



INVESTIGACIÓN ORIGINAL

HUMAN PAPILLOMAVIRUS DNA IN WOMEN HAVING LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESION

Tipificación del virus del papiloma humano en mujeres con lesión escamosa intraepitelial de bajo grado

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SUMMARY

Objective: to determine the prevalence and types of HPV in women with low-grade squamous intraepithelial lesions (LSIL).

Methods: ninety-six women were sequentially enrolled from the colposcopy clinic. The presence of HPV DNA in the biopsies was determined by L1 consensus primer polymerase chain reaction assay. The samples were co-amplified with biotinylated MY09/MY11/HMB01 and GH20/PC04 beta-globin primers. Reverse line blot hybridization was used for typing the samples.

Results: HPV DNA was detected in 28/96 (29.1%) patients. From these, 21/28 (75%) had a single HPV infection and 7/28 (25%) had multiple infection; 29/39 (74.3 %) had high-risk subtypes and 10/39 (25.7%) had low-risk. Thirteen different types were found; most of them were high-risk HPV subtypes (9/13, 69.2%). HPV 58 was the most frequently found subtype.

Conclusions: the HPV prevalence in women with LSIL was lower than expected; these results suggest a misclassification in the diagnosis.

Key words: papillomavirus, human, LSIL, cervix neoplasms, DNA probes, HPV.

RESUMEN

Objetivo: determinar la prevalencia y los genotipos de los papillomavirus humanos (PVH) en mujeres con lesiones escamosas intraepiteliales de bajo grado (LEIBG).

Métodos: noventa y seis mujeres de la clínica de colposcopia fueron incluidas. La presencia del ADN de los PVH en las biopsias fue determinada por ensayo de reacción en cadena de la polimerasa (RCP) con oligonucleótidos para el gen L1. Las muestras fueron coamplificadas con MY09/MY11/HMB01 e iniciadores para betaglobina GH20/PC04. Para tipificar se utilizó hibridación reversa en línea.

Resultados: el ADN de PVH fue detectado en 28/96 (29,1%) pacientes, de las cuales 21/28 (75%) tuvieron infección con un solo tipo y 7/28 (25%); infección múltiple. En 29/39 (74,3%) fueron de alto riesgo y 10/39 (25,7%) de bajo riesgo. Encontramos 13 diferentes tipos, 9/13 (69,2%) de ellos de alto riesgo. PVH 58 fue el tipo más frecuente.

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Conclusiones: la prevalencia de VPH en mujeres con diagnóstico citológico de LEIBG fue menor que la esperada; este resultado sugiere una clasificación inadecuada de los diagnósticos.

Palabras clave: papillomavirus humano, LEIB, neoplasmas del cuello uterino, sondas ADN HPV.

INTRODUCTION

Cervical cancer (CC) is the second most common cancer in women worldwide. 500,000 new cases are reported annually; 80% of them occur in developing countries.¹ CC is an important public health problem in México, it is a leading cause of death from cancer amongst Mexican women; more than 4,000 women die from it every year.¹ Cervical cancer is of particular interest in Yucatan (South East Mexico), since the mortality rates reported were higher than the national mortality rates, from 1997 to 2001.²

Cervical carcinomas represent the final stage of a continuous spectrum of epithelial alterations in which each stage imperceptibly leads to the next.³ Precursor lesions of invasive cervical cancer have been classified into being low and high grade squamous intraepithelial lesions (LSIL and HSIL).⁴ Human papillomavirus (HPV) is now recognized as the main etiologic factor of invasive cervical cancer. The best clinical evidence available has revealed 99.7% HPV DNA prevalence in invasive cervical cancer.⁵⁻⁷

A large number of HPV have been identified and about other 35 subtypes have been found in the female genital tract; these have been classified into high-risk or oncogenic types and low-risk types.⁸ Many studies regarding the natural history of intraepithelial lesions have reported that more than 40% of LSIL regress spontaneously and that high-risk human papillomavirus are associated with persistence and the development of cervical lesions which evolve to more aggressive forms.^{9,10} The aim of this report was to present the prevalence of HPV genotypes found in biopsies of women having low-grade squamous intraepithelial lesions (LSIL) in a population of 96 women at our colposcopy center.

MATERIALS AND METHODS

The study protocol was approved by the review committee of the participating institutions (Hospital O´Horan & Centro de Investigaciones Regionales). Written consent was obtained from all participants, ninety-six women having a cervical smear revealing LSIL were sequentially invited to participate in the study. All the women had a colposcopic examination at Hospital O´Horan, Merida, Yucatan, Mexico. A biopsy of the suspected LSIL area was taken for virological analysis.

Each woman in this study was interviewed and their personal record included: age, marital status, age of fist intercourse, number of pregnancies, deliveries, miscarriages and sexual partners, history of oral contraceptive use and active or passive smoking.

Data analysis was performed using Epi Info version 6.0, statistical software package.

Each fresh biopsy was placed in PBS and stored at -20°C. DNA was extracted by adding 200 μ l digestion solution to a final concentration of 200 μ g proteinase K per ml, 0.1% Laureth -12, 10 mM Tris PH 8.5 and 1 mM EDTA, followed by phenol-chloroform separation. The quality of the sample was assessed by PCR using GH20 and PCO4 beta-globin primers to confirm the presence of suitable DNA.

The presence of HPV DNA in the biopsies was determined by L1 consensus primer polymerase chain reaction assay. The samples were co-amplified with biotinylated MY09/MY11/HMB01 and GH20/PC04 beta-globin primers. Reverse line blot hybridization was used for typing the samples, containing 29 probe lines; detecting 27 individual HPV genotypes and two-globin control probe concentrations.¹¹

In brief, 35 μ l amplicon were denatured with 0.13N NaOH, added to genotyping strip and covered with hybridization buffer (4XSSPE/0.1% SDS) pre-warmed to 53°C. The typing trays were incubated in shaking water bath at 53°C for 60 minutes, followed by stringent washing (1xSSPE/0.1%) at 53°C for 15 min. After the wash, streptavidin-horseradish peroxidase conjugate was added to each

and incubated for 30 minutes. Colour development was activated by 4:1 (hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine). Samples were considered suitable if they hybridized with the -globin probe and positive if they hybridized with any papillomavirus probe. SiHa DNA was used as positive control and PCR mix without DNA was used as negative control. Every five reactions included one negative control.

RESULTS

All the samples were -globin positive. The HPV genome was detected in 28/96 (29.1%) patients.

With regard to the women's demographic characteristics, the range was 17-78 years; most of them (37.5%) were aged between 21 and 30. Forty-six percent of the women had had their first intercourse when they were younger than 18 years of age.

With regard to current smokers, 87.5% preferred not to smoke; 12.5% were smokers at the time of diagnosis. However, 42.7% lived with someone who smoked. Twenty-five percent of the women had been using an oral contraceptive during the last two years before diagnosis. With regard to the number of sexual partners, 93% referred to having had less than three during their lives.

Twenty-one out of these 28 (75.0%) had a single HPV infection and 7/28 (25.0%) had multiple infections. Multiple infections were as follows: 5 patients had two HPV subtypes, one had co-infection with three subtypes and one with five subtypes; 39 HPV subtypes

were therefore found in 28 women, where 29/39 (74.3%) were high-risk and 10/39 (25.7%) low-risk.

The frequency of the 13 subtypes among the 28 women was as follows: subtype 58 (21.4%), 16 (17.8%), 18 and 6 (14.2% each one), 31 (10.7%), 53 (10.7%), 59, 51 and 11 (7.1% each one) and 52, 45, 39, 60 (3.5% each one).

The highest frequency of HPV was found in women between 21-30 years old. There were more patients with high-risk HPV 21/28 (75%) than those with low-risk HPV 6/28 (21.4%); and only 1/28 (3.5%) had high and low-risk HPV (**Table 1**).

DISCUSSION

The Mexican National Cervical Cancer Screening Programme (CCSP) has established that every woman having human LSIL should be sent for a colposcopy study to any of the 28 colposcopy centers throughout the country. However, many studies have reported that more than 50% of these lesions regress spontaneously and that progress to more aggressive lesions is associated with high-risk human papillomavirus.¹²⁻¹⁴

According to these results, only women having a cervical smear revealing LSIL with a positive high-risk HPV test should be sent for colposcopy study. Given this context, cervical specimens from LSIL in a population of Mexican women having no social security were examined to ascertain HPV subtypes and prevalence.

Table 1. Molecular detection of HPV according to age

Age (years)	Patients with HPV	Patients with high-risk HPV	Patients with low-risk HPV	Patients with both
≤ 20	2 (7.1%)	1 (50 %)	1 (50%)	0
21-30	13 (46.4%)	9 (75%)	3 (25%)	0
31-40	5 (17.8%)	6 (100)	0	0
41-50	4 (14.2%)	3 (75%)	1 (25%)	0
51-60	3 (10.7%)	2 (66.6%)	1 (33.3%)	0
≥ 61	1 (3.5%)	0	0	1
Total	28 (100%)	21 (75%)	6 (21.4%)	1 (3.5%)

HPV DNA prevalence amongst women having LSIL varies substantially by age. Teenagers and young women have the highest frequency; the atypical squamous cells of undetermined significance/low squamous intraepithelial lesions triage study group (ALTS group) has reported 85% frequency in women aged 18-29.¹⁵

HPV DNA detection rates in women having LSIL decreases with age; only 30-50% of women having LSIL aged more than 40, are HPV DNA positive.¹⁶

The overall HPV DNA prevalence (29.1%) found in this study was lower than that reported for previous DNA HPV studies testing for LSIL using hybrid capture.¹²⁻¹⁴ However, it was similar to the frequency (33%) reported in another study carried out in Mexico in 1998 by Torroella-Kouri using the same methodology.¹⁷ HPV DNA detection increased in the group of women aged 21-30; however, detection rate (46.4%) was lower than expected.

It is remarkable that 78.5% of the positive women had high-risk HPV infection, because those women are at risk of developing high-grade squamous intraepithelial lesions and invasive cervical cancer; this agrees with the results published by the ATLS group.¹⁵

The number of sexual partners has been associated with HPV infection in many studies.¹⁸⁻²⁰ Women who have had less than three sexual partners have lower HPV DNA prevalence than women who have had more sexual partners. Ninety-three percent of all the women in our group had had less than three sexual partners. This may be the reason for the low DNA HPV prevalence found in our study group.

An aspect limiting this work was that the preparations were studied by a single cytotechnologist. However, this does not invalidate the results, as this is routine clinical practice and most therapeutic decisions are based on analysis performed by a single cytotechnologist.

Discordance between cytology and molecular detection found in women from Yucatan could be explained as a false-positive diagnosis of LSIL, agreeing with the well-documented observation that cervical screening programmes have not been very

efficient in developing countries, partially due to non-reproducible interpretation of the smears.²¹

Based on these results, we believe that there is an over-diagnosis of LSIL in cervical smears, resulting in increased healthcare cost, unnecessary surgical procedures and anxiety for the patients and their partners. More stringent criteria are necessary for diagnosing LSIL and there should be better cervical smear quality control.

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