Prevalence of Human Papilloma Virus in Oral lichen planus

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Abstract

Oral lichen planus (OLP) is a common chronic inflammatory and immune mediated disease. The diagnosis require clinical and histopathological examination to rule out possible other diseases.Viral infection has been hypothesized as a predisposing factor in the development of this disease. It is possible that oral mucosal viral infections including HPV infection may enroll as a causative agent in OLP pathogenesis. The study aimed to investigate the prevalence of human papilloma virus in oral lichen planus.The study was composed of thirty paraffinized samples of previously diagnosed oral lichen planus and thirty paraffinized samples of nonpathogenic mucosa were studied. In situ hybridizationwas used for detection of DNA HPV. The data were analyzed with SPSS software and chi square test was used to find the possible relation between HPV infection and oral lichen planus.Four out of 30 (13.40%) lichen planus samples and three out of 30 (10%) controls were HPV positive. No significant correlation (*P>0.05*) was observed between HPV infection and oral lichen planus.According to the findings we concluded there might be not relationship between human papilloma virus and incidence of oral lichen planus.

Keywords: Oral Lichen Planus Human papillomavirus, in situ Hybridization

Introduction

Oral lichen planus (OLP) is a chronic immunological mucosal disease (1). The etiology of OLP not well understoodinvolves the degeneration of the basal cell epithelial layer, induced by cell-mediated immunological reactions.The causative factors such as stress,trauma,hepatitis C anddiabetes have different degrees of support.Lichen planus affects abouttwo percent of the population (2). In spite of the disorder may occur in all age groups, the women are most commonly affectedtwice as often as men (3).

In contrast to cutaneous lichen planus, oral lichen planus lesions may persist for several years and tend to be difficult to treat, Atrophic and erosive lichen planus is associated with a risk of potential malignant transformation (3).

Human papillomaviruses are epitheliotropic viruses.The erosive and atrophied variants of OLP recordedthe highest prevalence of HPV DNAthan normal oral mucosa (4). The HPV viral protein increases the degradation of p53 (3). This leads to cell cycle dysregulation and eventually may lead to malignant transformation.Many studies, which included meta-analysis and review studies, suggest an important association between HPV and OLP (5).

In situ hybridization (ISH) is a type of hybridization has shown higher sensitivity that uses complementary DNA with different labelsto localize a specific DNA or RNA sequence in a portion or section of tissue. In situ hybridization (ISH), considered as a direct signal detection assay,it has the advantage of preserving the morphologic context of viral DNA signals;its application mainly in viral infectious diseases such as the typing ofhuman papillomavirus (HPV) (6).

Aim of study

The present study aimed to investigate the prevalence of HPVin tissue biopsies ofpatients with oral Lichen planus.

Materials and Methods

A total of 30 Iraqi patients with OLP will be enrolled in this case control study. Demographic and clinical data were collected, including patient's information concerning age, sex, site of the tumor, aswell as other information of occupation.Control group will be consisted of 30 healthy individualstheir age, gender matched with patients group.

Paraffin-embedded tissue blocks of both; study and control groups were collected. New sections were made from each of the paraffin embedded blocks which include the followings:

A. Four μm thick cross sections were made and fixed on ordinary slides to be stained by hematoxyline and eosin stain to confirm the diagnosis.

B. For the purpose of conducting in-situ hybridization procedure to detect the HPV other 4 μm thick sections were made on positively charged slides.

In situ hybridization is a method for localizing and detecting specific DNA or RNA sequences in morphologically preserved tissue sections. Briefly, the method involved deproteinization of fixed tissue sections mounted on slides; hybridization of a biotinylated probe to the target sequence, the hybridized probe is then detected by addition of a streptavidin – alkaline phosphatase (streptavidin-AP) conjugate (DNA probe hybridization/Detection system).

Upon addition of the single component BCIP/NBT solution (substrate) which is 5-brom-4 chloro-3 indolyl phosphate/Nitro blue tetrazolium, an intense blue signal appears at the specific site of the hybridized probe. This streptavidin-AP conjugate directly linked to the biotinylated probe provides a rapid and highly sensitive

detection method.Positive control was included for each run of in situ hybridization. The positive control was obtained by replacing the probe with housekeeping gene probe.To assess the statistical significance association of HPV and existence OLP, the Chi square test will apply. P values were considered statistically significant if P<0.05.

Results

The study groups consisted of 60 patients 30 with OLP and 30 individuals were control group. 60% of these patients were males and 40% were females in affected group. The mean age and HPV infection were 43y, 13.4% of affected (Table 1). Of thirty volunteers of control group 50% of each males and females mean age 45y only 10% was with HPV infection. There was no significant difference between males &females regarding HPV signals (P>0.05) as well, although males were 4 cases (13.4%) higher than females who were (10%) (Table 3-1).

Table(3-1):Sex distribution of positive HPV.

	Lichen planus		Control group	
Gender	Males	Females	Males	Females
	18 (60%)	12 (40%)	15 (50%)	15 (50%)
HPV infection	2 (6.7 %)	2 (6.7%)	2 (6.7 %)	1 (3.3%)
Mean age	43 Y		45 Y	

**Chi-square=1.623 P=0.084 P>0.05 Non significant*

The site of involvement showed the buccal mucosa was predominant site of

disease (23 case) while the gingiva and tongue showed (5, 2 cases) respectively (Figure 3-1).OLP showed positive ISH signals for HPVin (4) cases all were detected in nonkeratinized buccal mucosa. There was no significant correlation between the site&HPV positivity with P-value 0.43 (Table 3-2).

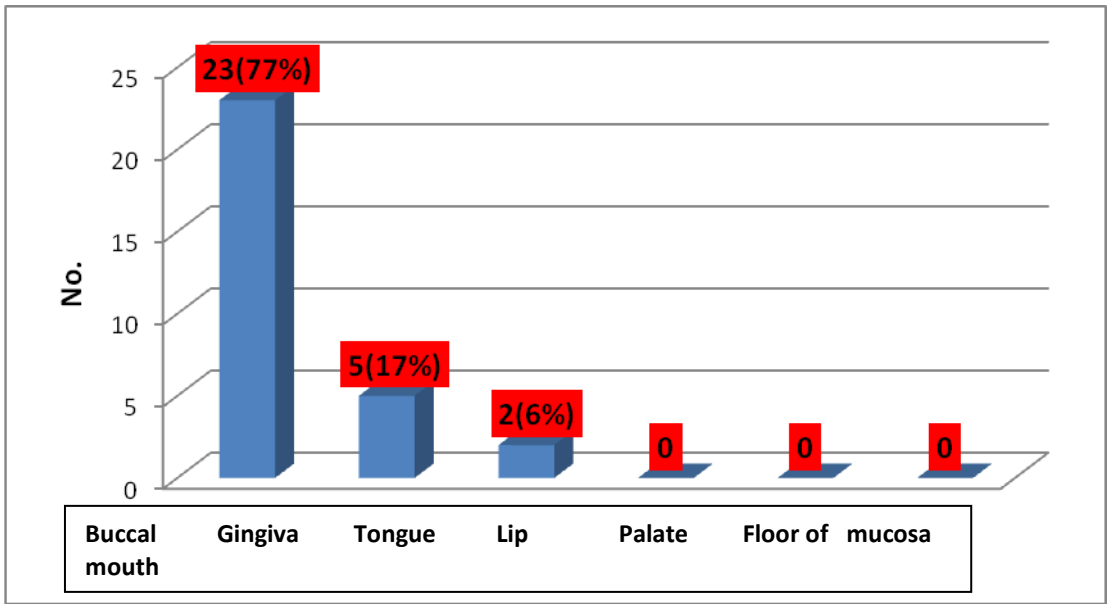

Figure (3-1). Distribution of the patients according to the localization of the OLP.

Table (3- 2): Site distribution of HPV-16 positive cases.

Site	Total No. %	HPV+Ve No. %
Buccal mucosa	23(77%)	4 (17%)
Gingiva	5(17%)	0
Tongue	2(6%)	0
Lip	0	0
Palate	0	0
Floor of mouth	0	0

P-value =0.43

Discussion

In this study most sensitive molecular method for in situ viral detection was applied.Ming*et al*. evaluated (ISH) for detection of HPVs compared to PCR assay showed that both methods detected HPV (DNA) without significant difference (Ming, 2008).

Santosh (2012), showed the buccal mucosa being themost commonly involved site.The finding consistent with our study the reticular form with bilateral posterior buccal mucosa was predominant site and account (77%) respectively. Others previous studies conflicts our results; Basma*et al* (2015) in Egyptian samples of patients similar results were also observed in study done by Carrozzo*et al*.(1996).

Great efforts in exploring the relation between OLP and HPV have mainly focused on many epidemiological studies of different populations. It is noticeable that there have beenwide variations in the results found among different geographicaldistributionofpopulations(11).

Inthepresent study, HPV DNA was found in four casesoforal lesions (13.4%) and three cases in control group (10%) without significant difference in both groups;Syrjänen*et al*(2011), done systematic review shows a statistically significant difference (11% versus 23%) between HPV associated with OLP lesions and HPV found in normal tissue. This difference might explain the regional differences in the distribution of risk factors other than HPV infection; The risk of oral HPV infection such as**history of sexually transmitted disease**,sexual behavior, HIV infection, and severity of immunosuppressionand difference in the accuracy and sensitivity methods of detection,sampling methodologies, the characteristic of patient and the types of studied specimens (e.g., biopsy tissue, saliva, oral mucosal scrapings) (Kreimer, 2010). Many Studies in the USA used in situ hybridization on paraffin embedded tissues did not find any relationship between HPV and OLP and these results coincided with our results.In contrast, patients with OLP in European countries have been reported to have high HPV

prevalence; in study from Italy the HPV found in 12 of 49 (25%). High risk-HPV was found in seven cases of sixteen OLP (43.7%) in Germany (12).

It has been foundthat HPVs infect the basal cell epithelial layer and in order for HPVsto reach and infect epithelial basal cells (13); viral particles must enter throughthe epithelium that was broken mucosal injury, and subsequently reach the basal cell layer.In present study the four cases with HPV infection (13.4%) were seen in buccal mucosa the reason may be the region lined by non-keratinizing epithelium and these regionof oral cavity more liable for injury and viral infection than keratinizing epithelium.Nishimura *et al*.(2004) also reported that HPV infection was frequently observed in the oral mucosa of patients wearing dentures, suggesting that chronic stimulation of the mucosa by the denture and subsequent erosion of the mucosa are the reason for the frequent infection (15).Many studiesfound high percentage of HPVcases in non-keratinizing oral mucosa, Giovannelli (2006) found that HPV infection can be affected by keratinization, so that the keratinized mucosa is more resistant to HPV infection. The proliferation rate of virus increase in non-keratinized tissue can make it more susceptible to HPV infection.

Based on the above data, it is necessary to furtherinvestigate the association between HPV and OLPby using other screening methods.

5. Conclusion

OLP is autoimmune disease. Infectious agents might be proposed as one of the causes of OLP. Our study showed a low prevalence of HPV infection in OLP lesions. The study concludes that HPV may be not play an actual role in patients with OLP.

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