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Human Papilloma Virus Infection Does Not Predict Response to Interferon Therapy in Ocular Surface Squamous Neoplasia

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Purpose: To identify the frequency of human papilloma virus (HPV) in ocular surface squamous neoplasia (OSSN) and to evaluate differences in clinical features and treatment response of tumors with positive versus negative HPV results.

Design: Retrospective case series.

Participants: Twenty-seven patients with OSSN.

Methods: Ocular surface squamous neoplasia specimens were analyzed for the presence of HPV. Clinical features and response to interferon were determined retrospectively and linked to the presence (versus absence) of HPV.

Main Outcome Measures: Clinical characteristics of OSSN by HPV status.

Results: Twenty-one of 27 tumors (78%) demonstrated positive HPV results. The HPV genotypes identified included HPV-16 in 10 tumors (48%), HPV-31 in 5 tumors, HPV-33 in 1 tumor, HPV-35 in 2 tumors, HPV-51 in 2 tumors, and a novel HPV in 3 tumors (total of 23 tumors because 1 tumor had 3 identified genotypes). Tumors found in the superior limbus were more likely to show positive HPV results (48% vs. 0%; $P = 0.06$, Fisher exact test). Tumors with positive HPV-16 results were larger (68 vs. 34 mm^2; $P = 0.08$, Mann−Whitney *U* test) and were more likely to have papillomatous morphologic features (50% vs. 12%; $P = 0.07$, Fisher exact test) compared with tumors showing negative results for HPV-16. Human papilloma virus status was not found to be associated with response to interferon therapy ($P = 1.0$, Fisher exact test). Metrics found to be associated with a nonfavorable response to interferon were male gender and tumors located in the superior conjunctivae.

Conclusions: The presence of HPV in OSSN seems to be more common in lesions located in the nonexposed, superior limbus. Human papilloma virus presence does not seem to be required for a favorable response to interferon therapy. *Ophthalmology 2015;■:1−6 © 2015 by the American Academy of Ophthalmology.*

Ocular surface squamous neoplasia (OSSN) represents a spectrum of disease ranging from mild dysplasia to invasive squamous cell carcinoma and is the most common nonpigmented tumor of the ocular surface.[1] Although human papilloma virus (HPV) has been implicated in the pathogenesis of a variety of cancers, most notably cervical cancer,[2] its association with OSSN has been a subject of much debate. Our group previously found HPV DNA and mRNA in 10 of 10 OSSN specimens (5 HPV-16 and 5 HPV-18); HPV was not detected in control specimens nor in any clinically uninvolved conjunctival specimens from OSSN eyes.[3] Other groups, however, have not replicated these findings, with some studies reporting no HPV and others reporting lower rates of HPV detection in OSSN[4−20] (Table 1). It is not clear why certain areas seem to have a very low frequency of HPV in OSSN tumors (e.g., India, Germany, Taiwan),[4−6] whereas others (e.g., Miami)[3] have a much higher frequency. The effect of latitude and sexual activity on HPV positivity in OSSN is worth further study.

Understanding the epidemiologic features of HPV in OSSN is important because in nonocular HPV-associated malignancies, prognosis and treatment may be altered based on viral presence.[2] For example, some HPV-positive tumors are treated with interferon in isolation or as part of combination therapy.[2,21] Although its exact mechanism is unknown, interferon is known to have both antiviral and antineoplastic properties.[22] For OSSN, interferon has become a popular treatment method and has been reported to be successful in 80% to 90% of OSSN tumors.[23,24] Conversely, 10% to 20% of tumors do not respond to therapy. It currently is unknown what tumor factors such as clinical features or viral presence may predict response to or lack of efficacy of interferon. This information is important because it can help to individualize therapy based on tumor characteristics. For example, physicians may proceed directly to surgery or may use a different agent in patients in whom interferon is unlikely to be effective. The aim of this study was to evaluate the frequency of HPV in our more recent OSSN specimens and to examine whether clinical characteristics, including response to interferon, were different based on HPV status.

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Table 1. Review of Published Epidemiologic Information on Human Papilloma Virus and Ocular Surface Squamous Neoplasia

Authors	Location	Population	Technique	Human Papilloma Virus Frequency
Auw-Haedrich et al[9]	Freiburg, Germany	12 OSSN patients, 15 controls	PCR	16.7% CIN (HPV-16), 0% controls
Chauhan et al[10]	New Delhi, India	64 OSSN patients, 15 controls	PCR	11% OSSN (HPV-16), 0% controls
Guthoff et al[5]	Würzburg, Germany	31 OSSN patients, 11 pterygia, 5 controls	IHC, PCR	0% OSSN, 0% pterygia, 0% controls
Lauer et al[11]	Louisiana	5 OSSN patients	PCR	80% OSSN (2 HPV-16, 2 HPV-16/18)
Manderwad et al[12]	Hyderabad, India	57 OSSN of 48 patients*	ISH, PCR	0% OSSN
McDonnell et al[13]	Los Angeles, California	48 OSSN of 44 patients,* 6 pterygium	PCR	88% OSSN (HPV-16), 0% controls
Mochizuki et al[14]	Gifu, Japan	1 OSSN	ISH, PCR	100% OSSN (HPV-16)
Moubayed et al[15]	Dar es Salaam, Tanzania	14 OSSN	ISH	93% OSSN (12 HPV-6, -11, -16, -18; 1 HPV-18)
Nakamura et al[7]	Japan	8 OSSN, 9 papillomas	IHC, ISH, PCR	50% OSSN (2 HPV-16, 2 HPV-18), 44% papillomas (HPV-6)
Saegusa et al[16]	Kitasato, Japan	3 OSSN, 16 papillomas	ISH, PCR	38% OSSN (HPV-16), 75% papillomas (HPV-16)
Scott et al[3]	Miami, Florida	10 OSSN, 5 controls	ISH, PCR	100% OSSN (5 HPV-16, 5 HPV-18), 0% controls
Sen et al[17]	New Delhi, India	30 OSSN, 35 papillomas, 30 controls	IHC	0% OSSN, 17% papillomas, 0% controls
Simbiri et al[18]	Gaborone, Botswana	28 OSSN, 8 pterygia	IHC, ISH, PCR	75% OSSN (6 HPV-6, 13 HPV-11, 17 HPV-16, 15 HPV-18, 7 HPV-31, 1 HPV-33); 75% pterygia (5 HPV-11, 6 HPV-16, 5 HPV-18)
Tuppurainen et al[19]	Kuopio, Finland	4 OSSN	ISH, PCR	0% OSSN
Woods et al[20]	Sydney, Australia	46 OSSN, 42 pterygia, 69 controls	IHC, PCR, sequencing	7% OSSN (HPV-16), 0% pterygia, 0% controls
Yu et al[8]	Uganda and Kenya	38 OSSN	PCR	61% OSSN (17 HPV-18, 6 HPV-16, -18)

CIN = conjunctival intraepithelial neoplasia; IHC = immunohistochemistry; HPV = human papilloma virus; ISH = in situ hybridization; OSSN = ocular surface squamous neoplasia; PCR = polymerase chain reaction.
*Some of the patients had tumors in both eyes.

Methods

Samples

Approval was obtained from the University of Miami Institutional Review Board, and the methods adhered to the tenets of the Declaration of Helsinki and complied with the Health Insurance Portability and Accountability Act. Twenty-eight OSSN specimens, collected from 27 patients between March 18, 1997, and February 14, 2013, underwent testing for HPV presence. Patient records were reviewed retrospectively for information on demographics and history of OSSN. Clinical features also were collected by chart review and photographs, when available. Clinical features studied included lesion location, clinical appearance (papillomatous, leukoplakic, gelatinous, flat, or nodular), and size. Furthermore, tumors were staged based on the American Joint Committee on Cancer clinical staging system.[25]

Human Papilloma Virus Testing

In situ hybridization was used to evaluate for HPV in OSSN tissue.[26,27] In brief, multiple 4-μm sections were placed on sequentially labeled silane-coated slides. The tissue was deparaffinized, proteased (30 minutes in 2 mg/ml pepsin), washed in sterile water and then 100% ethanol, and air dried. The probe cocktail containing the biotin-labeled genomic probe and the tissue DNA were codenatured at 95°C for 5 minutes, hybridized for 15 hours at 37°C, then washed at either low stringency (melting temperature [Tm] 30°C, for identification of HPV DNA) or high stringency (Tm 58°C, for identification of the specific HPV type). Streptavidin-conjugated alkaline phosphatase then reacted with the chromogen nitroblue tertrazolium and bromochloroindolyl phosphate to localize the probe—target complex. Nuclear fast red served as the counterstain. All samples were tested for HPV-2, -6, -11, -13, -16, -18, -26, -27, -30, -31, -32, -33, -35, -39, -40, -41, -42, -43, -44, -45, -51, -52, -56, -59, -68, and -70 as well as other novel types (an HPV detected that is related to but distinct from those included in the probe cocktail) as described previously.[26,27] An HPV-16-positive cervical intraepithelial neoplasia lesion and an HPV-6/11-positive genital condyloma lesion were used as positive controls. The negative controls were cervical and ocular squamous cell lesions that were histologically and molecularly negative for HPV infection.

p53 Detection

The immunohistochemistry detection of p53 was carried out using our previously published protocol.[26] In brief, the optimal conditions for detection of p53 using the antibody from Enzo Life Sciences (Farmingdale, NY) included antigen retrieval for 30 minutes and a dilution of 1:500. Omission of the primary antibody served as the negative control, and a cervical intraepithelial neoplasm known to express p53 was the positive control. The testing was performed using the automated Bond Max instrument (Leica, Buffalo Grove, IL).

Statistical Analysis

The Student *t* test, Mann—Whitney *U* test, chi-square test, and Fisher exact test were used, as appropriate, to evaluate for differences between HPV-positive and -negative tumors and to evaluate