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ORIGINAL ARTICLE

Correlation between Cytology, HPV-DNA Test and Colposcopy in Evaluation of Cervical Intraepithelial Lesions

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ABSTRACT

Objectives: The present study was undertaken to correlate cytology, HPV-DNA test and colposcopy in evaluation of cervical intraepithelial lesions.

Materials and methods: Patients were subjected to Pap smear, HPV-DNA detection, colposcopy and directed cervical biopsy if required. The various screening methods were correlated and evaluated by standard statistical methods.

Observations: A total of 324 patients were included in the study. Colposcopy was done in 263, Pap smear in 214, HPV-DNA in 100 and HPE in 116 patients. Sensitivity and specificity of Pap smear test, colposcopy and HPV-DNA testing were calculated to be 66.66, 93.54, 86.84, 86.32, 90 and 84.61% respectively. Their positive predictive value and negative value were 75, 90.60, 67.34, 95.28, 69.23 and 95.66% respectively. The percentage of false negative and false positive were calculated to be 33.33, 6.45, 8.95, 13.67, 10 and 15.38% respectively.

Conclusion: Various screening methods for evaluation of cervical intraepithelial lesions are complimentary to each other and need to be carried out depending on the clinical findings, patient's convenience and compliance, facilities and set-up available.

Keywords: Cytology, HPV-DNA test, Colposcopy, Cervical intraepithelial lesions.

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INTRODUCTION

Cancer of the cervix, in spite of being a preventable disease, continues to be a significant public health problem in females in India. To decrease the burden of this cancer, it is important that cervical intraepithelial lesions are timely diagnosed and treated. In the recent years much new development has taken place in the field of screening, diagnosis and management of cervical intraepithelial neoplasia. Primary cervical cancer screening by cytological examination of cervical cells with Pap smear has reduced the incidence of cervical cancer in countries with organized screening programs. However, several studies have shown that cytology has a limited sensitivity for detecting high grade CIN.[1,2] Several cross-sectional studies have reported that HPV-DNA testing is more sensitive than cytology in detecting high grade CIN.[2,3] On the other hand, several trials have raised concern about the lower specificity of HPV-DNA testing.[2,4] In spite of the introduction of HPV prophylactic vaccine, the various screening programs for carcinoma cervix will have to continue. The present study was therefore undertaken to evaluate and correlate the various methods of screening of cervical intraepithelial neoplasia including Pap smear, colposcopy, HPV-DNA detection. We also utilized this opportunity to counsel patients and create awareness regarding cervical cancer screening and its prevention.

MATERIALS AND METHODS

In the present study, we included patients from the gynecological outpatient department of SMI Hospital on the basis of following inclusion criteria:

1. Women with the complaints of postcoital bleeding, lower abdominal pain, intermenstrual bleeding, low backache, persistent vaginal discharge, vulval itching or burning, persistent dysuria, menstrual irregularities or other complaints.
2. Women with cervical erosions or unhealthy cervix on per speculum examination.
3. Women with history of infertility, abortions, STD/HIV, HSV or vulval warts.
4. Immunocompromised patients.
5. Patients with poor personal hygiene and very poor socioeconomic status.

Patients were briefed about the purpose of the tests to be done on them and written well-informed consent was obtained. These patients were subjected to Pap smear, HPV-DNA detection, colposcopy and directed cervical biopsy if required. If hysterectomy was performed, histopathological examination was done. The various screen-

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ing methods were correlated and evaluated by standard statistical methods.

RESULTS

A total of 324 patients were included in the study. Colposcopy was done in 263, Pap smear in 214, HPV-DNA in 100 and HPE in 116 patients. All four investigations were not done in all the patients due to ethical concerns. Histopathological examination was taken as the gold standard test and various statistical values derived accordingly. Majority (123 patients) of our patients belonged to the age group of 31 to 40 years as can be seen in Table 1. Out of 24 patients with cancer cervix, 14 (58%) were less than 50 years and 10 (42%) were more than 50 years of age. The youngest patient of cancer cervix was of 25 years age.

Out of 214 patients in whom Pap smear was done, the report was NILM in 189 patients, ASCUS in 3, AGUS in 1, LGSIL in 4, HGSIL in 6, invasive carcinoma cervix in 1, atrophic smear in 7 and Pap smear was unsatisfactory in 3 patients. Out of 263 patients in whom colposcopy was done, there were normal findings in 21 patients, 176 had chronic cervicitis, 20 had LGSIL, 8 had HGSIL, 19 had carcinoma cervix, 16 had senile vaginitis. Colposcopy was unsatisfactory in 3 patients. Out of 100 patients in whom HPV-DNA testing was done, 24 had positive result, 73 had negative result and 3 had borderline test result.

Table 2 shows correlation between the various methods. In 40 patients, all four investigations were done. It was seen that all four investigations correlated in 25 (62.5%) patients and did not correlate in 15 (37.5%) patients. Both Pap and HPE were done in 41 patients. Correlation was seen in 29 patients (70.73%), not seen in 10 patients (24.39%) and Pap smear was unsatisfactory in 2 patients (4.87%). Both colposcopy and HPE were done in 71 patients. Correlation was seen in 65 (91.55%) and not seen in 6 (8.45%). Both HPV-DNA and HPE were done in 72 patients. It was seen that there was correlation between HPV-DNA and HPE in 35 out of 41 (85.36%) patients with chronic cervicitis and 18 out of 20 (90%) patients with HGSIL and invasive cervical cancer. Both colposcopy and HPV-DNA were done in 95 patients, out of which correlation was seen in 85 (89.5%) and not seen in 10 (10.5%) patients. Both cytology and HPV-DNA were done in 61 patients. Correlation was seen in 50 (82%), not seen in 9 (15%) patients and cytology was unsatisfactory in 2 (3%) patients. Both cytology and colposcopy were done in 159 patients. Correlation was seen in 145 (92.36%), not seen in 12 (7.64%) patients and cytology was unsatisfactory in 2 patients.

Sensitivity, specificity, positive predictive value, negative predictive value, percentage of false negative and false positive of HPV-DNA testing, cytology and colposcopy have been shown in Table 3. HPV-DNA test was found to be most sensitive (90%) but less specific (84.61%) while Pap test was found to be less sensitive (66.66%) but most specific (93.54%). Sensitivity and specificity of colposcopy (86.84%; 84.32%) was almost comparable to that of HPV-DNA test (90%; 84.61%).

Table 1: Age wise distribution of patients

Age (years)	*No. of patients (%)*
20-30	55 (17)
31-40	123 (38)
41-50	94 (29)
51-60	35 (11)
>60	17 (5)
Total	324

DISCUSSION

Many studies have been carried out in the past regarding the accuracy of various screening tests of cervical cancer. Different authors have given variable opinion regarding sensitivity and specificity of these tests. SuFang et al evaluated the sensitivity and specificity of visual inspection, colposcopy, liquid based cytology and HPV-DNA assay.[5] Liquid-based cytology was superior in specificity (98.63%) and positive predictive value (92.86%) to visual

Table 2: Correlation between various methods

Correlation	*All four methods*	*Pap/HPE*	*Colpo/HPE*	*HPV/HPE*		*Colpo/HPV*	*Cyto/HPV*	*Colpo/Cyto*
				Chronic cervicitis	*HGSIL*			
Present	25 (62.5%)	29 (70.73%)	65 (91.55%)	35 (85.36%)	18 (90%)	85 (89.5%)	50 (82%)	145 (92.36%)
Absent	15 (37.5%)	10 (24.39%)	6 (8.45%)	6 (14.63%)	2 (10%)	10 (10.5%)	9 (15%)	12 (7.64%)
Total (patients)	40	41*	71	41	20	95	61*	159*

*Pap smear was unsatisfactory in two patients

Table 3: Statistical comparison of various methods

	Sensitivity	*Specificity*	*PPV*	*NPV*	*False (–ve)*	*False (+ve)*
Cytology	66.66%	93.54%	75%	90.60%	33.33%	6.45%
Colposcopy	86.84%	86.32%	67.34%	95.28%	8.95%	13.67%
HPV-DNA	90%	84.61%	69.23%	95.66%	10%	15.38%

inspection and colposcopy, while human papillomavirus DNA testing was superior in sensitivity (88.89%) and NPV (97.10%) to visual inspection and coloscopy. The best concordance with histologic findings was achieved by using both liquid-based cytology and viral DNA hybridization. The authors concluded that visual inspection and colposcopy should not be used when screening for early stage cervical lesions. The HPV-DNA assay is the best choice in primary screening, if available.

A study was carried out by Maria A et al on 62 women to evaluate the efficacy of four methods of risk assessment namely cytology, colposcopy, HPV molecular typing and detection of biomarkers (immunohistochemical identification of p16, p53, Bcl-2) in cervical biopsies in an attempt to define the most efficient combination.[6] Cytology and colposcopy showed very high sensitivity in detecting CIN and cancer (91.7% and 94.4%, respectively) but low specificity (34.6 and 50%, respectively). The detection of the 3 biomarkers reached an impressive sensitivity (83.3%) and a moderate specificity (65.4%). HPV detection and typing achieved 77.8% sensitivity, and the highest specificity of 80% in detecting CIN and cancer cases. Coupled HPV typing and colposcopy proved to be the most efficient combination, increasing sensitivity to 97.2% and negative prognostic value to 92.3%. Sensitivity and specificity of cytology and colposcopy in their study did not correlate with most of the other studies including our study.

In a cross-sectional study in India, naked eye visual inspection with acetic acid (VIA), magnified VIA (VIAM), visual inspection with lugol's iodine (VILI), cytology and HPV testing were evaluated as screening methods for the detection of high-grade squamous intraepithelial lesions (HSIL).[7] The sensitivities of cytology, HPV testing VIA,VIMA and VILI were 57.4, 62.0, 59.7, 64.9 and 75.4%, respectively (differences were not statistically significant). The specificities were 98.6, 93.5, 88.4, 86.3 and 84.3%, respectively. This is almost comparable to our study in which the sensitivity and specificity of cytology and HPV DNA testing were 66.66, 93.54, 90 and 84.61% respectively. The authors concluded that as a single test, cytology had the best balance of sensitivity and specificity. Visual tests are promising in low-resource settings. The use of both VIA and VILI may be considered where good quality cytology and HPV testing is not feasible. The sensitivity of cytology and HPV testing increased significantly when combined with VIA or VILI.

In another study, the efficacy of cytology was compared with colposcopy in detecting cervical lesions.[8] Cytology had a good specificity of 82.6% but sensitivity was 57.7%. False negative rate was 22.4% and false positive rate 17.4%. Colposcopy had a sensitivity of 84.6% and specificity of 43.4%. The false negative rate was 15.4% and false positive rate 56.4%. In this study all three modalities correlated in 36 of 78 cases (46.15%). Therefore, according to the author, there is a necessity for combination of tests to maximize the sensitivity of cervical screening. In our study, all four modalities ie cytology, colposcopy, HPE, and HPV-DNA correlated in 25 out of 40 (62.5%) patients.

Most of the initial studies concentrated on the role of cytology and colposcopy for screening against cervical cancer. But over the past decade, it has been seen that molecular testing for HPV has emerged as a highly sensitive screening method. In 2004, the International Agency of Research on Cancer (IARC) observed that an appropriate test is needed for low-resource settings and recommended that any such test should be carefully evaluated in demonstration projects.[9] Keeping this in mind, we included HPV-DNA testing also in our study, as most of the studies have not compared all the methods at a time. Most of the studies over the last decade have focused on HPV-DNA testing as a screening method.

Pajtler found that in comparison with repeat cytology, HPV-DNA test showed higher sensitivity (69.2% *vs* 61.5%) but significantly lower specificity (63.2% *vs* 93.0%) and positive predictive value (30.0% *vs* 66.7%), and comparable negative predictive value (90.0% *vs* 91.4%) in predicting histologically verified CIN3.[10] The authors concluded that HPV testing is of limited value in daily routine and should not be widely used until it is definitely demonstrated to be superior to conventional methods in improving the sensitivity, specificity and predictive value of CIN3 and invasive carcinoma detection. On the other hand, Kunh et al suggested that HPV-DNA testing should be considered for primary cervical cancer screening in low resource setting as HPV-DNA testing programs may be easier to implement than cytological screening.[11]

Our hospital caters to difficult hilly terrain and remote areas of Garhwal region. Most of the women are noncompliant and get lost to follow-up. Therefore, in our study many women had only colposcopy as this is the only test which gives immediate results and we did not want to miss the opportunity to screen our women. Sensitivity of a single Pap smear test in our study was 66.66% but sensitivity of repeat annual Pap test is considered to be more. Its specificity was 93.54% and percentage of false positive was only 6.45% thereby not leading to unnecessary treatment. Sensitivity of liquid based cytology is found to be better and this will gradually replace conventional testing. Moreover, HPV-DNA testing can be done with the same sample if indicated and inconvenience due to repeat sampling is avoided. In our study, colposcopy and HPV-DNA testing had comparable sensitivity and specificity of 86.84, 86.32; 90 and 84.61% respectively. We found that in patients with HGSIL results, majority (90%) had HPV-DNA test positive and required colposcopy. So,

in these patients, HPV-DNA test may be deferred and they can be directly referred for colposcopy. This is a more convenient and cost-effective approach. In view of its high negative predictive value, HPV-DNA test may be used for secondary screening in patients with abnormal (ASC) results as this will help in further management.

CONCLUSION

Various methods for evaluating cervical intraepithelial lesions are complimentary to each other and need to be carried out depending on the clinical findings, patient's convenience and compliance, facilities and set-up available.

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