Original Article

Expression of human papillomavirus 16 and 18 DNA in oral lichen planus using polymerase chain reaction

Aswathy K. Vijayan[1], Arvind Muthukrishnan[2], Vivek Velayudhannair[1], Jayanthi Varun[3], Mathew Vidyadharan[4], Joseena James[5]

[1]Department of Oral Medicine and Radiology, PMS College of Dental Science and Research, Vattapara, Thiruvananthapuram, Kerala, [2]Department of Oral Medicine and Radiology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, [3]Department of Oral Pathology, Azeezia Dental College, Meeyannoor, Kollam, Kerala, [4]Department of Pedodontics, PMS College of Dental Science and Research, Vattapara, Thiruvananthapuram, Kerala, [5]Department of Oral and Maxillofacial Surgery, PMS College of Dental Science and Research, Vattapara, Thiruvananthapuram, Kerala, India

Abstract

Introduction: Oral lichen planus (OLP) is a chronic inflammatory disease with cell-mediated immune dysregulation. The aetiology of OLP has been studied extensively for decades. Viruses like Hepatitis C Virus (HCV), human papillomavirus (HPV), herpes simplex virus (HSV), and stress have been hypothesized to play a role in the pathogenesis and malignant transformation of OLP. HPV has been proved to be an etiological agent in oropharyngeal cancers and non-tobacco-associated leukoplakia. The role of human papillomavirus in the pathogenesis of OLP has to be studied extensively.

Aim: This study aims to detect the presence of HPV 16 and HPV 18 DNA in the biopsy samples of OLP and also to determine the role played by the virus in the pathogenesis and malignant transformation of OLP.

Materials and Methods: Biopsy samples comprising 30 OLP tissues were collected. The DNA was extracted by the cetyltrimethylammonium bromide method. Polymerase chain reaction was performed by using general primers to amplify the HPV E6 gene.

Results: Twelve out of 30 (40%) OLP cases were positive for HPV DNA. A significant relation was found between HPV, site (buccal mucosa) and the type (reticular) of the lesion ($P = 0.007$). However, the difference between the percentage of HPV positive males and females was statistically insignificant ($P = 0.852$).

Conclusion: This study confirmed the presence of high-risk HPV 16 and HPV 18 DNA in OLP. The study showed a significantly higher expression of HPV in erosive OLP when compared to reticular OLP, suggesting a possible role of HPV in the malignant transformation of OLP.

Keywords: Human papillomavirus, oral lichen planus, PCR

Address for correspondence: Dr. Aswathy K. Vijayan, Department of Oral Medicine and Radiology, PMS College of Dental Science and Research, Vattapara, Thiruvananthapuram, Kerala - 695 028, India.
E-mail: aswathykvijayan4@gmail.com

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INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory condition characterised by white striations, papules and plaques on the buccal mucosa, tongue and gingiva.[1] The prevalence of OLP varies depending on geomorphology

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ranging from 0.5% to 2.2%.[2] Two major clinical forms of oral lichen plans have been described namely, the reticular and erosive types. OLP is considered to be a potentially malignant disorder (PMD) of the oral mucosa with a malignant transformation rate ranging between 0-6.25%.[3] Microscopically hyperkeratosis, basal layer liquefaction of the epithelium, and a strong infiltration of a band of lymphocytes are present.[4] OLP has recently been linked to viruses such as HPV and the human herpes virus. Previous studies have shown that apoptosis in OLP oral epithelial cells is similar to viral infection, in which the virus acts as a cytoplasmic antigen, resulting in a transfigured host cell protein profile.[5,6] In this regard, it is important to investigate the role of HPV in the pathogenesis of OLP.

HPV is a member of the papillomaviridea family, with no protective envelope and a diameter of 50–500 nm.[7] HPV types are distinguished based on the degree of homology among their nucleic acid sequences.[7,8] There has been an increased link between HPV16 and HPV18 and oral potentially malignant disorders and oral squamous cell carcinoma.[9,10] Many authors have suggested that HPV might play a role in the aetiology of various oral lesions, but the data provided in the literature addressing HPV infection in OLP are highly disputed. A conclusive link between HPV and OLP has yet to be discovered. This study sought to examine the presence of HPV DNA in OLP lesions and to determine their possible roles in the pathogenesis of OLP.

MATERIALS AND METHODS

Collection of biopsy specimens

The study sample consists of 30 histopathologically confirmed cases of OLP. The OLP biopsy specimens were obtained from the Department of Oral Medicine & Radiology, from patients clinically diagnosed with reticular and erosive types of OLP. Written informed consent was taken from all the participants and the study was approved by the Institutional Review Board. The samples were collected in a biopsy bottle containing DNA stabiliser solution. The specimens were divided into two parts, one for histopathologic examination to confirm the clinical diagnosis and the other part for identification and typing of HPV DNA. The OLP specimens were examined by two experienced oral pathologists. A consensus diagnosis was reached in all the cases after examination of haematoxylin and eosin-stained sections. The portion of the tissue subjected to HPV DNA analysis was immediately transferred to a deep freezer (-80°C) and stored until the tissues were taken for further processing.

For DNA extraction, specimens that were proven to be OLP positive based on histological assessment were chosen. Thirty tissue samples in a DNA stabiliser were provided for HPV detection and typing of HPV-16, HPV-18 using a conventional single polymerase chain reaction (PCR) assay. Ethical committee approval obtained: PMS/IEC/2018-19/40.

Deoxyribonucleic acid extraction

Genomic DNA extraction was done from the tissue sample using the cetyltrimethylammonium bromide method. PCR was done with Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers to check the quality of DNA (Product Size: 496bp; GAPDH F: TTCTGGGGACTGGCTTTCC; GAPDH R: AAAGTGGTCGTTGAGGGCAA). Bands were observed in all 30 samples. A no template control is included in the last lane to rule out contamination [Figure 1]. The DNA isolated was loaded into a 1.2% agarose gel electrophoresis to visualize the bands and their quality.

Quantification of isolated DNA

The quantity of the isolated DNA was checked in a UV-VIS spectrophotometer (Vivaspec Biophotometer, Germany). From the stock, 50 times dilution is obtained by mixing 1 μl DNA with 49-μl sterile distilled water. The A260/A280 ratio was recorded to check the purity of DNA preparation.

Single polymerase chain reaction assay

For the detection of target HPV DNA and typing of HPV-16 and HPV-18, a single polymerase chain reaction assay was used. HPV detection was done by PGMY09/11

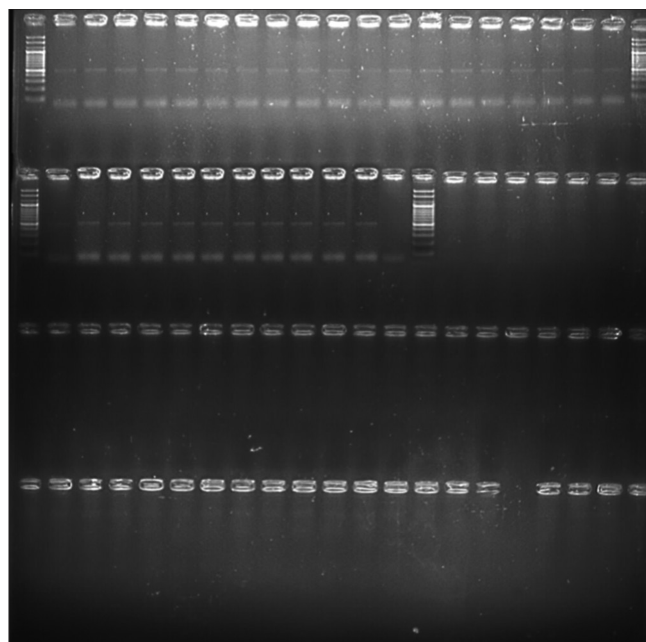

Figure 1: PCR by Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Primer

primers (200 bp HPV-18 and 500 bp HPV-16). The PCR was carried out using primers for the E6 region: HPV16F: 5'GTCAAAAGCCACTGTGTCCT3', HPV16R: 5'CCATCCATTACATCCCGTAC3;, HPV18F: 5'CCGAGCACGACAGGAACGCT3', HPV18R: 5'TCGTTTTCTTCCTCTGAGTCGCTT3'.

Each PCR reaction mixture contained 2 μL of 10 times PCR buffer with $MgCl_2$ (1.5 mM), 2 μL of dNTP mix (2.5 mM), 2 μL of oligonucleotide primer *F* (10 picomoles/μL), 2 μL of oligonucleotide primer R (10 picomoles/μL), 10.70 μL of H_2O, 1 μL of template DNA (50 ng/μL), 0.30 μL of Taq-polymerase (5 U) making the total amount of each reaction to 20.0 μL. Thermocycling conditions were as follows: Initial denaturation at 94°C for 3 minutes, 35 cycles of denaturation at 94°C for 50 seconds, annealing at 50°C for 40 seconds, extension at 72°C for 1.30 minute and final extension at 72°C for 40 seconds. The PCR product was loaded in 1.2% agarose gel to visualise the bands [Figure 2].

Statistical analysis

The data obtained were analysed using the Statistical Packages for Social Sciences (IBM SPSS Statistics, SPSS South Asia Pvt Ltd., Bangalore, for Windows, Version 20.0). Categorical variables were expressed as frequency (percentage). Prevalence of HPV in OLP was expressed using 95% CI. A Chi-square test was used to find the association of HPV in OLP with selected characteristics of OLP. For all statistical interpretations, $P < 0.05$ was considered the threshold for statistical significance.

RESULTS

Twelve samples (40%) out of 30 specimens were positive for HPV in PCR analysis [Table 1]. All the study subjects were between the age of 18 and 56 years, with 14 males and 16 females. In the HPV type-specific PCR assay, the frequency of positivity for both HPV-16 and HPV-18 genomes was 50% (6/30 and 6/30, respectively). The study sample included a 14:16 male to female ratio, with HPV positive rates of 21.4% (3/14) in males and 56.3% (9/16) in females, respectively. The frequencies of HPV positivity in reticular and erosive OLP were 14.3% (2/14) and 62.5% (10/16), respectively. Sixteen samples were obtained from the buccal mucosa and 14 from the labial mucosa. According to the OLP site, HPV positivity rates were 62.5% in the buccal mucosa (10/16) and 14.3% in labial mucosa (2/14), respectively. Samples from the buccal mucosa had a significantly higher HPV positivity rate compared to samples from the labial mucosa ($P = 0.007$). However, no significant difference was found between the percentage of HPV positivity between males and females ($P = 0.852$) [Table 2].

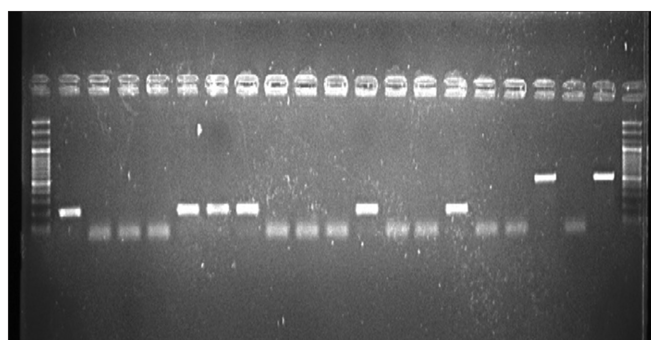

Figure 2: Agarose Gel electrophoresis of HPV by single PCR assay

DISCUSSION

OLP affects 0.5–2.2% of the global population.[11] However, prevalence varies according to geographic location.[11] The prevalence of OLP is 2.6% in the Indian population.[12] The risk of malignant transformation of erosive OLP is 1.2% to 3.2% in the follow-up of up to 10 years.[12] The mechanism of malignant transformation from OLP is not clearly understood, it could probably be a multifactorial mechanism.[12,13]

Table 1: Frequency distribution of gender, type and site of OLP, HPV positivity and HPV genome

	n (%)
Gender	
Male	14 (46.7)
Female	16 (53.3)
Type of OLP	
Reticular	14 (46.7)
Erosive	16 (53.3)
Site of OLP	
Buccal mucosa	16 (53.3)
Labial mucosa	14 (46.7)
HPV	
Positive	12 (40)
Negative	18 (60)
HPV genome	
HPV-16	6 (50)
HPV-18	6 (50)

Table 2: Association of HPV in OLP with selected characteristics of OLP

	Negative		Positive		χ^2	*P*
	Count	Percent	Count	Percent		
Gender						
Male	11	78.6	3	21.4	3.77	0.052
Female	7	43.8	9	56.3		
Type of OLP						
Reticular	12	85.7	2	14.3	7.23	0.007
Erosive	6	37.5	10	62.5		
Site of OLP						
Buccal mucosa	6	37.5	10	62.5	7.23	0.007
Labial mucosa	12	85.7	2	14.3		

HPV is a circular double-stranded DNA molecule belonging to the papillomaviridea family, and there are more than 150 recognised types of HPV.[14] Many types of infections are caused by HPV, including common warts, genital warts, recurrent respiratory papillomatosis, low grade and high grade squamous intraepithelial lesions, and cervical cancer.[15] Papillomavirus infections occur through micro-wounds of the epithelium, which allow virus entry into the basal layer. The basal layer is composed of stem cells that continually divide, which promotes the expression of viral genes. This virion is well maintained in undifferentiated basal cells all through the course of the infection. Due to the above-mentioned mechanism of infection, there can be a possible association between HPV and OLP.[16]

HPV proteins, in particular, the oncoproteins E6 and E7 of high-risk HPVs (HR-HPVs), interact with host cell proteins in various degrees of affinity to interfere with normal epithelial differentiation and apoptosis by stimulating cellular proliferation, DNA synthesis and inhibiting cell cycle regulators.[17] The continued and aberrant interactions between E7 and pRB and E6 and p53 lead to genomic instability and mutational events.[17,18] The high affinity of HR-HPV proteins for tumour suppressor gene and their tendency to stay in an abraded epithelium may result in malignant transformation of erosive OLP. Proteins of low-risk HPVs (LR-HPVs) have a low affinity for tumour suppressor proteins. As a result, these viruses have low oncogenic potential, and their infections are usually self-limiting. Cancer risks increase with persistent infection with high-risk HPVs, whereas benign lesions are associated with low-risk types.[17-19]

HPV 16 and 18 have been associated with potentially malignant disorders and oral squamous cell carcinoma.[20] Predominantly HPV is proven as the major etiological agent in uterine and cervical cancer.[21] Recent studies have shown an increase in presence of HPV-associated oropharyngeal cancers in non-tobacco and alcohol users.[21] HPV has been isolated from proliferative verrucous Leukoplakia which has a 95% chance of malignant transformation.[21]

In the present study, the association between OLP and HPV genotypes 16 and 18 was studied. A statistically significant difference was found between the site and the type of lesion. The prevalence of HPV varies markedly among geographically diverse populations.[3] OFlatharta *et al*.[22] reported a 26.3% (9/38) prevalence of HPV-16 in OLP samples, suggesting a possible aetiological role for HPV in OLP. The authors while stating the results highlighted that HPV in OLP is not a universal finding and it might just have been a chance finding. In a Swedish cohort, Sand *et al*.[23] reported HPV-18 positivity in five of 22 (27.3%) OLP patients; five (83%) of whom were HPV-18 -positive. The authors concluded that HPV infection was associated with HPV 18 in the study population; however, the pathogenic influence of HPV infection could not be determined. Sahebjamiee *et al*.[24] 2015 their study from Iran detected HPV DNA in 11 of 40 (27.5%) cases of OLP. Two studies conducted by Pol *et al*.,[25] and Debanth *et al*.,[26] from India, showed a strong association between OLP and HPV.

Arirachakaran *et al*.[27] and Khovidhunkit *et al*.[28] their study on Thai patients using PCR to detect HPV 16 in OLP demonstrated only 1.54% (1/65) 2.7% (1/37) positivity respectively, indicating no association of HPV in OLP in the Thai group of patients. Our study was in accordance with that of OFlatharta *et al*.[22] Sand *et al*.[23] and Sahebjamiee *et al*.[24] where the authors reported a possible association of HPV with OLP.

Another study conducted by Szarka *et al*.[29] on HPV DNA in non-erosive/atrophic OLP (Non-EA-OLP) and erosive/atrophic OLP (EA-OLP) showed higher prevalence in EA-OLP than Non-EA-OLP (22.4%). The authors also suggested that considering the HPV prevalence pattern found, it is tempting to hypothesize that HPVs may play some etiological role in the malignant progression of OLP.[29]

In another study, Mattila *et al*.[30] investigated 82 atrophic OLP for HPV DNA using Luminex-based assay and static cytometry. HPV DNA was found in 15.9% of OLPs. There was a significant correlation between HPV 6 and 11 DNA in OLP indicating an association with the subgroup of atrophic OLP. The significance of this observation remains obscure because HPV 6 and HPV 11 are the two most common low-risk HPV types. However, earlier literature has reported that low-risk types HPV6 or HPV11 have been associated with benign papillomas that progressed to carcinoma when treated by irradiation. One theory may be that HR- and LR-HPVs may behave differently in oral mucosa than in the genital tract because these two mucosal sites are exposed to different carcinogens and have a different microbial environment.[30]

The present study showed HPV positivity in 40% of samples of OLP indicating a definite presence of HPV in OLP. The frequencies of HPV positivity in reticular and erosive OLP were 14.3% (2/14) and 62.5% (10/16), respectively. Given the *P* value gained by the Chi-Square

test (P = 0.007), a significant relation was observed between HPV infection and erosive type of OLP, a finding consistent with that of Szarka *et al*.[29]

The present study findings suggest that HPV type 18 (50%) and HPV type 16 (50%) are observed equally in Asian patients. Sameera *et al*.[31] in their study to detect the presence of HPV DNA in OLP, confirmed the presence of HPV 18 in OLP. The authors were of opinion that high rates of HPV type 18 (86.6%) are observed in Indian patients compared to those of eastern and western continents. The authors strongly believed that HPV type 18 detection might be associated with OLP pathogenesis and may also provide information on its malignant potential. Razavi *et al*.,[32] in a case-control study in the Iranian population, examined the presence of HPV DNA in OLP samples. HPV 18 was detected in 9 out of 29 samples (31%) The authors concluded that there might be a coincidence between human papillomavirus type 18 and oral lichen planus. Contrary to the above studies in our study, both HPV 16 and HPV 18 were found equally in OLP samples.

Our preliminary study to analyze the HPV DNA in OLP patients showed a possible association of HPV in OLP samples. However, a significant relation was found between erosive OLP and HPV genotypes. In the present study, we used PCR, which is a sensitive method, for the detection of HPV. More prospective cohort studies are needed to establish a conclusive association between HPV DNA in OLP.

Limitation

The major limitation of the current study is the small sample size. The study should be conducted with a more number of cases to confirm the hypothesis of the relationship between HPV and OLP. In the present analysis HPV prevalence in clinically normal sites was not analysed. Thus, future studies comparing HPV infection in normally and pathologically oral sites are required to determine whether HPV is a factor that predisposes to the pathogenesis of OLP and its malignant transformation.

CONCLUSION

In the current study HPV type 16 and HPV 18 was detected in oral lichen planus which shows the presence of HPV in OLP patients. The high-risk types HPV 16 and 18 present in more erosive lichen planus in the present study augment the previous theory that ulceration is frequent in erosive OLP making it more susceptible to HPV infection. Adequate long-term follow-up of erosive OLP is essential taking into consideration the premalignant potential of OLP and the increased presence of high-risk genotypes 16 and 18. If HPV is proved to play a pathological role in OLP, then chronic use of steroids should be used with caution in OLP, which may induce immune suppression that could regulate HPV replication.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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