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# Role of human papilloma virus-16 in the pathogenesis of oral lichen planus – an immunohistochemical study

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**Background:** Oral lichen planus (OLP) is a common chronic inflammatory immune-mediated disease with an aetiopathogenesis associated with cell-mediated immunological dysfunction. It is possible that oral mucosal viral infections, including human papilloma virus-16 (HPV-16) infection, may have a causative role in OLP pathogenesis. **Aim:** To assess the prevalence of HPV-16 in histopathologically diagnosed specimens of OLP and to evaluate whether any clinical features (such as the localisation of specimens) or the age or gender of patients, are correlated with the presence of this virus. **Materials and methods:** This study was conducted on 30 specimens with a histopathological diagnosis of OLP, using the immunohistochemical marker HPV-16. Thirty normal oral mucosa specimens were also included as controls. Brown nuclear staining was accepted as positive for the HPV-16 antibody. The results were analysed using Fisher's exact test. *P* values <0.05 were considered to be significant. **Results:** Significant correlation ($P = 0.0001$) was observed between HPV-16 infection and samples with OLP. No statistical conclusions could be drawn regarding age, gender, localisation and HPV-16 positivity. **Conclusion:** Our study showed that HPV-16 may play a role in the pathogenesis of OLP. Taking into account the oncogenic potential of HPV-16, patients with OLP should be screened for the presence of this virus.

***Key words:*** *Clinical features, human papilloma virus-16, immunohistochemistry, oncogenic potential, oral lichen planus*

## INTRODUCTION

Oral lichen planus (OLP) is a chronic immunological inflammatory mucocutaneous disease[1]. OLP affects 0.1% to about 4% of the population, is a disease of the middle-aged and is more common among women[2]. The buccal mucosa, tongue and gingiva are commonly affected, whereas palatal localisation is uncommon. Lesions are typically bilateral with a variety of clinical presentations, including reticular, papular, plaque-like, atrophic and ulcerative[2].

OLP is generally considered to be an immunologically mediated process that histologically resembles a hypersensitivity reaction. It is characterised by an intense band-like T-cell infiltrate in the epithelium–connective tissue interface. The factors that initiate OLP are unknown, but the mechanism appears to involve several steps, such as: an initiating factor or event (exogenous/endogenous antigenic stimulation); focal release of regulatory cytokines; up-regulation of vascular adhesion molecules; recruitment and retention of T-lymphocytes; and basal keratinocyte cytotoxicity mediated by T-lymphocytes[3].

Recently, viruses, such as human papilloma virus (HPV) and human herpesvirus (HHV), have been found to play a role in the pathogenesis of OLP[4]. Existing data suggest that these viruses may alter host cell function by inducing the abnormal expression of cellular proteins, leading to disease development. This indicates that oral mucosal viral infections may play a role in the pathogenesis of OLP[5].

HPV is a member of papillomaviridea family, with no envelope and a diameter of 50–500 nm. Different types of HPV are distinguished based on the degree of nucleic acid sequence homology. Some types of HPV, such as HPV-16, HPV-18 and HPV-31, have been found to have an association with specific types of premalignant and malignant lesions[6].

The aim of the present study was to assess the prevalence of HPV-16 in histopathologically diagnosed cases of OLP and to evaluate whether any clinical features (age, gender and localisation) correlate with this virus.

## MATERIALS AND METHODS

The research was approved by Maharashtra University of Health Sciences (MUHS) Nashik Ethical Board

and was conducted in full accordance with the World Medical Association Declaration of Helsinki. It was a retrospective study performed on lesional and normal tissue embedded in preserved wax block, so consent was not needed from the participants/patients who donated the samples.

### Case selection

This case–control study was conducted on formalin-fixed paraffin-embedded tissue specimens from 30 patients with OLP (with minimum age 21 years and maximum age 58 years). The patients were classified according to age, gender and localisation of the lesion. All cases of OLP were of the reticular form and patients had no history of smoking, alcohol consumption or betel-nut chewing habit. Thirty normal oral mucosal tissue specimens, free of inflammation and necrosis (from subjects with a minimum age of 18 years and a maximum age of 52 years) were used as the control group.

### Immunohistochemical staining

Four-micrometer-thick sections of formalin-fixed and paraffin-embedded biopsy samples were processed using the poly-HRP method. Deparaffinisation and rehydration of the sections were followed by the blocking of endogenous peroxidase activity by incubating the sections in 3% hydrogen peroxide for 5 minutes. After rinsing with phosphate-buffered saline (PBS; pH 7.0), the sections were treated with antigen-retrieval solution (citrate buffer, pH 6.0) in a microwave for 15 minutes (Microwave setting used as per manufacturer suggestion) and then the slides were left to cool at room temperature for 30 minutes. Non-specific binding was reduced by incubation with protein-blocking serum for 20 minutes. The sections were incubated with HPV-16 primary antibody (mouse monoclonal antibody; Biogenic, Lab vision, Hyderabad, India) at room temperature for 30 minutes. After rinsing with PBS, the slides were incubated with poly-HRP secondary antibody for 30 minutes. The sections were washed with PBS and then the slides were incubated with DAB HRP substrate. DAB HRP substrate was used as a chromogen for visualisation of antibody binding. Finally, the sections were counterstained with Mayer's haematoxylin, cleared and mounted.

### Immunostaining results

Light microscopy was used to evaluate the immunohistochemical reactions. The brown nuclear staining was accepted as positive staining for HPV-16 antibody.

### Statistical analysis

We evaluated the histopathological features of the specimens and their localisation and the age and gender of the patients with OLP and conducted correlation analyses of these features with HPV-16 positivity. Statistical analyses were performed using Fisher's exact test.

## RESULTS

Twenty-one (70%) of 30 subjects with OLP were HPV-16 positive and all subjects in the control group were negative for HPV-16 (*Figures 1 and 2*). The *P* value obtained from Fisher's exact test showed a significant relationship ($P = 0.0001$) between HPV-16 infection and OLP (*Table 1*). There were no statistically significant correlations between age, gender, localisation of the lesion and HPV-16 positivity (*Tables 2 and 3*).

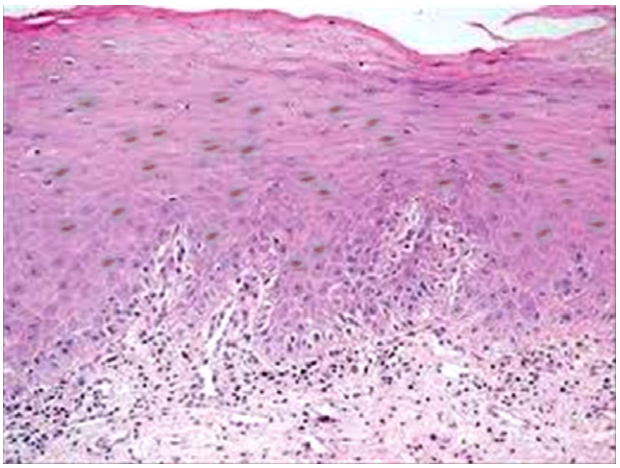

***Figure 1.*** Photomicrograph showing immunohistochemical staining of the human papilloma virus 16 (HPV-16) in oral lichen planus (magnification ×45).

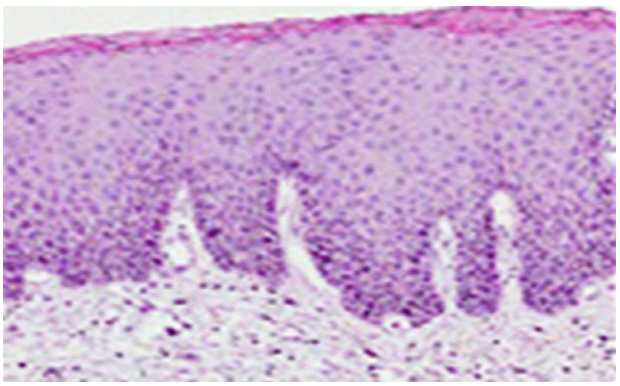

***Figure 2.*** Photomicrograph showing no immunohistochemical expression of human papilloma virus 16 (HPV-16) in normal oral mucosa (magnification ×45).

**Table 1** Prevalence of human papilloma virus 16 (HPV-16) in the group of patients with oral lichen planus (OLP) and in the control group (no OLP)

| Group | HPV-16 status | | Total no. of subjects in group | *P* value |
|---|---|---|---|---|
| | Positive(*n*) | Negative(*n*) | | |
| Patients | 21 | 9 | 30 | 0.0001 |
| Controls | 0 | 30 | 30 | (significant) |
| All | 21 | 39 | 60 | |

**Table 2** Prevalence of human papilloma virus 16 (HPV-16) according to gender

| Gender | HPV-16 status | | Total no.of subjectsin group | *P* |
|---|---|---|---|---|
| | Positive | Negative | | |
| Male | 9 | 16 | 25 | 0.944 |
| Female | 12 | 23 | 35 | (non-significant) |
| Overall | 21 | 39 | 60 | |

**Table 3** Relative prevalence of cases based on the localisation of lesion

| Site | Case | Control | All | *P* value |
|---|---|---|---|---|
| Mucosa of floor of mouth | 2 (6.67) | 0 (0) | 2 (3.33) | 0.199 (non- |
| Buccal mucosa | 18 (60) | 24 (80) | 42 (70) | significant) |
| Lip mucosa | 5 (16.67) | 2 (6.67) | 7 (11.67) | |
| Tongue | 3 (10) | 1 (3.33) | 4 (6.67) | |
| Gingiva | 2 (6.67) | 3 (10) | 5 (8.33) | |
| All | **30 (100)** | **30 (100)** | **60 (100)** | |

Values are given as *n* (%).

## DISCUSSION

OLP is a chronic inflammatory disease in which the immunopathogenesis involves cell-mediated immune dysregulation. OLP is classified as a potentially malignant lesion of the oral mucosa with a malignant transforming rate of 0–6.25%[7]. Molecular and epidemiological studies suggest that HPV infection in the upper respiratory tract may play a role in the pathogenesis of head and neck tumours[8]. The role of HPV in premalignant lesions has also been studied[9,10].

HPVs are epitheliotropic DNA viruses with more than 150 genotypes. HPV classification has been based on the degree of HPV DNA homology. HPV has been detected in various types of oral lesions, ranging from benign to malignant[11]. HPV-16 has increasingly been reported as being associated with potentially malignant lesions and with oral squamous cell carcinoma[12].

This high-risk HPV-16 produces two oncoproteins, E6 and E7, which are necessary for viral replication. The HPV E6 protein binds, and promotes the degradation of, the tumour-suppressor p53 by a ubiquitin-mediated pathway, diminishing the ability of the cell to undergo apoptosis. The HPV E7 protein binds, and degrades, the retinoblastoma protein (pRb), preventing it from inhibiting the transcription factor E2F, resulting in loss of cell-cycle control. The ultimate effect of the activity of the E6 and E7 proteins is dysregulated cell-cycle progression and HPV DNA replication in infected squamous epithelial cells, and eventual oncogenesis[13].

In 2000, Sand *et al.*[9] found the HPV genome in 27.3% of OLP lesions. In 2002, Oswald *et al.*[10] reported the presence of HPV-16 and HPV-18 in 9.4% of patients with OLP. In 2006, Giovannelli *et al.*[12] reported the presence of HPV-16, HPV-18, HPV-33 and HPV-35 in 22.4% patients with OLP. In 2010, Razavi *et al.*[14] found the HPV genome in 31% of OLP lesions and in 7.1% of controls. In 2011, Yildirim *et al.*[15] found the HPV-16 genome in 21% of OLP lesions. However, in the present study, a significant difference was observed between the case (70%) and the control (0%) groups regarding HPV-16 infection.

Based on this observation, it could be suggested that HPV-16 may play a role in the pathogenesis of OLP and thus could have potential benefit in the immunohistochemical detection of OLP.

In 2006, Giovannelli *et al.* reported that HPV infection can be affected by keratinisation, and that keratinised tissue is more resistant to HPV infection[12]. In the present study, HPV-16 was found in 85% of non-keratinised tissues (lip mucosa, mucosa of the floor of the mouth and buccal mucosa) and in 15% of keratinised tissues (tongue mucosa and gingiva). The increased rate of proliferation of non-keratinised tissue can make this tissue type more susceptible to HPV infection.

The *P*-value of Fisher's exact test in this study showed a statistically significant relationship between HPV-16 positivity and non-keratinised tissue but no statistical correlation between age, gender, lesion localisation and HPV-16 positivity.

## CONCLUSION

The prevalence of HPV-16-positive samples in this study was 70%, which is very high compared with other studies. The patients diagnosed with OLP in this study had no history of smoking, alcohol consumption or betel nut chewing habit; therefore, it is concluded that the HPV infections may play an important role in the pathogenesis of OLP. Taking into account the oncogenic potential of HPV-16, patients with OLP should be screened for the presence of this virus, and adequate long-term follow-up conducted of lesions that are found to be positive.

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## Conflict of interest

I declare that I do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

## Source of support

Nil.

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