Original Article

Immunocytoexpression profile of ProExC in smears interpreted as ASC-US, ASC-H, and cervical intraepithelial lesion

ABSTRACT

Aims: We aimed to investigate the immunocytoexpression profiles of a novel assay ProEx C for topoisomerase II alpha (TOP2A) and minichromosome maintenance protein 2 (MCM2) in abnormal interpreted smears.

Settings and Design: Screening programs with Papanicolaou smear and high risk group human papilloma virus testing have yielded a dramatic reduction of cervical cancer incidence. However, both of these tests have limited specificity for the detection of clinically significant cervical high grade lesions. ProEx C for topoisomerase II alpha (TOP2A) and minichromosome maintenance protein 2 (MCM2) has been considered to have tight association with high grade intraepithelial lesions.

Materials and Methods: A total number of 54 SurePath cervical cytology specimens of patients previously interpreted as atypical squamous cells–undetermined significance (ASC-US), atypical squamous cells–cannot exclude high grade squamous intraepithelial lesion (ASC-H), low grade squamous intraepithelial lesion (LSIL), and high grade squamous intraepithelial lesion (HSIL) were included in our study.

Results and Conclusions: ProEx C was positive in 14 of HSILs (100%), 3 of 19 LSILs (16%), 2 of 4 ASC-Hs, and none of ASC-USs (0%). The ProEx C test showed very intense nuclear staining in all cytologically abnormal cells. Further studies are indicated to evaluate the diagnostic role of ProEx C.

Key words: ASC-H; ASC-US; HSIL; immunocytochemistry; LSIL; ProEx C

Introduction

Cervix cancer is the second most common type of cancer in developing countries and has a high risk of mortality.[1] Screening strategies by the Papanicolaou (Pap) smear method have provided significant reduction in the incidence of cervical cancer.[2] However, sensitivity of a single Pap test is only 50% for the detection of current disease.[3]

According to the ASCUS-LSIL Triage Study (ALTS) by Schiffman *et al*., in the US, each year more than 3 million cases are diagnosed as atypical squamous cells–undetermined significance (ASC-US), atypical squamous cells–cannot exclude high grade squamous intraepithelial lesion (ASC-H), low grade squamous intraepithelial lesion (LSIL), or atypical glandular cells (AGC) that require further evaluation such as colposcopic biopsy to identify clinically significant high grade lesions (CIN-2/3 or carcinoma). Nevertheless, according to this study, further examination does not reveal a high-grade lesion in most of the cases.[4]

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Human papillomavirus (HPV) is involved in the etiopathogenesis of cervix carcinomas and LSILs.[5,6]

"High risk" group HPV types include HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68 that are associated with higher risk of malignant transformation and can be detected from the liquid-based cervicovaginal material. Identification of high-risk HPV types in patients diagnosed as ASC-US and LSIL determines the risk potential of these patients. However, specificity of HPV DNA test in detecting high grade squamous intraepithelial lesion (HSIL) is low.[7] Therefore, search for other useful molecular markers in the interpretation of high grade intraepithelial lesions have been considered in order to improve clinical management.

Minichromosome maintenance protein 2 (MCM2) and topoisomerase II-α (TOP2A) are two biomarkers that have been identified by DNA microarray. Both of them are involved in DNA replication. BD ProEx C (BD Diagnostics-Tripath, Burlington, NC) is a new immunohistochemical marker including these two monoclonal antibodies.[8,9] TOP2A and MCM2 gene expressions have been considered to be high in cervical carcinoma.[10-12]

TOP2A is an enzyme responsible for unlinking DNA strands during replication. MCM2 is a protein that is involved in the G1 phase of the cell cycle and maintains DNA synthesis via loading the prereplication complex onto DNA; it has helicase activity resulting in the unwinding of DNA.[13]

Limited number of studies concerning ProEx C have been reported. These studies showed that ProEx C assay in liquid–based cytology yielded a high sensitivity for biopsy-proven HSIL.[8,14] Diffuse ProEx C expression has also been reported in head and neck tumors, especially in respiratory squamous cell carcinomas.[15] In addition, strong ProEx C staining exhibited very high sensitivity and specificity for distinguishing normal/reactive hyperplasia from esophageal squamous intraepithelial neoplasia.[16] ProEx C immunoexpression has also been found to be significantly higher in melanomas compared to benign nevi.[17]

In the current study, we aimed to investigate the staining status of ProEx C in liquid-based smears, which were interpreted previously as ASC-US, ASC-H, LSIL, and HSIL.

Materials and Methods

We used 54 residual cervicovaginal cytology samples prepared by BD SurePath autostainer which were previously interpreted as ASC-US, ASC-H, LSIL, and HSIL between years 2013 and 2014. Case selection was performed by two pathologists from the two different institutes. Full agreement of diagnostic categorization by both of the observers and having a sufficient residual sample for preperation were used as inclusion criteria. Cases that fulfilled the two criteria were included in the study. All specimens had been stored at +4°C at refrigerator for less than 1 year.

A thin-layer slide was processed from the residual BD SurePath™ vial using the BD Prepstain™ instrument. The slides were treated with a pretreatment buffer for target retrieval using the BD SurePath slide preperation buffer (BD Diagnostics-Tripath). Immunocytochemical staining was performed with BD ProEx C using a detection reagent kit that includes prediluted antibody, a 3,3'-diaminobenzidine tetrahydrochloride-based chromogen, and hematoxylin-based counterstains (BD SurePath detection reagents and BD SureDetect counterstains), and an automated staining platform (Ventana Autostainer). The slides were than coverslipped and reviewed by the two pathologists.

A three-step algorithm was used for the interpretation of the slides.

1. The first step was to determine the adequacy of the specimen according to the 2001 Bethesda criteria. Then, the immunostained slide was compared with the Pap-stained original slide and examined for the existence of abnormal cells
2. The second step was to determine if there was dark brown nuclear staining in squamous cells. In some slides, a mild background staining was observed due to mucus. This type of staining was interpreted as negative
3. The third and the last step was to determine if these ProEx C stained cells were abnormal using the diagnostic criteria of ASC-US, ASC-H, and low or high grade squamous intraepithelial lesions. In some slides, a light immunostaining was observed in occasional nuclei of glandular cells and tubal metaplasia. This type of staining was also interpreted as negative. If all three criteria were met, the slide was interpreted as positive.

Results

In this study, a total of 54 patients with abnormal cytology interpretation results, including HSIL (n = 14), LSIL (n: 19), ASC-US (n = 17), and ASC-H (n = 4) were studied. All of the 14 cases interpreted as HSIL were scored as positive by ProEx C (100%) [Figure 1].

Three cases interpreted as LSIL (3/19) were scored as positive by ProEx C [Figure 2] whereas 16 were negative (84%).

No significant expression was observed in any of the 17 smears (0/17), which were interpreted as ASC-US (0%). Two of the smears interpreted as ASC-H (2/4) showed immunoreactivity by ProEx C (50%) [Table 1].

Ten colposcopic biopsy materials were available out of the 14 cases, which were previously interpreted as HSIL on cytology (10/14). No follow-up data was available in the remaining 4 cases. Five cases were reported as HSIL and the other cases were diagnosed as LSIL (5/10).

Of the 19 patients whose smears had been interpreted as LSIL, 18 had consequitive colposcopic biopsy materials. Nine cases resulted as low grade intraepithelial lesions; 8 LSILs and 1 vaginal intraepithelial neoplasia-1 (VAIN-1), whereas nine cases resulted as HSIL. Only 6 biopsy materials were present out of 17 cases, which were previously reported as ASC-US (6/17) on Pap-test. The biopsy results of these cases were LSIL (n = 4), endocervical polyp (n = 1), and chronic cervicitis (n = 1).

Three cases which were interpreted as LSIL on cytology showed immunoreactivity with ProEx C. The biopsy results of these cases were HSIL (1/3), VAIN-1 (1/3) and LSIL (1/3) seperately [Table 2].

Table 1: Distribution of the cases according to ProEx C immunoexpression and cytology interpretations

Pap Smear Interpretation Category	LSIL	HSIL	ASC-H	ASC-US
ProExC immunoexpression *n*/Total	3/19	14/14	2/4	0/17
%	16	100	50	0

The positive immunostaining occured in cells reflecting the atypia of at least low grade squamous intraepithelial lesion criteria. Ten cases were interpreted as HSIL and showed immunoreactivity by ProEx C. The immunoreactive cells were highly atypical and fullfilling the criteria of HSIL cytology. The biopsy results of these cases were HSIL (n = 5) and LSIL (n = 5).

Three of 4 ASC-H cases had colposcopic biopsies. One case that showed immunoreactivity by ProEx C was diagnosed as HSIL on biopsy material. Follow-up data was not available for the other positively immunostained case. The cases which were negative by ProEx C were resulted as LSIL (n = 1) and HSIL (n = 1) [Table 2].

ProEx C staining features were similar in biopsy confirmed HSIL and LSIL interpreted smears. Homogeneous dark brown nuclear staining was observed and intense staining was restricted in the nuclei of atypical cells in both HSIL and LSIL categories. No specific nuclear staining was present in normal appearing epithelial cells. On the other hand, no precipitate was observed in the nuclei of unstained cells. In addition, after cytologic examination, the biopsy materials from the same patients and normal cervical control tissues were also immunostained with ProEx C. Observation of similar nuclear staining features in the biopsies with low and high-grade squamous intraepithelial lesions provided the exclusion of storage changes.

Basal cell layer staining was observed in normal cervical epithelium. Lower one-third to full thickness epithelium

Table 2: Distribution of cases according to ProEx C immunoexpression and correlation of cytology interpretation and biopsy results of the cases

ProExC immunoexpression	Cytology Interpretation Result	Biopsy Result		
	LSIL	Low grade intraepithelial lesions*	HSIL	Follow-up unavailable
Positive	3	2**	1	0
Negative	16	7	8	1
Total	19	9	9	1
	HSIL	LSIL	HSIL	Follow-up unavailable
Positive	14	5	5	4
Negative	0	0	0	0
Total	14	5	5	4
	ASC-H	LSIL	HSIL	Follow-up unavailable
Positive	2	0	1	1
Negative	2	1	1	0
Total	4	1	2	1
	ASC-US	Benign histology***	LSIL	Follow-up unavailable
Positive	0	0	0	0
Negative	17	2	4	11
Total	17	2	4	11

*VAIN-1, LSIL; **Two ProEx C positive cases resulted as intraepithelial lesions; VAIN-1 (n=1) and LSIL (n=1); ***Endocervical polyp and chronic cervicitis

staining patterns were observed in LSIL [Figure 3] and HSIL lesions [Figure 4].

Overall sensitivity of a ProEx C test for detecting an intraepithelial lesion was 40% whereas specificity was 100%. Because all of the HSIL interpreted Pap-smears resulted as intraepithelial lesions in biopsies, sensitivity of ProEx C for detecting an intraepithelial lesion was 100% for HSIL interpretation category, seperately.

Discussion

All HSIL interpreted cases showed immunoreactivity by ProEx C (100%). This finding is similar to the study of Siddique *et al*., in which all of the ASC-H patients which were consequtively diagnosed as HSIL on biopsy had positive immunostaining by ProEx C.[18]

The staining pattern was invariably dark brown in color and highly intense without leaving any doubt, and all the stained cells were highly atypical and fulfilled the cytological HSIL criteria.

Sixteen percent of the cases which were interpreted as LSIL showed immunoreactivity by ProEx C, and all positive cases were diagnosed as at least low-grade squamous intraepithelial lesion on biopsy. We used the available residual samples of cervical cytology specimens for immunocytochemistry. During the case selection, some cases with intraepithelial lesion results could not be included in our study because their residual materials were insufficient because of reprocessing and additional Pap-stained slide preperation for diagnostic purposes. Larger series with high number of cases are necessary to reveal the predictive value of ProEx C for the detection of high grade lesions.

Artificial staining in the background of the smear due to mucus and focal mild level of staining in normal endocervical/metaplastic cell component can be observed. Similar problems have been declared in two other studies based on ProEx C immunocytochemistry.[18,19] Therefore careful investigation is essential for the detection of true abnormal cells. Hence, ProEx C stained slide should be simultaneously examined with the original Pap-smear preperate. Adequacy of the ProEx C stained slide was a limiting factor for the evaluation because repreperation was made using the remaining vials of the previously prepared smears by the Surepath method. Technical studies to decrease the nonspecific staining could help to improve the specificity of staining.

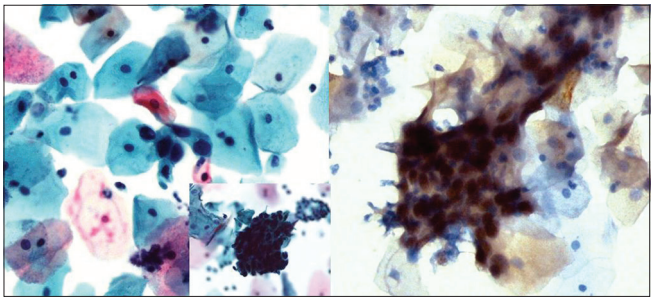

Figure 1: Left: A case interpreted as HSIL (Pap, x200), inset: Crowded group of atypical cells (Pap, ×400); Right: Positive ProExC staining in the same case (ProExC, x100)

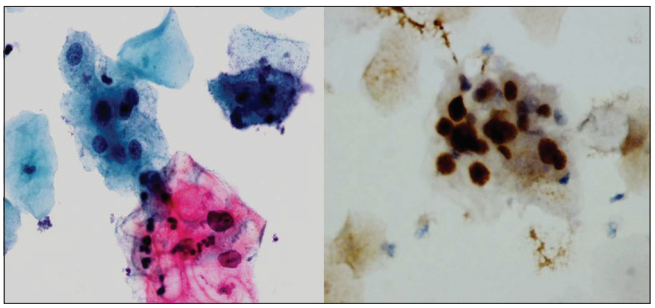

Figure 2: Left: A case interpreted as LSIL (Pap, ×200); Right: Positive ProExC staining in the same case (ProExC, x100)

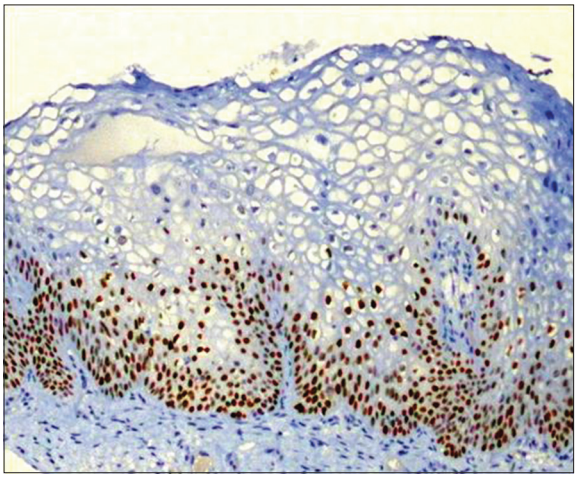

Figure 3: ProEx C immunoreactivity in lower one-third of cervical epithelium in a colposcopic biopsy resulted as LSIL (Pap, x100)

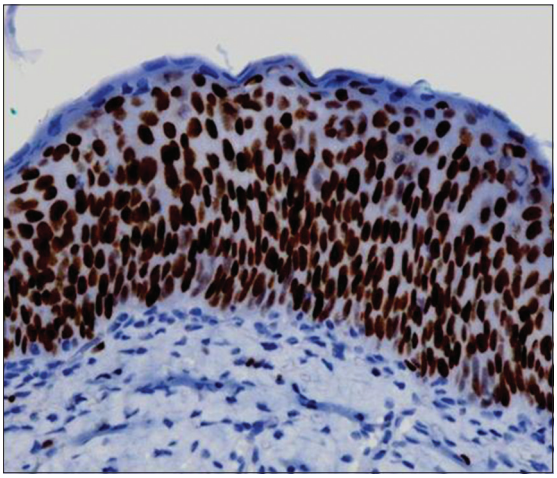

Figure 4: ProEx C immunoreactivity in full thickness cervical epithelium in a colposcopic biopsy resulted as HSIL (Pap, ×200)

All cases which were interpreted as ASC-US were negative by ProEx C. A significant majority of patients (65%) who were interpreted as ASC-US had no consequtive colposcopic biopsy and had lost to follow-up. Because the follow-up data and outcomes of these patients are unavailable, the negative ProEx C score correlation with diagnosis was impossible.

Discordances were determined between cytology and biopsy results of the patients. Half of the cytologically interpreted LSIL cases were consecutively diagnosed as HSIL, whereas half of the cytologically interpreted HSIL cases were consequtively diagnosed as LSIL. Sampling errors could be possible indicators for these discordances. A sampling error in cervical brushing could explain how LSIL interpreted smears achieved a final diagnosis of HSIL. Another possible factor could be progression of the lesion (from LSIL to HSIL) while dealing with underdiagnosis of HSIL. All of the HSIL interpreted smears had fulfilled the criteria of HSIL. Therefore, sampling error of colposcopic biopsy could be an indicator for the LSIL biopsy results of the HSIL interpreted smears.

In the study of Shroyer *et al*., no immunoexpression was determined in 10 pooled samples of NIL smears.[14] Studies investigating ProEx C immunoexpression status of a large number of smears interpreted as negative for intraepithelial lesion or malignancy (NIL), smears having reactive changes including atypical repair, and atrophic smears could help to evaluate the specificity of this test.

A limitation of our study is the lack of HPV DNA analysis. The reason of this limitation is the inadequacy of vial materials for HPV testing after the repreperations of ProEx C. Investigation of HPV DNA analysis combined with ProEx C expression in cervical smears and biopsies of patients with intraepithelial lesions could provide detailed information regarding cervical carcinogenesis.

Conclusion

Our study shows that ProEx C immunoexpression is evident in cases interpreted as HSIL in cytology. Studies with large number of cases would help to indicate the accurate sensitivity and specificity of this test. Nevertheless, regarding the high mortality rates of cervix carcinoma in developing countries, ProEx C immunocytochemistry could be used in cases suspicious for high grade lesions as a supportive method.

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Conflicts of interest
There are no conflicts of interest.

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