Using the Papanicolaou (Pap) smear for cervical cancer screening in a tertiary care hospital: a prospective study and clinical correlation

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Abstract

Background: Cervical cancer is the most frequent cause of death for women in developing countries. India, the second-most populous nation in the world, accounts for about 25% of cervical cancer deaths. Even in its premalignant stage, cervical cancer can be detected using a Papanicolaou (Pap) smear test.
The goal was to study the role of Pap smear in detecting premalignant, malignant, and non-neoplastic lesions of the cervix and to ascertain the frequency of different lesions.

Methods: All women who visited the obstetrics and gynecology outpatient department (OPD) at GMERS Medical College and Hospital, Navsari, India, in 1 year for different clinical problems were recruited. A total of 322 women who were sexually active and over 21 years of age were enrolled in the study. A Pap smear was used for all the women to screen for cervical cancer. The smear was obtained using an Ayre spatula and spread over a marked glass slide, which was placed in 95% ethyl alcohol and sent to the Department of Pathology for cytopathological examination. Women who had visible malignant cervical lesions were excluded. All the data were recorded in a predetermined form.

Results: A total of 322 patients were screened. Out of 322, 261 smears were NILM (negative for intraepithelial lesion or malignancy) and 126 (39.1 %) smears had inflammatory changes; 2.2 % and 1.2% smears showed changes as ASCUS (atypical squamous cells of undetermined significance) and ASC-H (atypical squamous cells cannot exclude high-grade squamous intraepithelial lesions), respectively; 4.7 % and 5.3% smears showed LSIL (low-grade squamous intraepithelial lesion) and HSIL (high-grade squamous intraepithelial lesion). Squamous cell carcinoma was observed in only 2 (0.6%) of the smears. A total of 16 (4.9%) smears were unsatisfactory for evaluation because they lacked sufficient squamous components or were obscured by inflammation.

Conclusion: The Pap smear test is a very easy, non-invasive, useful, simple, safe, and economical tool to detect preinvasive cervical epithelial lesions. It is evident and proven that every woman above the age of 30–35 years must be subjected to cervical screening, and this must be continued even in the postmenopausal period.

Highlights

What is current knowledge?

The gold standard for cervical screening programs has long been the Pap test, which is straightforward, safe, and affordable. We can identify the precancerous lesions of the cervix by doing routine exams using Pap smear tests.

What is new here?

Numerous educational initiatives that aid in reducing mortality and morbidity from cervical cancer should inform the population about the test. Every woman over the age of 30 years must get cervical screening in developing nations like India, and this screening must continue even after menopause. The sensitivity of cervical pathology identification can be improved by combining Pap tests with HPV-DNA testing.

Introduction

Cervical cancer is a prevalent ailment in developing nations. It is the seventh most frequent type of cancer overall and is the third among malignancies that affect women globally, after lung, colorectal, and breast cancers. In 2018, India bore the brunt of this disease, accounting for one-fifth of the total burden of cervical cancer, with 60,078 fatalities and 96,922 new cases. The second most frequent reason for death due to cancer among women in India is cervical cancer, which is mostly preventable (1).

The World Health Organisation (WHO) reports that 80% of cervical cancer fatalities take place in underdeveloped nations. Contrarily, affluent nations with successful screening programs have seen a decline in the incidence of cervical cancer (2).

Due to a lack of awareness and the difficulty in managing cytology-based screening programs, >80% of all cervical cancer cases are discovered in developing and low-resource countries, according to Global Cancer Statistics (3). With an estimated prevalence of about 23.29%, the second-most populous nation

in the world, India, is responsible for nearly 25% of cervical cancer deaths worldwide (4).

Due to the long pre-invasive stage, cervical cancer is a disease that can be prevented. Early detection and appropriate treatment are possible if robust screening is implemented (5). A Papanicolaou (Pap) smear test, the main screening test for the diagnosis of precancerous cervical intraepithelial neoplasia and the early stage of invasive cervical cancer, can detect early cervical epithelial alterations. Screening, detection, and preventative treatment have plenty of time due to the cervix's accessibility to Pap tests and visual inspection, as well as the gradual evolution from precursor lesions to invasive cancer.

Since its introduction in 1941, the Pap smear has served as the gold standard for detecting premalignant lesions and cervical cancer. Papanicolaou offered the initial suggestion for the test in 1928, but it took nearly 15 years for the medical world to accept it widely (6,7).

Numerous studies have demonstrated that screening and early detection have reduced the incidence and mortality from invasive cervical cancer globally. This is achievable because the Pap test enables early detection by detecting early cervical epithelial cell abnormalities, mild to severe dysplasia, and invasive malignancy (8). This test is essential for finding cervical cancer and its precursor lesions, but it also helps with the diagnosis of other illnesses, such as infectious and inflammatory disorders. There has to be a greater awareness within the medical community about this screening to provide cervical cancer tests to women in India's rural and urban areas. Because it is straightforward, efficient, and adaptable, the Pap smear forms a crucial component of routine clinical examinations and allows for screening of a sizable at-risk population (9,10). Pap smear screening has a sensitivity of 50%-75% and a specificity of 98%-99% (11). For a Pap test, cellular material from the squamocolumnar junction in the cervix is gently scraped off using a brush or spatula and spread onto a glass slide of approximately 25 mm by 50 mm. The cells are dyed, methanol-fixed, and subjected to microscopic visual examination.

There is a need to promote cervical cancer screening programs, inform women about cancer symptoms, and encourage them to go to the hospital for cancer screening. Counseling on the importance of cancer screening should be given to women and every member of the family. Women who have a positive

Pap test require appropriate care and ongoing monitoring. We must strengthen our health services and the healthcare system to integrate screening at primary health centers. The objectives of the current study were to assess women for precancerous lesions utilizing Pap smear results and look into the clinical association.

Methods

Place and type of study
This is a prospective descriptive study conducted at the Department of Pathology, GMERS Medical College and Hospital, Navsari, India. The study was conducted for outpatient department (OPD)-based patients of obstetrics and gynecology (OBG) departments between June 2021 to May 2022.

Sampling methods and sample collection
The screening procedure to find cervical cancer in its preclinical stage was explained to all of the participants. With an information sheet that was given to them, the women who volunteered to participate were re-informed about the Pap smear, biopsy (if necessary), and the obligatory follow-up in case of abnormal Pap test findings, and all their questions were addressed by the researchers. Then, fully informed consent was acquired. A thorough history was collected, including the patient's medical, menstrual, marital, obstetric, vaginal discharge, sexual, postcoital, intermenstrual, postmenopausal, and educational background. On the day of the test, we made sure that no local douche, antiseptic cream, or local interior examination was performed.

The cervix was viewed after the patient was positioned in the lithotomy position, and a Cusco's bivalve speculum was inserted via the vagina. The Ayre spatula's longer projection was inserted into the cervix close to the squamocolumnar junction and turned 360 degrees. An average of 2 smears were taken from each individual. On a spotless glass slide, the smeared substance was spread out carefully. The Coplin jar of 95% ethyl alcohol (fixative) was then filled with the glass slide, which was then promptly stained using the Pap smear technique.

The New Bethesda System for Reporting Cervical Cytology 2014 was used to analyze the smear results cytologically (12).

The 2014 Bethesda system categorizes lesions into 2 categories: epithelial cell abnormalities, including squamous and glandular cells, and lesions that are negative for intraepithelial neoplasia (NILM).

The squamous epithelial cell abnormality is categorized into the following categories:
1. Atypical Squamous Cells (ASC), including:
a. ASC-US (ASC of undetermined significance)
b. ASC-H (ASC that cannot exclude High-Grade Squamous Intraepithelial Lesions)
2. SIL (Squamous Intraepithelial Lesions):
a. LSIL (Low-Grade Squamous Intraepithelial Lesions)
b. HSIL (High-Grade Squamous Intraepithelial Lesions)
The LSIL and HSIL patients received counseling and were encouraged to get a biopsy for a histological analysis. Frank invasive malignancy was termed squamous cell carcinoma.

Similarly, glandular cell abnormalities were categorized into:
1. Atypical Endocervical Cell-NOS (Not Otherwise Specified)
2. Atypical Endometrial Cells-NOS
3. Atypical Glandular Cells-NOS
Inclusion criteria: All women who came to the gynecology OPD in the age group of 21 to 90 years who consented to Pap tests were included.
Exclusion criteria: The study excluded women who refused to consent to the Pap smear, had cervix cancer that had been diagnosed or treated, were pregnant, used a local douche or antiseptic cream, or had previously undergone Pap smear testing.

The WHO recommended follow-up and treatment for all the women who had abnormal Pap smear findings and did so in accordance with the recommended standards. The LSIL and HSIL patients received counseling and recommendations to get a biopsy for histological analysis and to have a colposcopic examination.

Statistical analysis: Data were inputted into an Excel sheet and Microsoft Word document.

Results

A total of 322 Pap smears were obtained from women between the ages of 15 and 90 years who visited the gynecology outpatient department at GMERS Hospital, Navsari, India. The majority (30.4%) of the women were in the 31–40 age range; 74.9 % of the women were multiparous, compared to 14.3 and 10.9 % of women who were primiparous or nulliparous. In contrast to the 34.0% of women who did not use any kind of contraception, 46.9% of the women underwent tubal ligations for contraception (Table 1).

Out of 322 women in the study, the majority (58.4%) reported no complaints, whereas 21.1% experienced asymptomatic vaginal hemorrhage and 8.0% experienced irregular bleeding. On the speculum inspection in our study, the majority of women (63.0%) had healthy cervixes, while 13.0% and 11.2% of the women showed white discharge per vaginum and cervical erosion, respectively (Table 2).

Table 1. Socioeconomic and demographic characteristics of the women

Sociodemographic characteristics	No. of cases (n=322)	Percentage (%)
Age group (y)		
21-30	79	24.5 %
31-40	98	30.4 %
41-50	87	27.0 %
51-60	35	10.9 %
61-70	17	5.3 %
>70	06	1.9 %
Marital status		
Married	322	100 %
Unmarried	Nil	00 %
Contraception		
None	113	34.0 %
Barrier	23	7.1 %
Hormonal	08	2.5 %
Intra-Uterine Contraceptive Device (IUCD)	23	7.1 %
Tubal	151	46.9 %
Others	04	1.2 %
Parity		
Nulliparous	35	10.9 %
Primiparous	46	14.2 %
Multiparous	241	74.9 %

Table 2. Complaints and per speculum findings of women attending the gynecology out-patient department

Complaints	No. of cases (n=322)	Percentage (%)
No complaints	188	58.4 %
Asymptomatic vaginal discharge	68	21.1 %
Postcoital bleed	06	1.9 %
Irregular bleeding	26	8.0 %
Postmenopausal bleed	09	2.8 %
Pain in the abdomen	14	4.3 %
Others	11	3.4 %
The finding of P/S examination		
Healthy looking cervix	203	63.0 %
White discharge per vaginum	42	13.0 %
Cervical erosion	36	11.2 %
Ectropion of the cervix	09	2.8 %
Bleeding on touch	11	3.4 %
UV prolapse/cystocele	18	5.6 %
Suspicious	03	0.9 %

Out of 322 cases, the vast majority (81.1%) were benign and included NILM, but 2.2% and 1.2% of the smears revealed ASCUS and ASC-H, respectively. Moreover, LSIL and HSIL were detected in smears at 4.7% and 5.3%, respectively. Besides, 2 (0.6%) cases of squamous cell carcinoma were found in our study. In the present study, 16 (4.9 %) smears were unsatisfactory for evaluation. Inadequate squamous components or obscuring inflammation were the main causes of inadequacy (Table 3).

Table 3. Cervicovaginal cytology interpretation/results by the Bethesda System (2014)

Interpretation /Results	No. of cases (n=322)	Percentage (%)
Unsatisfactory for evaluation	16	4.9 %
NILM*	261	81.1 %
ASC-US**	07	2.2 %
ASC-H***	04	1.2 %
LSIL****	15	4.7 %
HSIL*****	17	5.3 %
Squamous Cell Carcinoma	02	0.6 %
Adenocarcinoma	00	00 %

* NILM: Negative for Intraepithelial Lesion or Malignancy **ASC-US: Atypical Squamous Cells Undetermined Significance *** ASC-H: Atypical Squamous Cells cannot exclude High-Grade Squamous Intraepithelial Lesions **** LSIL: Low-grade Squamous Intraepithelial Lesions ***** HSIL: High-Grade Squamous Intraepithelial Lesion

In our study, 126 (39.1%) of the 261 cases with NILM had nonspecific inflammatory smears, which were followed by bacterial vaginosis in 18 (5.6%), Trichomonas vaginosis in 12 (3.7%), reactive changes in 9 (2.8%), and Candida infection in 7 (2.2%) smears. Besides, 84 (26.0%) of the cases revealed no additional changes (Table 4).

Table 4. Distribution of Negative for Intraepithelial Lesion or Malignancy (NILM) cases

Smear findings	No. of cases (n=261)	Percentage (%)
Nonspecific inflammation	126	39.1 %
Trichomonas vaginalis	12	3.7 %
Candida albicans	07	2.2 %
Bacterial vaginosis	18	5.6 %
Atrophy	05	1.6 %
Reactive changes	09	2.8 %
No other changes	84	26.0 %

Of the smears in our investigation, ASCUS and ASC-H were detected in 2.2% and 1.2%, respectively. Moreover, LSIL and HSIL were detected in smears at 4.7% and 5.3%, respectively. Also, 2 (0.6%) squamous cell carcinoma patients from the age range of 41-60 years were found (Table 5).

Table 5. Cervical epithelial abnormalities in relation to age

Age (y)	ASCUS*	ASC-H**	LSIL***	HSIL****	SCC*****
21-30	00	00	00	00	00
31-40	01	01	07	03	00
41-50	03	01	05	07	01
51-60	02	02	02	04	01
61-70	01	00	01	02	00
>70	00	00	00	01	00
Total	07 (2.2%)	04 (1.2%)	15 (4.7%)	17 (5.3%)	02 (0.6%)

*ASC-US: Atypical Squamous Cells Undetermined Significance ** ASC-H: Atypical Squamous Cells cannot exclude High-Grade Squamous Intraepithelial Lesions *** LSIL: Low-grade Squamous Intraepithelial Lesions **** HSIL: High-grade Squamous Intraepithelial Lesion ***** SCC: Squamous Cell Carcinoma

Discussion

The primary malignancies encountered in women in India, according to the National Cancer Registry Programme of India, are cancers of the uterine cervix and breast. There needs to be a successful mass screening program targeted at particular age groups to find precancerous diseases before they develop into invasive malignancies (13). It is well-known that the prevalence of cervical cancer has significantly decreased since the implementation of screening programs (14).

Cervical cancer prevention can be either primary or secondary. Changes in sexual behavior and immunization against the human papillomavirus (HPV) are two primary preventative strategies. Cervicoscopy, HPV testing, cytology, and visual examination of the cervix (VIA) are all methods of secondary prevention for cervical cancer. A Pap smear test is a secondary preventive measure used to find premalignant and malignant lesions that may require further investigation and/or treatment. (15,16).

In both industrialized and developing nations, cervical cancer is the cancer that is most frequently screened. Cervical cancer morbidities and fatalities still exist despite widespread screening and acceptance of the Pap smear. According to a study, 1 867 elderly women in the US passed away from cervical cancer in 1986 (17).

The prevalence of cytological abnormalities is significant overall, according to numerous studies in India. The variation in the prevalence of inflammatory changes and cervical dysplasia could be attributed to age, sexual activity level, the frequency of associated infections, and the existence or absence of cervical screening programs in various communities and societies. It may also be the result of social and cultural differences.

The present study found that 4.96% of the smears were inadequate, which is consistent with studies by Thomas et al. (22) and Manan et al. (18), which found that 5.88% and 5.186% of the smears were inadequate, respectively. In contras Shashidhar et al.'s (21) study, which comprised 15.64% of the participants, the incidence of atrophic changes (1.55%) was lower in the current study. (Table 6).

Table 6. Comparison of benign lesions findings of Pap smear cytology with other studies

Study	Total cases (n)	Inadequate	Normal	Atrophic	Inflammatory
Manan et al. (18)	487	5.186	40.24	1.23	54.35
Vaghela et al. (19)	400	13.25	1.50	-	53
Tailor et al. (20)	1425	-	-	1.33	-
Shashidhar et al.(21)	308	2.27	51.36	15.64	26.53
Thomas et al. (22)	85	5.88	58.82	-	-
Chauhan et al. (23)	5778	-	9.78	-	69.19
Mital et al. (24)	250	-	40.65	-	12.70
The present study	322	4.96	26.08	1.55	39.13

Out of 322 total cases, 2.17% of cases in the current study showed ASCUS, which is consistent with findings by Nayir et al. (26) and Tailor et al. (20). In the present study,4.65 % of the cases showed LSIL, which was comparable with the studies by Ushasarma et al. (32) and Shaki et al. (34). Moreover, HSIL was present in 5.27% of cases, which is consistent with the findings of Padmini et al. (33) and Shaki et al. (34). The present study found that 0.62% of the cases were malignant, which is consistent with previous studies conducted by Misra JS et al. (31) and Mandakini M Patel et al. (13), which found that the total number of malignant lesions was 0.6% and 0.7%, respectively. Additionally, the patients in this study visited an outpatient clinic at a tertiary care facility for gynecological problems rather than for Pap smear screening (Table 7).

The average age of the patients in Varghese C et al.'s (35) and Suma RK et al.'s (36) studies was 39.5 and 38.2 years, respectively, which is in line with our typical age range of 31 to 40 years. The present study's mean age is in accordance with the mean age reported by Shashidhar MR et al. (21), which is 31-40 years.

The sensitivity and specificity of the conventional Pap test vary widely, ranging from 30 to 80% and 86 to 100%, respectively. The mean sensitivity of cervical smear is 47%, and the mean specificity is 95%. (26)

There is an urgent need for community education on how to prevent the disease by giving free HPV vaccinations and cervical cancer screenings to adolescent girls to reduce the prevalence of cervical cancer in India. The advantages of Pap tests should be made known to the public by healthcare practitioners. This fact emphasizes the necessity of cytological screening in these populations and older patients, as well as the necessity of raising awareness about cervical cancer to encourage women to participate in the screening.

The following steps will help to improve Pap smear screening:
1. Women should be made aware of the significance of taking part in routine screening programs because prevention is always preferable to treatment.
2. Clinicians must ensure a sufficient sample is taken, regular follow-up is made, and appropriate patient care is provided.
3. Interpreting cervical smears requires the expertise of pathologists.
4. The infrastructure and funding provided by healthcare systems should be improved to perform screening programs.

Table 7. Comparison of Epithelial Cell Abnormalities with other studies

Study	Total cases (n)	Epithelial Cell Abnormalities (ECA)			
		ASCUS(%)	LSIL(%)	HSIL(%)	SCC (%)
Manan et al. (18)	487	0.20	0.20	0.41	0.41
Tailor et al. (20)	1425	1.4	00	0.35	0.14
Nayir et al. (26)	-	1.7	0.5	0.1	-
Bal et al. (27)	300	0.3	2.7	0.7	1.3
Sadhana Kothari et al.(29)	36740	0.11	0.83	0.31	0.05
Geethu G. Nair et al. (30)	2028	0.15	1.58	0.491	0.20
Kamna Gupta et al. (31)	4703	0.52	1.36	0.91	0.28
Mandakini M Patel et al. (13)	995	4.1	0.1	0.1	0.7
Preetha George et al. (33)	1000	0.3	2.0	0.9	0.3
Misra JS et al. (31)	36484	-	5.5	1.6	0.6
Ushasarma et al. (32)	242	1.32	3.53	3.52	3.53
Padmini et al. (33)	100	8.0	3.0	5.0	-
Shaki et al. (34)	1100	4.0	6.8	6.0	2.3
Present study	322	2.17	4.65	5.27	0.62

ASC-US: Atypical Squamous Cells Undetermined Significance; LSIL: Low-grade Squamous Intraepithelial Lesions; HSIL: High-grade Squamous Intraepithelial Lesion; SCC: Squamous Cell Carcinoma

In India, Pap smear screening has the following drawbacks: 1) The cultural taboo against discussing sexually transmitted diseases, the absence of familial support, and low socioeconomic level all contribute to women's lack of participation in routine screening programs. 2) Clinicians may not collect a sufficient smear, follow-up, and treat patients appropriately, or advise patients that prevention is preferable to treatment. 3) Smear interpretation by pathologists is subject to error, and many regions lack cytotechnologists for staining and smear interpretation. 4) The lack of resources and facilities makes it difficult for healthcare systems to run screening programs (37).

The limitation of this study is that it is a hospital-based study, which may not be a true reflection of the local population.

Conclusion

A very effective, easy-to-use, affordable, and secure method of identifying pre-invasive cervical epithelial lesions is the Pap smear test. Cervical screening for all women over the age of 30 years must be done regularly, and this screening must continue even after menopause. The government should organize regular health examination camps and health awareness campaigns in developing nations like India. For cervical screening programs, Pap tests have traditionally been recognized as the gold standard. We can identify the precancerous lesions of the cervix by doing routine exams using Pap smear tests. The sensitivity of cervical pathology identification can be improved by combining Pap tests with HPV-DNA testing. Widespread educational initiatives should inform the population about the test to reduce cervical cancer-related morbidity and mortality.

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Ethical statement

The Ethics Committee of the GMERS Medical College and Hospital, Navsari, India, gave its approval to the study protocol.

Conflicts of interest

The authors affirm that there is no conflict of interest regarding the publication of this work.

Author contributions

DD and SK collected the data, designed the experimental design, and contributed

to drafting and critically revising the article. The work was revised and expanded upon by all other authors after being first written by AP and DD and the data analysis. The final version of the manuscript was reviewed and approved by all authors.

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