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Comparison of Cluster Differentiation 44 expression in Oral Lichen Planus, Oral Leukoplakia and Oral Squamous Cell Carcinoma

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A B S T R A C T

Introduction: CD44 is a cell surface glycoprotein essential for the migration of cancer cells. The utilization of biomarkers in the early stages of OSCC diagnosis offers a preventive and therapeutic strategy. The aim of the study is to compare the expression of CD44 in OLP, OL, and OSCC.

Materials and Methods: This was a descriptive-analytical laboratory study performed on paraffin blocks from tissue biopsies of patients with OLP, OL, and OSCC available in the maxillofacial archive of the pathology department of Imam Khomeini Hospital, Urmia (Iran). The smears were stained by H&E and then stained by the CD44 antibody kit and cut into 5-micron dimensions. Four codes were used to evaluate the intensity of staining based on the average percentage of stained cells to total cells. Qualitative data were reported as frequency and percentage. Age and the level of expression of CD44 both had normal distribution; therefore, to analyze and compare the differences Parametric tests were used.

Results: There is a significant difference between the three lesions of OLP, OL, and OSCC in the expression level of CD44 ($p<0.001$), and expression of this molecule in the mucosa of OSCC was higher compared to the OLP and OL. Also, the expression of CD44 in three lesions of OLP, OL, and OSCC had a statistically significant difference with oral normal mucosa ($p<0.001$).
Conclusion: CD44 can probably be a suitable marker to confirm the prediction of dysplasia in oral premalignant lesions such as OLP and OL and invasion, metastasis, prognosis, and recurrence in OSCC

Keywords: Oral Squamous Cell Carcinoma, Lichen Planus, Oral leukoplakia, Oral CD44 Antigen

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Introduction

Oral cancer is a major health problem in many parts of the world. This malignancy is the sixth most common cancer in the world, the eighth in men and the fifth in women. Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity, which includes more than 90% of oral cavity malignancies. According to published information, more than 300,000 new patients and more than 140,000 new deaths due to oral squamous cell carcinoma are registered annually (1, 2, 3). Despite the advances in the treatment of this malignancy, the 5-year survival rate for patients is still below 50% (4,5). This malignancy has a strong relationship with risk factors such as tobacco and alcohol consumption, which cause genetic damage. As a result of this damage, the cells find uncontrolled proliferation, which leads to dysplasia and the development of premalignant and malignant lesions (1). Oral epithelial dysplasia (OED) is a diagnostic term to describe chronic, progressive, and premalignant histopathological changes in the oral mucosa. Among dysplastic tissues, oral lichen planus (OLP) and oral leukoplakia (OL) can be mentioned (1, 5).

Oral Lichen Planus is described as an autoimmune disease caused by T cells, which is often characterized by white stripes and has reticular, papular, plaque-like, atrophic, and ulcerative lesions. Different prevalences from 0.1% to 4% have been reported, and it is more common in women than men. The exact etiology of this lesion has not been definitively determined yet, but the changes in the immune response through cell mediation play a major role in its pathogenesis (6, 7). Oral leukoplakia is defined as white lesions of the oral mucosa that are not associated with any other lesions. This lesion grows in the form of thick, plaque-like white patches on the tongue, gingiva, and oral mucosa. It is an uncommon lesion that has been reported in different studies with different prevalence rates from below one percent to four percent, but it is the most common premalignant lesion. A clear etiology for the occurrence of OL has not been determined, but factors damaging to the mucosa, such as smoking, chronic trauma, and lack of vitamins A and B, are known as risk factors for it (8–13).

Despite good access to the oral cavity for clinical examinations, oral malignant lesions are detected in their advanced stages, which is 40% of all patients with oral cavity malignancy in developed countries (2). Predicting the behavior and examining the prognosis of oral lesions using traditional clinical and histopathological criteria, such as determining grading and staging or examining microscopic changes, is not performed well due to the heterogeneous nature of premalignant and malignant lesions. For this reason, the need for more specific markers is felt (4, 14). In recent years, interest in using more specific markers of malignancies for diagnosis and treatment, which are effective on cell division, progression, and prognosis of lesions, has increased (1). One of these markers is the Cluster Differentiation 44 (CD44) molecule. This molecule belongs to the family of membrane glycoproteins, which interacts with various ligands such as hyaluronan, fibronectin, type I and IV collagen, and affects cell-to-cell and cell-to-extracellular matrix communication, which seems to affect cell movement and transmission. (3,4,15). The nature of this intramembranous adhesion molecule and the role it can have in the development and prognosis of pre-malignant and malignant lesions have not only attracted the attention of basic science researchers but also pathologists and oncologists. The availability of different monoclonal antibodies against CD44 has increased the ease and accuracy of using this marker for immunohistochemically analyses (1). Few and contradictory studies have been published regarding the effect of the CD44 molecule in the development of premalignant and malignant lesions. Some studies report higher expression and others lower expression of this marker in these lesions (1, 4). Considering this issue, the study aimed to examine the expression of the CD44 marker in OLP, OL, and OSCC in order to determine its role in the pathogenesis of these lesions.

Materials and Methods

Setting

This study was a descriptive-analytical laboratory study designed to compare the expression of CD44 in three lesions of OSCC, OLP, and OL.

After extracting the files, the demographic information of the patients and the required information, such as the location of the lesion and the histopathologic diagnosis of the lesion, were recorded in tables. Patients who had incomplete files or a definitive diagnosis was not recorded in their records were excluded from the study. Finally, based on similar studies, 46 biopsy blocks were considered, which were divided into four groups (4):

1. Normal mucosa group including 10 biopsy blocks
2. 12 biopsy blocks of OLP (figures 1-4)
3. 12 biopsy blocks of OL (figures 5-7)
4. 12 biopsy blocks of well-differentiated OSCC (figures 8-11)

Measuring tools

1. Demographic form
Demographic variables included in this study were age and sex.

2. Slide assessment
The microscope slides were examined by two pathologists using an Olympus microscope at 400x magnification.

At first, the slides stained by hematoxylin and eosin were examined by the pathologist and confirmed with the diagnosis of normal mucosa without dysplasia, reticular OLP, OL, and Well Differentiated OSCC. Paraffin blocks of all cases were sectioned onto 5µ polylysin-coated slides. The avidin-biotin-peroxidase method was performed using the primary monoclonal antibodies against CD44s, standard isoform CD44 (Clone 156-3C11; HCAM, Diagnostic Biosystem, Pleasanton, CA, USA). Briefly, the sections were deparaffinized and washed in phosphate-buffered saline (PBS). Endogenous peroxidase activity was blocked using a 0.3% solution of hydrogen peroxidase at room temperature for 5 minutes. After microwave treatment for antigen retrieval, primary antibodies were applied for 60 minutes at room temperature and washed in PBS. Linking antibody and HR-peroxidase complex were added consecutively for 20 minutes at room temperature and washed in PBS. The peroxidase activity was visualized with diaminobenzidine (DAB), applied for 5 minutes. The most representative tumor areas were selected for scoring the immunostaining pattern. Then they were examined by two pathologists with a light microscope (Olympus, Cx31 LED, Tokyo, Japan). The number of stained cells compared to the total number of cells in ten consecutive high-power fields was counted, and the average was calculated as the percentage of stained cells. To evaluate the staining intensity, the following codes were defined based on the mean percentage of stained cells (1): Staining of less than five percent with a negative code, staining of five to 25% code +1, staining of 25% to 50% code +2, staining of 50% to 75% code +3, and staining of 75% and above code +4.

Ethical considration
The ethical considerations included compliance with the ethics code (IR.UMSU.REC.1400.450), maintaining integrity in the library collection and data reporting, obtaining written informed consent from all participants in accordance with the Declaration of Helsinki, and adhering to principles for conducting interventions involving human subjects.

Statistical and Data Analysis

Qualitative data were reported as frequency and percentage. The Kolmogorov-Smirnov test was performed, and it was observed that the quantitative variables of age and expression of CD44 both had normal distribution, so parametric tests were used to analyze and compare the differences. A P-value less than 0.05 was considered statistically significant. SPSS V.22 was used for statistical analysis.

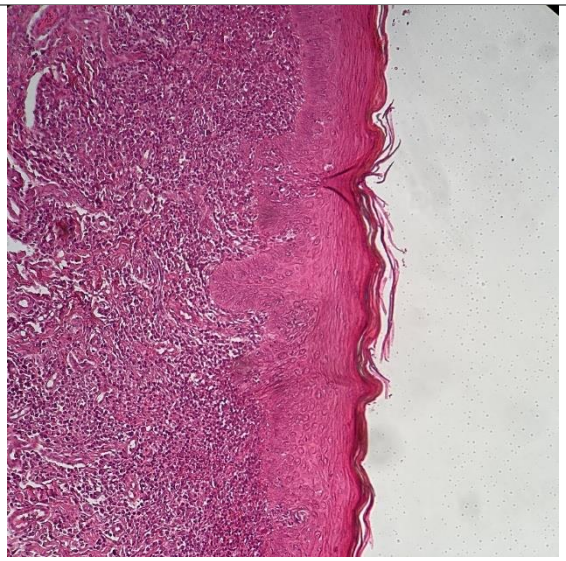

Figure 1. Hematoxylin and eosin staining of lichen planus with 10x10 magnification

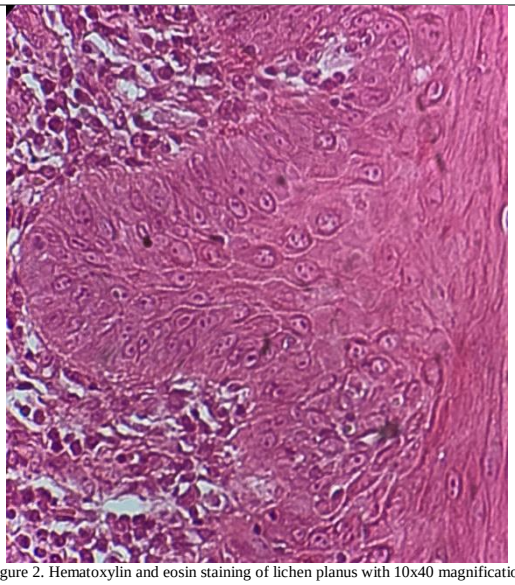

Figure 2. Hematoxylin and eosin staining of lichen planus with 10x40 magnification

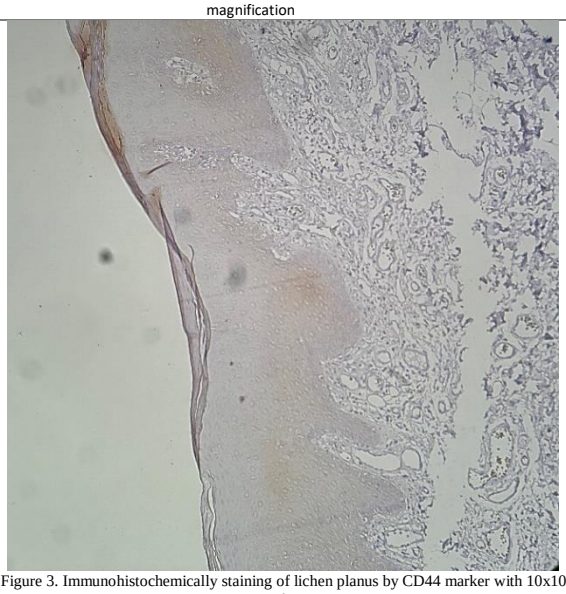

Figure 3. Immunohistochemically staining of lichen planus by CD44 marker with 10x10 magnification

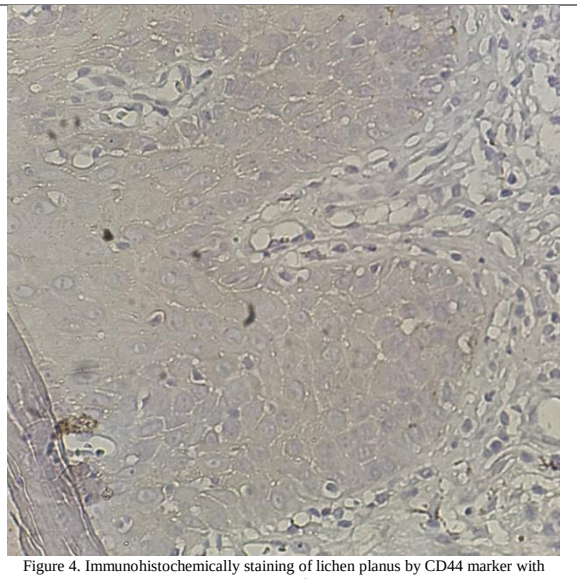

Figure 4. Immunohistochemically staining of lichen planus by CD44 marker with 10x40 magnification

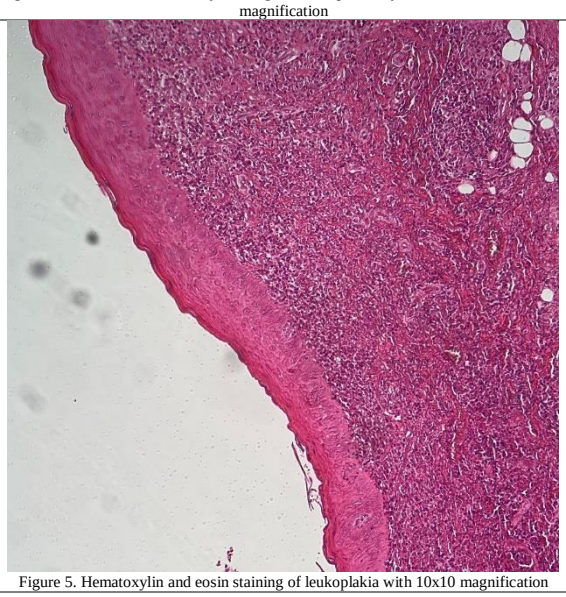

Figure 5. Hematoxylin and eosin staining of leukoplakia with 10x10 magnification

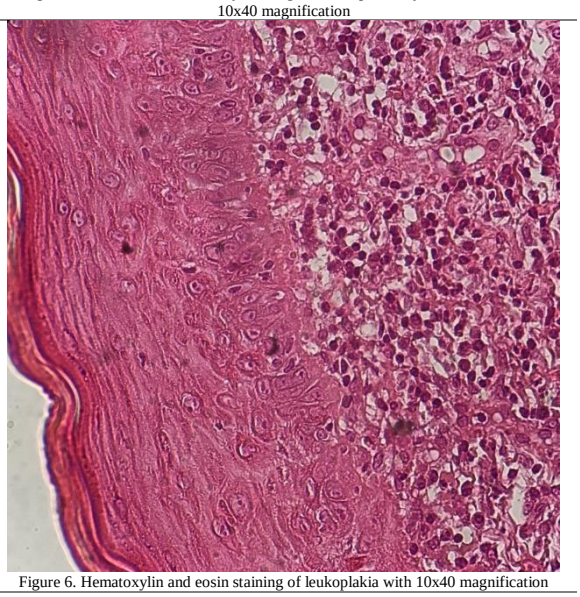

Figure 6. Hematoxylin and eosin staining of leukoplakia with 10x40 magnification

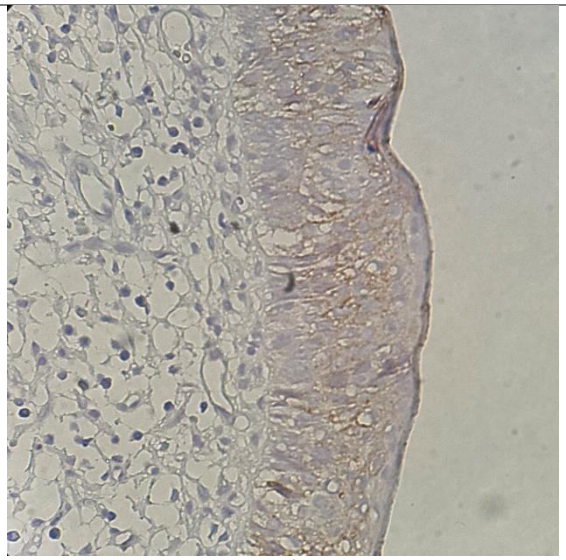

Figure 7. Immunohistochemically staining of leukoplakia by CD44 marker with 10x10 magnification

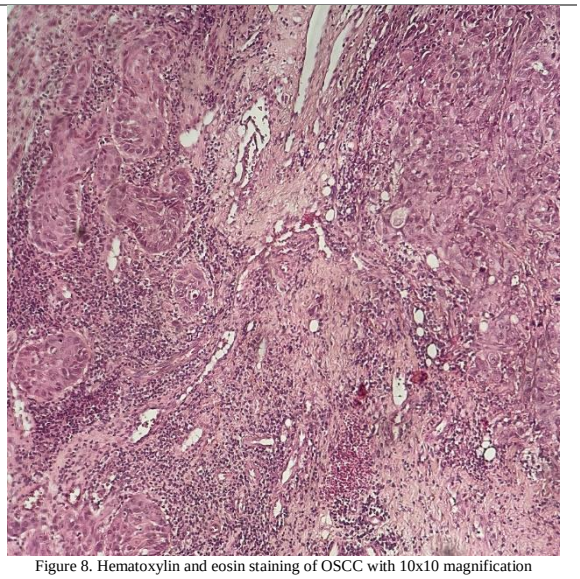

Figure 8. Hematoxylin and eosin staining of OSCC with 10x10 magnification

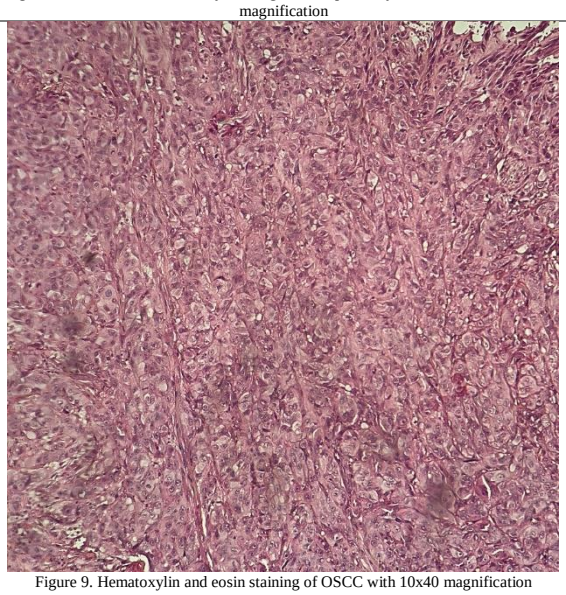

Figure 9. Hematoxylin and eosin staining of OSCC with 10x40 magnification

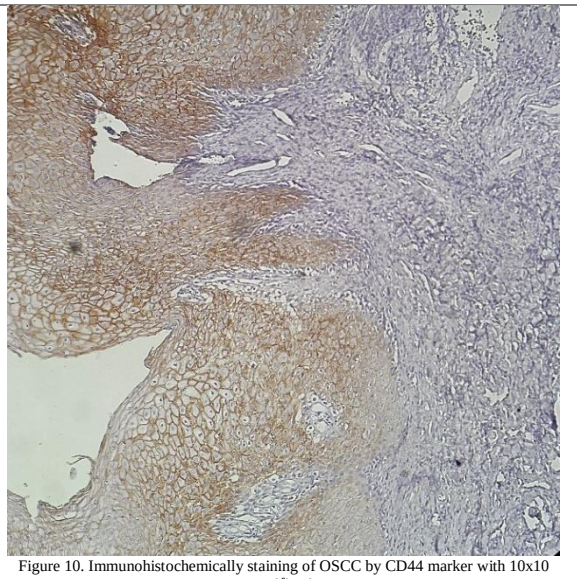

Figure 10. Immunohistochemically staining of OSCC by CD44 marker with 10x10 magnification

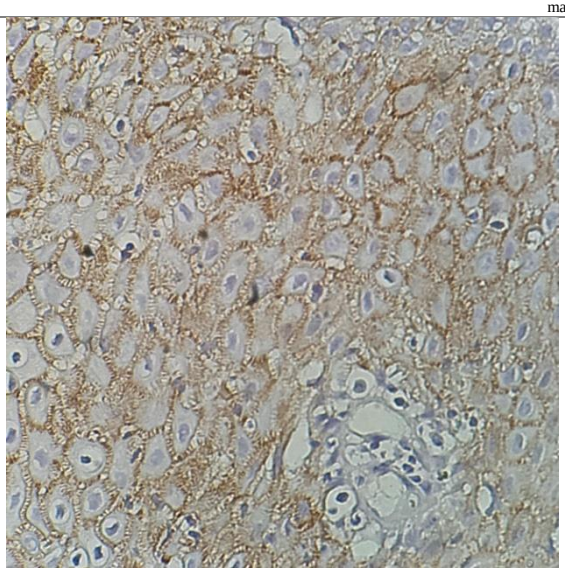

Figure 11. Immunohistochemically staining of OSCC by CD44 marker with 10x40 magnification

Results

In this study, 12 biopsy blocks from each of the lesions along with 10 biopsy blocks of normal mucosa from the maxillofacial archive of the pathology department of Imam Khomeini Hospital, Urmia, have been examined and compared for CD44 expression. The overall average age of the participants in this study was 54.17, and the standard deviation was 15.86. The youngest study sample was 12 and the oldest was 82 years old. The findings showed that 66.6% male and 33.3% female for the OSCC group; 41.7% male and 58.3% female for the OLP group; 58.3% male and 41.7% female for the OL group; and 50% male and 50% female for the normal mucosa group have been examined (Tables 1 and 2).

The average expression of CD44 in OLP was 23.16% with a standard deviation of 12.59%, and the lowest and highest expression of CD44 were 4% and 43%, respectively. The intensity of staining in the OLP was grade +1. Also, the average expression of CD44 in OL was 49.41% with a standard deviation of 18.58%, among which the lowest and highest expression of CD44 was 21% and 75%, respectively. And the intensity of staining in OL was +2 grade. The average expression of CD44 in OSCC was 74% with a standard deviation of 14.65%, among which the lowest and highest expression of CD44 was 52% and 92%, respectively. OSCC staining intensity was grade +3. And the average expression of the CD44 molecule in normal mucosal biopsies was 14.2% with a standard deviation of 9.01%, among which the lowest and highest expression of CD44 were 4% and 30%, respectively. In the current study, the intensity of staining in normal mucosal biopsies was grade +1 (Table 3). The results showed that there was a significant difference between OLP and OSCC for CD44 expression ($p = 0.031$), and the expression of this molecule in OSCC was much higher compared to OLP (74% versus 23.16%). Also, the findings showed that there was a significant difference between OL and OSCC in terms of CD44 expression ($p = 0.003$), and the expression of this molecule in OSCC was much higher compared to OL (74% versus 49.41%). Also, the results show that there was a significant difference between OLP and OL in terms of CD44 expression in the present study ($p = 0.001$), and the expression of this molecule in OL was much higher compared to OLP (49.41% against 23.16%) (Table 3).

The results of the study show that there is a significant difference between the expression of CD44 in each of the lesions compared to the normal mucosa. ($p=0.001$) (Table 4).

Table 1. The number of samples examined in this study by sex

Lesion	Sex	Number	Percentage
OLP	Male	5	41.7%
	Female	7	58.3%
	Total	12	100%
OL	Male	7	58.3%
	Female	5	41.7%
	Total	12	100%
OSCC	Male	8	66.7%
	Female	4	33.3%
	Total	12	100%
Normal	Male	5	50%
	Female	5	50%
	Total	10	100%

Table 2. Comparison of the average age of the study groups

Lesion	Age Average	Standard Deviation	youngest	Oldest
OLP	47.91	11.18	28	64
OL	61.25	14.64	29	82
OSCC	59.41	12.01	36	81
Normal	46.90	21.25	15	80
Total	54.17	15.86	12	82

Table 3. Comparison of CD44 expression in lesions examined in this study

Lesion	Average Expression	Standard Deviation	Lowest Expression	Highest Expression	Intensity of Staining
OLP	23.16%	12.59%	4%	43%	+1
OL	49.41%	18.58%	21%	75%	+2
OSCC	74%	14.65%	52%	92%	+3
Normal Mucosa	14.2%	9.01%	4%	30%	+1

Table 4. Comparison of CD44 expression in lesions

Lesion	Average	Standard Deviation	P-Value
OLP	23.16%	12.59%	0.031
OSCC	74%	14.65%	
OL	49.41%	18.58%	0.003
OSCC	74%	14.65%	
OLP	23.16%	12.59%	<0.001
OL	49.41%	18.58%	

Discussion

Researchers compared the expression of CD44 by gender, and the findings showed that there is no statistically significant difference between men and women for the expression of CD44 in any of the lesions. The mentioned molecule is expressed almost the same in both sexes. In confirmation of these results, the study of Chen et al. (16) and the study of Čēma et al. (12) were also published earlier.

In the study of Ratuava et al., unlike the findings of our study, they did not report a statistically significant difference in the level of CD44 expression between the lesions under investigation, and the researchers stated that the level of CD44 expression cannot be used to predict the clinical behavior of oral dysplastic lesions (17). However, Sawant et al. (18) showed that CD44 immunohistochemically staining increases with the progression of the disease. The authors concluded that this marker can be used to predict local recurrence and poor prognosis in patients with oral cancer or mucosal lesions. Abdul Majeed et al. also reported an increase in CD44 expression in OSCC compared to normal tissue, consistent with the results of the present study (19). Mannelli et al. (20) also observed an increase in the expression of CD44 in about 93% of OSCC biopsy blocks from patients under their study, which is higher compared to our study.

Since cell connections are reduced in dysplasia and in malignancies, the loss of cell-to-matrix connections seems necessary for cell migration and invasion, followed by metastasis. It seems logical that with the increase in the degree of dysplasia and also with the decrease in the

differentiation of cells in SCC, the expression of CD44 decreases, which is similar to the findings of Simionescu et al. (21).

In justifying the different results reported in other studies, there is a possibility that during dysplasia, due to the reduction of cell connections, cells try to produce more CD44 in a compensatory way. Since this protein has changes compared to the normal state due to possible mutations, it does not have the required efficiency, and despite the increase of this protein in the cell, effective connections are not established. Misra et al. (22), state that in fact many CD44 molecules do not bind to hyaluronic acid and matrix, and binding of CD44 to hyaluronic acid is very specific and depends on the state of CD44 activation. As a result, there may be enough CD44, but the connection is not established. Also, since CD44 has different isoforms and some studies, such as the study of Ratuava et al. (17), have been conducted on some specific isoforms, this issue can also be the source of some differences in the results with other studies as well as our study.

One of the limitations of this research was the lack of access to sufficient paraffin blocks of the samples. Additionally, the unavailability of immunohistochemically staining kits posed a significant constraint.

Conclusion

Based on the findings, a significant difference was observed between the three lesions of OLP, OL, and OSCC in terms of CD44 expression. So, this protein can probably be a suitable marker to confirm the prediction of invasion ability in oral premalignant lesions such as OLP and OL and the invasion ability, metastasis, and prediction of prognosis and recurrence in OSCC. Considering the critical importance of oral potentially malignant disorders, it is advisable to investigate and analyze additional markers within these lesions. This strategy aims to enable early diagnosis and treatment, thereby preventing the progression to malignant lesions in the oral cavity.

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Conflict of interest
The authors declare that no conflict of interest exists.

Authors' contributions
Methodology, Investigation, Visualization, Project Administration, Funding Acquisition: SM, Conceptualization, writing– Original Draft Preparation: SM, FA, Validation: MR, Formal Analysis: MR, AT, Resources, Data Curation: AT, Writing– Review & Editing, Supervision: FA.

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