Original Article

Immunohistochemical evaluation of Ki-67 as a predictive marker in atypical cervical metaplastic epithelium

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Abstract

Cervical cancer is the second most common cancer in women worldwide, studies in elucidating its pathogenesis still remains active areas of research. Infection of metaplastic epithelium at the cervical transformation zone followed by viral persistence, progression of persistently infected epithelium to cervical precancer, and invasion through the basement membrane of the epithelium remain the major steps identified in cervical cancer development. Though histologic assessment of colposcopy-guided biopsies remain the gold standard of assessment, a varying degree of inter-observer or intra-observer variability still leads to either overdiagnosis or underdiagnosis of the disease process. In our study, expression of Ki-67, a proliferation marker is used in classifying the various types of squamous metaplasia, thus predicting their potential for malignant transformation.

Key Words: Atypical immature metaplasia, Ki-67, Squamous metaplasia.

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INTRODUCTION

Metaplasia is the process by which one fully differentiated adult type of epithelium transforms into another differentiated adult type which is usually an adaptive change in response to chronic irritation or hormonal stimuli. Three different stages of metaplasia have been identified – reserve cell hyperplasia, immature squamous metaplasia and mature squamous metaplasia.[1] Atypical immature squamous metaplasia (AIM) was a term introduced in 1983 to describe cervical lesions involving an uniform intraepithelial full thickness basal cell proliferation with high nuclear density without maturation or without sufficient criteria for a diagnosis of high-grade cervical intraepithelial neoplasia.[2] The diagnosis of AIM has poor intra- and inter-observer reproducibility on haematoxylin and eosin (HandE)-stained sections because of its resemblance to CIN III. Hence there is an increasing need for surrogate markers which will help in further subclassifying these lesions either into metaplasia and high-grade SIL. Ki-67 is a proliferation marker that is confined to the parabasal cell layer of normal stratified squamous mucosa but shows expression in the stratified squamous epithelium in CIN lesions in correlation with the extent of disordered maturation. Although Ki-67 has been used as a diagnostic adjunct for the classification of cervical tissue specimens, the expression of Ki-67 alone does not discriminate HPV-mediated dysplasia versus benign proliferating cells in benign reactive processes. In our study, immunohistochemical evaluation of Ki-67 is used to classify squamous metaplasia and whether such a

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metaplastic epithelium has a significant potential for malignant transformation.

MATERIALS AND METHODS

The study comprised of 25 cases of cervical squamous metaplasia diagnosed by histopathological examination from our archival files were retrieved with the diagnosis of "cervicitis," "atypical immature squamous metaplasia," or "Mature squamous metaplasia." The study group consisted of 15 cases of IM, the negative control group consisted of 5 cases of benign cervical mucosa with inflammation and reactive changes, and the positive control group consisted of 5 cases of mature squamous metaplasia. In order to verify the histologic diagnoses, all cases were re-reviewed by 2 pathologists to obtain a consensus diagnosis.

The exclusion criteria: Invasive SCC and Glandular lesions.

The diagnostic criteria for immature metaplasia were described by Crum *et al*[2] and Park *et al*[3] has been taken into account.

Immunohistochemical evaluation of ki-67

Immunohistochemical staining using Ki-67 was performed on 4-µm sections of formalin-fixed, paraffin-embedded specimens. The sections were subjected to heat-induced antigen retrieval and were incubated in an automated stainer with Ki-67 antibody (Biogenex Labs) at a dilution of 1:50, and were stained with diaminobenzidine chromogen and counterstained with hematoxylin.

Presence of parabasal epithelial staining was used as a positive control. Presence of a cluster of 2 or more strongly staining nuclei in the upper two thirds of the epithelial thickness was interpreted as a positive result. While assessing the results of immunostaining, the immunostained slides were reviewed together with hematoxylin-eosin– stained slides to ensure that the location of the lesion was correctly identified on the immunostained slides and that the result of the staining was obtained only from the lesional area.

Statistical analysis was carried out using the Fischer exact test using SPSS 15.0 software for analysis. A p value of <0.05 is considered statistically significant.

RESULTS

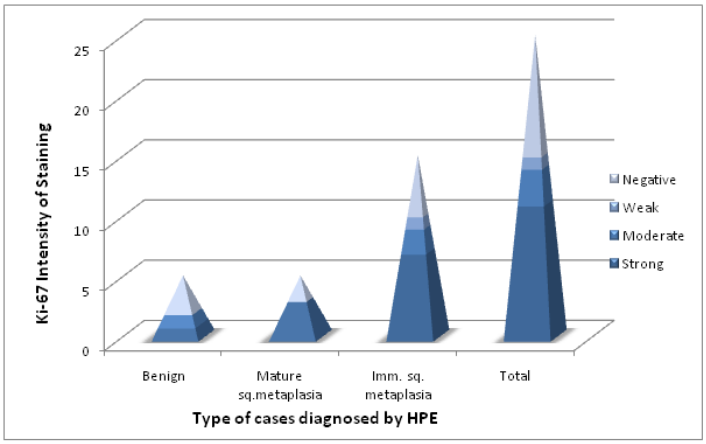
Of all the cases of mature squamous metaplasia 60% of the cases were strongly positive for ki67. Rest of 40% showed weak and patchy staining. Of the 5 cases of benign cervical mucosa, only 40% showed positivity for ki-67. They represented the regenerative epithelium. Of the 15 cases of immature squamous metaplasia, 46.67% showed a strong positivity, 13.33% showed moderate instensity of staining and only 6.67% of cases showed a weak positivity for ki-67. Most of these were adjacent to ulcer representing regeneration and reactive atypia. (Table 1)

However, there was no statistically significant association observed between the metaplastic epithelium and the staining intensity of Ki-67.

Table 1: Intensity of Ki-67 staining in cases

Diagnosis made by HPE	Ki-67 staining intensity				Total number of cases
	Strong	Moderate	Weak	Negative	
Benign cervical mucosa	1 (20%)	1 (20%)	0	3 (60%)	5
Mature squamous metaplasia	3 (60%)	0	0	2 (40%)	5
Immature squamous metaplasia	7(46.67%)	2(13.33%)	1(6.67%)	5(33.33%)	15
	11	3	1	10	25

Inference: Type of lesion shows no significant association with the intensity of staining by Ki-67 with p=0.154.



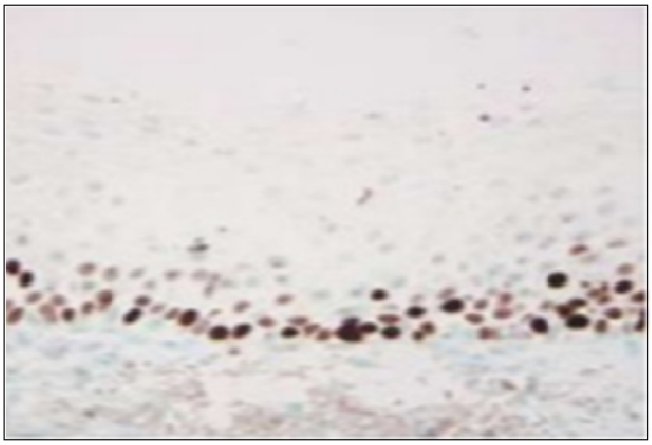


Figure 1

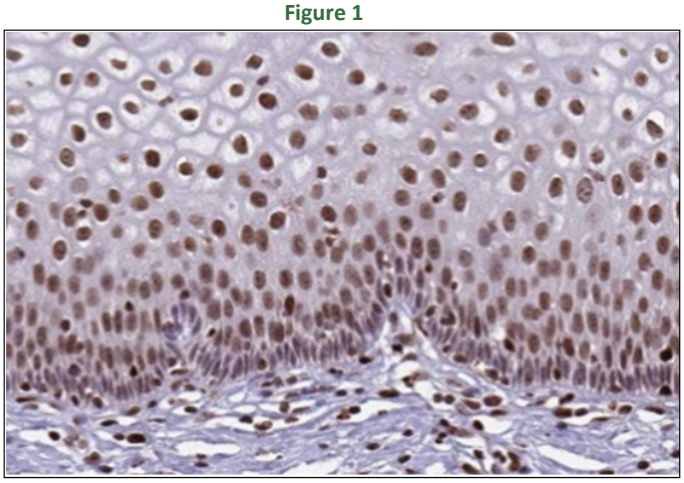


Figure 2

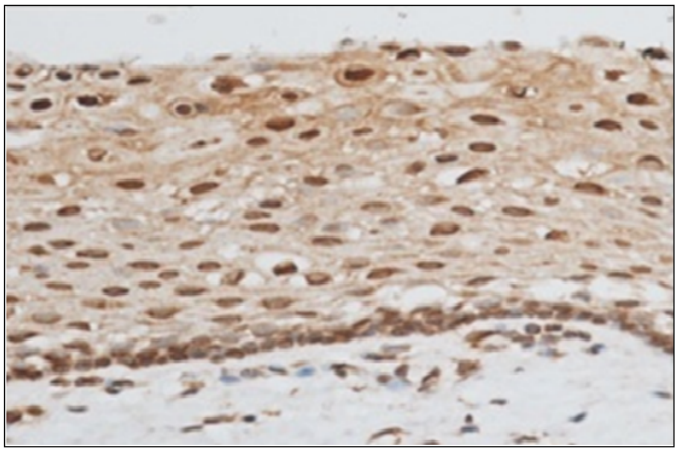


Figure 3

Figure 4

Legend

Figure 1: Squamous metaplasia cases Vs Ki-67 staining intensity**; Figure 2:** Ihc basal positivity with ki-67; **Figure 3:** Strong nuclear positivity with ki – 67; **Figure 4:** Moderate nuclear positivity

DISCUSSION

The physiologic replacement of endocervical surface and glandular epithelium by a newly formed squamous epithelium from the subcolumnar reserve cells is termed as squamous metaplasia. This process starts with the appearance and proliferation of reserve cells, first forming a thin layer of immature squamous cells without stratification or glycogen (immature squamous metaplasia), then differentiating into mature stratified squamous epithelium, which contains glycogen from the intermediate cell layer onwards. Most immature squamous metaplasia has no cytologic atypia (typical IM), while a small percentage can show mild atypia (atypical immature squamous metaplasia or AIM).

Those bearing resemblance to papillary lesions of AIM have been reclassified as immature condylomas. Non-papillary atypical immature squamous proliferations with cytological atypia sufficient for a diagnosis of high-grade dysplasia and some metaplastic features have been recently termed eosinophilic dysplasia.[4] Other non-papillary atypical immature squamous proliferations which contain both metaplastic features and cytological atypia that defy precise classification were termed as 'atypical immature metaplastic- like proliferation of the cervix'.[5]

Diagnosis of metaplasias on the basis of HandE staining alone is subject to a high level of intra-observer variability. Many studies show that IHC staining for Ki67 and p16 is a very useful adjunctive aid in the diagnosis of equivocal cervical biopsies.[6] In the previous studies, Ki67 expression has been found to be associated with the grade of dysplasia, indicating that IHC for Ki67 is a useful adjunctive test in the evaluation of low-grade lesions of the cervix.[7,8] Other advantages of this marker are simplicity, availability, reproducibility, and low-cost laboratory techniques.

According to the guidelines of the American Society of Colposcopy and Cervical Pathology (ASCCP), women with cervical biopsy–confirmed CIN 2 or CIN 3 should undergo an excisional treatment to prevent potential progression to invasive cancerous growth. These therapies may potentially affect reproductive outcomes, especially in young women; and most low-grade squamous intraepithelial lesions (CIN 1) regress spontaneously. Therefore, it is important to have accurate diagnostic interpretation of cervical biopsy specimen to distinguish between low-grade (CIN 1) and high-grade SIL (CIN 2 and CIN 3) to avoid overtreatment of false-positive cases and under treatment of false-negative cases.

Ki-67, a proliferation marker, is elevated in HPV-infected mature squamous epithelia and is useful for confirmation of the diagnosis in equivocal low-grade SIL. Ki-67, however, may be positive in HPV-negative squamous metaplasia or regenerating epithelium ; therefore, positivity of this marker in immature squamous epithelium is not specific for HPV infection.[9] In atypical immature squamous metaplasia, Ki-67 staining shows variable results, with a wide range of positivity and significant overlap between HPV-positive and HPV-negative cases.

Recommendations from the Lower Anogenital Squamous Terminology (LAST) Project, a collaboration between the College of American Pathologists and the ASCCP, state that Ki-67 should not be routinely added to p16 immunohistochemistry but may be considered in cases for

which p16 staining is inconclusive or technically inadequate.[10] The role of p16 and Ki67 immunohistochemistry in evaluation of cervical dysplasia is important for both pathologists and clinicians.

In the present study, the respective sensitivity and specificity of Ki67 were 95.6% and 85.1%. In problematic cases, Ki67 alone cannot differentiate between dysplasia and metaplasia. The current study revealed that Ki-67 can be positive in immature squamous metaplasia, and in some CIN lesions. For differentiation between metaplastic lesions with or without dysplasia, it would be helpful to consider another marker such as p16. Ki67 can be positive in some immature squamous metaplastic lesions.

Ki 67 is definitely a useful adjunct for interpreting the malignant potential of the metaplastic epithelium,but more interpretation criteria should be developed to avoid inter observer variability.

A complementary study including more cases and follow up examinations is warranted for better evaluation and definitive prognostic significance of this biomarker.

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