Journal of Clinical Virology 47 (2010) 43–48

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Prevalence of human papillomavirus genotypes, and mucosal IgA anti-viral responses in women with cervical ectopy

Olga L. Monroy[a], Cecilia Aguilar[a], Marcela Lizano[a], Fernando Cruz-Talonia[b], Rosa M. Cruz[b], Leticia Rocha-Zavaleta[a,*]

[a] Departamento de Biologia Molecular y Biotecnología, Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Circuito Escolar s/n, Ciudad Universitaria, Mexico D.F., C.P. 04510, Mexico
[b] Fundacion Cruz Talonia, Colegio Mexicano de Colposcopistas, Mexico D.F., Mexico

ARTICLE INFO

Article history:
Received 6 January 2009
Received in revised form 29 September 2009
Accepted 7 October 2009

Keywords:
Human papillomavirus infection
Cervical ectopy
Human papillomavirus prevalence
Endocervical cells

ABSTRACT

Background: Data on the prevalence of different human papillomavirus (HPV) genotypes and the associated mucosal immune response in women with cervical ectopy are scarce.
Objective: To assess the prevalence of different HPV genotypes and the mucosal anti-viral immune response in cervical ectopy.
Study design: Detection and typing of HPV DNA was determined in 141 women with cervical ectopy, 272 cytologically normal controls and 98 low-grade squamous intraepithelial lesions (LSIL) by PCR and direct sequencing. Mucosal IgA antibodies to HPV16 and HPV18 were evaluated in cervical mucus by ELISA.
Results: The prevalence of HPV in cervical ectopy was higher (73.7%) than that observed in control samples (30.5% in endocervix, and 1.8% in exocervix), but similar to the prevalence in LSIL (62.2%). Typing showed that the overall distribution frequency concerned 14 different genotypes, with HPV18 being the most prevalent in cervical ectopy (53.9%), whereas HPV16 predominated in LSIL (38.7%). High-risk HPV genotypes were 2.2 times more frequent in cervical ectopy than in the normal endocervix ($p < 0.0001$). HPV infection in cervical ectopy patients was accompanied by a mucosal IgA-antibody response. Antibody reactivity to HPV18 was significantly higher than the response to HPV16.
Conclusion: Cervical ectopy is a risk factor for infection with high-risk HPV genotypes, in particular HPV18. Our results emphasize the need of further studies to clarify the oncogenic potential of this virus in cervical ectopy.

© 2009 Elsevier B.V. All rights reserved.

1. Background

Infection with high-risk human papillomavirus (HPV) genotypes is associated with most cases of cervical tumors.[1] HPV types 16 (HPV16) and 18 (HPV18) are the most prevalent types found in cervical cancer. HPV16 is associated with infection of the squamous epithelium, and the progression to squamous cervical carcinoma.[2] In contrast, HPV18 is commonly associated with cervical adenocarcinomas[3] and glandular lesions,[4] suggesting that HPV18 has a greater tropism for cervical glandular tissue.[5]

Glandular epithelium lines the surface of the cervical canal (endocervix), and is composed of a single layer of mucin-secreting columnar cells that form cleft-like infoldings with a glandular appearance. During pregnancy and in adolescence the columnar cells of the endocervix extend beyond the external os onto the vaginal surface. This physiological process is called cervical ectopy, and it can also be induced by the intake of hormonal contraceptives. Although most women are asymptomatic, the condition can present with irregular discharge and postcoital and intermenstrual bleeding.[6,7] It has been suggested that cervical ectopy may be a potential risk factor for various sexually transmitted diseases, including *Chlamydia trachomatis*,[8] HIV,[9] and HPV infection.[10–12] In a previous work we showed that the detection of HPV DNA was more common in cervical ectopy than in cytology normal controls.[12] However, the prevalence of different HPV genotypes in cervical ectopy is yet unknown.

Humoral immunity in the female genital tract has been well characterised. It is known that IgA- and IgG-secreting plasma cells are abundant in the lamina propria of endocervix. However, little is known about the antibody-mediated immune response in women with cervical ectopy.[12]

The purpose of the present study was to define the prevalence of HPV genotypes in cervical ectopy. In addition, the presence of a

Abbreviations: HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion; PCR, polymerase chain reaction; HIV, human immunodeficiency virus; IgA, immunoglobulin A; IgG, immunoglobulin G; VLP, virus-like particle; ELISA, enzyme-linked immunosorbent assay; PRR, prevalence rate ratio; CI, confidence interval.

* Corresponding author. Tel.: +52 55 56229217; fax: +52 55 56229212.
E-mail address: lrochaz@correo.biomedicas.unam.mx (L. Rocha-Zavaleta).

1386-6532/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2009.10.008

mucosal antibody-mediated response to the virus in cervical ectopy was determined.

2. Objective

To assess the prevalence of different HPV genotypes, and the mucosal anti-viral immune response in patients with cervical ectopy.

3. Study design

3.1. Subjects and samples

The study population was selected from women attending the Clinic of Dysplasias of the Mexican College of Colposcopists, Mexico City. This clinic provides gynecological services to women referred for colposcopy because of either abnormal cytology or gynecological symptoms, and women without a history of cervical abnormalities who ask for a routine examination. In the present study, 511 women who consented to participate and provide data related to additional risk factors for cervical cancer were included. All women underwent cytological and histopathological analysis. Colposcopy-directed biopsies from 141 women with confirmed cervical ectopy, and 98 patients with low-grade squamous intraepithelial lesion (LSIL) were studied. All cervical ectopy cases complained of abnormal vaginal discharged and/or post-coital bleeding. Two independent, age-matched control groups were formed. From 272 subjects without a history of HPV infection, and no evidence of cervical abnormalities we obtained 105 exocervical biopsies ("normal exocervix" group) and 167 endocervical cell samples using a standard cytobrush ("normal endocervix" group). Consecutive exocervical biopsies were collected until the "normal exocervix" group was integrated. Afterwards, endocervical samples were consecutively obtained to constitute the "normal endocervix" group. Cervical mucus was collected by washing the uterine cervix with 1 mL of sterile, contaminant-free phosphate-buffered saline (PBS) (Roche Applied Science, Indianapolis, IN). Cell debris was eliminated by centrifugation at 13,000 rpm for 5 min and supernatant collected for analysis. Mucus samples were subsequently stored at −70 °C until tested.

3.2. HPV DNA detection

DNA was extracted and purified using the QIAamp DNA Micro kit (Qiagen Inc., Valencia, CA) according to the protocol of the manufacturer. HPV DNA was amplified using the general primers MY09-MY11,[13] which amplify a conserved 450-bp fragment from the L1 gene, and the L1C1-L1C2-1 consensus primers which amplify a 250-bp fragment of the L1 gene located upstream of the MY sequence.[14] The PCR protocol was: 95 °C for 5 min, 30 cycles of 45 °C (for MY09-MY11) or 49 °C (for L1C1-L1C2-1) for 30 s, and 72 °C for 60 s. PCR products were analysed by using agarose gel electrophoresis. An internal control β-globin gene fragment was co-amplified using the PC03 and PC04 primers as described elsewhere.[15] DNA from SiHa cells, containing 1–2 HPV16 copies per cell, was used as a positive control.

3.3. HPV typing

HPV genotypes were determined by two separate protocols; one using type-specific primers for HPV16 and HPV18, and the other using general HPV (MY09-MY11) primers followed by dideoxynucleotide sequencing. Specific amplification of HPV16 was achieved by using the Pr3-Pr4 primers that amplify a 499 fragment covering the HPV16-E7 gene plus fragments of the E6 and E1 genes.[16] Detection of HPV18 was accomplished by using the HPVE718F (5′-AAG AAA ACG ATG AAA TAG ATG-3′), and HPVE718R (5′-GGC TTC ACA CTT ACA ACA CA-3′) primers that amplify a 100 bp fragment of the HPV18-E7 gene. The PCR protocol was: 5 min at 95 °C, 38 cycles of 94 °C for 30 s, 54 °C (for HPV16) or 53 °C (for HPV18) for 60 s, and 72 °C for 90 s, followed by 10 min at 72 °C. Agarose electrophoresis was then performed. DNA from SiHa (HPV16 positive cell line) and HeLa (HPV18 positive cell line) cells were included as positive controls.

HPV genotypes were also identified by direct sequencing of positive MY09-MY11 PCR products. PCR products were purified with Exo-SAP-IT (USB Corp., Cleveland, OH), and subjected to cycle sequencing with an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA), using MY11 as the sequencing primer. The reaction mixture was analysed in the ABI Prism 3100-Avant Genetic Analyser (Applied Biosystems, Foster City, CA). Sequence alignments were performed against standard HPV genotypes available in GenBank using a BLAST server. HPV genotypes were identified on the basis of more than 98% sequence homology considering 350 nucleotides.

3.4. E6/E7 nested multiplex PCR

Multiple HPV infections were investigated by using a nested multiplex PCR based on the use of type-specific nested PCR primers that amplify the E6/E7 region as described elsewhere.[17] Briefly, DNA samples were subjected to a first-round amplification using the consensus forward GP-E6-3F (5′-GGG WGK KAC TGA AAT CGG T-3′), and reverse GP-E7-5B (5′-CTG AGC TGT CAR NTA ATT GCT CA-3′) and GP-E76B (5′-TCC TCT GAG TYG YCT AAT TGC TC-3′) primers to generate an amplicon ranging from 602 to 666 bp in size. The PCR protocol was: 10 min at 94 °C, then 40 cycles of 94 °C for 60 s, 40 °C for 60 s, 72 °C for 120 s, followed by 10 min at 72 °C. GP-E6/E7 PCR products were subsequently amplified with type-specific nested primers arranged in four cocktails for the following HPV genotypes: cocktail I (HPV16, 18, 31, 59, 45), cocktail II (HPV33, 6/11, 58, 52, 56), cocktail III (HPV35, 42, 43, 44), and cocktail IV (HPV68, 39, 51, 66). The PCR protocol was: 10 min at 94 °C, then 38 cycles of 94 °C for 30 s, 56 °C (for cocktails I and II) or 54 °C (for cocktail III) or 50 °C (for cocktail IV) for 30 s, 72 °C for 45 s, followed by 10 min at 72 °C. The identification of each HPV genotype was achieved by evaluating the size of the specific PCR product by agarose gel electrophoresis as described previously.[17]

3.5. Immunoassay for the detection of mucosal anti-HPV antibodies

HPV16 or HPV18 virus-like particles (VLP) were kindly donated by Dr. Martin Sapp (Feist Weiller Cancer Center, and Center for Tumor Virology, LSU Health Sciences Center, USA) and used as target antigen in a standard ELISA. Total protein content of mucus samples was determined using Protein Assay kit (Bio-Rad Lab., Hercules, CA). ELISA MaxiSorp plates (Nunc, Rochester, NY) were coated with 500 ng/well VLP diluted in PBS, at 4 °C overnight. Plates were washed and non-specific binding sites were blocked with 2% BSA for 2 h at 37 °C. After washing, 100 μL of cervical mucus diluted at a protein concentration of 100 μg protein/μL were added to the plate and incubated for 2 h at 37 °C. After washing, 100 μL of alkaline phosphatase-conjugated rabbit-anti human IgA (Dako Co., Carpinteria, CA), diluted 1:500 were added, and incubated for 1.5 h at 37 °C. After washing, alkaline phosphatase substrate Sigma 104 (Sigma–Aldrich Co., St. Louis, MO) was diluted in a 10% (w/v) diethanolamine solution, and added to the plates. The absorbance was read at 405 nm in an ELISA plate reader. All samples were tested in three wells, the assay was considered valid only when the coefficient of variation of the triplicates was ≤10%. Addition-

Download English Version:

https://daneshyari.com/en/article/3369515

Download Persian Version:

https://daneshyari.com/article/3369515

Daneshyari.com