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Prevalence of human papillomavirus types in North and Central regions of Mexico

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Abstract

Human papillomavirus (HPV) is a DNA virus linked to mucosal and cutaneous carcinogenesis. More than 200 different HPV types exist. We carried out a transversal study to investigate the prevalence of HPV types in two regions of Mexico. A total of 724 genital and non-genital samples from women (F) and men (M) were studied; 241 (33%) from North-Eastern (NE) and 483 (66%) from South-Central (SC) Mexico. The overall prevalence was 87%. In genital lesions from females, the NE group showed a prevalence of HPV types 16 (37%), 6 (13%), 59 (6%), 11, 18 and 66 (5.4% each); and the SC group showed types 6 (17%), 16 (15%), 11 (14.5%), 18 (12%) and 53 (6%). In the genital lesions from males, NE group showed types 16 (38%), 6 (21%), 11 (13%) and 59 plus 31 (7.5%) and the SC group showed types 6 (25%), 11 (22%), 18 (17%) and 16 (11.5%). When the two regions were compared, a higher prevalence of low-risk HPV 6 and 11 was found in the SC region and of high-risk HPV 59, 31 and 66 (the latter can also be present in benign lesions) in the NE region. Our findings complement efforts to understand HPV demographics as a prerequisite to guide and assess the impact of preventive interventions.

Introduction

Human papillomavirus (HPV) is a large and assorted group of mainly sexually transmitted double-stranded DNA viruses, grouped under the *Papillomaviridae* family [1]. They are causal agents of benign and malignant oropharyngeal-laryngeal mucosal lesions and of benign and malignant anogenital cutaneous and mucosal lesions [2, 3]. Among the various malignant neoplasms that can be caused by a persistent HPV infection, cervical cancer (CC) [2–5] is the fourth most common type of cancer in women globally (and the seventh overall), with a mortality rate of up to 50%. A large majority (around 85%) of the global burden occurs in the less developed countries of the world, where it accounts for almost 12% of all female cancers. In Mexico, CC ranks second in incidence and mortality (16.9% and 11.9%, respectively, in 2012) among malignancies occurring in the female population, only below breast cancer (24.8% in incidence and 14.2% in mortality) [6].

The development of CC is considered to be a multi-step process (where HPV is necessary but not a sufficient cause alone), resulting from a persistent cervical epithelium infection by high-risk viral types [5, 7]. Several studies have shown that HPV infection in men influences their wives' risk of CC besides contributing to the burden of genital warts and anal, penile and oropharyngeal cancer [8].

Different HPV types have been classified according to their biological and pathological properties. Types associated with low oncogenic risk include 2, 3, 6, 7, 10, 11, 13, 27, 28, 29, 32, 40, 42, 43, 44, 54, 57, 61, 62, 71, 72, 74, 78, 81, 83, 84, 86, 87, 89, 90, 91 and 94. Meanwhile, high-risk HPV types considered as oncogenic types, include 16, 18, 31, 33, 34, 35, 39, 45, 52, 58, 59, 67, 68, 70, 73 and 85. On the other hand, types 26, 30, 51, 53, 56, 66, 69 and 82 are considered probably oncogenic [9, 10].

HPV genotyping in different geographic regions would provide a better understanding of the prevalence and diversity of HPV in the Mexican population. The goal of this study was to know the prevalence of HPV in two different and separate geographical areas of Mexico: North-Eastern (NE) (Nuevo León and Tamaulipas states) and South-Central (SC) (Mexico City).

Methods**Study population**

A total of 724 samples from men (28.9%) and women (70.8%) taken from clinically evident (mostly genital) lesions were collected. A total of 241 HPV tests were carried out in our

laboratory at Vitagénesis SA, which serves a population from the NE Mexico; 483 HPV tests were performed in our collaborating laboratory, Clinigen SA, that mainly serves the population from Central Mexico. The age of the individuals in the study ranged from 5 to 95 years. Samples were taken with swabs from the genital regions (cervix, vulva and vagina in women; urethral, penis, scrotum and groin in men); and scrapings and warts were collected from the anus. In addition, swabs were taken from laryngeal lesions. All were gathered during a period of 4 years, between 2011 and 2015. In most cases, there was no access to clinical information other than sex, age and sample type. All samples were identified with an internal code to maintain patient confidentiality and anonymity during the entire process.

HPV genotyping

In the Vitagénesis laboratory, the analysis was carried out as follows: amplification by polymerase chain reaction (PCR) of the L1 region of the HPV genome was performed using the consensus universal primers MY09 and MY11. The resulting amplicon was subjected to a nested PCR, using the universal consensus primers GP5 and GP6 to increase the sensitivity and specificity of sample amplification [11, 12]. The success of PCRs was monitored by electrophoresis in 1.5% agarose gels. In the Clinigen Laboratory, the samples were analysed by PCR with three sets of universal primers: MY, L1C1 and GP. In both laboratories, positive target controls, as well as a PCR control with β -globin gene primers and a PCR negative control without DNA were included.

Most samples generated a visible band for β -globin on the agarose gel. All samples, whether or not rendering visible amplicons with MY primers, were subjected to amplification with L1C1 and GP primers and those with visible amplicons with GP followed the procedure of enzymatic purification and subsequent nucleotide sequencing. Finally, interpretation of the viral type was performed using NCBI-BLAST on the electropherogram obtained in the Sequencing Analysis 5.3.1* software on an ABI3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Patients were classified as HPV-negative or HPV-positive and the latter group was divided into four subgroups: females and males from the NE region (NE-F and NE-M, respectively) and females and males from the SC region of Mexico (SC-F and SC-M, respectively). Statistical analyses were performed regarding infection type, sample type, age, low/high-risk genotype and viral type. Among the four groups, a principal component analysis was carried out using SPSS for windows (IBM SPSS version 22 (IBM Corp., Armonk, NY, USA)) with the frequencies including three parameters: sample type, low/high-risk genotype and viral type.

Results

Of the 724 samples from clinically evident lesions, 92 (13%) were confirmed as HPV-negative and 632 (87%) as positive. From the latter, 71.2% (450/632) came from women, 28.5% (180/632) from men and in 0.3% (2/632) the gender was not provided.

HPV-positive cases were detected as single infection/co-infection in 98%/2% and 82%/18% of the NE-F and SC-F

cases, respectively; as a single infection in 100% of NE-M cases and as single infection/co-infection in 78%/22% of SC-M cases (Table 1).

Most of the positive specimens were from the genital region, nearly 99% in both female sub-groups and 90% (NE) and 82% (SC) in the male sub-groups. The rest of the cases in females came from laryngeal papillomas; and in males 3%/7% from anus/wart (NE) and 10%/3%/5% from larynx/anus/wart (SC) (Table 1).

Mean age \pm standard deviation of the subjects studied was 34 ± 10 years.

Approximately 73% of HPV positive cases belonged to subjects from 21 to 40 years old (76% in NE-F, 77% in NE-M groups and 72% in both groups of the SC region), followed by the subgroup of 41–50 years old with 15% in NE-F, 20% in SC-F, 12% in NE-M and 16% in the SC-M groups (Table 1).

Overall, 43 different viral types were detected, 20 of high-risk (HR) and 23 of low-risk (LR). The ratio of infections with high/low-risk viruses, including co-infections, was 74%/26% in NE-F, 62%/38% in SC-F, 54%/46% in NE-M and 41%/59% in SC-M subgroups. The frequencies of the infections according to their risk type and whether they were single infection or co-infection, are summarised in Table 2.

Frequency of viral types in the genital lesions

The anatomic origin of the samples was predominantly genital (95%) with 41 different viral types detected, of which 27 were present in the NE (14 HR and 13 LR) and 35 in the SC (19 HR and 16 LR). According to their subgroup viral types were present as follows: NE-F (24), SC-F (31), NE-M(12) and SC-M (18). Genotyping showed a predominant prevalence of HPV types 16 (37%), 6 (13%), 59 (6%) and 11, 18 and 66 (5.4%) in NE-F; HPV types 6 (17%), 16 (15%), 11 (14.5%), 18 (12%) and 53 (6%) in SC-F; HPV types 16 (38%), 6 (21%), 11 (13%) and 59 and 31 (7.5%) in NE-M; and HPV types 6 (25%), 11 (22%), 18 (17%) and 16 (11.5%) in SC-M. Thirty-three more HPV types were also detected, although at frequencies of less than 5% in each of the subgroups. Some viral types were found in only one region: HPVs 86, 43, 71, 89, 64 (LR) and 82 (HR) in the NE region; HPVs 4, 7, 32, 90, 61, 83, 55, 69 (LR) and 73, 30, 52, 28, 39, 48 (HR) in the SC region (Table 3).

Principal component analysis

For the two geographic regions (NE and SC), the first two main components accounted for 97% of total sample variation (Fig. 1). This analysis shows differences in HPV infections among both of the studied regions, according to sample type, low/high-risk genotype and viral type *per se*.

Discussion

This study focused on two geographically separate regions of México: NE and SC (approximately 700 km apart). In the USA, Europe, Sub-Saharan Africa, Latin America and the Caribbean [13–16], as well as in most Mexican populations investigated so far, HPV 16 has been found to be the most prevalent of the high-risk HPV types in oncogenic malignancies and in asymptomatic women [17]. Our results show this also to be the case in the NE region (with a prevalence of HPV type 16 of 37%/38% in women/men), but not in the SC region (where it was 15%/

Table 1. HPV infection frequencies among women and men from NE and SC regions of México

Description	Total cases (%)	Female		Male	
		NE-F (%)	SC-F (%)	NE-M (%)	SC-M (%)
HPV	n = 724	n = 173	n = 340	n = 68	n = 141
Negative	13.0	16.0	11.0	13.0	14.0
Positive	87.0	84.0	89.0	87.0	86.0
Infection type	n = 632	n = 146	n = 304	n = 59	n = 121
Single infection	86.4	97.9	81.6	100.0	77.7
Co-infection	13.6	2.1	18.4	0.0	22.3
Sample type	n = 566	n = 146	n = 267	n = 59	n = 94
Genital region	95.0	98.6	99.3	89.8	81.9
Larynx	2.0	0.7	0.7	0.0	9.6
Anus	1.0	0.0	0.0	3.4	3.2
Wart	2.0	0.7	0.0	6.8	5.3
Age (years old)	n = 577	n = 129	n = 290	n = 42	n = 115
5–10	0.3	0.0	0.0	4.8	0.0
11–20	4.5	4.7	4.8	4.8	3.5
21–30	36.6	39.5	36.2	47.6	29.6
31–40	36.7	36.4	36.2	28.6	41.7
41–50	17.2	14.7	19.7	11.9	15.7
51–60	3.5	3.9	2.4	2.4	6.1
> 60	1.2	0.8	0.7	0.0	3.5

NE, North-Eastern region; SC, South-Central region.

Table 2. HPV infection frequencies by risk type among women and men from NE and SC regions of México

Risk type	Total cases (%)	Female		Male	
		NE-F (%)	SC-F (%)	NE-M (%)	SC-M (%)
Single infection	n = 546	n = 143	n = 248	n = 59	n = 94
High-risk	62.5	73.4	66.1	54.2	40.4
Low-risk	37.5	26.6	33.9	45.8	59.6
Co-infection	n = 86	n = 3	n = 56	n = 0	n = 27
H/H	26.7	66.7	26.8	0.0	22.2
L/L	24.4	0.0	19.6	0.0	37.0
L/H	48.8	33.3	53.6	0.0	40.7
S + Co-infection	n = 718	n = 149	n = 360	n = 59	n = 148
High-risk	59.7	73.8	62.2	54.2	41.2
Low-risk	40.3	26.2	37.8	45.8	58.8

NE, North-Eastern region; SC, South-Central region; S, single; L, low-risk; H, high-risk.

11.5% in women/men). Despite this, it remained among the four most prevalent viral types (second in women and fourth in men).

HPV 18 is the high-risk viral type reported to be in second or third place of prevalence after HPV 16 in the great majority of Latin American countries [16] and in the different regions of Mexico [17–20]. In agreement with this result, in our study HPV 18 was the second most prevalent in the SC region

(12.8%); however, in the NE region, the prevalence was approximately three times less (4%), being the fourth in prevalence together with HPV 66.

The HPV 31 was the third most prevalent in our study, slightly more frequent in the NE region (5.5%) than in the SC region (3.2%). This viral type has also been found among the most prevalent in women with CC from the NC, C, S and W regions

Table 3. HPV infection frequencies by viral type detected in genital region among women and men from NE and SC regions of México

Viral type	NE						SC							
	Total cases		NE-F		NE-M		Total cases		SC-F		SC-M		Total cases	
	n = 607	%	n = 147	(%)	n = 53	%	n = 200	%	n = 311	%	n = 96	%	n = 407	%
16	133	21.9	55	37.4	20	37.7	75	37.5	47	15.1	11	11.5	58	14.3
6	106	17.5	19	12.9	11	20.8	30	15.0	52	16.7	24	25.0	76	18.7
11	81	13.3	8	5.4	7	13.2	15	7.5	45	14.5	21	21.9	66	16.2
18	60	9.9	8	5.4	–	–	8	4.0	36	11.6	16	16.7	52	12.8
31	24	4.0	7	4.8	4	7.5	11	5.5	9	2.9	4	4.2	13	3.2
59	21	3.5	9	6.1	4	7.5	13	6.5	8	2.6	–	–	8	2.0
51	20	3.3	5	3.4	1	1.9	6	3.0	12	3.9	2	2.1	14	3.4
53	20	3.3	2	1.4	–	–	2	1.0	18	5.8	–	–	18	4.4
45	16	2.6	3	2.0	–	–	3	1.5	10	3.2	3	3.1	13	3.2
33	14	2.3	2	1.4	–	–	2	1.0	10	3.2	2	2.1	12	2.9
58	14	2.3	2	1.4	1	1.9	3	1.5	11	3.5	–	–	11	2.7
35	12	2.0	1	0.7	–	–	1	0.5	10	3.2	1	1.0	11	2.7
56	11	1.8	6	4.1	1	1.9	7	3.5	3	1.0	1	1.0	4	1.0
66	9	1.5	8	5.4	–	–	8	4.0	–	–	1	1.0	1	0.2
4	7	1.2	–	–	–	–	–	–	7	2.3	–	–	7	1.7
73	7	1.2	–	–	–	–	–	–	7	2.3	–	–	7	1.7
81	6	1.0	2	1.4	–	–	1	1.0	4	1.3	–	–	4	1.0
7	5	0.8	–	–	–	–	–	–	3	1.0	2	2.1	5	1.2
90	4	0.7	–	–	–	–	–	–	2	0.6	2	2.1	4	1.0
39	3	0.5	–	–	–	–	–	–	1	0.3	2	2.1	3	0.7
70	3	0.5	1	0.7	–	–	1	0.5	1	0.3	1	1.0	2	0.5
62	3	0.5	–	–	1	1.9	1	0.5	2	0.6	–	–	2	0.5
54	3	0.5	1	0.7	1	1.9	2	1.0	1	0.3	–	–	1	0.2
44	2	0.3	1	0.7	–	–	1	0.5	–	–	1	1.0	1	0.2
30	2	0.3	–	–	–	–	–	–	2	0.6	–	–	2	0.5
32	2	0.3	–	–	–	–	–	–	2	0.6	–	–	2	0.5
52	2	0.3	–	–	–	–	–	–	2	0.6	–	–	2	0.5
68	2	0.3	1	0.7	–	–	1	0.5	1	0.3	–	–	1	0.2
42	2	0.3	–	–	1	1.9	1	0.5	1	0.3	–	–	1	0.2
86	2	0.3	2	1.4	–	–	2	1.0	–	–	–	–	–	–

(Continued)

Table 3. (Continued.)

Viral type	NE				SC			
	NE-F		NE-M		SC-F		SC-M	
	n = 147	(%)	n = 53	%	n = 311	%	n = 96	%
Total cases	n = 607	%	Total cases		Total cases		Total cases	
	n = 607	%	n = 200	%	n = 311	%	n = 407	%
55	1	0.2	-	-	-	-	1	1.0
69	1	0.2	-	-	-	-	1	1.0
28	1	0.2	-	-	1	0.3	1	0.2
48	1	0.2	-	-	1	0.3	1	0.2
61	1	0.2	-	-	1	0.3	1	0.2
83	1	0.2	-	-	1	0.3	1	0.2
43	1	0.2	1	0.7	-	-	-	-
71	1	0.2	1	0.7	-	-	-	-
82	1	0.2	1	0.7	1	0.5	1	0.2
89	1	0.2	1	0.7	-	-	-	-
64	1	0.2	1	1.9	-	-	-	-

NE, North-Eastern region; SC, South-Central region. Bold number correspond to high-risk viral type. All the frequencies correspond to single infection + co-infections cases.

[17, 20] and with high-grade squamous intraepithelial lesions in the USA (Arizona) and in Northwest (NW) Mexico [13] and with a lower prevalence in healthy women from the SW and W region and healthy men from the SC region [8, 19].

A study reported that among Latin-American countries, HPV 59 was only found in Mexico [16] and particularly in the NW and Northern region of Mexico [13,21]. We found that it was the second high-risk viral type with major prevalence (6%/7.5% in women/men) in the NE region but showed a low prevalence in the SC region (2.6% in women and undetected in men). These results are similar to the reported for asymptomatic women from the Northern region, where HPV 59 was the most common genotype [21] and the low prevalence (0–1%) found in SW and W regions of Mexico [19]. However, this viral type was the most prevalent (7.2%) in healthy men from the SC region [8].

Similarly, high-risk viral type HPV 66 was third in prevalence in the NE region (5%) and low in the SC region (1% in men and undetected in women). Likewise, HPV 66 was found in less than 1% of women from the SW and W region [19]. However, it was among the most prevalent in healthy men from the SC region of Mexico, Brazil and the USA [8] and also in healthy women from the USA, Cuba, Guatemala and Bolivia [13, 16].

Thus in keeping with previous studies in women from the US-Mexico border, HPV types 16, 59, 18, 31 and 56, are more common in the women of the NE region of Mexico.

With regard to low-risk HPVs, we found HPV 6 and 11, which cause nearly 90% of all external genital warts [22], to be the most prevalent in the four subgroups, as seen in other populations [17].

Notably, in the SC region low-risk HPV 6 and 11 were more prevalent (approximately 35% combined) compared with high-risk HPV 16 and 18 (27% combined). On the contrary, in the NE region, the high-risk HPV viral types were more frequent (41.5%) than these two low-risk viral types (22.5%) (Table 2). When comparing the two regions analysed, the prevalence of the high-risk HPVs 16 and 18 (combined) was 65% higher in the NE region than in the SC region and the prevalence of the low-risk HPVs 6 and 11 (combined) was 65% higher in the SC region than the frequencies found in the NE region. All the differences found related to viral type distribution and prevalence are reflected in the principal component analysis, in which the two regions analysed are clearly divided; in turn, it can be seen that the subgroups of males and females of the SC region differ more than those in the NE region.

With regard to the protection conferred by the bivalent vaccine against HPV 16 and HPV 18 (Cervarix®), the vaccine is known to provide cross-protection against phylogenetically related HPV types, such as 31, 33, 45, 52 and 58 (not included in the vaccine). Based on our results, we would expect the vaccine to provide protection against infections with these HPVs in approximately 51% and 39% of the population of the NE and SC region, respectively; and in approximately 74% of the population from both regions with the tetravalent vaccine (Gardasil®), which in addition to HPV 16 and 18, includes protection against the low-risk HPV 6 and 11.

This study provides information about differences in HPV types distribution and frequency in both women and men of NE and SC regions of México. This could be useful to adjust preventive measures, such as the application of region-specific vaccines and the inclusion of teenage boys, not just teenage girls, in the current immunisation scheme adopted in our country. It could also influence treatment and the implementation of follow-up strategies.

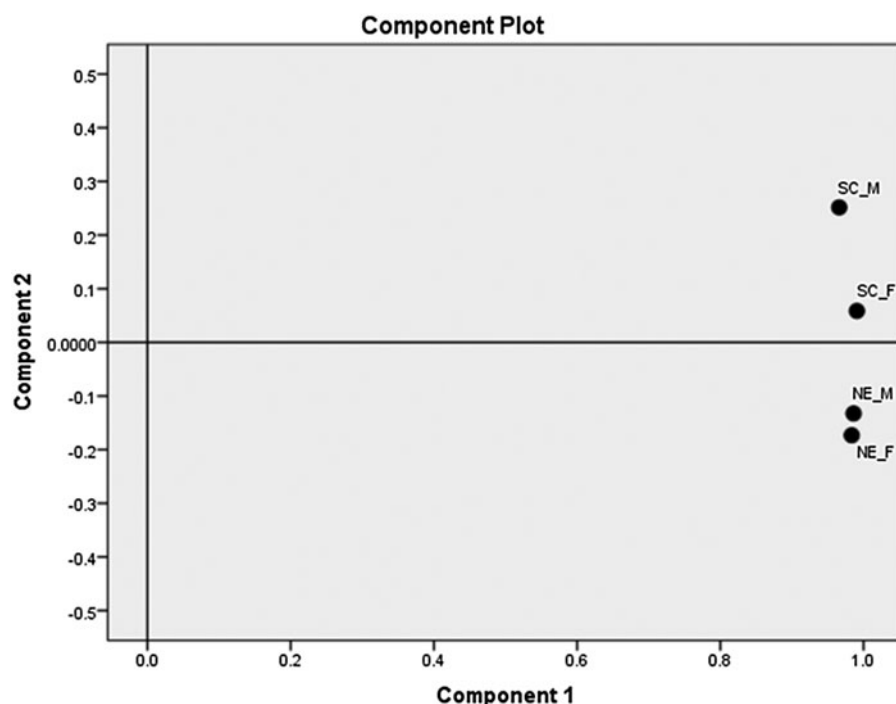


Fig. 1. Position of the four groups, female and male from NE (NE-F, NE-M) and SC (SC-F, SC-M) populations by the first two main components (PC1 and 2) of frequencies at sample type, low/high-risk genotype and viral type.

There were certain limitations in the comparison of both populations due to differences in sample processing in the two laboratories, e.g. the use of different DNA extraction methodology and the buffer in which the samples were preserved could influence samples with the low viral load. However, the use of PCR and Sanger sequencing-based genotyping in both laboratories, with the same reagents, primer sets and standardised methodology, which involves similar sensitivity, might minimise inter-laboratory variability, although the inherent risk of bias persists. Further studies with a larger cohort of women and men of different geographic regions, with longer follow-ups and with sociodemographic data are recommended in order to assess the demographic magnitude of this disease, as well as the potential impact on the effectiveness of current and future HPV vaccine campaigns.

Conclusions

The prevalence of HPV is heterogeneous in the diverse geographical populations of Mexico. In decreasing order of prevalence for genital lesions, high-risk HPV types 16, 59, 31, 18 plus 66 and 56 were the most common in the NE region; whereas types 16, 18, 53, 51, 31 and 45 were the most common in the SC region. With respect to low-risk HPVs, HPV 6 and 11 were the most prevalent in both regions, agreeing with previous reports [23].

Although previous studies have suggested that there is heterogeneity in the distribution of HPV types both in Mexico and other countries, oncogenic viral types 16, 18, 59 and 31 and non-oncogenic viral types 6 and 11, continue to predominate in various geographical regions.

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Conflict of interest. The authors declare that there are no conflicts of interest in this work.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national regulations and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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