Immunoflourescent assessment of Herpes Simplex Virus (HSV) type 1 in oral lichen planus

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ABSTRACT

Background: Oral lichen planus is one of the most common dermatological diseases presenting in the oral cavity. Hence, viral infection of the oral mucosa may be involved in the pathogenesis of oral lichen planus, Taking in to consideration the oncogenic potential of HSV-1, this study aimed to assess the presence of Herpes Simplex Virus type one by direct immunoflourescent in oral lichen planus. This study aimed to assess the presence of HSV type1 by direct immunofluorescent in histopathologically diagnosed OLP

Material and Method: Twenty formalin fixed embedded tissue blocks of oral lichen planus with 2 Positive control cases were taken from patients having infection with herpes labialis, US Biological herpes simplex virus-1 Glycoprotein C was used for detection of HSV-1 Ag by direct immunofluorescence assay

Results: One case of oral lichen planus showed positivity of HSV 1 with a non- statistical significance.

Conclusion: The present study couldn't find any correlation between HSV-1 positivity with clinical and histopathological features of OLP.

Key words: OLP, HSV, immunofloresant assay. **(J Bagh Coll Dentistry 2014; 26(1):103-107).**

INTRODUCTION

Lichen planus (LP) is a chronic mucocutaneous disease that affects the skin and the oral mucosa with unknown aetiology. Oral lichen planus (OLP) affects women more than men, and occurs predominantly in adults over 40, although younger adults and children may be affected [1].

OLP may arise anywhere in the oral cavity. The buccal mucosa, tongue and gingiva are commonly affected sites, whereas palatal localization is uncommon [2].

Lesions are typically bilateral and often appear as a mixture of clinical subtypes. Oral lichen planus (OLP) may present reticular, bullous or erosive form and occurs more frequently than the cutaneous form and tends to be more persistent and more resistant to treatment [2, 3].

Oral Lichen planus is probably of multifactorial origin, possibly induced by drugs or dental materials, psychological factors, infective agents, and often idiopathic. The etiopathogenesis appears to be complex, with interactions between genetic, environmental, lifestyle factors, and interesting new associations, such as with liver disease, have emerged [4].

Viral infections have recently been linked with OLP. Herpes Simplex virus-1 (HSV-1),

Cytomegalovirus (CMV), Human Herpes virus-6 (HHV-6) [5,6], Epstein- Barr virus (EBV) [5,7,8], Human Papilloma virus (HPV) [7] and Hepatitis C virus (HCV) [5,6] are virus types that have been studied in the etiopathogenesis of OLP.

Hence, viral infection of the oral mucosa may be involved in the pathogenesis of OLP.

Many DNA viruses are known to infect the oral and peri-oral mucosa. Herpes simplex virus (HSV: human herpesviruses types 1 and 2) causes an acute gingivostomatitis, herpes labialis (cold sores) and recurrent intra-oral herpes [9].

The specific demonstration of herpes simplex virus in smears from the oral lesion may be accomplished by immunofluorescent staining in which fluorescein-labeled antibodies to the virus react with viral antigen present in the infected epithelial cells. The infected cells showing characteristic yellow-green fluorescence are visualized by ultraviolet microscopy. This method has been quite successful in the specific diagnosis of oral lesions with herpes simplex virus [10].

The aim of the present study was to assess the presence of HSV type1by direct immunofluorescent in histologically diagnosed OLP cases and to correlate its presence with clinical variant, histopathological and demographic features.

MATERIALS AND METHODS

The study was conducted on formalin fixed paraffin embedded tissue specimens of 20 oral lichen planus. The cases were classified according to age, sex, localization and the histopathological type regarding (type of keratinization, degeneration of basal keratinocytes, inflammation intensity and thickness of epithelium). Two normal oral mucosal tissues were used as control group with two smears taking from patient having herpes labialis as positive control. US Biological herpes simplex virus-1 Glycoprotein C (Code No. H2033-08A) was used for detection of HSV-1 Ag by direct immunofluorescence assay according to manufacturer's protocol.

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In this study, Positive control was used Consisted of two patients having infection with herpes labialis, and a swab was taken from the site of infection put on a charged slide and the same procedure for IF was done. Also Two slides of Negative control were prepared as the procedure of IF to the whole samples but one slide was prepared by putting sample without using the substrate, but instead of that we used the bovine serum albumin, while the other slide was prepared by using distilled water instead of the sample.

Statistical analyses were performed using Chi-Square test.

RESULTS

A total of 20 cases of oral lichen planus were utilized in the study. Clinical and immunoflourescent analysis of HSV type 1 were given in table 1.

Out of 20 patients studied 10 (48%) were females and 11 (52%) were males.15 (75%) patients had classical white lesions mostly in the buccal mucosa followed by tongue and lower lip 3 (15%) then one case (5%) for each lower lip and gingiva.

Most of the lesions were described as reticular forms 12 (60%), followed by plaque 4 (20%), 3(15%) erosive lesions and 1 case (5%) was annular.

Table 1: Relation between the genders and the presence of HSV1

Genders		Presence of HSV1		Total	Relation			
		Negative	Positive		X^2	Continuity correction	d.f.	p-value
Females	No.	9	0	9	0.861	0	1	1 (NS)
	%	47.4%	0%	45%				
Males	No.	10	1	11				
	%	52.6%	100%	55%				
Total	No.	19	1	20				
	%	100%	100%	100%				

Table 2: Relation between the sites and the presence of HSV1

Site		Presence of HSV1		Total	Relation			
		Negative	Positive		X^2	Likelihood ratio	d.f.	p-value
Buccal mucosa	No.	14	1	15	0.351	0.591	3	0.898 (NS)
	%	73.7%	100%	75%				
Gingiva	No.	1	0	1				
	%	5.3%	0%	5%				
Lower lip	No.	1	0	1				
	%	5.3%	0%	5%				
Tongue & Lower lip	No.	3	0	3				
	%	15.8%	0%	15%				
Total	No.	19	1	20				
	%	100%	100%	100%				

Table 3: Relation between the age and the presence of HSV1

Age		Presence of HSV1		Total	Relation			
		Negative	Positive		X^2	Likelihood ratio	d.f.	p-value
20-29	No.	3	0	3	1.955	2.199	5	0.821 (NS)
	%	15.8%	0%	15%				
30-39	No.	5	0	5				
	%	26.3%	0%	25%				
40-49	No.	3	0	3				
	%	15.8%	0%	15%				
50-59	No.	6	1	7				
	%	31.6%	100%	35%				
60-69	No.	1	0	1				
	%	5.3%	0%	5%				
70-79	No.	1	0	1				
	%	5.3%	0%	5%				
Total	No.	19	1	20				
	%	100%	100%	100%				

Table 4: Relation between the clinical types and the presence of HSV1

Clinical types		Presence of HSV1		Total	Relation			
		Negative	Positive		X^2	Likelihood ratio	d.f.	p-value
Annular	No.	1	0	1	0.702	1.057	3	0.788 (NS)
	%	5.3%	0%	5%				
Erosive	No.	3	0	3				
	%	15.8%	0%	15%				
Plaque	No.	4	0	4				
	%	21.1%	0%	20%				
Reticular	No.	11	1	12				
	%	57.9%	100%	60%				
Total	No.	19	1	20				
	%	100%	100%	100%				

As far as histopathological features the results of this study showed that sub epithelial mononuclear infiltration, basal cell degeneration, parakeratinization acanthosis and aprominent granular layer were consistent finding in OLP figure 1(HE).

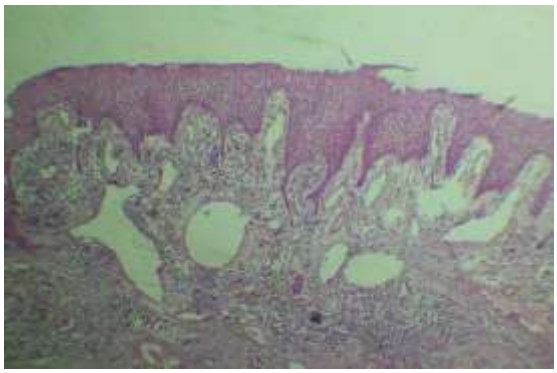

Figure 1: OLP 20X (HE)

The number of OLP cases that were positive for HSV was only one case (5%) and it was not statistically significant. (Fig. 2,3,4).

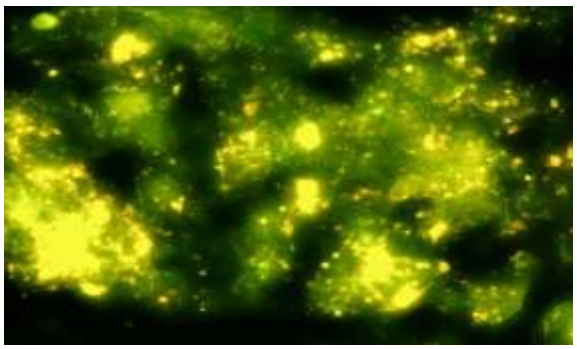

Figure 2: Positive Control Smear 20 X

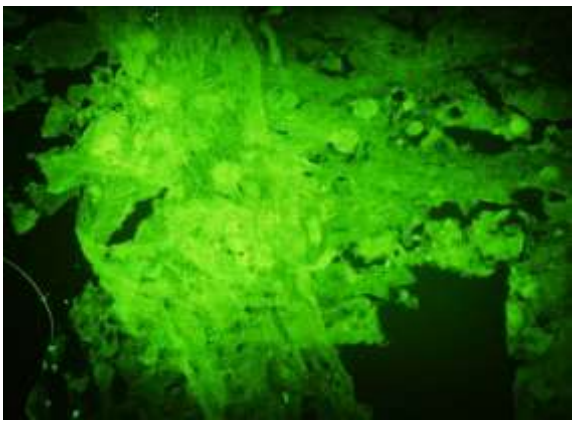

Figure 3: Negative IF picture in patient with O.L.P.

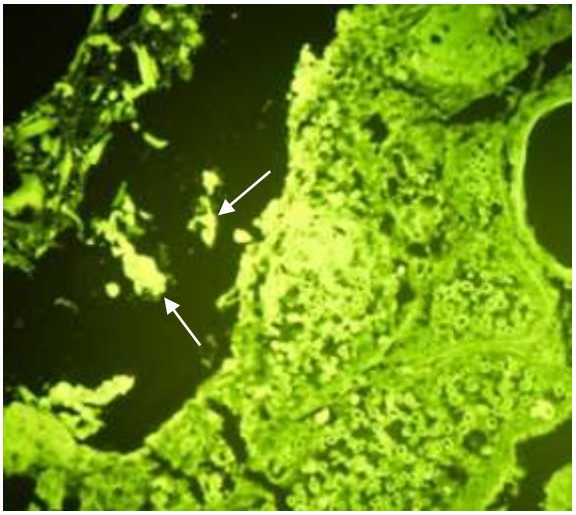

Figure 4: IF picture in patient with OLP 20 X

There was no any correlation between HSV positivity and age, sex, localization, clinical type and histopathological features.

DISCUSSION

The viruses play an important role in oral ulcerations and may therefore elicit activating effects upon immune response [11].

HSV-1 is adapted best and performs more efficiently in the oral, facial, and ocular areas [12]. Hence, viral infection of the oral mucosa may be involved in the pathogenesis of OLP. Many DNA viruses are known to infect the oral and peri-oral mucosa, HSV-1 was one of these oncogenic potential viruses [13].

HSV (human herpes viruses types 1 causes an acute gingivostomatitis, herpes labialis (cold sores) and recurrent intra-oral herpes. HSV-1 infections are common vesicular lesions of the skin and oral mucosa. HSV-1 has occasionally been found in the OLP, mainly in the erosive lesions in small series [13], however in the present study 1 of 20 OLP cases (5%) was positive for HSV-1 in which the infected cells swell to a large size leading to ballooning degeneration (fig2) and was not significant statistically and this result was compatible with other studies as Cox et al. [14], which reported HSV-1positivity in 4 cases [14], while De Vries et al. and Oflatharta et al. could not detect any HSV-1 DNA in OLP [15,16].

They all have concluded that HSV-1 virus has no causative role in the etiopathogenesis of OLP. The result of the present study couldn't find any correlation between HSV-1 positivity and clinical and histopathological features.

The low percentage of HSV-1presence in lesional OLP does not imply a causative relation between the two. The explanation of the presence of HSV-1virus in OLP could be secondary to a locally altered immune response or to a symptomatic shedding which defined as having HSV present without clinical lesions. Shedding often occurs at mucosal sites in the eyes, mouth, and genitalia [17]. Past estimates state that 5% of individuals demonstrate asymptomatic HSV shedding in the oral cavity, but detection methods have improved and sampling frequencies increased [17].

The shedding of HSV-1 in the oral cavity tends to be frequent and episodic. The inter-individual rates of viral shedding vary widely. Both seropositive and seronegative individuals demonstrate asymptomatic shedding. Factors that effect shedding include patient age, recent orofacial trauma, and inflammation. Most patients experience shedding for a limited time, generally 1to 3 days, but oral trauma or inflammation can prolong the episode. Infected saliva is a possible source of transmission of the virus. Recent data indicate that healthy individuals shed HSV-1 asymptomatically in the oral cavity for 1 to 2 days for an average of 13 days each month [17]

The examination of OLP specimens for other oncogenic viruses is certainly important and needed for further large sample studies.

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