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Original article

Human papillomavirus detection using in situ hybridization and correlations with histological and cytological findings

Corrélation cyto-histologique du diagnostic de l'infection par HPV avec la recherche du virus par hybridation in situ

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Abstract

Background and objective. – The human papillomavirus (HPV) is the main causative agent of cancerous and precancerous lesions in cervical cancers. The infection detection is performed by cytological and histological examination. However, molecular testing for HPV infection remains necessary to distinguish nonspecific inflammatory lesions from HPV-related lesions. We aimed to compare cytological and histological diagnosis of HPV lesions and to confront histological findings with molecular detection of HPV by in situ hybridization (ISH).

Material and methods. – A total of 2399 pap smears were collected between 1997 and 2002. Twenty-nine of them presented with lesions indicative of HPV infection. Colposcopy and biopsy were performed on 23 of these 29 pap smears, with a comparison of the cytological and histological findings. Molecular testing for HPV by ISH was performed on 11 pap smears.

Results. – A 79% cytological and histological agreement was observed. ISH revealed the presence of the virus in seven case patients, amounting to a 63% histological and molecular agreement. Seven HPV genotypes were categorized (6-11-16-18-31-33-51).

Conclusion. – HPV detection by ISH adds little to the diagnosis of HPV-associated lesions based on cytological and histological features. It, however, helps improve the specificity of the diagnosis and determine the viral genotype which, in turn, helps better define the lesion prognosis.

Keywords: Histology; Human papillomavirus; In situ hybridization

Résumé

Introduction et objectif. – Le virus du papillome humain (HPV) est étroitement lié aux lésions cancéreuses et précancéreuses du col utérin. La détection de l'infection à HPV repose sur la cytologie et éventuellement l'histologie. Toutefois, le recours au diagnostic moléculaire s'avère parfois nécessaire afin de distinguer les lésions inflammatoires non spécifiques des lésions dues à l'HPV. L'objectif de cette étude était de comparer le diagnostic cytologique et histologique, d'une part, et moléculaire, d'autre part, par hybridation in situ (HIS) de l'infection à l'HPV.

Matériel et méthode. – Notre étude a porté sur 29 frottis cervicovaginaux présentant des stigmates d'infection virale à HPV parmi les 2399 cas recueillis du service d'anatomie pathologique et cytologique de l'hôpital militaire de Tunis sur une période de cinq ans. Parmi les cas étudiés, 23 ont été suivis de biopsies avec examen histologique et confrontation cytohistologique. La recherche de l'infection par HPV a été réalisée par hybridation in situ sur 11 cas.

Résultats. – Une concordance de 79 % a été retrouvée entre le diagnostic cytologique et histologique des lésions condylomateuses. L'HIS a mis en évidence la présence du HPV dans sept cas, soit une concordance histomoléculaire de 63,63 %. Les génotypes d'HPV détectés étaient 6-11, 16-18 et 31-33-51.

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Conclusion. – La détection par HIS du virus HPV contribue faiblement à améliorer le diagnostic de ces lésions par les critères cytomorphologiques. Elle permet, cependant, d'améliorer la spécificité diagnostique et de déterminer le génotype du virus afin de mieux établir le pronostic de ces lésions.

Mots clés : Histologie ; Virus du papillome humain ; Hybridation in situ

1. Introduction

Cervical cancer is one of the most frequent female cancers. It is the second most frequently observed cancer in Tunisia and around the world, after breast cancer, and the third cause of cancer death in female patients [1,2]. Human papillomavirus (HPV) is closely associated with cancerous and precancerous cervical lesions observed in more than 90% of cervical cancers [3]. Condyloma refers to the benign cervical lesion, and is a crucial step in cancer progression. Detecting signs of viral infection from cervical and vaginal samples may be done by cytological examination and potentially by histological examination using cytomorphological and nuclear criteria. Molecular techniques are sometimes useful, including in situ hybridization, to confirm the presence of the virus and to prescribe an adequate curative or preventive treatment.

2. Material and methods

We conducted a five-year (1997–2002) retrospective study of 2399 pap smears. All pap smears were assessed by the anatomy and pathological cytology laboratory of Tunis Military Hospital. Signs of HPV infection were observed in 29 pap smears. We performed a comparative cytological and histological analysis as well as biopsies on 23 pap smears. The cytological and histological examinations were performed by two pathologists in compliance with the 2001 Bethesda system classification. We performed an in situ hybridization analysis of human papillomavirus strains on 11 cervical biopsies using the commercial kit Enzo to identify HPV genotypes 6/11, 16/18, or 31/33/51.

3. Results

3.1. Cytological and histological comparative analysis

Twenty-nine of the 2399 pap smears included in the study presented with cytological alterations due to HPV infection (Fig. 1). This figure amounts to a prevalence of 1.2% (29/2399). Patient age varied from 20 to 69 years, with a mean age of 42.48 years. A single peak of prevalence was observed in the 30–49 age group. The comparative cytological and histological analysis was performed on 23 pap smears, followed by a colposcopy with biopsy: 19 pap smears were classified as low-grade squamous intraepithelial lesions (LSIL) and four as atypical squamous cells of undetermined significance (ASCUS).

A total of 15 dysplastic pap smears presented with dysplastic lesions at histological examination (Fig. 2): eight low-grade lesions (53.33%) and seven high grade lesions (46.66%).

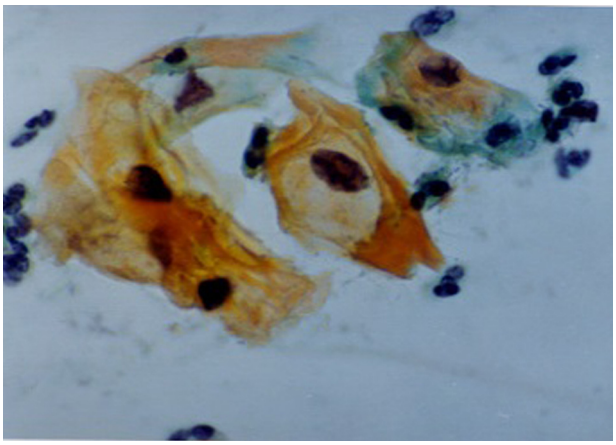

Fig. 1. Pap smear, Papanicolaou test × 100. Koilocyte showing hyperchromatic nucleus with irregular rims and a large perinuclear vacuole.
Frottis cervicovaginaux, test de Papanicolaou × 100. Cellule koïlocytaire avec noyau hyperchromatique à contours irréguliers entouré d'un halo clair périnucléaire.

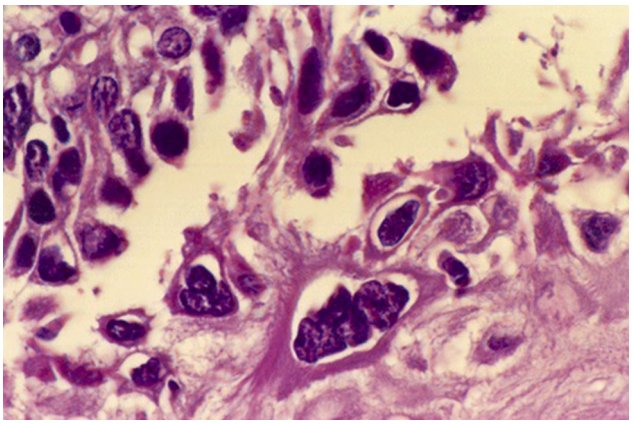

Fig. 2. HE × 100: epithelium of the exocervix showing a koilocyte with hyperchromatic nucleus and binucleation and a large perinuclear vacuole.
HE × 100 : épithélium exocervical montrant des koïlocytes munis de noyaux hyperchromatiques, parfois binuclés et entourés d'un halo clair périnucléaire.

Cytological and histological agreement was 79% (15/19). Four pap smears classified as false positive corresponding to non-specific lesions of atrophy, squamous metaplasia, and cervicitis were observed at histological examination.

Four of the 23 pap smears included in the comparative cytological and histological analysis did not have any sign of HPV infection. However, atypical signs were observed (probably reactional) and their biopsies were positive. A diagnosis of mild dysplastic lesions was established, with a discordance of 21% (Table 1).

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