Research Article

Role of p16, Ki67 and CK17 in differentiating benign lesions, cervical intraepithelial neoplasia(CIN) and atypical immature squamous metaplasia(AIM) of uterine cervix

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Abstract

Cervical intraepithelial neoplasia (CIN) is a premalignant lesion capable of progressing to cervical cancer. Despite the existing well-defined criteria, the histopathological diagnosis is subject to high rates of discordance among pathologists. **Aim:** To study the role of p16, Ki67 and CK17 in differentiating benign lesions, cervical intraepithelial lesions(CIN) and atypical immature squamous metaplasia (AIM)and to improve intra and interobserver reproducibility of diagnosis of cervical neoplasia. **Material and Methods:** In a cross sectional study, a total of 75 cervical biopsies including benign lesions (n=24), AIM (n=28), CIN (n=23) were studied and analyzed immunohistochemically using p16, Ki67 and CK17 immunomarkers. Data was evaluated using chi-square test. **Results:** p16 and Ki 67positivity were observed in 91.3% and 78.26% of CIN and 28.57% of AIM respectively. None of the benign lesions expressed p16 and Ki67while CK17 positivity was observed in 46.42% of CIN and 100% of AIM with 12.5% of benign lesions. **Conclusion:** The three biomarkers (p16, CK17 and Ki67) had a high degree of sensitivity and specificity and appear to be a useful and reliable diagnostic adjunct to improve the routine diagnosis and reduce interobserver variability in cervical biopsy specimens. Immunohistochemical markers such as p16 alone or with Ki67 represents important tool for the pathologists in distinguishing high grade cervical dysplasia from its benign mimics such as AIM and reactive inflammatory lesion thus avoiding overtreatment.

Key words: $p16^{ink4a}$, Cytokeratin17, Ki67, Cervical intraepithelial neoplasia, Cervix uteri

Introduction

Cervical cancer is the commonest cancer cause of death among women in developing countries [1]. Although introduction of pap in developed countries has been effective in reducing cervical cancer mortality and morbidity rates, the efficacy of pap test is hampered by high interobserver variability and high false negative and false positive rate, the range between 20-30% and 50-70% respectively [2,3]. Cervical cancer is caused by Human Papilloma virus (HPV), infection of which is acquired by about 80% of sexually active women by 50 years of age [4]. Almost all of the invasive cervical cancers are preceded by cervical intraepithelial neoplasia (CIN) [5,6]. Persistent infections with high risk human papilloma virus (hr-HPV) types lead to CIN and invasive cancer [7]. Despite well-defined criteria, the histopathologic diagnosis is subject to high rates of discrepancy among pathologists [8-10]. Supplementary methods using objective biomarkers are needed to achieve more accurate diagnosis.

The term atypical immature sqamous metaplasia (AIM) was initially introduced in 1983 to describe lesions in uterine cervix featuring a uniform intraepithelial full thickness basal cell proliferation with high nuclear density in absence of maturation but without sufficient criteria for diagnosis of high grade cervical intraepithelial neoplasia (CIN 3). [11]AIM has poor

Manuscript received: 18th December 2016
Reviewed: 26th December 2016
Author Corrected: 05th January 2017
Accepted for Publication: 12th January 2017

intra and interobserver reproducibility on routine H & E stained sections because of its resemblance to CIN 3[12].

P16INK4A, a sensitive marker of cells with active expression of E7 oncoprotein, has also shown high sensitivity and specificity to HSIL in adult women [13]. Interaction of high risk HPV E7 gene product with pRb, results in the liberation of E2F , inactivation of pRb and stimulation of the S-phase of the cell cycle. This is strongly associated with p16INK4A expression. P16 tends not to be expressed in either normal proliferative epithelium cells or inflammatory lesions [13.14].

A marker of proliferation Ki67 has been shown to be a sensitive and specific marker of HPV infection in mature squamous epithelia [15]. Ki67 usually expressed in the second or third parabasal layers and rarely in the basal layer of the cervical squamous epithelium.

Cytokeratin (CK) 17 is a marker for endocervical reserve stem cells which give rise to metaplasia and expression of CK17 decreases and disappears as the metaplastic epithelium matures. Antibody to CK17 is used to differentiate between immature sqamous metaplasia (ISM) and high grade CIN (CIN3) [16].

The aim of study is to analyse the staining patterns of p16, Ki67 and CK17 in benign cervical lesions, cervical intraepithelial neoplasia and atypical immature squamous metaplasia and to evaluate their utility in differentiating cervical intraepithelial neoplasia from benign lesions and atypical immature squamous metaplasia of uterine cervix.

Materials and Methods

This cross-sectional study was conducted in the Department of Pathology, Baba Raghav Das Medical College, Gorakhpur, UP on a total of 75 cases of formalin- fixed paraffin embedded cervical specimens of various cervical lesions comprising of benign lesions, cervical intraepithelial neoplasia (CIN) and atypical immature metaplasia(AIM) from August 2015 to October 2016. Inclusion criteria were all the female patients of age ranging from 21-70years presenting with various cervical lesions and who agreed to sign on consent form. Inadequate and autolysed tissue sample and the patients who did not adhere to the guidelines of protocols were excluded.

Histological sections were stained with Haematoxylin and Eosin stain for morphological diagnosis after concordance of a double blind evaluation by two independent pathologists. The cases were classified as benign lesions, CIN and AIM. Cases with either dissimilar diagnosis or with unsatisfactory material for evaluation were excluded from the study. Immunohistochemical staining for Ki-67, p16 and CK-17 antigens was performed on all the cases using avidin-biotin peroxidase complex method and their immunoexpression were evaluated.

The performance of the immunohistochemical tests for p16, Ki67 and CK 17, in the detection of above mentioned cervical lesions was statistically evaluated by means of conventional contingency tables to calculate sensitivity, specificity, positive and negative predictive values considering the histological diagnosis as gold standard.The data was analysed statistically by applying Z test in SPSS version 23. P value < 0.05 was considered statistically significant.

The present work has been conducted after getting ethical clearance from the institutional ethical committee.

Results

The patients ranged from 21-70 years with a mean of 41.26 ± 9.76**.** Out of total 75 cases, 24 cases (32%) were of benign lesions, 28 cases (37.33%) of atypical immature metaplasia and 23 cases (30.66%) were CIN. Among 23 cases of CIN, 4 cases were LSIL and 19 cases were HSIL.

Table-1: Comparative evaluation of p16, Ki67 and CK17 expression in benign, CIN and AIM lesions of cervix

IHC Markers	Benign (n=24 cases) [No. (%)]	CIN (n=23 cases) [No. (%)]	AIM (n=28 cases) [No. (%)]
p16	00 (00.00%)	21(91.30%)	08(28.57%)
Ki67	00 (00.00%)	18(78.26%)	08(28.57%)
CK17	03(12.50%)	13(46.42%)	28(100%)

Table-2: Comparative evaluation of p16, Ki 67 and CK17 in diagnosing AIM versus CIN

IHC Markers	AIM(N=28)	CIN(n=23)	Z Score	P value
p16	8	21	4.5010	<0.001
Ki 67	8	18	3.5321	<0.001
CK 17	28	13	3.8942	<0.001

Table-3: Sensitivity and Specificity of p16, Ki67 and CK17 in detection of CIN

IHC markers	Senstivity	Specificity	PPV	NPV
p16	**91.3%**	**84.61%**	**72.41%**	**95.65%**
Ki67	**78.26%**	**84.61%**	**69.23%**	**89.79%**
CK17	**56.52%**	**40.38%**	**32.5%**	**67.74%**

On analyzing the expression of p16, Ki 67 and CK 17 markers, out of 24 cases of benign lesions, none showed expression of p16 and Ki 67 while 3cases (12.5%) were positive for CK17.

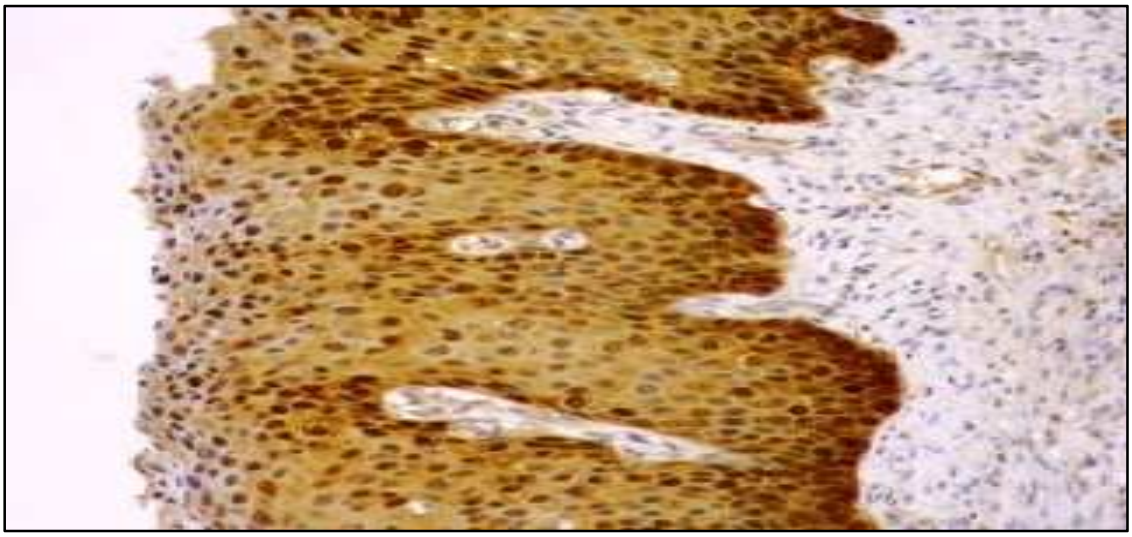

Fig.-1: Microphotograph of CIN 3 showing diffuse staining with p16

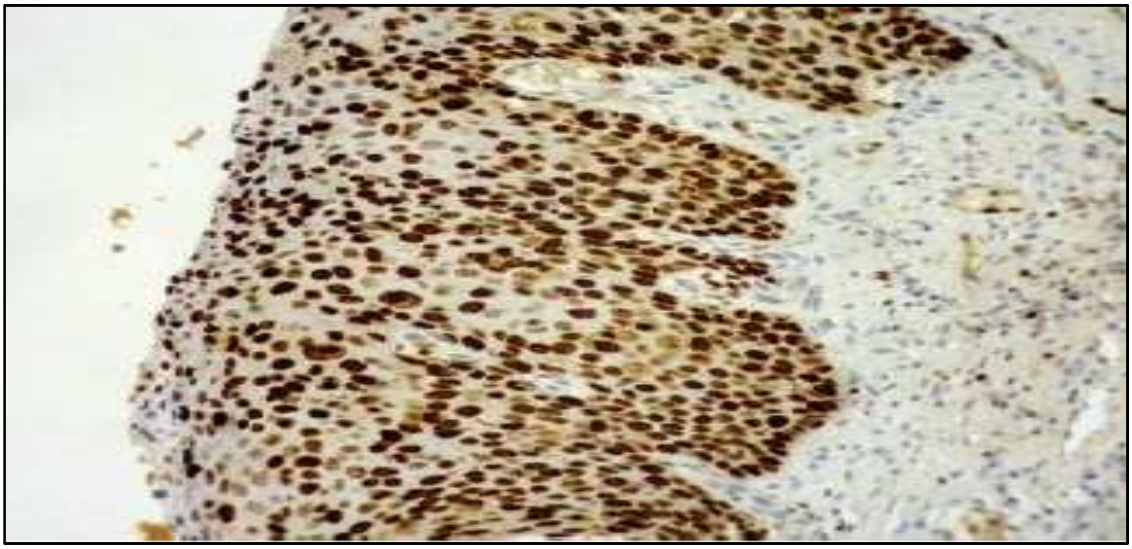

Fig.-2: Microphotograph of CIN 3 showing strong positivity with p16

Among 23 cases of CIN, all the cases of HSIL (19 cases, 100%) showed strong diffuse positivity while 2 out 4 cases of LSIL showed positive expression of p16. Ki 67 positivity was observed in 17 cases (89.47%) of HSIL and one case (25%) of LSIL respectively while CK17 expression was seen in 10 out of 19 cases (52.63%) of HSIL and 3 of 4 cases(75%) of LSIL.(Fig.1,Fig.2)

Among 28 cases of AIM, CK17 expression was observed in all cases while 8 cases (28.57%) expressed both p16 and Ki67. (Table 1) P value of all the three markers in differentiating AIM from CIN is <0.001, which is found to be highly significant. (Table 2)

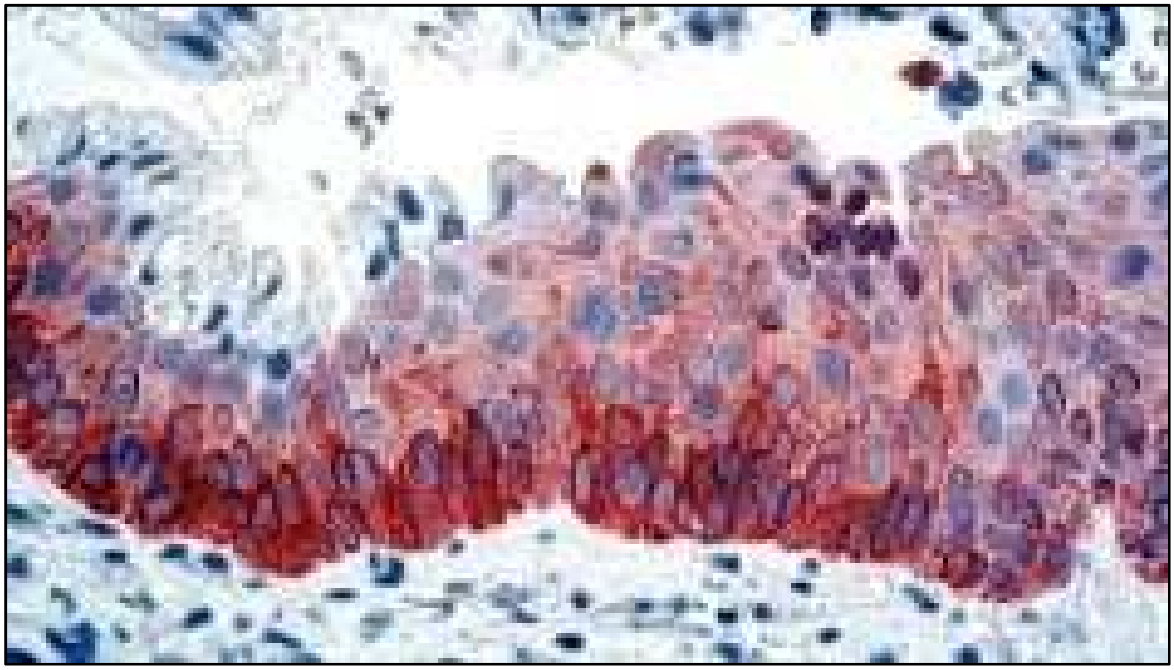

Fig.-3: Microphotograph of Atypical immature metaplasia showing positivity with CK17

The differences of expression of p16, Ki67 and CK17 between benign lesions versus CIN was <0.001, which was also found to be highly significant.

The sensitivity and specificity of p16 staining in detection of CIN were 91.3% and 84.61% respectively with positive predictive value (PPV) of 72.41% and negative predictive value (NPV) of 95.65 %.

The sensitivity and specificity of Ki67 were 78.26% and 84.61% with 69.23% PPV and 89.79%NPV respectively while the sensitivity and specificity of CK17 in detection of CIN were 56.52% and 40.38% respectively but its sensitivity in detection of atypical immature squamous metaplasia(AIM) were 100% in our study. (Table 3)

Discussion

Almost all of the invasive cervical cancers are preceded by cervical intraepithelial neoplasia (CIN).High rates of HPV infection is seen in adolescents and young women, persistence of which lead to development of premalignant lesions (CIN) and cervical cancer. Discordance on histological diagnosis of cervical cancer precursor lesions have been documented in several studies, suggesting a need to identify biological markers that could help the pathologist to make a correct diagnosis in equivocal lesions [9,17,18]. In adolescent and young adult women atypical immature metaplasia of the cervix, an immature metaplastic epithelium with mild cytological atypia but with strong reaction phenomenon, sometimes, mimics the morphology of high grade lesions. Therefore HSIL diagnosed lesions in this age group could in reality be a false positive. Testing for p16 expression appears to be a good addition to more accurately diagnose HSIL [10].

In the present study, p16 was not expressed in any of the benign lesions, while all cases of HSIL (19 out of 19 cases, 100%) showed strong positivity for p16. The staining was both nuclear and cytoplasmic and mostly involved full thickness of epithelium. Also p16 was positive in 2 of 4 LSIL cases (50%) of which one was diffuse basal and the other diffuse one third thickness. Out of 28 cases of AIM, 8 cases (28.57%) showed positive expression of p16, while 71.4% were negative for p16.

Reuschenbach et al found the similar results [7]. The results of present study are also in agreement with the study conducted by Benevolo et al and Focchi et al [20, 21]. In their study, they also reported 100% positivity in cases of HSIL while none of the benign lesions expressed p16.

The sensitivity and specificity of p16 staining were 91.3% and 84.61% respectively with 72.41% PPV and 95.65% NPV. Present study findings match with the study of Walts et al in which sensitivity and specificity of p16 in detection of CIN was 89% and 83% respectively. PPV was 86% while NPV was 94% while Aslani FS reported 91.30% sensitivity, 98.10% specificity with 95.40% positive predictive value (PPV) and96.30% negative predictive value (NPV) [22, 23].

Ki-67 is a cell proliferation marker demonstrated in some studies to aid in the diagnosis of HSIL. In the present study, Ki 67 expression was completely absent in benign lesions while 8 out of 28 (28.57%) cases of AIM showed positivity for Ki67. Among CIN cases, 17 (89.47%) of HSIL were positive for Ki67, while it showed positivity in 1 of 4 (25%) LSIL cases. The present study showed statistically significant positive relation between proliferative activity, distribution of Ki67 positive cells and increasing CIN grade.

Our findings were in close agreement with the observation of Aslani FS et al study [23]. In their study they found Ki67 expression in 100% cases of HSIL, 25% cases of LSIL, while none of benign lesions were positive. Kruse et al have found that there was a significant relation between CIN grade and number of Ki67 positive cells by using QPRODIT lineage analyzing system [24]. The distribution of Ki67 positive cells was related with CIN grade but there was overlap between CIN2 and CIN3 lesions similar to our study. Al-Saleh et al also found higher densities of Ki67 positive cells in HSIL than LSIL lesions [25].

The sensitivity and specificity of Ki67 staining were 78.26% and 84.61% respectively with 69.23% PPV and 89.79% NPV. This was in agreement with the Walts et al study [22].

On analyzing the expression of CK17 in AIM, all cases of AIM (28 cases, 100%) were positive. 3 out 24(12.5%) cases of benign lesions expressed CK17 , while among CIN , 3 out of 4 cases (75%) of LSIL and 10 out of 19 cases(52.63%) of HSIL showed positivity. These findings are well in accordance with the study of Aslani FS et al [23].

The sensitivity and specificity of CK17 in detection of CIN came out to be 56.52% and 40.38% respectively which are comparatively lower than p16 and Ki67 but its sensitivity in detection of AIM come out to be 100%.CK 17 is a marker for basal cell of complex epithelia whose expression doesn't correlate with HPV infection or dysplasia [18]. In our view, CK17 expression in pseudostratified epithelia merely reflects a metaplastic phenotype/process. The dual expression of CK17 and p16 in atypical squamous lesions with metaplastic features rather supports the hypothesis of Ma et al that CIN III alternatively may develop via HPV infection of metaplastic cell [26].

Conclusion

p16, CK17 and Ki67 immunomarkers had a high degree of sensitivity and specificity and can be used as reliable diagnostic adjuncts to improve the routine diagnosis and reduce interobserver variability in cervical biopsy specimens. Immunohistochemical markers such as p16 alone or with Ki67 proved to be an important tool for the pathologists in distinguishing high grade cervical dysplasia from its benign mimics such as reactive inflammatory lesion and AIM thus avoiding overtreatment. However, complementary study including more cases and follow up examinations is warranted for better evaluation and definitive prognostic significance of these markers.

Funding: Nil, **Conflict of interest:** None initiated,
Permission from IRB: Yes

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How to cite this article?

Mitra S.K., Misra R.K., Varshney B., Kumari A., Rai D. Role of p16, Ki67 and CK17 in differentiating benign lesions, cervical intraepithelial neoplasia(CIN) and atypical immature squamous metaplasia(AIM) of uterine cervix. Trop J Path Micro 2017;3(1):13-19.doi: 10.17511/jopm.2017.i1.03.

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