

## RESEARCH ARTICLE

# Role of immunomarkers (P16 and Ki67) in identifying pre-malignant and malignant lesion of cervix: a comparative study

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## ABSTRACT

**Background:** Cancer of the cervix is the second most common cancer among women worldwide. Although pap test has successfully reduced the incidence of cervical cancer, it is associated with high false positive and high false negative test results. In order to improve the efficacy of screening program, United States cervical cancer screening guidelines recommend use of combined testing (cytology + HPV-DNA) in women above 30-65 years of age. However, due to application of current HPV vaccines, cervical screening will face challenges due to declining trends in the prevalence of HPV genotypes covered by vaccines. **Aim:** The study is aimed at finding out the role of immunomarkers (P16 and Ki67) in identifying pre-malignant and malignant lesion of carcinoma cervix and their comparison with the co-testing (cytology + HPV-DNA) method of screening. **Methods:** A cross-sectional observational study was conducted on 32 cases after consent. The tests done were cytology + HPV-DNA, cytology+p16, cytology+ Ki-67 and were compared in terms of their sensitivity, specificity, positive predictive value and negative predictive value against the gold standard, histopathology. **Result:** Majority of cases were in 30-40 years of age group. The sensitivity, specificity, positive predictive value and negative predictive value of cytology + HPV-DNA testing was 94.44%, 50%, 77.27%, 83.33% while that of cytology + P16 was 88.88%, 60%, 80%, 75% and cytology + Ki-67 was 88.88%, 100%, 100%, 91.66% respectively. **Conclusion:** Ki67 immunomarker is a better screening tool in terms of specificity, positive predictive value and negative predictive value when compared to cytology + HPV/p16. Ki-67 can be considered as a potential screening tool in future for both vaccinated and unvaccinated population.

**Keywords:** Carcinoma cervix, premalignant lesions, screening, immunomarkers.

Cancer of the cervix, a potentially preventable disease<sup>1</sup>, is the second most common cancer among women worldwide<sup>2,3</sup> and the most common malignancy of the female genital tract in the developing countries. Worldwide, it accounts for approximately 273,000 deaths every year, of the new cases 80% occur in the developing countries<sup>2</sup>. The high burden of cervical cancer in developing countries is because of both high prevalence of HPV infection and the lack of effective cervical cancer screening programs<sup>4</sup>. Pap test introduced in 1950 has been successful in reducing the incidence of cervical cancer and associated mortality.

However, efficacy of pap smear is limited due to high false negative and false positive rate ranging between 20-30%<sup>2</sup> and 5-70 %<sup>3</sup> respectively. HPV DNA testing is a highly sensitive test with a higher sensitivity than cytology and is used in addition to cytology for primary screening in many developed countries. The effectiveness of HPV tests in cervical screening has been widely confirmed,<sup>5-7</sup> but the low specificity of HPV test may lead to unnecessary colposcopy referral and treatment, increases health costs and causes anxiety for women involved.<sup>8</sup> Furthermore, with the approval and application of current vaccines, cervical

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screening will face challenges due to declining trends in the prevalence of HPV genotypes covered by vaccines, and a key issue will be how to adapt screening algorithms for increasingly vaccinated cohorts<sup>9</sup>. In order to overcome limitations of the above screening test and improve predictive values, biomarkers Ki-67 and p16 have been investigated.

Ki-67 is a nuclear and nucleolar protein which is expressed during the G1, S, G2, and M phase of the cell cycle, while not being present in resting cells (G0 phase), and can, therefore provide an index of the cell growth fraction. While the exact function of the Ki-67 protein remains unclear, its expression appears to be an absolute requirement for progression through the cell-division cycle.<sup>10,11</sup> In normal human cervical squamous mucosa, expression of Ki-67 is limited to the proliferating basal and parabasal cells. In dysplasia and carcinoma, however, expression extends beyond the basal one third of the epithelium and the number of positive cells increase, with a significant positive correlation between ascending grade of squamous intraepithelial lesion and labelling index.<sup>12</sup>

p16 is a cyclin-dependent kinase inhibitor that prevents phosphorylation of retinoblastoma protein (RB) and thus regulates the cell cycle. p16 expression is negatively controlled by the RB1 gene product, and is at very low concentrations in normal cells, whereas it is strongly overexpressed in HPV- associated tumors, in which RB has been functionally inactivated by the hr-HPV E7 oncoprotein.<sup>13</sup> Therefore, p16 overexpression can be considered as a marker of HPV infection. Studies have shown them to be able to distinguish true dysplasia from mimics and hence have a clinical impact by decreasing the utilization of interventions like colposcopy, large loop excision of transformation zone (LLETZ), cone biopsy etc.<sup>14</sup>. The present study was done to compare the efficacy of Ki-67 and p16 as against the co-testing.

### Materials and methods

A cross-sectional analytical study was conducted in department of Obstetrics and Gynaecology, Dr Baba Saheb Ambedkar Hospital, a tertiary care hospital in Delhi, for duration of six months from January 2021 to June 2021. Prior approval from ethics committee was taken. A total of 32 asymptomatic married women accompanying patients in gynaecological OPD meeting the inclusion and exclusion criteria were included in the study after their written informed consent.

A detailed clinical history and examination was carried out and the following investigations were done: cytology + HPV-DNA, cytology+P16, cytology+Ki67 and histopathology. HPV-DNA was isolated from residual liquid based cytology sample by Pure Link Genomic DNA mini Kit (Invitrogen, USA) as per the manufacturer's instructions and was stored at -20°C, HPV detection was done using PGMY09/11 primers designed to amplify a 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 staining standard immunohistochemical (IHC) stains of p16 and Ki-67 were applied on cytology smears. The results for p16 and Ki-67 were scored by a semi-quantitative scoring system as mentioned in tables 1 and 2.

**Table 1: p16/INK4a scoring system (nuclear and/or cytoplasmic)<sup>15</sup>**

Intensity	Proportion
0 – No staining	0 – No staining
1 - Weak staining	1- <1% positive
2 – Moderate staining	2- 1-10 % positive
3 - Strong staining	3- 11-33% positive
	4- 34-66% positive
	5- >66% positive

**Table 2: Ki67 scoring system<sup>16</sup>**

Staining	Score
<10% positive staining	0
10-30% positive staining	1
30-50% positive staining	2
>50% positive staining	3

Reports of all the investigations were recorded. Descriptive data was presented in the form of frequencies and percentages with the help of tables. Sensitivity, specificity, positive predictive value and negative predictive value of each investigation were calculated taking histopathology as gold standard.

### Results

A total of 32 married adult asymptomatic women were included in the study. Four (4) women out of 32 were excluded from the study due to incomplete testing. The sociodemographic profile of participants is depicted in table 3. The age of the patient ranged from 22 years to 64 years, with mean age of 34.2 years. Maximum number of patients was in 30-40 years of age (32.14%). Majority of patients (53.57%) were having parity between P2-4.

Out of the total 28 participants, 18 (64.29%) had histology suggestive of pre-malignant lesions. Cytology was positive in 22(78.57%) participants while 20 (71.42%) and 17 (60.71%) tested positive for p16 and Ki-67 respectively. Comparison of various screening tests is tabulated in tables 4

and 5. Taking histopathology as gold standard, cytology with HPV-DNA showed highest sensitivity of 94.44% as compared to 88.88% each of p16 and Ki-67. However, Ki-67 had a specificity of 100% as compared to 60% in p16 and 50% in HPV-DNA.

testing on same group of patients. Outcomes of these tests are as follows.

Cytology + HPV-DNA: Out of 28 cases, the combined cytology and HPV DNA testing detected abnormal lesions in 22 cases. When biopsy of these 22 cases was done, 17 cases

**Table 3: Socio-demographic characteristics of study population**

Parameters		Negative for intraepithelial lesion or malignancy	Cervical intraepithelial lesion or higher lesions	Total
Age (in years)	18-29	05	01	06
	30-39	05	04	09
	40-49	02	06	08
	50-59	01	03	04
	>60	0	01	01
Parity	0-1	04	01	5
	2-4	07	08	15
	4-6	01	07	08
Religion	Muslims	09	07	16
	Hindus	05	07	12
Socio-economic status	Class – I	00	00	00
	Class- II	00	00	00
	Class-III	06	03	09
	Class-IV	04	07	11
	Class-V	03	05	08

**Table 4 : Comparison of cervical cancer screening test with histopathology**

Screening test	Report	Histopathology Positive	Histopathology Negative	Total
Cytology + HPV DNA testing	Positive	17	05	22
	Negative	01	05	06
	Total	18	10	28
Cytology+ p16	Positive	16	04	20
	Negative	02	06	08
	Total	18	10	28
Cytology+ Ki-67	Positive	17	0	17
	Negative	01	10	11
	Total	18	10	28

were found to show abnormal lesions and 5 cases showed normal histology. This led to 5 extra cases being subjected to unnecessary intervention. However, only one case was missed by combining the two tests. The sensitivity of the HPV + cytology was 94.44%, specificity was 50%, positive predictive value 77.77%, negative predictive value was 83.33%. Lorincz et al<sup>17</sup> also reported a higher sensitivity (100%) and slightly lower specificity on combining the two tests. Adding the two tests rather than cytology alone results in improving the sensitivity of any screening programme but does not improve its specificity. The false positives will be subjected to further unnecessary investigation increasing the

**Table 5: Efficacy of various cervical cancer screening tests with HPE**

Screening test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Cytology+ HPV DNA testing	94.44%	50%	77.27%	83.33%
Cytology+P16	88.88%	60%	80%	75%
Cytology + Ki-67	88.88%	100%	100%	91.66%

## Discussion

Primary screening test for cervical cancer in most countries who have implemented screening programs is pap cytology. However, cytology-based screening is challenging as it is associated with high false positive and false negative results. The effectiveness of HPV tests in cervical screening has been widely confirmed, but the low specificity of HPV test may lead to unnecessary colposcopy referral and treatment, increases health costs and causes anxiety for women involved. In the present study we have compared the efficacy of P16 and Ki67 as a screening test for pre-malignant lesion of cancer cervix when compared to co-

cost and stress to the patient. However as per American College of Obstetrics and Gynecology<sup>18</sup> 2016, current guidelines on cervical cancer screening advise the use of HPV-DNA testing in addition to cytology because of two reasons: firstly pap smear due to its low sensitivity and high inter-observer variability shows a suboptimal performance as a screening test and secondly as 99% of cervical cancers are associated with hr-HPV infection testing for HPV-DNA improves the sensitivity for detection of CIN1<sup>3</sup>.

Cytology + p16: Out of 28 cases, 20 cases showed abnormal lesion on combination of cytology + p16. When biopsy of these 20 cases was performed, 16 of them showed abnormal results (CIN or higher grade lesion). Four cases

diagnosed as abnormal by the combination were normal on biopsy. Hence the combination resulted in 4 false positive cases and unnecessary interventions and missed two cases one high grade (CIN 2) another low grade lesion (CIN 1).

A study conducted by Denton et al<sup>19</sup> showed a higher sensitivity (92.6% vs 92.2% for ASCUS vs LSIL respectively) and a low specificity of (63.2-71.1% vs 37.3-53.3% for ASCUS vs LSIL respectively). Tsoumpu et al<sup>20</sup>, who found that over expression of p16 in cervical smear increases with severity of cytological abnormality. Among normal smears, only 12% were positive for the immunomarker compared to 45% of ASCUS and LSIL and 89% of HSIL smears. In our study we found similar results: when cytology was normal only 33% were positive for the marker compared to 90% positivity for both low grade lesion and high grade lesion. Thus the addition of the marker decreases the likelihood of missing a high grade lesion. The combination of cytology + p16 showed a sensitivity of 88.88%, specificity of 60%, positive predictive value of 80% and negative predictive value of 75%. Some non-dysplastic cells also exhibit p16 immunoreactivity, therefore an additional criterion that can discriminate p16 staining in abnormal cells from atrophic or metaplastic cells is required to increase its specificity.

**Cytology + Ki67:** Out of 28 cytology slides on which the immuno-stain marker was applied, 16 showed abnormal results. When biopsy of these 16 cases was performed it was found that all the cases showed CIN or higher grade lesions. None of the cases were false positive. Two cases which showed normal results, when biopsied showed some abnormality. Two cases were missed, one of them was a low grade lesion (CIN 1) and another was a high grade lesion (CIN 2). When compared to the gold standard, HPE -the sensitivity of the test came out to be 88.88%, specificity was 100%, positive predictive value 100% and negative predictive value was 91.6%. In our study we find that the sensitivity as well as specificity of Ki-67 immuno-marker as an adjunct to cytology was very high, with a high positive predictive value. S Sahebali et al<sup>21</sup>, who found a test accuracy (area under curve) of 68%, 72%, and 86% for ASCUS, LSIL, and HSIL respectively. Zeng et al<sup>22</sup> found that Ki-67 is able to recognize cervical disease which was unobserved by cytologic screening; therefore it may work as an adjunct and complimentary tool to cervical cytology. Dunton et al<sup>23</sup> in the year 1997, found a sensitivity of 89%, specificity of 65%, positive predictive value of 60% and negative predictive value of 91% when using Ki-67 immuno-

stain for detection of high grade cervical intraepithelial neoplasia.

We find a higher specificity (100%), positive predictive value (100%) and negative predictive value (91.66%) of Ki-67 marker compared to cytology + HPV-DNA testing which showed a specificity of just (50%), positive predictive value of (77.27%) and negative predictive value of (83.3%). Ki-67 being a nuclear and nucleolus protein its expression seems to be an absolute requirement for progression through the cell division cycle thus indicating persistent HPV infection and higher chances of progression to carcinoma. Due to application of current HPV vaccines, cervical screening will face challenges due to declining trends in the prevalence of HPV genotypes covered by vaccines. In our study Ki-67 appears to be a better screening tool in comparison to cytology + HPV-DNA testing and therefore can be considered as one of the future option of screening for cervical cancer in both vaccinated and unvaccinated cohort. However further research is still required in this field, till then search for an ideal screening test which is sensitive, specific, cost effective and acceptable continues.

#### Conclusion

Till date in most of the low socio-economic countries pap smear, VIA and VILI based screening programs are being implemented. Ki-67 along with cytology showed a better specificity (100%) and positive predictive value (100%) and a good negative predictive value of (91.66%) when compared to cytology+ HPV-DNA and cytology+p16 testing. With increase in HPV vaccination coverage, effectiveness HPV-DNA based screening will decrease thus Ki-67+cytology can be considered as a screening tool for carcinoma cervix in the future.

**Conflict of interest:** None. **Disclaimer:** Nil.

#### References

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBACON 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No.11. Lyon, France: International Agency for research on cancer; 2013.
2. Fahey M, Irwig L, Macaskill P. Meta-analysis of Pap test accuracy. *Am J Epidemiol.* 1995; 141:680-9.
3. Sherman ME, Schiffman MH, Lorinez AT, Manos MM, Scott DR, Kuman RJ, et al. Toward objective quality assurance in cervical cytopathology: Correlation of cytopathologic diagnoses with detection of high-risk human papillomavirus types. *Am J Clin Pathol.* 1994; 102:182-7.

4. National Cancer Registry Programme. Time trends and cancer incidence rates: 1982–2005. Bangalore: National Cancer Registry Programme; 2009.
5. Ronco G, Dillner J, Elfstrom KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. *Lancet*. 2014; 383(9916): 524-32.
6. Gage JC, Schiffman M, Katki HA, et al. Reassurance against future risk of precancer and cancer conferred by a negative human papillomavirus test. *J Natl Cancer Inst*. 2014; 106(8): dju153.
7. Sroczynski G, Schnell-Inderst P, Mühlberger N, et al. Cost-effectiveness of primary HPV screening for cervical cancer in Germany—a decision analysis. *Eur J Cancer*. 2011; 47(11):1633-46.
8. Wentzensen N. Triage of HPV-positive women in cervical cancer screening. *Lancet Oncol*. 2013; 14(2):107-9.
9. Sundstrom K, Elfstrom KM. Advances in cervical cancer prevention: efficacy, effectiveness, elimination? *PLoS Med*. 2020;17(1):e1003035.
10. Endl E, Gerdes J. The Ki-67 protein: fascinating forms and an unknown function. *Experimental Cell Research*. 2000; 257(2), 231-37.
11. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. *Journal of Cellular Physiology*. 2000; 182 (3): 311-22.
12. Keating JT, Cviko A, Riethdorf S, et al. Ki-67, cyclin E, and p16INK4a are complimentary surrogate biomarkers for human papilloma virus-related cervical neoplasia. *American Journal of Surgical Pathology*. 2001; 25(7): 884-91.
13. McLaughlin-Drubin ME, Park D, Munger K. Tumor suppressor p16INK4A is necessary for survival of cervical carcinoma cell lines. *Proc Natl Acad Sci USA*. 2013; 110(40):16175-80.
14. Brown CA, Bogers J, Sahebali S, Depuydt CE, Prins FD, Malinowski DP. Role of Protein Biomarkers in the Detection of High-Grade Disease in Cervical Cancer Screening Programs. *J Oncol*. 2012; 2012: 2831.
15. Leong AS. Quantitation in immunohistology: fact or fiction? A discussion of variables that influence results. *Appl Immunohistochem Mol Morphol*. 2004; 12 (1): 1-7.
16. Shi Q, Xu L, Yang R, Meng Y, Qiu L. 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; 18:1351-55.
17. Löhrincz AT, Richart RM. Human Papillomavirus DNA Testing as an Adjunct to Cytology in Cervical Screening Programs. *Archives of Pathology & Laboratory Medicine*. August 2003; 127(8): 959-68.
18. Cervical cancer screening and prevention. Practice Bulletin No. 168. American College of Obstetricians and Gynecologists. *Obstet Gynecol*. 2016;128: e111-30.
19. Denton KJ, Bergeron C, Klement P, Trunk MJ, Keller T, Ridder R. The sensitivity and specificity of p16INK4a cytology vs HPV testing for detecting high-grade cervical disease in the triage of ASC-US and LSIL Pap cytology results. *American Journal of Clinical Pathology*. 2010 Jul 1; 134(1):12-21.
20. Tsoumpou, Arbyn M, Kyrgiou M, Wentzensen N, Koliopoulos G, Martin-Hirsch P, et al. p16<sup>INK4a</sup> immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. *Cancer Treat Rev*. 2009 May; 35(3): 210-20.
21. Sahebali S, Depuydt CE, Segers K, Vereecken AJ, Van Marck E, Bogers JJ. Ki-67 immunocytochemistry in liquid based cervical cytology: useful as an adjunctive tool? *Journal of clinical pathology*. 2003 Sep 1; 56(9):681-6.
22. 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;10:15-9.
23. Dunton CJ, Van Hoesen KH, Kovatich AJ, et al. Ki-67 antigen staining as an adjunct to identifying cervical intraepithelial neoplasia. *Gynecol Oncol*. 1997; 64: 451-5.

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