Comparison of biomarker expression in oral lichen planus and oral lichenoid lesions

Małgorzata Radwan-Oczko[1,A–F], Anna Lis-Nawara[2,C–D], Agnieszka Hałoń[3,B,C,F], Julia Bar[2,A,C–F]

[1] Department of Oral Pathology, Wroclaw Medical University, Poland
[2] Department of Immunopathology and Molecular Biology, Wroclaw Medical University, Poland
[3] Department of Pathomorphology and Oncological Cytology, Wroclaw Medical University, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2022;31(12):1327–1334

Address for correspondence
Malgorzata Radwan-Oczko
E-mail: malgorzata.radwan-oczko@umed.wroc.pl

Funding sources
None declared

Conflict of interest
None declared

Received on January 13, 2022
Reviewed on February 13, 2022
Accepted on July 27, 2022

Published online on Septemper 1, 2022

Abstract

Background. Oral lichen planus (OLP) and oral lichenoid lesions (OLL) comprise a group of oral mucosal disorders that have similar clinical and histological features.

Objectives. To compare the levels of investigated biomarkers in biopsied OLP and OLL, and to determine the pattern of biomarkers, which could be useful for the biological characterization of these 2 disorders.

Materials and methods. A total of 56 biopsy specimens in 2 groups were analyzed in this study. One group consisted of 25 idiopathic OLP lesions, and the other included 31 OLL from patients treated with antihypertensive and cardiac medications. The expression of protein p53, topoisomerase I (topo I), heat shock protein 90 (HSP90), and E-cadherin was analyzed using immunohistochemistry.

Results. The p53 protein expression showed a trend to a positive correlation with topo I expression in the total sample ($p = 0.067$, $R = 0.25$). The p53 protein and HSP90 expression was higher in the OLL group compared to the OLP group, but the difference was not statistically significant. No association was found between topo I and E-cadherin expression for either the OLP or OLL group.

Conclusions. The findings of this study suggest that the slightly higher protein p53 and HSP90 expression in the OLL group might be caused by the medications used. The slight association between p53 and topo I expression indicates that the cooperation between these proteins might be essential for the growth of OLP/OLL in general. We conclude that the overexpression of p53 protein and high expression of topo I found in both types of lesions might induce their biologically aggressive behavior.

Key words: p53, biomarkers, OLP, OLL

Cite as
Radwan-Oczko M, Bar J, Hałoń A, Lis-Nawara A. Comparison of biomarker expression in oral lichen planus and oral lichenoid lesions. *Adv Clin Exp Med*. 2022;31(12):1327–1334. doi:10.17219/acem/152429

DOI
10.17219/acem/152429

Background

Oral lichen planus (OLP) and oral lichenoid lesions (OLL) comprise a group of disorders of the oral mucosa. Oral lichen planus lesions are a chronic disorder most commonly affecting middle-aged adults, with a slight female predominance. Such lesions appear as white papules that coalesce to form reticular, annular or plaque-like patterns.[1,2] The etiology and pathogenesis of OLP are still not clear or fully explained. Studies suggest the presence of an immunological disorder in which $CD4^+$ (Th1) and $CD8^+$ (Tc) lymphocyte activation, as well as production of cytokines, such as interleukin-2 (IL-2), interferon gamma (IFN-γ) and tumor necrosis factor (TNF), can cause apoptosis of keratinocytes.[3–5] Recent studies have indicated that Th2 lymphocytes can also contribute to the pathogenesis of OLP.[6] The histopathological assessment of OLP lesions reveals hyperkeratosis, degeneration of the basal cell layer of the epithelium, hydropic degeneration of basal cells, infiltration of lymphocytes in the connective tissue, and acanthotic or atrophic epithelium.[1,2]

Oral lichenoid lesions are another type of lesion frequently observed in the oral mucosa. In certain patients, oral lichenoid drug reaction lesions can be caused by numerous medications, such as the majority of β-blockers, some angiotensin-converting enzyme inhibitors, some thiazide diuretics, nonsteroidal anti-inflammatory drugs, and oral hypoglycemic medications.[2,5,7] In a study by Balakumar et al. adverse drug reactions were identified in patients treated with combinations of β-adrenergic blockers and calcium-channel blockers in addition to β-adrenergic blockers and diuretics and coronary disease medications.[7] Similarly, Jinbu and Demitsu noted that nicorandil induced ulcerations on the sites typical of OLP lesions.[8] Farzin et al. found lichenoid reactions of linear striations on the buccal mucosa in 4.5% of 465 hypertensive patients being treated with antihypertensive medications.[9] An oral lichenoid drug reaction can occur at any time, even years after the beginning of treatment. In many cases, alternative medication options are not available. Oral lichenoid lesions often represent clinical and histological features of OLP, so diagnosis is often complicated.[2,9] Furthermore, there are no specific biomarkers helpful in distinguishing between OLP and OLL and predicting their behavior.[10–12]

Few studies have analyzed molecular markers that might be useful in identifying patients with progressive OLP growth.[11–13] They found that p53 protein and topoisomerase IIα expressions in OLP might be markers of proliferative activity.[10,11] Some data suggest that the overexpression of p53 protein in benign lesions might play a role in the early stages of oral carcinogenesis, and *TP53* mutations might be an important oncogenic event in malignantly transformed OLP.[3,11,14] A single study determined that focal loss of E-cadherin expression in OLP lesions might increase their progressive growth.[10] Similarly, it was noted that heat shock proteins are potentially involved in the pathogenesis of the inflammatory process, which is observed when OLP induces premalignant lesions.[10,13] There are no data on p53, topoisomerase I (topo I), heat shock protein 90 (HSP90), and E-cadherin expression in OLL, or comparative studies between OLP and OLL. Based on the data that OLP/OLL represent a heterogeneous group of inflammatory disorders that share common antigens and are characterized by similar clinical and histological features, it would be interesting to investigate biomarker patterns which would be useful for the biological characterization of these different lesions.[2]

Objectives

The aim of this study was to assess p53 protein, topo I, HSP90, and E-cadherin expression in OLP and OLL biopsy specimens, which could help distinguish between these 2 pathologies diagnostically.

Materials and methods

Patients and study group selection

A total of 56 patients with OLP who were referred to the oral pathology outpatient clinic between January 1, 2015 and May 31, 2019, were enrolled in the study. The clinical and histopathological diagnoses of OLP were made based on the World Health Organization (WHO) criteria defined in 1978 and modified in 2003.[15] Clinical investigation was performed by a specialist in periodontology and oral mucosal pathology, and histopathological investigation was performed by a specialist in pathomorphology. The patients were reviewed for demographic data, general health and type(s) of medication. A clinical investigation was carried out to record the clinical forms of the lesions and the sites involved.

Assuming that the lesions of some of the patients diagnosed with OLP could be related to medication side effects, 2 groups of patients were created. The 1st group (idiopathic OLP) consisted of 25 patients, and the 2nd group (OLL) of 31 patients whose lesions may have resulted from the treatment with antihypertensive and/or cardiovascular medications. Differences in the expression patterns of the 4 abovementioned biomarkers, which could be considered when making the diagnosis between these 2 pathologies, were evaluated.

The pathologist evaluating the molecular markers was blinded. All 56 biopsies were assessed without being divided into groups. After immunohistochemical evaluation, the data were described in accordance with the groups (OLP or OLL groups).

The study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethics Committee of the Wroclaw Medical University, Poland (approval No. KB230/2016).

Antibodies

Immunohistochemical staining was performed with the following antibodies: mouse monoclonal antibody DO-7 (clone DO-7) that reacts with both the wild and mutant forms of unphosphorylated human p53 protein (Novocastra, Newcastle, UK); anti-topo I that binds to a region within the middle of the topo I molecule (clone 1D6; Novocastra); anti-HSP90 protein that recognizes protein corresponding to 306 amino acids of the C-terminus of the HSP90 molecule (clone JPB24; Novocastra); and anti-human cadherin (clone NCH-38) that recognizes the 120 kD mature form of E-cadherin (Dako, Copenhagen, Denmark).

Immunohistochemical staining

Immunohistochemical staining for the analyzed proteins was performed on paraffin-embedded OLP tissue specimens using the Universal Dako REAL EnVision Detection System, Peroxidase/DAB+, Rabbit/Mouse (Dako); the primary antibodies were anti-p53 protein, topo I, HSP90, and E-cadherin. The 5-µm sections of OLP were deparaffinized and boiled 2 × 5 min in a citrate buffer (pH = 6.0) at 800 W in a microwave. After that, the OLP sections were slowly cooled for 30 min. Nonspecific tissue endogenous peroxidase reactivity was blocked with Dako REAL Peroxidase Blocking Solution (Dako). The OLP specimens were incubated overnight with primary antibodies at 4°C. After washing the OLP specimens with 0.1 M Tris-buffered saline (TBS; pH = 7.4), they were incubated with Dako REAL EnVision/HRP, Rabbit/Mouse (Dako) for 30 min at room temperature. After washing the antigen-antibody with TBS, the reaction was visualized using 3,3 diaminobenzidine (Dako) as a chromogen. The sections were counterstained with hematoxylin and mounted. The incubation buffer (TBS) without primary antibodies was used as a negative control. Positive controls for each antibody were performed according to the manufacturer's recommendations.

Interpretation of immunohistochemical staining

The assessment of protein expression in the OLP tissues was scored semiquantitatively, taking into account the intensity of immunostaining and the number of cells showing immunoreactivity for the analyzed proteins. The number of cells exhibiting staining for p53 protein and topo I antibodies was assessed by counting 1000 cells in 10–15 randomly selected high-power fields. Heat shock protein 90 and E-cadherin expressions were analyzed by determining cytoplasmic/membrane immunostaining based on the intensity of immunostaining and the percentage of the stained OLP tissue area. The cases were scored as negative for all protein expressions when there was no immunostaining or variable weak positivity (<5% of cells).

Statistical analyses

The statistical significance between the means for the different groups was calculated using the nonparametric Mann–Whitney U test; the frequencies were calculated using the χ^2 test or Fisher's exact test.[16] The associations between p53 protein and HSP90, topo I and E-cadherin were analyzed using Spearman's rank correlation. Differences were considered statistically significant when $p < 0.05$. Statistical analysis was performed using Statistica v. 13.0 software (StatSoft Poland, Kraków, Poland) and PAST v. 4.05 (https://past.en.lo4d.com/windows).

Results

Comparison of clinical parameters between groups

There were 21 men and 35 women with mean age of 57.7 ±13.6 years and 65.5 ±9.4 years, respectively. Following anamnesis, the OLP group included 25 patients with a mean age of 57.0 years, and the OLL group included 31 patients with a mean age of 68.0 years; the patients in the OLP group were significantly younger ($p = 0.0001$; Table 1). There was a significantly higher number of women when all 56 patients were considered ($p = 0.04$), as well as when the 31 OLL patients were considered separately ($p = 0.04$). The duration of the lesions was similar in the OLP and OLL groups (38.6 months and 39.3 months, respectively). A comparison of the parameters evaluated between the groups revealed a significant difference in the median age, which was higher in the OLL group. In the OLL group, more patients showed shorter disease duration (below 34 months), whereas in the OLP group, more patients presented with longer disease duration (above 34 months). The observed differences between the OLL group and the OLP group were statistically significant ($p = 0.018$; Table 1).

Two clinical forms among all 56 lesions were found: white lesions of striae in 43 cases and erosions in 13 cases; there were no statistically significant differences in the prevalence of clinical forms between the OLP and OLL groups. In the assessment of site involvement, lesions were present on the buccal mucosa in 27 patients, at other sites (such as the gingiva or tongue) in 4 patients, and at 2 or 3 sites in 25 patients. No statistically significant difference was seen in site involvement between the groups (Table 1).

Comparison of p53, topo I, HSP90, and E-cadherin expression between groups

The p53 expression was found in 19/56 (33.0%) of the oral lesions. Nuclear accumulation of p53 protein showed a trend to higher expression in the OLL group compared to the OLP group (Table 2). In the majority of cases

Table 1. Demographic parameters in oral lichenoid lesions (OLL) and oral lichen planus (OLP) groups

Parameters	OLL group (n = 31) median (25Q–75Q) mean ±SD	OLP group (n = 25) median (25Q–75Q) mean ±SD	Test value	p-value
Age [years]	68.0 (64.0 ±71.0)	57.0 (45.0 ±62.0)	3.46	0.0001*
Lesions presence duration [months]	38.6 ±19.9	39.3 ±18.9	0.87	0.386*
Lesions presence duration, patients (n)				
≤34 months	21	9	5.61	0.018**
>34 months	10	16		
Lesions presence clinical forms, patients with (n):				
White striae	23	20	0.26	0.609**
Erosive form	8	5		
Lesions presence – sites involved, patients with (n):				
Buccal mucosa involved	13	14	–	0.55***
Other site involved	3	1		
General involvement	15	10		

* U Mann–Whitney (test value – Z); ** χ^2 test; *** exact Fisher's test; 25Q – lower quartile; 75Q – upper quartile; SD – standard deviation.

in both groups, p53 expression was found in a low percentage of basal and suprabasal epithelial cells (Fig. 1A,B). Topoisomerase I expression was found in 42/56 specimens (75.0%), HSP90 in 28/56 specimens (50.0%) and E-cadherin in 37/56 specimens (66.1%); expressions were slightly higher in the OLL group than in the OLP group (Table 2).

Positive results for E-cadherin expression were obtained from 10–60% of immunoreactive tissue. Topoisomerase I expression revealed equal immunoreactivity in both groups and was observed in different tissue areas from 10–70% of tissue. The expression of HSP90 was slightly higher in the OLL group than in the OLP group (Fig. 1C–F). The intensity of topo I and HSP90 immunoreactivity was stronger in the OLL group than in the OLP group. Similarly, the range of E-cadherin immunopositivity was higher in the OLL group than in the OLP group (Fig. 1G,H).

Association between p53 protein and topo I, HSP90 and E-cadherin expression

The p53 protein expression showed a trend toward a positive correlation with top I expression only (of the biomarkers) when all patients were considered ($p = 0.067$, $R = 0.25$) (Table 3). Such a correlation between p53 and topo I expression was not observed when the 2 groups were considered separately. No correlation was observed between p53 protein expression and HSP90 and E-cadherin expression when the OLL and OLP groups were considered separately and when all cases were considered together (Table 3).

Discussion

Data on the identification of biomarkers of OLP and OLL, which could help explain their biological characteristics, are limited.[11–13] This study found no differences in terms of gender, forms and location of lesions between the OLP and OLL groups, which is consistent with other reports.[12,17] The differences in mean age between groups in this study are in contrast to the published data.[12,14] The reason why the patients in the OLL group were older might be that they had cardiac and vascular diseases. In the OLL group, a significantly higher number of patients had a longer duration of lesions, which could have been associated with the use of medications.

Table 2. P53, HSP90, topo I, and E-cadherin expression in oral lichenoid lesions (OLL) and oral lichen planus (OLP) groups

Biomarkers	Immunopositivity (% of positive cases)			
	OLL group (n = 31) [n, %]	OLP group (n = 25) [n, %]	Test value	p-value
p53	12 (38.7)	7 (28.0)	0.71	0.400
HSP90	18 (58.0)	10 (40.0)	1.81	0.177
E-cadherin	22 (70.9)	15 (60.0)	0.74	0.388
Topo I	24 (77.4)	18 (72.0)	0.64	0.642

HSP90 – heat shock protein 90; topo I – topoisomerase I. χ^2 test.

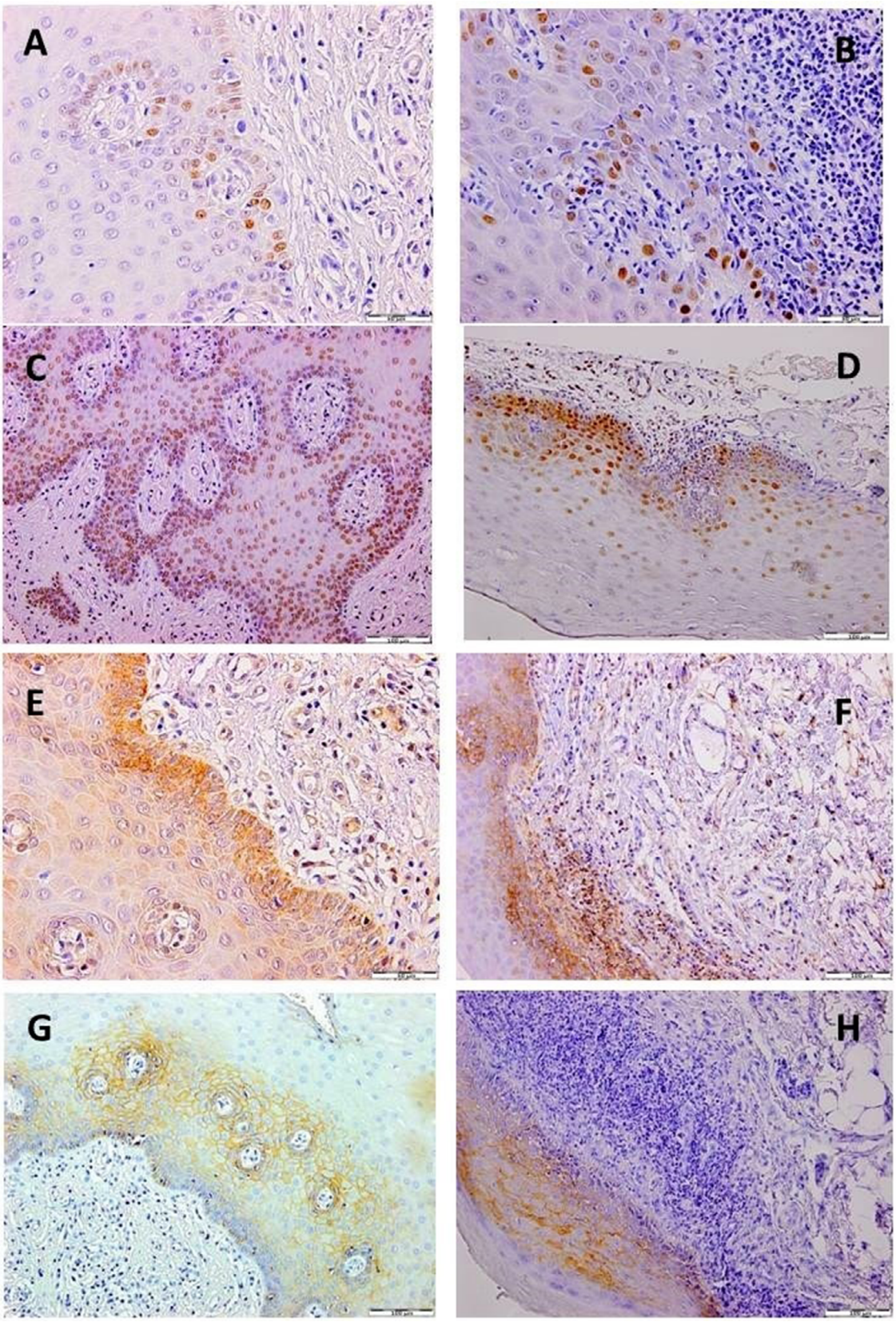

Fig. 1. Representative oral lichenoid lesions (OLL) and oral lichen planus (OLP) patients. A. Low number of keratinocytes and epithelial cells with p53 protein nuclear accumulation in OLP lesions; B. The p53 protein expression observed in a high number of cells in OLL; C. High number of cells showing topoisomerase I expression in OLP; D. Topoisomerase I immunostaining limited to deep layers of epithelial tissue in OLL; E. Heat shock protein 90 (HSP90) membrane/cytoplasmic expression detected in a high percentage of OLP tissue; F. The HSP90 membrane immunostaining observed in basal and suprabasal epithelial cells of patients with OLL; G. E-cadherin membrane expression in epithelial cells of untreated patients with OLP; H. E-cadherin expression in superficial epithelial tissue of OLL (the EnVision technique)

Fig. 1A,B,E: scale bar = 50 μm; Fig. 1C,D,F,G,H: scale bar = 100 μm.

Table 3. Correlation between p53 protein and HSP90, topo I and E-cadherin expression in the whole specimens, oral lichenoid lesions (OLL) and oral lichen planus (OLP) groups

Biomarkers	Number of cases	Spearman's R	p-value
P53 compared to HSP90			
Whole investigated group	56	0.16	0.225
OLP group	25	0.26	0.261
OLL group	31	0.07	0.671
P53 compared to topo I			
Whole investigated group	56	0.25	0.067
OLP group	25	0.15	0.509
OLL group	31	0.27	0.114
P53 compared to E-cadherin			
Whole investigated group	56	0.13	0.344
OLP group	25	0.34	0.139
OLL group	31	0.06	0.715

HSP90 – heat shock protein 90; topo I – topoisomerase I. Spearman's rank correlation.

In order to explore the differences between the OLP and OLL groups, the biomarkers p53, topo I, HSP90, and E-cadherin were analyzed.[10,11,18] As with previous reports, the expression of p53 was observed in a small number of cases in this study.[11,18] Consistent with existing studies, we consider that the heterogeneous pattern of p53 expression (ranging from weak to strong intensity of p53 immunostaining) observed in this study indicates that cases with strong nuclear accumulation of p53 protein are the result of gene alteration or protein structural changes which occur during the early stages of a premalignant lesion.[10,14,18] Our clinical observations of the OLP patients did not show any development of premalignant lesions. There are no reports in the literature regarding p53 protein expression in OLL patients taking antihypertensive or cardiac medications, which was analyzed in this study. The findings of this study revealed significant differences in p53 protein expression, which was higher in the OLL group; this could be explained as being related to ongoing medical treatment. Moreover, p53 protein expression might be a result of p53 protein activation after DNA damage by drugs used in the patients' therapy, which might reflect protective mechanisms, such as DNA repair, rather than gene alteration.[14] However, this observation needs to be confirmed by *TP53* gene status analysis.[16] Nevertheless, we postulate that the risk of progressive growth might be higher in the OLL group (that had greater p53 expression) than in the idiopathic OLP patients, due to loss of the suppressive role of p53 protein.[18,19] We found an association with high proliferative activity based on topo I expression and p53 protein nuclear accumulation in the sample as a whole but not when the OLL group alone was examined. We are unable to prove the prognostic value of the p53 protein due to the short time of clinical observation of the treated/untreated OLP patients. Nevertheless, the results of this study are supported by Bermejo-Fenoll et al. to an extext; their retrospective study found an association between p53 protein expression and malignancy of OLP lesions.[20] They found the development of squamous cell carcinoma (SCC) in 5 out of 550 patients with OLP; 3 of these 5 cases were being treated for hypertension, so they were diagnosed as OLL.[19] Giuliani et al. described overall malignant transformation in 1.4% of cases, including 1.37% for OLP and 2.43% for OLL in large groups, and concluded that p53 expression can cause progression from healthy oral tissue to malignancy; thus, p53 may be an early diagnostic sign of carcinogenesis.[21]

In this study, specimens showing the expression of topo I in a high number of cells indicated that this enzyme might characterize the subgroup of OLP that has high proliferative activity and leads to a progressive growth of these lesions. This hypothesis is supported by other studies which showed that high topo I expression in benign tumors increased the risk of malignancy.[22] As found in our study, similar topo I expression in specimens of both the OLP and OLL groups indicates that medications do not influence the cellular activity of oral epithelial cells. The lower expression of HSP90 in tissues from the OLP group might be associated with greater nuclear accumulation of p53 protein, which can form complexes with HSP90 in cells.[23,24] The lack of association between p53 protein and HSP90 expression in the study group as a whole, as well as in the OLP and OLL groups separately, suggests that HSP90 protein does not play a protective role against p53 protein in, for example, carcinoma tissue.[25]

The high E-cadherin expression observed in this study is consistent with other studies and indicates that high E-cadherin expression is important for tissue structure because this protein is highly expressed by normal oral epithelial cells in the spinous layer and basal layer.[26] Our results concerning equal E-cadherin expression in the OLP/OLL groups are somewhat in line with the the

study by Sargolzaei and Mohamadian, who did not observe significant differences in the expression of E-cadherin between OLP specimens with and without dysplasia.[12] However, there is research indicating that p53 protein expression reduces E-cadherin expression in oral SCC.[27] The lack of correlation between p53 protein and E-cadherin expression in the OLP specimens analyzed in this study suggests that there is no cooperation between these 2 proteins. This observation is consistent with a published report suggesting that the reduction of E-cadherin in OLP is associated with mild and moderate dysplasia.[26,27]

Limitations

The 1st limitation of this study is that the medications used by the OLL patients are not specified – not all classes of antihypertensive and cardiac medications cause OLL as a side effect. Moreover, there are new medications for which there are no published observations or investigations with regard to OLL. The 2nd limitation is the absence of a control group; tissue biopsies from healthy subjects would have been useful for comparison.

Conclusions

The results of this study suggest that high p53 protein and HSP90 expressions in the OLL of patients treated with cardiovascular medications could result not only from gene alterations but also from those medications. The impact of p53 protein on the biological behavior of oral cells might be different in idiopathic OLP compared to drug-related OLL. The association between p53 protein and topo I expression indicates that the association between these proteins might be essential for the growth and behavior of OLP/OLL. We conclude that the expression of p53 protein and topo I found in both types of lesion might induce their biologically aggressive behavior. However, to confirm these observations, future larger studies on the roles of these molecules in oral lesions are warranted.

ORCID iDs

Małgorzata Radwan-Oczko https://orcid.org/0000-003-4684-5722
Julia Bar https://orcid.org/0000-0002-3811-4834
Agnieszka Hałoń https://orcid.org/0000-0003-4240-7899
Anna Lis-Nawara https://orcid.org/0000-0003-4165-4652

References

1. Krupaa RJ, Sankari SL, Masthan KMK, Rajesh E. Oral lichen planus: An overview. *J Pharm Bioallied Sci*. 2015;7(Suppl 1):S158–S161. doi:10.4103/0975-7406.155873
2. Carrozzo M, Porter S, Mercadante V, Fedele S. Oral lichen planus: A disease or a spectrum of tissue reactions? Types, causes, diagnostic algorhythms, prognosis, management strategies. *Periodontol 2000*. 2019;80(1):105–125. doi:10.1111/prd.12260
3. Aghbari SMH, Abushouk AI, Attia A, et al. Malignant transformation of oral lichen planus and oral lichenoid lesions: A meta-analysis of 20095 patient data. *Oral Oncol*. 2017;68:92–102. doi:10.1016/j.oraloncology.2017.03.012
4. Akerzoul N, Chbicheb S. Pro-inflammatory cytokines and oral lichen planus. *Am J Clin Cancer Res*. 2018;4:1–6. http://www.ivyunion.org/index.php/ajcre/article/view/1245. Accessed October 1, 2020.
5. Cassol-Spanemberg J, Rodriguez-de Rivera-Campillo M, Otero-Rey E, Estrugo-Devesa A, Jane-Salas E, Lopez-Lopez J. Oral lichen planus and its relationship with systemic diseases: A review of evidence. *J Clin Exp Dent*. 2018;10(9):e938–e944. doi:10.4317/jced.55145
6. Yamauchi M, Moriyama M, Hayashida JN, et al. Myeloid dendritic cells stimulated by thymic stromal lymphopoietin promote Th2 immune responses and the pathogenesis of oral lichen planus. *PLoS One*. 2017;12(3):e0173017. doi:10.1371/journal.pone.0173017
7. Balakumar P, Kavitha M, Nanditha S. Cardiovascular drugs-induced oral toxicities: A murky area to be revisited and illuminated. *Pharmacol Res*. 2015;102:81–89. doi:10.1016/j.phrs.2015.09.007
8. Jinbu Y, Demitsu T. Oral ulcerations due to drug medications. *Jpn Dent Sci Rev*. 2014;50(2):40–46. doi:10.1016/j.jdsr.2013.12.001
9. Farzin M, Derafshi R, Ghapanchi J, Kafsh AZ, Rezaiee M. Oral manifestations of hypertension and rheumatic heart disease: A cross sectional study in elderly patients. *Asian J Med Pharm Res*. 2016;6(2):9–13. https://ajmpr.science-line.com/attachments/article/39/Asian%20J.%20Med.%20Pharm.%20Res.%206(2)%2009-13,%202016.pdf. Accessed June 15, 2020.
10. Sagari S, Sanadhya S, Doddamani M, Rajput R. Molecular markers in oral lichen planus: A systematic review. *J Oral Maxillofac Pathol*. 2016;20(1):115–121. doi:10.4103/0973-029X.180964
11. Farhadi S, Shahsavari F, Alf K. Comparison of expression of p53 and bcl-2 markers in oral lichen planus and oral squamous cell carcinoma. *J Res Dentomaxillofac Sci*. 2018;3(2):37–45. doi:10.29252/jrdms.3.2.37
12. Sargolzaei S, Mohamadian F. Immunohistochemical comparison of E-cadherin expression in oral lichen planus with and without dysplasia. *J Dental School*. 2017;35(1):26–31. doi:10.22037/jds.v35i1.24627
13. Chaiyarit P, Jintakanon D, Klanrit P, Siritapetawee M, Thongprasom K. Immunohistochemical analyses of survivin and heat shock protein 90 expression in patients with oral lichen planus: Immunohistochemical analyses of survivin and Hsp90. *J Oral Pathol Med*. 2009;38(1):55–62. doi:10.1111/j.1600-0714.2008.00713.x
14. Hadzi-Mihailovic M, Petrovic R, Raybaud H, Stanimirovic D, Ozar Koray M. Expression and role of p53 in oral lichen planus patients. *J BUON*. 2017;22(5):1278–1286. PMID:29135114.
15. van der Meij EH, van der Waal I. Lack of clinicopathologic correlation in the diagnosis of oral lichen planus based on the presently available diagnostic criteria and suggestions for modifications. *J Oral Pathol Med*. 2003;32(9):507–512. doi:10.1034/j.1600-0714.2003.00125.x
16. Hammer Ø, Harper DAT, Ryan PD. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol Electron*. 2001;4(1):9. https://palaeo-electronica.org/2001_1/past/past.pdf. Accessed May 10, 2022.
17. Irani S, Esfahani AM, Ghorbani A. Dysplastic change rate in cases of oral lichen planus: A retrospective study of 112 cases in an Iranian population. *J Oral Maxillofac Pathol*. 2016;20(3):395–399. doi:10.4103/0973-029X.190911
18. Agha-Hosseini F, Mirzaii-Dizgah I, Miri-Zarandi NS. Unstimulated salivary p53 in patients with oral lichen planus and squamous cell carcinoma. *Acta Med Iran*. 2015;53(7):439–443. PMID:26520632.
19. Lambrus BG, Holland AJ. A new mode of mitotic surveillance. *Trends Cell Biol*. 2017;27(5):314–321. doi:10.1016/j.tcb.2017.01.004
20. Bermejo-Fenoll A, Sanchez-Siles M, López-Jornet P, Camacho-Alonso F, Salazar-Sanchez N. Premalignant nature of oral lichen planus. A retrospective study of 550 oral lichen planus patients from south-eastern Spain. *Oral Oncol*. 2009;45(8):e54–e56. doi:10.1016/j.oraloncology.2009.01.009
21. Giuliani M, Troiano G, Cordaro M, et al. Rate of malignant transformation of oral lichen planus: A systematic review. *Oral Dis*. 2019;25(3):693–709. doi:10.1111/odi.12885
22. Nikitakis NG, Pentenero M, Georgaki M, et al. Molecular markers associated with development and progression of potentially premalignant oral epithelial lesions: Current knowledge and future implications. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2018;125(6):650–669. doi:10.1016/j.oooo.2018.03.012
23. Faraji Lamouki M, Abbaszadeh H, Gholinia H. Study of HSP27 expression in two types of oral lichen planus. *J Adv Biotech Res*. 2018;9(1):617–625. https://bipublication.com/files/ijabr20189189Mojtaba.pdf. Accessed January 1, 2021.

24. Chang WC, Tsai PT, Lin CK, Shieh YS, Chen YW. Expression pattern of heat shock protein 90 in patients with oral squamous cell carcinoma in northern Taiwan. *Br J Oral Maxillofac Surg*. 2017;55(3):281–286. doi:10.1016/j.bjoms.2017.01.008
25. Chatterjee S, Burns TF. Targeting heat shock protein in cancer: A promising therapeutic approach. *Int J Mol Sci*. 2017;18(9):1978. doi:10.3390/ijms18091978
26. de Freitas Silva BS, de Castro CA, von Zeidler SLV, de Sousa SCOM, Batista AC, Yamamoto-Silva FP. Altered β-catenin expression in oral mucosal dysplasia: A comparative study. *J Appl Oral Sci*. 2015;23(5):472–478. doi:10.1590/1678-775720150150
27. Fan CC, Wang TY, Cheng YA, et al. Expression of E-cadherin, Twist, and p53 and their prognostic value in patients with oral squamous cell carcinoma. *J Cancer Res Clin Oncol*. 2013;139(10):1735–1744. doi:10.1007/s00432-013-1499-9