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Original contribution

Human papillomavirus detection and p16^{INK4a} expression in cervical lesions: a comparative study

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Summary p16^{INK4a} expression in dysplastic cervical lesions is related to high-risk human papillomavirus (HR-HPV) infection. The immunohistochemical expression of this protein in these lesions allows an increase in diagnostic reproducibility in biopsies and the introduction of prognostic factors in low-grade lesions. Here, we studied the immunohistochemical expression of p16 in 86 dysplastic cervical lesions, 54 cervical intraepithelial neoplasms–grade 1 (CIN-I), 23 CIN-II, and 9 CIN-III. In addition, we performed HPV detection and genotyping. We detected HR-HPV in 19/54 CIN-I, 21/23 CIN-II and 9/9 CIN-III cases. p16^{INK4a} immunoreactivity was observed in 7/19 CIN-I HR-HPV–positive, 17/21 CIN-II HR-HPV–positive and all CIN-III cases. Immunoreactivity for p16^{INK4a} was found in 7/54 CIN-I and in 17/23 CIN-II cases. In the follow-up, we detected 3 p16-positive high-grade squamous epithelial lesions (CIN-II and CIN-III) in the CIN-I/p16-negative group and 5 p16-positive high-grade squamous epithelial lesions cases in the CIN-II/p16-negative group. We conclude that p16 negativity in CIN-I and CIN-II biopsies does not always imply regression of the lesion and that the diagnosis of CIN-II should not be based solely on p16 results.

1. Introduction

Routine cervicovaginal cytology examinations have led to a decrease in cervical cancer in developed countries with screening policies. Diagnostic criteria and protocols for the diagnosis and management of preneoplastic cervical lesions have been established. Referred to as cervical intraepithelial neoplasias (CIN), these lesions are classified into three grades on the basis of the thickness of the epithelium that shows alterations. These categories are as follows: CIN-I or low-grade squamous intraepithelial lesion (LSIL), which shows an alteration of maturation in the internal third of the epithelium [1-3]; CIN-III, or in situ carcinoma, which shows an alteration of maturation in the entire epithelial thickness [1-3]; and CIN-II, an intermediate state between CIN-I and -III defined as an alteration

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of epithelial maturation that affects two-thirds of the internal epithelium [1,2], or more than the internal third [3]. CIN-II and CIN-III can show mitosis in suprabasal cells of the epithelium [2].

CIN-II is the least reproducible diagnosis [1], with an interobserver correlation of around 50%, while for CIN-III this correlation is around 87% [4,5]. These 3 categories have their corresponding definitions in cytology: LSIL and HSIL (high-grade squamous intraepithelial lesion, CIN-II and CIN-III), which correlate with the capacity to progress to infiltrating squamous cell carcinoma [1-3]. At present, the cytological classification of LSIL and HSIL is also used for biopsies, correlating the former with CIN-I and the latter with CIN-II and CIN-III [2,6].

High-risk human papillomavirus (HR-HPV) infection is the "sine qua non" but not sufficient condition for developing cervical squamous cell carcinoma. HR-HPV infection produces the oncoproteins E6 and E7, which interact with p53 and pRb, respectively. This last interaction leaves E2F free, thus permanently stimulating p16^{INK4a} synthesis. In these circumstances, p16^{INK4a} can be detected by immunohistochemistry. Consequently, a relationship can be established between p16^{INK4a} immunoreactivity and HR-HPV infection.

In biopsies, p16^{INK4a} immunoreactivity can show focal or diffuse expression. Focal immunoreactivity of this protein has been observed more frequently in condylomatous lesions, while CIN-I is associated with low-risk (LR) HPV [7]. Diffuse p16^{INK4a} immunoreactivity is associated with HR-HPV infection [5,7-10], especially with infection by HPV-16 [9]. p16^{INK4a} is considered positive when expressed diffusely in the cervical epithelium. Furthermore, the diffuse pattern can be related to the severity of the lesion [5,7,8,11] and the degree of CIN [9,10,12-14]. For CIN-II/III, p16^{INK4a} positivity shows a sensitivity of 83.5% and a positive predictive value of 80.1% [10]. p16 overexpression shows 84% sensitivity, 98% specificity, 98% positive predictive value and 86% negative predictive value in detecting HR-HPV [14].

Between 22% and 88% of CIN-I cases express p16^{INK4a} diffusely [9,13-18]. In these circumstances, p16^{INK4a} positivity has been associated with progression of the lesion [11]. Almost all p16^{INK4a}-negative CIN-I cases show regression of the lesion (negative predictive value, 96%) [18]. Between 24% and 100% of CIN-II cases express p16^{INK4a} diffusely [8,9,13-16,18,19]. Some CIN-II cases show p16^{INK4a} immunoreactivity, but HR-HPV is not detected. Conversely, some of the cases do not show p16^{INK4a} immunoreactivity but HR-HPV is detected [20,21]. Between 54% and 100% of CIN-III cases express p16^{INK4a} diffusely [9,12-19]. In infiltrating squamous cell carcinoma cases, p16^{INK4a} immunoreactivity is detected in 100% of the cases [9,13]. Consequently, p16^{INK4a} is considered the best candidate for studying and grading cervical dysplasias [22-25]. Here, we performed a descriptive study of p16^{INK4a} and HR-HPV in all three grades of dysplasia.

2. Materials and methods

2.1. Cases

We selected 92 consecutive biopsy cases diagnosed with cervical dysplasia. All patients had a previous cytological study, and samples were tested by Hybrid Capture II (HC2). All biopsies were studied histopathologically and were tested for p16^{INK4a} immunoreactivity. HR-HPV was also examined.

2.2. Cytological study

The ThinPrep Platform (Hologic, Malborough, MA) was used in all cases. Liquid-based cytology and the Cervex-Brush (Therapak, Middlesex, UK) were used for collecting samples. Slide samples were obtained using the T-3000. Auto Stainer XL (Leica Microsystems, Wetzlar, Germany) was used to stain the slides. The automatic reading of the slides was performed using Imager (Hologic). The Bethesda criteria [26] were used to classify the lesions as low or high grade.

2.3. Histopathological study

Samples were processed using common methods, and hematoxylin and eosin (HE) staining was performed. CIN-I, CIN-II, and CIN-III cases were identified following classical criteria, [1-3,5], using HE stain without the p16^{INK4a} result.

2.4. HPV detection in cytology

The HR-HPV kit (Qiagen, Hilden, Germany) was used in the remaining cytological samples, following the manufacturer's instructions. HC2 is based on the hybridization of target HPV DNA with RNA probes to detect a total of 13 HR-HPV strains (16, 18, 31, 33, 35, 39, 45, 51, 56, 58, 59, and 68). Then antibodies against DNA-RNA hybrids are applied, and the signal is amplified. The final reaction is chemiluminescent, and it is measured by a luminometer with a reported sensitivity of 1 pg. A mean of 3 positive controls per plate was used as a cut-off. The result was reported as positive or negative with a semiquantitative value (relative light units [RLU]), which is the ratio between the RLU of each sample and mean RLU of positive controls. When RLU values fell between 0.8 and 2.7, the test was repeated with the remaining material.

2.5. Immunohistochemical study

p16^{INK4a} expression was studied using the CINtec Histology kit (Roche Diagnostics, Mannheim, Germany) in an automatic process using a Ventana Benchmark XT (Roche Diagnostics). The immunohistochemical positivity of p16^{INK4a} was classified as follows: (1) *isolated* (Fig. 1B),