Assessment of the Immunohistochemical expression of EBV in oral lichen planus

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ABSTRACT

Background: Oral lichen planus (OLP) is a chronic immunologic disease. The etiology of OLP is unknown, viral antigens (for example EBV) have been proposed as etiologic agents. OLP may get transformation to malignancy so research on the presence of these in OLP lesions seems to be necessary. The aim of this study was to evaluate EBV expression immunohistochemically in OLP.

Materials and Methods: Tissue specimens of 30 formalin fixed, paraffin-embedded tissue Blocks histologically diagnosed oral lichen planus was performed to evaluate EBV expression.

Results: Expression of EBV was detected in epithelium of (46.6%) in the study samples in (OLP). no statistically significant correlation was found with clinical parameters except for a significantly higher expression in females.

Conclusions: Epstein Barr viruses were present in considerable amounts in oral lichen planus. Taking into account the potential of viruses in OLP proving or disapproving or etiological role of viruses in OLP is continuously need to be examined in further studies.

Key words: EBV, lichen planus. (J Bagh Coll Dentistry 2015; 27(2):98-100).

INTRODUCTION

Oral Lichen planus (OLP) is a chronic mucocutaneous T-cell mediated immunoinflammatory disease which was first described by Erasmus Wilson (1869). Oral, genital, and some skin types of LP may predispose to the development of squamous cell carcinoma [(1)]. It is estimated to affect 0.5% to 2% of the general population [(2)], the most commonly affected peoples are middle- aged adults of both sexes with slight predilection for women, although it may occur at any age [(3)]

OLP may arise anywhere in the oral cavity. The buccal mucosa, tongue and gingiva are commonly affected sites, whereas palatal localization is uncommon. The lesions are usually symmetrical, bilateral or may be multiple. It has a variety of clinical presentations, including reticular, papular, plaque-like, atrophic, and ulcerative forms. [(4)]

The etiology of OLP is unknown. An alteration in the basal keratinocytes by certain stimuli that induce humeral and cell mediated immune response has been postulated as a mechanism [(5)].

Many variations in the clinical presentation of cutaneous Lichen Planus have been described [(5)]. Oral lesions are reported to have distinct clinical and histological features and characteristic distribution [(3)].

The histopathological features of OLP include liquefaction of the basal cell layer accompanied by apoptosis of the keratinocytes, a dense band-like lymphatic infiltrate between the epithelium and connective tissue, focal areas of hyper-keratinized epithelium (which give rise to the clinically apparent Wickham's striea) and occasional areas of atrophic epithelium, where the rete pegs may be shortened and pointed.[(6)]

Exogenous agents may also alter keratinocyte antigen expression. The response of these specific CD8+T cells is similar to what occurs during a viral infection where a virus can act as a cytoplasmic antigen or induce the expression of host cell proteins, resulting in an altered host cell protein profile [(8)].

Therefore, it is of interest to investigate the possibility of viral involvement in the pathogenesis of OLP. The Epstein-Barr virus, a member of the human. [(9)]

Approximately 90% of adults have demonstrable EBV antibodies. In-vivo, the infection is restricted to 2 target cells, the oronasopharyngealor the salivary gland epithelium and B-cell lymphocytes. EBV has the ability to establish a latent infection, which means a silent state of viral infection, characterized by a low expression of viral genes and minimal cytopathic effects or production of infectious virus. EBV is associated with infectious mononucleosis and oral hairy leukoplakia and with Burkitt lymphoma and nasopharyngeal carcinoma. [(10)].

MATERIALS AND METHODS

Tissue specimens

Thirteen tissue samples of paraffin embedded blocks histologically verified as oral lichen planus were randomly chosen from the archives of oral pathology department, College of Dentistry, Baghdad University.

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Immunohistochemical staining

Five-micrometer thick sections of formalin fixed and paraffin embedded biopsy samples were processed by the avidin-biotin-peroxidase complex (ABC) method. Deparaffinization and rehydratation of the sections were followed by the blocking of endogenous peroxidise activity with incubating the sections in 3 % H_2O_2 for 10

minutes. After rinsing with phosphate buffered saline (PBS, pH:7.0), Non specific binding was reduced with protein blocking serum for 10 minutes. Sections were incubated with Herpes Simplex virus type 1 primary antibody(HSV I polyclonal antibody) ,

Epstein Barr virus primary antibody (EBV/LMP1 monoclonal antibody at room temperature for 60 minutes. After rinsing with PBS, the slides were incubated with biotinylated secondary antibody for 30 minutes. The sections were washed with PBS and then the slides were incubated with label (streptavidin peroxidise, Lab Vision) for 30 minutes. .then used chromogen for visualization of the antibody binding. Finally, the sections were counter stained with Mayer's haematoxylin, cleared and mounted.

RESULTS

Evaluation of EBV Immunohistochemical expression

Immunostaining of EBV was detected as a brown staining in the cytoplasim of cells, in OLP cases positive IHC expression was found in 14 cases (46.66%).fig (1)

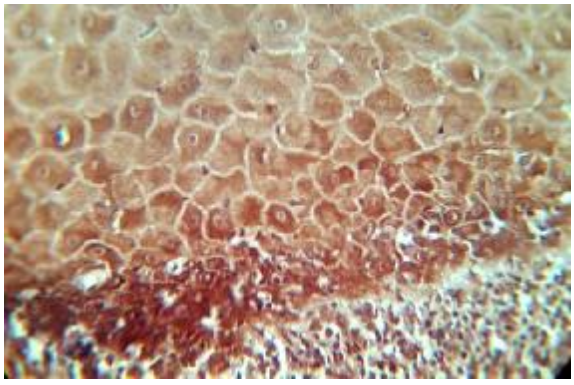

Figure 1: Positive brown cytoplasim immunostaining of EBV OLP (40X).

Three cases showed nuclear expression and were not counted as positive as recommended by the antibody manufacturer's data sheet. As shown in fig (2).

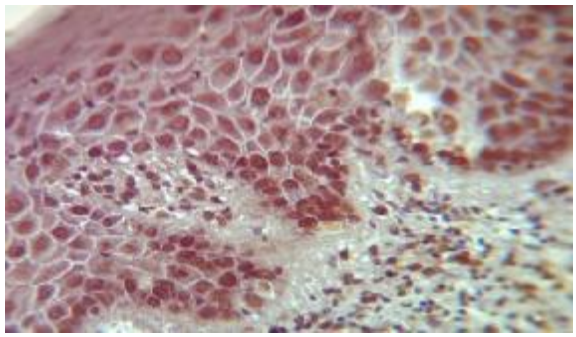

Figure 2: Positive brown nuclear immunostaining of EBV in OLP (40X).

According to spearman correlation and person correlation test , the results of this study in OLP showed statistically non - significant correlation regarding EBV expression in relation to the age (p-value =0.098) for person, (p-value =0.087) for spearman, and to the clinical presentation (p-value=0.127) for person,(p-value=0.174) for spearman statically non-significant.

While for sex statistically significant correlation (p-value=0.039) was showed as clarified in table (1.2.3).

Table 1: EBV expression as related to age

Age groups	Score 0	Score 1	Score 2	Score 3	Score 4
20-29	1	0	0	1	1
30-39	3	0	0	0	2
40-49	5	0	1	2	1
50-59	5	1	0	0	2
60	2	0	0	3	0
Total	16	1	1	6	6
Pearson correlation=0.654, p-value=0.098* NS Spearman correlation=0.662, p-value=0.087* NS					

Table 2: EBV positivity as related to sex

Gender	Score0	Score1	Score2	Score3	Score4
Male	7	0	1	0	0
Female	9	1	0	6	6
Total	16	1	1	6	6
Pearson correlation=0.478, p-value=0.039 *S Spearman correlation=0.462, p-value=0.028* S					

Table 3: EBV positivity as related to type.

Type	Score 0	Score 1	Score 2	Score 3	Score 4
Reticular	10	1	0	6	5
Erosive	6	0	1	0	1
Total	16	1	1	6	6
Person correlation=0.234, p-value=0.127* NS Spearman correlation=0.334, p-value=0.174*NS					

DISCUSSION

EBV has the ability to establish a latent infection, which mean a silent state of viral infection, characterized by a low expression of viral genes and minimal cytopathic effects or production of infectious virus, The association between EBV and premalignant and malignant disorders has been studied for the oral region. Some authors consider OLP to be premalignant lesions, but the premalignant potential of OLP remains controversial [10]. Regarding the possible premalignant potential of OLP, in any cases, the premalignant potential of OLP can not be ruled out.

In the present study the number of OLP cases that were positive for EBV was 14 (46%). High EBV prevalence in OLP in some studies [9,10] might be due to a decrease in the immune defense, locally or generally. Studies on immune-compromised patients seem to support this theory, because they show a higher prevalence of EBV, even in clinically normal oral mucosa [11].This study show higher positivity than Yildirim study in 2011

In this study show 3 cases with nuclear positivity, were not counted as positive as recommended by the antibody manufacturer's data sheet, this expression attributed to EBV LMP1 localization ,LMP1expressing show three patterns; aggregated patches in the membrane ,diffused expression in the nuclei ,and mixture of these three form [8], these results show that LMP1 has distinct plasma membrane and intracellular localization in different EBV- positive cell lines and that the heterogeneous distribution an inheretant feature [11].

Observed specific EBV in some OLP specimens suggested that EBV may be involved in the pathogenesis of some oral lesions. These high variability and inconsistency probably reflect geographical difference as well as differences in methodological sensitivity [12].

Horiuch et al 1995 shows that EBV could be one of the normal flora component of their mouth and higher prevalence of EBV in their country. Three theories for the presence of EBV in oral premalignant and malignant lesions have proposed: [i] EBV infection may be involved in the carcinogenesis of oral squamous cell epithelium; [ii] EBV easily infects squamous cell carcinoma cells [iii] EBV exists in cancer cells as a passenger. [13]

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الخلاصة

الخلفية: يعد الحزاز الفموي المنبسط من احد الامراض المناعية المزمنة غير معروفة الاسباب. و يفترض فايروس ال EBV كأحد الاسباب المرضية . تهدف الدراسة الى تقييم الظهور النسيج المناعي لفايروس EBV في عينات الحزاز الفموي المنبسط.

طرق البحث: تم تقييم الظهور الكيميائي النسيجي المناعي لفايروس EBV عن طريق الاجسام المضادة في ثلاثين عينة من الحزاز الفموي المنبسط محفوظة في الفورمالين ومغمورة بشمع البارافين.

النتائج: اظهرت الدراسة وجود فايروس EBV في 46,6% من الحالات المدروسة. لم يتم ايجاد اي علاقة لظهور الفايروس مع المتغيرات السريرية باستثناء وجوده بشكل اكبر في الاناث وبدلالة معنوية.

الاستنتاجات: يتواجد فايروس EBV بشكل ملحوظ في الحزاز الفموي المنبسط. من الممكن اجراء دراسات اخرى لتأكيد دور الفايروس في نشأة المرض.