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ORIGINAL ARTICLE

Analysis of oral lichen planus severity on micro-RNA linked with malignant transformation risks

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Abstract

Objective: The present study evaluated the oral tissue expression of micro-RNA (miRNAs) linked to the potential malignant evolution of oral lichen planus (OLP). Furthermore, the correlation between OLP severity and miRNAs expression was assessed, and possible predictors of miRNAs in OLP patients were identified.

Methods: The present study enrolled 41 patients with OLP (median age 58 years) and 42 healthy controls (median age 59 years). In each patient, miRNA levels (miR-7a-3p,-7a2-3p,-7a-5p,-21-3p,-21-5p,-100-3p,-100-5p,-125b-2-3p,-125b-5p,-200b-3p,-200b-5p) were assessed and analyzed through reverse transcription polymerase chain reaction. Clinical parameters and the eventual presence of OLP symptoms, signs, and disease severity scores in each patient were reported using an anamnestic questionnaire.

Results: In comparison with healthy controls, OLP patients showed significantly higher miR-7a-3p,-7a-2-3p,-21-3p, miR-21-5p and miR-100-5p levels ($p < 0.05$) and significantly lower miR-125b-2-3p,-125b-5p,-200b-3p, and -200b-5p levels ($p < 0.05$). Furthermore, OLP symptoms and signs and disease severity scores were significantly correlated and were also predictors of all analyzed miRNAs ($p < 0.05$).

Conclusions: In comparison with healthy subjects, OLP patients exhibited unbalanced oral miRNAs expression linked to the risk of potential malignant evolution of OLP. Furthermore, some miRNAs were correlated with OLP extent and were significant predictors of OLP symptoms, signs, and disease severity scores.

KEYWORDS
biomarker, disease severity, malignant disorders, microRNA, oral lichen planus, prognosis

1 | INTRODUCTION

Oral lichen planus (OLP) is a chronic, immuno-inflammatory, potentially malignant oral disease affecting the squamous epithelium and the underlying lamina propria (Adamo et al., 2022; Bermejo-Fenoll & López-Jornet, 2006). The worldwide prevalence is estimated to fluctuate between 0.22% and 5% (Gorouhi et al., 2014), and the female/male ratio is 2:1 (Farhi & Dupin, 2010). The etiology of OLP is unknown; however, the most relevant hypothesis explains OLP as an immune reaction elicited by T-lymphocytes, probably through exogenous or endogenous factors (Gueiros et al., 2018; Pippi et al., 2016). Furthermore, infectious agents such as hepatitis C virus (Lodi

et al., 2010; Petti et al., 2011), genetic factors (Gorouhi et al., 2014), autoimmunity (Warnakulasuriya et al., 2021), heat shock proteins (Payeras et al., 2013), and psychological factors (de Porras-Carrique et al., 2022; Rojo-Moreno et al., 1998) have been related with the etiology of OLP. Moreover, growing evidence has reported that the overall risk of OLP malignant transformation could be around 1.37% (Payeras et al., 2013), although several factors have been identified that could increase the possibility of cancerization, such as female sex, elderly age, atrophic-erosive lesions, tobacco, alcohol, and infections (Crincoli et al., 2011; Giuliani et al., 2019; González-Moles et al., 2019; Nunes et al., 2022). The first-line therapy of OLP relies on the use of topical corticosteroids (Lodi et al., 2012, 2020; Santonocito et al., 2021), miconazole (Lodi et al., 2007), while alternatively, calcineurin inhibitors such as topical tacrolimus (Polizzi et al., 2023) or systemic corticosteroids and immunosuppressants may be employed (Al-Hashimi et al., 2007; da Silva et al., 2021). However, there is no clear and resolutive therapy for OLP, with periods of exacerbation and quiescence (Thongprasom et al., 2011). Therefore, research efforts go toward the early identification of new biomarkers and potential therapeutic targets involved in the early risk of development, and the risk of malignant transformation of OLP lesions is of increasing interest.

Recently, a growing interest has attracted microRNAs (miRNAs) in oral tissue homeostasis and abnormal expression related to different oral inflammatory and autoimmune diseases such as OLP (Wu et al., 2015; Ziebarth et al., 2019). MiRNAs are small single-stranded noncoding RNA molecules which repress protein expression at a post-transcriptional level through imperfect base pairing with mRNA 3′ UTR, reducing translation or inducing degradation of the target mRNA (Li et al., 2022). Some miRNAs have been reported as being abnormally expressed in OLP; in this regard, miR-27b and miR-137 have been related to the potential malignant transformation of OLP lesions (Aghbari et al., 2018; Ahmadi-Motamayel et al., 2017). In this regard, significant changes were found in the expression of nine miRNAs (miR-21, 26b, 121, 137, 146a, 155, 203, 375, and 4484) in patients with OLP (Liu et al., 2015) and oral squamous cell carcinoma (OSCC) (Jakob et al., 2019). Furthermore, a recent preliminary study by Liang et al. (2016) showed an increased serum level of IL-17 and miR-155 m-RNA in patients with OLP compared to healthy controls; this study proposed the role of mi-RNAs in the erosive evolution of OLP. Therefore, based on the above-mentioned evidence, the aim of this study was to identify the oral tissue expression of miRNAs in patients with OLP. Furthermore, the correlation was assessed between OLP severity and miRNAs expression, and possible predictors of miRNAs expression in OLP patients were investigated.

2 | MATERIALS AND METHODS

2.1 | Study design

The present study was designed as a case–control study in which healthy controls and OLP patients were enrolled at the Dental School of the University of Catania, Italy. The local International Review Board approved the study protocol (121/20/PO), and written informed consent was obtained from each patient. The study was performed in accordance with the guidelines for strengthening the communication of observational studies (STROBE) (Table S1) and followed the declaration of Helsinki on medical research guidelines reviewed in 2016.

For OLP patients, in accordance with the updated WHO clinical and histopathological criteria of 2020 (Warnakulasuriya et al., 2021), the inclusion criteria were: (1) ≥ 18 years of age; (2) more or less symmetrical white lesions; (3) lace-like network of slightly raised white lines (reticular, annular or linear pattern) with or without erosions and ulcerations; (4) possible desquamative gingivitis; (5) well-defined, band-like zone of cellular infiltration consisting mainly of lymphocytes and confined to the superficial lamina propria; (6) vacuolar degeneration in the basal/suprabasal cell layer with keratinocyte apoptosis; (7) epithelial thinning with eventual ulceration and mixed inflammatory infiltrate in atrophic variant. The exclusion criteria for the OLP patients were: (1) use of anti-inflammatory drugs or antibiotics during the last 6 months preceding the study; (2) the state of pregnancy or breastfeeding; (3) the presence of contact or oral drug lichenoid lesions.

Healthy subjects had (1) no diagnosis of OLP, (2) the presence of any oral disease, (3) the presence of any systemic disease, and (4) medication intake in the 6 months prior to the study.

Each patient completed an anamnestic questionnaire reporting the eventual presence of any comorbidities (e.g. diabetes, hypertension, hypercholesterolemia, hypothyroidism, celiac disease, HBV, HCV, HPV, and EBV), anxiety, depression, and stress, all drugs usually taken, information on the lesion onset and the possible degree of limitation felt in oral functions. Furthermore, each subject underwent a scrupulous oral and general objective examination to assess the presence of oral mucosal OLP lesions and some possible skin and/or genital mucous membranes lichenoid lesions. Hypertension was assessed for blood pressure values ≥140/90 mmHg; diabetes was diagnosed with blood glucose >126 mg/dL and hypercholesterolemia with blood cholesterol >200 mg/dL with LDL >130 mg/dL. The presence of celiac and other possible systemic diseases was assessed during the medical examination of each enrolled patient.

2.2 | OLP symptoms, signs and disease severity scores

OLP symptoms and signs were assessed as previously described (Polizzi et al., 2023). More specifically, OLP symptom severity was classified in accordance with Raj et al. (2012) as follows: 0, no symptoms; 1, mild (occasional symptoms); 2, moderate (e.g., while eating spicy food); 3, severe (i.e., while eating any food); 4, intolerable (always present) symptoms. OLP signs were recorded with a scale formulated by Kaliakatsou et al. (2002) as follows: 0, no lesion present; 1, only white striae present; 2, white striae plus erosion of fewer than 1 cm^2; 3, white striae plus erosion of more than 1 cm^2; 4, white striae

plus ulceration of fewer than 1 cm^2; and 5, white striae plus ulceration of more than 1 cm^2. Finally, the OLP disease severity score was obtained through the combination of OLP symptoms and sign scores (Singh et al., 2017): 1–3, mild disease; 4–6, moderate disease; and 7–9, severe OLP disease.

2.3 | Tissue sample collection

In the OLP group, the tissue specimen was taken for OLP diagnosis, while in the healthy group, the oral mucosal specimens (without inflammation) were obtained from individuals who underwent oral surgery (e.g. third molar surgery, fractured teeth). Samples were dipped in formalin for regular histological diagnosis, while frozen fresh tissue was used for the analysis of the miRNA. A small 4-mm piece of biopsy fresh tissue was kept at −70°C immediately after surgery to analyze the expression of miRNAs levels.

2.4 | RNA extraction and reverse-transcriptase PCR (RT-PCR) analysis

Total RNA extraction was performed through TRIzol kit (Life Technologies). Complementary DNA (cDNA) was obtained by reverse-transcribing 10 ng RNA (in 10 μg) using the miRCURY LNA RT Kit (cat. no. 339340). PCR procedures were carried out on a QuantStudio™ 3 (Applied Biosystems Thermo Fisher Scientific) using 3 μL diluted 1:60 cDNA template of RT-product. The reactions were carried out in triplicate in three separate experiments. Triplicate values for real-time PCR were managed by calculating the triplicate mean. The miRCURY LNA SYBR® Green PCR kit (cat. no. 339346) was used to quantify the expression levels of the following miRNAs: miR-7a-3p, miR-7a-2-3p, miR-7a-5p, miR-21-3p, miR-21-5p, miR-100-3p, miR-100-5p, miR-125b-2-3p, miR-125b-5p, miR-200b-3p, and miR-200b-5p. The relative miRNAs expression levels were measured by the $\triangle\triangle$Ct method after normalization with reference control. The U6 snRNA was the reference gene, as previously described (Isola et al., 2023) and in agreement with previous studies (Cheng et al., 2022; Donati et al., 2019; Han et al., 2014; Luo et al., 2018; Tang et al., 2019) which recommended U6 snRNA as a reference gene for miRNA quantification by RT-PCR analysis in oral fluid specimens.

2.5 | Power sample analysis

The sample size was established considering a number of two groups, one tail, an effect size of 0.80 for the OLP disease severity score (which represented the primary outcome variable), a significance level of 0.05, a power of 80%, and an allocation ratio of 0.85. It was established that at least 36 patients per group were needed. In each group, over 41 patients were enrolled in order to achieve a good power sample.

2.6 | Statistical analysis

Numerical data were expressed as the median and interquartile range (Q1–Q3), while categorical variables were expressed as numbers and percentages. A non-parametric approach was used since analyzed variables were not normally distributed, as verified by the Kolmogorov–Smirnov test. The comparison between cases and controls was calculated using the Mann–Whitney test for numerical parameters and the Chi-Squared (or exact Fisher test) for categorical variables.

The Spearman correlation test was applied in order to assess the interdependence among miRNAs and variables related to the patient, such as age, comorbidities (yes/no), stress (yes/no), plaque index, depression (yes/no), anxiety (yes/no), OLP signs, OLP symptoms, and OLP disease severity score.

Furthermore, to identify possible significant predictors of miRNAs expression in the analyzed sample, a logarithmic transformation of each analyzed miRNA was performed and, conditionally to a state of obtained normality after a logarithmic transformation, a uni- and multivariable linear regression models were established in order to estimate the dependence of each miRNA by potentially explicative variables such as age, gender, smoking, BMI, comorbidities, stress, plaque index, depression, anxiety, OLP signs, OLP symptoms, and OLP disease severity score. Smoking, comorbidities, stress, depression, and anxiety were inserted as dichotomous variables (yes/no). Statistical analyses were performed using SPSS 22.0 for the Windows package. A p-value smaller than 0.05 was considered to be statistically significant.

3 | RESULTS

Both groups' demographics, clinical characteristics and miRNAs expression are shown in Table 1. The sample was composed of 42 healthy controls (22 males, 20 females, mean age 59 years old) and 41 patients with OLP (20 males, 21 females, mean age 58 years old); both groups were well matched for age ($p = 0.310$) and sex ($p = 0.591$). Figure 1 represents a case of ulcerative and symptomatic OLP.

In the OLP group, 63.4% of patients presented a red OLP form, while 36.6% had a white OLP form (Table 2). Moreover, patients with red OLP forms had atrophic OLP (29.3%) and ulcerative-erosive OLP (26.8%), and patients with white OLP forms had reticular OLP (21.9%) and plaque OLP (24.4%). The median OLP symptom score was 2 (1–3 IQR), and the OLP signs score was 3 (2–4 IQR). Moreover, one patient showed the presence of skin lichenoid lesions (Table 2).

3.1 | Primary outcome

Regarding miRNA concentrations (Table 3), in comparison with controls, OLP patients showed significantly higher levels of the miRNAs:

TABLE 1 Clinical characteristics of OLP and healthy controls.

Variables	Controls ($n=42$)	OLP ($n=41$)	p-value
Age, median (IQR)	59 (55–63)	58 (52–65)	0.310
Male sex, no. (%)	22 (52.4)	20 (48.8)	0.591
Plaque index, median (IQR)	1 (1–2)	2 (1–3)	<0.001
Diabetes, no. (%)	-	3 (9.1)	0.393
Hypertension, no. (%)	5 (11.9)	8 (19.5)	0.286
Hypercholesterolemia, no. (%)	3 (7.1)	9 (22)	<0.001
Hypothyroidism, no. (%)	2 (4.8)	6 (14.6)	0.076
Anxiety, no. (%)	3 (7.1)	11 (26.8)	<0.001
Depression, no. (%)	2 (4.8)	8 (19.5)	0.014
Stress, median (IQR)	1 (0–2)	2 (1–4)	<0.001

TABLE 2 Description of OLP forms, lichenoid lesions and types among OLP patients.

OLP ($n=41$)	No. (%)
OLP forms	
White	15 (36.6)
Red	26 (63.4)
OLP types	
Reticular	8 (19.5)
Plaque	10 (24.4)
Atrophic	12 (29.3)
Ulcerative	11 (26.8)
Systemic lichenoid lesions	
Skin mucous membranes	1 (2.4)
Genital mucous membranes	-

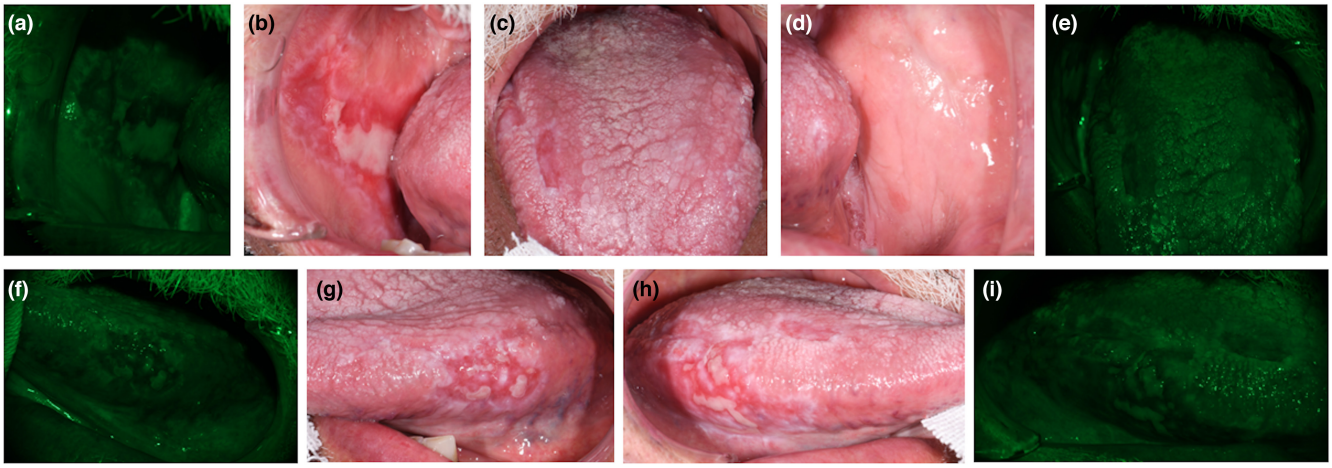

FIGURE 1 Ulcerative and symptomatic OLP male patient. (a) Loss of autofluorescence on the right buccal mucosa compared to (b) conventional photo on the same side, (c) tongue dorsum, (d) left buccal mucosa, (e) light-based autofluorescence photo on tongue dorsum, (f) autofluorescence photo on left tongue margin compared to (g) conventional photo on the same side, (h) right tongue margin, and (i) autofluorescence related photo. The erosive lesions around the ulcers, particularly in the left buccal mucosa and lateral margins of the tongue, showed loss of autofluorescence compared to the surrounding areas, indicating potential increased metabolic activity in the epithelium, blood flow and/or inflammation.

miR-7a-3p ($p<0.001$), miR-7a-2-3p ($p<0.001$), miR-21-3p ($p<0.001$), miR-21-5p ($p<0.001$) and miR-100-5p ($p=0.014$) and lower levels of miR-125b-2-3p ($p<0.001$), miR-125b-5p ($p=0.029$), miR-200b-3p ($p=0.046$), and miR-200b-5p ($p=0.009$).

The Spearman correlation analysis results are represented in Table 4. More specifically, the plaque index was positively correlated with miRNA 7a-3p (rs=0.365, $p=0.017$), 7a-2-3p (rs=0.394, $p=0.010$), 21-3p (rs=0.439, $p=0.004$), 21-5p (rs=0.364, $p=0.018$), and negatively with 7a-5p (rs=−0.404, $p=0.008$), and 125b-2-3p (rs=−0.471, $p=0.002$). Hypertension was positively correlated with miRNA 7a-3p (rs=0.704, $p<0.001$), 7a-2-3p (rs=0.545, $p<0.001$), 21-3p (rs=0.489, $p=0.001$), 21-5p (rs=0.549, $p<0.001$), and negatively with 125b-2-3p (rs=−0.541, $p<0.001$), 125b-5p (rs=−0.292, $p=0.060$), and 200b-5p (rs=−0.316, $p=0.042$). Hypercholesterolemia was positively correlated with miRNA 7a-3p (rs=0.582, $p<0.001$), 7a-2-3p (rs=0.533, $p<0.001$), 21-3p (rs=0.407, $p=0.008$), 21-5p (rs=0.362, $p=0.019$), and negatively correlated with miRNA 125b-2-3p (rs=−0.504, $p=0.001$), 200b-5p (rs=−0.342, $p=0.027$), while hypothyroidism was positively associated with miRNA 7a-3p (rs=0.383, $p=0.012$) and negatively with 125b-2-5p (rs=−0.336, $p=0.030$). Anxiety was positively associated with miRNA 7a-3p (rs=0.539, $p<0.001$), 7a-2-3p (rs=0.537, $p<0.001$), 21-3p (rs=0.376, $p=0.014$), 21-5p (rs=0.364, $p=0.018$), 100-5p (rs=0.307, $p=0.048$), and negatively with 125b-2-3p (rs=−0.384, $p=0.012$); depression was positively associated with miRNA 7a-3p (rs=0.351, $p=0.023$), 7a-2-3p (rs=0.393, $p=0.010$), and negatively associated with 7a-5p (rs=−0.343, $p=0.026$) while stress was positively associated with miRNA 7a-3p (rs=0.544, $p<0.001$), 7a-2-3p (rs=0.544, $p<0.001$), 21-3p (rs=0.409, $p=0.007$), 21-5p (rs=0.337, $p=0.029$), 100-3p (rs=0.385, $p=0.012$), and negatively associated with 125b-2-3p

TABLE 3 miRNAs expression in the analyzed sample.

	Controls ($n=42$)		OLP ($n=41$)		
miRNA	**Median**	**IQR**	**Median**	**IQR**	***p*-value**
miR-7a-3p	0.530	0.390–0.590	4.180	3.060–4.510	<0.001
miR-7a-2-3p	0.614	0.410–0.831	1.590	1.410–2.030	<0.001
miR-7a-5p	0.856	0.708–2.594	0.845	0.690–0.960	0.065
miR-21-3p	0.772	0.769–0.874	1.284	0.946–5.870	<0.001
miR-21-5p	1.033	0.993–1.380	1.822	1.321–3.890	<0.001
miR-100-3p	0.640	0.636–0.787	0.749	0.429–1.960	0.713
miR-100-5p	0.732	0.381–1.574	2.117	0.852–2.320	0.014
miR-125b-2-3p	1.210	1.190–1.263	0.590	0.348–0.622	<0.001
miR-125b-5p	0.960	0.800–0.960	0.435	0.260–1.650	0.029
miR-200b-3p	1.008	0.632–1.120	0.348	0.227–1.540	0.046
miR-200b-5p	0.729	0.562–0.729	0.350	0.210–1.320	0.009

(rs = −0.011, $p=0.945$). The OLP symptom score was positively associated with miRNA 7a-3p (rs = 0.493, $p=0.001$), 7a-2-3p (rs = 0.470, $p=0.002$), 21-3p (rs = 0.580, $p<0.001$), 21-5p (rs = 0.502, $p=0.001$), 100-3p (rs = 0.377, $p=0.014$) and negatively associated with 7a-5p (rs = −0.342, $p=0.027$), 125b-2-3p (rs = −0.573, $p<0.001$) The. OLP signs score was positively associated with miRNA 7a-3p (rs = 0.625, $p<0.001$), 7a-2-3p (rs = 0.642, $p<0.001$), 21-3p (rs = 0.608, $p<0.001$), 21-5p (rs = 0.551, $p<0.001$), and negatively associated with 7a-5p (rs = −0.364, $p=0.018$), 125b-2-3p (rs = −0.711, $p<0.001$). OLP disease severity was positively associated with miRNA 7a-3p (rs = 0.659, $p<0.001$), 7a-2-3p (rs = 0.624, $p<0.001$), 21-3p (rs = 0.574, $p<0.001$), 21-5p (rs = 0.497, $p=0.001$), and negatively associated with 7a-5p (rs = −0.359, $p=0.019$), 125b-2-3p (rs = −0.633, $p<0.001$). The other analyzed parameters were not significant (Table 4).

3.2 | Secondary outcome

The multivariate regression models, aimed at identifying possible predictors of miRNAs expression, highlighted that age was a significant predictor of miR-125-b5 ($p=0.021$); plaque index was a significant predictor of miR-7a-2-3p ($p=0.012$) and miR-7a-5p ($p=0.025$); diabetes was a significant predictor of miR-100-3p ($p=0.046$); hypercholesterolemia was a significant predictor of miR-21-5p ($p=0.001$) and miRb-200-5p ($p=0.023$); hypothyroidism was a significant predictor of miR-100-3p ($p=0.015$) and -200b-3p ($p=0.014$); anxiety was a significant predictor of miR-7a-3p ($p=0.022$) miR-7a-5p ($p=0.026$), miR-125b-2p ($p=0.024$), miR-200b-3p ($p=0.025$); stress was a significant predictor of miR-7a-3p ($p=0.026$), miR-21-3p ($p=0.015$), mir-125b-5p ($p=0.013$), and miR-200b-5p ($p=0.013$) (Table 5).

Furthermore, the analysis evidenced also that the OLP symptom score was a significant predictor of miR-7a-3p ($p=0.021$), and miR-21-5p ($p=0.009$), miR-100-5p ($p=0.045$), miR-125b-5p ($p=0.022$), and miR-200b-5p ($p=0.041$); The OLP signs score was a significant predictor of miR-7a-3p ($p=0.029$), miR-7a-2-3p ($p=0.002$), miR-21-3p ($p=0.041$), miR-100-5p ($p=0.011$), miR-125-b2 ($p=0.002$), and miR-200-b3 ($p=0.015$); OLP disease severity was a significant predictor of miR-7a-3p ($p=0.036$), miR-7a-2-3p (0.025), miR-21-3p ($p=0.012$), miR-100-3p ($p=0.023$), miR-125b-5p ($p<0.001$), and miR-200b-3p ($p=0.002$) (Table 5).

4 | DISCUSSION

The aim of this study was to identify the impact of OLP and its severity on miRNAs expression in oral tissues and to assess the correlation and possible predictors of local miRNAs concentration levels. It was found that patients with OLP exhibited different miRNA expression patterns of miR-7, -21, -100, -125, and -200 compared to healthy individuals, and a positive association among analyzed miRNAs and OLP disease extent.

These results are in agreement with the literature. A recent study conducted by Mehdipour et al. (2018) reported higher miR-21 and decreased miR-125a salivary levels in OLP and OSCC patients compared to healthy controls, indicating a potential role of these biomarkers for a bad prognosis of OLP. In addition, elevated miR-21 and decreased miR-125 expression in OLP tissues were also reported, and a negative correlation was observed between miR-21 and the tumor suppressor p53 expression in oral tissues (Danielsson et al., 2012).

In the present study, the worsening of OLP symptoms, signs, and disease severity scores were significant predictors of high miRNA-21-3p/-21-5p and reduced miR-125-b-2/-125b-5p expression levels. These results suggest that a decrease in miR-125 and an increase in miRNA-21 may predict more severe forms of OLP, potentially less responsive to drug treatment. Conversely, an increase in miR-125 and a decrease in miR-21 might be protective against OLP malignant risk evolution. In this regard, miRNA-21 salivary levels were found to be significantly increased in dysplastic OLP, while this did not occur in salivary miRNA-125a levels (Mehdipour et al., 2018; Mueller et al., 2021), and it is possible to hypothesize that miR-125-b tissue levels may represent more effective predictive and clinical severity biomarkers than miR-125a salivary levels. Therefore, while miR-125-b2 could act as a tumor suppressor and hinder the transformation of OLP into OSCC, miR-21 seems to act as an oncogene promoting the risk of OSCC

TABLE 4 Correlations between clinical variables and miRNAs concentrations.

	miR-7a-3p	miR-7a-2-3p	miR-7a-5p	miR-21-3p	miR-21-5p	miR-100-3p	miR-100-5p	miR-125b-2-3p	miR-125b-5p	miR-200b-3p	miR-200b-5p
Age	rs = 0.211 $p = 0.179$	rs = 0.059 $p = 0.710$	rs = −0.235 $p = 0.134$	rs = 0.125 $p = 0.429$	rs = 0.153 $p = 0.333$	rs = 0.005 $p = 0.973$	rs = −0.054 $p = 0.733$	rs = −0.164 $p = 0.299$	rs = −0.026 $p = 0.871$	rs = −0.024 $p = 0.881$	rs = −0.036 $p = 0.822$
Plaque index	rs = 0.365 $p = 0.017$	rs = 0.394 $p = 0.010$	rs = −0.404 $p = 0.008$	rs = 0.439 $p = 0.004$	rs = 0.364 $p = 0.018$	rs = −0.120 $p = 0.448$	rs = −0.031 $p = 0.847$	rs = −0.471 $p = 0.002$	rs = −0.091 $p = 0.566$	rs = −0.151 $p = 0.341$	rs = −0.162 $p = 0.304$
Diabetes	rs = 0.184 $p = 0.242$	rs = 0.097 $p = 0.540$	rs = 0.148 $p = 0.348$	rs = 0.081 $p = 0.611$	rs = 0.121 $p = 0.445$	rs = 0.026 $p = 0.665$	rs = −0.054 $p = 0.735$	rs = −0.182 $p = 0.250$	rs = −0.047 $p = 0.767$	rs = 0.007 $p = 0.966$	rs = −0.013 $p = 0.933$
Hypert.	rs = 0.704 $p < 0.001$	rs = 0.545 $p < 0.001$	rs = −0.075 $p = 0.638$	rs = 0.489 $p = 0.001$	rs = 0.549 $p < 0.001$	rs = 0.006 $p = 0.970$	rs = 0.287 $p = 0.065$	rs = −0.541 $p < 0.001$	rs = −0.292 $p = 0.060$	rs = −0.223 $p = 0.155$	rs = −0.316 $p = 0.042$
Hypercol.	rs = 0.582 $p < 0.001$	rs = 0.533 $p < 0.001$	rs = −0.179 $p = 0.256$	rs = 0.407 $p = 0.008$	rs = 0.362 $p = 0.019$	rs = −0.077 $p = 0.627$	rs = 0.244 $p = 0.120$	rs = −0.504 $p = 0.001$	rs = −0.277 $p = 0.076$	rs = −0.293 $p = 0.060$	rs = −0.342 $p = 0.027$
Hypothyr.	rs = 0.383 $p = 0.012$	rs = 0.216 $p = 0.169$	rs = −0.178 $p = 0.259$	rs = 0.178 $p = 0.260$	rs = 0.161 $p = 0.309$	rs = 0.031 $p = 0.845$	rs = 0.076 $p = 0.632$	rs = −0.336 $p = 0.030$	rs = −0.048 $p = 0.762$	rs = −0.144 $p = 0.363$	rs = −0.200 $p = 0.203$
Anxiety	rs = 0.539 $p < 0.001$	rs = 0.537 $p < 0.001$	rs = −0.237 $p = 0.132$	rs = 0.376 $p = 0.014$	rs = 0.364 $p = 0.018$	rs = 0.230 $p = 0.144$	rs = 0.307 $p = 0.048$	rs = −0.384 $p = 0.012$	rs = −0.190 $p = 0.229$	rs = −0.205 $p = 0.192$	rs = −0.303 $p = 0.051$
Depres.	rs = 0.351 $p = 0.023$	rs = 0.393 $p = 0.010$	rs = −0.343 $p = 0.026$	rs = 0.191 $p = 0.226$	rs = 0.186 $p = 0.238$	rs = 0.302 $p = 0.052$	rs = 0.186 $p = 0.239$	rs = −0.221 $p = 0.159$	rs = 0.010 $p = 0.949$	rs = −0.010 $p = 0.950$	rs = −0.101 $p = 0.526$
Stress	rs = 0.544 $p < 0.001$	rs = 0.544 $p < 0.001$	rs = 0.066 $p = 0.677$	rs = 0.409 $p = 0.007$	rs = 0.337 $p = 0.029$	rs = 0.323 $p = 0.037$	rs = 0.385 $p = 0.012$	rs = −0.396 $p = 0.009$	rs = 0.032 $p = 0.842$	rs = 0.081 $p = 0.609$	rs = −0.011 $p = 0.945$
Symptoms score	rs = 0.493 $p = 0.001$	rs = 0.470 $p = 0.002$	rs = −0.342 $p = 0.027$	rs = 0.580 $p < 0.001$	rs = 0.502 $p = 0.001$	rs = 0.377 $p = 0.014$	rs = 0.120 $p = 0.449$	rs = −0.573 $p < 0.001$	rs = −0.005 $p = 0.977$	rs = −0.046 $p = 0.770$	rs = −0.109 $p = 0.490$
Signs score	rs = 0.625 $p < 0.001$	rs = 0.642 $p < 0.001$	rs = −0.364 $p = 0.018$	rs = 0.608 $p < 0.001$	rs = 0.551 $p < 0.001$	rs = 0.220 $p = 0.161$	rs = 0.240 $p = 0.126$	rs = −0.711 $p < 0.001$	rs = −0.231 $p = 0.141$	rs = −0.242 $p = 0.122$	rs = −0.300 $p = 0.054$
Disease severity	rs = 0.659 $p < 0.001$	rs = 0.624 $p < 0.001$	rs = −0.359 $p = 0.019$	rs = 0.574 $p < 0.001$	rs = 0.497 $p = 0.001$	rs = 0.293 $p = 0.059$	rs = 0.165 $p = 0.296$	rs = −0.633 $p < 0.001$	rs = −0.147 $p = 0.352$	rs = −0.161 $p = 0.308$	rs = −0.228 $p = 0.146$

TABLE 5 Uni- and multivariable linear regression analysis for miRNAs expression in all enrolled patients. Significance was set as $p < 0.05$.

	miR 7a-3p				miR 7a-2-3p			
	Univariate		Multivariate		Univariate		Multivariate	
Variable	*B*	*p*-value	*B*	*p*-value	*B*	*p*-value	*B*	*p*-value
Age	0.229	0.218	-	-	−0.225	0.558	-	-
Plaque index	0.345	0.336	-	-	−0.214	0.029	0.156	0.012
Diabetes	−0.155	0.115	-	-	0.312	0.324	-	-
Hyperthyroidism	−0.432	0.568	-	-	0.336	0.248	-	-
Hypercholesterolemia	−0.371	0.257	-	-	0.458	0.369	-	-
Hypothyroidism	0.484	0.158	-	-	−0.332	0.557	-	-
Anxiety	0.257	0.196	-	-	0.214	0.115	0.277	0.022
Depression	0.332	0.419	-	-	−0.568	0.254	-	-
Stress	0.257	0.045	0.257	0.026	0.254	0.274	-	-
OLP symptoms score	0.236	0.036	0.189	0.021	0.214	0.041	-	-
OLP signs score	0.115	**0.029**	0.158	**0.029**	0.339	**0.032**	0.457	**0.002**
OLP disease severity	0.336	**0.036**	0.254	**0.036**	0.247	0.041	0.336	**0.025**
	miR 7a-5p				**miR 21-3p**			
	Univariate		**Multivariate**		**Univariate**		**Multivariate**	
Variable	***B***	***p*-value**	***B***	***p*-value**	***B***	***p*-value**	***B***	***p*-value**
Age	0.332	0.212	-	-	0.248	0.552	-	-
Plaque index	−0.441	**0.021**	−0.324	**0.025**	0.665	0.652	-	-
Diabetes	−0.257	0.235	-	-	−0.336	0.287	-	-
Hyperthyroidism	−0.665	0.258	-	-	0.236	0.547	-	-
Hypercholesterolemia	0.221	0.447	-	-	0.312	**0.045**	-	-
Hypothyroidism	0.236	0.554	-	-	−0.336	0.778	-	-
Anxiety	−0.254	**0.047**	0.009	**0.026**	0.354	0.446	-	-
Depression	−0.336	0.554	-	-	−0.287	0.317	-	-
Stress	0.218	0.332	-	-	0.554	0.105	0.258	**0.015**
OLP symptoms score	−0.331	0.046	-	-	0.215	0.886	-	-
OLP signs score	−0.458	**0.015**	-	-	0.284	**0.002**	0.158	**0.041**
OLP disease severity	0.365	**0.023**	-	-	0.336	**<0.001**	0.328	**0.012**
	miR 21-5p				**miR 100-3p**			
	Univariate		**Multivariate**		**Univariate**		**Multivariate**	
Variable	***B***	***p*-value**	***B***	***p*-value**	***B***	***p*-value**	***B***	***p*-value**
Age	0.258	0.265	-	-	0.248	0.125	-	-
Plaque index	0.645	0.458	-	-	0.588	0.195	-	-
Diabetes	0.365	**0.016**	-	-	−0.331	**0.029**	−0.057	**0.046**
Hyperthyroidism	0.667	0.332	-	-	0.123	0.334	-	-
Hypercholesterolemia	−0.257	**0.012**	−0.273	**0.001**	0.441	0.254	-	-
Hypothyroidism	0.336	0.428	-	-	0.285	**0.025**	0.293	**0.015**
Anxiety	−0.541	0.336	-	-	−0.336	0.665	-	-
Depression	0.109	0.685	-	-	−0.567	0.287	-	-
Stress	0.125	0.019	0.061	**0.013**	0.105	0.551	-	-
OLP symptoms score	−0.026	**0.037**	−0.013	**0.009**	0.259	0.285	-	-
OLP signs score	0.335	0.671	-	-	0.365	0.365	-	-

(Continues)

TABLE 5 (Continued)

	miR 21-5p				miR 100-3p			
	Univariate		Multivariate		Univariate		Multivariate	
Variable	*B*	*p*-value	*B*	*p*-value	*B*	*p*-value	*B*	*p*-value
OLP disease severity	−0.572	**0.009**	-	-	0.212	**0.012**	0.325	**0.023**
	miR 100-5p				miR 125b-2-3p			
	Univariate		Multivariate		Univariate		Multivariate	
Variable	*B*	*p*-value	*B*	*p*-value	*B*	*p*-value	*B*	*p*-value
Age	0.241	0.213	-	-	0.247	0.187	-	-
Plaque index	0.331	0.335	-	-	0.315	0.165	-	-
Diabetes	0.258	0.254	-	-	−0.025	0.254	-	-
Hyperthyroidism	0.574	0.541	-	-	0.107	0.668	-	-
Hypercholesterolemia	−0.361	0.055	-	-	−0.247	0.574	-	-
Hypothyroidism	0.354	**0.039**	-	-	−0.289	0.325	-	-
Anxiety	−0.512	0.224	-	-	−0.654	**0.047**	−0.254	**0.024**
Depression	0.115	0.274	-	-	0.369	0.254	-	-
Stress	0.258	0.158	0.231	**0.013**	−0.107	0.028	-	-
OLP symptoms score	−0.029	**0.028**	0.136	**0.045**	−0.265	0.185	-	-
OLP signs score	0.335	0.034	0.254	**0.011**	−0.374	**0.025**	0.365	**0.002**
OLP disease severity	0.554	**0.021**	-	-	−0.441	**0.006**	-	-
	miR 125b-5p				miR 200b-3p			
	Univariate		Multivariate		Univariate		Multivariate	
Variable	*B*	*p*-value	*B*	*p*-value	*B*	*p*-value	*B*	*p*-value
Age	0.470	**0.035**	0.225	**0.021**	0.254	0.245	-	-
Plaque index	0.541	0.416	-	-	0.117	0.336	-	-
Diabetes	−0.332	0.178	-	-	−0.255	**0.028**	-	-
Hyperthyroidism	0.254	0.128	-	-	−0.305	**0.025**	-	-
Hypercholesterolemia	−0.314	**0.001**	-	-	0.355	0.541	-	-
Hypothyroidism	−0.287	0.428	-	-	−0.247	**0.035**	0.214	**0.014**
Anxiety	−0.258	**0.045**	-	-	−0.244	0.045	0.136	**0.025**
Depression	0.111	0.936	-	-	−0.214	0.257	-	-
Stress	0.026	0.019	0.061	**0.013**	0.147	0.254	-	-
OLP symptoms score	−0.158	**0.037**	−0.013	**0.022**	0.198	0.336	-	-
OLP signs score	−0.255	0.671	-	-	0.278	0.012	0.247	**0.015**
OLP disease severity	−0.167	**0.009**	−0.505	**<0.001**	0.332	**0.012**	0.445	**0.002**
	miR 200b-5p							
	Univariate				Multivariate			
Variable	*B*		*p*-value		*B*		*p*-value	
Age	0.241		0.134		-		-	
Plaque index	0.332		0.416		-		-	
Diabetes	0.541		0.178		-		-	
Hyperthyroidism	0.552		0.128		-		-	
Hypercholesterolemia	−0.224		**0.011**		−0.115		**0.023**	
Hypothyroidism	0.365		0.428		-		-	

TABLE 5 (Continued)

	miR 200b-5p			
	Univariate		Multivariate	
Variable	*B*	*p*-value	*B*	*p*-value
Anxiety	−0.278	0.251	-	-
Depression	0.247	0.936	-	-
Stress	0.015	0.019	0.061	**0.013**
OLP symptoms score	−0.075	**0.028**	−0.015	**0.041**
OLP signs score	0.255	0.671	-	-
OLP disease severity	0.0258	**0.025**	-	-

development (Manzano-Moreno et al., 2021; Zheng et al., 2021). The present results also suggest a close correlation between OLP disease severity and the expression of these miRNAs in oral tissues. However, it should be noted that the expression of miR-125b-5p was not significantly correlated with any clinical parameters evaluated. In contrast, miR-125-b2 expression was negatively correlated with plaque index, hypertension, hypercholesterolemia, hypothyroidism, anxiety, and stress, miR-21-3p and miR-21-5p showed positive correlations. These data suggest the possibility of individualized expression based on specific miRNAs profile.

Literature data associated some miRNA levels with oral dysplasia and OSCC compared to healthy oral mucosa. Specifically, miRNA-7 and -21 families are known as OncomiR and are highly expressed during oral leukoplakia, OSCC, and OLP (Chattopadhyay et al., 2016; De Sarkar et al., 2014). In the present study, higher miR-7a-3p/miR-7a-2-3p and lower miR-7a-5p tissue expressions were significantly impacted by OLP symptoms, signs, and disease severity scores. Interestingly, miR-7a-5p appeared downregulated in lung cancer cells with highly metastatic capacity, and its overexpression induced suppression in lung cancer cell growth (Woo et al., 2020). This suggests that within the miR-7 family, not all are OncomiRs because miR-7a-3p and miR-7a-2-3p have been positively correlated with OLP and OSCC; instead, miR-7a-5p acted as tumor suppressor. In the present study, miR-7 was a significant predictor of the OLP severity extent in terms of OLP symptoms and signs; miR-7 expression levels were also correlated with various clinical parameters, such as plaque index and depression. In agreement, stress, depression and anxiety were significantly related to the expression of some miRNAs levels, in particular, miR-7 and miR-100, which were also significantly impacted by the OLP symptoms, signs, and disease severity scores, indices of erosive and symptomatic lesions, and this should be noted since a possible inflammatory status due to gingival inflammation or diabetes might have influenced the expression of the analyzed miRNAs. In this regard, miR-100-5p has been reported as a tumor suppressor downregulated in OSCC cell lines (Jakob et al., 2019); however, OLP patients expressed higher levels of this mediator, although there were no significant correlations with the disease severity.

However, the present study results have some limitations that need to be addressed. One is the study design; the lack of a longitudinal evaluation of the effects of the miRNAs could have better identified the miRNAs associated with the potential malignant evolution of OLP in the long term. The other limitation is the limited number of enrolled patients. Furthermore, the several significant correlations identified in the present study between miRNA expression and factors such as gingival plaque index, hypertension, hypercholesterolemia, hypothyroidism, anxiety and stress indicate which predictive biomarkers of disease should be carefully considered by evaluating all of the above when evaluating miRNA with regards to the individual profile of the patient's local (oral) and systemic health status.

5 | CONCLUSION

The results of the present study showed that patients with OLP presented a different pattern of all analyzed miRNAs compared to healthy subjects. Furthermore, OLP symptoms, signs, and disease severity scores were significant predictors of the several analyzed miRNAs. The present study provides interesting insights into miRNAs' potential utility as risk biomarkers of OLP disease progression. However, given the lack of longitudinal data in the present study, our results should be cautiously interpreted when associated with the developmental malignant risk transformation of OLP. Furthermore, the analysis of oral miRNA levels has proved to be a valuable tool for the prognostic evaluation of the risk profile of OLP patients; however, further studies with larger sample sizes and a longitudinal design are needed to understand better the impact of oral miRNAs during OLP and to test the predictive efficacy of miRNA levels in oral tissues as effective biomarkers for therapeutic monitoring of malignant evolution of OLP.

AUTHOR CONTRIBUTIONS

Alessandro Polizzi: Conceptualization; writing – original draft. **Simona Santonocito:** Investigation; methodology. **Alfio Distefano:** Methodology; formal analysis. **Rocco De Pasquale:** Validation; visualization; investigation. **Angela Alibrandi:** Formal analysis; software; data curation. **Amer M. Alanazi:** Validation; visualization; project administration. **Giovanni Li Volti:** Funding acquisition; validation; visualization. **Gaetano Isola:** Conceptualization; resources; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that may influence the work reported.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Adamo, D., Calabria, E., Canfora, F., Coppola, N., Lo Muzio, L., Spirito, F., Giuliani, M., Azzi, L., Maurino, V., Colella, G., Colella, C., Montebugnoli, L., Gissi, D. B., Gabriele, M., Nisi, M., Sardella, A., Lodi, G., Varoni, E. M., Giudice, A., … SIPMO. (2022). Where do you live? North versus Central-South differences in relation to Italian patients with oral lichen planus: A cross-sectional study from the SIPMO (Italian Society of Oral Pathology and Medicine). *BMC Oral Health*, *22*(1), 184. https://doi.org/10.1186/s12903-022-02181-7

Aghbari, S. M. H., Gaafar, S. M., Shaker, O. G., El Ashiry, S., & Zayed, S. O. (2018). Evaluating the accuracy of microRNA27b and microRNA137 as biomarkers of activity and potential malignant transformation in oral lichen planus patients. *Archives of Dermatological Research*, *310*(3), 209–220.

Ahmadi-Motamayel, F., Bayat, Z., Hajilooi, M., Shahryar-Hesami, S., Mahdavinezhad, A., Samie, L., & Solgi, G. (2017). Evaluation of the miRNA-146a and miRNA-155 expression levels in patients with oral lichen planus. *Iranian Journal of Immunology*, *14*(4), 316–324.

Al-Hashimi, I., Schifter, M., Lockhart, P. B., Wray, D., Brennan, M., Migliorati, C. A., Axéll, T., Bruce, A. J., Carpenter, W., Eisenberg, E., Epstein, J. B., Holmstrup, P., Jontell, M., Lozada-Nur, F., Nair, R., Silverman, B., Thongprasom, K., Thornhill, M., Warnakulasuriya, S., & van der Waal, I. (2007). Oral lichen planus and oral lichenoid lesions: Diagnostic and therapeutic considerations. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *103*(S25), e1–e12.

Bermejo-Fenoll, A., & López-Jornet, P. (2006). Familial oral lichen planus: Presentation of six families. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *102*(2), e12–e15.

Chattopadhyay, E., Singh, R., Ray, A., Roy, R., de Sarkar, N., Paul, R. R., Pal, M., Aich, R., & Roy, B. (2016). Expression deregulation of mir31 and CXCL12 in two types of oral precancers and cancer: Importance in progression of precancer and cancer. *Scientific Reports*, *6*(1), 32735. https://doi.org/10.1038/srep32735

Cheng, T.-Y., Zimmerman, J. J., & Giménez-Lirola, L. G. (2022). Internal reference genes with the potential for normalizing quantitative PCR results for oral fluid specimens. *Animal Health Research Reviews*, *23*, 1–10.

Crincoli, V., Di Bisceglie, M. B., Scivetti, M., Lucchese, A., Tecco, S., & Festa, F. (2011). Oral lichen planus: Update on etiopathogenesis, diagnosis and treatment. *Immunopharmacology and Immunotoxicology*, *33*(1), 11–20.

da Silva, E. L., de Lima, T. B., Rados, P. V., & Visioli, F. (2021). Efficacy of topical non-steroidal immunomodulators in the treatment of oral lichen planus: A systematic review and meta-analysis. *Clinical Oral Investigations*, *25*(9), 5149–5169. https://doi.org/10.1007/s00784-021-04072-7

Danielsson, K., Wahlin, Y.-B., Gu, X., Boldrup, L., & Nylander, K. (2012). Altered expression of miR-21, miR-125b, and miR-203 indicates a role for these microRNAs in oral lichen planus. *Journal of Oral Pathology & Medicine*, *41*(1), 90–95.

de Porras-Carrique, T., Gonzalez-Moles, M. A., Warnakulasuriya, S., & Ramos-Garcia, P. (2022). Depression, anxiety, and stress in oral lichen planus: A systematic review and meta-analysis. *Clinical Oral Investigations*, *26*(2), 1391–1408. https://doi.org/10.1007/s00784-021-04114-0

de Sarkar, N., Roy, R., Mitra, J. K., Ghose, S., Chakraborty, A., Paul, R. R., Mukhopadhyay, I., & Roy, B. (2014). A quest for miRNA biomarker: A track back approach from gingivo buccal cancer to two different types of precancers. *PLoS One*, *9*(8), e104839. https://doi.org/10.1371/journal.pone.0104839

Donati, S., Ciuffi, S., & Brandi, M. L. (2019). Human circulating miRNAs real-time qRT-PCR-based analysis: An overview of endogenous reference genes used for data normalization. *International Journal of Molecular Sciences*, *20*(18), 4353.

Farhi, D., & Dupin, N. (2010). Pathophysiology, etiologic factors, and clinical management of oral lichen planus, part I: Facts and controversies. *Clinics in Dermatology*, *28*(1), 100–108.

Giuliani, M., Troiano, G., Cordaro, M., Corsalini, M., Gioco, G., Lo Muzio, L., Pignatelli, P., & Lajolo, C. (2019). Rate of malignant transformation of oral lichen planus: A systematic review. *Oral Diseases*, *25*(3), 693–709.

González-Moles, M. Á., Ruiz-Avila, I., Gonzalez-Ruiz, L., Ayen, A., Gil-Montoya, J. A., & Ramos-Garcia, P. (2019). Malignant transformation risk of oral lichen planus: A systematic review and comprehensive meta-analysis. *Oral Oncology*, *96*, 121–130.

Gorouhi, F., Davari, P., & Fazel, N. (2014). Cutaneous and mucosal lichen planus: A comprehensive review of clinical subtypes, risk factors, diagnosis, and prognosis. *The Scientific World Journal*, *2014*, 1–22.

Gueiros, L. A., Arao, T., Souza, T., Vieira, C. L., Gomez, R. S., Almeida, O. P., Lodi, G., & Leao, J. C. (2018). IL17A polymorphism and elevated IL17A serum levels are associated with oral lichen planus. *Oral Diseases*, *24*(3), 377–383. https://doi.org/10.1111/odi.12718

Han, H. S., Jo, Y. N., Lee, J. Y., Choi, S. Y., Jeong, Y., Yun, J., & Lee, O. J. (2014). Identification of suitable reference genes for the relative quantification of microRNAs in pleural effusion. *Oncology Letters*, *8*(4), 1889–1895. https://doi.org/10.3892/ol.2014.2404

Isola, G., Santonocito, S., Distefano, A., Polizzi, A., Vaccaro, M., Raciti, G., Caccamo, D., Currò, M., Cannavò, G., Li Volti, G., Macaione, S., & Li Volti, G. (2023). Impact of periodontitis on gingival crevicular fluid miRNAs profiles associated with cardiovascular disease risk. *Journal of Periodontal Research*, *58*(1), 165–174. https://doi.org/10.1111/jre.13078

Jakob, M., Mattes, L. M., Kuffer, S., Unger, K., Hess, J., Bertlich, M., Haubner, F., Ihler, F., Canis, M., Weiss, B. G., & Kitz, J. (2019). MicroRNA expression patterns in oral squamous cell carcinoma: hsa-mir-99b-3p and hsa-mir-100-5p as novel prognostic markers for oral cancer. *Head & Neck*, *41*(10), 3499–3515. https://doi.org/10.1002/hed.25866

Kaliakatsou, F., Hodgson, T., Lewsey, J., Hegarty, A., Murphy, A., & Porter, S. (2002). Management of recalcitrant ulcerative oral lichen planus with topical tacrolimus. *Journal of the American Academy of Dermatology*, *46*(1), 35–41.

Li, Y., He, Y., Xiang, J., Feng, L., Wang, Y., & Chen, R. (2022). The functional mechanism of MicroRNA in oral lichen planus. *Journal of Inflammation Research*, *15*, 4261–4274. https://doi.org/10.2147/JIR.S369304

Liang, J., Xu, J., Zhu, Z., & Xu, X. (2016). Correlation of miRNA-155 and IL-17 mRNA expression in peripheral blood of female patients with

oral lichen planus. *International Journal of Clinical & Experimental Medicine*, *9*(10), 10569–10574.

Liu, F., Wu, J., & Ye, F. (2015). Expression of miRNA-155 and miRNA-146a in peripheral blood mononuclear cells and plasma of oral lichen planus patients. *Zhonghua kou qiang yi xue za zhi = Zhonghua kouqiang yixue zazhi = Chinese Journal of Stomatology*, *50*(1), 23–27.

Lodi, G., Carrozzo, M., Furness, S., & Thongprasom, K. (2012). Interventions for treating oral lichen planus: A systematic review. *The British Journal of Dermatology*, *166*(5), 938–947. https://doi.org/10.1111/j.1365-2133.2012.10821.x

Lodi, G., Manfredi, M., Mercadante, V., Murphy, R., & Carrozzo, M. (2020). Interventions for treating oral lichen planus: Corticosteroid therapies. *Cochrane Database of Systematic Reviews*, *2*(2), CD001168. https://doi.org/10.1002/14651858.CD001168.pub3

Lodi, G., Pellicano, R., & Carrozzo, M. (2010). Hepatitis C virus infection and lichen planus: A systematic review with meta-analysis. *Oral Diseases*, *16*(7), 601–612. https://doi.org/10.1111/j.1601-0825.2010.01670.x

Lodi, G., Tarozzi, M., Sardella, A., Demarosi, F., Canegallo, L., Di Benedetto, D., & Carrassi, A. (2007). Miconazole as adjuvant therapy for oral lichen planus: A double-blind randomized controlled trial. *The British Journal of Dermatology*, *156*(6), 1336–1341. https://doi.org/10.1111/j.1365-2133.2007.07883.x

Luo, M., Gao, Z., Li, H., Li, Q., Zhang, C., Xu, W., Song, S., Ma, C., & Wang, S. (2018). Selection of reference genes for miRNA qRT-PCR under abiotic stress in grapevine. *Scientific Reports*, *8*(1), 4444. https://doi.org/10.1038/s41598-018-22743-6

Manzano-Moreno, F. J., Costela-Ruiz, V. J., García-Recio, E., Olmedo-Gaya, M. V., Ruiz, C., & Reyes-Botella, C. (2021). Role of salivary microRNA and cytokines in the diagnosis and prognosis of oral squamous cell carcinoma. *International Journal of Molecular Sciences*, *22*(22), 12215.

Mehdipour, M., Shahidi, M., Manifar, S., Jafari, S., Mashhadi Abbas, F., Barati, M., Mortazavi, H., Shirkhoda, M., Farzanegan, A., & Elmi Rankohi, Z. (2018). Diagnostic and prognostic relevance of salivary microRNA-21,-125a,-31 and-200a levels in patients with oral lichen planus-a short report. *Cellular Oncology*, *41*(3), 329–334.

Mueller, R., Bajric, D., Keceli, H. G., Keller, A., Dommisch, H., Elsharawy, A., & Schaefer, A. S. (2021). hsa-miR-374b-5p regulates expression of the gene U2AF homology motif (UHM) kinase 1. *Journal of Periodontal Research*, *56*(6), 1028–1036. https://doi.org/10.1111/jre.12913

Nunes, G. P., Pirovani, B. O., Nunes, L. P., Silva, A. N. A., Morabito, M., Nunes-Junior, N. A., Delbem, A. C. B., & Ferrisse, T. M. (2022). Does oral lichen planus aggravate the state of periodontal disease? A systematic review and meta-analysis. *Clinical Oral Investigations*, *26*(4), 3357–3371. https://doi.org/10.1007/s00784-022-04387-z

Payeras, M. R., Cherubini, K., Figueiredo, M. A., & Salum, F. G. (2013). Oral lichen planus: Focus on etiopathogenesis. *Archives of Oral Biology*, *58*(9), 1057–1069.

Petti, S., Rabiei, M., de Luca, M., & Scully, C. (2011). The magnitude of the association between hepatitis C virus infection and oral lichen planus: Meta-analysis and case control study. *Odontology*, *99*(2), 168–178. https://doi.org/10.1007/s10266-011-0008-3

Pippi, R., Romeo, U., Santoro, M., Del Vecchio, A., Scully, C., & Petti, S. (2016). Psychological disorders and oral lichen planus: Matched case-control study and literature review. *Oral Diseases*, *22*(3), 226–234. https://doi.org/10.1111/odi.12423

Polizzi, A., Santonocito, S., Lo Giudice, A., Alibrandi, A., de Pasquale, R., & Isola, G. (2023). Analysis of the response to two pharmacological protocols in patients with oral lichen planus: A randomized clinical trial. *Oral Diseases*, *29*(2), 755–763. https://doi.org/10.1111/odi.13960

Raj, A., Sreelatha, K., & Balan, A. (2012). Dapsone in the treatment of resistant oral erosive lichen planus: A clinical study. *Journal of Indian Academy of Oral Medicine and Radiology*, *24*(1), 20–23.

Rojo-Moreno, J., Bagán, J., Rojo-Moreno, J., Donat, J. S., Milián, M. A., & Jiménez, Y. (1998). Psychologic factors and oral lichen planus: A psychometric evaluation of 100 cases. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *86*(6), 687–691.

Santonocito, S., Polizzi, A., de Pasquale, R., Ronsivalle, V., Lo Giudice, A., & Isola, G. (2021). Analysis of the efficacy of two treatment protocols for patients with symptomatic oral lichen planus: A randomized clinical trial. *International Journal of Environmental Research and Public Health*, *18*(1), 56.

Singh, A., Rai, A., Aftab, M., Jain, S., & Singh, M. (2017). Efficacy of steroidal vs non-steroidal agents in oral lichen planus: A randomised, open-label study. *The Journal of Laryngology & Otology*, *131*(1), 69–76.

Tang, F., Chu, L., Shu, W., He, X., Wang, L., & Lu, M. (2019). Selection and validation of reference genes for quantitative expression analysis of miRNAs and mRNAs in Poplar. *Plant Methods*, *15*(1), 1–15.

Thongprasom, K., Carrozzo, M., Furness, S., & Lodi, G. (2011). Interventions for treating oral lichen planus. *Cochrane Database of Systematic Reviews*, *7*, CD001168. https://doi.org/10.1002/14651858.CD001168.pub2

Warnakulasuriya, S., Kujan, O., Aguirre-Urizar, J. M., Bagan, J. V., González-Moles, M. Á., Kerr, A. R., Lodi, G., Mello, F. W., Monteiro, L., Ogden, G. R., Sloan, P., & Ogden, G. R. (2021). 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 Diseases*, *27*(8), 1862–1880.

Woo, S. Y., Lee, S. Y., Yu, S.-L., Park, S. J., Kang, D., Kim, J. S., Jeong, I. B., Kwon, S. J., Hwang, W. J., Park, C. R., & Son, J. W. (2020). MicroRNA-7-5p' s role in the O-GlcNAcylation and cancer metabolism. *Non-coding RNA Research*, *5*(4), 201–207.

Wu, D., Chen, X., Dong, C., Liu, Q., Yang, Y., He, C., Wang, J., Sun, M., & Wu, Y. (2015). Association of single nucleotide polymorphisms in MPO and COX genes with oral lichen planus. *International Journal of Immunogenetics*, *42*(3), 161–167.

Zheng, S., Yu, S., Fan, X., Zhang, Y., Sun, Y., Lin, L., Wang, H., Pan, Y., & Li, C. (2021). Porphyromonasgingivalis survival skills: Immune evasion. *Journal of Periodontal Research*, *56*(6), 1007–1018. https://doi.org/10.1111/jre.12915

Ziebarth, J. D., Bhattacharya, A., & Cui, Y. (2019). Functional analysis of genetic variants and somatic mutations impacting microRNA-target recognition: Bioinformatics resources. In *MicroRNA target identification* (pp. 101–120). Springer Nature.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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