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Research Article

Detection of Human Papillomavirus in Oral Lichen Planus Using Real Time PCR

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ABSTRACT

Background: Oral lichen planus (OLP) is a common chronic inflammatory mucocutaneous disease**.** It is possible that oral mucosal viral infections including HPV infection may have a causative role in OLP pathogenesis. The aim of the present study was to assess the prevalence of HPV DNA in tissue biopsies in patients with OLP and healthy controls.

Methods: This study was a case-control study. Fifty paraffinized specimens of previously diagnosed oral lichen planus and 30 paraffinized specimens of nonpathogenic mucosa were studied. real-time PCR used for detection of DNA HPV.The data were analyzed with SPSS software and $\chi 2$-test was used to find the possible relation between HPV infection and oral lichen planus.

Results: Seven out of 50 (14%) lichen planus samples and one out of 30(3.3%) controls were HPV positive. No significant correlation (P = 0.124) was observed between HPV infection and oral lichen planus**.**

Conclusion: In the current study, no significant relation was observed between HPV infection and OLP. Possibly, finding the effect of HPV in OLP lesions needs a larger study sample.

Keywords: Human papilloma virus,Oral Lichen Planus,real-time PCR

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory mucocutaneousdisease(1). OLP is a common disorder estimated to affect 0.5–2% of the general population and in a study from Iran, 0.5% of 1167 investigated Iranian textile workers had lichen planus(2,3). Oral lichen planus is adisease of the middle-aged and is more commonamong women(4). The buccal mucosa, tongue and gingiva are commonly affected, whereas palatallocalisation is uncommon. Lesions are typicallybilateral with a variety of clinicalpresentations,including reticular, popular, plaque-like, atrophic and ulcerative(5). OLP is generally considered to be an immunologically mediated process that histologically resembles a hypersensitivity reaction. It is characterized by anintense band-like T-cell infiltrate in the epithelium–connective tissue interface(6). The etiology and pathogenesis of OLP are unknown though several molecular hypotheses have been presented. The etiology of OLP involves the degeneration of the epithelial basal cell layer, induced by cell-mediated immunologic reactions.(1) Speculated causative factors such as stress, diabetes, hepatitis C, trauma and

hypersensitivity to drugs and metals have different degrees of support(7). Recently, viruses, such as human papilloma virus (HPV) and human herpes virus (HHV), have beenfound to play a role in the pathogenesis of OLP(4).Existing data suggest that these viruses may alter hostcell function by inducing the abnormal expression ofcellular proteins, leading to disease development. Thisindicates that oral mucosal viral infections may play arole in the pathogenesis of OLP(4). HPV is a member of papillomaviridea family, withno envelope and a diameter of 50–500 nm. Differenttypes of HPV are distinguished based on the degree ofnucleic acid sequence homology(8). Epidemiologic evidence suggests that HPV may be an independent risk factor for or pharyngeal cancer, being found three times more in many precancerous oral lesions and almost five times more in many or pharyngeal cancers as compared to normal oral mucosa(1). The aim of the present study was to assess the prevalence of HPV DNA in tissue biopsies in patients with OLP and healthy controls and to evaluate whether any clinical features(age, gender, residence,marital status and localisation) correlate with this virus.

MATERIALS AND METHODS

The current cross-sectional study included 50 formalin-fixed paraffin-embedded resection specimens with Oral lichen planushistopathologic diagnosis. Tissue specimens were collected from Department of Oral and Maxillofacial Pathology, School of Dentistry, affiliated to Babol University of Medical Sciences. The medical records of these subjects were reviewed in order to obtain demographic characteristics including age, gender,localization residence and marital status. All experiments were performed according to the relevant laws and guidelines in accordance with the ethical standards of the Declaration of Helsinki. This study was approved by the Ethical Committee of Babol University of Medical Sciences, and for all subjects, written informed consent was obtained

DNA Extraction

Ten µm thick tissue sections were deparaffinized according to a previously described procedure (9). DNA was extracted from each tissue sample, using DNA Extraction Mini Kit from Tissue (YektaTajhizAzma, Tehran, Iran) according to the manufacturer's instructions. The quality and quantity of purified DNA was determinedusing a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, USA). In addition, DNA integrity in each tissue sample was evaluated using human RNase P gene (RPP30) amplification based on a previously described procedure (10). Sterile micro centrifuge tubes containing only reaction mixtures were processed simultaneously with the tissue samples as a DNA extraction negative control.

HPV Detection by Real Time Polymerase chain reaction

HPV DNA detection was performed using the qualitative Real Time PCR with L1 General Primers(Table1) (GP5+ and GP6+) using a Rotor-Gene Q real-time PCR system (QIAGEN GmbH, Hilden,Germany). Real-time PCR was performed in a 25 µL reaction mixture containing 500 ng of extracted DNA, 12.5 µL SYBR Green qPCR Master Mix 2X (YektaTajhizAzma, Tehran, Iran) and 5pmol/µL of each primer. For amplification of HPV L1 region, the real-time PCR cycling conditions were as follows: 10min at 95˚C, followed by 40 cycles of 15 sec at 95˚C, 30sec at 48˚C and 30sec at 72˚C . To confirm amplification specificity melting curve analysis were carried out at temperatures between 65˚C and 95˚C, with temperature increasing at a rate of$0.5^{\circ}Cs^{-1}$

Table1.List of Primers used in this study

Target Gene	Primer Name	Sequences (5'-3')	Product Size
Human RNase P	RNP-F	5'-AGATTTGGACCTGCGAGCG-3'	65bp
Human RNase P	RNP-R	5'-GAGCGGCTGTCTCCACAAGT-3'	
HPV L1	GP5+	5'-TTTGTTACTGTGGTAGATACTAC-3'	150bp
HPV L1	GP6+	5'-GAAAAATAAACTGTAAATCATATTC-3'	

STATISTICAL ANALYSIS

Statistical analyses were done by SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). The $\chi 2$-test was utilized to assess associations between categorical variables. P value of ≤ 0.05 was considered to be statistically significant.

RESULTS

This study was performed on 50 samples of OLP and 30 OIF samples, In OLP group 12 (24%) were male and 38 (76%) were female. The mean age of the subjects was 47.06 ± 13.07 years. The mean age of men was 49.08 ± 15.4 and the mean age of women was 46.42 ± 12.4. In OLP group 5(10%) were single and 45(90%) were married, of which 100% (50 people) had urban life . The HPV was found in 7(14%) samples, which 4% were male and 10% were female. All HPV-infected people had urban living. The frequency of HPV in OLP is based on the demographic variables in Table 2.

Table2: The prevalence of HPV in OLP in terms of demographic variables

Variable	Categories	HPV+(%)	HPV-(%)	Total
Age	16-43	3(17.6)	14(82.3)	17
	44-71	4(12.1)	29(87.9)	33
Sex	Male	2(16.67)	10(83.33)	12
	Female	5(13.16)	33(86.84)	38
Marital status	Married	7(15.55)	38(84.45)	45
	Single	0(00.0)	5(100)	5
Residential Location	Urban	7(14)	43(86)	50
	Rural	0(00.0)	0(00.0)	0
City Location	Amol	0(00.0)	6(100)	6
	Babol	6(19.35)	25(80.65)	31
	Babolsar	0(00.0)	3(100)	3
	Ghaemshahr	0(00.0)	3(100)	3
	Sari	0(00.0)	4(100)	4
	Nur	0(00.0)	1(100)	1
	Freidunkenar	1(100)	0(00.0)	1
	Nowshahr	0(00.0)	1(100)	1

All biopsied specimens approved from OLP and OIF by the pathologist before doing experiment.The places in the mouth that biopsy performed, are listed in Table 3.

Table 3: Frequency of OLP in different oral regions in the study group and in the case of HPV

HPV+(cases)	Abundance	The number of patients	Lesion location
0	10%	5	Lebialmocousa
7	64%	32	Buccalmocousa
0	2%	1	Mandible buccal vestibule
0	2%	1	Mandibular interdental papila
0	2%	1	Tongue latral border
0	2%	1	Lingual lesion
0	10%	5	Gingiva
0	2%	1	Buccal vestibule
0	4%	2	Lower lip
0	2%	1	Upper lip

the most common site of OLP was buccalmocousa (64%). According to chi-square test, there was statistically significant correlation between different locations and OLP (p = 0.00). Additionally There were no statistically significant correlation between the presence of HPV and OLP(P=0.124)(Table 4).

Table4: Prevalence of human Papilloma virus in the group of patients with OLP and in the control group(no OLP)

Condition / HPV	Controls	Patients	P value	OR (CI)
Negative	29	43	0.124	0.212
Positive	1	7		

In the present study statistically significantcorrelation was found betweenage (P=0.005), gender (P=0.036), lesion localization (P=0.00),residence(P=0.00)andOLP.Therewas nosignificant difference between maritalstatusand OLP.

In OIF group 14(46.7%) were male and16 (53.3%) were female. The mean age of the subjects was 38.73 ± 13.22 years. The mean age of men was 37.86 ± 14.97and the mean age of women was 39.5 ± 11.94. In OIF group7(23.3%) were single and 23(76.7%) were married, which 13(43.3%) and 17(56.7%) had rural and urban life respectively . The HPV was found in 1 sample in OIF, which he has male and 46years old. Based on the results, HPV positive in the OLP (14%) was higher than the HPV positive in OIF (3.33%).

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%. 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. The role of HPV in premalignant lesions has also been studied. Human papillomaviruses 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(4). OLP affects 1.27% of the global population with prevalence varying according to the geographic location. Efforts to explore the correlation between HPV and OLP have mainly comprised epidemiological studies of various populations (1). In developing a screening procedure for oral precancer and cancer in Iran, we investigated HPV DNA in tissue specimens from the patients diagnosed with OLP. In the present study, No statistical relationship was observed between HPV infection and OLP .However, HPV was detected in 14% (7 of 50) of the OLP samples and 3.33%(1 of 30) of the normal controls (P = 0.124). There are profound variations in HPV prevalence among geographically different populations (11).

In 2009,Razavi et al. found the HPV genome in 31% of OLP lesions and in 7.1% of controls. In this study no significant correlation was observed between HPV infection and oral lichen planus(12). In two studies from the USA, Young and Miller found no relationship between HPV and OLP (13,14). In contrast, patients with OLP in European countries have been reported to have an HPV prevalence of 11.8–100%.(1).A study from Italy detected HPV DNA in 12 of 49 (25%) OLP lesions(15)High risk-HPV genotypes was found in three of seven (42%) cases of OLP in Germany and six of 22 (27%) in Sweden(1)In 2000, Sand et al. found the HPV genome in 27.3% of OLP lesions. In this study a significant association between HPV infection and oral lesions was demonstrated but the pathogenic influence of HPV infection remains unclear (16). In the study ofSahebjamiee et al OLP specimens were HPV‐positive in 11 of 40 (27.5%) cases. In this study, the prevalence of HPV DNA-positive samples in OLP patients with dysplasia (57%) was significantly higher than in OLP patients without dysplasia(15%)(1). In the other handin the study of latharta et ala statistical association was found between HPV presence and OLP(17). However, there are few current investigations of HPV as a risk factor for malignant transformation in the Iranian population.The variance in HPV DNA prevalence among these studies may be attributed to the differences in sensitivity of the applied molecular methods, sampling methodologies, patient characteristics and the types of studied specimens (e.g., saliva, oral mucosal scrapings or biopsy tissue(1). Furthermore in the present study significant statistical association was found between age, gender, lesion localization,and OLP. In disagreement with our finding ,Gichki et al was not found any association between age , sex and

patients outcome (18).In addition, in the study of Pol et al no statistical correlation was found between age, gender, lesion localization and OLP(4). These results are the first report from oral HPV status in the tissue of OLP patients from Northern Iran. However, further studies are needed to better understand the origin, pathogenesis of OLP.

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