ORIGINAL ARTICLE
ISSN 2645-4351

https://doi.org/10.22037/jds.v43i3.47062

Galectin-3, COX-2, and CD3 Expression in Oral Lichen Planus, Lichenoid Dysplasia, and Lichen Planus with Dysplasia

Fatemeh Mashhadiabbas[a], Mohammadreza Kashefi Baher[b*], Negar Karimi Hajishoreh[c], Sanaz Gholami[d], Maryam Mohammadalizadeh Chafjiri[d]

[a]Department of Oral and Maxillofacial Pathology, School of Dentistry, Dental Research Center, Research Institute of Dental Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[b]Health Research Center, Chamran Hospital, Tehran, Iran.
[c]Trauma and Surgery Research Center, AJA University of Medical Sciences, Tehran, Iran.
[d]Department of Oral & Maxillofacial Pathology, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
***Correspondence to Author, Mohammadreza Kashefi Baher, Email:** rezakashefi78@gmail.com

Submitted: 31 December 2024
Revised: 20 May 2025
Accepted: 21 May 2025
Published: Summer 2025

How to cite:
Mashhadiabbas F, Kashefi Baher M, Karimi Hajishoreh N Gholamic S, Mohammadalizadeh Chafjirid M. Galectin-3, COX-2, and CD3 expression in oral lichen planus, lichenoid dysplasia, and lichen planus with dysplasia. J Dent Sch 2025;43(3):128-136.

Abstract:

Objective(s): This research aimed to evaluate the immunohistochemical (IHC) expression of Galectin-3 (Gal-3), COX-2, and CD3 in oral lichen planus (OLP), lichenoid dysplasia (LD), and lichen planus with dysplasia (LPD). Analyzing these three markers can provide insight into the molecular role of inflammation in the differentiation and behavior of lichen planus and lichenoid lesions, as well as their potential for malignant transformation. **Methods:** This descriptive, cross-sectional study examined the paraffin blocks of OLP, LD, and LPD obtained from the archives of the Pathology Departments at Shahid Beheshti Dental School and Razi Hospital. A total of 17 OLP, 21 LPD, and 20 LD specimens were stained for Gal-3, COX-2, and CD3 markers using the En-Vision technique, and evaluated by two oral and maxillofacial pathologists. The expression of markers was compared and analyzed using the Chi-Square, Kruskal-Wallis, Mann-Whitney, and Fisher's exact tests, as well as Spearman's correlation coefficient at $p<0.05$. **Results:** High CD3 expression was observed in the connective tissue of all three groups with no significant difference ($P=0.889$). COX-2 expression was similarly low in both the connective tissue and epithelium of all three groups ($P=0.778$ and $P=0.979$, respectively). Gal-3 expression was moderately consistent in the connective tissue of all three groups ($P=0.278$), and weak in the epithelium ($P=0.515$). **Conclusion:** The findings suggested that CD3, COX-2, and Gal-3 play a similar role in inflammation in OLP, LPD, and LD, and are not associated with dysplastic changes.

Keywords: Lichen Planus; Oral; Lichenoid Eruptions; Immunohistochemistry; Galectin 3; Cyclooxygenase 2; CD3 Complex

Introduction

Oral Lichen Planus (OLP) is clinically identified by the existence of white lesions that are bilateral and symmetric. These lesions may or may not have erosions and ulcerations. Additionally, oral lichen planus can manifest as desquamative gingivitis. Histologically, it is characterized by the presence of a sub-epithelial band of lymphocytic infiltrate, vacuolar degeneration of basal and suprabasal layers, or epithelial thinning and ulcerations in the case of atrophic type.[1]

In situations involving dysplastic alterations like hyperchromatism and pleomorphism, the condition is identified as Lichen Planus with Dysplasia (LPD).[2] Lichenoid Dysplasia (LD) is frequently categorized as an oral leukoplakia linked with dysplasia, showing histological similarities to LPD. Nevertheless, it has been established that LD and OLP differ significantly in terms of their underlying causes.[3]

Dafar et al. revealed that the infiltration of T and B cells in the submucosa is more pronounced in OLP compared to oral leukoplakia.[4]

T cells consistently show positive expression of the CD3 marker, which can vary in its range of expression in malignancies, appearing either in the cytoplasm, surface membrane, or a combination of both.[5]

Cyclooxygenase 2 (COX-2), a 72-kDa inducible enzyme crucial for prostaglandin synthesis, is found to be upregulated in oral squamous cell carcinoma (OSCC) [6] and various chronic inflammatory conditions such as gingivitis, periodontitis, apical periodontitis, and OLP.[7, 8]

Galectin-3 (Gal-3), a distinct chimera-type member of the galectin family, demonstrates a variety of immunoregulatory impacts in inflammatory processes mediated by T-cells, autoimmune conditions, and the advancement of tumors.[9] Patients diagnosed with OLP exhibit elevated levels of Gal-3 in their serum when compared to individuals who are in good health.[10] Limited research exists on the presence of Gal-3 in OLP tissues.[11]

This research aimed to evaluate the presence of Gal-3, COX-2, and CD3 in OLP, LD, and LPD using IHC staining, as their combined impact on these conditions had not been

previously studied. Analyzing these markers provides valuable insight into the molecular role of inflammation in the differentiation, behavior, and potential malignant transformation of lichen planus and lichenoid lesions.

Methods

2.1. General procedure

This cross-sectional study assessed the paraffin blocks of OLP, LPD, and LD lesions obtained from the archives of the Pathology Departments at Shahid Beheshti Dental School and Razi Hospital in Tehran, Iran. A total of 17 OLP, 21 LPD, and 20 LD lesions were selected. The inclusion criteria required sufficient clinical information and definitive clinical and pathological diagnoses. Samples with inadequate tissue or missing demographic data were excluded.

Patient records were used to extract information such as age, gender (sex assigned at birth), lesion location, histopathological diagnosis, and presence/absence of dysplastic changes at the time of diagnosis. Two oral and maxillofacial pathologists re-evaluated the Hematoxylin and Eosin (H&E) stained slides to determine the consistency of the OLP and LD diagnoses with the modified WHO criteria.[2]

Approval for the study was obtained from the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.1398.157).

2.2. Immunohistochemical procedures

The tissue samples were subjected to IHC staining for Gal-3, COX-2, and CD3 markers using the En-Vision technique. Tissue sections were sliced to a thickness of 3 µm, placed on silicone-coated slides, and labeled. The slides were then deparaffinized in xylene for eight minutes (repeated three times), rehydrated in graded alcohols (100%, 99%) for five minutes, and rinsed in tap water. Antigen retrieval was carried out by immersing the slides in citrate buffer at 95°C for five minutes (repeated four times). Subsequently, the slides were cooled to room temperature and the staining process continued in a humid chamber. After washing the slides with wash buffer (PBS with a pH of 7.4), they were incubated with CD3 primary antibody (Clone LN10; Leica, USA), COX-2 primary antibody (Clone SP21; Vitro, Spain), and Gal-3 primary antibody (Clone 17C2; Leica, USA) for 60 minutes. Following another round of washing, a secondary antibody was applied for 30 minutes. This was followed by the application of poly HRP (horse radish peroxidase enzyme polymer) reagent for 30 minutes, after which the slides were washed in a wash buffer for 10 minutes. DAB was added to the slides for five minutes and then washed with buffer. The slides were counterstained with hematoxylin and rinsed under running water for five minutes. Finally, the slides were dehydrated in graded alcohols (70%, 80%, 90%, 100%) for five minutes each before being mounted. Tonsillar tissue[5] served as the positive control for CD3, renal tissue for COX-2 [12], and breast tissue for Gal-3[13]. For the negative control, serum was used in place of the primary antibody for the specimens. The IHC expression of the markers was assessed under a light microscope (Leica DM 500) by two oral and maxillofacial pathologists in a blinded fashion.

2.3. Semi-quantitative analysis of immunohistochemistry

The percentage of expression for each marker was calculated by counting the number of stained cells in 10 fields without overlap at x400 magnification. The obtained results were then categorized as follows: [5, 14, 15]

1) CD3 marker: 0%: negative, <10%: weak, 10-25%: moderate, 26-50%: intense, and >50%: very intense.
2) COX-2 marker: <20%: negative, >20% and <40%: weak, >40% and <60%: moderate, >60% and <80%: intense, and >80%: very intense.
3) Gal-3 marker: <5%: negative, 5-20%: weak, 20-50%: moderate, 50-80%: intense, and >80%: very intense.

Furthermore, the intensity of staining for the three groups was scored as follows: no brown cells = 0, light brown cells = 1, dark brown cells = 2, and black cells = 3. [14]

Next, the percentage scores and intensity of staining for the three markers were recorded for all three groups.

2.4. Statistical analysis

Fisher's exact and Chi-square tests were utilized to compare the qualitative nominal variables across the groups. Qualitative ordinal variables were compared using the Kruskal-Wallis test, followed by the Mann-Whitney test for pairwise comparisons. The age of patients was analyzed using one-way ANOVA, while gender distribution was assessed using the Chi-square test. Spearman's correlation coefficient was employed to evaluate the correlation of markers. All statistical analyses were conducted using SPSS version 26 at a significance level of 0.05. Inter-observer agreement was evaluated by calculating the kappa coefficient.

Results

3.1. Demographics, Dysplasia, and Biopsy Site Analysis

A total of 17 OLP, 21 LPD, and 20 samples of LD were chosen. Gender distribution did not show a significant difference among the three groups according to the Chi-square test (P=0.408). The average age of patients across all three groups was 52.45±13.08 years, with no

statistically significant difference observed (P=0.289, one-way ANOVA) (Table 1).

As moderate dysplasia was predominant in both the LPD and LD groups, no significant difference in dysplasia severity was observed between these two groups (Mann-Whitney, P = 0.437). The frequency distribution of the biopsy site did not significantly differ among the three groups (Fisher's exact test, P=0.128), with the buccal mucosa being the most common site. The kappa value for inter-observer agreement was determined to be 0.609, indicating an acceptable level of agreement.

3.2. Histopathologic findings

Figure 1 illustrates the histopathological features of OLP, LPD, and LD, stained with H&E.

Table 1- Demographic Characteristics of the Three Lesion Groups

Sample	Male (N/%)	Female (N/%)	Age (Mean ±SD)
Lichen planus	9 (52.9%)	8 (47.1%)	52.65 ± 11.27
Lichen planus with dysplasia	7 (33.3%)	14 (66.6%)	55.52 ± 15.76
Lichenoid dysplasia	10 (50%)	10 (50%)	49.05 ± 11.05
Total	26	32	52.45 ± 13.08

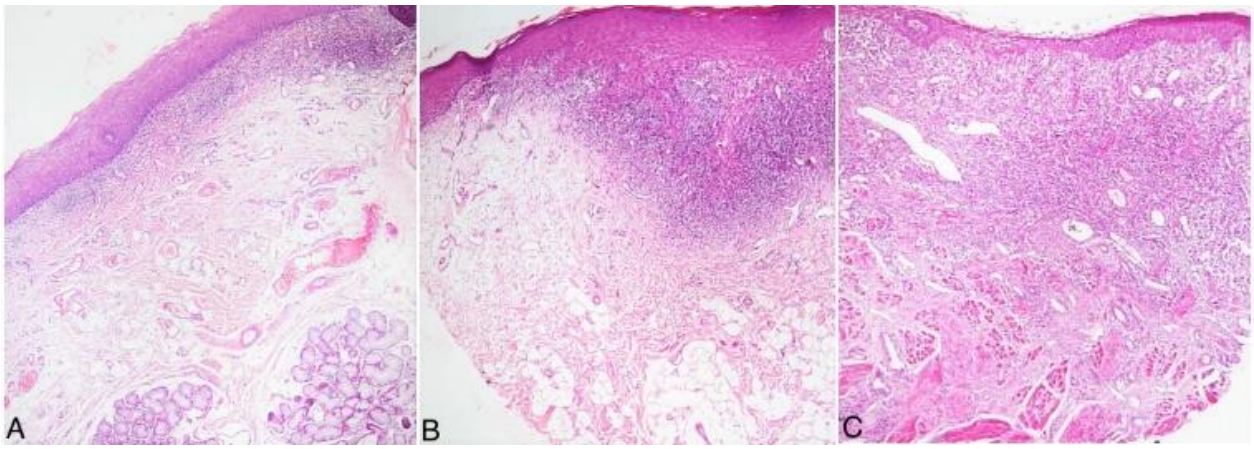

Figure 1: Light microscopic image of H&E staining at 40X magnification in A) oral lichen planus, B) oral lichen planus with dysplasia, and C) oral lichenoid dysplasia.

3.2.1. CD3 marker:

3.2.1.1.CD3 staining of the connective tissue:

In the LPD and LD groups, all specimens showed cytoplasmic staining, while 94.1% of specimens in the OLP group showed cytoplasmic staining. Fisher's exact test revealed no significant difference among the three groups (P = 0.293). The Kruskal-Wallis test showed no significant difference in staining percentage, with most specimens exhibiting very intense staining (P = 0.889) (Table 2).

Table 2- Percentage distribution of CD3 staining of the connective tissue in the three groups of lesions

P value = 0.889	Weak <10%	Moderate 10-25%	Intense 25-50%	Very Intense >50%	Total	Mean Rank
Lichen planus	1 5.9%	1 5.9%	6 35.3%	9 52.9%	17 100.0%	30.71%
Lichen planus with dysplasia	1 4.8%	1 4.8%	9 42.9%	10 47.6%	21 100.0%	29.69%
Lichenoid dysplasia	1 5.0%	2 10.0%	8 40.0%	9 45.0%	20 100.0%	28.28%
Total	3 5.2%	4 6.9%	23 39.7%	28 48.3%	58 100.0%	

3.2.1.2. Intensity of CD3 staining of the connective tissue:

All specimens of the LPD group, 94.1% of OLP specimens, and 75% of LD specimens showed dark brown cells. Fisher's exact test revealed a significant difference between OLP with/without dysplasia and LD in this respect (P=0.015). No significant difference in CD3 infiltration was observed among the groups (P=0.962), with diffuse infiltration being the most common pattern (63.8%) overall. Scattered infiltration was the second most common (24.1%). The mixed infiltration pattern was observed only in the OLP

group, where one sample (5.9%) exhibited both diffuse and scattered patterns, and another showed diffuse and patchy patterns.

3.2.1.3. Intensity of CD3 staining of lymphocytes, plasma cells, fibroblasts, and macrophages:

The Kruskal-Wallis test showed no significant difference in the intensity of CD3 staining of lymphocytes (P=0.734). Blood vessels were not stained for the CD3 marker in any group (P=0.0).

3.2.1.4. CD3 expression in the epithelium among the three groups:

CD3 staining was absent in epithelial cells, with cytoplasmic staining observed only in inflammatory cells within the epithelium (exocytosis). Fisher's Exact Test showed no statistically significant difference among the three groups regarding the location of CD3 staining (P=0.437), with the basal and parabasal regions being the most common locations across all groups. Overall, only one sample (4.8%) in the LPD group exhibited mid to superficial staining.

Figure 2 demonstrates the immunohistochemical view of CD3 expression in oral lichen planus, oral lichen planus with dysplasia, and oral lichenoid dysplasia.

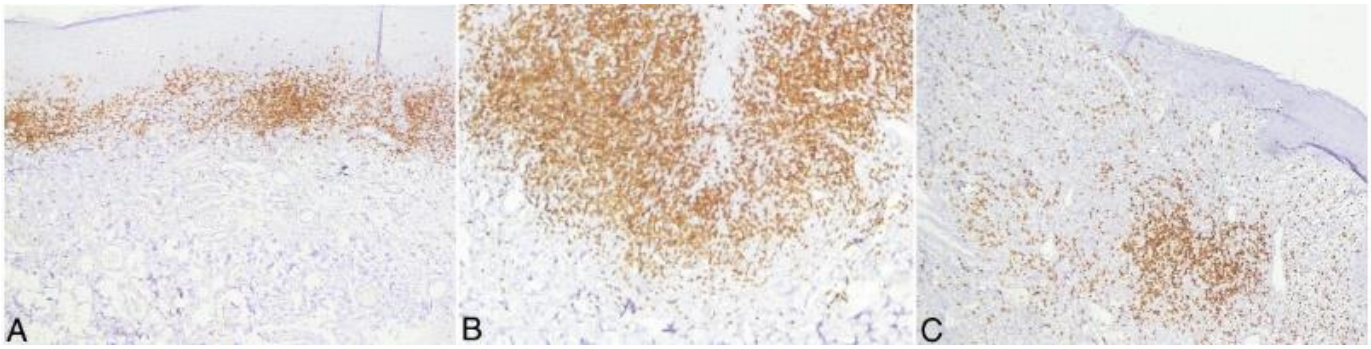

Figure 2: Light microscopic image of IHC staining for CD3 at 100X magnification in A) oral lichen planus, B) oral lichen planus with dysplasia, and C) oral lichenoid dysplasia.

3.2.2. COX-2 marker:

3.2.2.1. COX-2 expression in the connective tissue of the three groups:

All specimens showed cytoplasmic staining, and no significant difference was observed among the three groups in the percentage of COX-2 staining in the connective tissue (Kruskal-Wallis, P = 0.979), with weak staining detected in the majority of specimens (72.4%) (Table 3).

Table 3- Percentage of COX-2 staining of the connective tissue in the three groups

P value = 0.979	Negative <20%	Weak >20-<40%	Moderate >40-<60%	Intense >60%	Total	Mean Rank
Lichen planus	1 5.9%	12 70.6%	3 17.6%	1 5.9%	17 100.0%	28.85
Lichen planus with dysplasia	1 4.8%	16 76.2%	3 14.3%	1 4.8%	21 100.0%	27.83
Lichenoid dysplasia	0 0%	14 70.0%	5 25.0%	1 5.0%	20 100.0%	31.80
Total	2 3.4%	42 72.4%	11 19.0%	3 5.2%	58 100.0%	

3.2.2.2. Intensity of COX-2 staining of the connective tissue in the three groups:

Dark brown staining was observed in 64.7% of OLP specimens and 50% of LD specimens, while 71.4% of LPD specimens showed light brown staining. No significant difference was found among the groups (Chi-square, P = 0.091).

3.2.2.3. COX-2 infiltration pattern in the connective tissue:

Scattered staining was observed in 76.5% of OLP specimens, 81% of LPD specimens, and 50% of LD specimens. Fisher's exact test revealed no significant difference among the groups in this respect (P = 0.979).

3.2.2.4. Intensity of COX-2 staining of lymphocytes and blood vessels:

The Kruskal-Wallis test showed a significant difference

among the three groups in lymphocyte staining intensity for COX-2 (P = 0.036) but no significant difference in blood vessel staining intensity (P = 0.073). COX-2 expression in blood vessels was mainly weak, observed in 64.7% of OLP, 85.7% of LPD, and 95% of LD lesions.

3.2.2.5. COX-2 expression in the epithelium in the three groups:

Fisher's exact test showed no significant difference in the epithelial staining, which was mainly cytoplasmic (P = 0.840). Similarly, the Kruskal-Wallis test revealed no significant difference in staining percentages among the groups (P = 0.778), with the majority (52.2%) exhibiting weak staining. Moderate staining was the second most common (25.9%), and none of the OLP or LPD samples showed intense staining.

Regarding the COX-2 staining intensity in the epithelium, 76.5% of OLP, 90.5% of LPD, and 80% of LD specimens showed light brown staining, while the rest showed dark brown staining. Fisher's exact test found no significant difference among the groups (P = 0.466). Regarding the staining location, 52.9% of OLP lesions showed staining up to the middle of the epithelium, while 57.2% of LPD and 60% of LD specimens showed staining to the surface. This difference was also not significant (Fisher's exact test, P = 0.534). Figure 3 shows the immunohistochemical view of COX-2 expression in oral lichen planus, oral lichen planus with dysplasia, and oral lichenoid dysplasia.

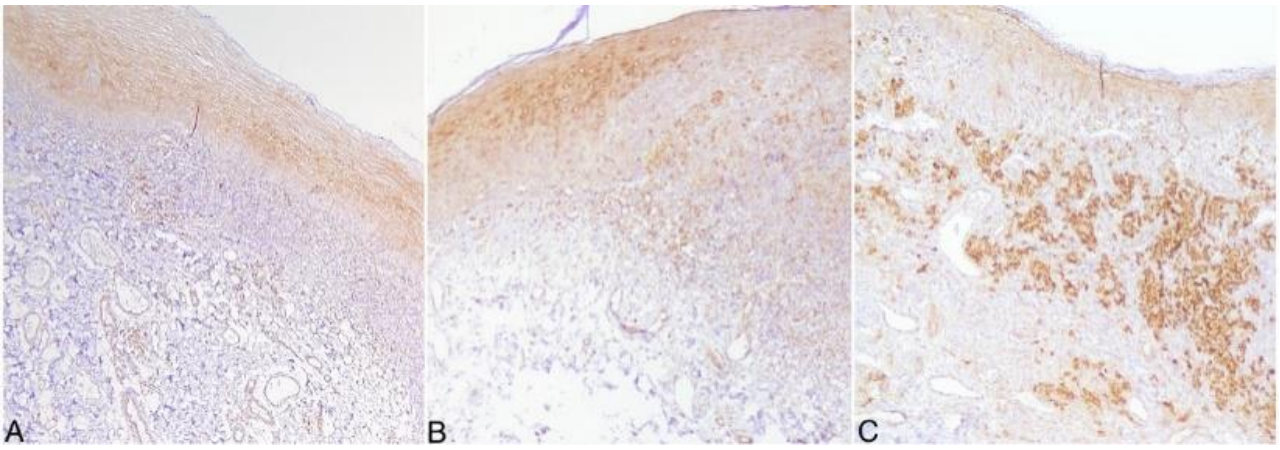

Figure 3: Light microscopic image of IHC staining for COX-2 at 100X magnification in A) oral lichen planus, B) oral lichen planus with dysplasia, and C) oral lichenoid dysplasia.

3.2.3. Gal-3 marker:

3.2.3.1. Expression of Gal-3 in the connective tissue:

Cytoplasmic staining occurred in all LPD, 82.4% of OLP, and 95% of LD specimens, while nuclear staining was found in three OLP and one LD specimen. Fisher's exact test revealed a significant difference among the groups, with lower expression in the OLP group (P = 0.014). The Kruskal-Wallis test revealed no significant differences in the percentage of staining among the groups, with moderate staining being the most common in all groups (P = 0.278). The percentage of staining was higher in the LD group compared to the others (Table 4).

Table 4- Percentage distribution of Gal-3 staining of the connective tissue in the three groups

P value = 0.278	Negative <5%	Weak 5-20%	Moderate 20-50%	Intense 50-80%	Very Intense >80%	Total	Mean Rank
Lichen planus	1 5.9%	6 35.3%	7 41.2%	3 17.6%	0 0%	17 100.0%	27.41
Lichen planus with dysplasia	0 0%	8 38.1%	11 52.4%	1 4.8%	1 4.8%	21 100.0%	27.50
Lichenoid dysplasia	0 0%	5 25.0%	10 50.0%	4 20.0%	1 5.0%	20 100.0%	33.38
Total	1 1.7%	19 32.8%	28 48.3%	8 13.8%	2 3.4%	58 100.0%	

Diffuse infiltration was observed in 52.9% of OLP specimens, while scattered infiltration was noted in 71.4% of LPD and 45% of LD specimens. Fisher's exact test showed no significant difference in this respect (P = 0.278).

The Kruskal-Wallis test showed no significant differences among the three groups in staining intensity (P = 0.862), with 75.9% of all specimens exhibiting light brown discoloration (Table 5).

Table 5 - Intensity of Gal-3 staining of the connective tissue in the three groups

P value =0.862	Light Brown Cell	Dark Brown Cell	Black Cell	Total	Mean Rank
Lichen planus	13 76.5%	4 23.5%	0 0%	17 100.0%	29.21
Lichen planus with dysplasia	15 71.4%	6 28/6%	0 0%	21 100.0%	30.64
Lichenoid dysplasia	16 80.0%	3 15.0%	1 5.0%	20 100.0%	28.55
Total	44 75.9%	13 22.4%	1 1.7%	58 100.0%	

3.2.3.2. Intensity of Gal-3 staining of lymphocytes and blood vessels:

The Kruskal-Wallis test showed no significant difference in lymphocyte staining intensity for Gal-3 among the groups (P = 0.828), with 77.6% of specimens showing no expression. Similarly, Gal-3 expression by blood vessels was not statistically significant across the groups (P = 0.213).

3.2.3.3. Expression of Gal-3 by the epithelium in the three groups:

Gal-3 expression by the epithelium differed significantly among the groups (Fisher's exact test, P= 0.002). However, the Kruskal-Wallis test showed similar staining percentages across the groups, with weak staining predominant (P = 0.515). Negative staining followed in the LPD and OLP groups, while moderate staining was second most common in the LD group. Only one OLP sample (5.9%) showed very intense staining, with the LD group having the highest mean rank.

The three groups showed no significant difference in staining intensity (Kruskal-Wallis test, P = 0.565), with most cells (75.9%) displaying light brown staining. Notably, only one sample (5%) in the LD group exhibited black cells, and one sample (5.9%) in the OLP group showed no brown cells.

The location of Gal-3 staining in the epithelium differed significantly among the groups (Fisher's exact test, P = 0.024). Staining in the basal, para-basal, and stratum spinosum layers was observed in 94.1% of OLP, 61.9% of LPD, and 50% of LD specimens.

Spearman's correlation coefficient showed a significant association between higher staining intensity of blood vessels for COX-2 and Gal-3 (r = 0.301, P = 0.022) and between lymphocyte staining intensity for COX-2 and CD3. No other significant correlations were found (P > 0.05).

Figure 4 shows the immunohistochemical view of Gal-3 expression in oral lichen planus, oral lichen planus with dysplasia, and oral lichenoid dysplasia.

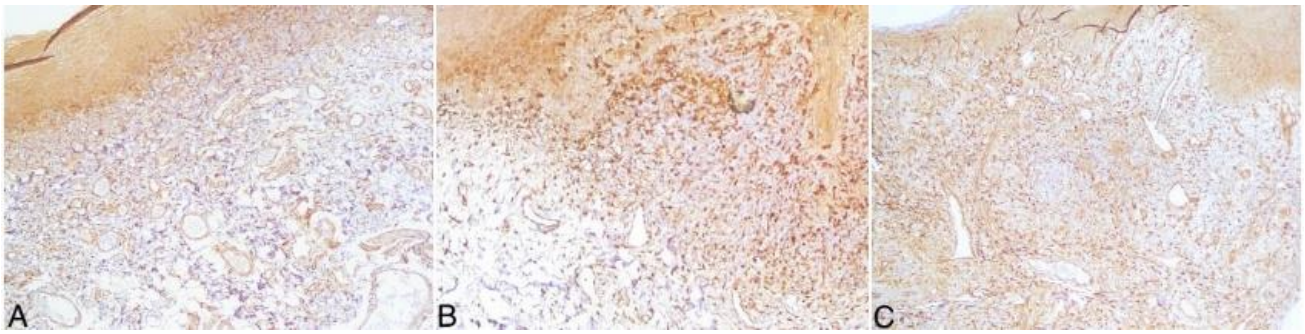

Figure 4: Light microscopic image of IHC staining for Gal-3 at 100X magnification in A) oral lichen planus, B) oral lichen planus with dysplasia, and C) oral lichenoid dysplasia.

Discussion

All three groups exhibited predominant cytoplasmic staining, which aligned with previous findings[16]. The connective tissue displayed a highly intense (>50%) CD3 staining in all three groups, with no significant variation. CD3 staining of cutaneous lichen planus lesions was also reported to be >50% by Ramazani et al. [5], suggesting the involvement of CD3 in the inflammatory process of both cutaneous lichen planus and OLP. Motta et al.[16] reported a CD3 staining rate of 142.2 cells/mm2 in OLP, which closely resembled the present findings despite employing different methodologies.

The rate of CD3 staining was reported to be 142.2 cells/mm^2 in OLP, which closely matched the present findings despite using a different methodology. The intensity of CD3 staining in the connective tissue did not show any significant difference among the three groups (dark brown or moderate intensity). Therefore, CD3 staining cannot be used as a marker to differentiate between OLP and dysplastic lesions. Ramezani et al. [5] and Maehara et al. [17] also observed dark brown and strong CD3 staining in OLP, respectively, which aligned with the present results. In the present study, all three groups exhibited similar CD3 infiltration in the connective tissue, with band-like infiltration observed in over 50% of specimens in each group, consistent with previous studies. Lymphocytes showed CD3 staining in 79.3% of specimens across the three groups, mainly with moderate intensity and no significant variation. This emphasizes the involvement of lymphocytes in the inflammatory process of all types of lesions. CD3 expression by plasma cells was consistent across all groups, with more than 50% of plasma cells not exhibiting CD3 staining, indicating their minimal role in the inflammatory or dysplastic processes of these lesions. Blood vessels in all groups did not show CD3 staining, suggesting their insignificant contribution to the inflammatory or dysplastic processes. Previous studies did not address this aspect for comparison. Epithelial cells did not exhibit CD3 staining, with only inflammatory cells in the epithelium showing cytoplasmic staining (exocytosis). In contrast, Popovska et al.[18] reported significantly higher CD3 staining in the epithelium of OLP compared to normal mucosa. CD3 was consistently positive in T cells, and its overexpression was associated with inflammatory or dysplastic lesions.[5]

The current investigation unveiled that the connective tissue exhibited cytoplasmic staining for COX-2 in all three groups of specimens, with a similar pattern observed in the epithelium. This staining was primarily cytoplasmic and supported previous findings.[19] The intensity of staining was predominantly weak across all three groups, with no significant variation. This emphasizes the role of COX-2 in the inflammatory process of all three types of lesions. Chankong et al.[20] reported a significant over-expression of COX-2 in OLP. However, Cortés-Ramírez et al.[14] demonstrated higher expression of COX-2 in OLRs compared to OLP in both the epithelium and connective tissue, which differed from our findings. Arreaza et al.[21] reported that 53% of OLRs and 81% of OLP cases tested positive for COX-2, which contrasted with the present results. This disparity may be attributed to their evaluation of reactive OLRs. Taghavi et al.[12] reported that 53.2% of OLP cases exhibited COX-2 expression in the connective tissue, with 59.6% of cases showing a moderate intensity of staining, which was higher than that observed in normal tissue and differed from our findings.

The pattern of COX-2 infiltration was predominantly scattered in all three groups, with no significant variation. Chankong et al. [20] reported a band-like infiltration of COX-2 in the lamina propria, while Arreaza et al.[21] demonstrated a band-like infiltration of COX-2 in the basal layer and submucosa of OLP and reactive OLRs, which closely resembled the pattern observed in this study.

In the present study, the LPD and LD lesions exhibited a higher intensity of staining for COX-2 in blood vessels compared to OLP. This suggests a potential role of angiogenesis in dysplastic transformation. Gately and Li [22] reported an increased expression of COX-2 in premalignant and malignant lesions, as well as in endothelial cells, immune cells, and tumoral cells, which aligns with the present results, despite their evaluation of different lesions. There were no significant differences observed among the three groups in terms of COX-2 staining intensity.

In the current investigation, a significant distinction was observed in the expression of Gal-3 in the connective tissue. Specifically, 82.4% of OLP cases, 100% of LPD cases, and 95% of LD cases exhibited cytoplasmic staining. Similarly, there was a notable difference in Gal-3 expression in the epithelium, with 70.6% of OLP cases, 95.2% of LPD cases, and 95.2% of LD cases showing cytoplasmic staining. These findings align with previous studies.[15, 23, 24] The three groups did not differ significantly in terms of the percentage or intensity of staining, both of which were predominantly moderate or light brown, respectively. These results suggest that Gal-3 may play a role in the inflammatory process. Additionally, the three groups exhibited similar patterns of Gal-3 infiltration in the connective tissue, with band-like infiltration observed in 52.9% of OLP cases, scattered infiltration in 71.4% of LPD cases, and a combination of band-like and scattered infiltration in 50% of LD lesions. These findings further support the involvement of Gal-3 in inflammation. Unfortunately, no similar study was available for comparison in this regard. The three groups also showed similar intensity of staining, predominantly low, and percentage of staining in the epithelium, predominantly weak and light brown. Therefore, it appears that this marker does not play a role in dysplastic changes of the epithelium. Previous studies have reported conflicting

results regarding the intensity of staining for this marker in OSCC.[15, 25] Gal-3 staining in the epithelium was primarily observed in the basal and para-basal layers, as well as the stratum spinosum. Again, no similar study was available for comparison in this field. The present findings suggest that this marker is involved in inflammation and angiogenesis. While the findings of the present study offer valuable insights, the results should be interpreted with caution due to the relatively small sample size and the use of convenience sampling, which may have introduced selection bias. Larger future studies employing random or stratified sampling methods are recommended to validate these observations and enhance representativeness.

Conclusion

The roles of CD3, COX-2, and Gal-3 in the inflammatory process of OLP, LPD, and LD are similar, but they do not play a role in dysplastic changes. Therefore, these markers cannot be utilized to distinguish dysplastic lesions.

Acknowledgement: We would like to extend our gratitude to everyone who contributed to the successful completion of this research.

Author Contributions: F.M: Conceptualization, Methodology, Supervision; and M.K.B: Writing – Original Draft, Revising, Data Curation; and N.K.H: Data Analysis, Review; and Sanaz Gholami: Investigation, Visualization, Corresponding author responsibilities; and M.M.C: Review & Editing, Experimental Design.

Funding: No funding was received for this research.

Ethical Approval Code: The study was approved by the Ethical Committee of Shahid Beheshti University of Medical Sciences with the ethical code IR.SBMU.DRC.1398.157, ensuring adherence to all ethical considerations throughout the research process.

Informed Consent Statement: This study utilized archived paraffin blocks and anonymized patient records, ensuring no additional procedures or interventions were performed on participants.

Data Availability Statement: The datasets generated during the current study are available from the corresponding author upon reasonable request.

Using AI: We would like to acknowledge the use of OpenAI's ChatGPT as a tool in revising and refining the content, for enhancing the clarity and quality of this manuscript.

Conflict of Interest: The authors declare no conflict of interest.

References

1. Warnakulasuriya S, Kujan O, Aguirre-Urizar JM, Bagan JV, González-Moles MÁ, Kerr AR, et al. Oral potentially malignant disorders: a consensus report from an international seminar on nomenclature and classification, convened by the WHO collaborating centre for oral cancer. Oral Dis. 2021;27(8):1862-80. doi: 10.1111/odi.13704

2. Rad M, Hashemipoor MA, Mojtahedi A, Zarei MR, Chamani G, Kakoei S, et al. Correlation between clinical and histopathologic diagnoses of oral lichen planus based on modified WHO diagnostic criteria. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107(6):796-800. doi: 10.1016/j.tripleo.2009.02.020

3. Mittal K, Jha M, Patil RS, Kapoor S. Lichenoid dysplasia–a case report with a review of differential diagnosis. J Clin Diagn Res. 2017;11(5):ZD01-2. doi: 10.7860/jcdr/2017/27360.9803

4. Dafar A, Siarov A, Mostaghimi Y, Robledo-Sierra J, De Lara S, Giglio D, et al. Langerhans cells, T cells, and B cells in oral lichen planus and oral leukoplakia. Int J Dent. 2022;2022:5430309. doi: 10.1155/2022/5430309

5. Ramezani M, Hashemi BS, Khazaei S, Rezaei M, Ebrahimi A, Sadeghi M. Diagnostic value of immunohistochemistry staining of Bcl-2, CD34, CD20 and CD3 for distinction between discoid lupus erythematosus and lichen planus in the skin. Indian J Pathol Microbiol. 2017;60(2):172-6. doi: 10.4103/0377-4929.208381

6. Pannone G, Bufo P, Caiaffa MF, Serpico R, Lanza A, Muzio LL, et al. Cyclooxygenase-2 expression in oral squamous cell carcinoma. Int J Immunopathol Pharmacol. 2004;17(3):273-82. doi: 10.1177/039463200401700307

7. Neppelberg E, Johannessen AC. DNA content, Cyclooxygenase-2 expression and loss of E-cadherin expression

do not predict risk of malignant transformation in oral lichen planus. Eur Arch Otorhinolaryngol. 2007;264:1223-30. doi: 10.1007/s00405-007-0346-5

8. Zhang F, Engebretson SP, Morton RS, Cavanaugh PF Jr, Subbaramaiah K, Dannenberg AJ. The overexpression of cyclo-oxygenase-2 in chronic periodontitis. J Am Dent Assoc. 2003;134(7):861-7. doi: 10.14219/jada.archive.2003.0284

9. Radosavljevic G, Volarevic V, Jovanovic I, Milovanovic M, Pejnovic N, Arsenijevic N, et al. The roles of galectin-3 in autoimmunity and tumor progression. Immunol Res. 2012;52(1-2):100-10. doi: 10.1007/s12026-012-8286-6

10. Ghapanchi J, Andisheh-Tadbir A, Torkaman P, Malekzadeh M, Mardani M. Evaluation of the serum levels of galectin-3 in patients with oral lichen planus disease. Oral Dis. 2019;25(2):466-70. doi: 10.1111/odi.13012

11. Muniz JM, Bibiano Borges CR, Beghini M, de Araújo MS, Miranda Alves P, de Lima LM, et al. Galectin-9 as an important marker in the differential diagnosis between oral squamous cell carcinoma, oral leukoplakia and oral lichen planus. Immunobiology. 2015;220(8):1006-11. doi: 10.1016/j.imbio.2015.04.004

12. Taghavi N, Mahdavi N, Shahla M. Correlation of Bcl-2 and COX-2 expression in oral lichen planus. J Iran Dent Assoc. 2014;26(2):114-21.

13. Fitzpatrick SG, Hirsch SA, Gordon SC. The malignant transformation of oral lichen planus and oral lichenoid lesions: a systematic review. J Am Dent Assoc. 2014;145(1):45-56. doi: 10.14219/jada.2013.10.

14. Cortés-Ramírez DA, Rodríguez-Tojo MJ, Gainza-Cirauqui ML, Martínez-Conde R, Aguirre-Urizar JM. Overexpression of cyclooxygenase-2 as a biomarker in different subtypes of the oral lichenoid disease. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;110(6):738-43. doi: 10.1016/j.tripleo.2010.08.005

15. Hossaka TA, Ribeiro DA, Focchi G, André S, Fernandes M, Lopes Carapeto FC, et al. Expression of galectins 1, 3 and 9 in normal oral epithelium, oral squamous papilloma, and oral squamous cell carcinoma. Dent Res J (Isfahan). 2014;11(4):508-12.

16. Motta A, Zhan Q, Larson A, Lerman M, Woo SB, Soiffer RJ, et al. Immunohistopathological characterization and the impact of topical immunomodulatory therapy in oral chronic graft-versus-host disease: a pilot study. Oral Dis. 2018;24(4):580-90. doi: 10.1111/odi.12813

17. Maehara T, Moriyama M, Kawano S, Hayashida JN, Furukawa S, Ohta M, et al. Cytokine profiles contribute to understanding the pathogenic difference between good syndrome and oral lichen planus: two case reports and literature review. Medicine (Baltimore). 2015;94(14):e704. doi: 10.1097/md.0000000000000704

18. Grossmann SdMC, de Oliveira Cde N, Souto GR, Góes C, Mesquita RA. Oral lichenoid lesion: a review of the literature. World J. Dent. 2015;4(2):103-7. doi: 10.5321/wjs.v4.i2.103

19. Hla T, Bishop-Bailey D, Liu C, Schaefers HJ, Trifan OC. Cyclooxygenase-1 and-2 isoenzymes. Int J Biochem Cell Biol. 1999;31(5):551-7. doi: 10.1016/s1357-2725(98)00152-6

20. Chankong T, Chotjumlong P, Sastraruji T, Pongsiriwet S, Iamaroon A, Krisanaprakornkit S. Increased cyclooxygenase 2 expression in association with oral lichen planus severity. J Dent Sci. 2016;11(3):238-44. doi: 10.1016/j.jds.2015.12.002

21. Arreaza AJ, Rivera H, Correnti M. Expression of COX-2 and bcl-2 in oral lichen planus lesions and lichenoid reactions. Ecancermedicalscience. 2014;8:411. doi: 10.3332/ecancer.2014.411

22. Gately S, Li WW. Multiple roles of COX-2 in tumor angiogenesis: a target for antiangiogenic therapy. Semin Oncol. 2004;31(2 Suppl 7):2-11. doi: 10.1053/j.seminoncol.2004.03.040

23. Newlaczyl AU, Yu LG. Galectin-3–a jack-of-all-trades in cancer. Cancer Lett. 2011;313(2):123-8. doi: 10.1016/j.canlet.2011.09.003

24. Hughes RC. Secretion of the galectin family of mammalian carbohydrate-binding proteins. Biochim Biophys Acta. 1999;1473(1):172-85. doi: 10.1016/s0304-4165(99)00177-4

25. Tokmak S, Arık D, Pınarbaşlı Ö, Gürbüz MK, Açıkalın MF. Evaluation and prognostic significance of galectin-3 expression in oral squamous cell carcinoma. Ear Nose Throat J. 2021;100(5_suppl):578S-83. doi: 10.1177/0145561319893861