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ORIGINAL PAPER

Evaluating the accuracy of microRNA27b and microRNA137 as biomarkers of activity and potential malignant transformation in oral lichen planus patients

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Abstract
Oral lichen planus (OLP) is a chronic inflammatory mucocutaneous disease with a potential malignant transformation, characterized by cytotoxic T cells against basal epithelial cells. MicroRNAs (MiRNAs) are short non-coding RNA that plays critical role in gene expression at post-transcriptional levels. Much evidence showed that miRNAs play an important role in regulating immune response and cancer development. The purpose of the present study was to compare the expression of miRNA 27b and miRNA 137 in tissues and saliva between OLP patients and controls by using RT-qPCR and to evaluate their use as biomarkers of disease activity and potential malignant transformation. Our results showed down expression of miRNA 27b and miRNA 137 in tissue and saliva of OLP patients compared to controls; among OLP subgroups, erosive-type miRNA 137 revealed the lowest level in tissue and saliva. In conclusion, alteration of miRNA 27b and miRNA 137 gene expression signify their use as biomarkers for diseases activity and tendency of malignant transformation, and down expression of miRNA 137 especially in erosive-type favors the use of saliva sample as a noninvasive method for monitoring a potential malignant transformation of OLP.

Keywords Oral lichen planus · MicroRNA · miRNA 27b and miRNA 137

Introduction

Oral lichen planus (OLP) is a chronic inflammatory mucocutaneous disease, that affects 1–2% of adult population with a female predilection [25]. It is classified as an oral premalignant lesion by the world health organization (WHO) with frequency of malignant transformation that ranges from 1.5 to 6.4% with a followup period of at least 6 months [5].

OLP has six clinical forms that may coexist with each other; white forms (papular, reticular and plaque) and red forms (atrophic, erosive and bullous). The etiology and pathogenesis of OLP are still unclear, although it has some autoimmune features such as autocytotoxic T-cell-mediated immunity against epithelial basement membrane, female predilection, a cyclic nature, chronicity and association with other autoimmune diseases [9].

MicroRNA (miRNA) is a short 22 nucleotides noncoding RNA, that regulate gene expression at post-transcriptional levels by binding to specific messenger RNA and promoting its degradation and/or translational inhibition [20]. miRNAs play critical roles in the control of cell division, proliferation, differentiation and death [4].

Alterations in miRNAs expression were reported to be involved in many physiological and pathological processes, including chronic inflammatory and autoimmune diseases as well as cancer [7]. miRNA alterations have also been found in patients with OLP [10].

In sera from 30 patients with multifocal mucosal OLP, Nylander et al. [19] identified 15 miRNAs as significantly differentially expressed compared to controls. Moreover, they found that miRNA-21, -223 and -143 were connected to

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epithelial cancer, while miRNA-21 and miRNA-181a were connected to different autoimmune diseases.

Arão et al. [3] stated that the expression of miRNA-146a and miRNA-155 in OLP lesions was increased in comparison with normal oral mucosa and blood samples. Danielsson et al. [9] investigated the levels of COX-2 and miRNA-26b in OLP lesions to see if there was any correlation between expression of COX-2 and its regulator miRNA-26b in OLP. They noted that the levels of COX-2 mRNA were higher while the expression of miRNA-26b was lower in OLP samples compared to normal oral epithelium samples.

In another study, Danielsson et al. [10] studied the correlation between expression of miRNA-21, miRNA-125b, and miRNA-203 and their potential targets, the tumor suppressor p53 and its relative p63, two important proteins both known to be deregulated in OLP. They found increased expression of miRNA-21 and miRNA-203, decreased expression of miRNA-125 and down regulation of p53 and DNp63 RNA in OLP compared to normal oral mucosa.

Zhang et al. [27] pointed out that miRNAs may be the novel candidate biomarkers for the implication of miRNAs in the pathogenesis of OLP as they found that miRNA 27b was significantly downregulated in OLP tissue, and miRNA 27b expression was even more suppressed in atrophic-erosive OLP than in reticular OLP. In addition, miRNA 27b was found to be expressed in the epithelial keratinocyte layer of both normal and OLP tissues.

Dang et al. [8] observed higher frequency of miRNA 137 promoter methylation in the mucosal tissues of patients with OLP than in the mucosal tissues from healthy individuals, but the frequency was significantly lower than in patients with oral squamous cell carcinoma (OSCC).

Collectively from the previous studies, it could be inferred that proper regulation of miRNA expression is important in maintaining normal immune functions and preventing autoimmunity, whereas dysregulation of miRNA is associated with the pathogenesis of autoimmune diseases, in particular, T-cell-mediated immune responses.

Therefore, studying different miRNA expression profiles might hopefully lead to the discovery of a novel biomarker for the diagnosis of OLP and for the detection of possible malignant transformation of this disease.

The aim of this study was to compare the expression of microRNA27b and microRNA137 in tissues and saliva between patients with OLP and controls, and to evaluate their use as biomarkers of disease activity and tendency for malignant transformation.

Materials and methods

Subjects, salivary samples and tissue specimens

Patients included in the present study were recruited from the outpatient clinic of Oral Medicine and Periodontology Department, and Oral Surgery Department, Faculty of Oral and Dental Medicine, Cairo University. The subjects included (40) individuals divided into: (20) patients diagnosed with OLP and (20) healthy individuals (control group). The OLP patients were systemically and orally free from any other diseases and did not receive any treatment for at least 3 months before taking the biopsy. Furthermore, OLP group was subdivided into three subgroups according to its clinical types [(a) papular, reticular or plaque, (b) atrophic and (c) erosive]. Clinical data were collected during the first visit, including age in years, sex distribution, duration of appearing lesion; OLP clinical types Table 1.

Salivary samples were collected on the same day of biopsy taking from the two groups. Whole unstimulated saliva (WUS) "Spitting method" was collected using standard technique described by Navazesh [18]. After collection, all samples were immediately stored at − 80 °C until assayed for detection of miRNA 27b and miRNA 137 in saliva.

Oral biopsy specimen samples were obtained from patients diagnosed according to the modified WHO diagnostic criteria for OLP [21] and from healthy individuals as control. The patients with OLP were requested to agree to take an oral biopsy and the study protocol was approved by the Local Committee of Research and Ethics of Cairo University.

Oral mucosa biopsy from control group was obtained from non-inflamed operculum during surgical third molar removal in Oral Surgery Department and also was obtained from non-inflamed gingiva for esthetic purpose in Periodontology Department.

Each biopsy specimen was divided into two parts; one part was fixed in formalin and embedded in paraffin for histopathological examination, and the other part was kept frozen and stored at − 80 °C until assayed for miRNA 27b and miRNA 137 extraction procedures.

Histopathologic examination

The tissue blocks were cut into 4 µm thick. The specimens were examined after staining with hematoxylin and eosin (H&E) to confirm the diagnosis of OLP according to the modified WHO diagnostic criteria and to show any dysplastic features.

Table 1 Descriptive data collection of OLP patients and control

Pts. no.	Sex	Age	Duration	Clinical types
1	F	40 years	Unknown	Papular
2	F	60 years	6 months	Atrophic
3	M	48 years	3 months	Erosive
4	M	44 years	–	Control
5	M	33 years	–	Control
6	M	60 years	4 months	Atrophic
7	M	47 years	More than 1 year	Plaque
8	F	58 years	More than 3 months	Atrophic
9	F	59 years	9 months	Atrophic
10	F	26 years	1 month	Atrophic
11	M	45 years	4 years	Erosive
12	F	43 years	2 months	Erosive
13	F	39 years	–	Control
14	M	53 years	–	Control
15	M	20 years	–	Control
16	M	25 years	–	Control
17	F	23 years	–	Control
18	M	32 years	–	Control
19	F	55 years	5 months	Bullous Erosive
20	M	20 years	–	Control
21	F	50 years	6 months	Atrophic
22	M	27 years	–	Control
23	M	23 years	–	Control
24	F	21 years	–	Control
25	F	34 years	1 month	Erosive
26	F	49 years	Unknown	Plaque
27	M	47 years	Unknown	Atrophic
28	F	49 years	5 months	Erosive
29	M	49 years	–	Control
30	M	47 years	–	Control
31	M	35 years	–	Control
32	M	26 years	–	Control
33	F	34 years	–	Control
34	F	42 years	2 months	Papular
35	F	28 years	–	Control
36	F	30 years	–	Control
37	F	39 years	–	Control
38	F	53 years	1 month	Erosive
39	M	55 years	Unknown	Reticular
40	F	57 years	4 months	Atrophic

M male, *F* female

Detection of miRNA 27b and miRNA 137 in saliva and tissue

MicroRNA extraction

microRNAs were extracted from oral biopsy and saliva by miRNeasy extraction kit (Qiagen, Valencia, CA, USA) using 750 µL QIAzol lysis reagent for extraction from biopsy and 1000 µL QIAzol lysis reagent for extraction from 200 µL saliva.

After homogenization of the tissue, the homogenate was incubated for 5 min at room temperature. Then, 200 µL chloroform were added, vortexed for 15 s, and incubated for 2–3 min at room temperature. This was followed by centrifugation at 12,000×*g* at 4 °C for 15 min. The upper watery phase was removed and 1.5 times of its volume (100%) ethanol was added. 700 µL of this mixture were placed in RNeasy Mini spin column in 2 ml collection tube and centrifuged at 8000×*g* at room temperature for 15 s. After the mixture had completely passed the column, 700 µL of buffer RWT were added to each column, and again centrifuged at 8000×*g* at room temperature for 15 s. 500 µL buffer RPE were added to the column and centrifuged at 8000×*g* at room temperature for 15 s. The previous process was repeated for 2 min at full speed. The column was transferred to new 1.5 mL collection tube and 50 µL RNase-free water was pipetted directly onto the column and centrifuged for 1 min at 8000 xg to elute RNA. The extracted micro-RNA was then stored at − 80 °C until use.

Reverse transcription (RT) and real-time quantitative PCR (qPCR) Reverse transcription was carried out on micro-RNA in a final volume of 20 µL RT reactions (incubated for 60 min at 37 °C, followed by 5 min at 95 °C) using the miScript II RT kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Real-time qPCR was performed using a MiScript SYBR Green PCR kit (Qiagen, Valencia, CA, USA) and miScript primer assay miR-27b, -137 (Qiagen, Valencia, CA, USA). 20 ng of cDNA was used as a template in a total volume of 20 µL reaction with the following conditions: denaturation at 95 °C for 15 min followed by 40 cycles of 94 °C for 15 s, 55 °C for 30 s, and 70 °C for 30 s, in which fluorescence was acquired and detected by Rotor-gene Q Real-time PCR system (Qiagen, USA). After the PCR cycles, melting curve analyses were performed to validate the specific generation of the expected PCR product. SNORD was used as an endogenous control.

The expression level of miR-27b and -137 was evaluated using the ΔCt method. The cycle threshold (Ct) value is the number of qPCR cycles required for the fluorescent signal to cross a specified threshold. ΔCt was calculated by subtracting the Ct values of SNORD from those of target micro-RNAs. ΔΔCt was calculated by subtracting the ΔCt of the control samples from the ΔCt of the OLP samples. The fold change in miR-27b, and -137 expression was calculated by the equation $2^{-\Delta\Delta Ct}$.

Statistical analysis

Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples in comparing two groups of normally distributed data and Mann Whitney *U* test for independent samples for comparing not-normal data. Comparison of numerical variables between more than two groups was done using Kruskal Wallis test with posthoc multiple two-group comparisons. Within each group, comparison of numerical variables was done using Wilcoxon signed rank test for paired (matched) samples. For comparing categorical data, Chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency was less than 5. Accuracy was represented using the terms sensitivity, and specificity. Receiver operator characteristic (ROC) analysis was used to determine the optimum cut off value for the studied diagnostic markers in diagnosing OLP. *P* values less than 0.05 were considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

Results

Histopathologic section

The histopathologic sections in subgroup: (a) all of papular, reticular and plaque types of OLP showed hyperparakeratosis of epithelial layer with mild band of inflammatory cells and basal cells degeneration. It was noticed that one case of subgroup (a)—plaque type—displayed melting of the rete ridge caused by the epithelial destruction in the highly inflamed connective tissue. This should not be misconstrued as an invasive process. This finding may be due to basal zone vacuolation by lymphatic cells-induced apoptosis of basal epithelial cells, Figs (1, 2).

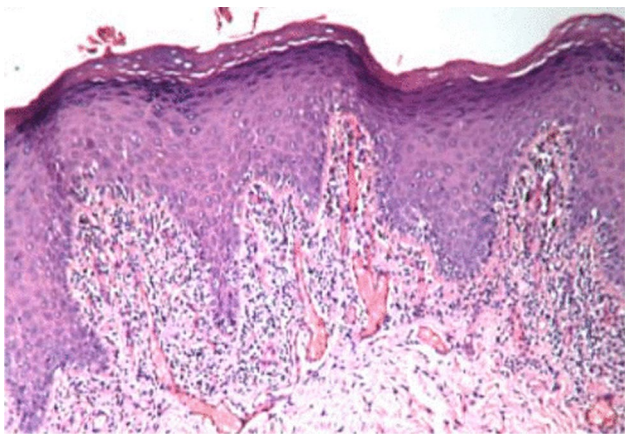


Fig. 1 Photomicrograph of papular OLP showing hyperplastic epithelium with hyperkeratosis and dense lymphocytic cells infiltration just underneath the epithelium. Notice the numerous dilated blood vessels in the connective tissue (CT) papillae, (H&E ×100)

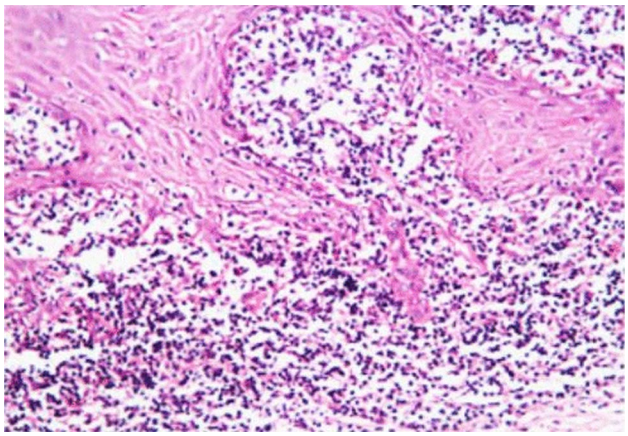


Fig. 2 Photomicrograph of plaque-type OLP showing the melting or fading of the rete ridge into the inflamed connective tissue. This finding may be caused by the epithelial destruction that has occurred. (H&E ×200)

Subgroup (b) demonstrated thinning of epithelial layer and moderate band of inflammatory cells with basal cell degeneration. Two cases of the atrophic type displayed active mitosis in the suprabasal cells of epithelium, Figs (3, 4).

Subgroup (c) showed ulceration with thin or even absent epithelial layer, massive band of inflammatory cells and basal degeneration. The bullous erosive OLP type in the H&E revealed the bulla separating the epithelium from connective tissue. Four cases of erosive type displayed

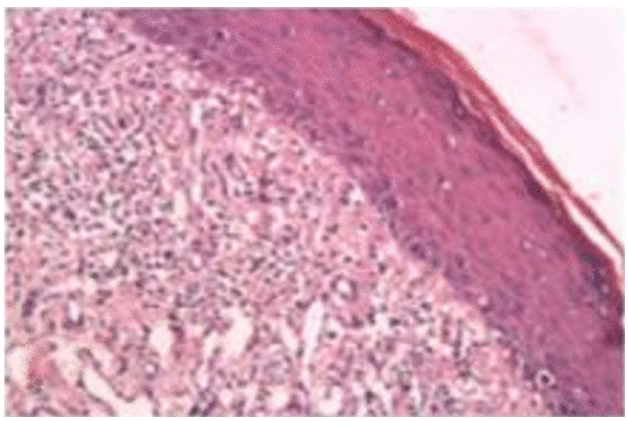


Fig. 3 Photomicrograph of atrophic OLP showing thin epithelial thickness and a band of inflammatory cells infiltration, (H&E ×200)

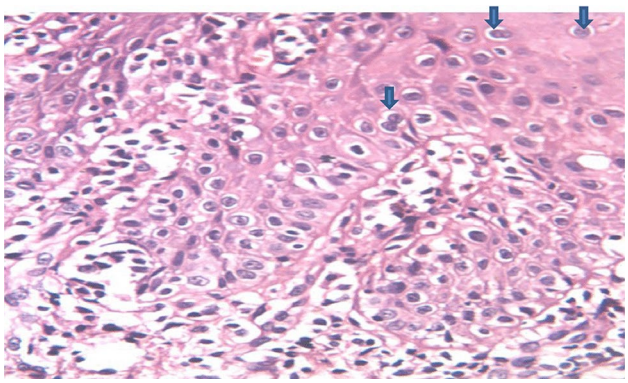


Fig. 4 Photomicrograph of atrophic OLP showing active mitotic figures (arrows), (H&E ×400)

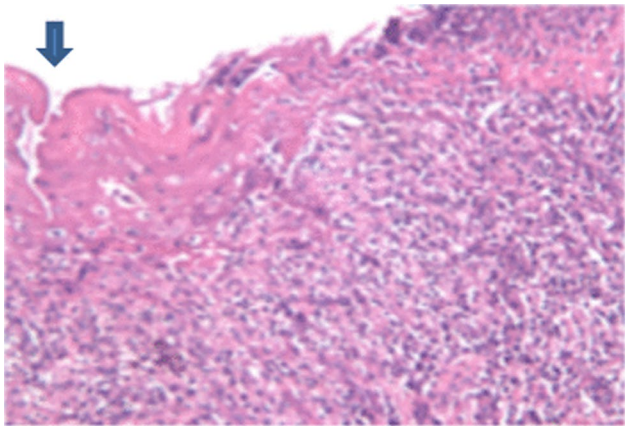


Fig. 5 Photomicrograph of erosive OLP showing epithelial ulceration (blue arrow) and massive band of cellular infiltration, (H&E ×200)

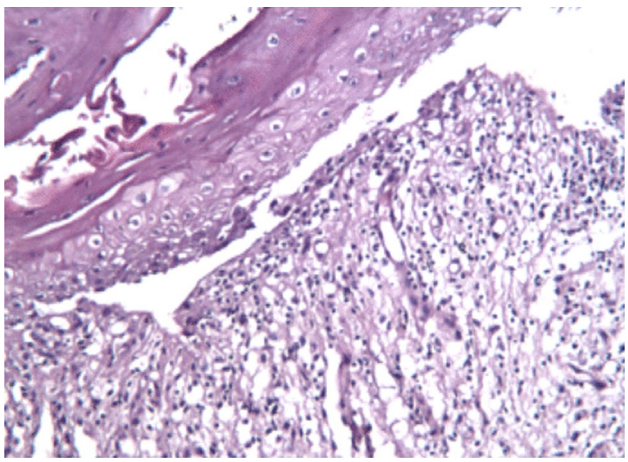


Fig. 6 Photomicrograph of bullous erosive OLP showing the separation of the epithelium. The base of the bulla consists of connective tissue, and the roof is composed of prickle cells, (H&E ×200)

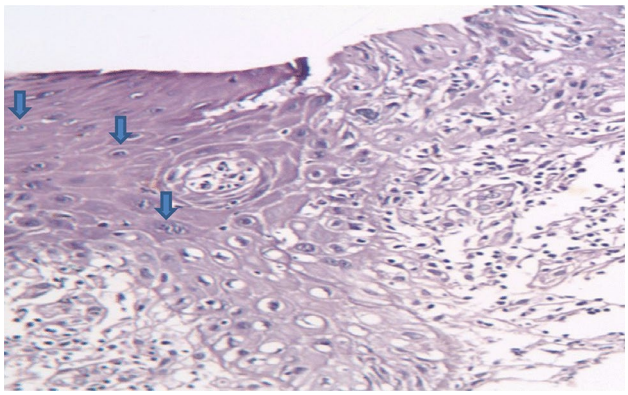


Fig. 7 Photomicrograph of erosive OLP showing high normal and abnormal mitotic figures (arrows), (H&E ×400)

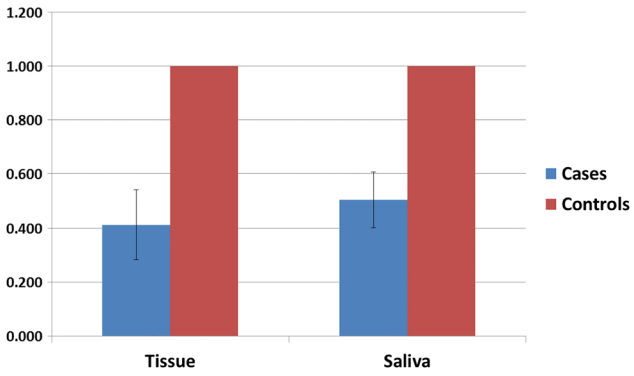


Fig. 8 Histogram showing miRNA 27b gene expression in tissue and saliva between OLP group and control group

Table 2 Comparison of miRNA 27b gene expression in tissue and saliva between OLP group and control group

Gene expression	OLP group		Control group		*P* value
	Mean	SD	Mean	SD	
miRNA 27b in tissue	1.589	1.129	4.674	1.002	0.01*
miRNA 27b in saliva	5.343	4.875	10.592	2.142	0.01*

*Significant at $P \leq 0.05$

aggressive attack of lymphocytes causing basal zone vacuolation of the epithelium. Notice the increase rate of mitosis in the suprabasal cells layers, Figs (5, 6, 7).

Comparison of miRNA 27b gene expression in tissue and saliva between OLP group and control group, Table (2); Fig. (8)

There was a statistically significant difference in miRNA 27b expression in tissue and saliva between both groups with *P* value 0.01. In tissue, OLP group showed lower

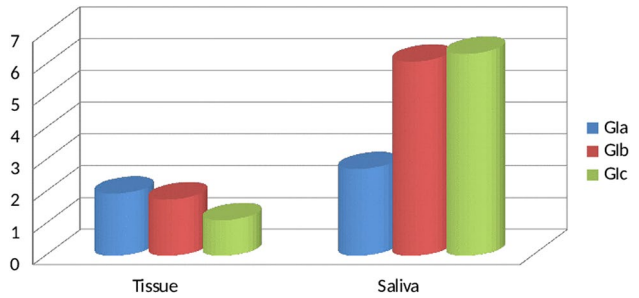


Fig. 9 Histogram showing miRNA 27b gene expression in tissue and saliva between OLP subgroups

expression of miRNA 27b with a mean value of 1.589 (± 1.129) while in the control group it reached 4.674 (± 1.002). The same was noted in saliva where miRNA 27b was lower in OLP group with the mean value of 5.343 (± 4.875) while in the control group it reached 10.592 (± 2.142).

Comparison of miRNA 27b gene expression in tissue and saliva between OLP subgroups, Table (3); Fig. (9)

There was no statistically significant difference in miRNA 27b expression between OLP subgroups in tissue and saliva with *P* value (0.535) and (0.581), respectively. In tissue, the miRNA 27b expression was highest in group (a) with a mean value of 1.953 (± 1.261) followed by group (b) with a mean value of 1.773 (± 1.228) and the lowest mean value was found in group (c) 1.120 (± 0.898). On the other hand, in saliva, miRNA 27b expression was highest in group (c) with a mean value of 6.347 (± 5.783) followed by group (b) with a mean value of 6.102 (± 5.139) and the lowest value was found in group (a) 2.724 (± 2.238).

Comparison of miRNA 27b gene expression in tissue and saliva between OLP subgroups (a) and (b), Table (4)

There was no statistically significant difference in gene expression between OLP subgroups (a) and (b) with *P* value (0.66) in tissue and (0.38) in saliva.

Table 4 Comparison of miRNA 27b gene expression in tissue and saliva between OLP subgroups (a) and (b)

Gene expression	Group (a) & (b) miRNA 27b in tissue	Group (a) & (b) miRNA 27b in saliva
P value	0.66	0.38

*Significant at $P \leq 0.05$

Table 5 Comparison of miRNA 27b gene expression in tissue and saliva between OLP subgroup (a) and (c)

Gene expression	Group (a) & (c) miRNA 27b in tissue	Group (a) & (c) miRNA 27b in saliva
P value	0.37	0.37

*Significant at $P \leq 0.05$

Table 6 Comparison of miRNA 27b gene expression in tissue and saliva between OLP subgroup (b) and (c)

Gene expression	Group (b) & (c) MiRNA27b in tissue	Group (b) & (c) MiRNA27b in saliva
P value	0.35	0.72

*Significant at $P \leq 0.05$

Comparison of miRNA 27b gene expression in tissue and saliva between OLP subgroups (a) and (c), Table (5)

There was no statistically significant difference in gene expression between OLP subgroups (a) and (c) with *P* value (0.37) in tissue and (0.37) in saliva.

Comparison of miRNA 27b gene expression in tissue and saliva between OLP subgroups (b) and (c), Table (6)

There was no statistically significant difference in gene expression between OLP subgroups (b) and (c) with *P* value (0.35) in tissue and (0.72) in saliva.

Table 3 Comparison of miRNA 27b gene expression in tissue and saliva between OLP subgroups

Gene expression	Group (a)		Group (b)		Group (c)		*P* value
	Mean	SD	Mean	SD	Mean	SD	
miRNA 27b in tissue	1.953	1.261	1.773	1.228	1.120	0.898	0.535
miRNA 27b in saliva	2.724	2.238	6.102	5.139	6.347	5.783	0.581

*Significant at $P \leq 0.05$

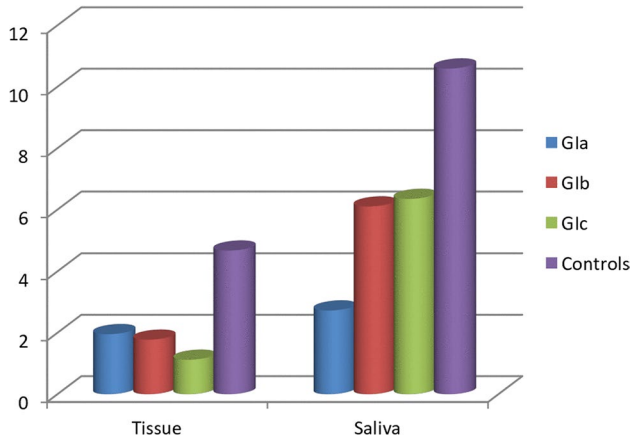


Fig. 10 Histogram showing Comparison of miRNA 27b gene expression in tissue and saliva between OLP subgroups and control

Comparison of miRNA 27b gene expression in tissue and saliva between OLP subgroups and control, Table (7); Fig. (10)

There was a statistically significant difference in miRNA 27b gene expression in tissue and saliva between OLP subgroups and control group except subgroup (c) in saliva.

Table 8 Comparison of miRNA 137 gene expression in tissue and saliva between OLP group and control group

Gene expression	OLP group		Control group		*P* value
	Mean	SD	Mean	SD	
miRNA 137 in tissue	2.027	1.171	6.478	1.534	0.01*
miRNA 137 in saliva	6.738	4.290	15.247	2.252	0.01*

*Significant at $P \leq 0.05$

In tissue, miRNA 27b expression was downexpressed in OLP subgroups (a, b and c) with *P* value (0.007, 0.000 and 0.000), respectively, compared to control group.

Whereas, in saliva, miRNA 27b expression was downexpressed in OLP subgroups (a and b) with *P* value (0.001 and 0.048), respectively, compared to control group, while, there was no statistically significant difference in subgroup (c) compared to control group.

Comparison of miRNA 137 gene expression in tissue and saliva between OLP group and control group, Table (8); Fig. (11)

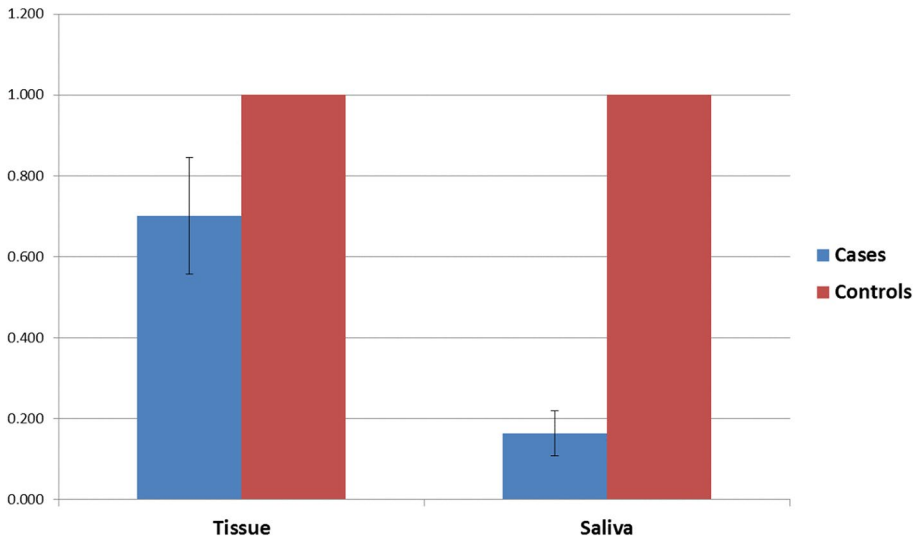
There was a statistically significant difference in miRNA 137 expression in tissue and saliva between both groups with *P* value 0.01. In tissue, OLP group showed lower

Table 7 Comparison of miRNA 27b gene expression in tissue and saliva between OLP subgroups and control

Control	Gene expression					
	Subgroup (a) miRNA 27b in tissue	Subgroup (a) miRNA 27b in saliva	Subgroup (b) miRNA 27b in tissue	Subgroup (b) miRNA 27b in saliva	Subgroup (c) miRNA 27b in tissue	Subgroup (c) miRNA 27b in saliva
P value	0.007*	0.001*	0.000*	0.048*	0.000*	0.107

*Significant at $P \leq 0.05$

Fig. 11 Histogram showing miRNA 137 gene expression in tissue and saliva between OLP group and control group



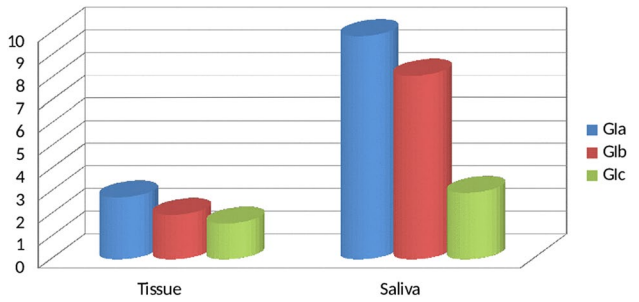


Fig. 12 Histogram showing miRNA 137 gene expression in tissue and saliva between OLP subgroups

expression of miRNA 137 with a mean value of 2.027 (± 1.171) while in the control group it reached 6.478 (± 1.534). The same was noted in saliva where miRNA 137 was lower in OLP group with the mean value of 6.738 (± 4.290) while in the control group it reached 15.247 (± 2.252).

Comparison of miRNA 137 gene expression in tissue and saliva between OLP subgroups, Table (9); Fig. (12)

There was no statistically significant difference of miRNA 137 expression between OLP subgroups in tissue with *P* value (0.168) while there was a statistical significant difference in saliva (0.002).

In tissue, the miRNA 137 expression was highest in group (a) with a mean value of 2.743 (± 0.558) followed by group (b) with a mean value of 1.966 (± 1.333) and the lowest mean value was found in group (c) 1.586 (± 1.186). The same was noted for the three subgroups in saliva where the highest mean value of miRNA 137 expression was noted in group (a) 9.859 (± 4.210) followed by group (b) 8.110 (± 3.903) and the lowest mean value was found in group (c) 2.941 (± 1.112).

Comparison of miRNA 137 gene expression in tissue and saliva between OLP subgroups (a) and (b), Table (10)

There was no statistically significant difference in gene expression between OLP subgroups (a) and (b) with *P* value (0.18) in tissue and (0.24) in saliva.

Table 10 Comparison of miRNA 137 gene expression in tissue and saliva between OLP subgroup (a) and (b)

Gene expression	Group (a) & (b) MiRNA137 in tissue	Group (a) & (b) MiRNA137 in saliva
P value	0.18	0.24

*Significant at $P \leq 0.05$

Table 11 Comparison of miRNA 137 gene expression in tissue and saliva between OLP subgroups (a) and (c)

Gene expression	Group (a) & (c) MiRNA137 in tissue	Group (a) & (c) MiRNA137 in saliva
P value	0.04*	0.00*

*Significant at $P \leq 0.05$

Table 12 Comparison of miRNA 137 gene expression in tissue and saliva between OLP subgroup (b) and (c)

Gene expression	Group (b) & (c) MiRNA137 in tissue	Group (b) & (c) MiRNA137 in saliva
P value	0.95	0.00*

*Significant at $P \leq 0.05$

Comparison of miRNA 137 gene expression in tissue and saliva between OLP subgroups (a) and (c), Table (11)

There was a statistically significant difference in gene expression between OLP subgroups (a) and (c) with *P* value (0.04) in tissue and (0.00) in saliva.

Comparison of miRNA 137 gene expression in tissue and saliva between OLP subgroups (b) and (c), Table (12)

There was no statistically significant difference in gene expression in tissue between OLP subgroups (b) and (c) with *P* value (0.95), while there was a statistically significant difference in gene expression in saliva with *P* value (0.00).

Table 9 Comparison of miRNA 137 gene expression between OLP subgroups in tissue and saliva

Gene expression	Group (a)		Group (b)		Group (c)		*P* value
	Mean	SD	Mean	SD	Mean	SD	
MiRNA137 in tissue	2.743	0.558	1.966	1.333	1.586	1.186	0.168
MiRNA137 in saliva	9.859	4.210	8.110	3.903	2.941	1.112	0.002*

*Significant at $P \leq 0.05$

Comparison of miRNA 137 gene expression in tissue and saliva between OLP subgroups and control, Table (13); Fig. (13)

There was a statistically significant difference in miRNA 137 gene expression in tissue and saliva between OLP subgroups and control group.

In tissue, miRNA 137 expression was in OLP subgroups (a, b and c) with *P* value (0.000) compared to control group.

Whereas, in saliva, miRNA 137 expression was in OLP subgroups (a, b and c) with *P* value (0.051, 0.001 and 0.000), respectively, compared to control group.

Receiver operating characteristic (ROC) curve analysis, Table (14)

The current data showed a cutoff value of 4.4 and 8.04 for gene expression of miRNA 27b in tissue and saliva, respectively. Therefore, cases below these cutoff values are diagnosed as having OLP, whereas cases above these values are diagnosed as negative (healthy) cases.

Another ROC curve was plotted to evaluate the diagnostic value of miRNA 137 in patients with OLP as biomarker of potential malignant transformation, and that showed a cutoff value of 4.6 and 13.7 in tissue and saliva, respectively. This means that cases below these cutoff values are diagnosed as having OLP with high malignant tendency, while cases above these values are diagnosed as healthy cases.

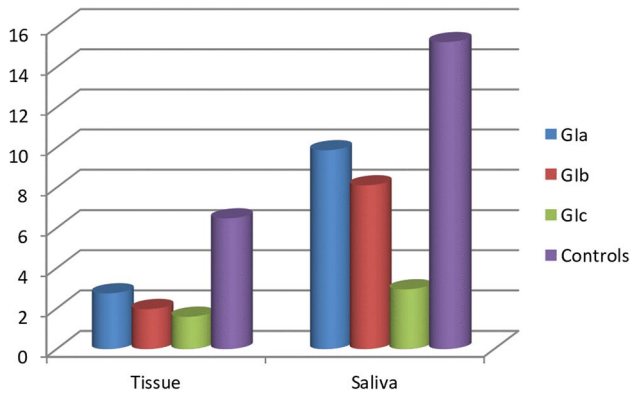


Fig. 13 Histogram showing miRNA 137 gene expression in tissue and saliva between OLP subgroups and control

Table 14 Results of the ROC curve analysis of T. miRNA 27b, S. miRNA 27b, T. miRNA 137 and S.miRNA 137

miRNA	Cutoff point	AUC	Sensitivity (%)	Specificity (%)
T.miRNA 27b	4.4	0.98	1.00	65
S.miRNA 27b	8.04	0.78	0.75	1.00
T.miRNA 137	4.6	1.00	1.00	1.00
S.miRNA 137	13.7	0.97	1.00	80

Furthermore, to assess the diagnostic accuracy of miRNA 27b and miRNA 137 as biomarkers for detecting OLP in tissue and saliva, the ROC curve analysis revealed an area under the curve (AUC) of 0.98, 0.78, 1.00 and 0.97 for Tissue miRNA 27b, Salivary miRNA 27b, Tissue miRNA 137 and Salivary miRNA 137, respectively.

Tissue miRNA 27b was the excellent test for diagnosis of OLP with AUC (0.98), 100% sensitivity and 65% specificity at cutoff value 4.4, whereas salivary miRNA 27b was the fair test with AUC (0.78), 0.75% sensitivity and 100% specificity at cut off value 8.04.

In addition, tissue miRNA 137 was found to be the perfect test for prediction of the potential malignant transformation of OLP with AUC (1.00), 100% sensitivity and 100% specificity at cutoff value 4.6, while salivary miRNA 137 was found to be the excellent test with AUC (0.97), 100% sensitivity and 80% specificity at cut off value 13.7.

Discussion

Currently, miRNAs biomarkers are being studied for their role in pathogenesis and prediction of the onset, severity and malignant tendency of OLP [17]. In the present study, we aimed to explore the potential of altered expression of miRNA 27b and miRNA 137 in tissues and saliva of OLP patients compared to controls.

The finding of the current study, using microarray analysis RT-qPCR method revealed that miRNA 27b and miRNA 137 were significantly down-regulated in tissues and saliva

Table 13 Comparison of miRNA 137 gene expression in tissue and saliva between OLP subgroups and control

Control	Gene expression					
	Subgroup (a) miRNA 137 in tissue	Subgroup (a) miRNA 137 in saliva	Subgroup (b) miRNA 137 in tissue	Subgroup (b) miRNA 137 in saliva	Subgroup (c) miRNA 137 in tissue	Subgroup (c) miRNA 137 in saliva
P value	0.000*	0.051*	0.000*	0.001*	0.000*	0.000*

*Significant at $P \leq 0.05$

of OLP patients compared to healthy control suggesting their role in pathogenesis and prognosis of OLP.

In the present study, among OLP subgroups, miRNA 27b showed different levels of expression. This is in agreement with Zhang et al.,[27] who reported that miRNA 27b in tissue sample of OLP patients were downregulated and were differently expressed in various clinical types of OLP.

Hildebrand et al. [13] found nine miRNAs including miRNA 27b that are associated with human keratinocyte differentiation in vitro and in vivo. Furthermore, previous studies showed that miRNA 27b regulated the expression of matrix metalloproteinase 13 and the transforming growth factor beta (TGF-beta)/bone morphogenetic protein signaling, immune response and chronic inflammation [2, 6, 11, 14, 22].

Wang et al. [26] demonstrated that miRNA 27b improved keratinocyte migration and that miRNA 27b inhibition increased keratinocyte apoptosis and mitochondrial reactive oxygen species. This may explain the downexpression of miRNA 27b in the different OLP subgroups which indicates its association with disease activity.

In the current study, we aimed to evaluate miRNA 137 in tissue and saliva to predict the risk of malignant transformation in OLP. It was selected because it serves as tumor suppressor gene as it is downexpressed in OSCC. It serves as a biomarker for early detection of cancer.

Dang et al. [8] found increased miRNA 137 methylation (down expression) in tissue biopsy of OLP patients but less than OSCC compared to healthy control. This positively correlates with our results, which found that miRNA 137 showed a statistically significant downexpression in tissue and saliva of OLP group compared to healthy control.

Prior evidence suggests that promoter methylation correlates with downexpression of miRNA 137 in OSCC relative to noncancerous oral tissue. MiRNA137 appears to play a role in cellular differentiation and cell cycle control, at least in part through negative regulation of Cdk6 expression [15, 16]. It is hypothesized that overexpression of Cdk6 may result in accelerated progression through the G1/S-phase checkpoint of the cell cycle, thus leading to increased proliferation and reduction in DNA repair capacity [23].

Moreover, Smith et al. [24] found that miRNA 137 act as a tumor suppressive miRNA in colorectal\cancer by negatively regulate oncogenic Musashi-1(MSI1) a stem cell regulator and it is downexpressed in colorectal cancer due to hyper-methylation of the promoter region. Also he showed that the induction of miRNA 137 significantly inhibited colon cancer xenograft tumor growth in mice.

Erosive type in the current study showed statistically significant down expression of salivary miRNA 137 in compared to other subtypes of OLP, that denotes the erosive type has the highest potential premalignant transformation which this result coordinate with recent a meta-analysis that showed the erosive types has risk for malignant transformation, Aghbari et al. [1].

Up to our knowledge, no previous studies evaluated miRNA 137 expression in saliva of patients suffering from OLP in its different clinical types. Langevin et al. [16] compared methylation in tumor tissue and mouthwash in patients suffering from SCCHN and who found that mouthwash has a high specificity (81.1%) than the tissue sample. This is in accordance with the results of the present study specially the erosive type of OLP predicting malignant transformation potential of the disease.

Overall, our finding shows a statistically significant down expression in miRNA 27b gene expression in tissue and saliva of OLP groups compared to control group, denoting the role of miRNA 27b in the pathogenesis of OLP. Furthermore, it showed the lowest level of miRNA 27b in erosive type followed by atrophic type and then papular, reticular and plaque types, denoting decrease in the activity of keratinocyte in atrophic and erosive types of OLP. Also, there is a statistically significant downregulation in miRNA 137 gene expression in tissue and saliva of OLP group compared to control group. Erosive type of OLP showed the more downexpression of miRNA 137 in tissue and saliva, denoting the tendency to malignant transformation and may serve as a biomarker for early malignant predilection of the disease.

In a recent systematic review, Guerra et al. [12] concluded that only few salivary biomarkers used to early diagnose head and neck cancer were diagnostically accurate. An ideal diagnostic biomarker should have high disease sensitivity and specificity, mandatory presence in all affected patients, and provide a cutoff value with minimal overlap between normal and disease states. ROC curve analysis of salivary miRNA 27b and miRNA 137 revealed a fair diagnostic and an excellent diagnostic performance, AUC (0.78 and 0.97) with (0.75%, 100%) sensitivity and (100%, 80%) specificity, respectively. These results favor the use of saliva sample especially miRNA 137 with excellent diagnostic accuracy as a noninvasive method for monitoring malignant transformation and disease activity of OLP.

Recommendation

Strict followup should be performed in the patients suffering from erosive OLP, as they have a high tendency of malignant transformation. Further studies should be performed to use miRNA (27b and 137) as a gene therapy in tissue regeneration and decrease potential for malignant transformation in erosive type of OLP.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article was approved by the Faculty of Oral and Dental Medicine Research Ethics committee, Cairo University in September 2013.

Informed consent Following an explanation of the study as well as information about the sampling procedures, each subject signed a written informed consent form approved by the Faculty Research Ethics committee.

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