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The association of HPV genotype with the regression, persistence or progression of low-grade squamous intraepithelial lesions☆

F.A. Silveira [a,b], G. Almeida [a,b], Y.L. Furtado [a,b,*], S. Cavalcanti [c], K.S. Silva [d], P. Maldonado [a], M.G.C. Carvalho [e]

[a] Institute of Gynecology, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
[b] Post-Graduate Program in Surgical Sciences, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
[c] Department of Microbiology and Parasitology, Universidade Federal Fluminense (UFF), Niterói, Brazil
[d] National Institute for Women's, Children's and Adolescents' Health, Instituto Fernandes Figueira, Fundação Osvaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil
[e] Pathology Department, Laboratory of Molecular Pathology, Clementino Fraga Filho Hospital, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

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ABSTRACT

Background: Human papillomavirus (HPV) is a highly prevalent sexually transmitted virus causing cytological alterations that precede cervical cancer. Approximately 130 genotypes have been sequenced. Low-grade squamous intraepithelial lesions (LSIL) are the most frequent cytological alteration and have an uncertain behavior.
Objectives: To analyze the frequency of HPV types in LSIL and their association with the regression, persistence or progression of these lesions.
Methods: A cohort study of forty patients with LSIL cytology was conducted from December 2007 to March 2011. The follow-up lasted two years and included cytology and colposcopy. HPV detection was performed using PCR, and genotyping was performed using PCR-specific and RFLP techniques.
Results: DNA-HPV was detected in 87% (35/40) of the cases, with oncogenic HPV accounting for 76%; type 16 in 32% (11/35) and type 18 in 20%. LSIL regression, persistence and progression rates at the end of the study were 60%, 23% and 17%, respectively. There was 50% regression in lesions in the high oncogenic risk group (types 16 and 18).
Conclusion: HPV 16 was the most frequent genotype found in LSIL. The persistence and progression of the LSIL were related to the persistence of oncogenic HPV. The longer the follow-up time, the lower the LSIL persistence rate and the higher its regression rate; the progression rate remained stable. In addition to the presence of oncogenic HPV, other factors are necessary for the progression of LSIL.

1. Introduction

Human papillomavirus (HPV) is an epitheliotrophic virus belonging to the *papillomaviridae* family. Approximately 130 types have already been sequenced, of which 40 infect the lower genital tract (Villiers et al., 2004; Stanley, 2010). There are 15 types classified as oncogenic, with types 16 and 18 accounting for 70% of cervical cancer cases (Smith et al., 2007). This is a sexually transmitted virus and, due to its high prevalence, women who are sexually active have a 50% to 80% chance of acquiring HPV infection during their lifetimes (Stanley, 2010). This infection is more frequent among young women. Most of these infections are transient and regress spontaneously (Stanley, 2010). A small number of women will present with persistent oncogenic genotype infections that portend a higher risk of progressing to precursor lesions and cancer.

Cytology identifies cell abnormalities that precede cervical carcinoma (Paesi et al., 2009). Low-grade squamous intraepithelial lesions (LSILs) account for most of the cytological anomalies for screening cervical cancer. In the USA, 2% to 3% of all cytology findings consist of LSIL (Clifford et al., 2005). In France, 1.12% of LSIL was identified in the screening cervical cancer study conducted in 2008 (Prétet et al., 2008). In Brazil, the prevalence of LSIL in 2009 reached 0.8% of the total cytology tests performed. When considering only abnormal exams, the prevalence of LSIL reached 31% (Ministério da Saúde (Brasil). Instituto Nacional de Câncer, 2012).

It is difficult to analyze the regression, persistence and progression rates of LSIL due to the heterogeneity of the studies. In the literature, regression rates from 7% (Campion et al., 1986) to 95% (Chuery et al., 2008) are reported, with persistence from 4% (Chuery et al., 2008) to 67% (Campion et al., 1986) and progression to high-grade lesions or cancer from 1% (Chuery et al., 2008) to 26% (Campion et al., 1986).

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* Corresponding author at: Instituto de Ginecologia, Rua Moncorvo Filho, No. 90, Centro, Rio de Janeiro, RJ, CEP 20211-340, Brazil.
E-mail address: yarafurtadorj@terra.com.br (Y.L. Furtado).

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The aim of this study is to identify HPV genotypes that are more prevalent in LSIL and study their association with the regression, persistence and progression of these lesions.

2. Methods

A cohort study was conducted from December 2007 to March 2011. Fifty-nine patients over 18 years of age with LSIL cytology were recruited from the Cervical Pathology and Colposcopy Unit at the Institute of Gynecology, Universidade Federal do Rio de Janeiro (UFRJ). The patients were interviewed and, after signing the informed consent, cervical swabs (ecto- and endo-cervix) were collected and stored in Eppendorf tubes. A specific Polymerase Chain Reaction technique (PCR) (Pestaner et al., 1994) and a Restriction Fragment Length Polymorphism (RFLP) technique (Walker et al., 2003) were used for the detection and typing of HPV-DNA.

The patients were followed in the outpatient clinic for two years and were subjected to semi-annual cytological controls and colposcopic examinations using conventional techniques with 5% acetic acid and Lugol's solution. Colposcopic changes were classified as major and minor alterations, which was compliant with the colposcopic terminology established by the International Federation of Cervical Pathology and Colposcopy (IFCPC) (Solomon et al., 2002). Cytological reports were created according to the 2001 Bethesda System (Furtado et al., 2010).

If a cytology report indicated a high-grade squamous intraepithelial lesion (HSIL), a colposcopy-guided biopsy was performed. This approach was used to verify the progression of the lesion. The regression of the lesion was assumed when the cytology was normal; persistence was documented when a report disclosed LSIL.

HPV genotyping was carried out at the first consultation and at 12 months follow-up.

For the molecular study, the cervical swabs were treated with K proteinase and were then subjected to a specific DNA extraction technique (Pestaner et al., 1994; Molijn et al., 2005).

To detect the presence of DNA-HPV, the PCR technique was used with the MY 09/11 and GP5+/GP6+ primers, expanding the DNA fragment of 450 bp and 140 bp respectively (Melgaço et al., 2010).

The typing of the DNA-HPV was conducted using the specific PCR technique (Melgaço et al., 2010), specific multiple primers and RFLP (Walker et al., 2003).

The study was approved by the Research Ethics Committee at the Maternity School of UFRJ in November 2007 and under number 07/2007.

3. Results

Of the 59 women, 40 completed the study. Nineteen women were excluded: twelve had to undergo biopsies; two became pregnant; one had HIV-positive serology; one underwent a kidney transplant; and three did not sign the informed consent form.

The mean age of the patients was 35 years old, ranging from 19 to 57 years old.

HPV DNA was detected in 87% (35/40) of the cases. An infection by a single type of HPV occurred in 82.8% (29 / 35) of the cases. The most frequent HPV types were 16 and 18, which were found in 32% (11/35) and 20% (7/35) of the cases, respectively. The least frequent oncogenic types (HPV 33, 45, 58 and 70), were detected in 18% (6/35) of the cases. The presence of low-risk HPV types 6 and 54 were detected in 15% (5/35), while the association of types 6 and 11 was found in 9% (3/35) (Fig. 1). In one case, despite a positive HPV DNA test, it was not possible to identify the genotype.

During the first round of genotyping, concurrent co-infection by types 16/18 and 6/11 was detected in 15% (5/35) of the cases (Molijn et al., 2005).

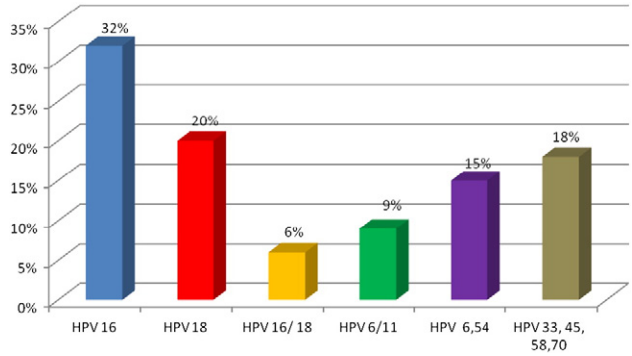

Fig. 1. HPV genotyping frequency in 35 cases of LSIL.

Viral persistence with the same HPV type for two genotypes occurred in 17% (6/35) of the cases, while the presence of different types was found in 12% (4/35) of the cases.

The persistence of LSIL cytology dropped over time, down from 74% (26/35) at 12 months to 40% (14/35) at 18 months and 23% (8/35) at 24 months. The LSIL cytological regression rate rose over time: up from 14% (5/35) at 12 months to 43% (15/35) at 18 months and 60% (21/35) at 24 months. The LSIL cytological progression rate was 14% (5/35) at 12 months, reaching 17% at 18 and 24 months (Fig. 2).

Four patients with lesion progression underwent a large loop excision of the transformation zone (LLETZ) and two patients underwent cold-knife conization. Analyzing the association between the genotype and the cytological progression of the lesion, it was noted that, among the six patients in which the lesions progressed, the second round of genotyping was negative for three of them; the same viral type was detected in one of them and different viral types were detected in two others. For the eight patients with persistent lesions, the second round of genotyping was negative for six of them; the viral type persisted for the other two patients.

For 16 out of the 21 patients who had regression, the second round of genotyping was negative. The viral type persisted in three of the cases. In two others, different viral types were detected.

When analyzing the association between the genotype and progression to more severe lesions, it was noted that 50% of the lesions in the high oncogenic risk group (types 16 and 18) regressed at the end of the study. For the less frequent oncogenic HPV group, the regression rate was high (71%). In the low-risk HPV group, the regression rate was even higher (75%), with no cases of progression (Fig. 3).

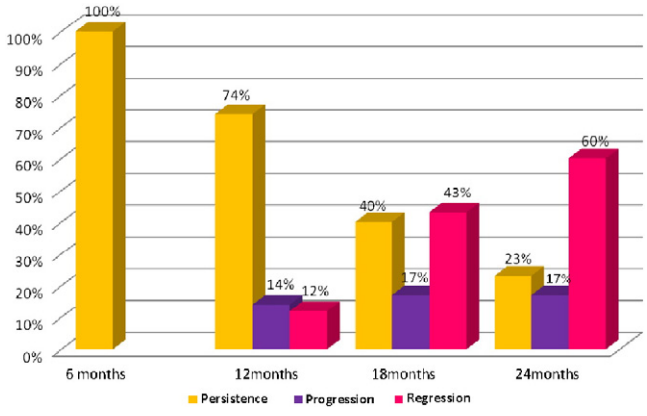

Fig. 2. Outcome of cytology findings in 35 cases of LSIL.