Original Study

EXPRESSION OF JUNB IN ORAL LICHEN PLANUS : A PRELIMINARY STUDY

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ABSTRACT

Aim: Lichen planus is a relatively common and chronic skin disease that affects the mucosa, too. The present study conducted to compare the expression of JunB marker in oral lichen planus and normal mucosa.

Materials & Method: In this experimental study, selected samples with oral lichen planus and normal mucosa were evaluated. Then, to study the expression of JunB, immunohistochemistry staining was performed according to the factory's order. The average percentage of JunB expression as well as the score for each sample was evaluated and recorded and analyzed by Mann-U-Whitney test.

Results: Of 55 samples investigated in this study, including 35 oral lichen planus (OLP) of Erosive type and 20 normal mucosal samples, average of JunB expression percentage was 41.8 $\pm$ 27% in OLP samples and 22 $\pm$ 8 % in normal mucosal samples. According to T-test, the percentage of expression of JunB in OLP and normal mucosa has a significant difference. ($p = 0.006$) Out of 35 existing OLP samples, 1 sample is score0, 22 samples are score1, 8 samples were score 2 and 4 samples were score 3. Of 20 normal mucosal samples, 1 sample had score 0, and 19 samples had score 1. Accordingly, according to Mann-U Whitney test, there was a significant difference in expression of JunB in OLP samples. ($p = 0.005$)

Conclusion: In this study, there was a significant statistical difference in the percentage of JunB expression and also related score between OLP samples and normal mucosa; as the percentage of JunB expression and its score was higher in OLP than the normal mucosa.

Key words: Angiogenesis, IHC, JunB, Oral Lichen Planus.

Introduction

Lichen planus is a relatively common and chronic skin disease that affects the oral mucosa, skin, genital mucosa and nails. It affects about 1% to 2% of the adult population but despite the extensive study, pathogenicity of oral lichen planus is still unknown.[1] The strange name of this disorder was introduced by an English physician, Erusmus Wilson, who first described the disease in 1986.[1] When the first case was reported in 1910, several studies were proposed that patients with lichen planus are at risk for cancer.[2] In some studies, the risk of malignancy of lichen planus in the 10-year-old follow up was reported to be 1.2% - 2.3%.[3] Since there are reports of increased risk of oral cancer in patients with OLP, these lesions are classified as lesions with oral malignant potential.[4,5] OLP is clinically appeared in several forms of Reticuler, plaque erosive, Atrophic, Papuler and Bolus, the most common form of which is Reticuler and about the types of erosive and atrophy, the possibility of dysplastic changes and becoming malignant (squamous cell carcinoma) is raised.[6,7] In various studies, depending on the patient's follow-up period, the percentage of malignancy has been reported between 6.5% and 65%.[8,9]

On the other hand, recent reports have shown that the density of blood vessels of lesions has effect in their biological behavior[10] to the extent that new therapies are based on reducing the vascular density of the lesions. Evaluation of Vascular density is made by various methods such as staining Immunohistochemistry and the occurrence of various vascular markers. In this regard, JUNB is known to be a regulator of VEGF, thereby affecting vascular proliferation and tissue angiogenesis.[11] JUNB is a member of the family of the activator protein AP-1 transcription factor and is known as a vital inflammatory regulator in fibroblasts and T lymphocytes.[12,13] A quantitative analysis of PCR has shown that JUNB of multiple genes regulates tumor invasion and angiogenesis, such as MMP2, MMP9 (matrix metalloproteinase), and CCL2 (ligand-2 (chemokine (cc motif))).

In addition, JUNB reduction significantly prevents the growth of tumor and the density of blood vessels in the xenograft tumor,[14] and the lack of JUNB in different types of cells, despite the stabilized HIF (hypoxia iducible factor), leads to a very poor expression of the induction of VEGF hypoxia. Therefore, JUNB is defined as an independent VEGF transcription regulator.[11]

Also, the effect of retardation and delay and fetal death was also observed due to the JunB's inability in lack of vascular connection with maternal blood flow through extra-embryonic tissues.[15]

So far, the occurrence of JUNB and its effect on biological agents have been studied in several pathological lesions, such as psoriasis[16] and melanoma and lung carcinoma, which have raised contradicted results between the occurrence of JUNB and more aggressive biologic agents in these lesions.[17]

Based on our knowledge of available information sources, the occurrence of this marker has not been studied in oral lichen planus. Therefore, the present study aimed to compare the expression of JUNB marker in oral lichen planus and normal mucosa.

Materials & Method

Paraffin blocks related to OLP samples referred to Oral Pathology Department, Dental branch of Islamic Azad University during 2006-2018 with definitive diagnosis of erosive lichen planus was performed based on patients' clinical records and modified WHO classification.[18]

Subjects with inadequate tissue and unsuitable fixation and bleeding were excluded from the study. These samples were examined by mouth pathologists and after re-approving entered the study.

Also, a number of normal mucosa samples that did not have pathologic lesion and were isolated for non-pathologic reasons such as surgical extraction of wisdom teeth were also included in the study to compare the expression of this marker. This is notable to mention that both case and control samples were matched through their sex and age.

In this experimental study, 5 micron sections were prepared from blocks of selected samples including OLP and normal mucosa of for hematoxylin-eosin staining. After observing the hematoxylin-eosin slides by oral pathologists, slides that have adequate tissue and fixation were selected. Samples with high bleeding and swelling and inadequate tissue related to recurrence of odontogenic lesions were excluded.

Subsequently, for the study of JUNB expression, the immunohistochemical staining method was performed using the DAKO Cham Mate horsedish peroxidase system and the DAKO DAB substrate system according to instruction order.[19]

In brief, the sections of deparaffinazed tissue were initially influenced by H_2O_2 for 10 minutes to inhibit endogenous peroxidase, and then in 700 watts for 18 minutes in a sodium citrate solution buffered were microwaved for 30 min (pH = 6). After washing the sections in a culture medium with monoclonal antibody against JUNB were placed at room temperature for one hour in the final antibody diluted in a serum blocking solution at a concentration of 2 mg / ml.

After further placement in the medium with the biotinylated antibody network and streptownin-labeled by peroxidase were continued by DAB substrate solution for 10 minutes. To evaluate the antibody response of JUNB, C11), the paraffin sections of colon adenocarcinoma, keratosis and normal lymph nodes (as positive control) and an immunoglobulin antibody of inhuman reaction of the rabbit were used as a negative control group.[20] The evaluation of the JUNB expression is as follows:

The expression of JUNB in less than 5% of the tumors of cells (score 0), in 5 to 50% (score 1), more than 50% to 90% (score 2) and more than 90% to 100% (score 3).[20]

Then, the information was recorded in the information form and used for statistical analysis. In order to compare the expression of JunB marker in two groups, independent T-test and to compare the expression score of JunB in two groups, Mann- U Whitney were used.

Figures 1-4 represent JunB expression in microscopic sections of OLP samples and normal mucosa.

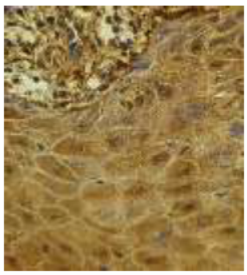

Figure 1: Expression of JunB (Score 3) in OLP by x400 magnification

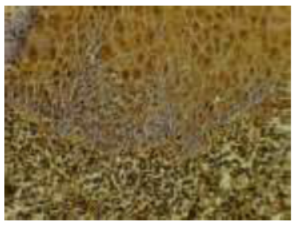

Figure 2: Expression of JunB (Score 2) in OLP by x200 magnification

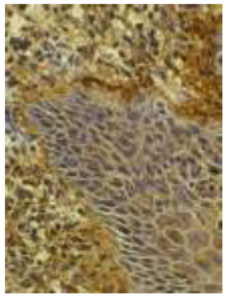

Figure 3: Expression of JunB (Score 1) in OLP by x200 magnification

Figure 4: Expression of JunB (Score 2) in normal mucosa by x200 magnification

Results

After IHC staining, the results obtained on 55 samples that included 38 women and 17 men with age average of 39.2 - 8.3 years old are presented and the results of the study are expressed using Tables 1 and 2.

	N	Minimum	Maximum	Mean	SD
OLP	35	0	97	41.8	27
Normal	20	5	39	22	8

Table 1: Comparison of the percentage of JunB expression in OLP and normal mucosa

As shown in Table 1, the lowest percentage of JunB expression in OLP is 0% and the highest is 97%, as well as the average percentage of JunB expression in OLP is 41.8% ± 27. The least percentage of expression of JunB in the normal mucosa was 22% ± 8.

T-test for comparing the percentage of JunB expression in OLP samples, all of which were the type of erosive, and normal mucosa was used. Accordingly, the percentage of JunB expression in OLP and normal mucosa has a statistical significant difference. (p value = 0.006)

The JunB Expression Score also based on Table 2 is as below:

	JunB SCORE				
	0	1	2	3	Total
OLP	1 (2.85%)	22 (62.85%)	8 (22.85%)	4 (11.45%)	35
Normal	1 (5%)	19 (95%)	0	0	20

Table 2: Comparison of JUNB SCORE in OLP and normal mucosa

Out of 35 available OLPs, 1 sample is reported score 0, 22 samples, score1, 8 samples, score2 and 4 samples, score3. Out of 20 normal mucosal samples, 1 sample, score 0 and 19 samples, score 1 have been reported. Also, none of the existing normal mucus samples is reported score 2 and 3. While about 1/3 OLP samples had scores of 2 and 3.

Based on Mann-U Whitney test, there was a significant difference in terms of JunB expression score in OLP samples, all of which were erosive and normal mucosa (p = 0.005)

Thus, OLP samples showed significantly higher score in terms of JunB expression than normal mucosa.

Discussion

In this study, there was a statistical significant difference between oral lichen planus, which all of them were the type of erosive, and the normal mucosa both in terms of the percentage and score of JUNB expression.

According to our knowledge about the available information on the expression of JunB in mucosal lesions, especially oral mucosa, no study has been conducted so far, and relevant studies are about other pathological lesions, including Xin Mao *et al*.[21] in a study who performed on the primary skin Tcell lymphoma, linked the amplification and expression of the JunB model to PCL, and suggested that JunB might be critical in the primary cutaneous B cell PCL. Also, Schmidt D *et al*.[11] in a study titled the critical role of JunB induced by NF-kappaB in regulating VEGF and angiogenesis, showed that JunB's lack of expression causes disorder in VEGF expression and finally concluded that angiogenesis of tumor is disrupted in teratocarcinomas that do not induce JunB.

On the other hand, Kanno T *et al*.[14] in a study titled JunB's role in promoting cellular invasion and angiogenesis in renal cell carcinoma concluded that JunB persuaded tumor invasion and increasing angiogenesis in this type of cancer. Eckhof K *et al*.[22] also in a study titled the significance of Jun transcription factors in the prognosis of ovarian cancer showed that JunD and pc-Jun proteins are effective in carcinogenesis and tumor progression, suggesting a predictive role in ovarian cancer, although there was no sign of correlation between JunB expression and general survival and non-dependent survival progression. In addition, JunB was clearly not related to any clinicopathologic parameters.

Chong Park *et al*.[16] also in a study titled comparing the expression of JunB, JunC and s100A8 in goutat's psoriasis showed the reduction of expression JunB in two types of psoriasis compared to normal mucosa.

In general, JunB, as a member of the Jun family is introduced as biomodulator function in the expression of inflammatory mediators.[12,13] Also, PCR analysis has shown that JunB adjusts multiple genes for tumor invasion and angiogenesis from the matrix metalloproteinase family, including MMP2.9. In addition, studies have shown that JunB reduction significantly prevents tumor growth and angiogenesis.

In this regard, JunB has been acting as a marker for cell proliferation, in particular angiogenesis regulator, by influencing VEGF vascular growth factor,[23,24] as reported the lack of JunB in various cell types, led to very poor expression of VEGF and delayed cell growth.[11] In previous studies, the effect of JunB expression on angiogenesis has been reported through the effect on VEGF in invasive growth of pathologic lesions, including malignant cells . On the other hand, in other sources, important results are found associated with increased expression of VEGF in pathologic lesions such as malignancies and other pathologic lesions, including cysts, and especially odontogenic cysts as well as mucosal lesions including lichen planus. In most studies, the researchers considered the angiogenesis process and its related factors to be necessary for the development of pathologic lesions.[25-27]

In this regard, in a study by Tao *et al* in 2007, on 30 lichen planus and 7 normal mucus samples, VEGF expression was used to evaluate angiogenesis and it was shown that the incidence of VEGF in Atrophic-erosive OLP was higher than samples Reticular and control significantly.[28] Scardina *et al* also conducted a study in 2009 on 30 lichen planus and 30 normal mucosal samples. In their study, it was also shown that the incidence of VEGF in OLP samples was significantly higher in comparison with normal mucosa.[29] Also, Farhadi *et al*., based on a study conducted in 2018 on 35 OLP samples, showed a significant difference in terms of score of VEGF expression in these samples to the normal mucosa.[30]

However, in the case of oral lichen planus, scientific information sources did not provide accurate and consistent reports on angiogenesis in the pathogenesis of the disease, although, based on these studies, oral lichen planus as a chronic inflammatory autoimmune disease has required scientific evidence on the hypothesis induced by inflammatory cells and as a result, the development of the

VEGF growth factor and the onset of the subsequent angiogenesis.[31]

Therefore, the mechanism of expression of VEGF in previous studies and according to the mechanism of the JunB marker effect through VEGF in available scientific reports and according to the results of the recent study, which, according to our knowledge of available information resources, is the first scientific report on the expression of JunB on oral lichen planus samples, it seems that the expression of this new marker can also be used to evaluate the pathogenicity of lichen planus. However, future studies with higher sample size and the use of more accurate evaluation methods include the genome evaluation of this marker seem necessary.

Conclusion

In this study, there was a statistical significant difference between the oral lichen planus samples and normal mucosa in both percentage and related score of JUNB expression; as, the percentage of expression of JunB and its score in OLP was higher than the normal mucosa.

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