

A PROSPECTIVE COMPARATIVE STUDY BETWEEN PAP SMEAR, VISUAL INSPECTION WITH ACETIC ACID, VISUAL INSPECTION WITH LUGOL'S IODINE, COLPOSCOPY AND HISTOPATHOLOGY FOR DIAGNOSIS OF CERVICAL INTRAEPITHELIAL NEOPLASIA AND EARLY CARCINOMA CERVIX

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Abstract

Carcinoma cervix is an important reproductive health problem in women and a leading cause of morbidity and mortality of women from cancer in the developing world. Invasive cervical cancers are preceded by a long phase of precancerous lesions which can be detected by screening and treated effectively by simple curative methods, thus preventing invasive cancer. The present study is aimed to compare the diagnostic accuracy of Pap smear, Visual Inspection with Acetic Acid (VIA) and Lugol's Iodine (VILI), colposcopy and histopathology for screening of premalignant and malignant lesions of the cervix. The study was a hospital based comparative observational study done on 130 married women in the age group of 18-60 years attending the Gynaecology ward. Papanicolaou's smear was taken of patients included in the study and the cervix was visually inspected using 5% glacial acetic acid and Lugol's iodine. The cervix of these patients was examined with colposcope. Biopsy was taken of patients showing acetowhite lesions on VIA, mustard yellow stained areas on VILI and cases with Swede score ≥ 5 on colposcopy. Sensivity, specificity and accuracy were calculated to analyze the diagnostic value of colposcopy, visual inspection methods and pap smear. The results were compared with that of histopathology of cervix, which is the gold standard.

Introduction

Carcinoma cervix is a grave gynaecological health problem in women. It is the fourth most common cancer worldwide with an estimated incidence of 5,28,000 new cases and 2,66,000 deaths. Cervical cancer ranks as the second most common cause of cancer deaths in Indian women and contributes 23.2% and 25.2% to the global cervical cancer incidence and mortality respectively. Despite its public health importance, there are no effective prevention measures in most developing countries and hence the risk of disease and death from cervical cancer remains largely uncurbed.

The most important risk factor for carcinoma cervix is Human Papilloma Virus (HPV) infection. There are over 100 different types of human papilloma viruses but not all types of HPV are carcinogenic. Most of the patients infected with HPV are asymptomatic. These infections are usually temporary and resolve within two years in

women with strong immunity. When the virus persists, there is a chance of developing pre malignancy and malignancy. However, it usually takes many years for HPV infection to cause cervical cancer. Invasive cervical cancers are preceded by a long phase of precancerous lesions which can be detected by screening and treated effectively by simple curative methods, thus preventing invasive cancer. Early detection and treatment of premalignant lesions results in down staging of cancer, which ultimately results in decreasing burden of cervical cancer in our country and the world. The first screening test widely used for cancer cervix was Papanicolaou's smear, the conventional cytological test. The American Cancer Society (ACS) promoted the Pap test during the early 1960s and achieved a decline in death rate due to cervical cancer by about 70% in the United States.

Several other screening tests are available for early detection of cervical cancer and its precursor lesions which include liquid based cytology, automated Pap smear testing, visual inspection methods and human papilloma virus (HPV) testing. Each screening test has its own advantages and disadvantages. Papanicolaou's smear has been the mainstay of cancer cervix screening since 1950s. But its sensitivity ranges from 30 to 87%. also requires multiple visits by the women. HPV testing is expensive and hence it cannot be used for screening programmes in low resource settings. Visual based screening methods, on the other hand, are low cost screening methods and require simple equipment and brief training. The present study is aimed to compare the efficacy of the Papanicolaou's smear, colposcopy, visual inspection with acetic acid (VIA), visual inspection with Lugol's iodine (VILI) and histopathology of cervical punch biopsy for screening of premalignant and malignant lesions of the cervix.

Methods

The study was a hospital based comparative observational study done on 130 married women in the age group of 18-60 years attending the Gynaecology OPD of the Dr. D.Y. Patil Hospital, Pimpri, Pune. Papanicolaou's smear was taken of each patient and the cervix was visually inspected using 5% glacial acetic acid and Lugol's iodine. The cervix was then examined with colposcope. Biopsy was taken of patients showing acetowhite lesions on VIA, mustard yellow stained areas on VILI and cases with Swede score ≥ 5 on colposcopy. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated to analyse the diagnostic value of colposcopy, visual inspection methods and Pap smear. The results were compared with that of histopathology of cervix, which is the gold standard.

Objectives:

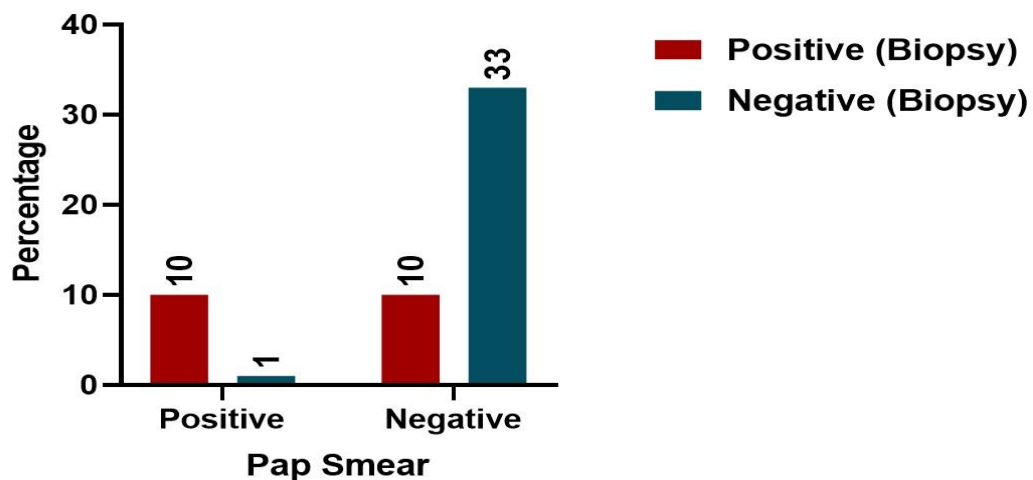
1. To do Pap smear of patients included in the study and to inspect the cervix visually using 5% acetic acid and Lugol's iodine followed by biopsy in patients showing acetowhite lesions on VIA, mustard yellow stained areas on VILI and cases with Swede score ≥ 5 on colposcopy.
2. To compare the results of pap smear, colposcopy, visual inspection of cervix with acetic acid and Lugol's iodine and histopathology for diagnosing premalignant and malignant lesions of cervix.

The study was approved by the Ethics and Scientific Committee of the hospital. A brief history of all the patients enrolled in the study was taken after obtaining an informed consent and ensuring them about confidentiality. Cervix was visualized under good lighting with an unlubricated Cusco's bivalve speculum and transformation zone was scraped with an Ayre's spatula. The sample was then smeared separately on two clean slides and fixed with 95% ethyl alcohol for at least 15 minutes before transportation to the laboratory for Papanicolaou staining. Reporting of Pap's smear was done as per 2014 Bethesda System. After taking the pap smear, the cervix was painted with a cotton swab soaked in 5% glacial acetic acid and it was examined 2 minutes after the application. Later the cervix was painted with Lugol's iodine. All suspicious or visible lesions which appeared after the application of acetic acid or Lugol's iodine abutting the squamocolumnar junction were then biopsied. Tissues were sent in formalin to the laboratory where they were processed and read by the pathologist. Histopathology of cervical biopsy was taken as the gold standard. Patients with abnormal results were referred for proper follow up and management in the gynaecology OPD. Sensitivity, Specificity, Positive Predictive Value, Negative Predictive

Value and Accuracy were calculated to analyse the diagnostic value of pap smear, colposcopy, visual inspection with acetic acid, visual inspection with lugol's iodine and histopathology of cervical biopsy.

Results

Out of the 130 patients in whom we did pap smear, there was a positive result in 11 of the patients, who's showed moderate dysplasia(HSIL).10 of these patients were biopsy positive and 1 was biopsy negative. 43 patients were pap negative out of which 10 were biopsy positive and 33 were biopsy negative. Thus, the sensitivity and specificity of pap smear were found to be 50% and 97% respectively. The positive and negative predictive values were 90.9% and 76.7% respectively. The overall accuracy of pap smear was found to be 79.6%.



Pap Smear	Biopsy		Total
	Positive	Negative	
Positive	10	1	11
Negative	10	33	43
Total	20	34	54

Sensitivity: $10/20 = 50\%$

Specificity: $33/34 = 97\%$

Positive Predicted Value: $10/11 = 90.9\%$

Negative predictive Value: $33/43 = 76.7\%$

False Positive: $1/34 = 0.02\%$

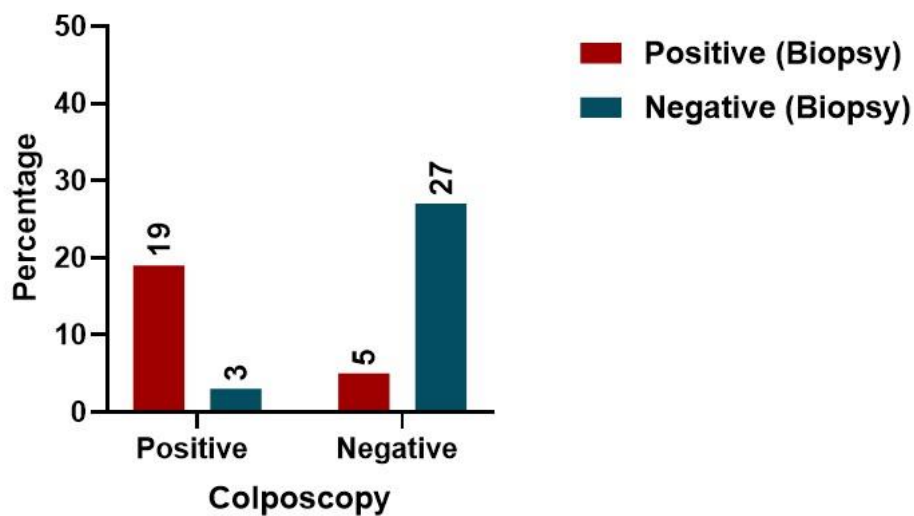
False Negative: $10/20 = 50\%$

Accuracy: 43/54 = 79.6%

Colposcopy was performed on all 130 women. Colposcopic findings were reported positive according to Swede's score (≥ 5). Biopsy was taken of patients showing acetowhite lesions on VIA, mustard yellow stained areas on VILI and cases with Swede score ≥ 5 on colposcopy. The commonest finding on colposcopy was cervical erosion. There were 2 cases with Swede score of ≥ 7 , which showed squamous cell carcinoma of the cervix on histopathological examination.

Swede's Score

Swede' score	Impression	Total
Upto 4	Low grade/normal(LSIL/WNL)	32
5-6	High grade/non-invasive cancer(CIN 2+)	17
7-10	High grade/suspected invasive cancer(CIN 2+)	5
		54



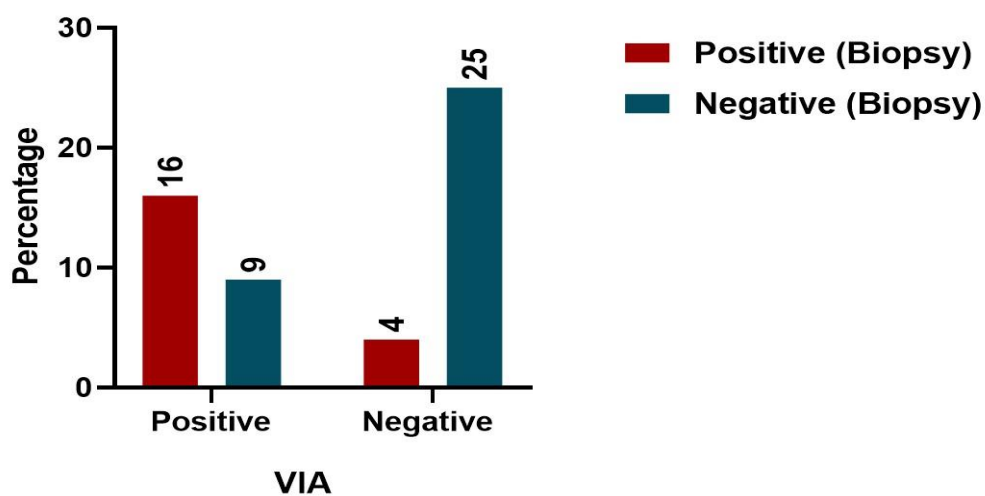
Colposcopy	Biopsy		Total
	Positive	Negative	
Positive	19	3	22
Negative	5	27	32
Total	24	30	54

Sensitivity: $19/24 = 79\%$
Specificity: $27/30 = 90\%$
Positive Predicted Value: $19/22 = 86.3\%$
Negative predictive Value: $27/32 = 84.3\%$
False Positive: $3/30 = 10\%$
False Negative: $5/24 = 20.8\%$
Accuracy: $46/54 = 85.1\%$

The sensitivity and specificity of colposcopy were found to be 79% and 90% respectively. The positive and negative predictive values were 86.3% and 84.3% respectively. The overall accuracy of colposcopy was found to be 85.1%.

VIA was done in all 130 patients. Out of the 54 patients in whom biopsy was taken, there was a VIA positive result in 25 of the patients, out of which 16 patients were biopsy positive and 9 were biopsy

negative. 29 patients showed negative VIA result out of which 4 were biopsy positive and 25 were biopsy negative. Thus, the sensitivity and specificity of VIA were found to be 80% and 73.5% respectively. The positive and negative predictive values were 64% and 86.2% respectively. The overall accuracy of VIA was found to be 75.9%.



VIA	Biopsy		Total
	Positive	Negative	
Positive	16	9	25
Negative	4	25	29
Total	20	34	54

Sensitivity: $16/20 = 80\%$

Specificity: $25/34 = 73.5\%$

Positive Predicted Value: $16/25 = 64\%$

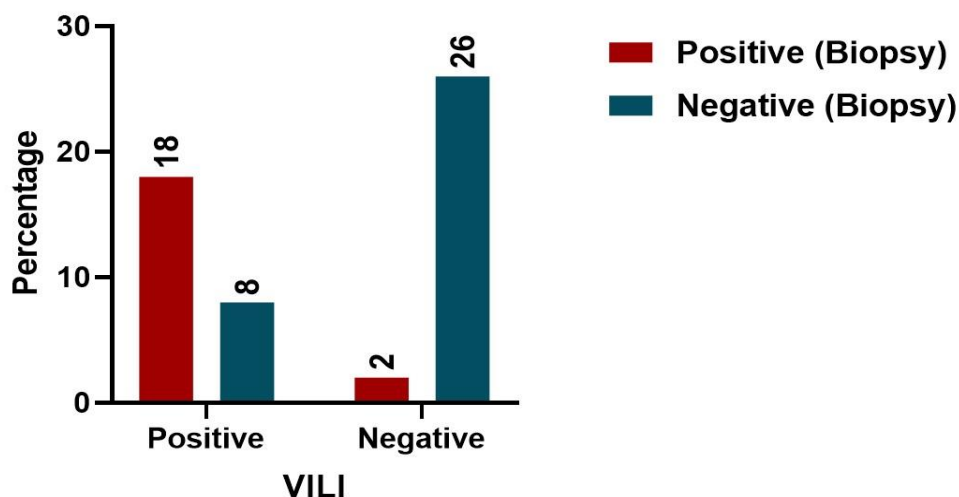
Negative predictive Value: $25/29 = 86.2\%$

False Positive: $9/34 = 26.4\%$

False Negative: $4/20 = 20\%$

Accuracy: $41/54 = 75.9\%$

VILI was done in all 130 patients. Out of the 54 patients in whom biopsy was taken, VILI was found to be positive in 26 patients out of which 18 patients were biopsy positive and 8 were biopsy negative. 28 patients showed negative VILI result out of which 2 were biopsy positive and 26 were biopsy negative. Thus, the sensitivity and specificity of VILI were found to be 90% and 76.4% respectively. The positive and negative predictive values were 69.2% and 92.8% respectively. The overall accuracy of VILI was found to be 81.4%.



VILI	Biopsy		Total
	Positive	Negative	

Positive	18	8	26
Negative	2	26	28
Total	20	34	54
Sensitivity: $18/20 = 90\%$ Specificity: $26/34 = 76.4\%$ Positive Predicted Value: $18/26 = 69.2\%$ Negative predictive Value: $26/28 = 92.8\%$ False Positive: $8/34 = 23.5\%$ False Negative: $2/20 = 10\%$ Accuracy: $44/54 = 81.4\%$			

Discussion

The sensitivity of pap smear in our study is 50% which is comparable to the sensitivity values in the studies of Bhattacharya et al(52%)and Sharma et al(54.5%).The specificity in our study is 97% which is almost equal to the specificity values in the studies done by Bhattacharya et al(95%), Karimi-Zarchi(98.5%),Sharma et al(100%).The sensitivity of pap smear in our study was low(50%) as compared to the high specificity(97%) owing to the large number of false negatives.

Pap smear	This study	Karimi-zarchi et al, 2015^[7]	Bhattacharya et al, 2015^[8]	Sharma et al, 2020^[9]
Sensitivity	50%	18.2%	52%	54.5%
Specificity	97%	98.5%	95%	100%
PPV	90.9%	85.5%	45%	100%
NPV	76.7%	71.3%	96%	93.1%
Accuracy	79.6%	72.2%	93%	89.1%

Majority of pap smears turned out to be inflammatory. Patients were considered to have persistent inflammatory cervical smear (PICS),if the cervical smear repeated after 3 months of antimicrobial treatment reported again as inflammatory. PICS seem to have some contributory effect in addition to other influencing factors studied like age, parity, duration of marital life and interplay of contraceptive non-usage for the causation of CIN. Hence, it is recommended that a woman with PICS should be subjected to cervical biopsy for histopathological scrutiny, especially if she is above 30 years, sexually active for 14 years and is at least a third para.¹⁰

Colposcopy	This study	Karimi-zarchi et al., 2015	Hol K Mishra et al, 2019^[11]
Sensitivity	79%	56.2%	83.33%
Specificity	90%	67.1%	78.57%
PPV	86.3%	16.9%	68.96%
NPV	84.3%		
Accuracy	85.1%		

Colposcopy in our study had a sensitivity of 79%, which was comparable to the work of Hol K. Mishra et al(83.3%). In contrast to the specificities in the investigations by Karimi-Zarchi et al. (67.1%) and Hol K Mishra et al. (78.57%), the specificity of colposcopy in our study was 90%. Colposcopy was shown to have a high NPV (84.3%) and accuracy (85.1%) according to our study. This suggests that colposcopy is a very specific test with a high value for diagnosing cervical premalignant lesions.

VIA	This study	Consul et al 2008^[12]	Bhattacharyya et al 2015	Sharma et al 2020
Sensitivity	80%	84.2%	89%	81.8%
Specificity	73.5%	55.2%	87%	88.1%
PPV	64%	55.2%	32%	52.9%
NPV	86.2%	84.20%	99%	96.7%
Accuracy	75.9%		87%	

Our study's VIA sensitivity of 80% is comparable to earlier studies' VIA sensitivities of 84.2%, 89.%, and 81.8% conducted by Consul et al., Bhattacharya et al., and Sharma et al respectively. Our study's VIA specificity(73.5%) is lower than that of other research, including Bhattacharya et al (87%)and Sharma et al(88.1%).When compared to the figure in the study by Bhattacharya et al. (87%), the accuracy of VIA was found to be less accurate at 75.9%.

VILI	This study	Consul et al,2008	Sharma et al,2020

Sensitivity	90%	89.5%	90.9%
Specificity	76.4%	75.9%	83.6%
PPV	69.2%	70.8%	47.6%
NPV	92.8%	91.7%	98.2%
Accuracy	81.4%		

The sensitivity of VILI was 90%, which is nearly on par with studies done by Consul et al. (89.5%) and Sharma et al. (90.9%). Also, VILI has a specificity of 76.4%, which is about the same as that of Consul et al. (75.9%). When compared to Sharma et al value, (83.6%), it is slightly lower. Be-cause VILI's overall accuracy was dete 81.4%,it is a good screening tool for premalignant

	Sensitivity	Specificity	PPV	NPV	Accuracy
Pap smear	50%	97%	90.9%	76.7%	79.6%
Colposcopy	79%	90%	86.3%	84.3%	85.1%
VIA	80%	73.5%	64%	86.2%	75.9%
VILI	90%	76.4%	69.2%	92.8%	81.4%

Conclusion

The sensitivity of Pap smear, colposcopy, VIA and VILI were 50%,79%,80% and 90% respectively. Thus, VILI was the most sensitive among the four methods. The sensitivity of pap smear is low (50%) when compared to colposcopy and visual inspection methods which can be attributed to the high number of false negatives.The sensitivity of pap smear can be improved by combining it with visual inspection methods such as VIA and VILI. Due to the high loss to follow-up rates following pap smear, performing VIA and VILI at the same session may be advantageous to the patient and time-saving. The specificity of Pap smear, colposcopy, VIA and VILI were 97%,90%,73.5% and 76.4% respectively. Thus, pap smear was the most specific amongst the four methods. The lower specificity of VIA when compared to pap smear can be attributed to the high incidence of suspected acetowhite epithelium which might be inflammation, immature metaplasia or latent HPV infection. The Pap test, colposcopy, VIA, and VILI had accuracy rates of 79.6%, 85.1%,75.9%, and 81.4%, respectively. Thus, colposcopy was the most accurate owing to its high sensitivity and specificity making it the best screening technique among the four compared in this study.While interpreting values from different studies, we might take into consideration that the performance of colposcopy largely depends on training, experience and skills of the colposcopist and accuracy of cytology requires laboratory services and skilled cytologists. The limitation of our study was the limited sample size. Hence, the inclusion of a larger number of cases would yield more accurate results.

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