10.21608/adjc.2024.305120.1586 Advanced Dental Journal
Volume7(2025) | Issue3 | Pages 555-562 ©Faculty of Dentistry-Cairo University

Original Article

Immunohistochemical Expression of EBP50 Protein in Oral Lichen Planus: A Retrospective Cross-Sectional Study

Arwa A. Eltohami[1], Heba Farag[2], Hatem Amer

[1]Oral Pathology Department, Faculty of Dentistry, the British University in Egypt.
[2] Oral and Maxillofacial Pathology, Faculty of Dentistry, Cairo University.

Email: arwa.eltohami @dentistry.cu.edu.eg

Submitted: 18-7-2024
Accepted: 14-8-2024

Abstract

Aim: The goal of this study is to investigate how the Na+/H+ Exchanger Regulatory Factor-1/Ezrin-radixin-moesin-binding phosphoprotein 50 protein is expressed in Oral Lichen Planus using immunohistochemistry.
Subjects and methods: This will be accomplished by gathering archival blocks of normal oral epithelium (control group) and oral lichen planus, applying the marker to the specimens, and subsequently quantifying the expression location, area percentage, and nuclear expression.
Results: The control group showed negative expression in basal cells, cytoplasmic in parabasal cells, membranous in spinous cells, and gradually disappeared in superficial cells until it became negative in the cornified layer. All oral lichen planus cases showed cytoplasmically positive basal cells, with sixty-four percent of them displaying prickle cell nuclear positivity and perinuclear cytoplasmic staining.
Conclusion: There was a significant difference in the marker's expression between the tested groups. This is owing to its different functions depending on its expression location and the type of tissue.

Keywords: Oral Lichen Planus, immunohistochemistry, Na+/H+ Exchanger Regulatory Factor-1/Ezrin–radixin–moesin-Binding Phosphoprotein 50.

Introduction

Oral lichen planus (OLP) is an immune-mediated illness that manifests in several clinical forms, such as reticular and erosive **(Neville et al, 2023)**. The exact cause of OLP is not well understood; however, several variables can trigger the disease, including immunological responses mediated by T cells, genetic predisposition, and bacterial or viral infections **(Giannetti et al, 2018).**

NHERF1/EBP50 is an abbreviation for Na+/H+ exchanger regulatory factor 1/ezrin–radixin–moesin-binding phospho-protein 50. The gene is situated on the 17q25 region of the human chromosome, and its activity is controlled by phosphorylation. It is one of the NHERF lineages that is a member of PDZ-scaffold proteins **(Wang et al, 2014)**.

The acronym PDZ stands for the initial three proteins that were found to possess this domain: Postsynaptic density protein (PSD95), Drosophila

disc large tumor suppressor (Dlg1), and Zonula occludens-1 protein (zo-1) **(Gianni et al, 2005)**. It has a crucial function in enhancing the effectiveness, selectivity, and accuracy of different cell signal transductions (**Zhang et al, 2003)**. An additional ezrin-radixin-moesin (ERM) binding domain was discovered in the C-terminal domain of the NHERF1/EBP50 protein. ERM proteins serve as a connection between proteins on the surface of cells and the inside actin cytoskeleton that supports the plasma membrane (**Vaquero et al, 2017)**.

Kristen L. Leslie et al found that EBP50 enhances the activation of nuclear factor-kappa B (NF-$_K$B) in systemic and vascular inflammation. Understanding the significance of this is crucial because inflammation significantly contributes to vascular disease, and NF-$_K$B is essential for conveying signals related to inflammation (**Leslie et al, 2013)**. Elevated NF-$_K$B signaling in OLP results in an excessive generation of inflammatory cytokines and the death of basal epithelial cells (**Zanetta et al, 2022)**. Thus, the researchers postulated that EBP50 controls the NF-KB signaling pathway (**Leslie et al, 2013)**.

The objective of this work is to assess the level of immunohistochemical expression of the EBP50 protein in Oral Lichen Planus and normal oral epithelium and to compare the expression between the two.

Subjects and Methods

Participants and staining

This study examined a total of 28 (14 per group) Formalin-fixed, paraffin-embedded tissue specimens collected from blocks stored in the archives of the Oral and Maxillofacial Pathology Department, Faculty of Dentistry at Cairo University Teaching Hospital. The specimens were obtained from the 10 years between 2012 and 2022. All relevant histopathological information was reviewed. The control group was collected from normal gingival mucosa of resected margins from different intrabony lesions.

Sections (4-5 microns thick) were cut for Hematoxylin and Eosin (H&E) and immunohistochemical staining. A positively charged (Opti-plus) slide was used for the immunohistochemical stain by the technicians in the Oral and Maxillofacial Pathology lab setting. The Immunostaining was performed at the National Cancer Institute using Ventana Benchmark auto-stainer. Commercially available concentrated monoclonal mouse antibody NHERF-1 (A-7) #sc-271552 was purchased from Santa Cruz Biotechnology, Inc. as well as a specialised ultraView Universal DAB (3,3'-Diaminobenzidine) Detection Kit, manufactured by Ventana Medical Systems in Tucson, AZ, USA. This kit is specifically developed for conducting immunohistochemistry on the Ventana® NexES and BenchMark automated systems. In every run, positive control of normal human gall bladder tissue was added. It was obtained from the National Cancer Institute. This work was accepted by the ethical committee of the Faculty of Dentistry, Cairo University.

Image analysis

Capturing microscopic images was done in Analytica research centre, Elharam, using SOPTOP EX20 biological microscope (China), HD camera (model No. XCAM1080PHB), and Imageview software at X40, X100, X200, and X400 magnification powers.

Area percent measurement and nuclear count: Immunohistochemical stained sections were examined using high power fields (X400) by light microscope and the most homogenous areas of the positive reaction were chosen for evaluation. The image analyzer computer system applying ImageJ 1.53e software (USA) was used for automated measurement of area percent of EBP50 positivity (Figure 1). Nuclear expression was counted manually using an image analyzer computer system applying QuPath-0.3.2 software, UK. It was performed in a standard frame area of 5.04X106 µm2. (Figure 2). A slide was classified as nuclear-positive if 25% of the cells exhibited nuclear-positive staining.

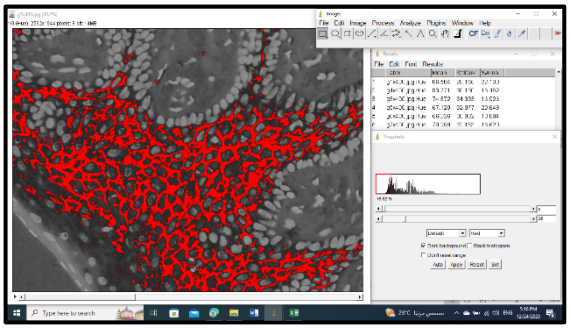

Figure 1: Copy display photo of ImageJ image analyser showing red binary colour marking the area percent of EBP50 positivity in the control group (X400, frame area= 5.04X106 µm2)

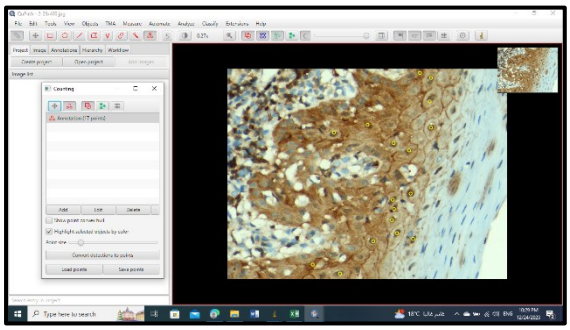

Figure 2: Copy display photo of QuPath-0.3.2 software showing yellow points marking the nuclear expression of EBP50 in OLP group (X400, frame area= 5.04X106 µm2)

Statistical methods

Statistical analysis of the results was performed using SPSS software. Shapiro-Wilk test of normality was used to test normality hypothesis of all continuous variables. One way-analysis of variance (ANOVA) test was used for the evaluation of statistically significant differences within the studied groups, followed by the Tukey Kramer Post hoc test for the statistically significant results. An unpaired T-test was used for the evaluation of statistical significance between the two groups. P-values ≤ 0.05 were considered statistically significant.

Results

Demographic data:

The demographic data of the cases under study are recorded in table 1. The age range was from 28 to 74 years old. Among the cases reviewed, females were more frequently affected than males, accounting for 71%. Regarding the prevalent location, it was observed that the buccal mucosa was the most often affected site, accounting for 29%. Finally, the predominant clinical variety was the reticular type, which affected 29% of all cases.

Table 1: Demographic data of studied cases.

		OLP
Age Range	-	28-74
Sex	Male	14%
	Female	71%
	NA*	15%
Site	Alveolar mucosa	-
	Lateral border of the tongue	-
	Floor of the mouth	-
	Retro-molar area	-
	Lip	-
	Palate	-
	Buccal mucosa	29%
	Labial mucosa	7%
	Oral commissures	7%
	NA	57%
Clinical Variant	Reticular	29%
	Erosive	14%
	NA	57%

Histopathological and Immunohistochemical findings

Microscopic examination of Haematoxylin & Eosin (H&E) stained sections for the control group revealed a normal para-keratinized stratified squamous epithelium with normal thickness. There were a few scattered melanocytes in the basal cell layer and the connective tissue stroma. The underlying

stroma was normal containing fibroblasts, collagen fibres, and thin blood vessels. (Figure 3.A).

The expression location of EBP50 varied between different striae. It was negative in the basal cells, cytoplasmic in the parabasal cells, and changed to be membranous in the spinous cell layer then gradually disappears in the superficial cells until negative in the cornified layer. None of the striae showed nuclear expression. (Figure 3.B).

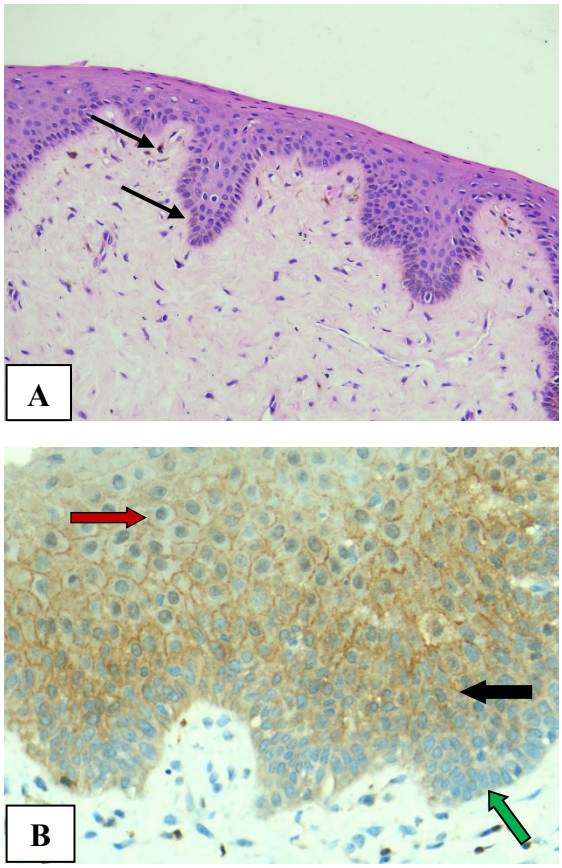

Figure 3: (A) Microscopic image for the control group showing normal oral epithelium with scattered melanocytes (black arrow) (H&E stain, magnification x200). **(B)** Microscopic image of EBP50 expression in the control group showing that it's negative in basal cells (green arrow), cytoplasmic in parabasal (black arrow), and membranous in prickle cells (red arrow). (Immunohistochemistry, magnification x400).

In OLP, the sections stained by H&E showed a dense band-like subepithelial chronic inflammatory infiltration. The basal cell layer showed hydropic degeneration. Some Civatte bodies were seen in the parabasal layer representing degenerating keratinocytes as seen in **Figure (4).**

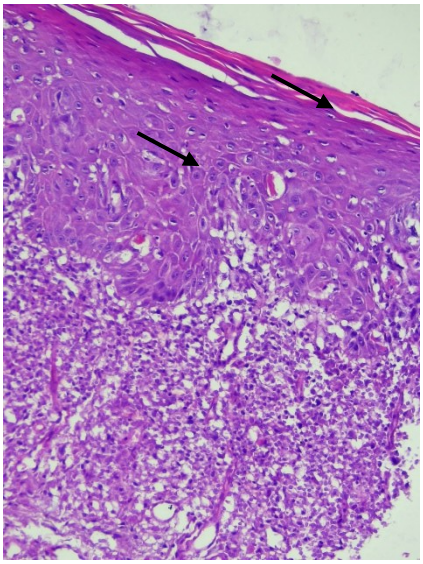

Figure 4: Microscopic image for OLP showing Civatte bodies (black arrows) (H&E stain, magnification x200).

Regarding the immunohistochemistry, there was a great resemblance with the control group in gradually shifting to be membranous till it fades away in the superficial layers. In contrast to the normal group, all cases of OLP had their basal cells cytoplasmically positive. Figure (5). Sixty-four percent of OLP cases had nuclear positivity in the prickle cell layer with perinuclear cytoplasmic staining. Thirty-six percent of cases had negative nuclear staining. Figure (6).

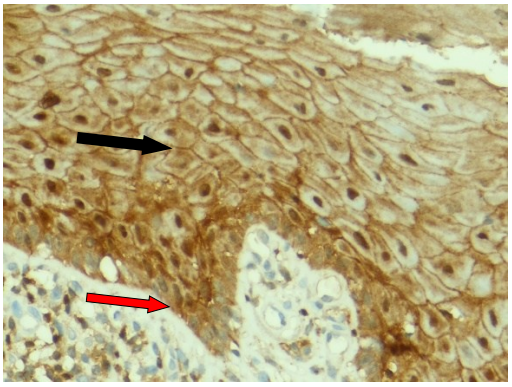

Figure. (5). Microscopic images for EBP50 expression in OLP group showing nuclear and cytoplasmic positivity in the prickle cell layer (black arrow) as well as cytoplasmic positivity in the basal cell layer (red arrow). (Immunohistochemistry, magnification x400).

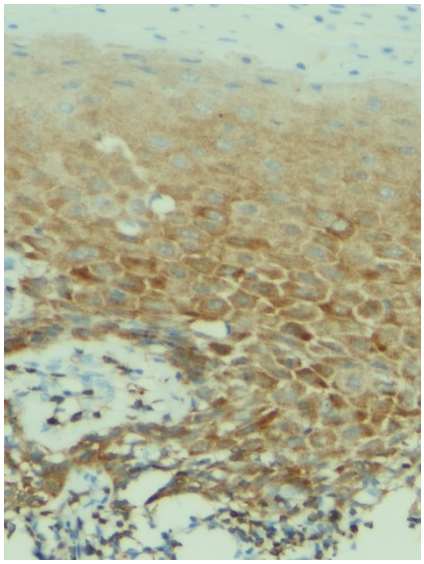

Figure. (6). Microscopic image of the OLP group showing cytoplasmic expression of EBP50 but nuclear negative. (Immunohistochemistry, magnification x400).

Statistical analysis

Area percent of EBP50:

When comparing the two clusters, the greatest mean area percent was recorded in the OLP group, whereas the lowest value was recorded in the Control group. One way-ANOVA test revealed that the difference between them was statistically significant ($P<0.001$). Tukey's post hoc revealed a significant difference between the two groups (Table 2).

Nuclear count of EBP50

When comparing the nuclear count, the control group was found to be negative. On the other hand, the mean nuclear count in the OLP group was 15.9. Its standard deviation was 8.4 with minimum value 2 and maximum value 35.

Table 2: Area percent of EBP50 in the tested groups and significance of the difference using (ANOVA) test.

	Control	**OLP**
Mean	15.39 ^{b}	40.75 ^{a}
SD	5.35	7.2
Min	6.688	25.793
Max	22.13	54.871
P-value	< 0.00001*	

*Significant at $p<0.05$

Tukey's post hoc test means sharing the same superscript letter are not significantly different.

Discussion

Oral lichen planus (OLP) is one of the immune-mediated illnesses that have the potential to progress into oral squamous cell carcinoma (OSCC) **(Neville et al, 2023 & Giannetti et al, 2018)**. The incidence of OLP malignant transformation was determined to be 2.28% **(Cheng et al, 2022)**. The present research conveys the preliminary and the first results on the expression level of EBP50 in normal oral epithelium and OLP.

The current study used the automated BenchMark Ultra IHC/ISH Ventana Medical Systems. This is due to its several advantages over manual staining, including enhanced reproducibility and hence greater quality, in addition to increased standardisation **(Nitta et al, 2022)**. In addition to utilising a monoclonal antibody because of its ability to specifically target a single epitope, therefore, producing more specific results. Unlike polyclonal antibodies, which can bind to many epitopes **(Taylor et al, 2010)**.

The present study analyses the results using three approaches: area percentage, nuclear count, and expression location. A slide was categorized as nuclear-positive if at least 25% of the cells displayed nuclear-positive staining. It didn't follow a scoring system. This is because the function of EBP50 varies depending on where it is expressed. As a result, the expression site of EBP50 might differ between different lesions and even within the same lesion. Furthermore, the existing literature lacks a consistent and dependable scoring system, as it is always subjective and varies among different pathologists. Thus, these three methodologies were employed to obtain a reliable and precise dataset regarding that marker.

Regarding the immunohistochemical findings of EBP50 expression in the control group, it exhibited a staining pattern in line with that described by Shankar et al and WANG et al but differed from them by being negative in the basal cell layer **(Shankaret al, 2019 & Wang et al, 2014)**. Taking into consideration that they used a polyclonal antibody in their research, this can be ascribed to the link between EGFR, E-cadherin, and EBP50. Ramírez Moreno and Bulgakova discussed the interaction between EGFR signalling and E-cadherin-mediated cell-cell adhesion pathways, known as crosstalk. Activation of EGFR can cause the reduction of E-cadherin expression, resulting in a decrease in the adhesion between cells and an increase in the movement of cells **(Ramírez et al, 2022)**. Additionally, Vaquero et al. highlighted the link between EBP50 and the interaction between EGFR and E-cadherin. They demonstrated that the lack of EBP50 results in the stimulation of EGFR and consequent cellular proliferation **(Vaquero et al, 2017)**.

In the OLP group, the expression of EBP50 was similar to the normal control group, except that it also stained the basal cell layer making it the highest area percent. Furthermore, a significant amount of nuclear staining was observed in the majority of patients, with an average value of 15.9. This is because there may be a correlation between nuclear factor-kappa B (NF-KB) and EBP50. It is established that NF-KB signaling is increased in OLP, resulting in excessive production of

inflammatory cytokines and the programmed cell death of the basal epithelial cells **(Zanetta et al, 2022)**. Kristen L. Leslie et al in their study on systemic and vascular inflammation found that EBP50 enhances NF-KB activation due to the significant role inflammation plays in vascular disease and the crucial necessity of NF-KB in transmitting inflammatory signals. Consequently, they postulated that EBP50 governs NF-KB signalling **(Leslie et al, 2013)**.

Conclusion

The expression of EBP50 differs dramatically among the normal oral epithelium and Oral Lichen Planus, exhibiting unique patterns in basal and prickle cells.

Conflict of Interest:

The authors declare no conflict of interest.

Funding:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Ethics:

This study protocol was approved by the ethical committee of the faculty of dentistry- Cairo university on: 27/9/2022, approval number: 4922.

Data Availability:

Data will be available upon request

Clinical trial registration:

Not applicable for this type of study.

CRediT statement:

Author 1: Data curation, Writing - review & editing, Writing - original draft, Methodology, Conceptualization, Resources.

Author 2: Data curation, Conceptualization, Project administration, Supervision, Methodology, Writing - review & editing, Writing - original draft.

Author 3: Methodology, Writing - original draft, Writing - review & editing, Investigation, Formal analysis, Supervision, Data curation.

References

Neville BW, Damm DD, Allen CM, et al. *Oral and maxillofacial pathology*. 4th edn. Missouri, USA: Elsevier, **2023**; p. 788.

Giannetti L, Dello AD and Spinas E. Oral lichen planus. *J Biol Regul Homeost Agents* **2018**;32(2):391–395.

Wang L, Du YR, Ji MY, Wang W, Zhan N, Zhou QS and Dong WG. Reduced EBP50 expression or mis-localization of the EBP50 protein is associated with the malignant progression of esophageal squamous cell carcinoma. *Eur Rev Med Pharmacol Sci* **2014**;18(24):3854–3863.

Gianni S, Engström Å, Larsson M, Calosci N, Malatesta F, Eklund L, Ngang CC, Travaglini-Allocatelli C and Jemth P. The kinetics of PDZ domain-ligand interactions and implications for the binding mechanism. *J Biol Chem* **2005**;32(4):391–395.

Zhang M and Wang W. Organization of signaling complexes by PDZ-domain scaffold proteins. *Acc Chem Res* **2003**;36(7):530–538.

Vaquero J, Nguyen Ho-Bouldoires TH, Clapéron A and Fouassier L. Role of the PDZ-scaffold protein NHERF1/EBP50 in cancer biology: From signaling regulation to clinical relevance. *Oncogene* **2017**;36(22):3067–3079.

Leslie KL, et al. Ezrin-radixin-moesin-binding phosphoprotein 50 (EBP50) and nuclear factor-KB (NF-KB). *J Biol Chem* **2013**;288(51):36426–36436.

Zanetta P, et al. Probiotics as potential biological immunomodulators in the management of oral lichen planus: What's new? *Int J Mol Sci* **2022**;23(7):3489.

Cheng LL. The proportion of oral lichen planus cases with malignant transformation may be higher, than reported to date. *J Evid Based Dent Pract* **2022**;22(2):101717.

Nitta H, Robida MD and Polaske N. *Handbook of Practical Immunohistochemistry*. 3rd ed. Gewerbestrasse, Cham: Springer Nature, **2022**; p. 77–78.

Taylor CR, Shi SR and Barr NJ. Chapter 1, Techniques of immunohistochemistry: principles, pitfalls, and standardization. In: *Diagnostic immunohistochemistry: theranostic and genomic applications*. 3rd edn. Philadelphia: Saunders, **2010**; p. 1–41.

Shankar A, Crouch DH and Macluskey M. Ezrin–Radixin–Moesin Binding Phosphoprotein 50: A Potential Novel Biomarker in Human Papilloma Virus Associated Head and Neck Squamous Cell Carcinomas. *Head Neck Pathol* **2019**;13(2):188–197.

Ramírez Moreno M and Bulgakova NA. The cross-talk between EGFR and E-Cadherin. *Front Cell Dev Biol* **2022**;9(3):828673.

Zanetta P, et al. Probiotics as potential biological immunomodulators in the management of oral lichen planus: What's new? *Int J Mol Sci* **2022**;23(7):3489.