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ORIGINAL ARTICLE

Efficiency of immunohistochemical p16 expression and HPV typing in cervical squamous intraepithelial lesion grading and review of the p16 literature ☆

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Abstract

Diagnosing and grading cervical cancer precursors is challenging. This study investigates the presence of HPV infection, the expression of p16, and any correlation between these two findings.

H&E-stained slides of cervical loop excision materials diagnosed as LSIL and HSIL were reviewed. An immunohistochemical panel consisting of p16 as well as of all HPV types and HR-HPV types was applied. Staining of p16 was evaluated according to distribution extent and degree of intensity.

All HSIL cases and 80% of LSIL cases were positive for p16. In HSIL cases, the staining distribution was as follows: 50% full thickness, 45% basal, and 5% rare. The staining intensity for the same cases was strong in 70%, variable in 20%, and weak in 10% accordingly. In LSIL cases, staining distribution was basal in 58.3% and rare in 41.7%. None of the LSIL cases showed full thickness of p16 positivity. The staining intensity of the same cases was strong in 25%, variable in 16.7%, and weak in 58.3%. Of all cases, 48.6% were positive for screening kit (all HPV types), and 31.4% of all cases were positive for HR-HPV. The distribution of this positivity was 35% for HSIL and 26.6% for LSIL cases. The total HPV-type positivity rate was 48.6%, the distribution being 50% for HSIL and 46.6% for LSIL cases.

p16 is a highly sensitive marker for cervical epithelial dysplasia. Strong and full thickness staining of p16 in the cervix epithelium is highly supportive of HSIL, while weak and basal/rare staining favors LSIL. All HPV-positive cases were also p16-positive, but no statistically significant relationship between HPV infection positivity and the intensity and distribution of p16 was found. HPV is not helpful in the grading of SIL, as an unignorable rate of HR-HPV positivity (26.6%) was detected in LSIL group.

Keywords: HSIL; LSIL; HPV type; P16

Introduction

Occasionally, it is challenging to render an accurate histopathologic diagnosis of cervical cancer precursors, which is an essential determiner in prognosis and survival. In particular, the differential diagnosis between reactive states and LSIL, and LSIL and HSIL may be

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difficult [6,7,9,20]. At this point, the need for objective and reliable diagnostic tools cannot be ignored.

p16, a tumor suppressor protein and also a cyclin-dependent kinase inhibitor, whose overexpression has repeatedly been reported to be typical of dysplastic and neoplastic epithelium of cervix [29,1,15], slows down the cell cycle by inactivating the cyclin-dependent kinases that phosphorylate retinoblastoma protein (pRb) [26,16]. The viral oncogenes E6 and E7 of HPV, whose expression is associated with malignant transformation of the cervical epithelial cells [27,31], have the ability to bind and inactivate pRb. The pRb status has a remarkable influence on the expression of p16. p16 expression in cervical lesions is hypothesized to be caused by the functional inactivation of pRb by HPV-E6 and E7 proteins.

Many studies have proposed that p16 is a useful biomarker especially for HR (high risk)-HPV type-related cervical neoplasia [16, 18, 33, 35, 39, 45, 56, 58], and also for predicting SIL progression [13,22,10]. Moreover, a few other studies have recently concluded that there exists a significant association between cervical lesion grade and p16 staining distribution and intensity [17,12,11]. As for the localization of p16 staining, there are studies that report predominant cytoplasmic staining [15] as well as predominant nuclear staining [30], and a combination of both [23].

In this study, we used immunohistochemistry to investigate the staining pattern of p16 on the basis of distribution and intensity, and the presence of HPV in squamous intraepithelial lesions of the cervix. In this way, it was expected to define the role of p16 expression and HPV typing in the diagnosis and grading of the precursor lesions of the cervix.

Materials and methods

Hematoxylin-eosin stained slides from formalin-fixed, paraffin-embedded tissue sections from cervical loop excision materials, formerly diagnosed as LSIL and HSIL, were reviewed by the same pathologist. From the group of cases with confirmed diagnosis, we selected a LSIL group of 15 cases and a HSIL group of 20 cases. From each chosen paraffin-embedded tissue block, three serial sections were taken.

HPV and p16 immunohistochemistry

Viroactiv® HPV Screening Kit and Viroactiv® HPV High Risk Kit were used for HPV immunohistochemistry. Also, we used the CINtec™ p16 (INK4a) Histology Kit (DakoCytomation) (1:25 concentration). For the purpose of antigen retrieval, the slides were rehydrated in decreasing concentrations of alcohol (96%, 70%, 50%, respectively) for 2 min each. Afterwards, the slides were completely covered with citrate buffer and boiled for 5 min in a pressure cooker, and then left to cool down at room temperature in the citrate buffer for 20 min. During the procedure of immunostaining, the slides were placed in washing buffer for 5 min, drained off, and covered with blocking solution. Then, cover slip was added, and the slides were left for incubation at room temperature for 5 min. After cover slip removal, we added one drop of High Risk Antibody VAHP or Screening Antibody VASP and 200 μL of freshly diluted Mouse Anti-Human p16 (INK4a) reagent onto each slide. The slides were then left for incubation at room temperature in humid chamber for 30 min. In a next step, the slides were placed in washing buffer for 5 min. One drop of detection reagent (enzyme-linked AB-conjugate) and cover slip was again added onto each slide and left for an incubation time of 30 min at room temperature. The slides were left in fresh washing buffer for 5 min. Immediately following removal from the washing buffer, one drop (50 μl) of the chromogen solution and cover slip was added, and incubated at room temperature for 10 min. The slides were placed in distilled water for 1 min. After brief counterstaining with hematoxylin and mounting, the slides were examined microscopically.

Nuclear staining was interpreted as positive for all HPV types and HR-HPV types.

Taking the previous reports [15,23,30] into consideration, nuclear staining with or without cytoplasmic staining was regarded as positive for p16 evaluation. Staining distribution was grouped into three categories: rare, basal, and full thickness. The intensity was also subdivided into three groups: weak, variable (containing weak and strong areas of intensity), and strong. It was also monitored if any staining for p16 occurred in the normal squamous or glandular epithelium.

Statistical analysis

The χ^2-test and Fisher's Exact Test were used for statistical analysis.

Results

All HSIL cases (20/20) and 80% of LSIL cases (12/15) were positive with p16. Staining distribution in the HSIL cases was as follows: 50% full thickness, 45% basal, and 5% rare (Fig. 1k, l). The staining intensity of the same cases was strong in 70%, variable in 20%, and weak in 10%, accordingly. Of p16-positive LSIL cases, staining distribution was basal in 58.3% and rare in 41.7%. None of the LSIL cases showed full thickness of p16 positivity (Fig. 1m, n). The staining intensity of the