

PAP SMEAR AS A SCREENING TOOL FOR EVALUATING CERVICAL DYSPLASIA AND MALIGNANCY- A HOSPITAL-BASED STUDY

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ABSTRACT

BACKGROUND

In women, cervical cancer is one of the most common cancers and the most researched disease. Pap smear is a cost-effective method for regular screening of population for early detection of pre-neoplastic and neoplastic lesions of cervix. We wanted to assess the usefulness of the Pap test as an initial screening tool for examination of the cervix by comparing the results in biopsy specimens and recording the data as positive and negative for dysplastic changes. We also wanted to subject the 'suspicious of malignancy' biopsy tissues to IHC for p16INK4a, a diagnostic marker for all degrees of cervical dysplasia and cervical cancer to correlate the findings.

METHODS

Women visiting the Department of Obstetrics and Gynaecology, presenting with complaints of pain abdomen, white discharge P/V, irregular menstrual bleeding, postmenopausal bleeding, mass per vagina or for regular health check-up were included in the study. Suspicious of malignancy cases were followed by biopsy or hysterectomy. The cytological findings were correlated with histopathological findings in such cases. Finally, these suspicious of malignancy cases were subjected to IHC- p16INK4a marker.

RESULTS

In the present study, a total of 2234 Pap smear cases were studied and 96 out of 2234 cases were followed by histopathological study. Out of 2234 cases, 41 (1.83%) were suspicious of malignant lesions, 1686 cases (75.47%) were inflammatory, 495 cases (22.15%) were normal and 12 cases (0.53%) smears were unsatisfactory for evaluation. Among 41 suspicious of malignant cases, 11 cases (26.83%) were HSIL and 27 cases (65.85%) were LSIL. The sensitivity of cervical cytology was 91.6% and specificity was 86.9% in the present study. Out of 41 cases of suspicious of malignancy, only 22 cases were histopathologically correlated and subjected to IHC p16INK4a marker. Out of 22 histopathologically correlated cases, 11 cases turned out to be cervicitis (50%) were negative for HPV infection. Remaining 11 cases were premalignant and malignant (50%) and were positive for HPV infection.

CONCLUSIONS

The outcome of this study reveals sensitivity of 91.6% suggesting that Pap smear is an essential primary tool for screening of cervical cancer. It is also simple and cost-effective procedure that can be performed in the outpatient department. Thus, combination of Pap smear screening test and confirmation with p16INK4a IHC marker will certainly reduce the high mortality caused due to cervical cancer with underlying HPV infection, as well as alleviate the suffering caused by the disease.

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BACKGROUND

Cervical cancer is the 4th most common cancer among women worldwide.¹ About 5, 27,624 new cases are being registered out of which 2, 75,000 deaths are occurring per year due to cervical cancer.¹ About 1, 22,844 new cervical cancer cases are diagnosed in India annually (estimations made for 2012).¹ In India cervical cancer is the second most common cancer usually affecting women aged 15 to 44 years of age.¹ The incidence of cervical cancer rises in 30-34 years of age and peaks at 55-65 years, with median age of 38 years.² An Indian woman faces 2.5% of cumulative lifetime risk and 1.4% of cumulative death risk from carcinoma cervix.³ The most important risk factor for cervical intraepithelial neoplasia and invasive cervical cancer is human papilloma virus infection.⁴

Majority of cervical cancers are squamous cell carcinoma followed by adenocarcinoma.⁴ Early screening of disease through Pap test has considerably reduced morbidity and mortality from the disease in the developed countries.^{5,6} The benefits of Pap test in preventing cervical cancer have been demonstrated through national screening programs in countries like Finland and Sweden.^{5,6} As a result these countries are reported to have low incidence and prevalence of cervical cancer in the world.^{5,6} Complete and accurate assessment of cervical lesion relies on three methods of investigation which includes the colposcopic examination of cervix, cervical cytology and histopathological examination of biopsies.

The current study is designed with the intention of evaluating the efficacy and impact of Pap test for screening of cervical cancer mainly as a hospital-based study as well as to assess the importance of p16INK4a marker for identification of dysplastic and malignant lesions of cervix and also as a surrogate marker for HPV detection.

Aim & Objectives

- To assess the usefulness of the Pap test as an initial screening tool for examining the cervix, by comparing the results in available biopsies and recording the data as positive and negative for dysplastic changes.

To subject "suspicious of malignancy" biopsy tissues to

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Immunohistochemistry using p16INK4a, a diagnostic marker in cases of cervical dysplasia and cervical cancer, as a surrogate marker in HPV detection and correlating the inference.

METHODS

This is a Hospital based Prospective observational study. All women who were willing to give an informed valid consent were included in the study who were attending outpatient clinic of Department of Gynecology, Chennai Medical College Hospital and Research Centre, Trichy. The study was conducted from July 2013 to March 31st, 2015 (One year and 8 months).

Inclusion Criteria

All women attending Gynecology OPD with or without specific complaints consenting for Pap smear examination

Exclusion Criteria

- Patient not willing for Pap smear test and cervical biopsy.
- Known case of carcinoma cervix.
- Women who had total hysterectomy.
- Pregnant women.

Statistical Analysis

The data was collected and analysed using standard statistical chi – square test, P < 0.05 statistically significant. Data was entered in Microsoft excel and analysis was done using SPSS version 22.

Method of Collecting PAP Smear

After obtaining the informed consent from the patient, the patient is placed in dorsal lithotomy position. With the use of Cusco’s self-retaining speculum without the use of lubricant, the cervix was exposed and visualized for any gross pathological findings and were recorded.

After the inspection of the cervix, the Pap smear was obtained with the use of Ayer’s spatula. The Squamocolumnar junction was scraped by rotating to 360 degrees and smeared onto a glass slide. The smear was fixed by dipping in a Coplin jar containing isopropyl alcohol.

Interpretation of Results

Smears were visualized and their impressions were reported by the senior pathologist according to The Revised 2001 Bethesda System.⁴

Cervical Biopsy

All patients with Pap smears reported other than “Negative for intraepithelial lesion” who consented for cervical biopsies were subjected to it and their tissues were microscopically examined, following which cytohistopathological correlation were made.

After explaining the procedure to the patient, the consent of the patient pertaining to the cervical cone biopsy or hysterectomy was taken accordingly. Cervical biopsy and hysterectomy procedure were performed by the gynecologist.

The specimen excised is fixed in 10% formalin were sent to the Department of Pathology. The tissue were processed

and embedded in paraffin blocks, sections made and hematoxylin and eosin staining were performed. The cervical cytology and hysterectomy specimen findings were evaluated and correlated with the Pap smear findings.

IHC

All cases “suspicious of malignancy” by Pap smear were subjected to study the presence of HPV as an important causative factor in causing cervical dysplasia and cervical cancer by using p16INK4a as an immunohistochemical marker. IHC was done for all the suspected cases along with positive and negative control.

p16INK4a Marker⁷

p16INK4a is a diagnostic marker for correlating the degrees of cervical dysplasia and neoplasia. It is a surrogate marker for HPV infection, due to its relationship with HPV E7 inactivated RB protein.

p16INK4a Scoring Protocol⁷

p16INK4a expression was associated with distinct nuclear and cytoplasmic staining of epithelial cells and was evaluated using semi quantitative immunohistochemical score (0 to 8points) according to the intensity of staining and the proportion of epithelial cells stained. The total score was the sum of score for stain intensity and the score for proportion of epithelial cells stained.

Interpretation of Results⁷

Nature of Staining	Score
No Staining	0
Weak Staining	1
Moderate Staining	2
Strong Staining	3

Table 1. Depending on The Intensity of Staining (0-3 points)

Score	% Positivity
0	Nil
1	<1% nuclei positivity
2	>1% - 10% nuclei positivity
3	>11% - 33% nuclei positivity
4	34% - 66% nuclei positivity
5	>66 % nuclei positivity

Table 2. Depending on The Proportion of Epithelial Cells Stained (0-5 Points)

RESULTS

In the present study, a total of 2234 Pap smears were studied in the Department of Pathology, Chennai Medical College Hospital and Research Centre, Trichy during the period between July 2013 to July, 2015.

Out of these 2234 Pap smears, the diagnoses “suspicious of malignancy” was given in 41 cases and 1686 cases was diagnosed as inflammatory smears, 495 cases were given as Normal study and 12 cases were unsatisfactory smears.

Sl. No	Lesions	No. of Cases	Percentage
1	NILM	495	22.15%
2	Inflammatory	1686	75.47%
3	Suspicious for Malignancy	41	1.83%
4	Unsatisfactory	12	0.53%

Table 3. Cervical Cytology Diagnosis in 2234 Cases

Sl. No.	Age in Years	No. of Cases	Percentage
1	0-19	1	0
2	20-29	176	8.1%
3	30-39	576	26.5%
4	40-49	769	34%
5	50-59	484	21.4%
6	60-69	188	8.4%
7	70-79	35	1.4%
8	80-89	5	0.2%

Table 4. Age Wise Distribution of Cases

Clinical Presentation	No. of Cases	Percentage
White Discharge Per Vagina (WDPV)	675	30.21%
Postmenopausal Bleeding (PMB)	28	1.25%
Pain Abdomen (PA)	396	17.73%
Nothing Significant (NS)	991	44.36%
Mass Per Vagina (MPV)	74	3.31%
Irregular Bleeding Vagina (IBV)	70	3.13%
Total	2234	100%

Table 5. Various Clinical Presentations in The Present Study

Sl. No.	Lesions	No. of Cases	Percentage
1	Suspicious for Malignancy	41	2.37%
2	Non-Neoplastic	1686	97.63%
	Total	1727	100%

Table 6. Types of Cervical Lesions Based on Pap Smear Study

Sl. No	Lesions	No. of Cases	Percentage
1	LSIL	27	65.85%
2	HSIL	11	26.83%
3	ASCUS	3	7.32%
	Total	41	100%

Table 7. Distribution of 'Suspicious for Malignancy' Lesions

Sl. No	Age in Years	Non-Neoplastic Lesions	
		No. of Cases	Percentage
1	0-19	-	-
2	20-29	133	7.89%
3	30-39	430	25.50%
4	40-49	558	33.1%
5	50-59	372	22.06%
6	60-69	159	9.43%
7	70-79	30	1.78%
8	80-89	4	0.24%
	Total	1686	100%

Table 8. Age Wise Distribution of Non-Neoplastic Lesions on Pap Smear

Sl. No.	Age in Years	Suspicious for Malignancy	
		No. of Cases	Percentage
1	0-19	-	-
2	20-29	-	-
3	30-39	2	4.88%
4	40-49	13	31.71%
5	50-59	17	41.46%
6	60-69	5	12.19%
7	70-79	3	7.32%
8	80-89	1	2.44%
	Total	41	100%

Table 9. Age Wise Distribution of 'Suspicious for Malignancy' on Pap Smears

Sl. No.	Lesions	No. of Cases	%
1	Non-Specific Inflammation	1091	64.71%
2	Reactive Atypia	68	4.03%
3	Atrophic Vaginitis	289	17.14%
4	Endo Cervical Glandular Hyperplasia	2	0.12%
5	Specific Infections	236	14%
	Total	1686	100%

Table 10. Percentage Distribution of Non-Neoplastic Lesions on Cytology

Sl. No.	Lesions	No. of Cases	Percentage
1	Bacterial Vaginosis	139	58.90%
2	Candidiasis	81	34.32%
3	Trichomonas Vaginalis	16	6.78%
	Total	236	100%

Table 11. Percentage Distribution of Specific Infections on Cytology

Sl. No.	Age in Years	Premalignant Lesions					
		LSIL		HSIL		ASCUS	
		No. of Cases	%	No. of Cases	%	No. of Cases	%
1	0-19	-	-	-	-	-	-
2	20-29	-	-	-	-	-	-
3	30-39	1	3.7%	-	-	1	33.33%
4	40-49	9	33.33%	4	36.36%	-	-
5	50-59	12	44.44%	3	27.27%	2	66.67%
6	60-69	2	7.41%	3	27.27%	-	-
7	70-79	2	7.41%	1	9.09%	-	-
8	80-89	1	3.7%	-	-	-	-
	Total	27	100%	11	100%	3	100%

Table 12. Age Wise Distribution in Relation to Neoplasia

Sl. No	Lesions	No. of Cases	Percentage
1	Neoplastic	11	50%
2	Non-Neoplastic	11	50%
	Total	22	100%

Table 13. Histopathological Diagnosis In 22 Cases

Sl. No	Lesions	No. of Cases	Percentage
I	Malignant		36.36%
1	Squamous Cell Carcinoma	3	27.27%
2	Endocervical Adenocarcinoma	1	9.09%
II	Premalignant		
1	CIN I	4	36.36%
2	CIN II/III	3	27.27%
	Total	11	100%

Table 14. Distribution of Neoplasia on Histopathology

p16INK4a	Cervical Lesions			Total
	Cervicitis	CIN	Carcinoma	
Positive %	-	7 31.82%	4 18.18%	11 50%
Negative %	11 50%	-	-	11 50%
Total	11 50%	7 31.82%	4 18.18%	22 100%

Table 15. Incidence of HPV In Different Cervical Lesions on Histopathology

Cytological Diagnosis	Nonspecific Cervicitis	CIN I	CIN II/III	Squamous Cell Carcinoma	Adenocarcinoma	Total
Nonspecific Cervicitis	73	1	-	-	-	74
LSIL	9	4	2	-	-	15
HSIL	1	-	1	3	1	6
ASCUS	1	-	-	-	-	1
Total	84	5	3	3	1	96

Table 16. Comparison of Cytological Diagnosis with Histopathological Diagnosis

DISCUSSION

For the detection of pre-invasive and invasive cervical epithelial abnormalities cervical Pap smears has become a very important screening test. This screening test is a simple, inexpensive and reliable method for screening of female population which greatly reduces the morbidity and mortality associated with cervical carcinoma, if detected in its precancerous stage.⁸

The present study was conducted to investigate the efficacy of Pap test as an initial screening test and confirm the diagnosis by histopathological evaluation which is gold standard in order to assess the presence of changes for cervical cancer. Then the biopsies which were done subsequent to Pap test were subjected to IHC studies for HPV screening and incidence of malignancy related changes.

Two thousand two hundred and thirty-four cases including neoplastic and non-neoplastic lesions were studied and among them 41 cases were suspicious of malignancy, 19 were lost for follow up. 22 cases the Pap smear screening test was compared with the histopathological diagnosis.

CONCLUSIONS

The outcome of this study reveals sensitivity of 91.6% stating that Pap smear is an essential primary tool for screening of cervical cancer. It is also simple and cost-effective procedure that can be performed in the outpatient department.

p16INK4a marker used in this study shows over expression which is noted in all dysplastic lesions of cervix as well as in invasive cervical carcinoma, thus depicting it as a reliable marker for identification of dysplastic and malignant lesions of cervix and also as a surrogate marker for HPV detection.

Thus, combination of Pap smear screening test and confirmation with p16INK4a IHC marker will certainly reduce the high mortality caused due to cervical cancer with underlying HPV infection, as well as alleviate the suffering caused by the disease.

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