Human papillomavirus infection and ocular surface disease (Review)

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Abstract. Human papillomavirus (HPV) infection has been implicated as a primary cause of lesions in the anogenital region, skin, oropharynx and respiratory tract. Additionally, the role of HPV in the pathogenesis of ocular surface disease has also been extensively studied. Conjunctival papilloma development has been strongly associated with the HPV infection of certain subtypes. On the other hand, the role of HPV in conjunctival pterygium, conjunctival intraepithelial neoplasia (CIN) and ocular surface squamous neoplasia (OSSN) remains controversial. Genetic predisposition and environmental factor is important in HPV hosts as regards the pathogenesis of ocular surface disease. Several studies have indicate a synergic role of HPV with ultraviolet radiation in pterygium establishment. A higher recurrence risk rate and more aggressive disease of ophthalmic pterygium is observed in cases of HPV infection. The purpose of this review was to provide a systematic review of the literature and to assist in a better understanding of the role of HPV in ocular surface disease.

Contents

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Abbreviations: HPV, human papillomavirus; CIN, conjunctival intraepithelial neoplasia; OSSN, ocular surface squamous neoplasia; SCCC, squamous cell carcinoma of the conjuctiva; UV, ultraviolet; MMC, mitomycin C; EV, Epidermodysplasia verruciformis

Key words: human papillomavirus, virus, conjunctiva, papilloma, neoplasia

1. Introduction

Papillomaviruses (HPV) belong to the virus family of *Papovaviridae*. The papillomaviruses are highly host species-specific and tissue-restricted, and present epithelial tropism. More than 100 genotypes of human papillomaviruses have been fully sequenced, and more HPV types are continuously being found (1,2).

All recognized viruses require terminal differentiation for replication and virion production. HPVs cause a wide range of diseases from benign lesions to invasive tumors (3). They are grouped in 3 groups as follows: Cutaneous, mucocutaneous and associated with the autosomal recessive epidermodysplasia verruciformis (EV). According to their propensity for malignant progression in the cervix, mucocutaneous HPVs have been divided into low- and high-risk types (4).

Papillomaviruses are small, non-enveloped viruses with icosahedral symmetry and contain a double-strained circular DNA with approximately 8 open reading frames which are divided into 3 functional regions: The early (E) region encoding proteins E1- E7 responsible for viral replication, the late (L) region encoding structural proteins L1-L2, and the long control region (LCR) responsible for transcription and replication (5,6). Only the E6 and E7 proteins of high-risk HPV strains present transforming properties by interacting with the tumor suppressor genes p53 and with the retinoblastoma family of proteins pRb, involved in controlling cell proliferation (7,8). The viral proteins may also contribute to potentially higher number of abnormalities in the cell genetic material (9).

In recent years, HPV has been associated with benign and malignant lesions of not only the anogenital region, but also of the skin, oropharynx, respiratory tract and ocular surface with variation of different genotypes tropism in the various anatomical sites (10-12). The epithelium of the ocular surface is exposed to the environment and therefore it is susceptible to infections, particularly in cases when protective barriers of mucin, tears and superficial cellular layer are compromised. HPV has mainly been shown to be involved in the pathogenesis

of ocular surface diseases, such as conjunctival papillomas, papillomas and carcinomas of the lacrimal sac, conjunctival intraepithelial neoplasia (CIN), ocular surface squamous neoplasia (OSSN) and conjunctival pterygium and even squamous cell carcinoma of the conjunctiva (SCCC) (12-14).

2. Data collection methods

For this review article, a thorough search on MEDLINE (through PubMed), EMBASE (through OVID) and SCOPUS was performed, from inception to February, 2019, in order to identify studies addressing the association between infection with HPV and ocular surface disease.

3. HPV detection in ophthalmic pterygium

Conjunctival pterygia are fibrovascular lesions of the bulbar conjunctiva, that can display an aggressive clinical behavior and, occasionally, threaten vision. They represent a proliferative disorder of the conjunctiva, characterized by the overgrowth of altered limbal cells centripetally towards the cornea in a wing-shaped manner. In advanced stages, they present Bowman's layer dissolution, epithelial and mesenchymal transition and stromal inflammation, neovascularization and matrix remodeling under the action of cytokines, growth factors matrix metalloproteinases and vascular endothelial growth factors. They have a predilection for nasal limbus and their growth can obscure the visual axis, and can cause irregular astigmatism and chronic inflammation (15). Its pathogenesis is considered a multifactorial process where ultraviolet (UV) radiation (16) and other environmental factors, genetic predisposition and oncogenic viruses may play a role (17). Increased UV-associated oxidative stress has been reported in pterygium, compared with normal conjunctiva, leading to the induction of proteins, such as survivin (18). The latter has been associated with DNA oxidation and the downregulation of p53 (18). Detorakis *et al* (19) previously identified potential viral co-morbidity in pterygium development and proposed a 'two-hit' theory for its establishment. The first hit is a damaging reaction mediated by UV radiation exposure that leads to genetic alterations or mutations, and the second hit is an oncogenic event mediated by viral infection in the compromised ocular barriers.

HPV has been extensively studied as a possible pathogenetic co-factor; however controversy exists between different studies (Table I). In their studies, McDonnell *et al* (20), Dushku *et al* (22), Chen *et al* (26), Schellini *et al* (28), Kuo *et al* (29), Otlu *et al* (32), Guthoff *et al* (34) and Hamed-Azzam *et al* (38) did not detect HPV in pterygia, while Sjo *et al* (13), Takamura *et al* (30) and Hsaio *et al* (36) noted a very low prevalence of the virus ranging from 3-4,8%.

On the contrary, a number of previously published studies have successfully detected HPV (21,23-25,27,31,33,35,37,39) The average HPV prevalence in human pterygium was found to be 18.6% (range 0-100%) (40). The lack of consensus between studies could reflect differences in methodology and sampling. Piras *et al* (25) proposed that geographic differences in the prevalence of the virus in the various countries may explain these findings, supporting its multifactorial pathogenesis.

The diagnosis of viral infection is based on the detection of HPV-DNA. The application of various detection techniques with varied sensitivity and specificity can significantly compromise the results (41). HPV-DNA can be directly isolated from a biopsy specimen with *in situ* hybridization (ISH), Southern blotting and dot blot hybridization. These techniques however, are laborious and lack sensitivity. By contrast, polymerase chain reaction (PCR) is highly associated with false-positive results due to its high sensitivity (42). Real-time PCR permits the rapid detection and quantification of the viral load (42). Reverse transcriptase-PCR is a qualitative assay that permits the identification of viral gene expression with the use of reverse transcriptase (42). A combination of the previous techniques can be applied in order to acquire qualitative and qualitative information of viral gene expression.

Pterygium treatment is based on surgical excision and the topical use of antimetabolites, such as mitomycin C (MMC) or 5-fluorouracil. Several surgical techniques have been described, such as bare sclera closure and sliding conjunctival flaps (43). The additional use of conjunctival autografts or amniotic membrane grafts, has significantly lowered the need for repetitive surgery, although recurrences may still occur (44).

4. HPV detection in conjunctival papilloma

Conjunctival papillomas represent one of the most common benign tumors of the squamous epithelium of the conjunctiva. They may display dysplasia, but rarely undergo malignant transformation (45). Despite their tendency to recur following surgical excision, spontaneous regression is possible. According to their growth pattern, conjunctival papillomas may present as exophytic, mixed or more rarely inverted. The exophytic growth pattern may be sessile or pedunculated (12). Although they can appear in both children and adults (46), they most frequently occur in adults aged 20-39 years, which also corresponds to the peak age of genital HPV infection in sexually active adults. Papillomas seem to appear with a male preponderance and their incidence declines with increasing age. They are usually detected medially and inferiorly on the conjunctiva (45).

Their pathogenetic role has not been fully clarified. HPV infection by auto-inoculation from contaminated fingers has been strongly associated with their development. Fetal passage through an HPV-infected birth canal may explain the presence of conjunctival papilloma in children. Several studies have investigated the role of HPV in the pathogenesis of conjunctival papilloma (Table II). In the majority of these cases, the low-risk HPV types 6 and 11, typically found in condylomata accuminata, are predominant among HPV-infected conjunctival papillomas with an incidence varying from 50-100%. Egbert and Kersten (61) also refer the detection of HPV 6 and 11 in a conjunctival papilloma of an infant whose mother suffered from vulvar HPV infection during pregnancy, indicating a possible vertical transmission during delivery. The detection of low-risk HPV types in conjunctival papillomas may explain the benign nature of these lesions. In addition, HPV infection has been also detected in epithelial lacrimal sac papillomas and carcinomas. The tear

Table I. HPV in ophthalmic pterygium.

Author (Refs.)	Date of publication	HPV prevalence	HPV type	Country of the study	Method of detection	Sample size
McDonell *et al* (20)	1992	0%	-	USA	PCR	6
Varinli *et al* (21)	1994	64%	-	Turkey	IHC	25
Dushku *et al* (22)	1999	0%	-	USA	PCR	13
Detorakis *et al* (23)	2001	24%	18	Greece	PCR	50
Gallagher *et al* (24)	2001	50%	6, 11, 16	UK	PCR	10
Piras *et al* (25)	2003	100% 21%	Types 52, 54, *cand*HPV90	Italy/ Equador	PCR, sequencing	41
Chen *et al* (26)	2003	0%	-	Taiwan	PCR	65
Ateenyi-Agaba *et al* (27)	2004	50%	11, 37	Uganda	PCR, Southern blotting	10
Schellini *et al* (28)	2006	0%	-	Brazil	PCR	36
Kuo *et al* (29)	2006	0%	-	Taiwan	PCR	4
Sjö *et al* (13)	2007	4.4%	6	Denmark	PCR, ISH	90
Takamura *et al* (30)	2008	4.8%	-	Japan	PCR-HC II	42
Rodrigues *et al* (31)	2008	58.3%	1, 2, 16	Brazil	PCR	36
Otlu *et al* (32)	2009	0%	-	Turkey	Real-time PCR	40
Tsai *et al* (33)	2009	24%	16, 18	Taiwan	Nested PCR	129
Guthoff *et al* (34)	2009	0%	-	Germany	PCR, IHC	
Piecyk-Sidor *et al* (35)	2009	27.6%	5, 6, 11, 16, 18, 31, 52, 59	Poland	PCR	58
Hsiao *et al* (36)	2010	3%	18	Taiwan	PCR, ISH	65
Chong *et al* (37)	2014	64.4%	16, 18, 58, 59	Malaysia	Nested PCR	45
Hamed-Azzam *et al* (38)	2016	0%	-	Israel	IHC	100
Chalkia *et al* (39)	2018	42.86%	33, 39, 45, 56, 59, 66	Greece	Real-time PCR	21

HPV, human papillomavirus; IHC, immunohistochemistry; ISH, *in situ* hybridization; HC II, hybrid capture II.

flow on an HPV infected conjunctiva may be responsible for the development of such lesions.

Treatment options of conjunctival papillomas include mainly surgical excision, cryotherapy and carbon dioxide laser (62). Additionally, oral cimetidine, topical MMC and topical interferon-α have been implicated in the management of these benign lesions (63). However, despite the various therapies, the recurrence rate in conjunctival papilloma remains high (6-27%).

5. HPV detection in ocular surface squamous neoplasia

OSSN encompasses a wide range of conjunctival lesions that range histologically from dysplasia and carcinoma *in situ*, generally termed CIN, to invasive SCCC and is considered the most common ocular-surface malignancy (64). It involves more commonly the interpalpebral area, arising from the limbus and may extend to involve cornea (65). The incidence of OSSN varies widely with increased incidence in countries where HIV infection is an epidemic (66). In fact, immunosuppression due to HIV-infection has been strongly associasted with OSSN, as various studies have demonstrated a 10-fold increase for OSSN in these patients. Other risk factors associated with OSSN are an advanced age, the male sex, UV exposure, immunosuppression, atopic eczema and xeroderma pigmentosum (66,67). Conjunctival SCC represents the most severe form of OSSN, which if left untreated, can result in mortality (68). Metastasis to lymph nodes is common; thus, aggressive treatment with enucleation or exenteration should be considered (69).

A number of studies have been conducted in order to identify the presence of HPV in OSSN (Table III). However, the reported presence of the virus varies widely with a range from 0-100%. From the studies reported to date in Table III, an average prevalence rate of 33.8% (range, 0-100%) has been observed. It should also be noted that in the majority of these studies, the HIV status of the patients has not been disclosed, leaving the role of HIV as enhancer or confounder of HPV carcinogenicity in OSSN unclear. In a number of studies, mucosal, mainly high risk types 16 and 18 have been detected. Scott *et al* (75) isolated HPV16 and mRNA corresponding to the E6 region in CIN specimens. Notably, Ateenyi-Agaba *et al* (14) detected cutaneous HPV types in nearly half of OSSN cases in HIV-positive patients, but rarely in HIV-negative patients, and thus no association was found with mucosal types in both groups.

Table II. HPV in conjuctival papilloma.

Author (Refs.)	Date of publication	HPV prevalence	HPV types	Method of detection	Sample size
Lass *et al* (47)	1983	50%	11	SB	2
Naghashfar *et al* (48)	1986	0%	-	SB/ISH	1
McDonnell *et al* (49)	1987	65%	6, 11	ISH	23
Mäntyjärvi *et al* (50)	1989	0%	-	ISH	1
Fierlbeck *et al* (51)	1990	0%	-	ISH	1
Mincione *et al* (52)	1992	50%	6, 11	ISH	4
Saegusa *et al* (53)	1995	100%	16	PCR	5
Michel *et al* (54)	1996	0%	-	ISH	1
Nakamura *et al* (55)	1997	50%	6	PCR	8
Assadoullina et al (56)	2000	0%	-	PCR	1
Sjo *et al* (57)	2001	92%	6, 11, 16	PCR	52
Minchiotti *et al* (58)	2006	100%	11	PCR	4
Sjö *et al* (59)	2007	81%	6, 11, 45	PCR	106
Takamura *et al* (30)	2008	100%	-	PCR/HC-II	8
Annadanam *et al* (60)	2017	100%	6, 11	ISH	1

HPV, human papillomavirus; SB, Southern blotting; ISH, *in situ* hybridization; HC II, hybrid capture II.

Chauhan *et al* (84) also reported an improved disease-free survival of patients with HPV-infected OSSN. On the other hand, De Koning *et al* (81) noted a low prevalence of cutaneous HPV cases and no evidence of association of mucosal HPV types with OSSN. Tornesello *et al* (79) also noted an absence of high-risk types and low detection of EV-related types in their study and yet, another study (74) did not note significant correlation between abnormal p53 gene-product expression in OSSN and HPV infection. These discrepancies between the different studies render the role of HPV in OSSN uncertain.

Margin-free excision remains the treatment of choice for OSSN. The additional use of topical mitomycin, 5-fluorouracil, interferon and cryotherapy and radiation may be used to reduce the risk of recurrence and metastasis (87).

6. Conclusions and future perspectives

HPV is a known cause of intraepithelial damage which leads to squamous neoplasms on mucosal surfaces (88). Many types of cervical carcinoma and precancerous lesions have been attributed to HPV infection. In addition, HPV has been linked with dysplastic and malignant squamous lesions of the oropharynx (89). The associatoin between HPV and squamous neoplasms of the ocular surface and the conjunctiva is not completely understood. It appears that the HPV genotype as well as the presence of associated risk factors play a significant role in lesion pathogenesis (13,45).

Koilocytosis is the histological hallmark of HPV infection (90). The koilocyte is a superficial or intermediate mature squamous cell characterized by perinuclear vacuolation, densely staining peripheral cytoplasm, and a nucleus with an undulating nuclear membrane and a rope-like chromatin pattern (91). Viral antigen has been demonstrated in nuclei of koilocytes using broad spectrum papillomavirus antibodies (47).

HPV has been identified in several lesions of the ocular surface. A strong association between HPV types 6 and 11 and conjunctival papilloma has been established (49,52,57,59,60). The varied percentages of papillomas associated with HPV presence could be attributed to differences in genetic predisposition, lifestyle and environmental exposure (73). In cases of pterygium, the association between the virus presence is not clear. Based on current data, HPV appears to function as a pathogenetic co-factor in addition to genetic factors like p53 gene mutation (92), as well as environmental factors, such as UV radiation and HIV co-infection (18) and chemical exposure (73). Despite the controversies in the literature, HPV infections seem to be a crucial co-morbidity in susceptible hosts (33). Frequent recurrences of a pterygium following excision may be associated with the presence of HPV (24). The proposed pathogenesis involves p53 inactivation (33).

While there is a strong association between HIV and the risk of OSSN (66), the role of HPV is less conclusive. Previous studies have suggested that only the cutaneous HPV subtype, and not the mucosal, is correlated with the presence of OSSN (14,81). Furthermore, older individuals seem to be more prone to the development of the described lesions (40).

The discrepancies in the reported results may be attributed to the selection bias of different regions (25) and the different methods for HPV isolation. While there is no gold standard for measuring HPV, PCR is generally considered to be the most sensitive method (76). It should also be noted that the newer HPV subtypes are continuously sequenced; thus, the range of possible genotype identification is incomplete. Some unknown types of HPV could be involved in the pterygium pathogenesis (93). Chalkia *et al* (39) described PCR-mediated

Table III. HPV in OSSN.

Author (Refs.)	Date of publication	HPV prevalence	HPV types	Method of detection	Sample size	HIV status
Lauer *et al* (70)	1990	80%	16, 18	PCR	5	-
Tuppurainen *et al* (71)	1992	0%	-	PCR/ISH	4	-
McDonnell *et al* (20)	1992	88%	16	PCR	42	-
Tabrizi *et al* (72)	1997	39%	6, 11, 16, 18	PCR	88	-
Karcioglu *et al* (73)	1997	55.6%	16,18	Nested PCR, SB	45	-
Dushku *et al* (22)	1999	0	-	Nested PCR	8	-
Toth *et al* (74)	2000	22%	16, 18	PCR/IHC	23	-
Scott *et al* (75)	2002	100%	16, 18	PCR/ISH	10	-
Eng *et al* (76)	2002	0%	-	Nested PCR	20	-
Tulvatana *et al* (77)	2003	0%	-	PCR/dot hybridization	30	-
Ateenyi-Agaba *et al* (27)	2004	86%	EV-HPV types	PCR	21	-
Moubayed *et al* (78)	2004	93%	6, 11, 18	ISH immunomax	14	64,2%
Tornesello *et al* (79)	2006	19.8%	6, 18, EV-related HPVs, CJ198	PCR	86	65,1% (25% HPV^+)
Kuo *et al* (29)	2006	100%	6, 11, 16, 18, 33, 37, 58, 72	Nested PCR	9	-
Sen *et al* (80)	2007	0%	-	IHC	30	-
De Koning *et al* (81)	2008		38% Genital (both high and low risk). 22% cutaneous types	PCR	81	(48% HPV^+)
Manderwad *et al* (82)	2009	0%	-	PCR/ISH-CARD	57	-
Guthoff *et al* (34)	2009	0%	-	PCR/IHC	31	No HIV patients
Ateenyi-Agaba *et al* (14)	2010		Mucosal HPV: 6,4% SCC; 7,7% dysplasia cutaneous HPV: 44,7% SCC; 41% dysplasia	PCR	94 SCC 39 dysplasia	Uncertain role of HIV
Asadi-Amoli *et al* (83)	2011	88%	No type found	Nested PCR	50	-
Chauhan *et al* (84)	2012	11%	16	PCR	64	-
Woods *et al* (85)	2013	6.5%	16	Nested PCR	50	-
Afrogheh *et al* (86)	2016	30%	16	IHC, ISH, PCR	43	?

HPV, human papillomavirus; OSSN, ocular surface squamous neoplasia; ISH, in situ hybridization; CARD, catalyzed reporter deposition; IHC, immunohistochemistry; SCC, squamous cell carcinoma.

exfoliative cytology as a valuable detection method for HPV in ophthalmic pterygium, while others have used exfoliative cytology for OSSN and conjunctival papilloma (94). The use of an easy-applicable, reliable and cost effective method may offer a more detailed investigation of the role of HPV in ocular surface, that may permit the use of topical antiviral treatment in HPV related ocular surface diseases. In fact, two recent studies (95,96) refer the efficient topical use of Cidofovir in OSSN. Cidofovir is a nucleoside analog with activity against a broad spectrum of DNA viruses. It has been used efficiently in squamous papilloma of the oropharynx, condylomata acuminata, molluscum contagiosum, and Kaposi's sarcoma (97,98). Finally, it would be of interest to evaluate the potential effect of HPV vaccination on the prevalence of these diseases in the future.

To conclude, HPV infection seems to play an important role in several aspects of ocular surface disease. Further research is required to elucidate the specific pathogenetic mechanisms of HPV in various ocular surface disease entities and findings may be clinically important in view of the potential development of targeted therapies or preventive measures, such as HPV vaccines.

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AKC was involved in the design of the study, and in the acquisition of data, drafting and writing of the manuscript. GB was involved in the acquisition and analysis of the data from studies to be included in this review, and in the drafting and writing of the manuscript. ETD and DAS were involved in the conception of the study, and in the revision of the manuscript. All authors have read and approved the final version.

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Not applicable.

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Competing interests

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