High-risk papillomavirus (hr-HPV) L1 capsid protein and cervical lesions

INTRODUCTION

Immunohistochemistry has become, in the last decades, the most useful adjunct method in surgical pathology, enabling the "in situ" detection of antigens important in the diagnosis of poorly differentiated neoplasms, selection of possible original site for metastatic neoplasms, and, more recently, assessing prognostic or predictive tumor markers. Immunohistochemistry has also proved to be a very useful tool for the histological detection of microbial infectious in tissue samples, including viruses such as cytomegaloviruses, measles, and hepatitis B. In this study, immunohistochemical detection of HPV was used as a simple tool for identification HPV infection in cellular and tissue samples. Initial work from Jarosh et al. in 1985 was based on immunohistochemistry directed against bovine papillomavirus, which was shown related to L1 protein of the papillomavirus. The immunoreaction of such antigens is resisted to high-replication episomal infections, since integration of viral DNA sequences within the genome of infected cells has been associated to truncation of expression of several viral genes, including those of L1 region.

METHODS AND MATERIAL

Penfill blocks of 153 cervical biopsies (110 CIN1-3, 43 cervicitis) were retrieved from the files of Instituto Adolfo Lutz, Sao Paulo, corresponding to a series of patients with a previous report of epithelial lesions on a Paperoxular smear. In all cases, colposcopy performed at Hospital Laonor Mendes de Barros from 1996-1997 was considered satisfactory, and biopsies were obtained from the abnormal areas of the lesions. Biopsies were fixed in formalin and routinely stained by hematoxylin-eosin. Histological diagnosis was obtained from the same core by M. de L. Lab.(rtM). Non-ionic in situ hybridization (NISH) had been previously carried out, as reported by Roteli-Martins et al., 2001, using a digoxigenin-labeled sense or antisense probe (75111, 18, 31353) coated probe with biotinylated tyramide-catalyzed signal amplification system (CIA: DAKO, USA).

The finding of 19 cases of low-risk papillomavirus (hr-HPV L1) was determined by the presence of cervical lesions, as the finding of 19 cases of high-risk papillomavirus (hr-HPV L1) was determined by the presence of cervical lesions.

RESULTS

Out of 153 samples submitted to the High Risk HPV L1 protein (HR-HPV L1), 25 were positive, 121 were negative. Positive reaction was characterized by a strong staining of the whole nucleus, surrounded by a cytoplasmic with no background, except for rare cases with a granular cytoplasmatic staining, possibly due to endogenous blood or other endogenous component unmasked by the heat-induced epitope retrieval. In negative reactions with HPV lesions, the staining was absent or present only in a few cells, for instance in the cases of CIN 3 (fig. 11B, 11C, 11D). The distribution of immuno-reactivity for HR-HPV L1 and HPV-DNA detection by NISH according to the histological diagnosis is depicted in Table 1, where it can be seen that some lesions with histologically non-associated were also positive, as well as some lesions with positive histological diagnosis, but negative for HPV-DNA detection.

There were 3 uses of a series of hr-HPV L1 protein, and HR-HPV DNA by Hybrid Capture. The results from the two methods agreed in 65 negative and 26 positive reactions. Negative results allowed to HPV-DNA detection both for cervical and vaginal canal, as well as positive results by Hybrid Capture, as were classified as positive for cervical and vaginal canal, as well as positive results by Hybrid Capture.

High-risk HPV DNA was also detected by Hybrid Capture in 72 out of 146 samples. Table 2 shows the relation between the immunohistochemical detection of HPV L1 protein and HPV-DNA by Hybrid Capture. The results from the two methods agreed in 65 negative and 26 positive reactions. Negative results allowed to HPV-DNA detection both for cervical and vaginal canal, as well as positive results by Hybrid Capture, as were classified as positive for cervical and vaginal canal, as well as positive results by Hybrid Capture.

The result of the present study is to assess the immunoreactivity of L1 capsid protein of HPV in a series of biopsies of patients representing the spectrum of squamous intraepithelial lesions of uterine cervix, as compared to the detection of HPV DNA by In Situ Hybridization and by Hybrid Capture.

DISCUSSION

This study, designed to assess the immunoreactivity of L1 capsid protein of HPV in a series of uterine cervix biopsies, showed nuclear immunoreactivity of this antigen in 28.2% of lesions with CIN1, contrasting to 9.3% in samples with CIN2. Since it is well documented that the vast majority of cases of CIN harbor HPV DNA, our findings show a low sensitivity of immunohistochemistry which, thus, should not be used for screening for HPV infection. However, since morphological methods could fail to reveal the virus under normal conditions, the "in situ" detection of viral markers may offer useful information on biological behavior of the tumor and its histology. In this study, we also evaluated the potential value of this approach in "in situ" localization of HPV, which was demonstrated to be a reliable tool for the identification of the viral genome.

The finding of 19 cases of CIN 1 or CIN 2 for HPV-DNA by NISH, but not immunohistochemistry hr-HPV L1 protein could be observed either to a "latent phase" of infection or, more probably, to a lower sensitivity or the IHC amplification method used here. Since NISH has become a more sensible method when the catalysing signal amplification (CSA/CARD) was introduced further studies should assess the potential sensitivity of monoclonal antibody (CSA) with this amplification system, preferably in more recently collected tissue samples.

The rather low immunoreactivity of hr-HPV L1 protein in CIN 3 cases should possibly correspond to the integration of HPV DNA sequences in the genome of infected keratinocytes, thus truncating the expression of this gene. However, the immunodetection of L1 protein in 4 cases of cervical lesions in this series of women submitted to biopsy due to recent finding of HPV-associated cervical lesions could possibly represent a finding of replicative HPV infection not yet followed by a cytological effect in this area, thus deserving further studies. In several cases studied here in HPV infection was only detected by one of the methods, NISH and IHC should preferentially be used combined.

A major potential clinical use of this immunodetection is its application in the initial biopsy of cohorts of cases initially diagnosed as CIN1, CIN 2 or as with "equivocal lesions". If this approach proves our hypothesis that this immunodetection corresponded to a true active infection with a hr-HPV with a higher rate of evolution to higher-degree epithelial lesions, then its use as an adjunct method for selecting cases for a closer follow-up and earlier intervention should be considered.

REFERENCES