Original Research Article

Analysis of abnormal cervical cytology and its Clinicopathological correlation in Papanicolaou smears at tertiary care center - A prospective observational study

Lakshmi Salodia[1], Medhavi Sharma[2*], Rajrani Sharma[3]

[1,2]Senior Resident, [3]Senior Professor and HOD, Department of Obstetrics and Gynaecology, Pacific Meical College and Hospital, Udaipur, Rajasthan, INDIA.
Email: dr.lakshmi04@gmail.com

Abstract

Objective: To study the role of Pap smear in detecting premalignant and malignant lesions as well as non-neoplastic lesions of cervix. **Materials and Methods**: This prospective, observational study was conducted in the Department of Obstetrics and Gynecology, Pacific Medical College and Hospital, Udaipur, Rajasthan. A total of 840 patients were included in the study within the duration of one year period. Pap smears were taken from women between ages of 18 to 75 years presenting with different Gynaecological complaints and as a routine beyond the age of 45 years by using Ayres Spatula. Smears were reported as per the Bethesda system. **Results:** A total of 576 abnormal pap smears were found, with only 58cases reported to have epithelial cell abnormalities. In this study, ASCUS(Atypical squamous cells of undetermined significance) was most common with 41 cases followed by LSIL(Low grade squamous intraepithelial lesion) with11 cases than HSIL (High grade squamous intraepithelial lesion) with 4 cases. Squamous cell carcinoma diagnosed on 2 cases. **Conclusion:** Pap smear is a simple, cheap,safe and practical diagnostic tool for early detection of cervical cancer , so it should be established as a routine screening procedure

Key Words: Papsmear, cervical cancer, The Bethesda system.

[*]**Address for Correspondence:**
Dr. Medhavi Sharma, Senior Resident, Department of Obstetrics and Gynaecology, Pacific Meical College and Hospital, Udaipur, Rajasthan.
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INTRODUCTION

Cervical carcinoma is more common in developing countries than in developed ones, particularly in parts of Asia, South America and Africa.[1] Although worldwide cervical cancer rates have decreased dramatically with the increase in screening efforts, incidence and prevalence in developing countries remains high due to lack of screening programs, with approximately 80% of all cervical cancer deaths occurring in the developing world. [2] In India, the annual incidence of carcinoma of cervix is estimated to be 5,00,000 new cases per year. Carcinoma of cervix accounts for 25-50% of total cancers while for 86-90% of all genital cancers in Indian women.[3] Cervical infections are one of the commonly encountered problems in women of reproductive age group. They usually present with white discharge, foul smelling odor and pruritis.[4] Also vaginal discharge or leucorrhoea is the commonest complaint seen in women reporting to gynecological clinic. The common agents causing vaginitis include anaerobic bacteria leading to bacterial vaginosis (BV), vulvovaginal candidiasis and trichomonal vaginitis.[5,6] The mainstay of cervical cancer screening for the last 7 decades has been the Papanicolaou test. Most of these

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infections can be easily diagnosed on routine Pap smear examination. It is a simple test. The Papanicolaou test, also known as the Pap test or the Pap smear, was developed in the 1940's by Georgios Papanikolaou. It involves exfoliating cells from the transformation zone of the cervix to enable examination of these cells microscopically for detection of cancerous or precancerous lesions. The Pap test is indicated to screen for malignant and premalignant lesions of the cervix. The recommended age at initiation of cervical cancer screening has undergone significant revision over time as the natural history of HPV infection and subsequent cervical dysplasia has been elucidated. Although former guidelines recommended starting Pap smear screening at age 18 or the onset of sexual activity, these guidelines were revised in 2006 to recommend initiation 3 years after the onset of sexual activity or age 21, whichever comes first. In 2009, these were further revised to recommend that cervical cancer screening begin at age 21, regardless of sexual history. This recommendation was confirmed in 2012 and again in January 2016. [7]Pap smear screening has its own drawbacks. It has low sensitivity i.e. between 47-62%, low predictive value and the subjective interpretation of the results.[8] On the other hand, Pap smear testing also has strengths, such as wide acceptance, meeting most of the criteria for a good screening test in settings with adequate resources, obtaining a permanent record of the test in the form of a slide, and having a high specificity of 60-95%.[8,9]

M ATERIAL AND METHODS

This prospective, observational study was conducted in the Department of Obstetrics and Gynecology, Pacific Medical College and Hospital, Udaipur, Rajasthan. A total of 840 patients were included in the study within the duration of one year period. Pap smears were taken from women between ages of 18 to 75 years presenting with different Gynaecological complaints and as a routine beyond the age of 45 years by using Ayres Spatula. Smears were reported as per the Bethesda system.

Evaluation was done by Cytology using Bethesda Classification[9]

Within normal limits	Negative
Infection (specify organism)	Negative
Reactive/reparative changes	
Atypical squamous cells of undetermined significance (ASCUS) or	Atypical cells Atypical squamous cells of undetermined
Atypical glandular cells of undetermined significance (AGUS)	HPV atypia
Low grade squamous intraepithelial lesion (LSIL)	CIN I
High grade Squamous intraepithelial lesion(HSIL)	CIN II, CIN III
Invasive carcinoma	Invasive carcinoma

Inclusion criteria

- Women between 18 to 75 years of age with sexual history.

Exclusion criteria

- Women below 18 years.
- Women without sexual exposure.
- Women above 75 years.

Procedure

Paps smears were taken by using an Ayres spatula and cervical brush after exposing the cervix by a Cusco's speculum. The broad end of spatula was placed on the cervix and rotated through 360˚ and the collected material was spread over a glass slide. The oblong relabelled narrow end of spatula was used to take smear from posterior vaginal fornix and spreaded over a second glass slide. The endocervical sample was collected using a cytobrush and was spread over labelled third glass slide. The samples were gently smeared on pre-labelled glass slides. The glass slides were fixed by mixture of equal amount of ether and 95% ethanol, and by fixating spray from out-reach camp. The slides were sent to the pathology department for cytological study. Cytotechnologist stains the slides with Papanicolaou stain. Each slide was then meticulously examined by pathologist. All the findings were recorded in the database and classified according to the 2001 Bethesda System of reporting Pap smear cytology. Analyzable data from cytopathology database were analysed using SPSS 16.0 version. If on examination, multiple small coccobacilli with conspicuous absence of lactobacilli were found, then the smear was reported as positive for bacterial vaginosis. While taking Pap smear a simultaneous wet film examination was also performed. For this, cervical smear was taken with Ayre's spatula and spread on clean glass slide. A drop of normal saline was put on it and covered with cover slip, followed by examination first under low power and then under high power for identification of Trichomonas vaginalis (motile and flagellated), Candida (branched or pseudo- hyphae) and viruses (HPV).

RESULTS AND OBSERVATIONS

In our study we analysed 830 pap smears taken from women presenting to Gynecology OPD of Pacific medical college and hospital, Udaipur, Rajasthan. Women presenting with different Gynecological complaints between 18 to 75 and as routine beyond the age of 45 years. In our study we have taken 830 women, in them 15 were less than 20 years, 234 women were between 21 to 30 years, 313 women were between 31 to 40 years, women between 51 to 60 years were 41, 23 women were between 61 to 70 years and 4 women were above 65 years. (Table 1)

Table 1: Age distribution of patient

Age (years)	No.of patient	Percentage
<20years	15	1.807%
21-30 years	234	28.19%
31-40years	313	37.71%
41-50years	200	24.09%
51-60 years	41	4.93%
61-70 years	23	2.77%
>70yrs	4	0.48%

Table 2 shows that patients who attended OPD had come with various complaints like lower abdominal pain (44.56%), white discharge (57.83%), bleeding per vagina (13.49%), post coital bleeding (3.73%) and post menopausal bleeding (1.32%). Some patients had single complaints and some patients had multiple complaints.

Table 2: Distribution of symptoms amongst patients

Symptoms	No.of patient	Percentage
White discharge per vaginum	480	57.83%
Menstrual irregularities	112	13.49%
Post coital bleeding	31	3.73%
Lower abdominal pain	370	44.56%
Post menopausal bleeding	11	1.325%

Table 3 shows distribution of patients according to vaginal examination. Out of 830 patients 156 (18.79%) were having no obvious abnormal pathology in cervix on per speculum examination. Rest of the patients had vaginitis (8.31%), nabothian follicles (6.75%), cervical erosion (43.01%), ectropion (1.33%), senile vaginitis (0.96%), bleeding on touch (1.81%) and cervical polyp (2.04%) like cervical changes present.

Table 3: Distribution of patients according to vaginal examination

Findings	No.of patient	Percentage
Cervical erosion	357	43.01%
Vaginitis	69	8.31%
Ectropion	11	1.33%
Senile vaginitis	08	0.96%
Bleed on touch	15	1.81%
Nabothian follicles	56	6.75%
Cervical polyp	17	2.04%

Table 4 shows cervical cytology findings There were abnormal Pap smears (epithelial cell abnormalities as well as benign cellular changes of inflammation, repair, etc.), with normal cases. Microscopy of inflammatory smears showed bacterial vaginosis, specific infections like trichomoniasis, candidiasis, herpes zoster and other non specific infections.

Table 4: Cervical cytology findings

Cervical cytology findings	No.of cases	Percentage(%)
Negative for intraepithilial lesions	732	88.19%
Normal	156	18.79%
Inflammatory	576	69.39%
Infections		
Candidiasis	52	6.26%
Trichomoniasis	20	2.41%
Bacterial vaginosis	23	2.77%
Herpes zoster	01	0.12%
Atrophic smear	21	2.53%

Table 5 shows Epithelial cell abnormalities and were categorized according to revised Bethesda system 2001, 11(1.33%) smears showing LSIL and 41(4.94%) showing ASCUS (Atypical squamous cells of undetermined significance) representing maximum number among the screening. Abnormal cell has enlarged, hyperchromatic nuclei and altered N:C ratio. Cells of LSIL(Low grade squamous intraepithelial lesion)has perinuclear clearing which is termed as koilocytes. HSIL (High grade squamous intraepithelial lesion) reported in 04 (0.48%) patients. Squamous cell carcinoma clear cut diagnosed on 02(0.24%) clinical cases.

Table 5: Epithilial cell abnormalities

Diagnosis	No. of cases	Percentage
ASCUS(3a)	41	4.94%
LSIL(3b)	11	1.33%
HSIL(4)	04	0.48%
SCC(in ca)	02	0.24%
Total	**58**	

Table 6 shows Epithelial cell abnormalities which are classified on the basis of age. 225 patients (27.10%) of age group 21-30 yrs, 293 patients (35.30%) of 31-40 yrs, 192 patients (23.13%) of 41-50 yrs , 41 patients (4.93%) of 51-60 yrs and 17 patients (2.04%) of age group 61-70 yrs. Most of the pap smear detected ASCUS and LSIL. Pap Smear Screening show that Pap smear screening is very important to detect pre-cancerous cell in cervix.

Table 6: Age distribution pattern in different types of lesion

Age (yrs.)	Inflammatory smears (IL)	ASCUS	LSIL	HSIL	SCC	Total
21-30	217	06	02	00	00	225(27.10%)
31-40	277	15	01	00	00	293(35.30%)
41-50	175	11	06	00	00	192(23.13%)
51-60	027	09	02	03	01	041(4.93%)
61-70	015	00	00	01	01	017(2.04%)
Total	711	41	11	04	02	
Percentage						

DISCUSSION

In our study we analysed 830 pap smears taken from women presenting to Gynecology OPD of Pacific medical college and hospital, Udaipur, Rajasthan. Women presenting with different gynecological complaints between 18 to 75 and as routine beyond the age of 45 years. In our study we have taken 830 women, in them 15 were less than 20 years, 234 women were between 21 to 30 years, 313 women were between 31 to 40 years, women between 51 to 60 years were 41, 23 women were between 61 to 70 years and 4 women were above 65 years (Table 1) . Whereas, Sharma *et al*[10] studied abnormal smears were seen mainly among the age group more than 21 and less than 65 years of age. In a study done by Pradhan[11] in 800 samples, the abnormal smears were seen mainly among age group of 21-40 years and carcinoma was also found mainly in this group. Lakshmi *et al*[12] in their study found that maximum number of women were between 45 to 55 years age group (34%). In study conducted by Sunita *et al*[13] maximum number of women were between 31 to 40 years age group (32.68%). In study conducted by Mandakini *et al*[14] between 15 to 30 years maximum number of women were studied. Table 2 in our study shows that patients who attended OPD had come with various complaints like lower abdominal pain (44.56%), white discharge (57.83%), bleeding per vagina (13.49%), post coital bleeding (3.73%) and post menopausal bleeding (1.32%). Some patients had single complaints and some patients had multiple complaints. The most common clinical presentation in all cases in study conducted by Sabina *et al*[15] were lower abdominal pain 763(76.3%) followed by abnormal vaginal bleeding 352(35.2%) and whitish discharge per vagina 391(39.1%). Moghal[16] in his study observed menorrhagia (41%) as the most common symptoms. Table 3 in our study shows distribution of patients according to vaginal examination. Out of 830 patients 156 (18.79%) were having no obvious abnormal pathology in cervix on per speculum examination. Rest of the patients had vaginitis (8.31%), nabothian follicles (6.75%), cervical erosion (43.01%), ectropion (1.33%), senile vaginitis (0.96%), bleeding on touch (1.81%) and cervical polyp (2.04%) like cervical changes present. Table 4 in our study shows cervical cytology findings. There were abnormal Pap smears (epithelial cell abnormalities as well as benign cellular changes of inflammation, repair,etc.), with normal cases. Microscopy of inflammatory smears showed bacterial vaginosis, specific infections like trichomoniasis, candidiasis, herpes zoster and other non specific infections. Lakshmi *et al*[12] found abnormal Pap smear reports which were 187(93.5%), whereas in study conducted by Sunita *et al*[13] 433(77.32%) reports were abnormal. In study conducted by Mandakini *et al*[14] abnormal Pap smear reports were 689(69.2%). In study conducted by Sabina *et al*[15] 106(10.6%) cases of abnormal epithelial lesions. The percentage of epithelial

abnormalities is 2.3%to 6.6% in USA, from 1.6% to 7.9% in the Middle East and 1.87% to 5.9% in India.[17] Inflammatory smear reports were 134(67%) in study conducted by Lakshmi *et al*12, whereas in study conducted by Sunita *et al*13 403(71.96%) reports were inflammatory and in study conducted by Mandakini *et al*[14] inflammatory Pap smear reports were 572(57.5%). In study done by Sabina *et al*[15], a total 1000 patients were included in the study. Predominance of inflammatory smears was noticed in 894 (89.4%) cases. Cronje *et al*[18] (2001) reported similar preponderance of inflammatory smears in their study. In another study conducted by Sharma S[19] (2000) higher frequency of inflammatory smears of 302 cases (45.3) was noted out of 667 cases. Microscopy of inflammatory smears in our study showed bacterial vaginosis, specific infections like trichomoniasis, candidiasis, herpes zoster and other non specific infections. The incidence of candidiasis in study by Naina *et al*[20] was 2.50% of all cases. Another similar study reported candidiasis in 11.16% of total population observed.[21] The overall incidence of infectious vaginitis on Pap smear in study by Naina *et al*[20] was 22.57% (1292/5725) as compared to 50.07% reported by a similar study.[21] They also found similar pattern of infections on Pap smear with bacterial vaginosis (68.78%) being most common followed by Trichomonas infection (20.05%) and candidiasis (11.07%) of total 1292 cases with infections on Pap smear. HPV infection related changes were observed in only two cases (0.15%). Another study also reported same pattern of infections with predominance of bacterial vaginosis on Pap smear.[21] In the study by Jha *et al*[22] five cases had bacterial vaginosis and in three cases it was candidiasis. In the study by Ranabhat *et al*[23] candidiasis, trichomonisis, bacterial vaginosis and herpes simplex infections were detected in nine cases (1%), four cases (0.45%), 67 cases (7.6%) and in three cases (0.34%) respectively unlike present study. In the study Shurbaji *et al*[24] Candida was identified in 309 (3.0%) of the 10,370 smears. Ninety-nine (72%) patients were asymptomatic, 29 (21%) had symptoms typical of candidasis, and nine (7%) had nonspecific symptoms.[24] In present study although the number of cases was not comparable to the study of Shurbati *et al*[24], however, the percentage of detection of candida in cervical smears was similar and patient was asymptomatic. Epithilial cell abnormality in the conducted by Kalpana singh *et al*[25] negative for intraepithelial lesions were found in 156(83.9%) and epithelial cell abnormality were seen in 30 (16.1%). Another study conducted by Sarma U *et al* (2012)[26] showed epithelial cell changes in 11.95% of cases. In study by Jha *et al*[22] 10% of the cervical smears showed epithelial cell abnormalities. In our study Table 5 shows epithelial cell abnormalities which were categorized according to revised Bethesda system 2001, 11(1.33%) smears showing LSIL and 41(4.94%) showing ASCUS (Atypical squamous cells of undetermined significance) representing maximum number among the screening. Abnormal cell has enlarged, hyperchromatic nuclei and altered N:C ratio. Cells of LSIL(Low grade squamous intraepithelial lesion) has perinuclear clearing which is termed as koilocytes. HSIL (High grade squamous intraepithelial lesion) reported in 04 (0.48%) patients. Squamous cell carcinoma clear cut diagnosed on 02(0.24%) clinical cases. Smears showing ASCUS (Atypical squamous cells of undetermined significance) were 5(2.5%) in study conducted by lakshmi *et al*[12]. In study conducted by Sunita *et al*[13] 13(2.3%) reports showed ASCUS and in study conducted by Mandakini *et al*[14] reports showing ASCUS were 41(4.1%). Sharma *et al*10 in their study found proportion of ASCUS (3.2%) is similar to the report given in Evidence Based Medicine (4%)27 and Vaghela *et al*28 (2.8%). In study conducted by lakshmi *et al*[12] smears show LSIL (Low grade squamous intraepithelial lesion) as [15](7.5%). In study conducted by Sunita *et al*[13] 11(1.9%) reports gave LSIL and in study conducted by Mandakini *et al*[14] reports showing LSIL were 41(0.1%). Lakshmi *et al*[12] found HSIL (High grade squamous intraepithelial lesion) from reports which were 12(6%), whereas in study conducted by Sunita *et al*[13] 2(0.3%) reports gave HSIL. In study conducted by Mandakini *et al*[14] HSIL reports were 1(0.1%). Smears shown in study conducted by Lakshmi *et al*[12] revealed squamous cell carcinoma amongst 2(1%). Whereas, study conducted by Sunita *et al*13 3(0.5%) reports gave squamous cell carcinoma and in study conducted by Mandakini *et al*[14] reports showing squamous cell carcinoma were 7(0.7%). Table 6 in our study shows epithelial cell abnormalities which are classified on the basis of age. 225 patients (27.10%) of age group 21-30 yrs, 293 patients (35.30%) of 31-40 yrs, 192 patients (23.13%) of 41-50 yrs , 41 patients (4.93%) of 51-60 yrs and 17 patients(2.04%) of age group 61-70 yrs. Most of the pap smear detected ASCUS and LSIL. Pap Smear Screening show that Pap smear screening is very important to detect pre-cancerous cell in cervix. Among the patients examined by the study conducted by Kalpana *et al*[25], 30-39 yrs age group 12(11.11%) showed LSIL and 10 (9.25%) ASCUS , 40-49 yrs age group 10 (9.25%) showing LSIL and 8 (7.4%) showed ASCUS in their smears. HSIL present in higher age group patients 45 to 60 years. Study conducted by Asotic A *et al* [29] (2014) showed the highest percentage of patients with LSIL and HSIL findings was in the age group under 29 yrs, not similar to our study. In another study done by Gupta *et al*[30] (2013), maximum cases 40.37% in age group of 30-39 yrs followed by 35.96% in age group of

20-29 yrs .LSIL found in largest no. 1.36% while HSIL found 0.91%11.

CONCLUSION

Cervical cancer is one of the most common malignancy in women of developing country. Pap smear is a simple, cheap, safe and practical diagnostic tool for early detection of cervical cancer, so it should be established as a routine screening procedure.

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