DOI:10.24041/ejmr2022.02

A COMPARATIVE STUDY ON CERVICAL CANCER SCREENING METHOD: IS IMMUNO-MARKER A BETTER OPTION?

Tradeep Saluja*, Tamkin Khan, Shaista Manan Vasenwala***, Eram Ali****, Ayesha Ahmad*****

Department of Obstetrics & Gynaecology, Delhi Cantonment General Hospital, Delhi, India-110010*.

Department of Obstetrics & Gynaecology, JN Medical College, AMU, Aligarh, U.P., India-202002**.

Department of Pathology, JN Medical College, AMU, Aligarh, U.P., India-202002***.

Department of Obstetrics & Gynaecology, Rao Tularam Memorial Hospital, Delhi, India-110073****.

Department of Obstetrics & Gynaecology, Era's Lucknow Medical College & Hospital, Era University, Sarfarazganj, Lucknow, U.P., India-226003*****.

Received on : 22-04-2022
Accepted on : 16-06-2022

Address for correspondence

Dr. Ayesha Ahmad
Department of Obstetrics & Gynaecology
Era's Lucknow Medical College &
Hospital, Era University, Lucknow-226003.
Email: docayeshaahmad@gmail.com
Contact no: +91-8800807090

ABSTRACT

Cervical cytology by Papanicolaou (PAP) staining has been the backbone of primary screening of cervical cancer. For low resource countries, the major constraints in running a successful screening program are paucity of experienced personnel, requirement for multiple visits, economic considerations and inherent attributes of the test including a low sensitivity and specificity. The present study was designed to compare the efficacy of commonly available screening tests and feasibility of immuno-markers (p16 & Ki-67) as a primary screening tool. This was a cross-sectional analytical study. 100 patients were approached and agreed to participate in this study. They underwent cervical screening by multiple modalities including PAP smear, Liquid based cytology (LBC), HPV- DNA testing, cytology + HPV- DNA/p16/ Ki-67 and dual markers. Screening test results were compared with histopathology and statistical analysis done. The sensitivity and specificity of conventional cytology was 61.11% and 70%; LBC: 88.88% and 50%; Cytology + HPV DNA: 94.44% and 50%; Cytology + p16: 88.88% and 60%; Cytology + Ki-67: 88.88% and 100%; P16+Ki67: 88.88% and 60%. Combining Ki-67 with Conventional cytology improved specificity and positive predictive value of cervical cancer screening.

KEYWORDS: Carcinoma cervix, Immuno-markers, Screening tests, p16, Ki-67, Cervical screening.

INTRODUCTION

Cervical cancer screening is commonly done by Papanicolaou (PAP) smear and Human Papilloma Virus (HPV) DNA assay. PAP smear has been credited with reducing the incidence of cervical cancer by 79% and mortality by 70%, its efficacy is hampered by inter observer and intra-observer bias which leads to high false negative and false positive rates (1). On the other hand, HPV DNA testing though shows better sensitivity in comparison to PAP smear, it fails to discriminate between transient and chronic infection thus leading to poor specificity. This differentiation is of vital importance as persistent infections predispose to cervical cancer.

In order to overcome these limitations and improve predictive values, Ki-67 and p16 biomarker assays have been investigated. Ki-67 is a nuclear and nucleolar protein which is expressed during G1, S, G2, and M phase of cell cycle, while not being present in resting cells (G0 phase). Therefore, it serves as a surrogate marker of cell growth fraction. While its exact function remains unclear, the expression appears to be mandatory for progression through cell cycle (2-3). Increased epithelial cell proliferation in HPV infected cells leads to increased Ki-67 staining.

p16 is a cyclin-dependent kinase inhibitor which has been noted to prevent phosphorylation of retinoblastoma protein (*RB*). Its expression is maintained at low concentration in normal cells by negative control of *RB1* gene product. In HPV-associated tumors, RB gene is functionally inactivated by hr-HPV E7 oncoprotein, thus resulting in over expression of p16 (4). This has been utilized by researchers in distinguishing true dysplasia from mimics.

Aim of the Study

The present study was done to find out if addition of biomarkers to traditional screening tests improves efficacy of cervical cancer screening. We compared results of conventional PAP smear, HPV DNA, LBC, Ki-67 and p16 in a sub-set of North Indian population.

Rationale for the Study

Cervical cancer screening requires development of an effective test with high levels of sensitivity and specificity. The most commonly used tests - Conventional PAP and LBC- have low sensitivity and specificity thus leading to frequent missed diagnosis. HPV test has high sensitivity but low specificity due to which there is an increase in diagnostic testing, unnecessary colposcopy referral and over-treatment of benign lesions. Addition of biomarkers such as Ki-67 and p16 has been theorized to help in differentiation of true dysplasia from benign mimics.

METHODOLOGY

Research Design

The present study was a cross-sectional analytical pilot project, conducted at Jawaharlal Nehru Medical College, Aligarh over a period of two years. Prior institutional ethical clearance was obtained.

Inclusion and Exclusion Criteria

All sexually active women more than 18 years of age, attending Gynaecology OPD were offered the choice of recruitment. The exclusion criteria included vaginal bleeding, frank cervical malignancy, and pelvic organ prolapse.

Recruitment of Subjects

Using purposive sampling techniques, 100 women were recruited to the study after detailed informed consent. As it was a pilot study, the sample size was not predetermined.

Sample Collection and Testing

- PAP smear was graded by Bethesda system. Histopathology of formalin fixed cervical biopsy was carried out, paraffin embedded tissue processed and stained with Haematoxylin and Eosin (H&E) and studied under magnification.
- HPV DNA was isolated from residual LBC sample by 'Pure Link Genomic DNA mini Kit (Manufacturer: Invitrogen, USA) stored at -20ºC. HPV detection was done using PGMY09/11 primers which can amplify 450bp HPV L1 gene fragment. PCR products were confirmed for their respective amplicon size on 2% Agarose Gel Electrophoresis and visualized by Gel Documentation System (Biorad, USA).
- For Ki-67 and p16, sample was collected with Ayre's spatula and Cytobrush, slides prepared and fixed. p16 and Ki-67 scoring was done by a semi-quantitative scoring system. Table 1 (5-6).

nuclearand/orcytoplasmic	**Intensity of staining**	**Proportion of positive staining**
	0–None	0–None
	1-weak	1-<1%
	2–moderate	2-1-10%
	3-strong	3-11-33%
		4-34-66%
		5->66%
K167scoringsystem (6)	**% of positive staining**	**Score**
	<10%	0
	10-30%	1
	30-50%	2
	>50%	3

Table 1: p16 and K167 Scoring Systems[5]

Analysis of Data:

Descriptive data is presented as frequencies and percentages. The screening test results are compared with histopathological results and presented as sensitivity, specificity, PPV and NPV.

Results

Socio-demographic profile showed that maximum subjects ranged from 31 to 40 years of age, belonged to lower socio-economic status and had a parity between 2-4. (Table 2)

Age	Negative for intraepithelial Lesion or Malignancy	Cervical intraepithelial Lesion or Higher Lesions	Total
20-30	05	01	06
30-40	05	04	09
40-50	02	06	08
50-60	01	03	04
60-70	0	01	01
Parity			
P0-1	04	01	5
P2-4	07	08	15
P4-6	01	07	08
Religion			
Muslims	09	07	16
Non-muslims	05	07	12
Socio-economic status			
Class-lll	06	03	09
Class-lV	04	07	11
Class-V	03	05	08

Table 2: Socio-demographic Characteristics of Study Population

Figure 1 details the recruitment process. 68 subjects with a normal PAP smear who had a negative result on Visual Inspection of Cervix after application of acetic acid (VIA) were excluded for the purpose of evaluation. Out of the remaining 32 cases, 4 women who had normal PAP and negative VIA but clinically suspicious cervix did not agree for further screening. Of the remaining 28 cases, the following tests were done: Liquid Based Cytology (LBC), HPV DNA testing, Immuno-markers (p16 and Ki-67) and Histopathology (HPE). 18 cases were found abnormal on HPE.

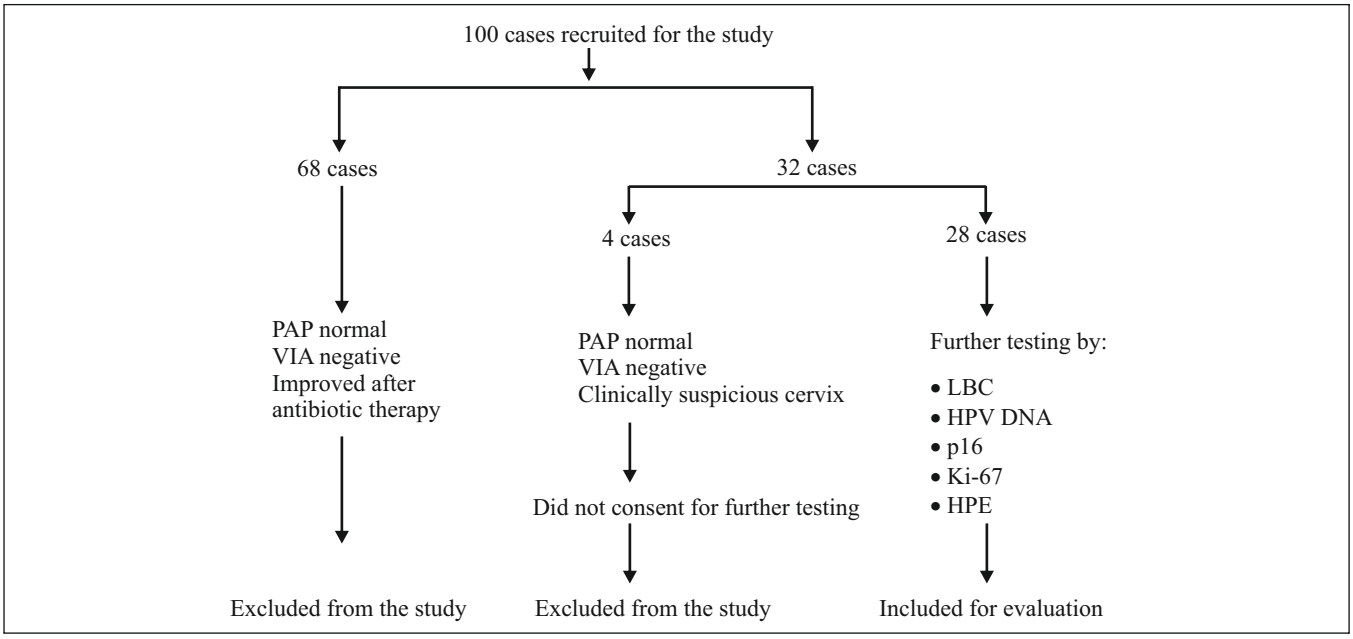

Fig. 1: Recruitment of Cases to the Study

Table 3 and 4 summarize the statistical findings. HPE was taken as the gold standard for diagnosis.

HPE	**Screening Test**	**True Positive**	**False Positive**
HPE Positive n=18	Conventional Cytology	11	03
	Liquid Based Cytology	16	0
	HPV-DNA	15	05
	HPV+cytology	17	05
	Cytology+p16	16	04
	Cytology+Ki-67	16	0
	Dual markers (p16+Ki-67)	16	04
HPE Negative n=10	**Screening Test**	**True Negative**	**False Negative**
	Conventional Cytology	07	07
	Liquid Based Cytology	10	02
	HPV-DNA	05	03
	HPV+Cytology	05	01
	Cytology+p16	06	02
	Cytology+Ki-67	10	02
	Dual markers (p16+Ki67)	06	02

Table 3: Comparison of Cervical Screening Tests with HPE

Screening test	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Conventional Cytology	61.11	70	78.57	50
LBC	88.88	100	100	83.33
HPV DNA Testing	83.33	50	75	62.5
Cytology and HPV DNA Testing	94.44	50	77.27	83.33
Cytology and P16	88.88	60	80	75
Cytology and Ki-67	88.88	100	100	83.33
Cytology and P16 and Ki67	88.88	60	80	50

Table 4: Comparing Efficacy of Screening tests with HPE

***Conventional cytology*:** 14/28 cases were detected positive out of which 11 cases were abnormal on HPE. 7/14 cases found normal on conventional cytology had CIN (4 CIN-1, 3 CIN-3). The sensitivity was calculated as 61.11%, specificity 70%, PPV 78.6% and NPV 50%.

***Liquid Based Cytology*:** 16/28 cases were detected positive. 2 cases found negative on LBC showed CIN on HPE (1 case CIN-1, 1 case CIN-2). The sensitivity and specificity were calculated as 88.8% and 100% respectively with a PPV of 100% and NPV of 83.3%.

HPV DNA: 20/28 cases were found abnormal. 3/28 cases found negative had CIN (2 cases of CIN-2; 1 case of CIN-1). 15/20 were true positives. The sensitivity and specificity were calculated as 83.3% and 50% respectively with a PPV of 75% and NPV of 62.5%.

***Cytology + HPV DNA*:** 22/28 cases were found abnormal. 17/22 were abnormal on HPE. The sensitivity was 94.4%, specificity 50%, PPV 77.7% and NPV 83.3%.

***Cytology + p16*:** 20/28 cases were abnormal. 16/20 had abnormal HPE whereas 4/20 were normal. The sensitivity was calculated as 88.8%, specificity 60%, PPV 80% and NPV 75%.

***Cytology + Ki67*:** 16/28 cases were abnormal. All cases had abnormal HPE. There were no false positive cases. 2 cases were missed; one was positive for CIN-1 and one for CIN-2. The sensitivity was calculated as 88.8%, specificity 100%, PPV 100% and NPV 83.3%.

Cytology + P16 + Ki67: 20/28 cases were abnormal; 16 were confirmed abnormal on HPE. 2 cases were missed: one had CIN-1 and one had CIN-2. The sensitivity was found to be 88.8%, specificity 60%, PPV 80% and NPV 50%.

DISCUSSION

Cytology based screening is challenging in low-resource settings due to requirement of multiple visits and limited number of trained personnel involved in sampling, interpretation and treatment. Immuno-biomarkers are attractive potential point-of -care (POC) tests in resource-constrained settings, with minimal requirement of reagents and technical equipment. The present study compared the efficacy of p16 and Ki-67 as stand alone, primary screening tests for cervical cancer.

When using conventional cytology, our findings were comparable with existing literatures. Park et al (7), Sherwani et al (8) & Karimi et al (9) found conventional cytology to have a sensitivity of 50%, 53.7% and 51% respectively. Nurunnabi et al (10) found PPV of cytology as 71.42%. Low sensitivity was attributed to

inadequate sampling technique, inhomogeneous distribution of abnormal cells, presence of obscuring blood and mucus, inflammation or thick areas of overlapping epithelial cells and the technical expertise of cyto-pathologist evaluating the slide specimen.

Sherwani et al (8) and Abulafia et al (11) found sensitivity of LBC as 97.6% and 76% and specificity as 50% & 86% respectively. We found LBC to be 88.8% sensitive and 100% specific. We feel this discrepancy could be due to limitations of a small sample size and interpretation of slides by a single experience cyto-pathologist.

HPV DNA has been studied extensively and uniformly found to have a better sensitivity than conventional cytology. The present study similarly found a high sensitivity of HPV DNA. However, we found a low specificity (50%) which is probably due to the fact that the test has restricted ability in differentiating between transient or persistent HPV infections.

We found that combining cytology with HPV DNA improved the sensitivity of detection with no change in specificity. Our results were comparable with findings reported in literature (12). Most developed countries incorporate testing for high risk (Hr) HPV DNA along with cytology in evaluation of smears (13) However, for resource - limited countries, this is neither practically nor economically feasible. A poor specificity despite combination of the two tests translates into unnecessary burden of investigation, increasing cost and patient anguish.

As found by other investigators, we also found that a combination of cytology with p16 increased both the sensitivity and specificity (88.8% and 60% respectively) (14) Tsoumpou et al (15) found that over expression of p16 in cervical smear increases with severity of cytological abnormality. The reason why specificity is still low could be because of small sample size or p16 immunoreactivity of non-dysplastic cells resulting in a decrease in specificity. We, therefore agree that an additional parameter which can differentiate p16 staining in abnormal cells from atrophic or metaplastic cells, is required to increase its specificity.

Ki-67 is another marker which improved the sensitivity and specificity of cytology in the present study. Our findings were in agreement to Sahebali et al[16] who found a test accuracy of 68%, 72%, and 86% for ASCUS, LSIL, and HSIL respectively. Zeng et al[17], found that Ki-67 is able to recognize cervical disease which was unobserved by cytologic screening; they suggested that combining Ki-67 to cytology needs to be studied. Ki-67 is a nuclear protein and its expression seems to be a mandatory requirement for progression through cell cycle. Its expression indicates persistent HPV infection and high rate of cellular proliferation suggesting higher chances of progression towards carcinoma**.**

The combination of p16 and Ki-67 did not offer any added advantage for screening in the present study. This finding was different from other studies where dual stain cytology was found to have better sensitivity as compared to PAP smear. Ikenberg et al (18) found the sensitivity of dual-stain cytology significantly different from PAP smear (86.7% vs 68.5%; $P < .001$) for detecting CIN2+, with comparable specificity (95.2% vs 95.4%; $P = 15$). The investigators found that HPV DNA testing in women 30 years or older was more sensitive (93.3%) than dual- stain cytology (84.7%) (P value = 0.03) but less specific (93.0% versus 96.2%; $P < 0.001$). Uijterwaal et al (19) also found a better sensitivity and specificity of dual markers as compared to Hr-HPV testing. We feel that our findings are at variance probably due to the small sample size and limited duration for the study as it was a part of post graduate program, further work up is required to find out reasons for difference.

CONCLUSION

A combination of Ki-67 with PAP smear showed improved specificity and positive predictive value of cervical screening.

There was no gain in terms of sensitivity or specificity of cervical screening with the use of dual immuno-markers.

REFERENCES

1. Petry KU, Menton S, Menton M, et al. Inclusion of HPV testing in routine cervical cancer screening for women above 29 years in Germany: results for 8466 patients. Br J Cancer. 2003; 88(10): 1570-1577.
2. Endl E, Gerdes J. The Ki-67 protein: fascinating forms and an unknown function. Exp Cell Res. 2000; 257(2): 231-237.
3. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol. 2000; 182(3): 311-322.
4. McLaughlin-Drubin ME, Park D, Munger K. Tumor suppressor p16INK4A is necessary for survival of cervical carcinoma cell lines. Proc Natl Acad Sci U S A. 2013; 110(40): 16175-16180.
5. Leong AS. Quantitation in immunohistology: fact or fiction? A discussion of variables that influence results. Appl Immunohistochem Mol Morphol. 2004; 12(1): 1-7.
6. Shi Q, Xu L, Yang R, et al. Ki-67 and P16 proteins in cervical cancer and precancerous lesions of young women and the diagnostic value for cervical cancer and precancerous lesions. Oncol Lett. 2019;18(2):1351-1355.

7. Park IA, Lee SN, Chae SW, et al. Comparing the accuracy of Thin Prep Pap tests and conventional Papanicolaou smears on the basis of the histologic diagnosis: a clinical study of women with cervical abnormalities. Acta Cytol. 2001; 45(4): 525-531.
8. Sherwani RK, Khan T, Akhtar K, et al. Conventional Pap Smear and liquid based cytology for cervical cancer screening-A comparative study. J Cytol. 2007; 24: 167-172
9. Karimi-Zarchi M, Peighmbari F, Karimi N, et al. A Comparison of 3 Ways of Conventional Pap Smear, Liquid-Based Cytology and Colposcopy VS Cervical Biopsy for Early Diagnosis of Premalignant Lesions or Cervical Cancer in Women with Abnormal Conventional Pap Test. Int J Biomed Sci. 2013; 9(4): 205-210.
10. Nurunnabi ASM, Sultana T. Visual inspection using acetic acid and Pap smear as methods of cervical cancer screening: An experience of Dhaka Medical College Hospital, Dhaka, Bangladesh. International Journal of Human and Health Sciences. 2020; 4(3): 189-193.
11. Abulafia O, Pezzullo JC, Sherer DM. Performance of ThinPrep liquid-based cervical cytology in comparison with conventionally prepared Papanicolaou smears: a quantitative survey. Gynecol Oncol. 2003; 90(1): 137-144.
12. Lörincz AT, Richart RM. Human papillomavirus DNA testing as an adjunct to cytology in cervical screening programs. Arch Pathol Lab Med. 2003; 127(8): 959-968.
13. ACOG Practice Bulletin No. 168: Cervical Cancer Screening and Prevention. Obstet Gynecol. 2016;128(4): e111-e130.
14. Denton KJ, Bergeron C, Klement P, Trunk MJ, Keller T, Ridder R; European CINtec Cytology Study Group. The sensitivity and specificity of p16(INK4a) cytology VS HPV testing for detecting high-grade cervical disease in the triage of ASC-US and LSIL pap cytology results. Am J Clin Pathol. 2010; 134(1): 12-21.
15. Tsoumpou I, Arbyn M, Kyrgiou M, et al. p16 (INK4a) immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. Cancer Treat Rev. 2009; 35(3): 210-220.
16. Sahebali S, Depuydt CE, Segers K, et al. Ki-67 immunocytochemistry in liquid based cervical cytology: useful as an adjunctive tool? J Clin Pathol. 2003; 56(9): 681-686.
17. Zeng Z, Del PG, Cohen JM, et al. MIB-1 expression in cervical Papanicolaou tests correlates with dysplasia in subsequent cervical biopsies. Appl Immunohistochem Mol Morphol. 2002; 10(1): 15-19.
18. Ikenberg H, Bergeron C, Schmidt D, et al. Screening for cervical cancer precursors with p16/Ki-67 dual-stained cytology: results of the PALMS study. J Natl Cancer Inst. 2013; 105(20): 1550-1557.
19. Uijterwaal MH, Witte BI, Van Kemenade FJ, et al. Triaging borderline/mild dyskaryotic Pap cytology with p16/Ki-67 dual-stained cytology testing: cross-sectional and longitudinal outcome study. Br J Cancer. 2014;110(6):1579-1586.

Orcid ID:

Tradeep Saluja - http://orchiod.org/0000-0003-3686-7776

Tamkin Khan - http://orchiod.org/0000-0002-8837-7931

Shaista Manan Vasenwala - https://orcid.org/0000-0001-9814-6507

Eram Ali - https://orcid.org/0000-00020317-823X

Ayesha Ahmad - https://orcid.org/0000-0003-2324-158X

How to cite this article:

Saluja T., Khan T., Vasenwala S.M., Ali E., Ahmad A. A Comparative Study On Cervical Cancer Screening Method: Is Immuno-marker A Better Option?. Era J. Med. Res. 2022; 9(1): 9-14.

Licencing Information

Attribution-ShareAlike 2.0 Generic (CC BY-SA 2.0) Derived from the licencing format of creative commons & creative commonsmay be contacted at https://creativecommons.org/ for further details.