Research Article

Utility of Papanicolaou test in diagnosis of cervical lesions: a study in a tertiary care centre of western Uttar Pradesh

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ABSTRACT

Background: Cervical cancer is the most common cancer among females in India. It is also one of the major causes of deaths among them. Papanicolaou (Pap) cytological test helps in detecting the early epithelial abnormalities in cervical cells. The morbidity and mortality due to cervical cancer has come down in countries with well-established screening programmes at national level. This study was conducted to emphasize the value of cervical screening as a tool for early detection of non-neoplastic and neoplastic lesions of cervix. Slide positivity rate was computed and clinico-pathological correlation was done.
Methods: This cross-sectional study was conducted from January 2012 to December 2014 (3 years), on 2202 women aged between 20-70 years coming for a Pap smear examination in a tertiary care hospital in Bareilly, India. After staining with conventional Papanicolaou technique, all cases were classified as per Bethesda nomenclature (2001).
Results: Out of 2202 cases, 85.16% slides were found to be negative for intraepithelial abnormalities and 14.84% slides were labelled as positive for epithelial lesions. 97.67% of high grade lesions were found in women more than 40 years. Smears with epithelial cell abnormality were found more common in older age groups whereas smears negative for epithelial cell abnormality were found more common in young age groups.
Conclusion: Pap test is a cost effective cancer screening and is a simple method to detect various lesions of cervix, non-neoplastic as well as neoplastic. High risk screening programmes should be directed to all women >40 years. Pap test is especially useful to diagnose precancerous lesions of cervix, thereby early detection of these lesions & subsequent proper treatment can be helpful in prevention of cervical cancer.

Keywords: Cervical smear, Bethesda system (2001), Pap smear, Screening

INTRODUCTION

The Papanicolaou (Pap) test is a screening test which is performed by scraping the cells from the uterine cervix. This test was introduced in 1941, by George Papanicolaou as a cervical cytology screening test.[1] Pap smear test is a rapid, cost effective outpatient department procedure which is capable of detecting neoplastic as well as non-neoplastic cervical lesions at the earliest. It has led to a fall in incidence and mortality of cervical cancer.

Unfortunately, some developing countries lack this facility to carry out widespread Pap screening.[2] Cervical cancer is the second most common cancer in women worldwide.[3] In India, it is estimated that the number of new cases are 126000 per year.[4] I is the most common cancer of women in developing countries, even then, it is

estimated that only about 5% of women have been screened for the disease with pap smear, compared to 40-50% in developed countries.[5] Because of poor access to screening and treatment services, the vast majority of deaths still occur in women living in low- and middle-income countries.

The easy accessibility of the cervix and the propensity of the cancer cells to exfoliate from its surface have enabled us to study the process of malignant transformation in the cervix in very early stage.[6] The Pap test is done by taking samples from the transformation zone, an area where physiologic transformation from columnar endocervical epithelium to squamous (ectocervical) epithelium takes place, where dysplasia and cancer arises.

Nowadays, cytology along with Human Papilloma Virus (HPV) testing is being assessed as a screening test for cervical cancer in the developed countries. Similar to other DNA viruses,[7] HPV is detected by DNA testing with Polymerase Chain Reaction (PCR) or Hybrid Capture (HC).

In a developing country the Pap test may be useful as a screening modality, which is ultimately lead to the reduction of cervical cancer-related mortality by the earlier possible detection of premalignant lesions. So, greater awareness among clinicians regarding this test is required to carry out screening for cancer of cervix among women in both rural and urban areas in India.

The present study was conducted to analyse the results of Pap screening tests in order to determine the slide positivity rate and clinico-pathological correlation laying emphasis on the importance of conventional Pap smears for the diagnosis of inflammatory, premalignant and malignant lesions of the cervix.

METHODS

This cross-sectional study was conducted at Rohilkhand medical college and hospital (A tertiary care centre in western UP). A total of 2214 clinically suspected females were screened by pathology department from the period of January, 2012 to December, 2014. After taking informed consent, proper history of the patient was noted regarding menopausal age, socioeconomic status and literacy level, age of marriage, obstetric history, history of used oral contraceptives and history of sexual partners. Cervical scrape smears were collected using Ayer's spatula or endocervical brush. The smears were fixed in absolute alcohol and stained by trained cytotechnologists using Papanicolaou's method. Specimen adequacy was assessed and the epithelial abnormalities were classified according to the Bethesda system 2001[8] by two pathologists independently. Any discrepancy was sorted out through evaluation by third independent pathologist. Slides which were labelled as unsatisfactory by any of two pathologists were revised wherever possible. All the women where revision of slide was not possible were

excluded by labelling as unsatisfactory slides for evaluation and were excluded from the study. Blinding was maintained at every stage of study and during evaluation of slides by different pathologists. Colposcopy and biopsy was advised according to standard recommendations. Ethical clearance from institutional ethical committee was obtained beforehand.
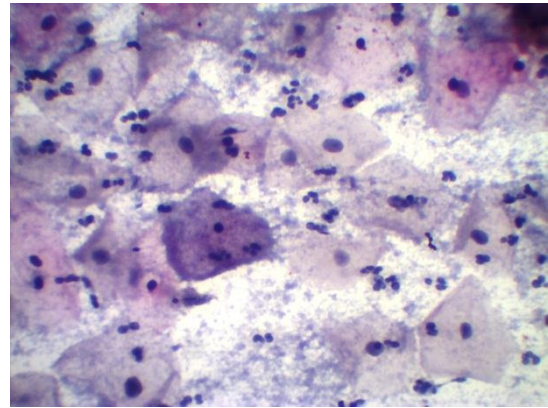

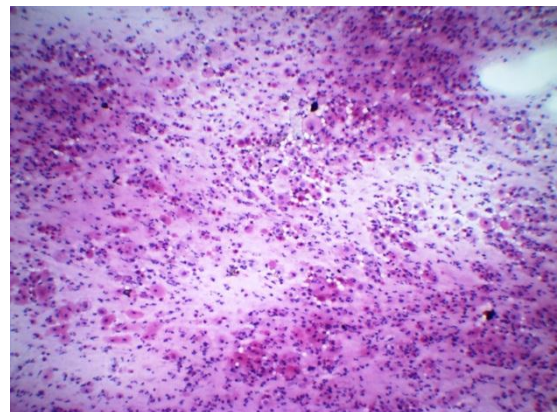**Figure 1: Showing the characteristic "clue cells" of bacterial vaginosis.**

Figure 2: Showing numerous basal and parabasal cells in case of atrophic vaginitis (Pap 100x).
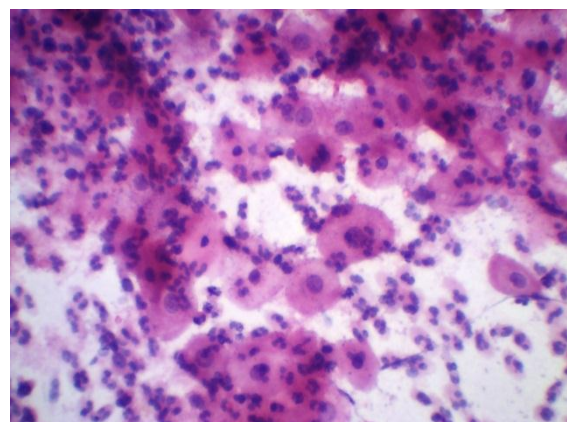

Figure 3: Showing reactive nuclear changes in epithelial cells and marked inflammation by neutrophils (Pap 400x).
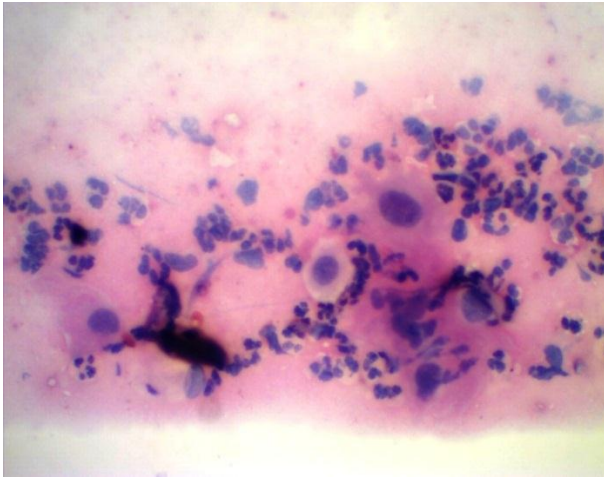
Figure 4: Showing intermediate cell having size >3 times that of normal (ASCUS) (Pap 400x).
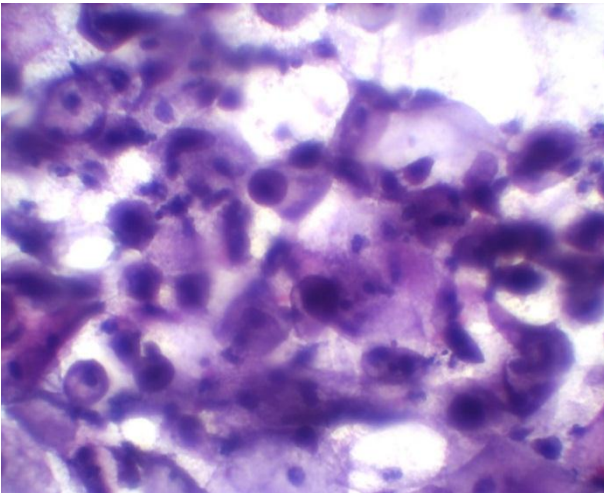

Figure 5: Showing low grade squamous intraepithelial lesion (Pap 400x).
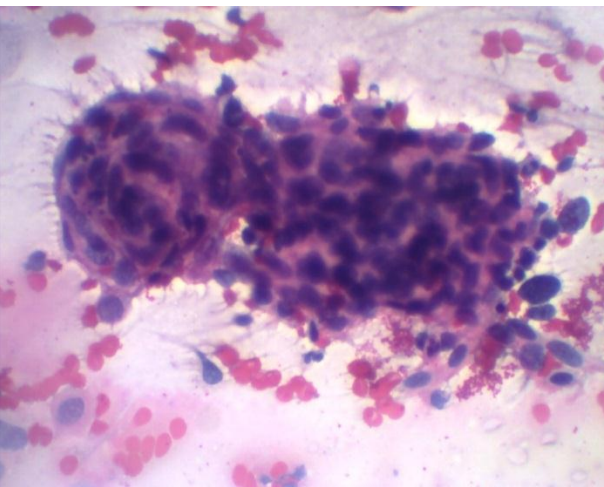

Figure 6: Showing HSIL - Cluster of cells, showing increased N/C ratio, hyperchromatic nuclei and coarse chromatin (Pap 400x).
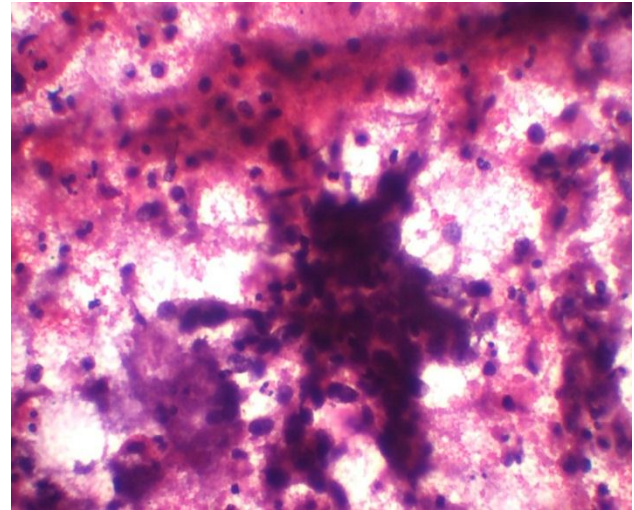

Figure 7: Showing squamous cell carcinoma with pleomorphic, overcrowded malignant cells having tumor diathesis in the background (Pap 100x).

RESULTS

Out of 2214 women evaluated during the period of study, 12 slides were labelled as unsatisfactory for evaluation. Therefore, rest 2202 women were included in the study. Out of 2202 women maximum i.e. 42.23 % were in the age group of 31-40 years followed by 29.75 % women in 21-31 years age group and 18.39 % were in 41-50 years age group. 6.13 % women and 3.5 % women were in fifth decade and sixth decade respectively (Table 1).

Table 1: Distribution of patients according to age groups.

Age group	Number of subjects	Percentage
21-30	655	29.75 %
31-40	930	42.23 %
41-50	405	18.39 %
51-60	135	6.13 %
61-70	77	3.5 %
Total	2202	100%

Most of the women had multiple symptoms. Vaginal discharge was the most common presentation (56.37%) followed by pruritus (36.31%), burning micturition (24.85%), pelvic pain (20%) and backache (17.98%) (Table 2).

Among 2202 slides, 2.10% slides were found to be normal, 85.16% slides were found to be negative for intraepithelial abnormalities (inclusive of acute inflammatory, reparative/reactive cellular changes of inflammation, infections and atrophic smear) and 14.84% slides were labelled as positive for epithelial lesions (Table 3).

Table 2: Distribution of patients on the basis of symptoms*

Symptoms	No. of patients	Percentage
Per vaginal discharge	1248	56.68%
Pruritus	804	36.51%
Burning micturition	550	24.98%
Pelvic pain	442	20.07%
Backache	398	18.07%
Menstrual abnormalities	342	15.53%
Infertility	110	5.00%
Dyspareunia	100	4.54%
Post-menopausal bleeding	85	3.86%

*Multiple answers were allowed

Among the smears reported negative for intraepithelial abnormalities, reactive/inflammatory changes were seen in maximum smears i.e. 84.06% cases out of all smears examined (Table 3).

Among the epithelial cell abnormalities, most frequent was Atypical Squamous Cells of Undetermined Significance (ASC-US) reported in 10.08% cases followed by LSIL reported in 1.10% patients. Out of all smears examined, 0.56% Pap smears revealed high grade lesions and malignancy (0.45% HSIL and 0.09% malignancy) (Table 3).

Although only 0.56% smears showed high grade lesions, but majority (97.67%) of these (11 out of 12) were found in women more than 40 years. whereas Maximum cases of HSIL (50%) were seen in 40-50 years age group, all the smears positive for malignancy belonged to women >50 years of age. Distribution of different epithelial lesions among different age groups was not skewed (Table 4).

Table 3: Distribution of smears according to cyto-pathological diagnosis.

Cyto-diagnosis	No. of cases	% (Out of 2204)
Normal smear	**46**	**2.10**
Negative for intraepithelial lesions		
Reactive / inflammatory	1851	83.56
Atrophic smear	35	1.60
Total	1886	85.16
Epithelial lesions		
ASCUS	222	10.00
AGC	1	0.05
ASC-H	10	0.50
LSIL	25	1.10
HSIL	10	0.50
Malignancy	2	0.09
Total	270	14.84
All total	2202	100

Among the smears showing atrophic changes, mostly (85.71%) belonged to patients aged >50 years where as smears showing inflammatory changes mostly (92.38%) belongs to <50 years. This difference in distribution was found to be statistically significant (Table 4).

Regarding the distribution of abnormal smears, smears with epithelial cell abnormality were found more common in older age groups whereas smears negative for epithelial cell abnormality were found more common in young age groups. This difference in distribution was found to be statistically significant (Table 4).

Table 4: Distribution of smears according to cyopathological diagnosis in relation with age groups.

Age group	Epithelial cell abnormality (N=268)						Total	Negative for intra-epithelial cell abnormality (N=1886)		Total
	ASCUS	ASC-H	AGC	LSIL	HSIL	Malignant		Reactive / inflammatory changes	Atrophic changes	
21-30	28	0	0	3	0	0	31	598	0	598
31-40	72	0	0	11	1	0	84	740	0	740
41-50	60	1	1	4	5	0	71	372	5	377
51-60	47	1	0	3	3	2	56	81	13	94
61-70	15	8	0	4	1	0	28	60	17	77
Total	222	10	1	25	10	2	270	1851	35	1886
P value	>0.05 (Fisher exact test)							<0.05 (Chi square test)		

P value <0.05 (Chi square test)*
*For distribution of cases among epithelial cell abnormalities & negative for intraepithelial cell abnormality

DISCUSSION

Screening for any disease aims to detect the disease in its earliest possible stage and treat it so that it prevents the morbidity and mortality due to its advanced stage which also decreases the economic burden to the person, family as well as to the country.

Uterine cervix is ideal for screening due to easy accessibility of cervix for inspection, palpation, exfoliative cytology, and screening. Cervical cancer is the most commonly screened disease in female and if it is carried out effectively, it significantly decreases the deaths due to it. Cervical cancer screening represents one of the great success stories in cancer prevention. In India incidence rates of cancer of the cervix is very high especially in rural areas.[9] The age-standardized incidence rates have ranged from 16-55 per 100000 women in different regions of India.[2] Although control of cervical cancer by early detection and treatment remains a priority of the national cancer control programme of India, organized cytology screening programmes are definitely lacking. One possible reason is the technical and financial constraints to organize cytology screening.[4]

Introduction of conventional Pap screening services reduces cervical cancer rates by 60% to 90% within 3 years of implementation; and these reductions in incidence and mortality are consistent.[10] Therefore the Pap test is designated as the "single best cancer screening procedure".[8] The past failures of cervical screening in developing countries are attributable to failures in programme quality rather than to technological limitations of the screening test.[10] In fact successful implementation of Pap test screening in southern Vietnam, which recorded reductions in cervical cancer incidence from 29.2 per 100000 in 1998 to 16 per 100000 in 2003 reiterates these view.[11]

The percentage of epithelial abnormality came out to be 14.84% in our study while the inflammatory lesions accounted for 85.16% cases.

Table 5: A comparison regarding rates of abnormal Pap smears (excluding inflammatory lesions) with previous studies.

Study	Abnormal Pap smears (%)
Misra et al., 2006[13]	5.9
Banik et al., 2011[14]	8.2
Neelima et al., 2012[15]	9.5
Imai et al., 2012[16]	1.2
Jena et al., 2012[17]	6.3
Present study	12.10

In a study conducted at Lucknow by Misra et al.,[12] incidence of SIL was found to be 5.9%. The study by

Banik et al.[13] was conducted in Bangladesh and showed a high prevalence of epithelial cell abnormality of 8.2% possibly because the patients included in the study visited the tertiary care hospital for specific gynaecological complaints. Result similar to our study was found by Neelima and colleagues[14] with 80% inflammatory and 9.5% epithelial abnormality. A study conducted in Japan by Imai et al.[16] found out to be low (1.2%) as it was carried out on asymptomatic females.

Lastly ratio of non-neoplastic to neoplastic lesions in our study came out to be app. 50:1, the neoplastic lesions including malignant as well as premalignant lesions (LSIL and HSIL). Wide variation in the ratio of non-neoplastic to neoplastic lesions in different studies is observed indicating the geographical influence on occurrence of cervical carcinoma. The results in the present study were similar to that reported by Van der Graaf et al.

Table 6: A comparison regarding ratio of non-neoplastic to neoplastic lesions with previous studies.

Study	Non-neoplastic	Neoplastic	Ratio
Van der Graaf et al.[18] (1987)	162626	2559	63.5:1
Murthy et al.[19] (1990)	84889	2125	39.9:1
Mostafa et al.[20] (2000)	112091	815	137.5:1
Bhatia et al.[21] (2001)	100	16	6.25:1
Robyr et al.[22] (2002)	740	27	27.4:1
Kulkarni et al.[23] (2013)	258	43	6:1
Present study	1851	37	50:1

In the present study, the ratio of premalignant (LSIL and HSIL) to malignant lesions was 17.5:1, which was comparable to study of Kulkarni et al. where the ratio was 20.5:1. However, the rate of premalignant lesions was much higher as reported by Lozowski et al.[24] with ratio of 62.5:1, possibly because there is an early detection of pre malignant lesions in developed countries, so malignant lesions were less common. Whereas, Mostafa et al. had found a lower rate of premalignant lesions, the ratio being 3.2:1.

CONCLUSION

From the above study we conclude that more than 95% of Pap Smears examined on patients with suspicion were of abnormal pattern either of inflammatory or having any atypical epithelial lesions. Thus Pap smear should be advised in all patients with suspicion. All medical practitioners are well aware of this.

As most of the pre malignant and malignant lesion were found in women >40 years. Therefore, screening programmes should be directed to all women >40 years. Cervical cancer is one such ideal disease for screening. Hence for a country like India with great burden of carcinoma cervix, population based screening programs by the government will prove fruitful to reduce the mortality and morbidity of cervical cancer.

As most of inflammatory lesions were in young women and these inflammatory lesions may be precursors for malignant lesions, therefore early detection of these lesions with the help of screening test like Pap smear & subsequent proper treatment can be helpful in prevention of cervical cancer.

ACKNOWLEDGEMENTS

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