



A Clinicopathological study of uterine cervical lesions in a Tertiary Care Centre

Sujata Kumbhar*, Sujata Kanetkar, Avinash Mane, Akanksha Gore

Department of Pathology, Krishna Institute of Medical Sciences 'Deemed To Be' University, Karad, Maharashtra, India



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ABSTRACT

Pap test is a simple yet effective screening method to detect cervical cancer in its early stage, and thus it helps to reduce morbidity and mortality from cancer. A total of 430 cases were studied after taking ethical clearance. The cytological diagnoses were made by Cytopathologist using Bethesda 2014 system for categorization of cervical smears. Cytological results were correlated with clinical features and histopathological results wherever biopsy was available. The total of the 430 cases studied 24 (5.58%) were inadequate to report, excluding these, of the total 406 cases 260 (64.04%) cases were symptomatic, and 146 (35.96%) were asymptomatic. Cytologic diagnoses were NILM –normal- 140 (55.23%) with a mean age of 38.48 years, NILM- inflammatory 128 (47.76%) with a mean age of 35.96 years, ASC-US 36 (8.86%) with a mean age of 36.69 years, ASC-H 18 (4.43%) with a mean age of 45 years, LSIL 42 (10.34%) with a mean age of 41.90, HSIL 22 (5.42%) with a mean age of 51.22 years, and invasive cancer 20 (4.92%) with a mean age of 61.8 years. Sensitivity, specificity, and positive predictive value, negative predictive value and accuracy of Pap test were 89.79%, 95.34%, and 95.65%, 89.13% and 83.33% respectively. Pap smear test is a simple, rapid, economical, still practical screening test to detect cervical intraepithelial lesions and cancer.

*Corresponding Author

Name: Sujata Kumbhar
Phone: 9272325081
Email: drsujatakumbhar@yahoo.in

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INTRODUCTION

In 1941 Greek doctor George Papanicolaou introduced a test for screening cervical premalignant and malignant lesions which were named after his name as Pap test or Pap smear (Lakshmi and Gouri, 2016; Shaki et al., 2018).

Cervix cancer is the second most common cancer in women worldwide and is a significant public health problem in developing countries like India, where it is leading cancer (Rana et al., 2013). In the western world before the introduction of this test cancer of the cervix was the leading cause of morbidity and mortality in women. Its incidence and mortality had markedly reduced by effective implementation of this test (Olusegun et al., 2017; Shuaibu et al., 2018). However, because of lack of awareness, resources and organized screening program in developing countries like India the burden of cervical cancer is still high being >80 % of the cases were from developing countries in the world statistics (Sachan et al., 2018; Arul et al., 2017).

Cervix cancer is a preventable malignancy as it has a long latent or precancerous phase during which it can be easily detected by cost-effective and straightforward Pap test and treated as well effectively and thereby its dreaded complications are avoided (Bal

et al., 2012; Kabaca *et al.*, 2013).

Many risk factors are associated with the pre-malignant and malignant lesions of the cervix (Rose, 2016). Cervical infection is one amongst them. Abnormal vaginal flora can produce carcinogenic nitrosamines. Bacterial vaginosis, the vaginal discharge might have a role in the development of cervical intraepithelial neoplasia. There have been several studies on this but with inconsistent results (Salih *et al.*, 2017).

The common presenting complaints of the women having intraepithelial neoplasia or malignancy are abnormal vaginal discharge and postmenopausal bleeding (Bukhari *et al.*, 2012).

Pap test has 50-75% sensitivity and 98- 99% specificity to detect precancerous and cancerous lesions (Shaki *et al.*, 2018).

HPV is the primary etiologic agent of cervical cancer. HPV DNA test used in conjunction with a Pap test increases the sensitivity of Pap screening. Along with the detection of precancerous and cancerous lesions, Pap test also helps in the detection of non-neoplastic lesions (Sachan *et al.*, 2018).

Cytology reports by Bethesda system categorize epithelial cellular abnormality into squamous or glandular cells which are further classified according to the degrees of abnormality (Nayar and David, 2015).

As Pap test is an easy, simple, effective and versatile tool to detect precancerous and cancerous lesions of the cervix, it becomes an integral part of routine check-up in OPD as well as it helps in conducting Pap smear camps to screen large populations at risk (Sachan *et al.*, 2018).

We carried out this study to analyze the magnitude of the problem of precancerous and cancerous lesions of the cervix in women who underwent Pap test for cervical screening at our hospital which caters mostly to women in the rural area and with low socioeconomic status and thereby to improve the screening strategy to make it still more effective. In this study, we have done a correlation of cyst diagnosis with histopathological diagnosis as it is the gold standard to assess the efficacy of cytology screening. Clinical presentation was also studied. The results of this study will add some more information to the existing data.

MATERIALS AND METHODS

This was a descriptive, analytical and comparative study carried out in the department of pathology at tertiary care centre during a period from January

2018 to Dec 2018. A total of 430 Pap smears were studied.

Patient's socio-demographic and clinical details were obtained from the patient's datasheet. After taking the written informed consent from patients, by using conventional method sample was obtained on labelled microscopic slides by Gynecologist from the cervix using Ayre spatula and cervical cytobrush. Two smears were prepared from the sample from each patient and fixed in wet condition by immediately dipping it in a fixative which is a mixture of 50% ethanol and 50% ether in Coplin's jar for at least 20 min. After fixation, the smears were stained by Pap's stain and mounted by DPX mountant.

The Pap smears were reported according to standard Bethesda 2014 system for reporting cervical cytology smears (Nayar and David, 2015; Tangjitg-amol *et al.*, 2016).

Cervical biopsy for histopathology was available in 92 cases. Correlation of cytology with gold standard histopathology was done in these cases.

Diagnostic parameters were calculated using the formulae as— (Joshi *et al.*, 2015; Warpe *et al.*, 2016).

Sensitivity = $TP/TP+FN$,

Specificity = $TN/TN+FP$,

Positive predictive value (PPV) = $TP/TP+FP$,

Negative Predictive value (NPV) = $TN/TN+FN$,

Accuracy = $TP+TN/TP+TN+FP+FN$,

(T. P. - True positive, T. N. - True negative, F. P. - False positive, F. N. - False negative)

Statistical analysis

Significance of correlation was tested by calculating p-value. $P < 0.05$ was considered as statistically significant.

RESULTS

In the present study, Pap smears of a total of 430 cases were studied for age group, clinical presentation, cytological diagnosis and its correlation with histopathology.

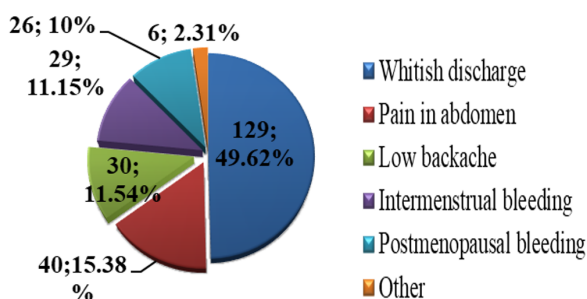
Of the total 430 smears, 24 (5.58%) were inadequate to report by Bethesda criteria, therefore not studied further. The reasons for inadequacy were either sparse cell population, > 75% of the smeared area obscured by inflammatory exudate, mucin or blood.

Of the total 406 cases, 260 (64.04%) cases were symptomatic, and 146 (35.96%) were asymptomatic.

In the symptomatic group of 260 cases, the most common complaint was whitish discharge per vaginam

Graph 1 shows the clinical presentation of the symptomatic group of 260 cases. The most common complaint was whitish discharge per vaginam in 129 (49.62%) of 260 cases, followed by pain in abdomen in 40 (15.38%), low backache in 30 (11.54%), intermenstrual bleeding in 29 (11.15%), postmenopausal bleeding in 26 (10%) and other complaints like mild fever, weakness, decreased appetite, loss of weight etc. in 6 (2.31%) cases.

Maximum cases were of NILM, and minimum cases were of ASC-H. (NILM -Negative for intraepithelial lesion or malignancy, ASC-US - Atypical Squamous Cells of Undetermined Significance, ASC-H- Atypical Squamous Cells cannot exclude HSIL, LSIL- Low-grade Squamous Intraepithelial Lesion, HSIL -High-grade Squamous Intraepithelial lesion, IC- invasive cancer.)

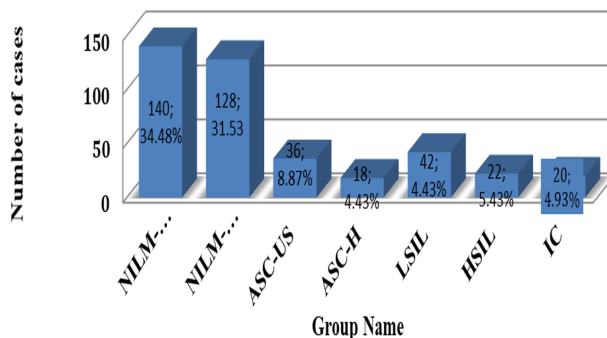


Graph 1: Clinical presentation of symptomatic patients

The above Graph 2 maximum cases were of NILM category, i.e. 268 (66%) of the total 406 cases and the total NILM cases 140 (52.23% of 268) cases were normal (unremarkable), and 128 (47.76%) cases were inflammatory.

The mean age for invasive carcinoma category was high compared to other categories.

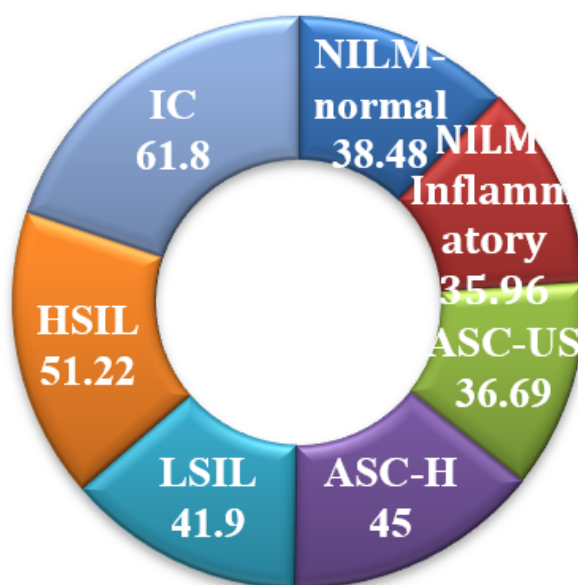
From Graph 3 of the total 406 cases, a total of 268 (66%) were of NILM cases including inflammatory and 138 (34%) cases were showing epithelial atypia. Among the 268 NILM cases 140 (55.24% of 268 and 34.48% of 406) were of normal (unremarkable) having an age range of 22 - 65 with a mean age of 38.48 years, 128 (47.76% of 268 and 31.53% of 406) cases of NILM- inflammatory with an age range of 22 - 60 years and mean age of 35.96 years, 36 (8.86%) cases of ASC-US with an age range of 25 - 54 years and mean age of 36.69 years, 18 (4.43%) cases of ASC-H with age range 36 - 65 years and mean age of 45 years, 42 (10.34%) cases of LSIL with



Graph 2: Group wise distribution of number of cases

age range 30 - 71 and mean age of 41.90, 22 (5.42%) cases of HSIL with an age range of 28-65 years and mean age of 51.22 years, and 20 (4.92%) cases of IC (invasive cancer) with an age range of 45 -80 years and mean age of 61.8 years. The mean age for invasive carcinoma category was high compared to other categories.

Amongst the inflammatory smears, 4 cases were positive for candidal infection, 2 cases were with trichomonas vaginalis, 20 cases were of atrophy with inflammation, 29 cases were of inflammation with cells with koilocytic change suggestive of HPV effect showing no atypia, 5 cases were of cervical erosion, 2 cases of cytopathic changes suggestive of HSV, remaining 66 of the inflammatory smears were of non-specific inflammation.



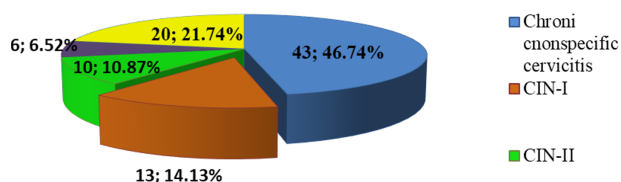
Graph 3: Mean age wise distribution of Groups

Maximum cases were of chronic non-specific cervicitis, and minimum cases were of CIN-III (CIN- cer-

vical intraepithelial neoplasia)

As in Graph 4 biopsy was available in a total of 92 cases. On histopathology of these 92 cases, 43 (46.74%) cases showed chronic non-specific cervicitis, 13 (14.13%) cases showed CIN-I, 10 (10.87%) showed CIN-II, 6 (6.52%) showed CIN-III and 20 (21.74%) showed invasive carcinoma.

In the case of carcinoma, all the cases (Asotic et al., 2014) showed the same result on both cytology and histopathology.



Graph 4: Histopathology Diagnosis

As in Table 1 biopsy was available in 46 cases of inflammatory smears, out of which 41 cases showed chronic cervicitis, four cases turned out to be CIN-I, and one case was CIN-II. A biopsy was available in 6 cases of ASCUS diagnosed cytologically, which showed one case as chronic cervicitis, 3 cases as CIN-I and 2 cases as CIN-II. In the case of ASC-H biopsy was available in 5 cases which showed CIN-I in 1 case, CIN-II in 1 case, CIN-III in 2 cases and carcinoma in 1 case. In LSIL, a biopsy was available in 8 cases which showed chronic cervicitis in 1 case, CIN-I in 4 cases, CIN-II in 2 cases and CIN-III in 1 case. Amongst HSIL cases biopsy was available in 10 cases which showed histopathological diagnosis as 1 case of CIN-I, 4 cases of CIN-II, 3 cases of CIN-III and 2 cases of carcinoma. In the case of IC, group biopsy was available in 17 cases, all of which showed invasive carcinomas histopathologically.

In the inflammatory group, 29 cases diagnosed on cytology as chronic inflammation with koilocytic change without cytological atypia showed on histopathology as koilocytic hyperplasia with chronic non-specific cervicitis.

Total invasive carcinomas reported on histopathology were 20. Of these, 17 were reported as squamous cell carcinoma, one case as well-differentiated invasive villoglandular papillary adenocarcinoma, one case as invasive papillary squamous-transitional cell carcinoma and one case as high-grade malignant tumour –small cell carcinoma.

From Table 2, of the 92 biopsy available cases, 46 cases showed a positive result, and the other 46 showed negative diagnoses histopathologically. By

Comparing cytology with histopathology and considering the histopathology as the gold standard for diagnosis, the cytology showed 44 true positives, 2 false positive, 5 false negative and 41 true negatives.

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100 = \frac{44}{44+5} = \frac{44}{49} \times 100 = 89.79\%$$

$$\text{Specificity} = \frac{TN}{TN+FP} = \frac{41}{41+2} = \frac{41}{43} \times 100 = 95.34\%$$

$$\text{Positive predictive value (PPV)} = \frac{TP}{TP+FP} = \frac{44}{44+2} = \frac{44}{46} \times 100 = 95.65\%$$

$$\text{Negative Predictive value (NPV)} = \frac{TN}{TN+FN} = \frac{41}{41+5} = \frac{41}{46} \times 100 = 89.13\%$$

$$\text{Accuracy} = \frac{TP+TN}{TP+TN+FP+FN} = \frac{44+41}{44+41+2+5} = \frac{85}{102} \times 83.33\%$$

DISCUSSION

Cervical cancer incidence is decreasing markedly in developed countries compared to developing because of organized, effective Pap screening camps with increased awareness in the female population. So the Cervix cancer is only cancer, the incidence and mortality of which is reduced markedly without medical intervention (Asotic et al., 2014). In the developed countries, the incidence of cervical cancer and mortality is reduced by up to 80% due to a population-based cervical cancer screening program by Pap smear testing every 3-4 years. In a developing country like India also cervix cancer is on the declining trend because of increase in population-based Pap screening programs (Shaki et al., 2018).

Though Pap screening has become a standard technique to diagnose the lesions on cytology, definitive diagnosis is always made by histopathology as a gold standard (Asotic et al., 2014; Mainali et al., 2018). With cytology, false positive and false negatives are there at the rate of 10-35% (Asotic et al., 2014).

We conducted this study to categorize the cytological Pap smears by Bethesda 2014 system of reporting cervical cytology and to study the correlation with clinical features and histopathology thus to assess the efficacy of cytology to diagnose the lesions.

We studied 430 Pap smears that were collected from patients with different Gynaecological complaints as well from women coming for routine preventive pap screening in Gynaecology OPD and Pap smear camps conducted in rural areas.

In this study, 24 (5.58%) smears were inadequate to report. In this category, the smears included were those who were scanty in cell population according to Bethesda system 2014, smears obscured by

Table 1: Correlation between cytological with histopathological diagnosis of the same

Cytology diagnosis (n)	Histopathological diagnosis					P value
	Chronic cervicits n (%)	CIN-I n (%)	CIN-II n(%)	CIN-III n (%)	IC n (%)	
Inflammatory(46)	41(95.35)	4 (30.77)	1 (10)	0 (0)	0 (0)	0.00
ASC-US (6)	1 (2.33)	3 (23.07)	2 (20)	0 (0)	0 (0)	
ASC-H (5)	0 (0)	1 (7.7)	1 (10)	2 (33.33)	1 (5)	
LSIL (8)	1 (2.33)	4(30.77)	2 (20)	1 (16.67)	0 (0)	
HSIL (10)	0 (0)	1 (7.7)	4 (40)	3 (50)	2 (10)	
IC (17)	0 (0)	0 (0)	0 (0)	0 (0)	17 (85)	
Total (92)	43	13	10	6	20	

Table 2: Pap smear and histopathological diagnosis chart for calculating diagnostic parameters

Cytology	Histopathology		Total
	Positive	Negative	
Positive	T. P. 44	F. P. 2	46
Negative	F. N. 5	T. N. 41	46

inflammatory exudates, blood or mucin >75% area. Our result was comparable to result by [Bamanikar et al. \(2016\)](#) and [Sachan et al. \(2018\)](#) who reported the inadequacy rate as 5.99% and 6.42% respectively. In contrast, the inadequacy rate by [Kothari et al. \(2014\)](#) was low- 1.09% and that by [Warpe et al. \(2016\)](#) was little higher than us - 7.43%. Our little higher rate might be attributed to the majority of the smears collected in camp, and as these camps were in rural areas where facilities for sample collection might not be that much satisfactory as required.

Unsatisfactory smears (24 cases) were not studied further. Of the remaining total, 406 cases 260 (64.04%) cases were symptomatic, and 146 (35.96%) cases were asymptomatic. These asymptomatic cases were those that had come in Pap smear camps conducted in rural areas and some cases that came in the hospital for routine Pap screening. Our findings were comparable with findings by [Sachan et al. \(2018\)](#); [Bamanikar et al. \(2016\)](#); [Ramadevi et al. \(2017\)](#), in their study they observed that these symptoms were associated with CIN and cervical cancer. In our study, we also observed similar findings.

Our findings were comparable with findings by [Warpe et al. \(2016\)](#) who reported 75.14% of total NILM out of which 61.22% were NILM-normal, i.e. without inflammation and 38.77% cases were NILM- inflammatory. In a similar study by NILM - standard cases were 26.53%, and NILM - inflamma-

tory were 60.13 %. More number of normal cases in our study might be attributed to cases that had come to the hospital as well as in Pap smear camps voluntarily, having no complaint just for routine Pap smear screening.

Of the total 128 inflammatory smears, 4 (3.13%) cases were positive for candidal infection, 2 (1.56%) cases were with trichomonas vaginalis, 20 (15.62%) cases were of atrophy with inflammation, 29 (22.65%) cases were of inflammation with cells with koilocytic change suggestive of HPV effect showing no atypia, 5 (3.90%) cases were of cervical erosion 2 (1.56%) cases with cytopathic effect suggestive of HSV infection. Remaining 66 (51.56%) of the inflammatory smears were of chronic non-specific inflammation. Our findings were comparable to that by [Warpe et al. \(2016\)](#). In their study they found chronic non-specific inflammation in 61.2%, Candida infection in 0.8%, Trichomonas vaginalis in 7.35%, bacterial vaginosis in 12.24%, mixed infection in 5.3%, other mixed infection 3.67% and increased lactobacilli 3.23%. Concerning HPV infection and HSV infection, our findings are comparable with that of [Rana et al. \(2013\)](#). Significant proportion of cases of Trichomonas vaginalis infection are associated with ASCUS. Pap smear examination is a valuable tool in the detection of cervical infections as well ([Rana et al., 2013](#)). Some studies have reported that persistent chronic infection if not treated in time it

can increase the possibility of getting intraepithelial lesion in that individual. A repeat Pap smear is advisable after appropriate treatment in such cases (Sachan *et al.*, 2018).

In the inflammatory group, 29 cases diagnosed on cytology as chronic inflammation with koilocytic change without cytological atypia showed on histopathology as koilocytic hyperplasia with chronic non-specific cervicitis. Our finding was comparable with findings by Rana *et al.* (2013). HPV effect on cytology range from koilocytosis without atypia to HSIL with which the risk of progression to cancer is there. For this transformation to take place, it takes years. Therefore, at this stage, cytology diagnosis by Pap smear has a vital role in detecting the precancerous and cancerous lesions in time (Rana *et al.*, 2013).

In our study, epithelial abnormality (cytological atypia) was found in 138 (34%) of the total cases of 406. Tiwari *et al.* (2011) found 40% smears with abnormal findings. In their study, they studied perceptions and concerns of women undergoing Pap smear examination. They concluded that factors such as poor awareness, shyness, poor hygiene and old age could be responsible for abnormal Pap smears, and this needs special attention in cancer prevention activities of the government. Another study by Shuaibu *et al.* (2018) carried out to study Pap smear awareness, utilization and pattern of cervical intraepithelial neoplasia revealed that there is low awareness and utilization of Pap screening in the population, therefore, there is a need to address factors such as poverty and illiteracy which predispose women to cervical cancer due to their lack of awareness of screening tests and this highlights the importance of girl child education.

In the present study, LSIL cases were more, and ASCH cases were least in number whereas in a study by Kabaca *et al.* (2013). ASCUS cases were more in number and IC cases were least in number. Mean age was highest in IC group patients in our study as well as in the study by Kabaca *et al.* (2013). Mean ages in other remaining categories were also comparable with that in the study by Kabaca *et al.* (2013) the out of total 406 cases of Pap smear, 92 (22.66%) underwent cervical biopsy for further diagnosis on histopathology as the cervical biopsy is the gold standard for detection of carcinoma cervix provided biopsy must be obtained from representative area. In similar studies by Warpe *et al.* (2016); Bamanikar *et al.* (2016), a biopsy was available in 17.77%, 5.27% and 3.13% cases respectively. Of these 92 cases, 43 (46.74%) cases showed chronic non-specific cervicitis, 13 (14.13%)

cases showed CIN-I, 10 (10.87%) showed CIN-II, 6 (6.52%) showed CIN-III and 20 (21.74%) showed invasive carcinoma.

There was a significant correlation/association between cytological diagnosis on Pap smear and histopathology of the same. In our study, sensitivity, specificity, and positive predictive value, negative predictive value and accuracy of Pap smear in diagnosing epithelial cell abnormality and malignancy were 89.79%, 95.34%, and 95.65%, 89.13% and 83.33% respectively.

Sensitivity and specificity of Pap smear can be increased by adopting proper technique and adequate sampling from the transformation zone. Various methods of Pap smear sampling, such as conventional and liquid-based, are used. In our study, Pap smears were sampled by a conventional method. Use of liquid-based cytology is advised to improve the sensitivity and specificity of Pap smear. Pap smear screening, coupled with HPV testing, will improve the overall detection rate of carcinoma cervix (Bamanikar *et al.*, 2016).

False-negative reports on cytology might be due to sampling error, non-representative sample, poor staining, misinterpretation by Cytopathologist (Lakshmi and Gouri, 2016; Ramadevi *et al.*, 2017).

CONCLUSIONS

Pap smear is a simple, rapid, economical, still effective screening test for cervical intraepithelial lesions and cervical cancer well before it can cause any harm to the patient thereby, it helps to reduce the morbidity and mortality associated with it. It also helps to decrease the incidence of cervical cancer. It is also useful in detecting non-neoplastic lesions as well. Because of its high sensitivity, it can be considered as an effective tool in the early detection of cervical cancer at least in the low resource area.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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