

Beclin-1 as a potential prognostic marker in correlation with E-cadherin in oral lichen planus and oral squamous cell carcinoma: an immunohistochemical analysis

Heba N. Shalash^{a,b}, Radwa R. Hussein^c, Fatma Seragel-Deen^d

^aDepartment of Basic Dental Science, Oral and Dental Research Institute, National Research Centre, ^bDepartment of Oral and Maxillofacial Pathology, Faculty of Dentistry, Galala University, Suez, Egypt, ^cOral Medicine, Periodontology and Oral Diagnosis, ^dOral Pathology, Faculty of Dentistry, Ain Shams University, Cairo

Correspondence to Heba N. Shalash, PhD, Department of Basic Dental Science, Oral and Dental Research Institute, National Research Centre, PO Box 12622, Dokki, Giza, Egypt. Tel: +20 100 501 6380; fax: +20 233 387 803; e-mail: hebashalash111@gmail.com

Received: 8 May 2024

Revised: 5 June 2024

Accepted: 9 June 2024

Published: 24 December 2024

Journal of The Arab Society for Medical Research 2024, 19:135–147

Background/aim

Oral lichen planus (OLP) is a chronic, unexceptional, frequent disease of inflammatory origin, autoimmune background with unspecified etiology. Also it has been regarded as an oral potentially malignant disorder and reflects the potential hazard of malignant transformation into oral squamous cell carcinoma (OSCC). Beclin-1 happens to be one of the proteins regulating autophagy, where its dysfunction has been involved in various disorders. The function of Beclin-1, as per a marker for autophagy, still needs to be verified in oral premalignant lesions and their progression to OSCC. E-cadherin is an integral intercellular epithelial component responsible for intercellular adhesion, in which its down-expression denotes diminished cellular adhesion and propensity for invasion. Our work aimed to explore the levels of Beclin-1 and E-cadherin in erosive and nonerosive OLP and OSCC to assess the possible role of autophagy in the pathogenesis of OLP and estimate the malignant potential in each OLP type.

Materials and methods

This retrospective study was carried out in the pathology unit Ain Shams University Specialized Hospital. Sixty formalin-fixed paraffin-embedded tissue blocks, along with their clinicopathologic records, were retrieved from the archives of the Department of Oral Pathology, Faculty of Dentistry, Ain Shams University, Egypt. They were classified into three groups (20 each) as follows: group 1: the negative control group; group 2: comprised OLP cases, subdivided into two groups (10 each): group 2A, the erosive type and group 2B, the nonerosive type and group 3: comprised the OSCC cases, subdivided into two groups (10 each): group 3A, well-differentiated OSCC and group 3B, moderately differentiated OSCC. Immunohistochemistry was utilized to assess the expression levels of both Beclin-1, as a marker of autophagy and E-cadherin, as a marker of invasiveness and aggressiveness, to validate the malignant transformation potential.

Results

The present result obtained significant increases ($P < 0.05$) in the levels of Beclin-1 in a group of patients with OLP, the erosive type (G2A), and both groups of OSCC cases (G3A and B), while G3B was the highest level. Regarding E-cadherin, a significant decrease ($P < 0.05$) was found in their levels in all groups of OLP and OSCC cases, compared to the control group; however group G3B exhibited the lowest reduction. Correlation between Beclin-1 and E-cadherin revealed an insignificant correlation between the two markers in all groups, except a significant negative correlation ($r = -0.9$, $P < 0.03$) was found in a group of OLP patients with erosive type.

Conclusion

Beclin-1 could potentially be an important prognostic marker in OLP and OSCC. Low levels of E-cadherin expression in erosive OLP indicate greater potential for invasiveness, migration capability and a higher tendency to malignant transformation, which was found comparable to OSCC.

Keywords:

autophagy, Beclin-1, E-cadherin, oral lichen planus, oral squamous cell carcinoma

J Arab Soc Med Res 19:135–147

© 2024 Journal of The Arab Society for Medical Research
1687-4293

Introduction

Oral lichen planus (OLP) is an inflammatory disease of chronic onset that is mediated by cytotoxic T-lymphocyte assault on the basal cell layer of the oral epithelium. Histopathologically, OLP is typically manifested as a subepithelial lymphocytic band along with some basal cell liquefaction degeneration. As

regards the clinical presentation, OLP is usually manifested as a white lesion with a fine lacy pattern,

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

which may or may not be associated with raw, erythematous, erosive or even ulcerative lesions. Therefore, OLP, by consensus, can be, in general, categorized into erosive and nonerosive types [1]. OLP is considered a prevalent disease, with 1.01% worldwide affection. The frequency has been found to escalate noticeably from the fifth decade of life [2].

The malignant potential of OLP has been heavily questioned for the past 10 years, especially for the long-standing, persistent erosive lesions of OLP. A growing body of empirical evidence has substantiated this speculation, and the WHO has already categorized OLP as an oral potentially malignant disorder in the latest two editions [3]. OLP can progress to oral squamous cell carcinoma (OSCC) with an estimated transformation rate and malignant risk of 1.14 and 2.28%, respectively [4,5].

OSCC owns the lion's share of 90% of all head and neck cancers. Unfortunately, the incidence rate of OSCC has been drastically escalated over the last 10 years. The most common oral locales to be involved by OSCC are the tongue, lips, buccal mucosae, floor of the mouth, retromolar trigone, and palate. With respect to the causal relationship, tobacco consumption (in any form, especially when accompanied by alcohol), persistent inflammation, infection by oncogenic viruses, certain bacteria and fungi, genetic aberrations, immunosuppressed state and malnutrition are thought to be the main etiopathological factors of OSCC [6].

Autophagy is a conserved cellular apparatus utilized for the removal of harmful microorganisms and unwanted damaged organelles via a specialized lysosomal degradation system that primarily aims to maintain the equilibrium of the internal body habitat. Not only does autophagy perform a great function under physiological conditions, but it is also associated with various maladies like infection, autoimmune disorders, cancer, and metabolic diseases, upon its dysregulation. The underlying mechanism of autophagy is intertwined with other biological pathways and is mediated by specific factors, for example, ATG family of proteins, one light chain 3B tube-associated protein (microtubule-associated protein one light chain 3B, LC3B), p62/SQSTM1, and Beclin-1 [7].

Basic autophagy essentially serves to maintain the survival of cells. However, its excessive, permanent activation can induce self-degradation of cells, leading to death, as there is a cross-talk with

apoptotic pathways [7]. Regarding its contribution to tumorigenesis, autophagy has a dual role; at first, it performs an integral function of being tumor-suppressive in the early phases of cell transformation, but once the cell acquires fully established malignant characteristics, autophagy incites its progression into malignancy through further promoting the process of epithelial-mesenchymal transition (EMT) and conveying multidrug resistance [8].

Beclin-1 is a key regulatory scaffold protein that has many functional domains that help to integrate the signals required for protein-protein interactions throughout the autophagic pathway, as it is directly involved in the earliest stage of autophagolysosome formation [7]. Questioning its role in tumorigenesis, Aita *et al.* [9] in 1999 first revealed that Beclin-1 might be an oncosuppressive protein as it inhibits the proliferation of breast cancer MCF7 cells. Moreover, it has also been found that Beclin-1 expression in human solid cancers is decreased, supporting its tumor-suppressive assumption [10].

Nevertheless, Beclin-1 immunoexpression in some other malignancies, including gastric carcinomas and colorectal cancers, has been proven to be higher, while there was an insignificant level of expression in normal native cells, suggesting that increased Beclin-1 expression is blamed for the tumor progression [11]. Furthermore, Jung *et al.* [12] implied that Beclin-1 has an oncopromoter role under cellular stressful conditions like hypoxia and advanced nutrient deprivation.

In the same vein, levels of expression regarding autophagy markers Beclin-1, LC3B, ATG7 together with p62 have been found to mirror the aggravated histopathological grade of a spectrum of oral lesions starting from normal to premalignant (mild, moderate, and severe dysplasia) and ending up with OSCC [13]. Even paralleling the clinicopathologic parameters, in a study comprising 90 primary OSCCs, the upregulated expression of Beclin-1 was reflected in the tumor grade and nodal metastasis, with a compromised overall survival rate [14].

E-cadherin is a transmembrane intercellular adhesion molecule in all types of epithelia. Via its cytoplasmic domain, E-cadherin complexes with β -catenin stabilize the actin cytoskeleton and deliver intracellular tumor-inhibiting signals. E-cadherin is responsible for the maintenance of apicobasal epithelial polarity and cell-cell cohesion, thus sustaining the epithelial tissue integrity [15].

Therefore, diminished expression of E-cadherin interferes with this function, resulting in gradual loss of epithelial characteristics and rather acquiring migratory capacity, in a process known as EMT, which is considered the initial milestone for invasion and metastasis [16]. During EMT, autophagy can help the transforming cells to survive metabolic stress such as nutrient starvation and hypoxia. Also, the malignant cells become dependent on autophagy to overcome the obstacles faced during vascular dissemination and distant metastatic spread. Therefore, it is believed that switching on the autophagic pathway induces the process of EMT [17,18].

Our research aimed to explore the immunohistochemical expression of Beclin-1 and E-cadherin to assess the autophagy and invasion levels in erosive and nonerosive OLP and compare them with those of both normal mucosa and different grades of OSCC to assess the possible role of autophagy in pathogenesis of OLP and estimate the malignant potential in each OLP type.

Materials and methods

Materials tissue blocks

Formalin-fixed, paraffin-embedded tissue blocks normal oral mucosa (20 blocks), OLP (20 blocks; 10 for erosive and 10 for nonerosive OLP) and OSCC (20 blocks; 10 for well-differentiated and 10 for moderately differentiated OSCC) along with their clinicopathologic records, were retrieved from the archives of the Department of Oral Pathology, Faculty of Dentistry, Ain Shams university. Tissue sections were stained with hematoxylin and eosin and reviewed to confirm the diagnosis.

Reagents

- (1) Beclin-1 rabbit polyclonal antibody (2-BE002-10) (3 ml ready-to-use) from BIOCYC Gesellschaft für Biotechnologie, Kosmetik und Recyclingverfahren mbH & Co. Entwicklungs KG Am Mühlenberg 11, Potsdam, Germany.
- (2) E-cadherin mouse monoclonal antibody [4A2] (ab231303, a concentration of 1 µg/ml) Abcam (UK).

Study design

This retrospective study was carried out in the pathology unit Ain Shams University Specialized Hospital. Sixty paraffin blocks, along with their clinicopathologic records, were retrieved from the

archives of the Department of Oral Pathology, Faculty of Dentistry, Ain Shams University, Egypt. The studied cases were divided into three groups as follows:

Group 1: negative control group G1, where normal oral mucosal tissue blocks were used as negative control.

Group 2: comprising OLP cases, subdivided into group 2A, the erosive type and group 2B, the nonerosive type.

Group 3: comprising the OSCC cases, subdivided into group 3A, a well-differentiated OSCC and group 3B, a moderately differentiated OSCC.

Ethical approval

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. This study has been approved by the Medical Research Ethics Committee (FDASU-REC), Faculty of Dentistry, Ain Shams University, Cairo, Egypt, with registration number FDASU-Rec ER072308.

Sample size calculation

We designed a power analysis to maintain adequate power for applying a statistical test of the null hypothesis in order to have no difference existing between different tested groups regarding the level of the immunohistochemical markers. By adopting an alpha level of (0.05), a beta of (0.2), that is power=0.8, and an effect size of 1.09 calculated based on the results of former research (20), the predicted sample size (n) was a totally of 60 samples. The χ^2 test for the calculation of sample size was performed using G*Power software, version 3.1.6 [19].

Methods

Tissue blocks were cut in 4 µm thickness and mounted on positively charged glass slides (Opti-Plus, BioGenex Laboratory, USA) for tissue processing, histopathological examination, and immunohistochemical staining.

Histopathological examination

Sections were stained with hematoxylin and eosin to confirm the diagnosis and to select the representative tissue blocks.

Immunohistochemical analysis

Sections were dewaxed and labeled with Beclin-1 and E-cadherin antibodies using the biotin-streptavidin method for immunohistochemical staining of OLP,

OSCC, and normal mucosal tissue sections. The immunostaining was performed by an automated stainer (Thermo Scientific Lab Vision Corporation, USA). The immunostained sections were examined in the Basic Dental Science Department, Oral and Dental Research Institute, National Research Centre using Ordinary Light Microscope to assess the immunopositivity of Beclin-1 and E-cadherin in our designated cases in four fields per slide. An Image Analysis Computer System was applied to evaluate the area percentage of the positive expression using LC microimaging software (Olympus, Germany). Analysis of images was performed using image analysis software, Image J (1.50i, USA).

Statistical analysis

Analysis of statistical results was carried out by SPSS 16 (Statistical Package for Scientific Studies, Munich, Germany), GraphPad Prism and Microsoft Office Excel 2007. Data of all groups were presented as minimum, maximum, range, median, mean, and SD. Given data was explored using the Shapiro–Wilk test and the Kolmogorov–Smirnov test. Accordingly, comparison between different groups was performed

by using one way analysis of variance test followed by Tukey's post-hoc for intergroup comparison and Pearson's correlation coefficient to evaluate the correlation between the two markers. *P* values less than 0.05 were considered statistically significant.

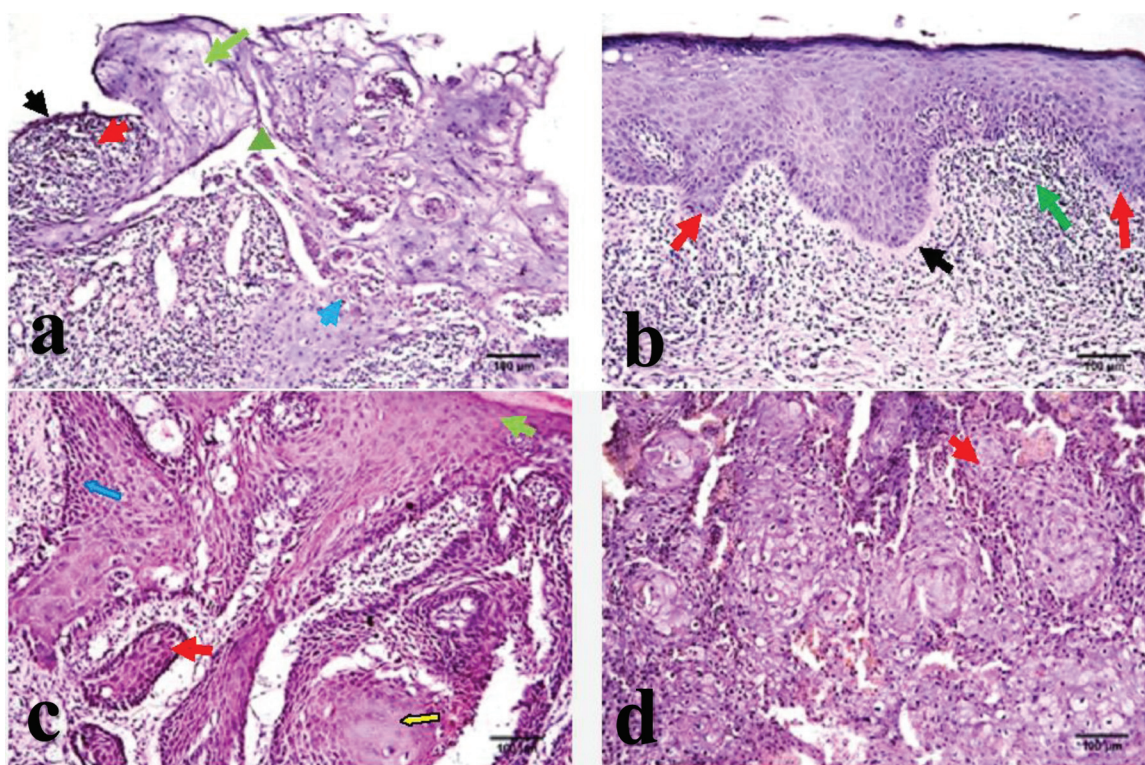
Results

Histopathological evaluation

Sections from G2A subgroup showed areas of ulcerated epithelium along with a hyperplastic component showing hydrobic degeneration and intraepithelial clefting. A subepithelial lymphocytic band was evident. Basal cell degeneration was present with the formation of hyaline Civatte bodies. On the other hand, sections from G2B subgroup showed a hyperplastic keratinized stratified squamous epithelium with focal areas of basal cell degeneration. A prominent subepithelial lymphocytic band was seen. A narrow subbasal hyaline band was shown in the tissue sections (Fig. 1a and b).

Sections from the G3A subgroup showed invasion of the connective tissue by masses of epithelial cells

Figure 1



Histopathological staining of OLP and OSCC (a) erosive OLP case displaying an area of ulcerated epithelium (black arrow) showing hydrobic degeneration (green arrow), intraepithelial clefting (green arrow head), subepithelial lymphocytic band (red arrow) and basal cell degeneration with hyaline Civatte bodies (blue arrow). (b) Reticular (nonerosive) OLP case showing a hyperplastic keratinized stratified squamous epithelium basal cell degeneration (red arrows), prominent subepithelial lymphocytic band (green arrow) and narrow subbasal hyaline band (black arrow). (c) Well-differentiated OSCC case showing invasion by masses of malignant epithelial cells (green arrow) with some undergoing keratinization (yellow arrow) and solid ones (cell nests) (red arrow) with cells showing loss of intercellular cohesion (blue arrow). (d) Moderately differentiated OSCC case showing cell nests, nuclear pleomorphism and hyperchromatism (red arrow) (H&E, original magnification x200). OLP, oral lichen planus; OSCC, oral squamous cell carcinoma.

forming keratinized masses and cell nests. Malignant cells also showed signs of epithelial dysplasia. Other sections from the G3B subgroup showed extensive formation of cell nests invading the connective tissue (Fig. 1c and d).

Immunohistochemical results

Expression of Beclin-1 and E-cadherin in G1, normal control group

Normal oral mucosal tissue sections showed positive nuclear Beclin-1 immunostaining in basal and suprabasal cells. A few scattered prickle cells demonstrated positive immunostaining throughout the surface epithelium. Some stromal cells also exhibited positive nuclear immunoreactivity. Concerning E-cadherin expression, positive, intense circumferential membranous immunostaining throughout the whole epithelium was noted. Moreover, positive immunoreactivity in the vascular endothelial cells was also noted (Fig. 2a and b).

Expression of Beclin-1 in oral lichen planus and oral squamous cell carcinoma

Regarding the expression of Beclin-1 in the OLP, G2A subgroup, cases showed prominent positive immune expression of Beclin-1 in most of the epithelial cells, with predominant nuclear and some cytoplasmic staining along with obvious abundant immunostaining in most of the underlying stromal cells nuclei. However, in subgroup G2B, positive nuclear immune expression of Beclin-1 was seen in the basal epithelial cells and extending to a few prickle cells in the middle third. The immunostaining was seen as more conspicuous and diffuse in the nuclei of underlying subjacent inflammatory cells and less pronounced in the remaining connective tissue

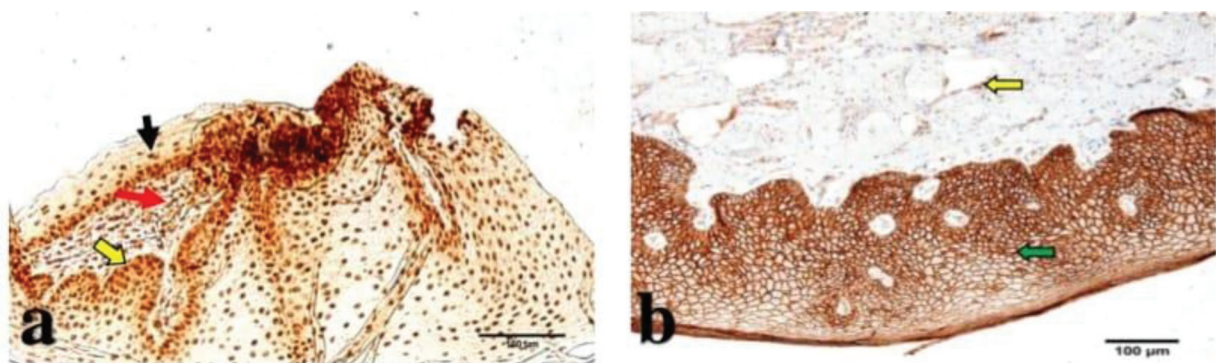
stroma. Some endothelial cells were positively expressed with Beclin-1 (Fig. 3a and b).

As for the expression of Beclin-1 in the OSCC, G3A subgroup, our results showed abundant positive immunostaining of Beclin-1 in both the cytoplasm and nuclei of the malignant squamous cells. The reactivity was seen more evidently at the periphery of the tumor masses, as the center was mainly occupied by Beclin-1 immunonegative cells. The stromal, as well as the endothelial cells, showed immunopositivity. On the other hand, G3B subgroup displayed diffuse immunopositivity to Beclin-1 in the vast majority of the tumor cells, with fewer immunonegative ones. The immune expression was predominantly nuclear within the cell nests; however, it was both nuclear and cytoplasmic within the loose cellular masses and dispersed malignant cells in the stroma. The positive reaction was obvious in the endothelial and stromal cells (Fig. 3c and d).

Expression of E-cadherin in oral lichen planus and oral squamous cell carcinoma

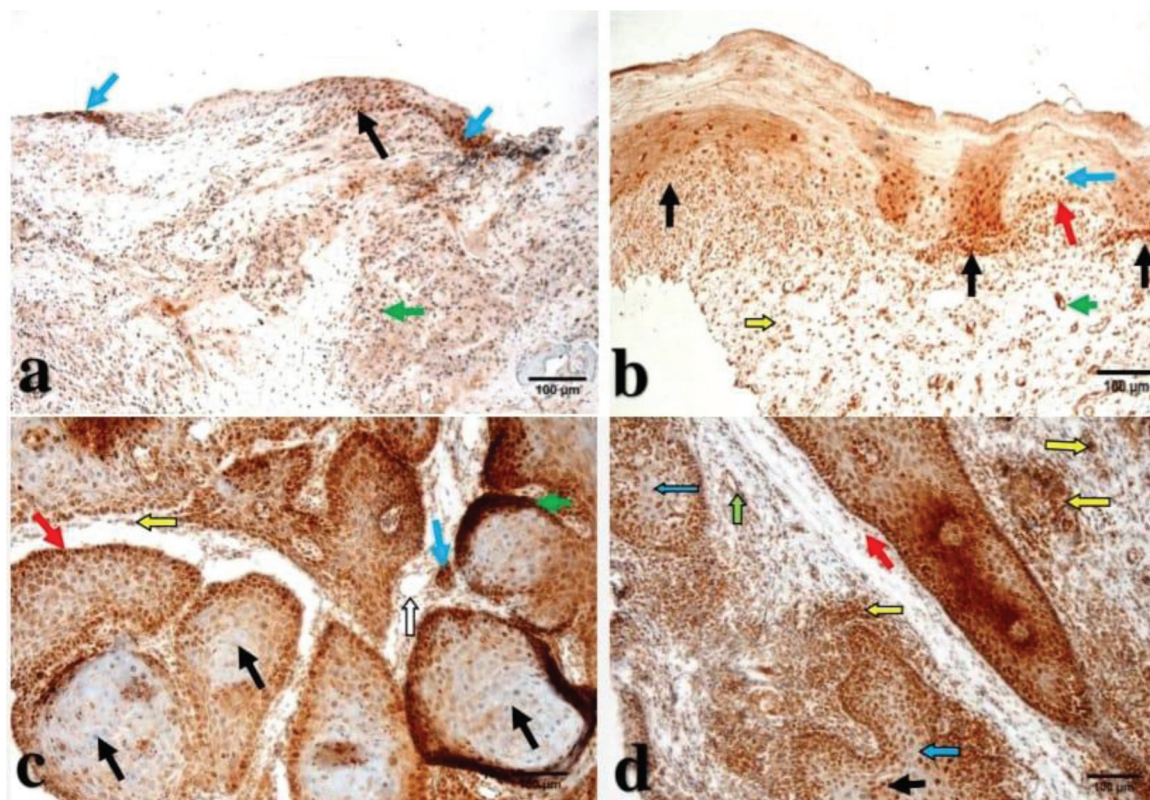
Regarding the expression of E-cadherin in OLP, the studied cases in the G2A subgroup showed strong positive membranous immune expression in all of the atrophic epithelium. There was some positive cytoplasmic immunostaining of some prickle cells as well as focal circumnuclear immunoreactivity. Positive immunostaining was observed in the endothelial cells, and some stromal cells were probably mast cells. Cases from the G2B subgroup showed positive, strong membranous immunostaining of E-cadherin in all the epithelial layers. There was also an evident immunoreactivity in the endothelial cells (Fig. 4a and b).

Figure 2



Immunohistochemical expression of Beclin-1 and E-cadherin in G1 control group. (a) Positive nuclear Beclin-1 immunostaining in basal and suprabasal cells (yellow arrow) with few scattered prickle cells demonstrating positive immunostaining throughout the surface epithelium (black arrow). Some stromal cells also exhibit positive nuclear immunoreactivity (red arrow) (Beclin-1, original magnification x20). (b) Positive intense circumferential E-cadherin membranous immunostaining throughout the whole epithelium (green arrow), with positive immunoreactivity in the vascular endothelial cells (yellow arrow) (E-cadherin, original magnification x20).

Figure 3



Immunohistochemical expression of Beclin-1 in OLP and OSCC. (a) G2A case showing predominant positive nuclear immunoreexpression in epithelial cells (black arrow), few cytoplasmic staining (blue arrows) and abundant nuclear immunostaining in most of the underlying stromal cells (green arrow). (b) G2B case showing positive nuclear immunoreexpression in the basal epithelial cells (red arrow), few prickle cells in the middle third (blue arrow), underlying inflammatory cells (black arrows), endothelial cells (green arrow) and in the remaining connective tissue stroma (yellow arrow). (c) G3A case showing abundant positive cytoplasmic (blue arrow) and nuclear (red arrow) immunostaining in malignant squamous cells at the periphery of the tumor masses (green arrow), as the center shows negative immunoreexpression (black arrows). The stromal cells (yellow arrow) and the endothelial cells (white arrow) exhibit immunopositivity. (d) The G3B case shows diffuse predominant nuclear immunopositivity, as few cells are spared from the immunoreactivity (blue arrows). The immunoreexpression is nuclear within the cell nests (black arrow), while it is both nuclear and cytoplasmic within the loose cellular masses and dispersed malignant cells in stroma (yellow arrows). The positive reaction is also quite obvious in the endothelial (green arrow) and stromal cells (red arrow) (Beclin-1, original magnification $\times 20$). OLP, oral lichen planus; OSCC, oral squamous cell carcinoma.

Concerning expression of E-cadherin in OSCC, cases from the G3A subgroup showed prominent positive membranous immunostaining of E-cadherin within the invading malignant masses. Focal nuclear/cytoplasmic staining of some sporadic cells could be seen. The positive immunoreactivity among the stromal cells is minimal. Cases from G3B subgroup showed positive membranous E-cadherin immunostaining within the broadsheets of the malignant epithelial cells. Some areas showed attenuation of the well-defined strong membranous immunoreactivity, where the staining is rather granular cytoplasmic. Focal cells demonstrated positive immunostaining along the nuclear membrane (Fig. 4c and d).

Comparison between different groups regarding Beclin-1

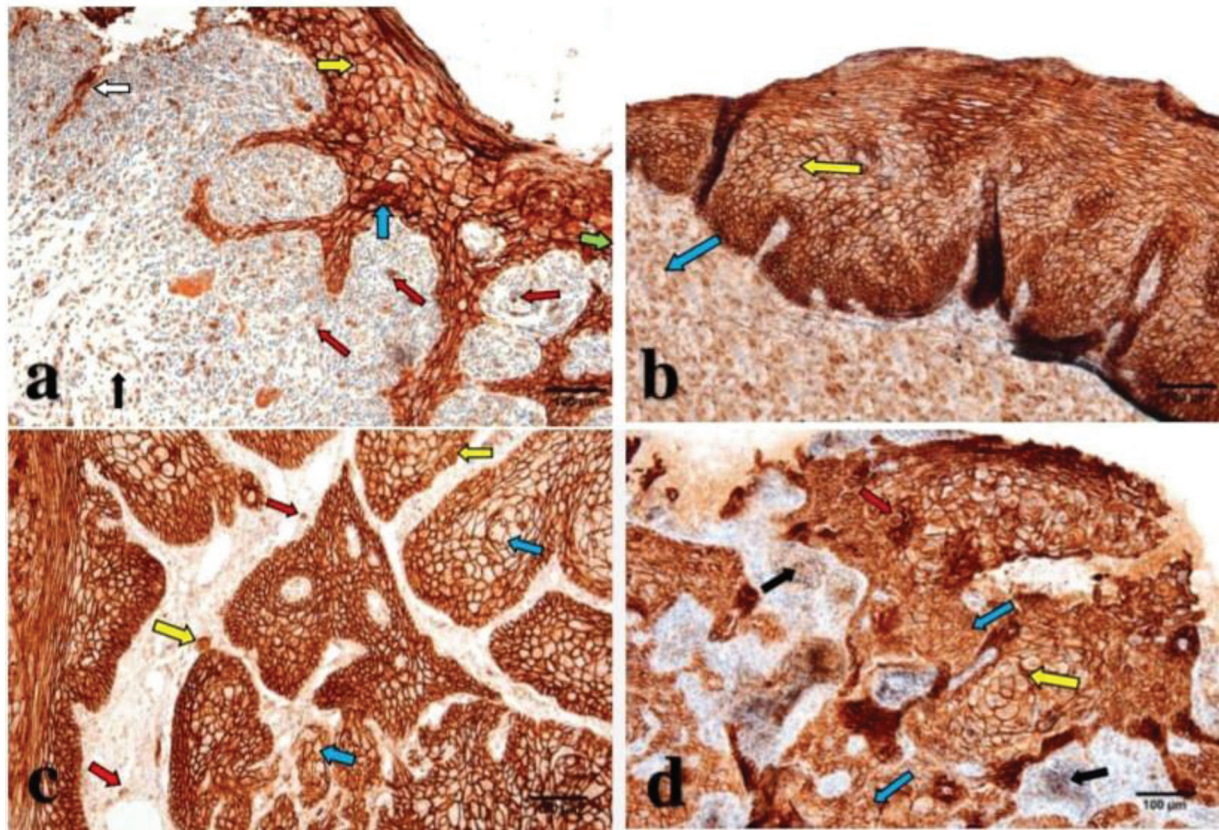
The present result found a significant increase ($P < 0.05$) in Beclin-1 expression levels in G2A and both groups G3A and B, while G3B exhibited the

highest of them. E-cadherin expression showed a significant decrease ($P < 0.05$) in all groups of OLP and OSCC cases compared to the control group. However group G3B revealed the lowest reduction. Comparison between different groups has shown significant differences among them as P value less than 0.05, as group 1 (3.03 ± 1.22) was significantly the lowest, while G3B (25.42 ± 1.49) was significantly the highest, as presented in Table 1. Intergroup comparison (post-hoc analysis) of the mean of the different groups was calculated and revealed that there was a significant difference between all groups as P value less than 0.05, except (G1 vs. G2B as $P = 0.06$) and (G3A. vs. G3B as $P = 0.053$).

Comparison between different groups regarding E-cadherin

Comparison between different groups revealed a significant difference between them as P value less than 0.05, as G3B (3.19 ± 0.51) was significantly the

Figure 4



Immunohistochemical expression of E-cadherin in OLP and OSCC. (a) G2A case showing strong positive membranous immunoreexpression in the atrophic epithelium (yellow arrow) with positive cytoplasmic immunostaining in some prickly cells (blue arrow), focal circumnuclear immunoreactivity (green arrow) at the area of ulceration (white arrow), endothelial cells (black arrow) and some stromal cells probably mast cells (red arrows). (b) The G2B case showed positive, strong membranous immunostaining in all the epithelial layers (yellow arrow) with evident immunoreactivity in the endothelial cells (blue arrow). (c) the G3A case shows prominent positive membranous immunostaining in the malignant masses (blue arrows) with focal nuclear/cytoplasmic staining in some sporadic cells (yellow arrows). (d) G3B case showing positive membranous immunostaining (yellow arrow) and granular cytoplasmic staining (blue arrows) in malignant epithelial cells. Focal cells demonstrate positive immunostaining along the nuclear membrane (red arrow) with stromal patchy positive immunoreactivity (black arrows) (E-cadherin, original magnification $\times 20$). OLP, oral lichen planus; OSCC, oral squamous cell carcinoma.

Table 1 Descriptive results of Beclin-1 expression levels in all groups

			Minimum	Maximum	Mean $\pm$ SD
Beclin-1	G1 (Control group)		1.32	4.91	3.03 $\pm$ 1.22 ^a
	G2 (OLP cases)	A	8.48	12.99	10.35 $\pm$ 2.35 ^b
		B	3.55	8.13	5.41 $\pm$ 1.82 ^a
	G3 (OSCC cases)	A	21.90	23.37	22.56 $\pm$ 0.70 ^c
		B	23.89	27.13	25.42 $\pm$ 1.49 ^c

OLP, oral lichen planus; OSCC, oral squamous cell carcinoma. All data with different superscript letters (a, b, c) are significantly different at P value less than 0.05, using one way analysis of variance analysis.

lowest, while G1 (19.36 $\pm$ 1.34) was significantly the highest, as presented in Table 2. Intergroup comparison (post-hoc analysis) of the mean of the different groups was calculated and revealed that there was a significant difference between all groups as P value less than 0.05, except (G2A vs. G2B as $P=0.214$), (G2A vs. G3A as $P=0.99$) (G2A vs. G3B. as $P=0.052$), and (G2B vs. G3A as $P=0.37$).

Correlation between Beclin-1 and E-cadherin

Pearson's correlation coefficient was used to evaluate the correlation between the two markers which revealed that there was an insignificant correlation between the two markers in all groups as P value more than 0.5, except G2 of a patient with OLP erosive which exhibited a negative significant correlation ($r=-0.9$, $P<0.03$) as presented in Table 3.

Table 2 Descriptive results of E-cadherin expression levels in all groups

			Minimum	Maximum	Mean±SD
E-cadherin	G1 (Control group)		16.68	21.16	19.36±1.34 ^a
	G2 (OLP cases)	A	5.06	6.31	5.80±0.53 ^b
		B	6.60	9.80	7.76±1.53 ^b
	G3 (OLP cases)	A	4.04	10.06	6.12±2.36 ^b
		B	2.63	3.88	3.19±0.51 ^c

OLP, oral lichen planus; OSCC, oral squamous cell carcinoma. All data with different superscript letters (a, b, c) are significantly different at *P* value less than 0.05, using one way analysis of variance analysis

Table 3 Pearson's correlation coefficient between Beclin-1 and E-cadherin regarding all groups

Pearson's correlation		<i>r</i>	<i>P</i> value
G1 (Control group)	0.04	0.90	
G2 (OLP cases)	A	−0.90	0.03*
	B	0.80	0.08
G3 (OSCC cases)	A	−0.40	0.45
	B	0.20	0.73

OLP, oral lichen planus; OSCC, oral squamous cell carcinoma.

*Significant difference as *P* value less than 0.05.

Discussion

OLP is a chronic, enduring inflammatory autoimmune disease that poses a huge influence on patients' well-being and level of affluence. The critical outcome of this disease is the progression of OLP into OSCC [20].

OSCC is considered a type of cancer commonly found in the head and neck region. Even though extraordinary advancement has been reached in treatment strategies, it shows a lower survival rate than other solid cancers [21]. The reason for this could be attributed to the deficiency in the early markers of diagnosis and patients' resistance to chemotherapy [22].

Still vague is the underlying molecular mechanism associated with the transformation of OLP into cancer. Nevertheless, they both appear to be connected, like in other conditions of autoimmune etiology. This could be attributed to the oncogenic outcomes related to chronic inflammation recognized in such a lesion [23].

Some studies showed the risk of cancer development from OLP which had been thoroughly underrated [5]. Moreover, a sequence of measures was recommended to follow in OLP malignancy research in order to enhance their methodological eminence and design [24].

Basic autophagy essentially serves to maintain the survival of cells. It is essential and valuable for the cells and living organisms since it blocks the

accumulation of toxic metabolites, degrades damaged organelles and provides living organisms with bioenergetic substrates required for survival [25]. However, its excessive, permanent activation can induce self-degradation of cells, leading to death, as there is a cross-talk with apoptotic pathways [7].

Using a yeast two-hybrid screen, it was demonstrated that the crucial autophagy protein Beclin-1 directly acts together with the protein Bcl-2. The Beclin-1 autophagy-related gene (BECN1) encodes Beclin-1, which was identified in 1998 [26]. Beclin-1 is crucial to the development of tumors. However, depending on the situation, Beclin-1 can act as a booster or a suppressor [27]. On the other hand, the integrity of the epithelial tissue is maintained by E-cadherin, which is also in charge of maintaining cell–cell cohesion and apicobasal epithelial polarity [15]. Consequently, reduced E-cadherin expression disrupts this function, leading to a progressive loss of epithelial properties and an acquisition of migratory ability, a process called EMT that is thought to be the first step toward invasion and metastasis [16].

Concerning OLP expression of Beclin-1, our study showed significant differences between different groups, as group 1 was significantly the lowest, while G3B was significantly the highest. In OLP subgroups, Beclin-1 showed an increase in expression, which was significantly different from the control group, with the G2A subgroup being the highest. That agrees with a study by Azouz *et al.* [28], who observed a significant upsurge in the level of the Beclin-1 gene in the studied groups compared to the control group. However, they discovered no significant difference between oral and cutaneous LP. The presence of higher expression of Beclin-1 in atrophic OLP could be a reflection of the higher risk background of this OLP subtype of malignant transformation. This is in agreement with a search by Shen *et al.* [29], who detected the autophagy level higher in erosive OLP in comparison with nonerosive subtype, which contributes to the higher lesion

destruction locally, which might implicate the fact that atypical autophagy of cells might have a crucial part in the pathogenesis of OLP.

The expression was detected mainly in most of the epithelial cells, with predominant nuclear staining as well as some cytoplasmic staining. The immunostaining was also detected in most of the underlying stromal cell nuclei. Some cases from G2B subgroup showed positive nuclear immunoexpression of Beclin-1 in the basal epithelial cells. In addition to the presence of positive Beclin-1 immunostained endothelial cells. The presence of predominant nuclear staining was reported in another study of Beclin-1 by Xu *et al.* [30], who showed that the subcellular localization of Beclin-1 in mice varied according to the level of development, where it is localized primarily during embryonic stage in the cytoplasm and plasma membrane, while later postnatal life, largest part of it re-localized into the nucleus with very fewer portion remained in the cytoplasm. This also explains the dual presence of Beclin-1 expression in both the nuclei and the cytoplasm, with the nuclear localization taking the lion's share.

This finding could be attributed to the fact that OLP lesions are frequently nonremissive and persistent and may progress to malignant transformation or other causes of morbidity. While the precise cause of the disease is still unknown, immunological dysregulation has been shown to be a significant factor [31]. The condition is a result of the impact of several extrinsic or modified self-antigens [32]. According to Zhou *et al.* [33], antigen presentation, T cell activation and migration, and keratinocyte apoptotic dysregulation may all be components of the immunopathogenesis of OLP. In summary, many cytokine mediators may be used by activated T cells (CD4+ helper T cells and CD8+ cytotoxic T cells) to mediate the apoptosis of keratinocytes [34]. Autophagy plays a vital role in sustaining the homeostasis of cells by breaking down long-lived or defective proteins.

There is also another finding seen in another study, in OLP, which is the deficiency of apoptosis, that could be explained by the actively established machinery for anti-apoptosis, such as Bcl-2 that was found over-expressed [35] in OLP. The erosions seen in atrophic OLP are a result of loss of epithelium due to autoimmune aggression, leaving the submucosal structural elements such as nerves, salivary glands and blood vessels without protection. The resulting counter-back action of the epithelium hence will be

hyperproliferation in addition to halted apoptosis, favoring an excellent medium and extreme risk for malignant transformation. However, it requires an additional event, which should be a mutation in the TP53 gene [36].

Generally speaking, the transformation of OLP into OSCC could be attributed to changes on the molecular level upon the basal cells of the targeted oral epithelium exerted by the inflammatory infiltrate, in addition to the consequences brought up by the growth factors and chemokines formed by the inflammatory infiltrate upon the basal cells, that sequentially react by increasing their rate and activity of proliferation and apoptosis inhibition processes. Collectively, this creates sufficient mutations that predispose to cancer development, in adjunct with the inactivation of p53, the tumor-suppressor gene which plays the final role in the transformation mechanism [37]. The danger of a concluding consequence on the molecular level, culminating in the manifestation of a malignant clone, extends to the entire surface of the oral mucosa distressed by OLP. This would account for the higher proportion of malignant OLP cases that develop multiple OSCCs [38] and deliver molecular support for the existing classification of OLP as a malignant field [39].

Beclin-1, an autophagy-promoting regulator, has contradictory effects on carcinogenesis. By eliminating faulty or damaged organelles and other cellular components, Beclin-1 helps reduce tumors; nonetheless, its activity can also promote cancer onset and development. Beclin-1 overexpression in head and neck cancer increases autophagy, which affects apoptotic and autophagic pathways to increase necrosis and inflammation while limiting DNA damage and chromosomal instability [28].

Our study of Beclin-1 expression in OSCC showed abundant positive immunostaining of Beclin-1 in the G3A subgroup in both the cytoplasm and nuclei of the malignant squamous cells with abundant positive immunostaining of Beclin-1 in both the cytoplasm and nuclei of the malignant squamous cells, while subgroup G3B, displayed diffuse immunopositivity to Beclin-1 in the vast majority of the tumor cells, with fewer immune-negative ones. Yet, there was an insignificant difference between the two subgroups.

Data on Beclin-1 role in and development of OSCC is rather limited. Our study was in accordance with Ahn *et al.* [11], who revealed that beclin-1 showed increased expression in gastric and colorectal malignant cells in

contrast to control normal epithelial cells, which proposes that Beclin-1 might be a part of the carcinogenesis process. Another study by Fujii *et al.* [40] showed that tumorigenesis was promoted by autophagy, which, in turn, gave protection for cancer cells against apoptosis. Also, another study by Barca *et al.* [41] found a significant correlation between Beclin-1 expression and poor prognosis of OSCC. Our data, however, was contradictory to some studies, as Qiu *et al.* [42], studied HCC and concluded that its progression and pathogenesis are accompanied by decreased autophagy.

E-cadherin is a transmembrane protein representing an important member in the family of cell adhesion molecules “cadherins” whose primary function is to maintain epithelial integrity through stabilization of epithelial cell-cell junction, inhibiting cell motility and migration [15]. Therefore, E-cadherin acts as a tumor-suppressor protein, reduced or lost expression of which can result in drastic consequences in the form of commencing a program on a cellular level called EMT, in which the epithelial cells gain a mesenchymal phenotype, signaling the invasive phase [16]. As a result, E-cadherin can serve as an excellent prognostic biomarker that helps predict possible progression by monitoring its differential expression levels among normal and different stages of epithelial dysplasia up to frank malignancies.

As regards the expression of E-cadherin in the different groups, our study demonstrated that G1 was significantly the highest, while G3B was significantly the lowest. In OLP subgroups, G2A and G2B, E-cadherin showed a significant reduction in expression compared to the control group G1, with the G2A subgroup level being lower than that of G2B but with no statistically significant difference. In line with our results is that of the study conducted by Du and Li [43], Hämäläinen *et al.* [44], and Rauf *et al.* [45], who showed a significantly different expression between normal mucosa and OLP. In contrast to our study, Sridevi *et al.* [46] revealed a significantly weak E-cadherin expression in atrophic and erosive OLP in relation to the nonerosive type and still, expression of all was significantly lower than that of the normal control tissue. However, in agreement with our findings, comparing erosive to nonerosive OLP types, Bar *et al.* [47] found that the fraction of E-cadherin immunostained cells was similar in both OLP types.

The expression pattern of E-cadherin in normal tissue is intense, continuous homogeneous membranous

staining throughout the whole epithelium (sparing the most superficial keratinocytes), and that finding is also highlighted by Sridevi *et al.* [46] and Rauf *et al.* [45]. Concerning OLP in our study, both erosive and nonerosive cases showed strong positive membranous staining in the whole epithelium. Moreover, some erosive OLP cases exhibited some cytoplasmic immunostaining as well as focal circumnuclear immunoreactivity. Nevertheless, unlike our findings, Sridevi *et al.* [46] showed weak E-cadherin expression in basal keratinocytes, which might be attributed to focal destruction and degeneration resulting in loss of intercellular adhesion. Supporting our results, Puneeta *et al.* [48], upon comparison to the normal tissue with typical membranous reactivity, reported a progressive cytoplasmic shift in immunostaining in the cases of oral epithelial dysplasia paralleling the increasing grades from mild to severe. Aberrant nuclear localization of E-cadherin has been detected and explained in cases of colorectal cancer where there was re-shuttling from the cell membrane to the cytoplasm, which might be a result of posttranscriptional modification [49].

Positive E-cadherin immunostaining observed in some endothelial cells can be explained by the fact that the vascular type of E-cadherin is an integral part of endothelial cellular adherens junctions, serving to maintain vascular integrity [50]. As for the positive immunostaining in a few stromal cells, it has been demonstrated that E-cadherin is expressed in a subset of CD34+ cells [51]. Besides, positive E-cadherin expression in mast cells has been determined in previous studies [52,53].

As concerns E-cadherin expression in OSCC, our study demonstrated that G3B subgroup was significantly the lowest. E-cadherin also showed a significantly reduced expression in G3A compared to the control group G1. Comparing the two OSCC grades to each other, G3B exhibited a significant reduction in E-cadherin expression in relation to G3A. The present findings concerning OSCC are also in accordance with those of many previous studies, with a consensus considering that downregulation of E-cadherin expression is directly associated with poor prognosis, as concluded from the meta-analysis study performed by Lorenzo-Pouso *et al.* [54]. The descending reduction in E-cadherin expression from well to moderately differentiated OSCC was well depicted in a study conducted by Ali *et al.* [55], who confirmed the strong correlation between the declined E-cadherin expression and the advanced histopathologic grade of cancer. Discordant with this concept, Ukpo *et al.* [56] implied that

cadherin expression in oropharyngeal SCC is unrelated to the histologic grade and metastatic potential, negating the fact regarding E-cadherin a reliable prognostic factor for cancer progression.

The immunostaining of E-cadherin in the G3A subgroup was more intense along the cell membranes of the malignant cells, with less evident cytoplasmic staining, while in the G3B subgroup, the membranes of the cancer cells were not as deeply stained as those of G3A, with more obvious cytoplasmic staining of some cells as well as an aberrant nuclear reactivity. These findings concerning the intensity of the membranous staining are compatible with those of the study of Ali *et al.* [55]. A mixed pattern of immunostaining (membranous +cytoplasmic) was also found in some OSCC cases by Khan *et al.* [57] and D'Souza *et al.* [58], with no reported significance of this cytoplasmic localization or presence of any correlation with a other clinicopathologic parameters in the study. In alignment with our findings, Puneeta *et al.* [48] found that cytoplasmic reactivity noticeably escalated in the OSCC group from well to moderately to poorly differentiated OSCCs.

Explaining the more prominent cytoplasmic E-cadherin localization in moderately differentiated OSCC than the well-differentiated cases, Kaur *et al.* [59] postulated that the cytoplasmic shift in E-cadherin expression indicates an advanced stage of malignancy and the explanation was based on the deranged formation of a cadherin- β -catenin complex in which the catenin protein normally acts as a cytoplasmic adaptor that helps anchorage the cadherin to the cell membrane from one side and to the actin cytoskeleton from the other side. So, with such derangement or improper binding, the E-cadherin protein might shuttle to the cytoplasm. Basically, it has been established that E-cadherin is constantly in a condition of turnover as a result of trafficking between both the cell membrane and the cytosol, where it can undergo temporary storage, degradation or redirection to the cell surface [60].

The current study aimed at exploring a possible correlation between the autophagic process, represented by Beclin-1 protein and the invasiveness potential constituted by the EMT process with its cardinal marker E-cadherin, in all study groups. The statistical analysis disclosed an insignificant correlation between the two proteins in all groups/subgroups except in G2A (erosive OLP), where there was a strong, significant inverse correlation between the

upregulated levels of Beclin-1 and the downregulated expression of E-cadherin. In agreement with our findings regarding the relationship between autophagy and EMT, Shen *et al.* [61] dictated that Beclin-1-mediated autophagy in colon cancer cells prompted EMT through upregulating the expression of vimentin and twist and downregulating E-cadherin levels. Contradictory to ours, the results of Li *et al.* [62] concluded that Beclin-1, in the context of thyroid cancer, is a tumor-suppressor protein, so the knockdown of which resulted in the downregulation of E-cadherin in cancer cells, suggesting a positive correlation between Beclin-1 and E-cadherin.

A growing body of evidence reveals that there is a cross-talk between autophagy and EMT as the autophagic process involves the degradation of E-cadherin among other cell adhesion macromolecules, thereby inducing EMT; at the same time, cells undergoing EMT turn on autophagy program to be capable of surviving the metabolic challenges within the tumor microenvironment [63].

Conclusion

Upregulated levels of autophagy, as indicated by higher expression of Beclin-1 and downregulation of E-cadherin expression in erosive OLP and OSCC imply that it might be very comparable to well-differentiated OSCC concerning the disruption of epithelial organization and intercellular cohesion, as represented by the deteriorated E-cadherin expression. Interestingly, the presence of a strong inverse relation between Beclin-1 and E-cadherin in erosive OLP indicates a great potential for invasiveness as well as migration capability and hence higher tendency to malignant transformation. However, we recommend further studies using more biomarkers with Beclin-1 to assess the malignant potential of erosive OLP.

Financial support and sponsorship
Nil.

Conflicts of interest

There are no conflicts of interest.

References

- 1 Krupaa R, Sankari S, Masthan K, Rajesh E. Oral lichen planus: an overview. *J Pharm Bioallied Sci* 2015; 7(Suppl 1):S158–161.
- 2 González-Moles M, Warnakulasuriya S, González-Ruiz I, González-Ruiz L, Ayén Á, Lenouvel D, *et al.* Worldwide prevalence of oral lichen planus: a systematic review and meta-analysis. *Oral Dis* 2020; 27:813–828.
- 3 Warnakulasuriya S, Kujan O, Aguirre-Urizar J, Bagan J, González-Moles M, Kerr A, *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; 27:1862–1880.
- 4 González-Moles M, Ruiz-Ávila I, González-Ruiz L, Ayén Á, Gil-Montoya J, Ramos-García P. Malignant transformation risk of oral lichen planus: a systematic review and comprehensive meta-analysis. *Oral Oncol* 2019; 96:121–130.
 - 5 González-Moles M, Ramos-García P, Warnakulasuriya S. An appraisal of highest quality studies reporting malignant transformation of oral lichen planus based on a systematic review. *Oral Dis* 2021; 27:1908–1918.
 - 6 Zou Z, Li B, Wen S, Lin D, Hu Q, Wang Z, Fang J. The current landscape of oral squamous cell carcinoma: a comprehensive analysis from clinical trials.gov. *Cancer Control* 2022; 29:1–14.
 - 7 Cao W, Li J, Yang K, Cao D. An overview of autophagy: mechanism, regulation and research progress. *Bull Cancer* 2021; 108:304–322.
 - 8 Kang R, Zeh H, Lotze M, Tang D. The multifaceted effects of autophagy on the tumor microenvironment. *Adv Exp Med Biol* 2020; 1225:99–114.
 - 9 Aita V, Liang X, Murty V, Pincus D, Yu W, Cayanis E, *et al.* Cloning and genomic organization of beclin-1, a candidate tumor suppressor gene on chromosome 17q21. *Genomics* 1999; 59:59–65.
 - 10 Valente G, Morani F, Nicotra G, Fusco N, Peracchio C, Titone R, *et al.* Expression and clinical significance of the autophagy proteins BECLIN-1 and LC3 in ovarian cancer. *Biomed Res Int* 2014; 2014:462658.
 - 11 Ahn C, Jeong E, Lee J, Kim M, Kim S, Kim S, *et al.* Expression of beclin-1, an autophagy-related protein, in gastric and colorectal cancers. *APMIS* 2007; 115:1344–1349.
 - 12 Jung Y, Lee Y, Koo J. The potential of Beclin-1 as a therapeutic target for the treatment of breast cancer. *Expert Opin Ther Targets* 2016; 20:167–178.
 - 13 Wu J, Li L, Wang S, Pang X, Wu J, Sheng S, *et al.* Autophagy is positively associated with the accumulation of myeloid-derived suppressor cells in 4-nitroquinoline-1-oxide-induced oral cancer. *Oncol Rep* 2018; 40:3381–3391.
 - 14 Tang J, Fang Y, Hsi E, Huang Y, Hsu N, Yang W, *et al.* Immunopositivity of Beclin-1 and ATG5 as indicators of survival and disease recurrence in oral squamous cell carcinoma. *Anticancer Res* 2013; 33:5611–5616.
 - 15 Coopman P, Djiane A. Adherens Junction and E-Cadherin complex regulation by epithelial polarity. *Cell Mol Life Sci* 2016; 73:3535–3553.
 - 16 Bure I, Nemtsova M, Zaletaev D. Roles of E-cadherin and Noncoding RNAs in the epithelial-mesenchymal transition and progression in gastric cancer. *Int J Mol Sci* 2019; 20:2870.
 - 17 Gugnoni M, Sancisi V, Manzotti G, Gandolfi G, Ciarrocchi A. Autophagy and epithelial-mesenchymal transition: an intricate interplay in cancer. *Cell Death Dis* 2016; 7:e2520.
 - 18 Gundamaraju R, Lu W, Paul M, Jha N, Gupta P, Ojha S, *et al.* Autophagy and EMT in cancer and metastasis: who controls whom? *Biochim Biophys Acta Mol Basis Dis* 2022; 1868:166431.
 - 19 Faul F, Erdfelder E, Lang A, Buchner A. G* Power 3. a flexible statistical power analysis program for the social, behavioral and biomedical sciences. *Behav Res Methods* 2007; 39:175–191.
 - 20 Tampa M, Caruntu C, Mitran M, Mitran C, Sarbu I, Rusu L-C, *et al.* Markers of Oral Lichen Planus Malignant Transformation. *Disease Markers* 2018; 2018:1959506. doi: 10.1155/2018/1959506.
 - 21 Henderson-Jackson EB, Helm J, Strosberg J, Nasir NA, Yeatman TJ, Kvols LK, *et al.* Palladin is a marker of liver metastasis in primary pancreatic endocrine carcinomas. *Anticancer Res* 2011; 31:2957–2962.
 - 22 Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics. *CA Cancer J Clin* 2007; 57:43–66.
 - 23 De Porras-Carrique T, Ramos-García P, Aguilar-Diosdado M, Warnakulasuriya S, González-Moles MÁ. Autoimmune disorders in oral lichen planus: a systematic review and meta-analysis. *Oral Dis* 2022; 29:1382–1394.
 - 24 Ramos-García P, Gonzalez-Moles MA, Warnakulasuriya S. Oral cancer development in lichen planus and related conditions-3.0 evidence level: a systematic review of systematic reviews. *Oral Dis* 2021; 27:1919–1935.
 - 25 Doherty J, Baehrecke EH. Life, death and autophagy. *Nature cell biology* 2018; 20:1110–1117.
 - 26 Liang XH, Kleeman LK, Jiang HH, Gordon G, Goldman JE, Berry G, *et al.* Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. *J Virol*. 1998; 72:8586–8596.
 - 27 Hu YJ, Zhong JT, Gong L, Zhang SC, Zhou SH. Autophagy-Related Beclin 1 and Head and Neck Cancers. *Onco. Targets Ther.* 2020; 13:6213–6227.
 - 28 Azouz T, Rashed L, Fayed S, Mounir R, Elwan Y. Level of autophagy in lichen planus: role of endoplasmic reticulum stress proteins. *Egypt Acad J Biol Sci* 2022; 14:265–275.
 - 29 Shen MX, Hu WY, Cai Y. Expression and significance of microtubule associated protein 1 light chain 3B, p62 and Beclin1 in lesion tissues of oral lichen planus patients. *Zhonghua Kou Qiang Yi Xue Za Zhi* 2022; 57:1217–1224.
 - 30 Xu F, Fang Y, Yan L, Xu L, Zhang S, Cao Y, *et al.* Nuclear localization of Beclin 1 promotes radiation-induced DNA damage repair independent of autophagy. *Sci Rep* 2017; 7:45385.
 - 31 Alrashdan MS, Cirillo N, McCullough M. Oral lichen planus: a literature review and update. *Arch Dermatol Res* 2016; 308:539–551.
 - 32 Conrotto D, Barattero R, Carbone M, Gambino A, Sciannameo V, Ricceri F, Arduino PG. Can atrophic-erosive oral lichen planus promote cardiovascular diseases? A population-based study. *Oral Dis* 2018; 24: 215–218.
 - 33 Zhou G, Zhang J, Ren XW, Hu JY, Du GF, Xu XY. Increased B7-H1 expression on peripheral blood T cells in oral lichen planus correlated with disease severity. *J Clin Immunol* 2012; 32:794–801.
 - 34 Zhang N, Zhang J, Tan YQ, Du GF, Lu R, Zhou G. Activated Akt/mTOR-autophagy in local T cells of oral lichen planus. *Int Immunopharmacol* 2017; 48:84–90.
 - 35 González-Moles MA, Bascones-Ilundain C, Gil Montoya JA, Ruiz-Avila I, Delgado-Rodríguez M, Bascones-Martínez A. Cell cycle regulating mechanisms in oral lichen planus: Molecular bases in epithelium predisposed to malignant transformation. *Arch Oral Biol* 2006; 51:1093–1103.
 - 36 González-Moles MÁ, Keim-Del Pino C, Ramos-García P. Hallmarks of cancer expression in oral lichen planus: a scoping review of systematic reviews and meta-analyses. *Int J Mol Sci* 2022; 23:13099.
 - 37 Gonzalez-Moles MA, Gil-Montoya JA, Ruiz-Avila I, Esteban F, Bascones-Martínez A. Differences in the expression of p53 protein in oral lichen planus based on the use of monoclonal antibodies DO7 and pAb 240. *Oral Oncol* 2008; 44:496–503.
 - 38 González-Moles MÁ, Warnakulasuriya S, González-Ruiz I, González-Ruiz L, Ayén Á, Lenouvel D, *et al.* Clinicopathological and prognostic characteristics of oral squamous cell carcinomas arising in patients with oral lichen planus: a systematic review and a comprehensive meta-analysis. *Oral Oncol* 2020; 106:104688.
 - 39 Gonzalez-Moles MA, Scully C, Ruiz-Avila I. Molecular findings in oral premalignant fields: update on their diagnostic and clinical implications. *Oral Dis* 2012; 18:40–47.
 - 40 Fujii S, Mitsunaga S, Yamazaki M, Hasebe T, Ishii G, Kojima M, *et al.* Autophagy is activated in pancreatic cancer cells and correlates with poor patient outcome. *Cancer Sci* 2008; 99:1813–1819.
 - 41 Barca I, Mignogna C, Novembre D, Ferragina F, Cristofaro MG. Immunohistochemical analysis of the Beclin-1 expression predicts the progression of oral squamous cell carcinoma. *Int J Environ Res Public Health* 2021; 18:11125.
 - 42 Qiu DM, Wang GL, Chen L, Xu YY, He S, Cao XL, *et al.* The expression of beclin-1, an autophagic gene, in hepatocellular carcinoma associated with clinical pathological and prognostic significance. *BMC Cancer* 2014; 9:327.
 - 43 Du Y, Li H. Expression of E-cadherin in oral lichen planus. *Exp Ther Med* 2015; 10:1544–1548.
 - 44 Hämäläinen L, Soini Y, Pasonen-Seppänen S, Siponen M. Alterations in the expression of EMT-related proteins claudin-1, claudin-4 and claudin-7, E-cadherin, TWIST1 and ZEB1 in oral lichen planus. *J Oral Pathol Med* 2019; 48:735–744.
 - 45 Rauf M, Shahab S, Khadija S, Ahmad A, Azmat H, Waseem H, Sheikh A. Decrease expression of E-cadherin in oral lichen planus- a marker of premalignant lesion. *Int J Pathol* 2021; 19:167–171.
 - 46 Sridevi U, Jain A, Nagalaxmi V, Kumar U, Goyal S. Expression of E-cadherin in normal oral mucosa, in oral precancerous lesions and in oral carcinomas. *Eur J Dent* 2015; 9:364–372.
 - 47 Bar J, Cierpikowski P, Lis-Nawara A, Duc P, Hatoń A, Radwan-Oczko M. Comparison of p53, HSP90, E-cadherin and HPV in oral lichen planus and oral squamous cell carcinoma. *Acta Otorhinolaryngol Ital* 2021; 41:514–522.
 - 48 Puneeta N, Santosh T, Mishra I, Gaikwad P, Sahu A. Evaluation of E-cadherin and vimentin expression for different grades of oral epithelial dysplasia and oral squamous cell carcinoma – an immunohistochemical study. *J Oral Maxillofac Pathol* 2022; 26:285–286.

- 49 Su Y, Chang Y, Lin W, Liang C, Lee J. An aberrant nuclear localization of E-cadherin is a potent inhibitor of Wnt/ β -catenin-elicited promotion of the cancer stem cell phenotype. *Oncogenesis* 2015; 4:e157.
- 50 Giannotta M, Trani M, Dejana E. VE-cadherin and endothelial adherens junctions: active guardians of vascular integrity. *Dev Cell* 2013; 26:441–454.
- 51 Ohgami R, Chisholm K, Ma L, Arber D. E-cadherin is a specific marker for erythroid differentiation and has utility, in combination with CD117 and CD34, for enumerating myeloblasts in hematopoietic neoplasms. *Am J Clin Pathol* 2014; 141:656–664.
- 52 Tegoshi T, Nishida M, Ishiwata K, Kobayashi T, Uchiyama F, Nabeshima K, *et al.* E-cadherin and cadherin-associated cytoplasmic proteins are expressed in murine mast cells. *Lab Invest* 2000; 80:1571–1581.
- 53 Klewer T, Bakic L, Müller-Reichert T, Kiewisz R, Jessberger G, Kiessling N, *et al.* E-Cadherin restricts mast cell degranulation in mice. *Eur J Immunol* 2022; 52:44–53.
- 54 Lorenzo-Pouso A, Silva F, Pérez-Jardón A, Chamorro-Petronacci C, Oliveira-Alves M, Álvarez-Calderón-Iglesias Ó, *et al.* Overexpression of E-Cadherin is a favorable prognostic biomarker in oral squamous cell carcinoma: a systematic review and meta-analysis. *Biology (Basel)* 2023; 12:239.
- 55 Ali A, Ghoneim S, Ahmed E, El-Farouk L, Anis S. Cadherin switching in oral squamous cell carcinoma: a clinicopathological study. *J Oral Biol Craniofac Res* 2023; 13:486–494.
- 56 Ukpo O, Thorstad W, Zhang Q, Lewis J. Lack of association of cadherin expression and histopathologic type, metastasis, or patient outcome in oropharyngeal squamous cell carcinoma: a tissue microarray study. *Head Neck Pathol* 2012; 6:38–47.
- 57 Khan S, Hegde V, Shrivastava D, Azamulla M, Alam M, Srivastava K. Comparative assessment of E-cadherin expression between the metastatic and non-metastatic oral squamous cell carcinoma: an immunohistochemical study. *Pesqui Bras Odontopediatria Clín Integr* 2023; 23:e220077.
- 58 D'Souza Z, Tupkari J, Joy T, Rathi D, Nagar S, Mehta V, Jadhav G. A prospective immunohistochemical study using E-cadherin and Ncadherin to depict cadherin switch in oral squamous cell carcinoma. *J Popul Ther Clin Pharmacol* 2023; 30:365–374.
- 59 Kaur J, Sawhney M, DattaGupta S, Shukla N, Srivastava A, Walfish P, Ralhan R. Clinical significance of altered expression of β -catenin and E-cadherin in oral dysplasia and cancer: potential link with ALCAM expression. *PLoS One* 2013; 8:e67361.
- 60 Niño C, Sala S, Polo S. When ubiquitin meets E-cadherin: plasticity of the epithelial cellular barrier. *Semin Cell Dev Biol* 2019; 93:136–144.
- 61 Shen H, Yin L, Deng G, Guo C, Han Y, Li Y, *et al.* Knockdown of Beclin-1 impairs epithelial mesenchymal transition of colon cancer cells. *J Cell Biochem* 2018; 119:7022–7031.
- 62 Li S, Zhang H, Du Z, Li C, An M, Zong Z, *et al.* Induction of epithelial-mesenchymal transition (EMT) by Beclin-1 knockdown via posttranscriptional upregulation of ZEB1 in thyroid cancer cells. *Oncotarget* 2016; 7:70364–70377.
- 63 Santarosa M, Maestro R. The autophagic route of E-cadherin and cell adhesion molecules in cancer progression. *Cancers (Basel)* 2021; 13:6328.