

PAP Smear and HPV Co-Testing - Need of the Hour

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ABSTRACT

Introduction: Cervical cancer is one of the major causes of mortality among the women in developing countries. Pap smear is a method of cervical screening used to detect premalignant and malignant conditions of cervix. Present research aimed to study and analyze the utility of pap smear and HPV screening in women.

Material and Methods: We screened 9900 women in the age group of 20 to 70 years between 1st January 2017 to 31st December 2019 presenting with different gynecological complaints and as a part of preventive health check-ups. Smears were reported according to 2001 Bethesda. Also, the HPV testing was carried out in the cases as advised by clinician.

Results: Out of 9900 cases, 80.8% cases were benign comprising of Negative for intraepithelial neoplasia(NILM), 16.2 % cases were inflammatory, 1.5 % cases were of atypical squamous cells of undermined significance, 0.7 % cases low grade squamous intraepithelial lesion, 0.6 % cases high grade squamous intraepithelial lesion, 0.1% cases atypical glandular cells of undermined significance, 0.02 % cases were of squamous cell carcinoma. Out of 1420 cases which underwent HPV testing, 7.1% came out to be positive for HPV infection.

Out of the HPV positive cases, 68.62% were high risk category and 31.43% were low risk category.

Conclusion: Pap smear is a proven strategy. Even asymptomatic women in age group of 20 to 35 years must be subjected to cervical screening. But HPV along with cytology should now be new the gold standard for follow up and screening of cervical cancer. High risk category HPV positivity, will give better prediction about cervical cancer.

Keywords: Papanicolaou smear, Atypical squamous cells of the cervix, Squamous intraepithelial lesions, Squamous cell carcinoma, Squamous cell cancer.

INTRODUCTION

The Papanicolaou test is also known as the pap test, pap smear is a screening method to detect precancerous and cancerous lesions in cervix, ^[1]. Greek doctor Georgios Papanicolaou invented this test and it was named after him.

Cancer of cervix is the second most common cancer among women in the world. It accounts for 12 % of all cancers in females, ^[2]. In India, every year 122,844 women are diagnosed with cervical cancer and 67,477 die from the disease, ^[3].

In developing countries where genital hygiene is poor, non-specific

infections of cervix and vagina are commonly encountered.

Cervical screening program has successfully reduced the burden of disease in many developing countries, ^[4,5]. Cervical screening is able to detect the premalignant lesions before the development of invasive cervical cancer. Epidemiological studies suggest that HPV is associated with 10 -fold or greater risk of cervical neoplasia than controls, ^[6]. It is now known that certain strains (16 and 18) are present in most cervical cancers, several newer strains as etiological factor are under investigation, ^[7].

Pap smear involves collection of exfoliated cells from the squamocolumnar

junction, i.e. transformation zone, from posterior vaginal wall and endocervical canal using brush or spatula. The collected cells are fixed in methanol, stained and are examined under microscope for presence of cervical premalignant lesions.

The U.S. Preventive Services Task Force and the American Cancer Society recommends pap smear screening starting around 21 years of age and can be repeated at every 3 years interval, [8,9]. Colposcopy guided cervical biopsy are needed to diagnose and prevent further progression to cervical cancer.

The aim of the present study is to study and analyze the pap smear reports in symptomatic and asymptomatic women and to study the role of HPV co-testing by HPV DNA PCR.

MATERIAL AND METHODS

The study was carried out in a tertiary care hospital at Pune from 1st January 2017 to 31st December 2020 in the age group of 20-70 years. Ethical approval was obtained from the institution. Patient's consent for taking pap smear was obtained in a structured proforma. Patient data in the form of name, age, clinical complaints, per vaginal findings, per speculum findings were collected from the requisition form and noted.

Exclusion criteria:

- Women below 20 years of age
- Women who are pregnant
- Women not willing to give consent for pap smear
- Women who use local douche

Procedure: The patient was placed in lithotomy position. A Cusco's speculum was introduced through the vagina and the cervix was visualized. The cyto brush was rotated 360° near the squamocolumnar junction and immediately put into the preserved cyto-solution. The gynecologists specify whether only LBC or HPV co-test was required. If an HPV co-testing was

requested the sample was tested in molecular biology section.

The sample is processed by *Nanocyte* processor by fully automated filtration technique and uniform and thin pap smear of 16mm diameter is prepared. Then the pap smear was stained by *Rapid-Pap* kit.

The 2001 Bethesda system was followed for reporting. The adequacy of the specimen is evaluated by well preserved and well visualized 5000 squamous cells for liquid base preparation. The main focus of the study was to detect squamous and glandular cell abnormalities.

- Within normal limit
- Inflammatory
- Atypical squamous cells of undetermined significance (ASCUS)
- Atypical glandular cells of undetermined significance (AGUS)
- Low Grade Squamous intraepithelial invasion (LSIL)
- High grade Squamous intraepithelial invasion (HSIL)
- Invasive carcinoma

False negative results can be due to:

- Inadequacy of sample
- Bloodstained sample
- Artifacts in the staining

RESULTS

In this study 9900 pap smears were studied with respect to age group, clinical signs and symptoms, parity, PS findings, PV findings.

Most of the patients were in the age group of 31-50 years (64.8 %) with mean age group of 40.2 years.

Out of 9900 cases studied 5400 cases (54.5 %) were asymptomatic and 4500 cases were symptomatic (45.5%). Asymptomatic group comprised of patients who came for routine check-up and pap screening camps conducted by the Institute.

Among the symptomatic patients 4500 cases, the whitish discharge per vagina was the most common symptom followed

by the second most common symptom of pain in lower abdomen. Other symptoms of intermenstrual bleeding, Postcoital bleeding,

Postmenopausal bleeding and dyspareunia were also observed. (Table 1).

Table 1. Chief complaints:

CHIEF COMPLAINTS	NUMBER OF PATIENTS	PERCENTAGE
Whitish discharge per vaginum	1998	44.4 %
Pain in lower abdomen	927	20.6 %
Intermenstrual Bleeding	693	15.4%
Postcoital Bleeding	459	10.2%
Post-Menopausal Bleeding	378	8.4 %
Dyspareunia	45	1.0%

Per-speculum and per vagina findings were analyzed. In per speculum findings, the most common finding was that of normal looking cervix in women.

Cervical erosion is the second most common finding followed by cervical hypertrophy and bleeding on touch.

Atrophic vagina and cervical polyp were also observed. (Table 2)

On per vaginal examination, the most common finding was that of normal looking cervix. Mass in uterus or adnexa and cervix which bleeds on touch was also observed. (Table 2).

Table 2: Per speculum and per vagina findings.

Sr No	Per Speculum/ Per vagina	Findings	Number of patients	Percentage
A	Per Speculum	Normal cervix	5198	52.5 %
		Cervical erosion	2594	26.2 %
		Cervix hypertrophy	1466	14.8 %
		Bleeds on touch	307	3.1 %
		Atrophic vagina	257	2.6 %
		Cervical polyp	79	0.8 %
B	Per vagina findings	Normal Cervix	9513	96.1 %
		Mass in uterus or adnexa	178	1.8%
		Cervix bleeds on touch	207	2.1%

Out of 9900 cases, 4500 cases were symptomatic.

Among them, (2%) were reported as unsatisfactory smear either due to inadequate material or hemorrhagic smear. In symptomatic patients, Negative for intraepithelial lesion or malignancy (NILM) was the most common finding followed by inflammatory smear. Atypical squamous cells of undermined significance (ASCUS),

Low grade squamous intraepithelial lesion (LSIL), and High Grade Squamous Intraepithelial Lesion (HSIL) were reported in significant number of cases. Atypical glandular cells of undermined significance (AGUS) and the squamous cell carcinoma (SCC) were reported in very few cases

Total epithelial cell abnormalities accounts for 2.82% of the cases. (Table 3)

Table 3: Pap smear analysis (symptomatic patients).

Pap smear analysis report	No. Of patients	Percentage (%)
Unsatisfactory	90	2
Negative for intraepithelial lesion or malignancy (NILM)	3636	80.8
Inflammatory smear	639	14.2
Atypical squamous cells of undermined significance (ASCUS)	68	1.5
Low grade squamous intraepithelial lesion (LSIL)	32	0.7
High Grade Squamous Intraepithelial Lesion (HSIL)	27	0.6
Atypical glandular cells of undermined significance (AGUS)	5	0.1%
Squamous cell carcinoma (SCC)	2	0.04%

In the patients who do not have any complaint i.e. asymptomatic patient (5400), the most common finding was negative for intraepithelial lesion or malignancy (NILM)

followed by inflammatory smear .Though the patients were asymptomatic , atypical squamous cells of undermined significance (ASCUS) and Low grade squamous

intraepithelial lesion (LSIL) were reported in few cases (Table 4).

Table 4: Pap smear analysis (asymptomatic patients).

Pap smear analysis report	No. Of patients	Percentage (%)
Negative for intraepithelial lesion or malignancy (NILM)	5238	97
Inflammatory smear	125	2.3
Atypical squamous cells of undermined significance (ASCUS)	27	0.5
Low grade squamous intraepithelial lesion (LSIL)	3	0.05

Table 5: HPV analysis.

Type of HPV	No of cases	Percentage %
HPV 16	35	34.3
HPV 18	30	29.4
HPV 59	2	1.96
HPV31	2	1.96
HPV 39	1	1
HPV 6	18	17.64
HPV 11	14	13.7

HPV was carried out in 1420 cases as advised by gynecologist by HPV DNA PCR DETECTOR. Out of which 102 cases (7.1%) came positive for HPV infection.

Out of 102 cases, 70 cases (68.6%) were positive for high risk group and 32 cases were positive for low risk group.

Among the high-risk group, the most common infection was that of HPV 16 followed by HPV 18. Few cases of HPV 59, HPV 31, HPV 39 also contribute in infection of high-risk group. (Table 5). Among the low risk group, HPV 6 predominates followed by HPV 11. (Table 5)

DISCUSSION

Cervical cancer is the most screened cancer in developed and developing countries. Our study emphasizes the importance of pap smear screening and HPV co testing for early detection of premalignant and malignant lesions of cervix even in asymptomatic patients.

Nayir et al, [10] in their study observed that the population based cervical cytology screening program using pap smear testing every 3-4 years have reduced cervical cancer incidence and mortality by up-to 80 % in developed countries in the last five decades.

In our study, maximum numbers of patients were between 31 to 50 years (64.8 %) with mean age of 40.2 years. Cervical cancer usually occurs between 40 and 50 years and its precursor lesion usually occur 5-10 years earlier as shown in the study by

Shanmugham et al, [11]. It is recommended that women should have at least one pap smear test done before the age of 45 years, [12]. The women who started their first smear after age of 45 years might miss the chance of cancer prevention all together.

In the study by Vijayalaxmi et al, [1] maximum number of patients are in the age group of 45 to 55 years and in the study by Sunita et al, [13] maximum number of patients are in the age group of 31 to 40 years.

In our study, out of 9900 cases, 4500 cases were symptomatic.

In symptomatic patients reports of NILM (Negative for intraepithelial lesion and malignancy) 80.8%, inflammatory in 16.2%, ASCUS (Atypical squamous cells of undermined significance) 1.5 %, LSIL (Low grade squamous intraepithelial lesion) 0.7%, HSIL (High grade squamous intraepithelial lesion) 0.6%, AGUS (Atypical glandular cells of undermined significance) 0.1% and squamous cell carcinoma 0.02 %.

Also, in asymptomatic patients, reports of NILM were 97 %, Inflammatory were 2.3 %, ASCUS 0.5 %, and LSIL 0.05 %.

These findings are comparable with Sunita et al, [13] who reported 88.02 % NILM, 2.98 % ASCUS, 1.19 % LSIL, 0.66 % HSIL, 0.21 % AGUS and 0.95 % malignancy.

More or less same are the findings with Shaikhali et al, [14] who reported 97.57 NILM, 0.82 % ASCUS, 0.92 % LSIL, 0.30% HSIL, 0.03 % AGUS, and 0.03 % of squamous cell carcinoma.

On the other hand, Adepiti et al, [15] found more cases as compare to our study of LSIL (10.2 %), ASCUS (5.7 %), HSIL (5.4 %) and less cases as compare to our study of NILM (40.6%), and Inflammatory (29.9%).

The difference in prevalence of inflammatory changes and cervical dysplasia could be the result of social and cultural differences, age, hygiene of women,

sexual activity, relationship and presence or absence of cervical screening program in the locality.

Comparison of epithelial cell abnormality and other findings in our study with other studies is as follows.

STUDY	NILM (%)	INFLAMMATORY (%)	ASCUS (%)	LSIL (%)	HSIL (%)	AGUS (%)	SCC (%)
Sunita ^[13]	88.02	-	2.98	1.19	0.66	0.21	0.95
Ashok ^[3]	56	32.5	1	5.5	2.5	-	-
Shaikhali ^[14]	97.51	-	0.82	0.92	0.30	0.03	0.03
Adepiti ^[15]	40.6	29.9	5.7	10.2	5.4	-	-
Omna	52.8	18.4	4	6.8	6	-	-
Our study (symptomatic)	80.8	16.2	1.5	0.7	0.6	0.1	0.02
Our study (Asymptomatic)	97	2.3	0.5	0.05	-	-	-

The 2012 USPSTF, ASCCH and ACOG guidelines recommend HPV co-testing with cytology for women aged 30-65 years every 5 years and only cytology every 3 years,^[17,18]

In our study 7.1% cases were reported as HPV positive. Shaikhali et al,^[14] had reported 10.34 % cases.

In our study HPV 16 was the commonest genotype detected (34.3 %), followed by HPV 18 (29.4 %). These findings are comparable with Shaikhali et al,^[14] who reported HPV 16 (45 %) and HPV 18 (15%). Bhatla et al,^[19] also reported the similar findings.

PCR is a sensitive technique to detect HPV and has low false negative rate,^[20]

CONCLUSION

1. Although Pap smear is effective, economical, safe, easily applicable and very useful test.
2. And efforts should be intensified to encourage women to screen at an earlier age, so as to reduce the precancerous and cancerous lesions of cervix in future.
3. But Molecular pap smear i.e. cytology and HPV co-testing facilitates the identification women at risk for cervical cancer and allows for stratification of patients, thereby allowing better risk assessment.
4. Molecular PAP is very useful in dilemma cases or borderline results.

5. Universal Co-testing strategy will lead to better compliance and
6. Will allow us to increase screening interval thereby reducing the burden on the system.

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