Original Article

CD44 Expression in Oral Lichen Planus and Related Lesions—An Immunohistochemical Study

Abstract

Background: Cluster of differentiation 44 (CD44) is a cell surface adhesion protein involved in the progression and metastasis of oral squamous cell carcinoma. The current study aims to evaluate the expression of CD44 in oral lichen planus and related lesions and thereby assess the relative risk of malignant transformation of these lesions. **Materials and Methods:** Formalin-fixed paraffin-embedded tissue blocks of 10 oral lichen planus (Group 1), 10 oral lichenoid lesions (Group 2), 8 with oral lichen planus with dysplasia (Group 3), and 5 with lichenoid dysplasia (Group 4) were included in the study. Immunostaining was done for the tissue sections using CD44 mouse monoclonal antibody. Staining density, staining intensity, and immunoreactive scores of CD44 were evaluated in all four groups. Statistical analysis was done by Statistical Package for the Social Sciences® software and the Kruskal–Wallis test was used. **Results:** CD44 staining pattern of lichenoid dysplasia and lichen planus with dysplasia changed from membranous to cytoplasmic. The membranous CD44 immunoreactivity was mild with a score of 2.25 for Group 3 and 1.6 for Group 4 whereas moderate for other groups with a *P*-value of 0.009. The cytoplasmic immunoreactivity was significantly high in Group 3 (5.3 ± 2.6) followed by Group 4 (3.2 ± 1.2), Group 2 (1 ± 1.8), and Group 1 (0.7 ± 1.3) with a *P*-value of 0.001. **Conclusion:** The CD44 membranous immunoreactivity scores were low while the cytoplasmic immunoreactivity was high in oral lichen planus with dysplasia and oral lichenoid dysplasia when compared to oral lichen planus and oral lichenoid lesions. CD44 immunostaining pattern can help in assessing the malignant transformation of oral lichen planus or lichenoid lesions.

Keywords: *CD44, dysplasia, lichen planus, lichenoid lesions*

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Introduction

Lichen planus is an autoimmune T cell-mediated disease affecting the skin, nails, hair, and mucous membranes. The global prevalence of oral lichen planus is 1.01%.[1] Mucosal lichen planus tends to follow a chronic clinical course with acute exacerbations while cutaneous lichen planus has a milder course.[2] Oral lichen planus is a potentially malignant disorder with varying rates of malignant transformation which can be accounted for by the lack of universally accepted diagnostic criteria. According to the workshop convened by WHO in 2020, oral lichen planus is characterized clinically by the presence of bilateral, symmetric white lesions with/without erosions and ulcerations or presenting as desquamative gingivitis and histologically by the presence of a sub-epithelial band of lymphocytic infiltrate, vacuolar degeneration of basal, suprabasal layers or epithelial thinning and ulcerations in case of atrophic type.[3] Currently, there is no effective treatment due to the recalcitrant nature of this disease.[4]

Oral lichen planus-related lesions like lichenoid lesions and lichenoid dysplasia resemble lichen planus clinically and histologically but with different etiopathogenesis and biologic behavior. Oral lichenoid lesions do not exhibit typical clinical and/or histopathological features of oral lichen planus and have been associated with identifiable causative factors like contact with dental restoration, drugs, betel quid, intake of food or some substances, like cinnamon or oral graft versus host disease.[5] Recently oral lichenoid lesions have been newly added to the WHO 2020 classification of oral potentially malignant disorder. The term oral lichenoid disease was proposed to include both oral lichen planus and lichenoid lesions

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since both had similar malignant potential.[3] Two types of oral lichen planus-like lesions with epithelial dysplasia have been reported.[6] Lesions exhibiting dysplasia with clinical features of oral lichen planus have been described as the malignant transformation of oral lichen planus, while lesions without the typical clinical features of lichen planus but histologically exhibiting dysplasia and lichenoid features like a subepithelial band of inflammatory infiltration have been termed as oral lichenoid dysplasia.

Malignant transformation is connected to the loss of epithelial phenotypes and a reduction in differentiation. Loss of epithelial features, such as loss of epithelial cell polarity, reduced cellular adhesion, and greater mobility, is driven by the expression of mesenchymal genes.[7] The cluster of differentiation 44 (CD44) is a cell surface adhesion protein involved in cell-to-cell and cell-to-extracellular matrix interactions. CD44 can also act as a stem cell marker and has been associated with the progression and metastasis of oral squamous cell carcinoma.[8] The current study aims to evaluate the expression of CD44 in oral lichen planus and related lesions and thereby assess the relative risk of malignant transformation of these lesions.

Materials and Methods

Study design

The current retrospective study was conducted after obtaining ethical clearance from the Institutional Review Board of SRM Dental College (SRMU/M&HS/SRMDC/2022/PG/007). All procedures performed in the study were conducted as per the ethical standards given in the 1964 Declaration of Helsinki, as revised in 2013.

Study setting

The formalin-fixed paraffin-embedded tissue blocks were retrieved from the archives of the department of oral pathology during the period of 11 years from January 2010 to December 2021 which were previously diagnosed as lichen planus/lichenoid mucositis/lichenoid dysplasia/lichen planus with dysplasia/lichenoid reaction/lichenoid lesions were reevaluated by three oral pathologists. The clinical and histopathological data obtained from the records were reviewed by oral pathologists independently and 10 with lichen planus (Group 1), 10 patients with lichenoid lesions (Group 2), 8 with lichen planus with dysplasia (Group 3), and 5 with lichenoid dysplasia (Group 4) were included in the study. Oral mucosa adjacent to the extracted impacted tooth was considered the normal control group. Diagnosis of oral lichenoid lesions and oral lichen planus was given based on the criteria given by Warnakulasuriya *et al*., 2020.[3] Cases with a clinical diagnosis of oral lichen planus exhibiting dysplasia histopathologically were included under Group 3 and cases clinically resembling oral lichen planus/leukoplakia with histopathological lichenoid features and epithelial dysplasia were included under Group 4. Cases without patient details or clinical pictures were excluded from the study.

Immunostaining

Formalin-fixed paraffin-embedded blocks were obtained and from each block, 3- to 4-micron thick sections were cut and air dried on poly-L-lysine coated slides. The sections were deparaffinized, rehydrated, and then put in a pressure cooker with TRIS buffer (pH 6.0) for antigen retrieval. CD44 mouse monoclonal antibody, Path Insitu biologicals™ polymer, which served as the primary antibody, was diluted to a concentration of 1:300 before being applied to the slides for 1 hr and 30 mins. After that, the segments were carried out on phosphate-buffered saline (PBS) for two changes of 5 mins each. For 30 mins, the sections were treated with a secondary antibody. Each section received 25 l of the diaminobenzidine working solution for color development, and Harris hematoxylin was utilized as a counterstain. The stained slides were dehydrated followed by clearing in xylene and mounted, examined under a light microscope.

Based on the thickness of the epithelium stained with CD44, scoring was done.[9] Score 1 denoted staining up to 1/3rd of the epithelium, score 2 implied staining till 2/3rd of the epithelium, and score 3 indicated staining of the entire thickness of the epithelium. A score of 0 was given for negative CD44 immunostaining. Cytoplasmic and membranous immunostaining of CD44 was evaluated by measuring staining density and staining intensity.[10] Five fields were selected for each case at a magnification of 400x, and in each field, the number of stained cells per 100 cells was determined as the staining density. Using the basal cells of normal mucosa [Figure 1] as the positive control, the staining intensity was assessed as mild, moderate, or severe. The immunoreactive score of each specimen was calculated by multiplying the mean percentage scores of staining density and intensity across these five fields. To avoid interobserver bias, the slides were assessed independently by three oral pathologists, who were blinded to clinical records and histopathological diagnosis.

Statistical analysis

Statistical analysis of data was done using Statistical Package for the Social Sciences® software (version 22.0). The Kruskal–Wallis test was done to compare the scores within the four groups and a *P*-value of <0.05 was considered to be statistically significant.

Results

The demographics of the patients under each group are mentioned in Table 1. The mean age of the patients under Group 4 was higher (56.2 ± 3.3 years) than the other Groups. A greater proportion of females were found in groups 1 and 2. In 90% of the control group CD44 was

expressed in 1/3rd of the epithelium, while in Group 1 equal number of samples had score 1 and score 2 [Figure 2]. Score 3 was evident only in 20% of Group 4 samples. Eighty percent of Group 4 and 75% of Group 3 samples had a score of 2. Membranous staining density and staining intensity were more in Group 1 [Figure 3] and Group 2 [Figure 4] while cytoplasmic staining density and staining intensity were greater in Group 3 [Figure 5] and Group 4 [Figure 6] when compared to other groups. The membranous CD44 immunoreactivity was mild with a score of 2.25 for Group 3 and 1.6 for Group 4 whereas moderate for other groups with a *P*-value of 0.009 [Table 2]. The cytoplasmic immunoreactivity was significantly high in Group 3 (5.3 ± 2.6) followed by Group 4 (3.2 ± 1.2), Group 2 (1 ± 1.8), and Group 1 (0.7 ± 1.3) with a *P*-value of 0.001 [Table 3].

Discussion

A family of transmembrane glycoproteins known as CD44 are found in a variety of cells and tissues, including hemopoietic, endothelial, mesenchymal, and epithelial lineages. CD44 participates in a variety of biological activities by acting as a growth factor-presenting molecule, a signal transmitter, and a receptor for cell-to-cell or cell-to-matrix adhesion. Abnormal cell surface expression of CD44 appears to be associated with tumor metastasis and the progression of various carcinomas. Only a few studies have evaluated their role in the malignant transformation of oral lichen planus and oral lichenoid lesions.

It is a diagnostic challenge to clinically distinguish oral lichenoid lesions from oral lichen planus. Aguirre-Urizar *et al.* proposed a common term oral lichenoid disease to include both oral lichen planus and oral lichenoid lesions.[5] Oral lichenoid disease is considered to be an oral potentially malignant disorder with a low malignant transformation rate of <3%. In our study, the age distribution of patients with lichen planus was found to be similar to those with lichenoid lesions. The mean age was highest for the patients with lichenoid dysplasia. A similar trend was also observed by Czerninski *et al*.[11] Lichen planus and lichenoid lesions were common in females which is in accordance with the literature.[11-13] In the study by Czerninski *et al*., the lowest percentage of males was distributed in the lichenoid reaction group, similar to our study.

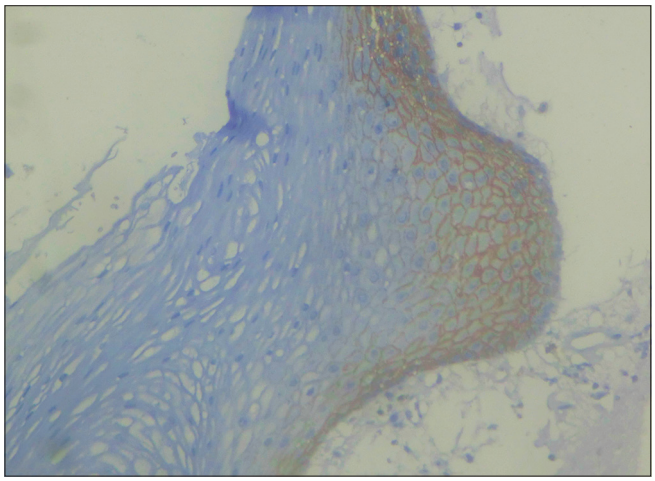


Figure 1: CD44 expression in the control group

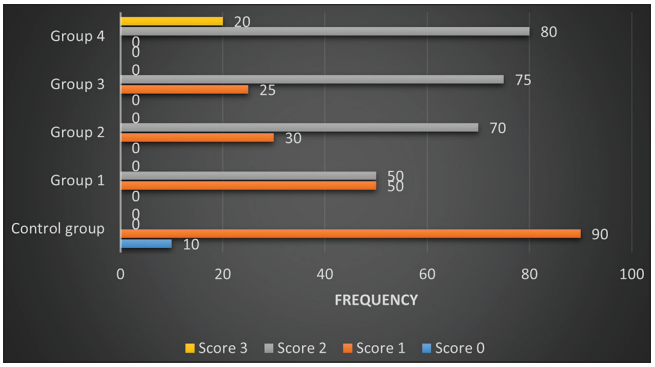


Figure 2: Comparison of the thickness of the epithelium stained by CD44 among different groups

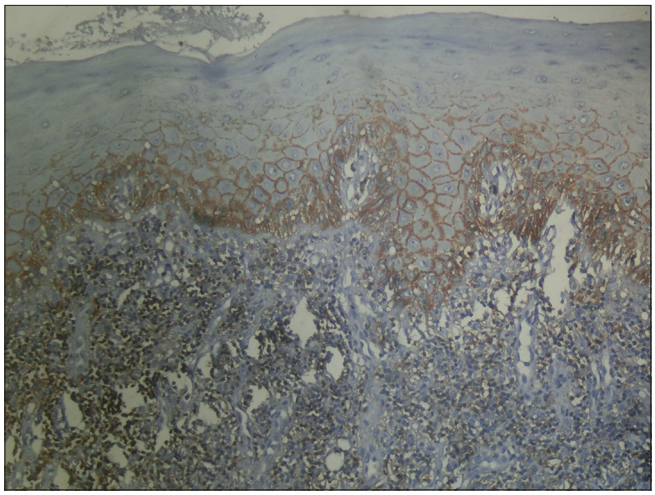


Figure 3: CD44 expression in oral lichen planus

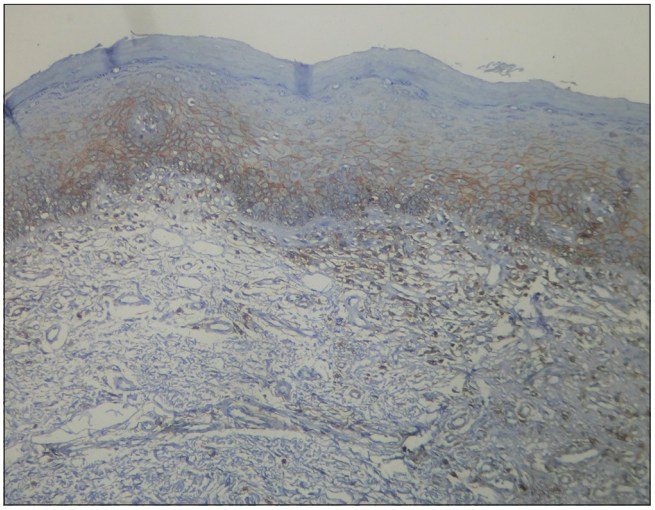


Figure 4: CD44 expression in oral lichenoid lesions

Table 1: Demographics of the patients

Demographics		Group 1 (*n*=10)	Group 2 (*n*=10)	Group 3 (*n*=8)	Group 4 (*n*=5)
Age (in years)	Mean	46.8	47.1	53.1	56.2
	SD	5.1	3.9	3.2	3.3
Gender	Male, *n* (%)	3 (30)	4 (40)	4 (50)	4 (80)
	Female, *n* (%)	7 (70)	6 (60)	4 (50)	1 (20)

Table 2: Intergroup comparison of membranous staining density, staining intensity, and immunoreactive scores

Groups	Staining density		Staining intensity		Immunoreactive scores	
	Mean±standard deviation	*P*	Mean±standard deviation	*P*	Mean±standard deviation	*P*
Group 1 (*n*=10)	2.1±0.83	0.36	2.2±0.6	0.003	4.8±2.74	0.009
Group 2 (*n*=10)	1.9±0.53		2.4±0.49		4.4±1.11	
Group 3 (*n*=8)	1.6±0.7		1.3±0.48		2.25±1.48	
Group 4 (*n*=5)	1.4±0.49		1.2±0.4		1.6±0.49	

Table 3: Intergroup comparison of cytoplasmic staining density, staining intensity, and immunoreactive scores

Groups	Staining density		Staining intensity		Immunoreactive scores	
	Mean±standard deviation	*P*	Mean±standard deviation	*P*	Mean±standard deviation	*P*
Group 1 (*n*=10)	0.9±0.7	0.000	0.6±1.2	0.04	0.7±1.3	0.001
Group 2 (*n*=10)	0.5±0.7		1.4±1.2		1±1.8	
Group 3 (*n*=8)	2.6±1.0		2±0.9		5.3±2.6	
Group 4 (*n*=5)	2.4±1.0		1.4±0.5		3.2±1.2	

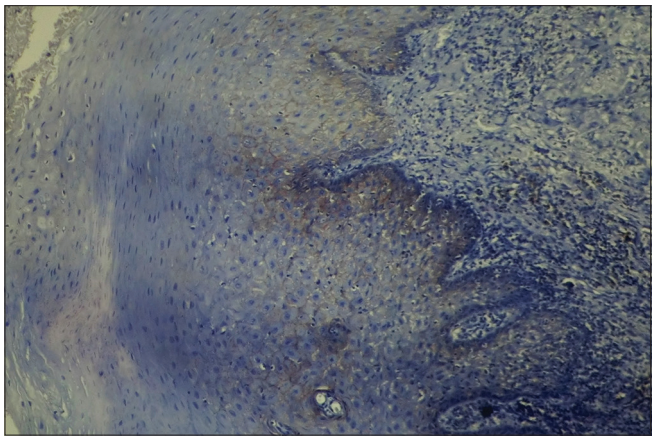


Figure 5: CD44 expression in oral lichen planus with dysplasia

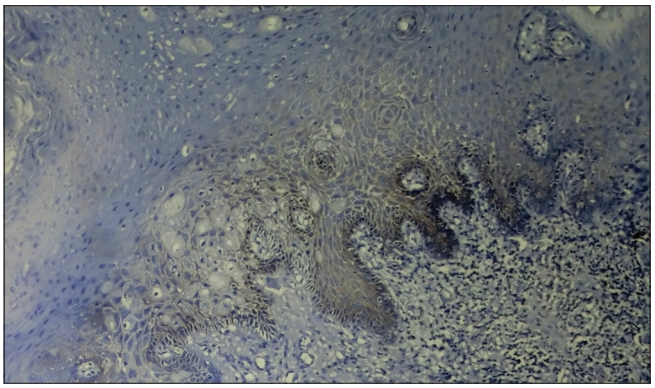


Figure 6: CD44 expression in oral lichenoid dysplasia

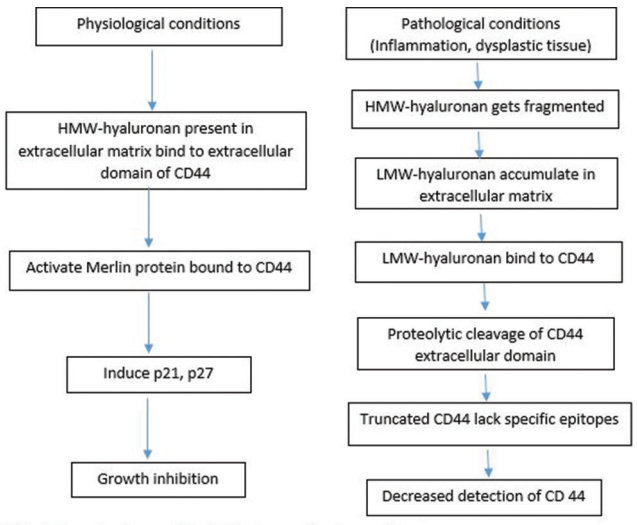
In the majority of the lichenoid dysplasia (80%) and lichen planus with dyaplasia (75%) cases, two-thirds of the epithelium was stained, whereas only one-third of the epithelium was stained in 90% of the normal mucosa. In a study by Asareh *et al*., CD44 positively stained two-thirds of the epithelium in 100% of erosive lichen planus and 80% of epithelial dysplasia. They also observed that the staining pattern was mostly membranous in erosive lichen planus whereas it changed from membranous to cytoplasmic in epithelial dysplasia.[14] Similarly, in the current study, the staining pattern of lichenoid dysplasia and lichen planus with dysplasia changed from membranous to cytoplasmic. Čēma *et al*.[15] propounded that CD44 expression in the membrane as well as in the cytoplasm of dysplastic epithelium could be due to the interaction of CD44 antigen with the cytoskeleton. It has also been suggested that cytoplasmic expression of CD44 expression in oral epithelium can serve as a predictive factor for the malignant transformation of non-homogenous leukoplakia.[16]

In the connective tissue stroma of lichen planus and lichenoid lesions, CD44 expression was positive in inflammatory cells suggesting the role of CD44 in leucocyte adhesion, rolling, aggregation, and activation.[17] Liu *et al*. demonstrated that most of the T cells in the lamina propria of lichen planus expressed CD44 suggesting the role of CD44 in homing T cells to the sites of inflammation.[18]

The transmembrane adhesion molecule, CD44 is a receptor for hyaluronic acid. Under normal conditions, CD44 interacts with hyaluronan to activate merlin protein, thereby inhibiting cell growth [Figure 7]. In inflammatory and dysplastic merlin protein conditions, proteolytic cleavage

of the extracellular domain of CD44 occurs, resulting in truncated CD44 lacking specific epitopes.[17] In accordance with this fact, low membranous immunoreactivity scores were noted in lichenoid dysplasia and lichen planus with dysplasia. Downregulation of CD44 in dysplasia indicates the cleavage of the extracellular domain and a possible increase in low molecular weight heparin. In the study done by Naga *et al*., greater downregulation of CD44 was observed with severe grades of dysplasia. They have proposed that CD44 is essential for signaling epithelial cells to migrate upward. In dysplasia, the altered CD44 expression due to pathological cell adhesion could contribute to invasion and early malignant transformation.[19] A similar result was also evident in the study by Godge *et al*.,[20] wherein CD44v6 isoform expression reduced with an increase in the severity of dysplasia. In our study, the CD44 membranous immunoreactivity score was reduced in lichenoid lesions when compared to lichen planus, reflecting the inflammatory status. The study by Zargaran *et al*.[17] revealed that the membranous staining of CD44 was lower in oral squamous cell carcinoma when compared to oral lichen planus.

CD44 is a marker for cancer stem cells and they play a role in maintaining the phenotype and stemness of cancer stem cells. Ghazi *et al*.[21] investigated the role of CD44 as a cancer stem cell marker in dysplastic and non-dysplastic lichen planus. CD44 expression was high in dysplastic oral lichen planus when compared to non-dysplastic lichen planus implying their involvement in carcinogenesis and malignant transformation of lichen planus which is an oral potentially malignant disorder. Similarly, in our study, the cytoplasmic immunoreactivity of CD44 was more in lichen planus with dysplasia and lichenoid dysplasia when compared to lichen planus and lichenoid lesions. The present study has some limitations, such as its retrospective methodology, limited sample size, and focus on a single center.



HMW - High-molecular-weight; LMW - Low-molecular-weight

Figure 7: Mechanism of action of CD44

Conclusion

The CD44 staining pattern of oral lichenoid dysplasia and lichen planus with dysplasia changed from membranous to cytoplasmic. CD44 membranous immunoreactivity scores were low in lichenoid dysplasia and lichen planus with dysplasia while the cytoplasmic immunoreactivity was high in lichen planus with dysplasia and lichenoid dysplasia when compared to lichen planus and lichenoid lesions. Within the limitations of the study, it can be concluded that CD44 expression can help in assessing the malignant transformation of lichen planus or lichenoid lesions.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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