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Evaluation of the Immunohistochemical Expression of Serotonin in Oral Lichen Planus

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ABSTRACT

Background: Oral lichen planus (OLP) is a T-cell-mediated disorder of oral mucosa and is mainly observed in middle-aged adult women. The etiology of OLP is unclear.

Objective: This study investigates the potential role of serotonin in the pathogenesis of OLP, thourgh evaluation of the immunohistochemical expression of serotonin (5-HT) in biopsies from patients with OLP and its association with clinicopathological findings.

/Patients and methods: Paraffin-embedded tissue samples were histologically confirmed as OLP. Monoclonal antibody serotonin was used for immunohistochemical staining. The patterns of positively stained cells were analyzed using semiquantitative techniques.

Results: A total 40 biopsies from 24 female and 16 male OLP patients were involved. The mean age was 49.15 (SD 11.39) years. Using an immunohistochemical method, the results showed that the intensity of expression of 5-HT at the basal layer was established in 11 (27.5%) cases who had grade 0, 18 (45%) cases had grade 1, and 11 (27.5%) cases had grade 2, with a median H-index of 55 ranging between 0-200. The intensity of 5-HT expression at the suprabasal layer showed that 7 (17.5%) cases had grade 0, 15 (37.5%) cases had intensity grade 1, 16 (40%) cases had intensity grade 2, and only two (5%) cases had grade 3, with a median H-index was 80, ranging between 0-300. The median total H-index was 153.5 (SD 111.5), ranging between 0-500. **Conclusions:** Serotonin has been proven in the epithelium of oral lichen planus in most studied cases, which is considered an important marker in its pathogenesis.

Keywords: Serotonin, 5-HT, Oral lichen planus, Paraffin-embedded tissue sample, Immunohistochemical staining, University of Baghdad.

INTRODUCTION

Oral lichen planus (OLP) is the most common noninfectious oral mucosal disorder. It is a chronic immune-mediated mucocutaneous condition [(1)], and the pathogenesis of OLP and its triggering factors are unknown [(2)]. Women are more likely than men to get OLP, primarily affecting people over 40 years. However, it can also affect younger adults and children [(3)].

Serotonin, (5-HT**),** is a neurotransmitter and hormone whose effects on the central nervous system (CNS) and the corresponding organ systems help regulate several physiological functions. It's interesting to note that a significant amount of serotonin is also produced outside the CNS. Serotonin receptors are likewise widely expressed in a diversity of peripheral organs [(4)]. Intestinal chromaffin cells in the gastrointestinal tract are the generators of peripheral serotonin, which is released into the bloodstream, then occupied by platelets, and attracted to the site of inflammation upon activation. Additionally, a potent immunological regulator, peripheral 5-HT, affects various immune cells through the immune cells expressed at receptors (5-HTRs), comprised of the innate and adaptive immune systems [(5)]. Therefore, it makes sense to suppose that 5-HT contributes to the pathophysiology of autoimmunity disorders, which could openup new avenues for dealing with them [(6)].

This study investigates the potential role of serotonin in the pathogenesis of OLP, thourgh evaluation of the immunohistochemical expression of serotonin (5-HT) in biopsies from patients with OLP and its association with clinicopathological findings.

PATIENTS AND METHODS

Study design: The study was based on paraffin-embedded tissue blocks and their accompanying histopathological inspection reports for 40 confirmed OLP samples collected, between 2012 and 2020.

Study samples: The samples were from the archives of the Oral Pathology Department, College of Dentistry, University of Baghdad. Cases with adequate tissue samples and available clinical information confirmed by histopathological features based upon modified WHO diagnostic criteria with the relevant casereports' data on age, gender, and site were taken.

Sample processing: Two sections of 4 μm thickness were cut and put onto microscopic charge slides from each block. To reassess the histological picture of OLP and ensure enough tissue, the slide was stained with hematoxylin and eosin and an IHC analysis was carried out on the remaining slide.

The histopathological assessment of inflammation:

Two pathologists subjectively reviewed the OLP cases' microscopic slides to establish the level of inflammation. To undertake a semiquantitative evaluation of inflammation, cases were classified into

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1 of 4 groups:
- Score 0, 0–10% of the total cells were stained.
- Score (+), 10–29% of the total cells were stained.
- Score (++), 30–49% of the total cells were stained.
- Score (+++)>50% of the total cells were stained [7].

Immunohistochemically, Deparaffinization, rehydration, antigen retrieval, blocking endogenous peroxidase, and then the addition of primary and secondary antibodies to the slide constitute the majority of the IHC procedure. The main antibody was a monoclonal anti-serotonin antibody (MBS530895, 1:50 dilution) obtained from Mybiosource Biotechnology.

The scoring system:

The scoring system for the 5-HT marker was applied semi-quantitatively Histo-score (H-score) in the epithelium, and the intensity of stainingwas only for inflammatory cells and blood vessels in the connective tissue [8] Semiquantitative, combining both the intensity and the percentage of immunoreactivity using the formula: H-score = Σ (intensity of staining X percentage of stained area), where (Grade 0) no staining (−), (Grade 1) is mild staining (+); (Grade 2) moderate staining (++); and (Grade 3) strong staining (+++). The H-score ranged from 0–300 [8].

Serotonin cytoplasmic immunoreactivity was considered positive in any number of cells [9]. Evaluation of serotonin staining localization was conducted, and cases were classified as suprabasal staining, basal staining, or both [9].

Ethical Considerations:

Ethical approval was obtained for this study by the Ethics Committee in the Department of Oral Diagnosis, College of Dentistry, University of Baghdad (Ref # 272, date: 25/3/2021).

Statistical Analysis

The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 25 for windows. Qualitative data were defined as numbers and percentages.

Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test.

Normal distribution of variables was described as means and standard deviation (SD), and independent sample t-test/Mann-Whitney U test and ANOVA/Kruskal-Wallis test were used for comparison between groups. P value ≤0.05 was considered to be statistically significant.

RESULTS

This study included 40 cases of oral lichen palnus. **Table 1** summarizes the sociodemograhic data of the included patients.

Table (1): Sociodemographic findings of the studied cases.

Sociodemographic characteristics		Number	Percentage	
Age (years)	<40 years	7	17.5%	
	40-49 years	13	32.5%	
	50-59 years	10	25%	
	≥60 years	10	25%	
Mean ± SD (Range)		49.15 ± 11.39 (28-69)		
Gender	Male	16	40%	Mean 45.81 (SD 10.41)
	Female	24	60%	Mean 51.37 (SD 11.68)
Total		40	100%	

The clinical presentation of the patients under study revealed that, as shown in **Table 2**, 19 (47.5%) cases had the reticular form of oral lichen planus, and 21 (52.5%) cases had the erosive type. Regarding the observed site distribution, 30 (75%) of the analyzed cases with OLP were in the buccal mucosa site, while the remaining cases were dispersed in smaller numbers.

Table (2): Clinical types and sites of OLP in the studied group

Clinical and Sites	Classes	No.	%
Clinical	Reticular	19	47.5%
	Erosive	21	52.5%
Total		40	100%
Sites	Buccal Mucosa	26	65%
	Tongue	4	10%
	Gingiva	3	7.5%
	Palatal Mucosa	3	7.5%
	Upper lip+Buccal mucosa	2	5%
	Retromolar aera+ Buccal mucosa	1	2%
	Crest of Alveolar Ridge+ Buccal mucosa	1	2.5%
Total		40	100%

The histopathological assessment of inflammation was taken into account when classifying OLP cases. In this study, the inflammatory score + among 11 (27.5%) cases, score ++ among 14 (35.0%) cases, and score +++ among 15 (37.5%) cases, **Table 3**.

Table (3): The score for the inflammatory assessment of OLP among studied groups

Histopathological assessment of inflammation		Number	Percentage
Inflammatory score	**Score +**	11	27.5%
	Score ++	14	35%
	Score +++	15	37.5%
Total		40	100%

Evaluation of IHC expression of serotonin (5-HT) marker

The 5-HT marker's IHC expression was assessed; regarding the intensity of 5- HT expression in the basal layer, findings revealed that of the studied cases, 11 (27.5%) cases had grade 0, 18 (45%) cases had grade 1, and 11 (27.5%) cases had grade 2, with a median H-index of 55 ranging between 0-200. The intensity of 5- H.T. expression at the suprabasal layer showed that 7 (17.5%) cases had grade 0, 15 (37.5%) cases had intensity grade 1, 16 (40%) cases had intensity grade 2, and only two (5%) cases had grade 3, with a median H-index was 80, ranging between 0-300. The median total H-index was 153.5 (SD 111.5), ranging between 0-500 (**T able 4 and Figure 1**).

Table (4): Expression of 5-HT among studied cases.

Expression of 5-HT			Number	Percentage
Basal	**Intensity**	Grade 0	11	27.5%
		Grade I	18	45.0%
		Grade 2	11	27.5%
	H. score index	Median= 55, Ranging between 0-200		
Suprabasal	**Intensity**	Grade 0	7	17.5%
		Grade I	15	37.5%
		Grade 2	16	40.0%
		Grade 3	2	5.0%
	H. score index	Median=80 Ranging between 0-300		
Total H. score		Median= 153.5 (SD 111.5), Ranging between 0-500		

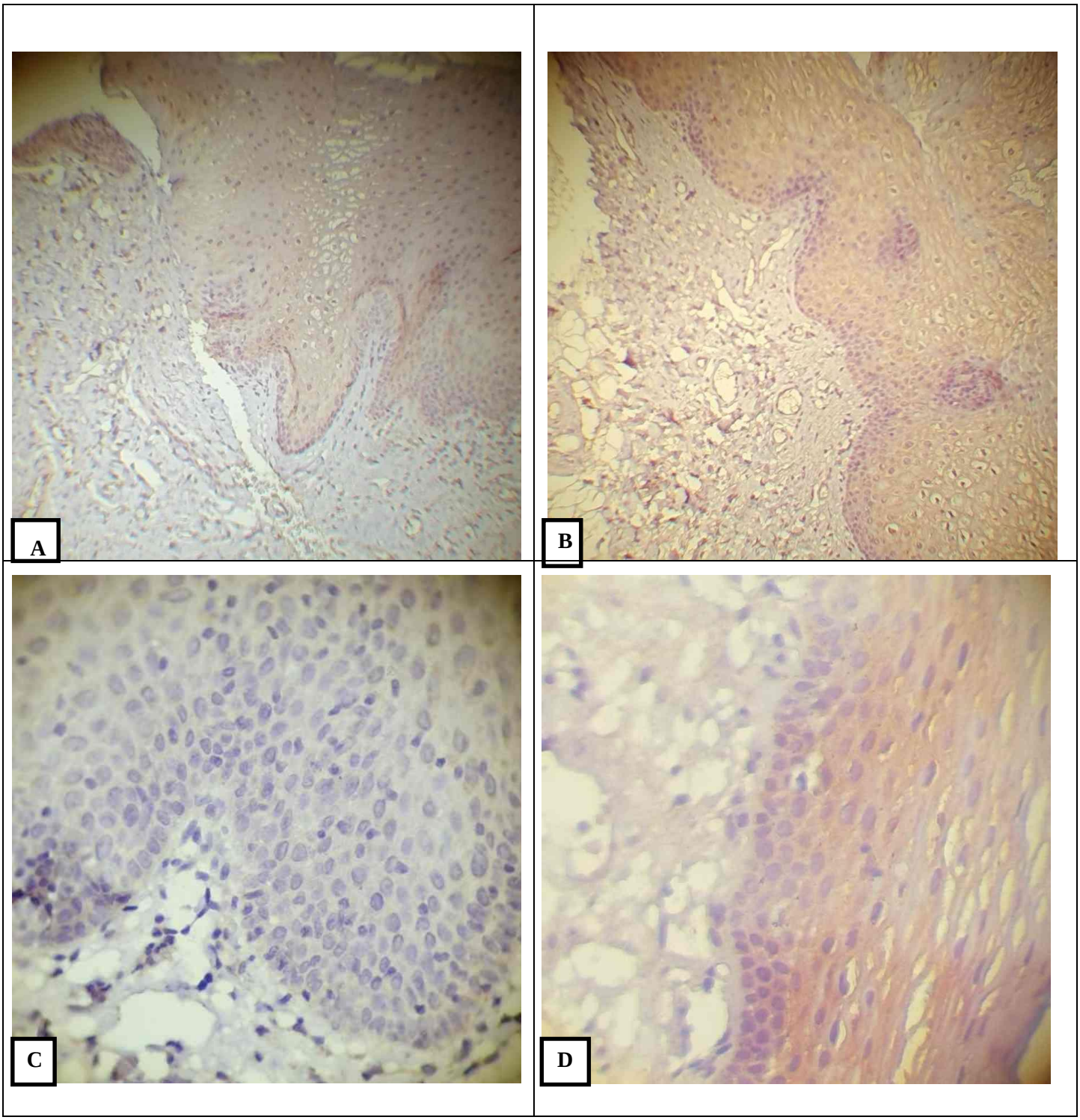

Figure (1): Immunohistochemical expression of serotonin (5-HT) marker in OLP cases. (A& B):-Diffuse cytoplasmic staining of serotonin expression in all epithelial layers (x100) (C&D):- High-power view of the previous section (×400).

Additionally, inflammatory cells and blood vessels showed positive immunoreactivity for serotonin, as described in **Table 5** and **Figure 2**.

The current study indicated that serotonin expression in inflammatory and capillary endothelial cells was in 31 (77.5%) and 32 (80%) cases, respectively. Strong serotonin expression appeared in 13 (32.5%) and 15 (37.5%) cases, respectively.

Table (5): Expression of the 5-HT marker regarding the intensity of staining in inflammatory and capillary endothelial cells in OLP cases

Expression of 5-HT	Intensity	Number	Percentage
Inflammatory cells	**Negative**	9	22.5%
	Mild	3	7.5%
	Moderate	15	37.5%
	Strong	13	32.5%
Total positive		31	77.5%
Capillary endothelial cells	**Negative**	8	20%
	Mild	1	2.5%
	Moderate	16	40%
	Strong	15	37.5%
Total positive		32	80%

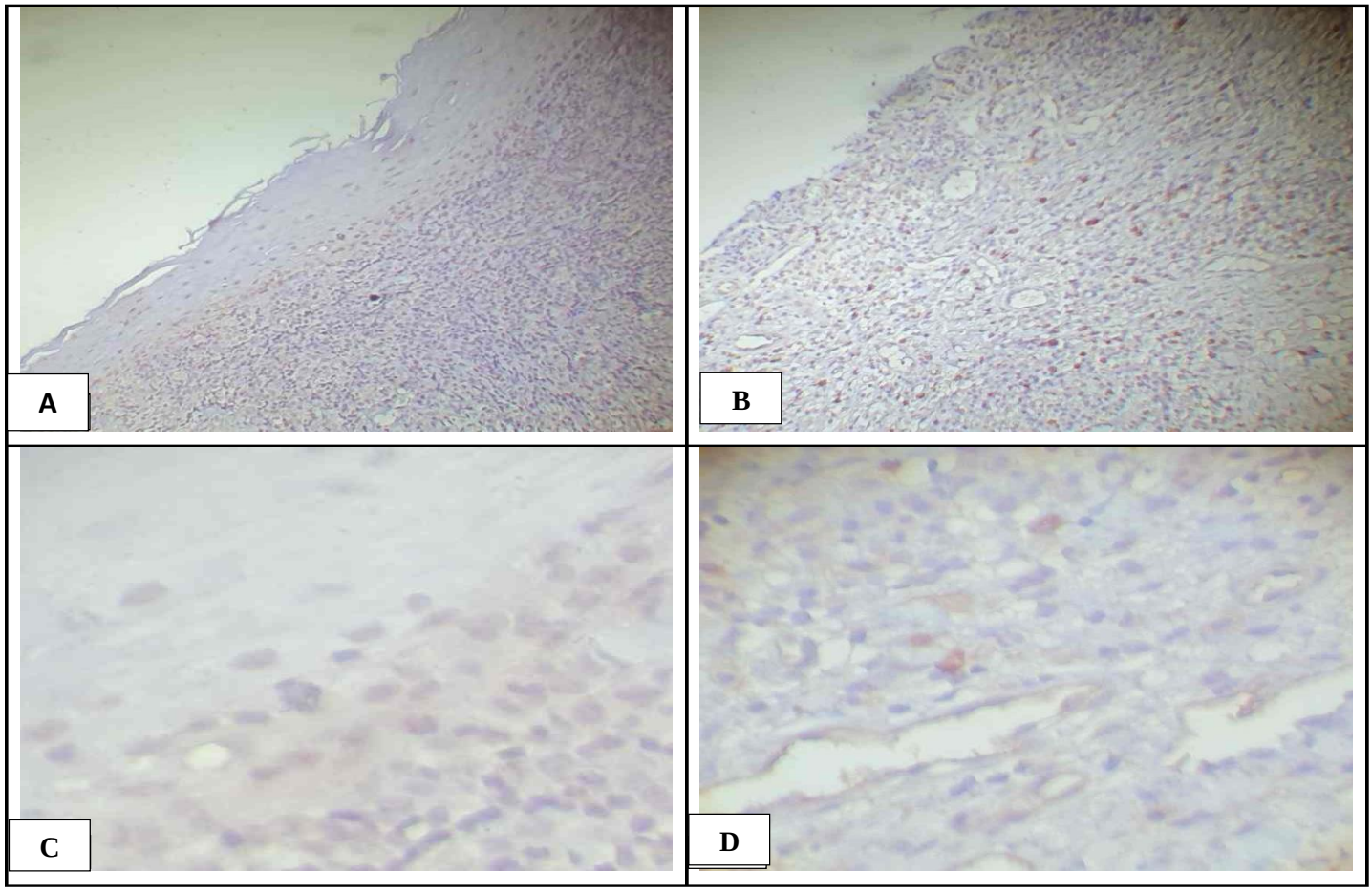

Figure (2): Immunohistochemical expression of serotonin (5-HT) marker in OLP cases. (A& B):-Cytoplasmic staining of serotonin expression in the epithelial layer, inflammatory cells, and blood , vessels (x100) (C&D):- High-power view of the previous section (×400).

The 5-HT marker expression in regards to clinicopathological findings:

The H-score index of serotonin at the basal layer revealed no statistically significant differences with a demographic, inflammatory score, and clinical presentation of disease (P >0.05). However, the mean rank was higher among the samples of females, erosive type, and inflammatory score +++ than males, reticular type, and score + and ++. **The H-score index for serotonin at the suprabasal layer was significantly higher among the samples of females, and the inflammatory score was +++ (P values 0.037 and 0.03, respectively). Age and clinical types showed no significant difference in the mean rank of the H-score (P >0.05). The total H-score index was lower among males and patients with reticular-type samples. Still, that difference was non-significant (P values 0.196 and 0.106, respectively), while the inflammatory score +++ was significantly higher than the other scores (P= 0.036), Table 6.**

Table (6): The H- score index at basal layer, suprabasal layer, and total H-score index for 5-HT with P-value according to clinicopathological findings.

Variable		H-score Index at the basal layer Mean rank	P-value	H-score Index at suprabasal layer Mean rank	P-value	Total H-score Index for 5-HT Mean rank	P-value
Age in years	<50	17.95	0.163	21.5	0.57	20.4	0.96
	≥50	23.05		19.4		20.5	
Gender	Male	19.38	0.61	17.3	0.037*	18.5	0.198
	Female	21.25		25.1		23.4	
Clinical types	Erosive	22.11	0.403	23.8	0.079	23.6	0.106
	Reticular	19.05		17.4		17.6	
Inflammatory score	Score +	15.8	0.135	14.7	0.03*	14.3	0.036*
	Score ++	22.8		21.8		23.8	
	Score +++	23.6		26.6		24.5	

*Mann-Whitney U test, * Kruskal-Wallis test, Significant ≤0.05.

DISCUSSION

In this study, patients with oral lichen planus ranged in age from 28 to 69 yearsold, with a mean age of 49.15 (SD 11.39). Previous investigations found comparable results [10,11,12,13,14,15]. As far as gender is concerned, the results of this investigation demonstrated that 24 (60%) cases of OLP were females; 1:1.5 was the male-to-female ratio, which agrees with previous studies [16,17,18,19] but disagrees with **Bandyopadhyay *et al*. [20].**

According to our knowledge, this is the first study to examine serotonin expression in OLP disease via immunohistochemistry. We compare this study with those conducted on cutaneous lichen planus (CLP) and psoriasis. These diseases were considered to have an immunological link among them (OLP, CLP, and psoriasis), as suggested by **Vajaitu *et al*. [21]**, as well as considering those diseases are inflammatory and immune-mediated diseases.

In this study, the serotonin expression of each biopsy sample was evaluated; 29 out of 40 (72.5%) were positive in the basal layer, and eleven cases were negative (27.5%). As for the suprabasal layer in the OLP studied cases, the serotonin expression was negative in 7 (17.5%) cases, while in 33 (82.5%) cases was positive. Consequently, The investigation's results are compatible with many researchers [8] who studied serotonin IHC expression in CLP and discovered that the intensity of serotonin staining was much higher in L.P tissue than in control, where 31 of 40 cases (77.5%) were positive for serotonin, suggesting that serotonin might have a role in the development of L.P. Additionally, concluded that L.P patients with depression and anxiety had increased serotonin expression.

Oral mucosa is described as a highly complex and vulnerable area susceptible to psychological effects. Additionally, these writers included OLP as one of the psychosomatic disorders [22]. **Kronfol and House [23]** and **Maes (2011) [24]** revealed an altered CD4 and CD8 profile and immunological activation in patients with severe depression.

Inflammation might trigger disruptions in the neuro-immuno-cutaneous system (NICS), which may contribute to more inflammation and increase serotonin expression; this may drive T-cell-mediated inflammation, which is involved in the pathophysiology of LP. It is widely known that depression and anxiety are common among OLP patients [25].

The median H- index was 55, ranging between 0-200 in the basal layer, whereas the median H- index in the suprabasal layer was 80. Ranging between 0-300, this was in agreement with **Lundeberg *et al.* [26],** who found that cells in people withallergic contact dermatitis are more dendritic and located in the epidermis' top layer, where they may produce serotonin. According to research by **Welz-Kubiak and Reich [27],** serotonin is thought to promote T lymphocyte proliferation and the generation of IL-6 and TNF via a variety of receptors.

The current study found serotonin in inflammatory and capillary endothelial cells in 31 (77.5%) and 32 (80%) cases, respectively. A positive, strong serotonin expression appeared in 13 (32.5%) and 15 (37.5%) cases, respectively; similar findings were found by **Kurmuş *et al*. [8].** They claimed that 21 out of 31 (70%) in the L**P** groups were positive. According to the findings above, through inflammatory cells, serotonin may aid in the

development of LP by changing capillary permeability.

The H-score index for the basal layer revealed no statistically significant differences with demographic, clinical presentation, and inflammatory score (P >0.05), even though the mean rank was higher among the samples of females, erosive types, and inflammatory score +++ than males, reticular types, and inflammatory score + and ++.

The H-score index for serotonin at the suprabasal layer was significantly higher among the samples of females, and the inflammatory score was +++ (P values 0.037 and 0.03, respectively). It was noticed that women's mean rank H-score was 25.1, which was significantly higher than the mean rank H-score index of males, which was 17.3. It is elucidated by frequent earlier surveys that established that women were more vulnerable than men to stress; this was noted by **Weisberg *et al.* [28].** There was no statistically significant association between the clinical type and H-score index for serotonin at the suprabasal layer.

The total H- score index of serotonin was higher among samples from femalesand with erosive types. Still, this difference was not statistically significant, whereas the inflammatory score +++ was significantly higher than other scores (+ and ++) (P= 0.036). This could be explained by **Ahern [29]**, who studied the correlation between serotonin and inflammation and reported that serotonin is a mediator of inflammation and a stimulant of T cell activation, attracting T cells to areas of inflammation.

CONCLUSIONS

It has been demonstrated that serotonin is present in the epithelium of OLP in most cases, which is considered an important marker in its pathogenesis. The total H-score index among the samples with the inflammatory score +++ was significantly higher than the other scores, which showed that serotonin was the mediator of inflammation and a stimulant of T cell activation, where it recruits T cells to sites of inflammation.

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