

# Association of high-risk human papillomavirus with ocular surface squamous neoplasia: a case-control study in Mexico

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## Abstract

**Objective.** To investigate the association of high-risk human papilloma virus (HR-HPV) and other risk factors with ocular surface squamous cell neoplasia (OSSN). **Materials and methods.** We obtained DNA from 22 fresh frozen OSSN tissues and 22 pterygia as controls, we used a broad-spectrum HPV DNA amplification short PCR fragment to identify HPV infection in all specimens and then genotyped HPV by a reverse hybridization line probe assay. We also obtained demographic, sun exposure, and tobacco consumption information. **Results.** HR-HPV frequency was 40.9% in the OSSN group and 4.5% in the pterygia group ( $p=0.009$ ). After covariate adjustment, OSSN was associated with HR-HPV (OR=16.3, 95%CI=1.2,218.1,  $p=0.03$ ) and sunburn (OR=10.8, 95%CI=1.8,86.0,  $p=0.02$ ). **Conclusions.** Ocular surface squamous cell neoplasia is a multifactorial disease. The strong association between HR-HPV and OSSN, suggests that HR-HPV could play an etiological role in OSSN development.

**Keywords:** squamous cell neoplasms; human papillomavirus; conjunctiva; pterygium; sunburn

## Resumen

**Objetivo.** Investigar la asociación del virus del papiloma humano de alto riesgo (VPH-AR), así como de otros factores, con neoplasia escamosa de la superficie ocular (NESO). **Material y métodos.** Se obtuvieron 22 especímenes de tejido fresco de NESO y 22 de pterigión como controles; se utilizó una técnica molecular altamente sensible para identificar la infección por VPH en todos los especímenes, así como la genotipificación del VPH. También se obtuvo información demográfica sobre exposición a la luz solar y tabaquismo. **Resultados.** La frecuencia de infección por VPH-AR fue de 40.9% en el grupo de NESO y de 4.5% en el grupo control ( $p=0.009$ ). Después de ajustar por covariables, NESO se asoció con el VPH-AR (OR=16.3, IC95%=1.2,218.1,  $p=0.03$ ) y el eritema solar (OR=10.8, IC95%=1.8,86.0,  $p=0.02$ ). **Conclusiones.** La neoplasia escamosa de superficie ocular en una neoplasia multifactorial. Los presentes resultados sugieren que el VPH-AR podría tener un papel etiológico en el desarrollo de NESO.

**Palabras clave:** neoplasias de células escamosas; infecciones por papilomavirus; conjuntiva; pterigión; quemadura solar

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Ocular surface squamous neoplasia (OSSN) is the most prevalent non-pigmented neoplasia affecting the ocular surface.<sup>1</sup> OSSN is a tumor that affects the squamous epithelial cells of the conjunctiva and the cornea, and most frequently originates in the interpalpebral limbal area of the ocular surface. It is believed that limbal stem cells in the ocular surface, having a high mitotic rate, are more prone to suffer DNA damage. Ultraviolet radiation has been the primary risk factor associated with OSSN development,<sup>2,3</sup> but also increased age, fair skin, tobacco consumption and oncogenic viruses, such as human immunodeficiency virus (HIV) and human papillomavirus (HPV),<sup>4</sup> have been implicated.<sup>2,5,6</sup>

The role of HPV persistent infection as a risk factor for the development of OSSN has been controversial. Some studies have reported HPV detection in 100% of OSSN samples,<sup>6-8</sup> whereas others have reported no detection at all.<sup>9-11</sup> The large variability in HPV prevalence in OSSN can be explained by differences in the molecular techniques employed. Also, to the quality and quantity of biopsy samples, their storage conditions, and to potential sample contamination. Furthermore, most studies investigating the presence of HPV in OSSN lacked a control group, with adequate histopathological analysis of the epithelial characteristics to rule out epithelial dysplasia.<sup>6-8,12-15</sup>

We aimed to investigate the presence of HR-HPV in fresh frozen biopsies of proven OSSN lesions, and to compare it to the presence of HR-HPV in a group of epithelial dysplasia-free pterygium samples, using a highly sensitive molecular technique. We also investigated the potential association of OSSN with tobacco consumption, sun exposure and other sociodemographic variables.

## Materials and methods

### Study population

This study was conducted in a single center, third level health institution; *Instituto Nacional de Rehabilitación Luis Guillermo Ibarra Ibarra* (INRLGII) in Mexico City, Mexico, between February 2016 and March 2018. We prospectively included 22 patients that had histopathological confirmation of OSSN. Also, we prospectively included, during the same period, a group of 22 patients with primary pterygium who underwent surgical resection and had histopathological confirmation of an epithelium without dysplasia. Exclusion criteria for both groups were being younger than 18 years-old, HIV seropositivity (given that HIV-1 associated immunodeficiency may increase the probability of HPV infection to become persistent) and having an OSSN recurrent lesion (since previous topical

or subconjunctival chemotherapy could have impacted HPV infection status). Our study followed the tenets of the Declaration of Helsinki and was approved by our institutional review board. All patients agreed to participate and provided written informed consent.

### Data collection

Data on age, sex, education level and occupation were recorded. To evaluate differences in solar exposure between groups we used a validated questionnaire, previously designed to evaluate skin cancer risk in Mexican population.<sup>16</sup> Briefly, this questionnaire consists of 10 questions to evaluate known risk factors for skin cancer: pigmentation of skin, hair and iris; skin redness after solar exposure (sunburn); amount of time under direct solar exposure; type of job; practice of recreational outdoor activities; and family or personal history of skin cancer, and personal history of radiotherapy (full questionnaire available in supplementary appendix).<sup>17</sup>

To evaluate differences in tobacco consumption, we used a set of validated questions from the National Addiction Survey 2011 in Mexico.<sup>18</sup>

### Samples and DNA extraction

Fresh specimens of OSSN and pterygia were surgically excised and dissected in half. For OSSN biopsies, care was taken to dissect the surgical border of the half that was cryopreserved, so that it could be analyzed by the pathologist. One half and the surgical border of the other half were inserted in formalin for histopathological analysis, the other half was preserved in sterile microtubes and immediately transported to the laboratory on ice to be cryopreserved until processed. For DNA extraction we used the phenol-chloroform-isoamyl alcohol method; briefly, each frozen specimen was resuspended in 500  $\mu$ L of cellular lysis solution (100 mM Tris HCl pH 7.5, 100 mM NaCl, 10 mM EDTA, 1% sarkosyl), with 5  $\mu$ L of proteinase K (20 mg/  $\mu$ L) and was incubated at 55°C for 12 hours. After incubation, 500  $\mu$ L of phenol/chloroform/isoamyl alcohol (25:24:1) was added. Samples were mixed thoroughly and centrifuged at 12 000 rpm for 10 minutes to obtain two phases. The upper aqueous phase was transferred to a new microtube, and NaOAc 2m (50 $\mu$ L) and 100% ethanol (1.5 mL) were added. It was mixed until DNA precipitation occurred. The DNA pellet was washed with 80% ethanol, then dried at room temperature, and resuspended in 50  $\mu$ L of sterile distilled water for 24 hours before quantification. DNA quantification was performed with a UV-spectrophotometer (BioDrop  $\mu$ LITE, BioDrop Ltd., UK).

## Detection and typing of HPV

All HPV detection tests were performed blinded to the histopathological results and other clinical data. All samples were analyzed with a broad-spectrum HPV DNA amplification short PCR fragment and then genotyped by a reverse hybridization line probe assay to detect a broad spectrum of HPV genotypes (INNO-LiPA HPV Genotyping Extra II, Furijebo Europe, Belgium) as previously described.<sup>19</sup> This system allows the identification of 32 HPVs that infect mucous membranes, including high risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68; probable high risk types 26, 53, 66, 70, 73 and 82; and low risk types 6, 11, 40, 42, 43, 44, 54, 61, 62, 67, 81, 83 and 89. Briefly, the protocol amplifies through PCR a 65-bp fragment from the HPV L1 open reading frame. Then, the HPV genotyping assay is performed by hybridization of the biotinylated amplified product on a strip, followed by an alkaline wash. Addition of a conjugate and a substrate produces color development on the strip and finally visual interpretation of the reactivity pattern is performed.

## Histopathological analysis

Half of each dissected biopsy, as well as the surgical border of the other half in OSSN lesions, were formalin-fixed and paraffin embedded in blocks. Care was taken to orient the tissue over a thin filter paper, with the epithelium facing the external zone, right before formalin fixation. A section from each paraffin block was placed over a slide, stained with hematoxylin and eosin and examined by a single experienced anatomical pathologist who evaluated each slide to confirm the absence of epithelial dysplasia in pterygia samples as well as to confirm the diagnosis and classification of OSSN. Epithelial dysplasia was diagnosed if abnormally shaped cells, cells of unequal size and loss of normal maturation (disorganization), were seen affecting primarily the basal epithelial cells. Of notice, two pterygia samples were excluded from the study because of mild dysplastic changes. Histological classification of OSSN was reported according to the degree of involvement of the dysplastic epithelium.<sup>20</sup> Mild dysplasia was diagnosed when only the lower third of the squamous epithelium was affected, moderate when two thirds were compromised, and severe or *in-situ* carcinoma with full-thickness dysplasia. Invasive ocular surface squamous cell carcinoma was diagnosed when the presence of infiltrating nests of tumor cells had penetrated the epithelial basement membrane.

## Statistical analysis

Sample size was calculated to estimate the difference of proportions in two independent samples using the 33% meta-analytical estimate for HPV in OSSN provided by Di Girolamo and colleagues,<sup>1</sup> and the 0% in pterygia (controls) as reported by Woods and colleagues.<sup>21</sup> The estimated sample size was 25 cases and 25 controls with an alpha of 0.05 and 80% power. Differences in socio-demographic characteristics, sun exposure and tobacco smoking between groups, were calculated using Chi-square tests or t-tests depending on the type of data. To determine the proportion of HR-HPV in each group, we calculated the frequency of positive HR-HPV samples. The association between HR-HPV in OSSN was evaluated comparing the proportion of HR-HPV in cases and in controls, estimating an odds ratio. Fisher's exact test was used to compare differences between proportions.

Finally, to evaluate the association between OSSN and the variables of interest we fitted adjusted bivariate logistic regression models, to estimate odds ratios and 95% confidence intervals. Afterwards, we fitted a multivariate logistic regression model to evaluate the association between OSSN and the variables that resulted statistically significant in the bivariate analysis. All statistical analyses considered *p*-values under 0.05 to be significant and were performed using Stata 14.0.\*

## Results

Sociodemographic characteristics, sun exposure and tobacco smoking data are shown in table I. In the OSSN group we found a male to female ratio of 1.4:1, whereas in the pterygia group it was a 1.2:1 ratio. The average age in patients with OSSN was  $66 \pm 16.2$  years, in comparison to  $57.6 \pm 12.6$  years in the pterygia group. Education level was higher in the OSSN group, where 32% had high school or college level, whereas in the pterygia group only 13.6% had high school level. We observed no difference in tobacco smoking between groups. A higher proportion of patients in the pterygia group reported outdoor jobs, mainly farming and street trading; while OSSN patients reported more recreational outdoor activities. The OSSN group had lower accumulated sun exposure ( $7.3 \pm 7.7$  hours) than the pterygia group ( $11.3 \pm 9.7$  hours), yet this difference was not statistically significant. Sunburn was more common

\* StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.

**Table I**  
**SOCIODEMOGRAPHIC CHARACTERISTICS, SUN EXPOSURE AND TOBACCO SMOKING**  
**IN THE STUDIED POPULATION. MEXICO CITY, 2018**

	Total (n=44)		Cases (n=22)		Controls (n=22)		P value*
	n	%	n	%	n	%	
Level of education							
Illiterate	3	6.8	1	4.5	2	9.1	0.226
Elementary school	22	50.0	8	36.4	14	63.6	
Middle school	9	20.5	6	27.3	3	13.6	
High school	5	11.4	2	9.1	3	13.6	
Technician	2	4.5	2	9.1			
College or above	3	6.8	3	13.6			
Sex							
Male	25	56.8	13	59.1	12	54.6	0.761
Female	19	43.2	9	40.9	10	45.5	
Age							
Mean in years (SD)		61.8 (14.9)		66 (16.2)		57.6 (12.6)	0.063
Tobacco smoking							
Yes	22	50.0	13	59.1	9	40.9	0.228
No	22	50.0	9	40.9	13	59.1	
Outdoor job							
Yes	30	68.2	13	68.4	17	77.3	0.66
No	11	25.0	6	31.6	5	22.7	
Outdoor recreative activities							
Yes	19	43.2	13	59.1	6	27.3	0.033
No	25	56.8	9	40.9	16	72.7	
Radiotherapy history <sup>‡</sup>							
Yes	4	9.1	4	19.1	0	0	0.032
No	39	88.6	17	81	22	100	
Family history of skin cancer							
Yes	2	4.5	2	9.1	0	0	0.148
No	42	95.5	20	90.9	22	100	
Skin cancer							
Yes	1	2.3	1	4.5	0	0	0.312
No	43	97.7	21	95.5	22	100	
Sunburn							
Yes	13	29.5	10	45.5	3	13.6	0.021
No	31	70.5	12	54.5	19	86.4	
Accumulated sun exposure <sup>§</sup>							
Mean in years (SD)		9.46 ( 9.00)		7.3 (7.73)		11.3 (9.75)	0.078

SD: Standard deviation

\* P value comparing cases vs. controls

<sup>‡</sup> Radiotherapy history for any type of cancerous tumor other than ocular surface squamous neoplasia

<sup>§</sup> Accumulated sun exposure unit of measure is years of exposure

in the OSSN group, reporting more skin redness with sun exposure than the pterygia group (45.5% vs 13.6%, respectively;  $p=0.021$ ).

Table II shows data on histopathological diagnosis and HPV genotyping results in both groups. HR-HPV proportion in OSSN samples was 40.9%, whereas in

**Table II**  
**HISTOPATHOLOGIC DIAGNOSIS AND HUMAN PAPILLOMAVIRUS GENOTYPING RESULTS IN OCULAR SURFACE SQUAMOUS NEOPLASIA AND PTERYGIA GROUPS. MEXICO CITY, 2018**

Group	Age	Sex	HPV genotype	Histopathology diagnosis
Ocular surface squamous neoplasia				
1	92	Male		In situ carcinoma
2	51	Female	58, 66	Mild dysplasia
3	59	Male		Mild dysplasia
4	75	Male	58, 66	Moderate dysplasia
5	71	Male	16, 58	Moderate dysplasia
6	79	Male	16, 52, 53, 58, 66	In situ carcinoma
7	86	Female		Moderate dysplasia
8	30	Male	16	Invasive carcinoma
9	63	Male		Moderate dysplasia
10	80	Male		Moderate dysplasia
11	41	Female		In situ carcinoma with acantholysis
12	73	Female		Invasive carcinoma
13	66	Female	16, 52, 58	Moderate dysplasia
14	44	Male		Mild dysplasia
15	54	Male	16	Moderate dysplasia
16	76	Female	11, 16, 18, 53	In situ carcinoma with intense koilocytotic atypia
17	85	Male	16, 18	In situ carcinoma
18	79	Male		Moderate dysplasia
19	60	Female		Mild dysplasia
20	62	Female		Mild dysplasia
21	77	Male		Mild dysplasia
22	49	Female		In situ carcinoma
Pterygia				
1	46	Male		Epithelium without dysplasia
2	47	Female		Epithelium without dysplasia
3	67	Female		Epithelium without dysplasia
4	43	Female		Epithelium without dysplasia
5	47	Male		Epithelium without dysplasia
6	46	Male		Epithelium without dysplasia
7	53	Male		Epithelium without dysplasia
8	54	Female		Epithelium without dysplasia
9	76	Male		Epithelium without dysplasia
10	65	Female		Epithelium without dysplasia
11	45	Female		Epithelium without dysplasia
12	67	Female		Epithelium without dysplasia
13	66	Male		Epithelium without dysplasia

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14	68	Female		Epithelium without dysplasia
15	65	Male		Epithelium without dysplasia
16	69	Female		Epithelium without dysplasia
17	67	Male	53, 58, 66	Epithelium without dysplasia with koilocytotic atypia
18	37	Male		Epithelium without dysplasia
19	36	Male		Epithelium without dysplasia
20	57	Female		Epithelium without dysplasia
21	71	Male		Epithelium without dysplasia
22	76	Male		Epithelium without dysplasia

HPV: Human papillomavirus

pterygia samples was 4.5% (data not shown in table). The most prevalent genotypes in the OSSN group were HPV 16 (32%), HPV 58 (23%), and HPV 66 (19%). In the OSSN group, six cases had mild dysplasia, eight cases had moderate dysplasia, six cases had in situ carcinoma, and two cases had invasive carcinoma. The latter cases were referred to the National Institute of Cancerology to get a comprehensive evaluation by an oncologist. In the pterygia group, only one case had histological features compatible with koilocytotic atypia without other findings of epithelial dysplasia. Furthermore, this was the only subject, in the control

group, with a positive result for HPV infection, where multiple genotypes were involved.

As shown in table III, bivariate logistic regression analysis showed an association between HR-HPV and OSSN (OR=14.54, CI95%=1.65,128.44,  $p=0.016$ ), which became stronger after covariate adjustment (OR=16.35, CI95%=1.23,218.11,  $p=0.03$ ). We found no association between OSSN and sex, age, tobacco smoking, nor accumulated sun exposure. We observed a significant association between sunburn and OSSN (OR=5.28, CI95%=1.20,23.15,  $p=0.02$ ), which increased after covariate adjustment (OR=10.77, CI95%=1.85,86.01,  $p=0.02$ ).

**Table III**  
**BIVARIATE AND ADJUSTED ASSOCIATIONS BETWEEN OCULAR SURFACE SQUAMOUS NEOPLASIA, HIGH RISK HUMAN PAPILLOMA VIRUS AND OTHER RISK FACTORS. MEXICO CITY, 2018**

	Bivariate		Multivariate	
	OR (95%CI)*	P value	OR (95%CI)	P value
High-risk human papillomavirus				
No	REF		REF	
Yes	14.54 (1.65, 128.44)	0.016	16.35 (1.23, 218.11)	0.035
Sex				
Male	REF			
Female	0.83 (0.25, 2.74)	0.761		
Age (years)	1.04 (1.00, 1.09)	0.069	1.06 (1, 1.13)	0.061
Tobacco smoking				
No	REF			
Yes	1.73 (0.52, 5.72)	0.367		
Sunburn				
No	REF		REF	
Yes	5.28 (1.20, 23.15)	0.027	10.77 (1.35, 86.01)	0.025
Accumulated sun exposure (hr/year)	0.93 (0.86, 1.01)	0.071		

\* Odds ratio (95% confidence interval)



## Discussion

We found that OSSN patients had 16.3 times the odds of having HR-HPV, compared to patients with pterygia (OR=16.35, CI95%=1.23,218.11,  $p=0.03$ ). When we compared our results with previous reports, we found that very few studies included a control group, and fewer conducted a statistical analysis to control for potential confounders. De Koning and colleagues reported a proportion of HR-HPV in OSSN samples of 38%, and also included low risk OSSN lesions.<sup>22</sup> Nevertheless, even though they had a control group, they found no association between HR-HPV and OSSN (OR=1.0, CI95%=0.4,2.7). Several differences between our study and theirs can be noticed. They used a group of patients with different diseases as controls (pinguecula, chronic inflammation, pyogenic granuloma, cavernous angioma, and other non-disclosed diagnoses), which could imply different degrees of participation of HPV in these lesions, given that no histopathological analysis to discard epithelial dysplasia was performed. The study was done in Uganda, which is a country with a high prevalence of HIV seropositivity.<sup>23</sup> The mean age in their OSSN cases was 35 years, while the mean age in our study was 66 years, indicating that their OSSN cases occurred in younger adults. They did not exclude HIV seropositive participants. HIV infection could simultaneously increase the probability of OSSN development<sup>23-25</sup> and HPV persistent infection,<sup>26</sup> which is an important confounder.

Previous studies show a great variability in the molecular techniques employed. These differences could explain, in part, the inconsistency in the HR-HPV prevalence observed across studies. Many studies employed a single-round PCR method to generate relatively long fragments, that may not be sensitive enough in samples with a low copy number of viral DNA, or in samples with multiple HPV genotypes.<sup>27</sup> Previous reports using tissue derived from genital lesions, showed that short fragment amplimers, such as SPF<sub>10</sub>, allow ultrasensitive detection of a broad spectrum of HPVs under these circumstances.<sup>19,28</sup> We only found one previous study using the same molecular technique employed in our study, the SPF<sub>10</sub> HPV-LiPA system.<sup>22</sup> Their results, in terms of HR-HPV prevalence in OSSN, are comparable to ours. In Mexico, two previous studies have analyzed HPV DNA from paraffin embedded samples of squamous cell carcinoma.<sup>14,15</sup> Even though, both studies lacked a control group, did not include low risk OSSN lesions and had DNA fragmentation in several samples; they reported a 22 to 50% prevalence of HPV 16, which is also similar to our findings.

We did not find an association between OSSN and solar exposure. Sun exposure is a common risk factor for

OSSN and pterygia; thus, our controls likely had a high level of solar exposure, which could explain why we did not observe an association with OSSN. However, we did observe a difference between people who experienced sunburn and people who did not. Our results showed that sunburn is associated with the development of dysplastic changes and carcinoma development in the conjunctival epithelium (OR=10.77). Only one previous study conducted in Australia,<sup>2</sup> have reported that fair skin and sunburn were associated with OSSN. This is interesting given that people with more pigmented skin also have more pigment in the conjunctiva.<sup>29</sup> Melanocytes in the limbal conjunctiva are interspersed in the epithelial basal cells within Vogt palisades. Higa and colleagues have shown that melanin granules within these melanocytes have a polarized location towards the apical area of the cell where they function as a "solar shade" for limbal stem cells.<sup>30</sup> Probably, our finding is explained by the fact that people with less pigmentation have a higher risk of suffering DNA damage secondary to UV light exposure. Unrepaired DNA damage, especially in the limbal stem cells, can trigger dysplastic changes in the epithelium. To our knowledge this is one of the first studies to describe this association, yet further studies are needed to support this finding.

Despite the fact that some authors have reported the presence of HR-HPV in pterygium,<sup>31,32</sup> a causal relationship of HR-HPV with pterygium seems improbable. First, pterygium consists of a fibrovascular abnormal growth with elastotic degeneration of the collagen fibers and fibroblastic hyperplasia in the substantia propria of the conjunctiva with epithelial hypertrophy or atrophy,<sup>33</sup> but without epithelial dysplastic changes. Given that HPV is an epitheliotropic virus, evidence has shown that the oncogenic changes induced by HR-HPV always happen in the epithelial cells of the affected tissues such as anogenital, laryngeal and esophageal,<sup>34</sup> which is not the case in pterygium. Second, OSSN and other neoplasms, such as primary acquired melanosis, may coexist with pterygium.<sup>35</sup> Unfortunately, the majority of studies have not performed histological analysis on the pterygia samples to rule out dysplastic changes in the epithelium. Several studies have found evidence of concurrence with OSSN in pterygia samples, with a frequency that ranges from 5 to 24%.<sup>35,36</sup> In a recent study from Mexico the frequency of OSSN in pterygia was 11.2%,<sup>37</sup> highlighting the importance of a careful histological evaluation to rule out the concurrence of epithelial dysplasia in these lesions.

To our knowledge this is the first case-control study where HIV seropositive patients were excluded and fresh-frozen tissue samples from OSSN and pterygium were used for detection of HR-HPV through a highly

sensitive molecular technique. Even more, we also documented many variables associated with OSSN, which allowed us to employ inferential statistical analysis to adjust for confounding factors, increasing the validity of our results. We excluded HIV seropositive patients, given the potential confounding role of HIV infection in the association between OSSN and HPV persistent infection.<sup>4</sup> One limitation of our study is that only a single experienced anatomical pathologist made the histologic diagnosis of our samples, which potentially could be a source of information bias. Another limitation in our study, is that HR-HPV prevalence in pterygia and in healthy conjunctiva is unknown in Mexico. Nevertheless, if HR-HPV had an etiological role in pterygium the HR-HPV prevalence in these patients would need to be higher than in healthy conjunctiva, making our estimates for the association between OSSN and HR-HPV conservative. A final limitation of our study is that given the low frequency of OSSN we did not reach the initial estimated sample number; however, our expected HR-HPV proportion in OSSN (33%) was lower than the observed proportion (40.9%), giving us sufficient statistical power to detect the association.

Our findings must also be interpreted from a public health perspective. Previous cancer studies in Mexico have highlighted the importance of early detection,<sup>38</sup> and that is also relevant for ocular surface neoplasms. In the case of different types of eye cancerous lesions, including ocular surface neoplasms, few epidemiological studies have been conducted in Mexico. The availability and use of a national cancer registry could improve the epidemiological characterization of ocular surface neoplasms.<sup>39</sup> Finally, if HPV were to be confirmed as an etiologic agent for OSSN, HPV vaccination could substantially reduce the incidence of the disease; unfortunately, recent analyses suggest that HPV vaccination is decreasing in Mexico, which could affect the incidence of all HPV-related cancers.<sup>40</sup>

## Conclusions

Our study findings suggest that HR-HPV could play an important role in OSSN by itself or as a cofactor, added to epithelial cell damage induced by UV light or to other immunological factors. Further studies are needed to confirm the potential etiological role of HR-HPV in the development of OSSN lesions. Increasing our understanding of this potential causative agent could lead to improved therapeutic approaches, such as using interferon alpha-2b (INF $\alpha$ -2b),<sup>41,42</sup> and to take advantage of the potential preventative role of HR-HPV vaccination.

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