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Short Communication

Genotyping of Human Papillomavirus in Cervical Squamous Intraepithelial Lesions in Mexican Women

María del Carmen Colín-Ferreyra[1,2]*, Ma. Victoria Domínguez-García[1], Hugo Mendieta-Zerón[2], María del Socorro Camarillo-Romero[1], Michelle Pérez-Rogel[1], Migdania Martínez-Madrigal[3], Sergio Martínez-Pérez[1], and María del Socorro Romero-Figueroa[4]

[1]Center for Research in Medical Sciences and [2]Faculty of Medicine, Autonomous University of Mexico, Toluca; [3]Hospital Monica Pretelini. Institute of Health of Mexico, Toluca; and [4]Mexican Institute of Social Security of West Delegation, Toluca, Mexico

SUMMARY: Approximately 40 genotypes of the human papillomavirus (HPV) have been identified in cervical mucosa. In particular, HPV-16 and HPV-18 have been associated with cervical neoplasia. Squamous intraepithelial lesions (SILs) are precursors of cervical cancer. This study aimed to identify the HPV by genotype in SILs using a linear array genotyping test in a population in Mexico. We performed a cross-sectional study of 129 female patients with or without SILs, as determined by colposcopy, who completed a risk factor questionnaire. Cervical swab samples were obtained and genotyped using a Linear Array HPV Genotyping assay. Forty-nine (37.98%) samples were positive for HPV, and 24 genotypes were identified among these samples. The most common genotype was HPV-16. Twelve genotypes were found in both high- and low-grade SILs (HPV-6, 16, 31, 39, 51, 52, 53, 58, 59, 61, 67, and 84), of which seven were high-risk SILs (HPV-16, 31, 39, 51, 52, 58, and 59). Among the populations studied, the most frequent genotype was HPV-16, multiple infections were found, and four patients without injury tested positive for HPV.

Human papillomavirus (HPV) is a virus from the Papillomaviridae family of non-enveloped double-stranded DNA viruses. Sixteen HPV genera have been identified according to a classification scheme based on biological and genetic properties. Of these, only three are found in humans: alpha-papillomavirus, beta-papillomavirus, and gamma-papillomavirus. Viruses belonging to the alpha-papillomavirus group infect mucosal surfaces and are found in the cervix. This genus contains 15 species (1–15), of which species 7 and 9 contain the genotypes HPV-18 and HPV-16, respectively. In particular, genotypes of species 9 are associated with the development of malignant lesions (1).

Some HPV strains can infect the stratified squamous epithelium in the cervical mucosa. Approximately 40 HPV genotypes have been identified, of which 15 are considered high-risk and 25 are considered low-risk. In particular, HPV-16 and HPV-18 are associated with cervical neoplasia, making the HPV the third leading cause of cancer related deaths worldwide (2,3).

Squamous intraepithelial lesions (SILs) are precursors of uterine cervical cancer (CC). According to the current Bethesda classification, cervical lesions are classified as low-grade SILs (LSILs) and high-grade SILs (HSILs) (4). The reported prevalence of HSILs in Mexico is 18.8%, and 51.92% of patients with these lesions had HPV infection (5).

The aim of this study was to determine the HPV genotypes in SILs in a population in Mexico using the Linear Array® HPV Genotyping test (Roche, Mannheim, Germany). This cross-sectional study was approved by the local Research Ethics Committee. One hundred twenty-nine patients who met the inclusion criteria agreed to participate in the study by signing an informed consent letter.

Samples from female patients diagnosed with SILs by colposcopy were obtained during a visit to a dysplasia clinic at the 221st Mexican Social Security Institute of the Maternal and Perinatal Hospital “Monica Pretelini” at Mexican Health Institute, and the Center for Research in Medical Sciences of the Autonomous University of Mexico. Cervical samples without SILs diagnosed by colposcopy were obtained from women who were patients at the gynecology service centers. The samples were collected by cervical scraping and were preserved in ThinPrep® PreservCyt® Solution (Hologic Inc., Marlborough, MA, USA).

Patients completed a questionnaire to provide information on their age, area of residence, education level, numbers of sexual partners, participation in anal sex, age at first intercourse, smoking, use of hormonal contraception, use of condoms, number of pregnancies, and number of vaginal deliveries.

For HPV genotyping, DNA was extracted from 129 cervical swab samples using the Amplicor kit (Roche) and the gene for β-globin, along with HPV, were simultaneously isolated. Specimens that tested positive

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*Corresponding author: Mailing address: Center for Research in Medical Sciences, Faculty of Medicine. Autonomous University of Mexico, Paseo Tollocan esq. Jesus Carranza s/n, C.P. 50180, Toluca, Mexico. Tel: + 722- 2194122(ext. 156); Email: mdcolinf@uaemex.mx

for HPV were subsequently genotyped using the aforementioned Linear Array HPV Genotyping assay. The test amplifies target DNA using the polymerase chain reaction and uses nucleic acid hybridization to detect 37 anogenital HPV DNA genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39, and CP6108) and includes a β-globin probe to check for sample integrity and to reduce the risk of false negatives. Thirteen genotypes have been defined as HPV types with a high-risk of carcinogenesis (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).

For statistical analysis, chi-square testing for SILs were calculated using SSPS 17.0 software (SPSS Inc., Chicago, IL, USA). Frequencies and percentages were used for the presence of genotypes.

One hundred and twenty-nine cervical samples were obtained (one from each patient). Patient characteristics are shown in Table 1. Ninety-nine samples were

Table 1. Characteristics of patients with and without squamous intraepithelial lesions

	Total (n = 129) f (%)	SIL (n = 99) f (%)	Without SIL (n = 30) f (%)	p
Age				
30 years or less	34 (26.4)	27 (27.3)	7 (23.3)	0.432
Older than 30	95 (73.6)	72 (72.7)	23 (76.7)	
Occupation				
Housewife	66 (51.2)	57 (57.6)	9 (30)	0.007
Work away from home	63 (48.8)	42 (42.4)	21 (70)	
Level of education				
Less than high school	66 (51.2)	59 (59.6%)	7 (23.3)	0.000
From high school onward	63 (48.8)	40 (40.4%)	23 (76.7)	
Sexual partners				
0–1	68 (52.7)	53 (53.5)	15 (50)	0.447
2 or more	61 (47.3)	46 (46.5)	15 (50)	
Anal sex				
Yes	27 (20.9)	26 (26.3)	1 (3.3)	0.004
No	102 (79.1)	73 (73.7)	29 (96.7)	
Age at first intercourse				
Under 16 years	21 (16.3%)	20 (20.2)	1 (3.3)	0.020
16 or more	108 (83.7%)	79 (78.8)	29 (96.7)	
Smoking				
Yes	26 (22.5%)	21 (21.2)	5 (16.7)	0.399
No	103 (77.5%)	78 (78.8)	25 (83.3)	
Hormonal contraceptive use				
Yes	21 (16.3)	15 (15.2)	6 (20)	0.352
No	108 (83.7)	84 (84.8)	24 (80)	
Condom use				
Yes	28 (20.2)	20 (20.2)	8 (26.7)	0.399
No	101 (79.8)	79 (79.8)	22 (73.3)	
Pregnancies				
Nulliparous	18 (14)	15 (15.2)	3 (10)	0.353
With pregnancy	111 (86)	84 (84.8)	27 (90)	
Vaginal births				
Have had less than 3 partos vaginales	49 (38)	40 (40.4)	9 (30)	0.209
Have had 3 or more vaginal deliveries	80 (62)	59 (59.6)	21(70)	
Age of first pregnancy				
Under 16 years	70 (54.3)	58 (56.6)	12 (40)	0.57
16 or more	59 (45.7)	41 (41.4)	18 (60)	

f (%), frequency (percentage); value of p, Chi-square.

Table 2. HPV types in Mexican woman without SIL and with LSIL and HSIL

	Total $N = 129$		Without SIL $N = 30$		LSIL $N = 65$		HSIL $N = 34$	
	n	%	*n*	%	*n*	%	*n*	%
High-risk								
16	19	15	1	3.3	5	7.7	13	38
18	1	0.8	0	0	1	1.5	0	0
31	4	3.1	0	0	3	4.6	1	2.9
33	1	0.8	1	3.3	0	0	0	0
39	4	3.1	1	3.3	1	1.5	2	2.9
45	2	1.6	0	0	2	3.1	0	0
51	5	3.9	0	0	3	4.6	2	5.9
52	4	3.1	0	0	2	3.1	1	2.9
56	2	1.6	0	0	2	3.1	0	0
58	3	2.3	0	0	1	1.5	2	5.9
59	3	2.3	0	0	1	1.5	2	5.9
Low-risk								
6	3	2.3	0	0	1	1.5	2	5.9
11	1	0.8	0	0	0	0	1	2.9
53	4	3.1	0	0	2	3.1	2	5.9
54	1	0.8	0	0	1	1.5	0	0
61	2	1.6	0	0	1	1.5	1	2.9
62	2	1.6	0	0	2	3.1	0	0
66	1	0.8	0	0	1	1.5	0	0
67	2	1.6	0	0	1	1.5	1	2.9
71	2	1.6	1	3.3	1	1.5	0	0
81	1	0.8	0	0	0	0	1	2.9
83	2	1.6	0	0	2	3.1	0	0
84	5	3.9	0	0	3	4.6	2	5.9
CP6108	1	0.8	0	0	1	1.5	0	0
Overall patients	49	38	4	13.3	20	31	25	74

obtained from women with SILs and 30 were obtained from women without SILs.

The 129 samples were genotyped for HPV and 49 (38%) were positive for HPV (Table 2). The 24 HPV genotypes found in these samples included 11 high-risk genotypes and 13 low-risk genotypes. Notably, four women without SILs were infected with HPV. Three of these women were infected with high-risk genotypes (HPV-16, HPV-33, and HPV-39) and one with a low-risk genotype (HPV-71). The most prevalent high-risk genotype in both patients with infections with a single genotype and patients with infection with more than one genotype was HPV-16, followed by HPV-51 and HPV-84.

In the patients with HPV-positive samples, 15 patients (30.6%) were infected with multiple genotypes and 13 were infected with high-risk genotypes (86.6%). The more frequent combination was of a high-risk genotype with a low-risk genotype, which was found in six cases (40%). A maximum of eight genotypes was obtained in a single sample (four high-risk and four low-risk). The presence of more genotypes was observed in patients with HSILs (Table 3).

Genotypes 26, 35, 42, 55, 64, 68, 69, 70, 72, 73,

Table 3. Infection multiple genotypes regarding high risk and intraepithelial lesion type

Multiple infection	Risk	SIL
54, 66,84	3 LR	LSIL
62, CP6108	2 LR	LSIL
31, 84	HR, LR	LSIL
51, 53	HR , LR	LSIL
16 ,83	HR , LR	LSIL
18 ,84	HR , LR	LSIL
16, 52	2 HR	LSIL
6, 39	HR , LR	LSIL
51, 59	2 HR	LSIL
56,81,84	HR , 2 LR	HSIL
16, 52, 53	2 HR , LR	HSIL
11, 16, 59	2 HR , LR	HSIL
39, 67	HR , LR	HSIL
6,16,51,53,56,59,61,84	4 HR , 4 LR	HSIL
16,31	2 HR	HSIL

HR, high risk; LR, low risk; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

82, and 1S39 were not identified in the patients. The samples from the 129 patients were analyzed for the presence of HPV; 38% were positive, and the most prevalent genotype was HPV-16. These findings are consistent with other studies on SILs from Tunisia, China, Gambia, Turkey, and Japan (6–10).

Presently, 30.6% of the patients were positive for multiple infections, which is consistent with another study on SILs, which reported a value of 29.6% (10). We identified more high-risk genotypes than low-risk genotypes, most often HPV-16 (33.3%) combined with HPV-52 and HPV-53. In Japan, HPV-16 and HPV-52 have been reported most often (9.9%); the same study had identified one patient with five genotypes (10), and interestingly, we detected one patient with eight genotypes. A study in Benin reported that HPV-59 was the genotype most frequently identified in multiple infections in LSILs (26.32%) (11). The present findings confirm that the sampled Mexican population had infections caused by high-risk genotypes, which may influence the development of CC in these individuals. These data highlight the importance of studies that determine the genotypes infecting the Mexican population, and the importance of a proper vaccination system.

The population studied here may be at risk of developing CC, as it has been suggested that HPV-16 and HPV-18 are major risk factors for the development of CC and SILs (12).

In conclusion, in the population studied, the most frequent genotype was HPV-16, multiple infections were found, and four patients without injury tested positive for the HPV.

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Conflict of interest None to declare.

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