		
		Positive	**Negative**	
Pap smear findings	Positive	91 (TP)	19 (FP)	110
	Negative	38 (FN)	86 (TN)	124
	Total	129	105	234

Sensitivity= TP/(TP+FN) = 70.54%
Specificity= TN/(TN+FP) = 81.9%
PPV=TP/(TP+FP) =82.72%
NPV=TN/(TN+FN) =69.35%
Diagnostic Accuracy= (TP+TN)/(TP+FN+FP+TN) = 75.6%

Table 5: Comparison of frequencies of pap smears findings with previous studies

Study	**Unsatisfactory (%)**	**NILM* (%)**	**Epithelial cell abnormalities**				
			ASC-US$^{\$}$ (%)	**ASC-H$^{\$\$}$ (%)**	**LSIL$^{!}$ (%)**	**HSIL$^{\&}$ (%)**	**SCC$^{\#}$ (%)**
Nair GG. et al[12]	2.7	94.8	0.2	-	1.5	0.5	0.2
Bamanikar SA et al[13]	5.99	88.02	2.98	-	1.19	0.66	0.95
Rana R. et al[14]	7	91.2	1.24	-	0.83	0.4	-
Singh M et al[15]	-	41.02	33.34	-	15.38	7.70	2.56
Goel NM[16]	6	91	1.9	-	0.77	0.3	0.20
Lahari NA. et al[17]	10.4	77.2	4.4	-	6	1	0.4
Present Study	1.21	74.47	6.23	0.76	11.86	3.79	1.68

*-NILM: negative for intraepithelial lesion or malignancy
$^{\$}$-ASC-US: Atypical squamous cell of undetermined significance
$^{\$\$}$- ASC-H: Atypical squamous cell, cannot rule out HSIL
$^{!}$- LSIL: Low-grade squamous intra-epithelial lesion
$^{\&}$- HSIL: High-grade squamous intra-epithelial lesion
$^{\#}$- SCC: Squamous cell carcinoma

Table 6: Comparison of Pap smear cytology results for the diagnosis of cervical lesions with previous studies.

Study, Year	**Sensitivity %**	**Specificity %**	**PPV* %**	**NPV** %**	**Accuracy %**
Verma I et al[4]	78.5	86.7	64.7	-	81
Bamanikar SA et al[13]	89.47	88.70	82.92	89	89.5
Demir F et al[18]	69	98	89	93	-
Goel NM[16]	87.5	98.9	94.9	97.1	96.7
Present Study	70.54	81.9	82.72	69.35	75.6

*- PPV- Positive predictive value
**- NPV- Negative predictive value

al,[4] Bamanikar SA et al[13] and Goel NM[16] found the diagnostic accuracy of pap smears as 81%, 89.5% and 96.7% respectively (Table 6). This difference may be as conventional methods of pap smear preparation were used in the current study, whereas some of the previous studies used the more accurate liquid-based cytology techniques.[25]

As cervical carcinoma has a long natural history of progression from low-grade cervical lesion to invasive malignant cervical lesion, screening can be effectively used to detect lesions in early stage of cervical cancer.[7] In our study, we found diagnostic accuracy of pap smear to detect low-grade cervical lesions (LSIL) was 84.21% (48/57), whereas it is 100% for high-grade cervical lesions (Table 3). This indicates, that early and accurate detection of cervical lesions is possible by non-invasive screening method (pap smear cytology), which can help the clinicians to treat the patients as early as possible, conclusively, can increase the survival rate of the patients with cervical lesions.

5. Conclusion

As cervical malignancy is the worldwide major problem of the women of reproductive and post-reproductive age group, so, early diagnosis of cervical lesions is necessary to impede the progression of pre-malignant cervical lesions to malignancy. The present study showed that, pap smear is able to detect the various cervical lesions according to the 2014 revised Bethesda system for cervical cytology. It also highlighted, that the diagnostic accuracy of pap smear

cytology was 75.6% for overall cervical lesions. Also, the recent CHC protocol can be an applicable tool for specialists to determine whether results are concordant or discordant and to evaluate errors in cytologic screening. It indicates that non-invasive pap smear technique can detect the cervical lesions accurately in early or pre-malignant stage, which can help the clinicians to decide the management at an earlier stage.

6. Conflict of Interest

None.

7. Source of Funding

None.

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