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Research Article

Cytological patterns of cervical pap smears with histopathological correlation

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ABSTRACT

Background: Cervical cancer is the most common, preventable cancer and does not develop suddenly from normal epithelium but is presented by a spectrum of intraepithelial neoplasia. Pap smear is an ideal screening and low cost effective test to detect intraepithelial neoplasia especially in developing countries but has limitations and needs confirmation by histopathology. The study aimed to determine the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of pap smears as screening test in cervical lesions by comparing with histopathology.
Methods: This is a prospective study conducted at a tertiary care centre in Department of pathology from October 2012 to September 2014. A complete clinical history and demographic status of the subjects was recorded. Conventional pap smears were reported adopting Bethesda system and biopsies were taken in cases of epithelial cell abnormality, routinely processed and stained with H&E.
Results: Total number of pap smears analysed were 356**.** NILM (negative for intraepithelial lesions or malignancy) constituted major group 248/356 (69.64%) and epithelial cell abnormality were 98/356 (27.5%). In the present study overall sensitivity was 94.11% specificity 64.28% positive predictive value 82.75%,negative predictive value 85%,diagnostic accuracy 83.33%,concordance rate 78.2% and P value <0.001. Concordance rate for CIN I was 78.9%, for CINII and CINIII 60% and for squamous cell carcinoma 100%.
Conclusion: Pap smear is simple, inexpensive and can be performed in the outpatient department. Hence, it should be recommended routinely as a method of improving reproductive health, early detection of premalignant and malignant cervical lesions.

Keywords: NILM, Epithelial cell abnormality, Pap smears, Screening test

INTRODUCTION

Cervical cancer is common world-wide and ranks third among all malignancies for women. Eighty percent of cancer cervix are seen in developing countries, where it is the commonest cancer in woman.[1] In India women at risk for cervical cancer are 432.20 million, annual number of cervical cancer cases is 122,844, annual number of deaths is 67, 4773.[2] It has been estimated that an average woman under 40yrs of age has 2% chance of developing cervical carcinoma. Studies show that cervical carcinoma does not develop suddenly from normal epithelium but is presented by a spectrum of intraepithelial neoplasia, if these lesions were untreated; up to one third of them would develop into carcinoma.

No form of cancer better documents the remarkable effects of prevention, early diagnosis, and curative therapy on the mortality rate than cancer of the cervix. The accessibility of the cervix to pap testing and visual examination (colposcopy) as well as the slow progression from precursor lesions to invasive carcinoma (typically

over the course of years) provides ample time for screening, detection, and preventive therapy.

Our centre caters to the need of rural, tribal and semi urban population. The present study is about knowing the proportion of negative for intraepithelial lesions and epithelial cell abnormality, role of demographic parameters in the causation of these lesions and role of pap smear as a screening test in detecting these lesions.

METHODS

This is a prospective study conducted in tertiary care centre at department of pathology from October 2012 to September 2014. A complete clinical history and demographic status of the subject was recorded as per the proforma, with respect to age at presentation, age at marriage, parity, first child birth, socioeconomic status and literacy. The inclusion criteria were**:** Symptomatic patients with normal cervix on clinical examination and symptomatic patients with suspicious cervical lesion. Cases excluded from the study were**:** pregnant women, patients who have undergone hysterectomy, excessive vaginal bleeding at the time of examination and patients on therapy.

Conventional pap smears were taken with the help of the gynaecologists at the squamo columnar junction using Ayer's spatula in clockwise direction for 360°.The endocervical smears were made by rotating the endocervical brush. Two separate smears were made from the squamocolumnar junction and endocervical area on a single glass slide. The smears were immediately fixed in 95% Isopropyl alcohol for 30 minutes.

Biopsy was taken from cervix mainly in cases with epithelial cell abnormality on pap smears and badly eroded cervix, by applying 3% acetic acid (acetowhite areas).They were fixed in 10% formalin, routinely processed and stained with haematoxylin and eosin.

The pap smears were reported by adopting "The Revised Bethesda System of Classification (2001 TBS)" and histopathology "WHO classification of tumours of uterine cervix (2003)". Correlation of cytological findings with histopathology was done considering histology as gold standard. Sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy, concordance rate and P value was calculated. HPV tests were not done due to restriction of cost effectiveness of kits.

RESULTS

Total number of pap smears received during this period were 380.Inadequate smears were-24 (6.31%).Total number of pap smears available for analysis were 356.Cervical biopsies available for cyto-histological correlation were 78. Pap smears revealed epithelial cell abnormality in 98 subjects, and smears were negative for intraepithelial lesions or malignancy (NILM) in 248 subjects, remaining 10 smears were normal on examination. In 248 cases of NILM, non specific inflammation seen in100cases, reactive cellular changes seen in 53 cases, squamous metaplasia seen in 25cases, 6 smears showed atrophy. Sixty four cases showed specific infections in smears. Candida species infection (Figure 1) was most common comprising 7.86% of cases, followed by Trichomonas vaginalis (7.3%) (Figure 2).

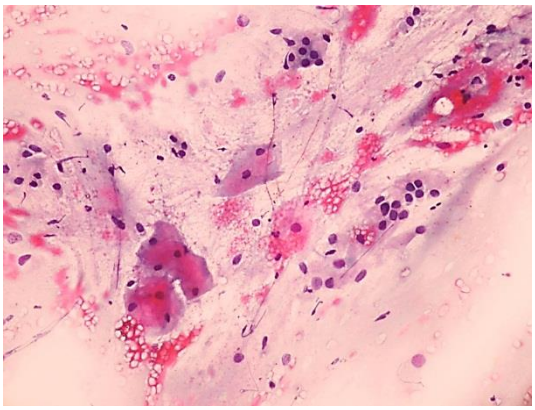

Figure 1: Photomicrograph showing Negative for intraepithelial lesion or malignancy: Candida species (PAP stain; 100x).

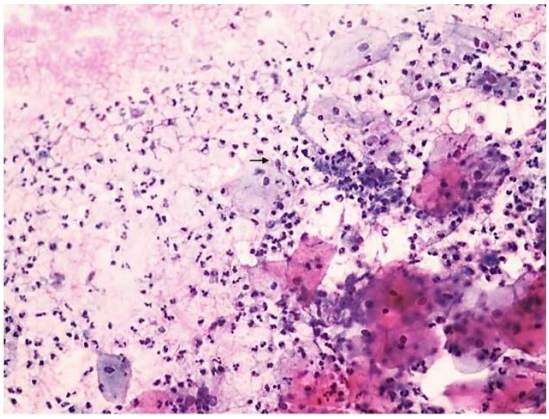

Figure 2: Photomicrograph showing Negative for intraepithelial lesion or malignancy: Trichomonas vaginalis (PAP stain; 100x).

Epithelial cell abnormality (ECA) was commonest in the age group 31-40 years. Maximum number of NILM cases and premalignant lesions were in third and fourth decades, malignant lesions were observed more than 50 years of age. Commonest symptom was white discharge in 197/356 (55.33%) of subjects. None of the women had any history of multiple sexual partners, smoking, and usage of oral contraceptive pills. Mean parity was 2.43 with squamous cell carcinoma seen in subjects with more than 5 children.

The age of marriage for NILM and epithelial cell abnormality was between13-18. Most of the cases had first child birth between 15-19 years-196/356 (55.05%). Majority of the subjects (75.56%) were belonging to the low socioeconomic group. 58.7% of the women were illiterates, 83.14%were from rural area.

Amongst the subjects with ECA, Atypical squamous cells of undetermined significance (ASCUS) were 30 (Figure 3) , Atypical glandular cell not otherwise specified(AGC NOS): 2 (Figure 4), Low grade squamous intraepithelial lesion(LSIL):50 (Figure 5, 6 & 7) ,High grade squamous intraepithelial lesion (HSIL):14 (Fig 8 & 9) and Squamous cell carcinoma 2 (Fig 1 & 11).

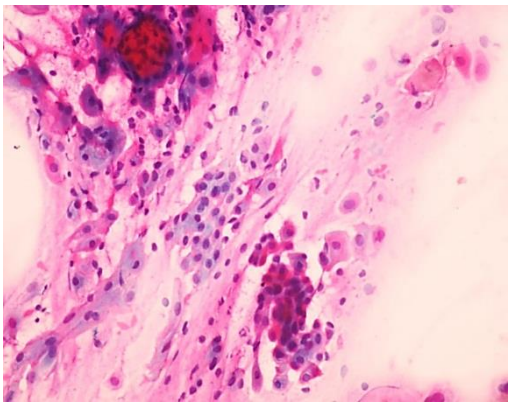

Figure 3: Photomicrograph showing nucleomegaly is2-3 times of the intermediate squamous cell nucleus with regular nuclear membrane and fine chromatin: ASCUS (PAP stain; 100x).

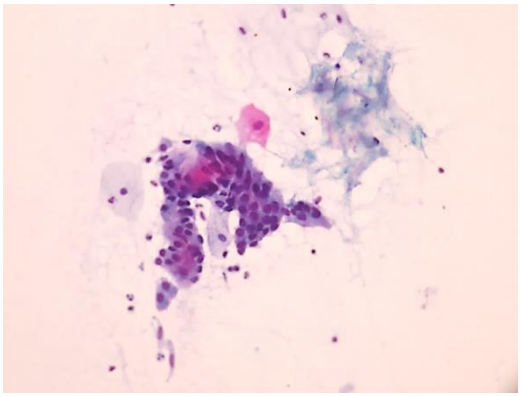

Figure 4: Photomicrograph showing Nuclear enlargement, up to three to five times the area of normal endocervical nuclei, with mild hyperchromasia, AGC-NOS (PAPstain; 100x).

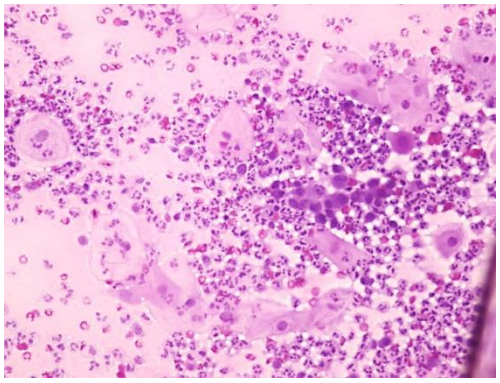

Figure 5: Photomicrograph showing sheet of cells with nuclear enlargement three times of normal intermediate cells LSIL (PAPstain; 100x).

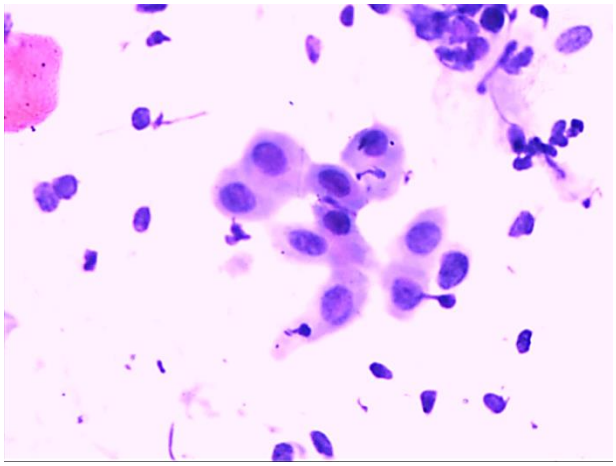

Figure 6: Photomicrograph showing sheet of cells with nuclear enlargement two to three times of normal intermediate cells LSIL (PAPstain; 400x).

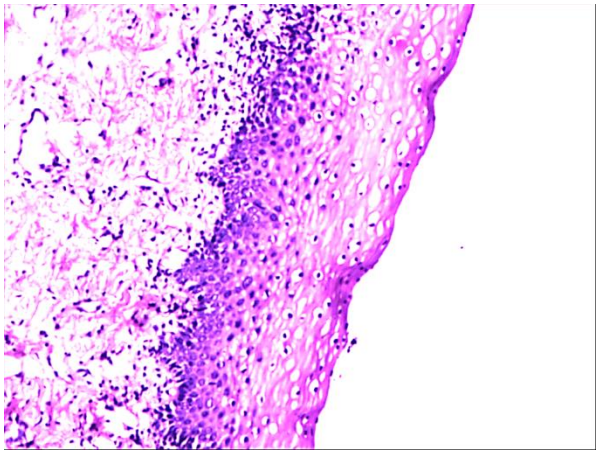

Figure 7: Photomicrograph showing dysplasia<1/3 of epithelium CIN I (H&Estain; 100x).

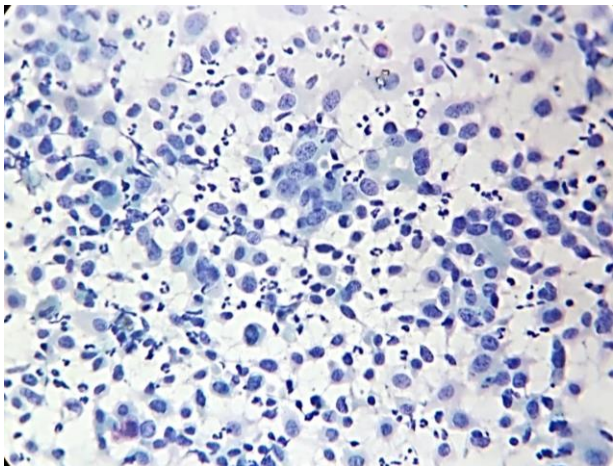

Figure 8: Photomicrograph showing HSIL with marked increase in nuclear/cytoplasmic ratio, irregular nuclear membrane (PAPstain; 100x).

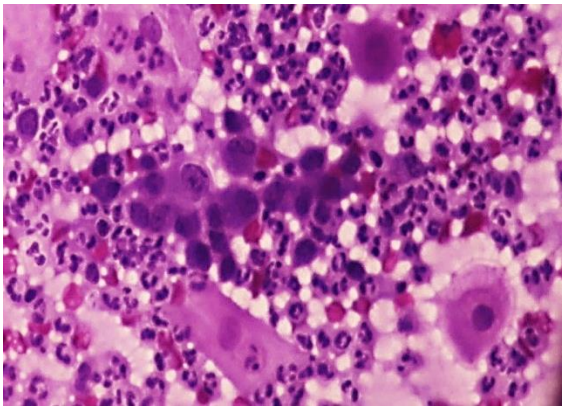

Figure 9: Photomicrograph showing HSIL with marked increase in nuclear/cytoplasmic ratio, irregular nuclear membrane (PAPstain; 400x).

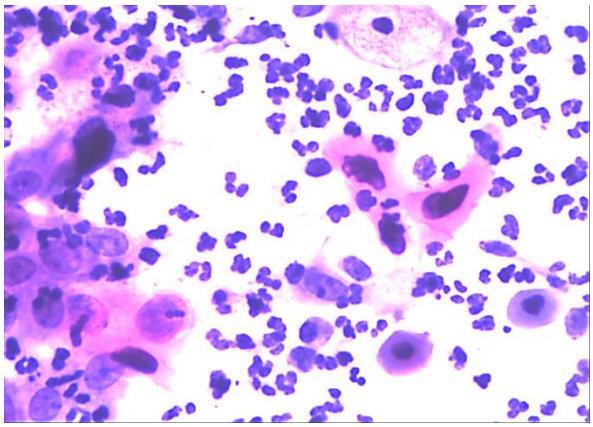

Figure 10: Photomicrograph showing Squamous cell carcinoma with dyskeratotic cells (PAP stain; 400x).

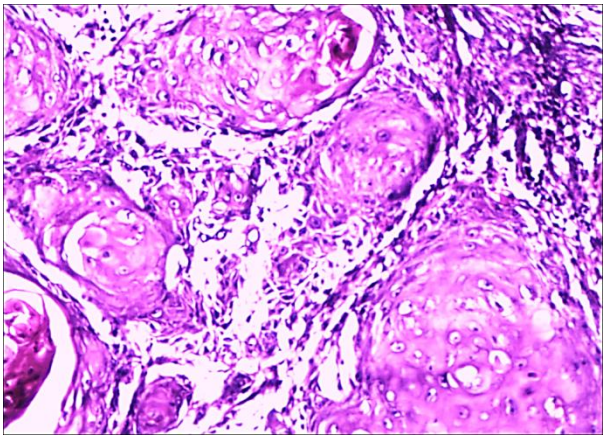

Figure 11: Photo micrograph showing Keratinising squamous cell carcinoma with keratin pearls (H&E stain;100x).

In the present study NILM cases 17/20 (85%), ECA cases 44/58(75.86%), ASCUS cases 6/12(50%), LSIL cases 30/35(85.7%), HSIL cases8/9(88.88%) and squamous cell carcinoma 2/2(100%) were correlated on histopathology. (Table 1&2)

Out of 17 deferred cases, six cases of ASCUS on cytology were given the diagnosis of non specific cervicitis on histopathology. Out of 3 cases with diagnosis of HSIL on pap smears, two turned out to be squamous cell carcinoma on histopathology. (Table 3)

Table 1: Cyto-histopathological correlation: 78 cases.

Cytological patterns on pap smears	Number of cases on PAP smears	Histopathologial diagnosis					
		Non neoplastic	CIN I	CIN II	CIN III	CA in situ	SCC
NILM	20	17	3	-	-	-	-
ASC-US	12	6	4	1	-	1	-
LSIL	35	3	30	2	-	-	-
HSIL	9	-	1	5	1	-	2
SCC	2	-	-	-	-	-	2
Total	78	26	38	8	1	1	4

Table 2: Correlated and non correlated cases on histopathology (N=78).

Cytological patterns on pap smears	Number of cases on PAP smears	Correlated	Non-correlated
Negative for intraepithelial lesion or malignancy(NILM)	20	17	3
Epithelial cell Abnormality	58	44	14
• ASCUS	12	6	6
• Premalignant lesions	44	36	8
• Squamous cell carcinoma	2	2	0

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Table 3: Deferred cases on histopathology (N=17).

Cytodiagnosis	Deferred cases	Histopathological diagnosis
Negative for intraepithelial lesion or malignancy	3	CIN 1
Epithelial cell abnormality	14	
ASCUS	6	NILM
LSIL	5	NILM-3,CIN II -2
HSIL	3	CINI-1,SCC -2

The overall Sensitivity of study was 94.11%, Specificity was 64.28 % Positive predictive value was 82.75%, Negative predictive value was 85% and Diagnostic accuracy was 83.33%. (Table 4)

P value for correlation between pap smears and histopathology through chi square test is 0.001 in present study, which is significant. Concordance rate for CIN I was 78.9%, for CIN II and CIN III 60%, for squamous cell carcinoma 100%, and overall concordance rate was 78.2%.

Table 4: Senstivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of pap smears.

Lesions		Senstivity %	Specificity %	Positive predictive value%	Negative predictive value%	Diagnostic accuracy
NILM		65.8	94.23	85	84.48	84.61
Epithelial cell abnormality		93.6	54.83	75.8	85	78.2
	LSIL	78.94	95	93.75	82.60	87.17
	HSIL	60	95.58	66.66	94.2	91
	Carcinoma	50	100	100	94.87	97.43
Pap smears as a screening test for all cervical lesions		94.11	64.28	82.75	85	78.2

DISCUSSION

Women in India face constraints not only in obtaining health services, but also in expressing reproductive health needs. Lack of awareness, cultural barriers and economic factors prevent them from seeking timely care.

The age range in the present study was 20-70 years which was comparable to the study of; Jain et al.,[3] Robyr et al.,[4] Mostafa et al.[5] Well-established risk factor of cervical cancer is multiparty. Mean parity in present study was 2.48, which was comparable with study by Saha R et al.[6]

College of American Pathologists (CAP) uses the ratio ASCUS / SIL as a quality indicator of management and recommends maintaining it <2-3 (Jones et al.[7] 2000). In present study the ratio was 0.45 which was comparable with study by Jain et al.[3]

McGrath et al.[8] in his article states that, since the introduction of the cytological category atypical squamous cells of undetermined significance (ASCUS) by the Bethesda System (TBS) in 1988, ASCUS has been problematic for pathologists, clinicians, patients, and society. ASCUS on pap smears show nuclear enlargement in squamous cells, nuclei are enlarged 2.5 to 3 times the size of a normal intermediate squamous cell nucleus and may have slight hyperchromasia but finely granular, evenly distributed chromatin. Nuclear membrane irregularities should not be present. The nuclear changes may be suggestive of human papillomavirus (HPV) but quantitatively or qualitatively short of a definitive diagnosis. The main differential diagnosis is low-grade SIL (LSIL) vs. marked reactive changes.

High interobserver and intraobserver variability is well documented in the literature, regardless of years of training or level of expertise. Perimenopausal changes and changes related to hormone replacement therapy can represent diagnostic challenges and may lead to over diagnosis of ASCUS in older women. Reluctance to diagnose LSIL in peri-menopausal and postmenopausal women may also lead to more interpretations of ASCUS.

In present study out of 12 cases diagnosed as ASCUS on cytology, 6 were non-neoplastic, 4 were CINI, 1 was CIN II and 1 was carcinoma in situ in histopathology. Fifty percent of ASCUS on cytology showed diagnosis of cervical intraepithelial neoplasia on histopathology stating that all cases of ASCUS should be followed up periodically and biopsy is a must. Out of 35 cases diagnosed as LSIL on cytology, 3 were non-neoplastic, 30 were CINI, and 2 were CINII in histopathology. The 6 false positive cases of ASCUS on Pap smear were probably due to regenerative changes and their evaluation therefore becomes more important. It is suggested in literature that regenerative changes can be divided into typical and atypical and the atypical regeneration group in particular be added to the ASC-US group. To decrease false positive rate adequate sampling, avoiding technical errors like air drying, fixation artefacts is necessary.

According to Abli et al.[9] differentiation of regenerative changes from neoplastic lesions in the smear test requires taking a biopsy after 3 positive results in cases where ASCUS is found. Due to the low socioeconomic level of the patient population, follow-up is not possible usually proceed to endo-ectocervical biopsy with colposcopy even after a single positive result. This leads to an increased false positivity rate.

Only 2% of patients with HSIL have invasive cancer well documented in literature, however, up to 20% with HSIL will develop cancer if it is left untreated. To prevent HSIL from developing into cancer, the precancerous cells are removed or destroyed. In the present study out of 4 HSIL cases on pap smears, 2 cases (50%) were diagnosed as CIN II & CIN III, other 2 cases (50%) were diagnosed as squamous cell carcinoma on biopsy, hence colposcopic guided biopsy would minimize the errors.

In present study sensitivity was 94.11% which was comparable with Tamboli et al.[1] with the sensitivity 90.65%.However the sensitivity was lower as in study by Jain Vet al[3]which was 78%. The specificity was 64.28% which was comparable to study by Anschau et al.[10] Diagnostic accuracy in present study was 83.33%. In study by Nasreen et al.[11], Jain et al.[3], Tamboli et al.[8] diagnostic accuracy was 79.09%, 73.2%, 90.4% respectively. In present study P value is <0.001which is comparable with Nasreen et al.[11] and highly significant.

CONCLUSION

A number of new technologies have emerged in diagnosing various lesions of cervix which are costly and cannot be easily implemented in rural and tribal areas. In the present study, majority of the subjects belonged to rural and tribal areas (94.94%) with illiteracy rate of 58.7%.

In the present setup conventional pap smears still remain the most dependable screening and diagnostic test. Diagnosing infective lesions, reactive / regenerative changes and malignancy by pap smears is highly accurate.

The problematic zone is ASCUS, LSIL and HSIL, which is also documented in the present study. In ASCUS we have atypical squamous cells, sometimes showing cytopathic effects mimicking LSIL. In such cases besides biopsy, HPV DNA testing would help us in further follow up of the cases and management.

In cases of LSIL on Pap smear, colposcopy guided biopsy would help us in taking biopsy from a representative area to rule out HSIL. Regular interval screening and stringent quality control are necessary to improve the efficacy of Pap smear screening test.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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