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## Original Research Article

# Study of cervical cytology in pap smears in a tertiary care hospital of North Maharashtra

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## ABSTRACT

**Background:** Cervical cancer is the fourth leading cause of death in females worldwide. In India cervical cancer is the leading cause of morbidity and mortality. Cancer of cervix is preventable, and can be diagnosed at the pre-malignant stage with adequate and repetitive cytological screening by Papanicolaou (Pap) smears. Aim of this study was to study the role of Pap smear in detecting premalignant and malignant lesions as well as non-neoplastic lesions of cervix.

**Methods:** It is a retrospective study of 240 pap smears studied from January 2022 to June 2022 and received in pathology department of a tertiary care hospital of north Maharashtra. Samples are collected from women between 21 to 65 years presenting with some gynecological problems. Smears were reported as per the 2014 Bethesda system.

**Results:** Out of 240 women, 216 were having normal cytology and 146 cases with inflammatory changes. 10 cases were unsatisfactory. 5 cases of ASCUS, 1 case of SCC, 2 cases of HSIL and 6 cases of LSIL were observed.

**Conclusions:** Pap smear test is a simple, safe, noninvasive, economical OPD based procedure to detect pervasive cervical epithelial lesions. Every woman should undergo Pap test at least once in her life before the age of 45 years.

**Keywords:** Cervical cancer, Pap smear, Screening

## INTRODUCTION

Cervical cancer is the fourth most common cancer and the fourth leading cause of cancer death among women worldwide. It is the second most common cancer in India with 1,23,907 new cases and 77,348 deaths per year.<sup>1</sup>

Screening a woman just one time in her life after the age of 35 decreases her risk of dying from cervical cancer by 70%. Her risk of dying from cervical cancer drops by more than 85% if she is screened every 5 years. However, more than 1.5 billion women worldwide have never been screened for cervical cancer.<sup>2</sup> As per Globocan 2020, in India, cervical cancer accounted for 9.4% of all cancers and 18.3% (123,907) of new cases.<sup>3</sup> About 70% of those

diagnosed with cervical cancer are at the advanced stages, with nearly 60,000 deaths each year.<sup>4</sup> 86% of all deaths due to cervical cancer are in developing countries, low- and middle-income countries.<sup>5</sup> According to ACS guidelines for cervical cancer screening, it is recommended that screening should begin in average-risk individuals at age 25 years and cease at age 65 years.<sup>6</sup>

ACOG recommends Pap smear screening starting at 21 years of age until the age of 65 years and should be repeated at 3 years interval. In addition, human papilloma virus (HPV) test may be performed for abnormal Pap smear tests or as dual testing. In case of abnormal Pap smear report, depending on the type of abnormality the test may need to be repeated in 6 to 12 months.<sup>7</sup>

The situation of cancer prevalence is alarming in rural population where the majority of women are illiterate and are ignorant about the factors that contribute to the development of cervical cancer. In addition, medical facilities, advice and awareness programmes are almost non-existent.<sup>8</sup>

A number of risk factors have been associated with cervical cancer, namely; illiteracy, low socioeconomic status, long duration of married life, early menarche, early marriage, early first childbirth, age at last child birth, multiparity, multiple sexual partners, late menopause, genital infection, poor genital hygiene, tobacco use, passive smoking and contraceptive use.<sup>9</sup>

### **Morphogenesis**

The ectocervix is covered by mature squamous mucosa, whereas the endocervix is lined by mucus-secreting endocervical epithelium. The latter undergoes squamous metaplasia. Most cervical squamous cell carcinomas likely originate from the metaplastic squamous epithelium located between the original and new squamocolumnar junctions, the transformation zone. Adenocarcinomas typically occur within the endocervical canal.<sup>10</sup>

### **Etiological factors**

Human papilloma virus (HPV) infection has traditionally been considered to be a necessary condition for the development of most types of squamous cell cervical carcinomas. 13 types of HPV with oncogenic risk have been identified. HPVs of high oncogenic risk are of the greatest importance in cervical carcinogenesis; more often in patients with cervical cancer, HPV types 16 and 18 are identified. Nevertheless, in recent years, there has been an increase in data indicating that some cervical tumors, mainly adenocarcinomas, are HPV-negative.<sup>11</sup>

The knowledge that the cancer is caused by a virus infection has also opened up the possibility of vaccination against the HPV virus. If such vaccination programs became globally comprehensive, the prevalence of the cancer could be decreased to the level where screening would be no longer necessary.<sup>12</sup>

Cervical cancer screening cytology- choices available i) Cytology: Cervical cytology is based on the examination of cells obtained from cervical transformation zone for any cellular and nuclear abnormality, ii) HPV testing: Primary HPV testing is increasingly used as the standard test for cervical cancer screening worldwide. It can also be done as a reflex test following abnormal cytology report (ASCUS or higher) and can be used as co-test where HPV testing and cytology are done simultaneously and management decision is based on the combined report, iii) Co-testing: Co-testing is combined testing with both cytology and HPV testing, iv) Visual inspection tests: A satisfactory alternative to cytology is visual inspection tests with 3-5 per cent acetic acid and/or Lugol's iodine.

The abnormal area appears to be dense acetowhite and Lugol's negative, v) New strategies- High risk (HR) HPV E6/E7 mRNA test, tests for DNA integration, genome mutation and DNA methylation.<sup>1</sup>

The papanicolaou test also known as Pap test is a screening method used to detect potentially precancerous and cancerous processes in the cervix.<sup>13</sup>

Pap stain was first developed by Dr GN Papanicolaou in 1947. The stain has undergone various modifications from regressive conventional method to progressive rapid Pap staining where the time taken for staining has reduced. Further the stain was modified as ultrafast Pap stain and modified ultrafast Pap stain. Later the other modifications were Enviro-Pap stain, REAP stain and cytocolor. Each method has its own advantages and disadvantages.<sup>14</sup>

Reporting of pap smears using revised Bethesda System has unified various overlapping terminologies and created a standardized framework for laboratory reports.<sup>15</sup>

Hence, we have undertaken the present study using the revised Bethesda system 2014, with the intention to estimate the prevalence of cervical epithelial abnormalities.

## **METHODS**

The study is a six month retrospective observational study conducted in Department of Pathology of a private medical college of north Maharashtra to evaluate all the pap smears reported during January 2022 to June 2022.

As this was a retrospective study, no separate informed consent was required and the study was approved by Institutional Ethics Committee.

### **Sampling methods and sample collection**

The patients were instructed by the gynecologists prior to the procedure to avoid coitus, use of local douching and antiseptics before the cytological examination. Patients were placed in the lithotomy position, and a sterile bivalve speculum was inserted into the vagina.

The posterior vaginal wall was retracted posteriorly and the anterior vaginal wall anteriorly to allow proper visualization of the cervix and vaginal wall. After per speculum examination of the patient, the longer projection of Ayre's spatula was inserted in cervix near squamocolumnar junction and rotated through 360°. The cytological smears were taken by gynecologists for routine screening by conventional method.

All the slides were labelled immediately and dipped in 95% ethyl alcohol jars. Pap staining was done by trained cytotechnologists followed by light microscopy and slide interpretation by cytopathologists.

### Inclusion criteria

All sexually active women coming to gynecology department in the age group from 21 to 65 years with the complaints of vaginal discharge, intermenstrual bleeding, postmenopausal bleeding, abdominal pain, irregular menses and something coming out of vagina and who consented for Pap smear test were included in the study.

### Exclusion criteria

Women aged less than 20 years, pregnant females, previous history of cervical cancer treatment, women without sexual history, women who have undergone hysterectomy, who have used local antiseptic, women with menstrual bleeding, cervical growths and who were not willing to do the Pap test were all excluded from the study.

The conditions interfere with cytological examination are improper fixation or drying of a smear before fixation, failure to obtain adequate cellular sample, excessive use of lubricating jelly on the vaginal speculum, excessive mucus, blood, or purulent exudates.

### Statistical analysis

Data was entered in Excel sheet and using Microsoft word.

## RESULTS

This study was conducted at a tertiary care hospital in north Maharashtra. Study includes participation of 240 women. Demographic data like age, marital status, education, parity and contraception method usage of all the study subjects was noted and tabulated in Table 1.

Highest number of participants (79-32.91%) were in the age group of 31-40 years, followed by 41-50 years (72-30%) and least number of participants (18-7.5%) were above 60 years age. The mean age of the study population is 38.9 years.

Out of 240 women, 208 (86.7%) were parous and 32 women (13.33%) were nulliparous. Of all the study participants 104 women (43.33%) had no formal basic education, 70 (29.16%) had primary school education, 26 (10.83%) had completed higher secondary education and 40 (16.66%) women were graduates and above.

All the participants were married with a monogamous relationship. The most common family planning method was barrier method used by 133 (55.41%) women while 82 (34.16 %) were not using any form of family planning methods. Most of the women were of low socioeconomic strata and none of them gave history of smoking or tobacco use in any form.

None of the women who participated in the study had Pap smear testing earlier in their life. 40 women (16.67%) knew that there are tests available that can detect the

cancer of the cervix. But none knew about the test that can detect the precancerous lesions. In our study 93 (38.75%) had vaginal discharge, 38 (15.83%) had irregular menses, 37 (15.41%) had complain of something coming out of vagina, 30 (12.5%) had pain in abdomen, 15 (6.25%) were asymptomatic, 10 (4.16%) had post menopausal bleeding, 09 (3.75%) had urinary problems. 04 (1.67%) had post coital bleeding and 04 (1.67%) had dyspareunia.

**Table 1: Socio demographic data of the study participants.**

Socio-demographic characteristics	Number	Percentage
<b>Age group (years)</b>	21-30	40
	31-40	79
	41-50	72
	51-60	31
	Above 60	18
<b>Parity distribution</b>	Nulliparous	32
	Primipara	58
	Multipara	150
<b>Education level</b>	Uneducated	104
	Matric	70
	Higher secondary	26
	Graduate	40
<b>Marital status</b>	Married	240
	Unmarried	-
<b>Contra-ception usage</b>	None	82
	Barrier	133
	Tubal ligation	18
	OCP	07

All the participants were categorized into different groups depending on their age. Group 1 consists of participants aged between 21-30 years, group 2 consists of participants aged between 31-40 years, Group 3 consists of participants ranged between 41-50 years, Group 4 consists of 51-60 years and Group 5 consist of females above 60 years age.

**Table 2: Chief complaints of the study participants.**

Chief complaint	Number	Percentage
<b>White discharge</b>	93	38.75
<b>Irregular menses</b>	38	15.83
<b>Something coming out of vagina</b>	37	15.41
<b>Pain in abdomen</b>	30	12.5
<b>Asymptomatic</b>	15	6.25
<b>Post menopausal bleeding</b>	10	4.16
<b>Urinary problems</b>	09	3.75
<b>Dyspareunia</b>	04	1.67
<b>Post coital bleeding</b>	04	1.67
<b>Total</b>	240	100

**Table 3: Per speculum findings of the study participants.**

Per speculum findings	Number	%
Normal healthy looking cervix	101	42.08
White discharge	82	34.16
UV prolapse	34	14.16
Cervical erosion	16	6.67
Congested hypertrophied cervix	04	1.67
Friable with bleed on touch	03	1.25
Total	240	100

**Table 4: Spectrum of cytodiagnosis on pap smear reporting by Bethesda system 2014 of the study participants.**

Pap smear diagnosis	Number	Percentage
Unsatisfactory	10	4.17
NILM	216	90
A) inflammatory	146	60.83
A. Non specific	128	53.34
B. Candida	13	5.41
C. Bacterial vaginosis	14	5.83
B) atrophic smear	10	4.17
C) No other changes	60	25
Epithelial cell abnormalities	14	5.83
A) ASCUS	05	2.17
B) LSIL	06	2.60
C) HSIL	02	0.86
D) SCC	01	0.43

**Table 5: Cervical epithelial cell abnormalities in relation to age of the study participants.**

Age group (years)	ASCUS (%)	LSIL (%)	HSIL (%)	SCC (%)	%
21-30	-	-	-	-	-
31-40	-	-	-	-	-
41-50	03	04	-	-	50.0
51-60	01	02	01	-	28.57
>60	04	00	01	01	21.42
Total	05 (35.71)	06 (42.85)	02 (14.28)	01 (7.14)	14 (100)

On per-speculum examination 101 women (42.08%) had normal healthy looking cervix, 82 (34.16%) had white discharge, 34 (14.16%) had prolapse, 16 (6.67%) had cervical erosion, 04 (1.67%) had congested and hypertrophied cervix, 03 (1.25%) had friable cervix which bled on touch.

Out of 240 cases, 10 cases (4.17 %) were unsatisfactory for evaluation, 216 cases (90%) were reported as negative for intraepithelial lesion/malignancy and 14 cases (5.83%) had epithelial cell abnormality. In cases with negative for intraepithelial lesions, 146 cases (60.83%) were

inflammatory, 60 cases (25%) showed no other changes and 10 cases (4.17%) were atrophic smears. In inflammatory cases 27 cases (11.25%) showed presence of microorganisms. Among 14 of intraepithelial lesions - 05 (2.17%) were ASCUS, 06 cases (2.60%) were LSIL, 2 cases (0.86%) were HSIL and one case (0.43%) was of squamous cell carcinoma cervix. Most common age group showing epithelial cell abnormality was above 60 years of age. Out of 05 cases (2.17%) of ASCUS, 03 were in the age group of 41-50 years, 06 cases (2.60%) of LSIL were maximum in the age range 41-50 yrs. Both cases of HSIL and single case of squamous cell carcinoma cervix was above 60 years of age. The most frequent epithelial abnormality was LSIL. The mean age of ASCUS was 45 years, LSIL was 43 years, 58 years for HSIL and 62 years for carcinoma cervix. Nearly half of the patients with abnormal pap smears presented with a normal looking cervix.

## DISCUSSION

Cancers of uterine cervix is one of the leading malignancies in Indian females cancer cervix is considered to be an ideal gynaecological malignancy for screening as it has a long latent phase which precede the invasive disease.<sup>16,17</sup>

It is a well-known fact that the burden of cervical cancer has been reduced dramatically after the introduction of screening programmes.<sup>15</sup> In order to detect the efficiency of the screening tests and to plan strategic programmes, objective data are required from various hospital studies. Here lies the importance of our study, conducted in a tertiary care hospital of North Maharashtra, where there are limited published data concerning the pattern of epithelial cell abnormality in Pap smears.

The mean age of the participants was 36.4±6.29 years. It is thought that the average age of women population in study was appropriate considering the fact that the common age to develop cervical cancer is between 40 and 50 years and its precursor lesion usually occurs 5-10 years earlier. It is recommended that the women should have at least one smear test before the age of 45 years. The women who had their first smear after age of 45 years might miss the chance of cancer prevention all together.<sup>5</sup>

In our study majority of patients were in the third decade (32.91%) followed by fourth decade (30%). Verma et al (2016), Vedvathi et al (2019) and Vijaya et al (2021) also had similar findings in their studies.<sup>5,18,19</sup> This reveals that Pap smear cytology is still delayed by many years in majority of women of this country. The physicians or healthcare professionals should request Pap smear testing and should educate people about the benefits of the Pap smear test.

In our study most of the women had Pap test for the first time in their life and none of the women knew that cervical cancer can be detected in precancerous state by Pap test.



An effective population screening method in community settings has to be embraced extensively to increase the detection of cervical cancer in precancerous stage.

Majority of the women in our study were multiparous and uneducated. A study by Abraham et al reported that women with better education and easy accessibility to healthcare services were more likely to seek treatment for gynecological symptoms at initial stages and hence, show reduced prevalence of gynecological morbidities.<sup>20</sup> Our study participants constituted of a vulnerable population with low literacy level, low socio-economic status and distinct socio-cultural practices such as early age at marriage and pregnancy. This might also have contributed to the higher proportion of symptoms suggestive of gynecological morbidities among these women.

The most common presenting complaint in our study was white discharge followed by irregular menses. Manjit et al (2012), Pushplata et al (2018) and Singh et al (2018) also recorded white discharge as the most common presenting complain in their respective studies.<sup>21,22,17</sup>

On per speculum examination majority of the women had a normal looking healthy cervix (42.08%) followed by white discharge (34.16%). This correlated with the study of Umarani et al (2015).<sup>23</sup> However study done by Laxmi et al (2020), Pushplata et al (2018) and Vedvathi et al (2019) had discharge as the most common per speculum

finding.<sup>24,22,18</sup> The least common finding in our study was bleed on touch found only in 1.25% which correlated with all the above four studies.

Out of 240 smears, 10 (4.17%) were unsatisfactory for evaluation due to various reasons like scant cellularity, inflammation and hemorrhagic background. Supriti et al in her study had 2.3% unsatisfactory smears.<sup>25</sup> Proper sampling and transportation of slide to the cytopathology laboratory is recommended for a better cellularity.

In our study inflammatory smears comprised 60.83%. This was comparable with various other studies like Laxmi et al (2020), Manjit et al (2012) and Manan et al (2019) who in their studies found 69%, 71.3% and 54% inflammatory smears respectively.<sup>24,21,26</sup> Non specific inflammation was 53.34% in our study. However this was 97% and 85% in the studies conducted by Umarani et al (2015) and Honey et al (2018) respectively.<sup>23,13</sup> Among specific inflammation, bacterial vaginosis was the leading cause in our study. Similar finding was seen in the study done by Geethu et al (2016), Honey et al (2018) and Majumdar et al (2020).<sup>15,13,27</sup> This was followed by candidiasis seen in 5.41% smears. Candidiasis ranged between 0.34% - 6.26% in different studies.<sup>13,24,27</sup> We did not find any case of trichomonas vaginalis and herpes. Majority of the studies also did not report any case of these two parasites considering its rarity.

**Table 6: Studies comparing prevalence of epithelial abnormalities in the study participants.**

Author	Year	Place	No. of cases	Total prevalence (%)	ASCUS (%)	LSIL (%)	HSIL (%)	SCC (%)
Nair et al <sup>14</sup>	2016	Kerala	2028	2.42	0.15	1.58	0.49	0.20
Arul Anne <sup>27</sup>	2016	T.N.	630	3.8	0.9	0.5	0.6	0.8
Verma et al <sup>5</sup>	2016	H.P.	200	9.0	1.0	5.5	2.5	0
Sarala et al <sup>7</sup>	2017	T.S.	1000	1.2	0.6	0.2	0.3	0.1
Pushplata et al <sup>22</sup>	2018	U.P.	1650	8.48	2.9	5.09	0.48	0
Sharma et al <sup>12</sup>	2018	U.P.	450	6.9	3.3	1.2	0.4	0.2
Jadhav et al <sup>25</sup>	2019	Gujarat	487	1.23	0.20	0.20	0.41	0.41
Vedvathi et al <sup>17</sup>	2019	Karnataka	200	9.0	4.0	3.5	1.0	0
Laxmi et al <sup>24</sup>	2020	Rajasthan	576	10.06	4.94	1.33	0.48	0.24
Majumdar et al <sup>26</sup>	2020	Tripura	600	9.0	3.8	6.7	5.2	2.3
Present study	2021	Maharashtra	240	5.83	2.17	2.60	0.86	0.43

In our study, when we analysed the smears, 90% were negative for intraepithelial lesions or malignancy and 5.83% smears were classified as epithelial lesions. This was comparable with the study of Arul Anne (2016) who in her study found 93.4% NILM cases and 6.6% epithelial lesions.<sup>28</sup>

ASC-US, LSIL, HSIL and squamous cell carcinoma were found in 2.17%, 2.6%, 0.86% and 0.43% respectively in our study. Most cases of ASCUS were in the age range of

41-50 years and single case of SCC was seen in above 60 years of age. LSIL was seen in the age group of 40 to 60 years. HSIL was seen in females above 50 years of age. This data was compared with various other studies which showed the following results.

The total prevalence of epithelial cell abnormalities varied between 1.2-10.06%. Our study had a prevalence of 5.83% which falls in the range of aforementioned studies. The

prevalence of LSIL, HSIL and SCC are comparable with all other studies.

In age group of 21-40 years, majority of PAP smears showed no epithelial cell abnormality. The finding was similar to the findings of K.Sarala (2017) et al.<sup>7</sup> This shows that the prevalence of epithelial cell abnormalities increases with age.

In this study, most women with LSIL belonged to the 41-50 year-old age group, followed by 51-60 year-old age group. HSIL was most common among women more than 50 years. This indicates that multiparity (>3) is a significant risk factor for cervical carcinoma.

Limitations of this study are few but important. Being a retrospective study, eventual outcome of all patients could not be known and hence no consistent pattern of the disease could be established. Use of liquid based cytology methods may reduce the number of unsatisfactory smears, but is not cost effective in our set up.

The sensitivity of the pap smear test could have been improved if co-testing were done with HPV DNA and also with serial pap smears as per recommendations. All abnormal pap smears should be followed with colposcopy guided biopsies. Pap smear has certain limitations, such as false-negative rate of 20.9%, sensitivity rate of only 37-84% for CIN 1 or higher.<sup>7</sup> It is also vulnerable to subjective interpretation, and has low predictive value. The notification of results to women as well as the visits required for serial cytological screening also pose programmatic and logistic challenges.

## CONCLUSION

Our study indicates the need and importance of cervical screening. It also directs and gives us the scope to conduct further studies regarding the early detection of cervical cancer. The ultimate goal of the PAP-smear screening process is to find women with precancerous lesions, so that they can be treated before the malignancy develops into potentially lethal invasive cancer.

The Pap smear as a screening tool has great importance and is widely used routine test which enables proper management at an early stage by detecting the early cervical changes.

Even though our study represented only a small part of our country, the results emphasized that we have to encourage and motivate the women for active screening. It should be a continuous process in order to prevent people dropping out for follow up visits and treatment procedures. Screening programmes should be established, data collection should be improved on screening, protocols should be established for patient follow up and guidelines should be identified for patient referrals. By doing so, morbidity and mortality due to cervical cancer will definitely reduce in near future. Taking into account the

presence of women who had never undergone Pap test, the community should be enlightened about Pap smear test, including its aim, the required frequency of application, by diffuse educational activities, and media programmes.

We propose that larger studies are required to estimate the pattern of cervical cytological abnormalities along with detection of common HPV strains in our area, as this knowledge would be useful for prevention of HPV infection either by vaccines or future targeted therapy.

Thus, we have to strengthen our health services to spread cervical cancer screening programs, educate and motivate women to visit the hospital for cancer screening.

Although PAP smears are recommended to perform extensively to screen for cervical cancer, the preventive power of PAP smear lies in regular serial screening.

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