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Research article

Dynamic changes of Th1/Th2/Th17 cytokines and hBD-2/3 in erosive oral lichen planus patients saliva before and after prednisone acetate treatment

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ABSTRACT

Objective: This study aimed to investigate the expression of T helper 1 (Th1)/Th2/Th17- related cytokines and human beta defensins 2 and 3 (hBD-2 and -3) in the saliva of patients with erosive oral lichen planus (EOLP) and to explore their role in the pathogenesis of EOLP and the effects of glucocorticoids on EOLP.

Methods: A total of 30 patients with EOLP and 20 age- and sex-matched healthy individuals were included in this study. The patients were treated with prednisone at a dose of 0.4 mg/(kg·d) for 1 week and examined before and after treatment. Unstimulated whole saliva samples were collected to determine the levels of cytokines (interleukin 1 beta [IL-1β], tumour necrosis factor alpha [TNF]-α, interferon gamma [IFN-γ], IL-4, IL-6, IL-10 and IL-17) by cytometric bead array and those of hBD-2 and -3 b y enzyme-linked immunosorbent assay. In addition, oral rinse samples were collected to detect *Candida* load.

Results: The levels of salivary IL-1β, IL-6, hBD-2 and hBD-3 were higher and the IFN-γ/IL-4 and IL-1β/IL-6 ratios were lower in patients with EOLP than in healthy individuals. In patients with EOLP, hBD-2 levels were positively correlated with IFN-γ levels and negatively correlated with IL-17 levels, whereas hBD-3 levels were negatively correlated with IL-17 and IL-10 levels. In addition, the prevalence of EOLP was positively correlated with IL-6 levels and negatively correlated with the IFN-γ/IL-4 ratio. The levels of IL-1β, TNF-α, IFN-γ, IL-6, hBD-2 and hBD-3 and the IFN-γ/IL-4 ratio decreased after treatment with prednisone for 1 week. The levels of IL-6, hBD-2 and hBD-3 were significantly higher in EOLP patients than in healthy individuals; while TNF-α levels and the IFN-γ/IL-4 ratio were significantly lower in EOLP patients than in healthy individuals. Furthermore, the oral counts of *Candida* spp. (colony forming unit [CFU]) were negatively correlated with TNF-α levels. Numerical Rating Scale(NRS) and Sign scores decreased in EOLP patients after treatment. Approximately 80 % of patients were effectively treated. Salivary TNF-α levels were significantly higher in the treatment-ineffective group than in the treatment-effective group before treatment with prednisone, and differences in salivary IL-6 levels

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before and after treatment were significantly higher in the treatment-effective group than in the treatment-ineffective group.
Conclusions: High expression of IL-1β, IL-6, hBD-2 and Th1/Th2 imbalance in saliva may be associated with the pathogenesis of EOLP. IFN-γ/IL-4 balance may serve as a protective factor for EOLP. Glucocorticoids significantly alleviate the symptoms of EOLP and inhibit the expression of Th1/Th2 cytokines.

1. Introduction

Oral lichen planus (OLP) is a chronic inflammatory disease involving the skin, nails and oral mucosa [1]. It commonly affects any site of the oral mucosa, buccal mucosa, tongue and gingiva and usually has a bilateral, symmetrical distribution [2,3]. The reported prevalence rates of OLP vary from 0.5 to 2.2 %, with its incidence being more common among middle-aged women [3]. OLP manifests as white keratotic lesions that are either erosive or non-erosive. Erosive lesions may cause significant pain and indicate a more severe stage of disease development. The World Health Organization (WHO) has classified OLP as an oral potentially malignant [4].

The aetiology and pathogenesis of OLP remain inconclusive. Immunity, genetics, psychophysiology, infection, endocrine and other factors have been associated with OLP [5]. However, the majority of studies have suggested that OLP is an immune-related disease mediated by T lymphocytes. The immunopathological mechanism underlying the development of OLP is mainly related to the activation of $CD4^+$ helper T (Th) lymphocytes [6]. Th1/Th2 imbalance is considered a major cause of OLP [7]; but the results are controversial [7,8]. It was reported that OLP is represented by Th1 cytokine shift [9,10]; meanwhile other research showed a dominance of Th2 response in OLP patients [10,11]. In addition, some researchers observed that the IFN-γ/IL-4 ratio in saliva and tissues of OLP patients showed no statistically significant difference from that of the control group. In recent years, numerous studies have reported that Th17 cells inducing IL-17 play an essential role in the development of OLP [12]. Th17 cells were present in OLP lesions, and there was an increased infltration of Th17 cells in EOLP tissues in comparison with non-erosive OLP , with the Th17 proportion in OLP patients (both types) higher than in the control group [13]. Besides, Th17 may play the role of promoting inflammation in EOLP [14]. In addition, Human beta-defensins (hBDs, −1, 2, 3) are a family of epithelial cell derived antimicrobial peptides (AMPs) that protect mucosal membranes from microbial challenges [15]. HBD-2, a major antimicrobial peptide produced by many types of epithelial cells, is vigorously induced by lichen planus-related inflammation [16] , suggesting the involvement of hBD-2 in the pathogenesis of OLP [17]. Similar to hBD-2, the signal of hBD-3 was strongly detected in the spinous and suprabasal layers, stronger than those of either normal oral epithelium [18]. HBD-2 and -3 are involved in the development of OLP [16], but there are few studies on the changes of hBDs content in saliva of OLP patients and its correlation with proinflammatory cytokines.

Glucocorticoids have strong anti-inflammatory and immunosuppressive effects and can be used for the first-line treatment of OLP with more evident inflammatory reactions such as congestion or erosion [19]. However, they often produce some side effects, such as increased susceptibility to fungal infections in humans. *Candida albicans* infection or overgrowth may lead to aggravation or deterioration of OLP [20], whereas a preventive antifungal is beneficial for EOLP treatment [21].

In this study, we determined the expression of Th1/Th2/Th17-related cytokines and hBD-2 and -3 in the saliva of patients with EOLP and examined the correlation between EOLP development and oral *Candida* load in patients with EOLP. In addition, we investigated whether low doses and short courses of glucocorticoids for the systemic treatment of EOLP caused changes in the oral *Candida* load.

2. Subjects and methods

2.1. Study population

In this prospective study, a total of 30 patients with EOLP were recruited from the Department of Mucosa of the Affiliated Stomatology Hospital of Guangxi Medical University from December 2014 to August 2015. The diagnosis of EOLP was confirmed based on medical history, clinical presentation and pathological examination, and the patients were undergoing prednisolone acetate treatment. A total of 20 age- and sex-matched healthy volunteers were included in the control group. All participants signed an informed consent form, and the study was approved by the Research Ethics Committee of Guangxi (2023038). The exclusion criteria were as follows: patients with other oral mucosal diseases, severe periodontal diseases, serious systemic diseases (such as diabetes, hepatitis and uncontrolled hypertension), autoimmune diseases and tumours; patients who used antibiotics locally in the mouth or systemically in the past 1 month; patients who used immunosuppressants, immunomodulatory drugs or herbal medications in the past 3 months; pregnant and lactating women and patients who were not eligible for hormone therapy.

2.2. Clinical data

Demographic data (age, sex and ethnicity) were obtained through history taking. Oral mucosal lesions were diagnosed according to the WHO diagnostic criteria for lichen planus [22] and confirmed based on pathological examination: White plaques (reticular, annular, or plaque pattern) have a symmetricl distribution with radiating Wickham's striae and erosions; and the histopathologic features are characteristic with atrophic epithelium, sawtooth rete processes, bandlike lymphocytic infiltrate in the superficial part of

the lamina propria and close to the epithelium. The 30 patients diagnosed with moderate-to-severe EOLP were prescribed oral prednisone acetate at a dose of 0.4 mg/(kg·d) for 1 week. The three principle parameters evaluated by two clinicians with good inter-examiner consistency were pain, specific congested area and surface area of atrophic and erosive lesions. The surface area of lesions was measured using a sterile flexible periodontal scale probe and expressed in squared millimetres. The Numerical Rating Scale (NRS) is an 11-point scale consisting of integers from 0 through 10; 0 representing "No pain'' and 10 representing "Worst imaginable pain.'' Respondents select the single number that best represents their pain intensity [23]. The specific scoring criteria are shown in Table 1.

2.3. Sample collection

The passive drool method was used to collect unstimulated whole saliva samples from all patients at each follow-up visit [24]. Saliva collection took place between 8 and 10 a.m. and volunteers were asked to refrain from eating, drinking and smoking, 2 h prior to sample collection. Saliva was collected using the spitting method with the subjects in a sitting position and after a 5 min adaptation period, with the head slightly inclined downwards and minimizing facial and labial movements. The saliva accumulated at the base of the oral cavity was spat into a 50 mL sterile centrifuge tube with a volume of 5 mL, within no more than 10 min. The samples were centrifuged (3500 rpm) at 4 °C for 20 min to determine the levels of cytokines (interleukin 1 beta [IL-1β], tumour necrosis factor alpha [TNF]-α, interferon gamma [IFN-γ], IL-4, IL-6, IL-10 and IL-17) by cytometric bead array and those of hBD-2 and -3 b y enzyme-linked immunosorbent assay.

In addition, 5 mL of oral rinse samples were collected and centrifuged at 8000 rpm for 5min. The pellets were used for the culture of *Candida* spp.

2.4. Detection of candida load

The pellets of oral rinse samples were re-suspended in 500 μL of phosphate-buttered saline (PBS; pH, 7.2–7.4). Thereafter, 50 μL of the precipitated sample was inoculated on Sabouraud medium (Beijing Luqiao Technology Co., Ltd., China) for the culture of *Candida*. Each sample was cultured in duplicate. The average colony forming units (CFUs) of *Candida* were counted 48 h after incubation at 37 °C.

Detection of Th1-, Th2- and Th17-related cytokines in saliva via flow-through microsphere technology.

A cytometric bead array (CBA) kit was used to detect the expression of Th1-, Th2- and Th17-related cytokines in saliva according to the manufacturer's instructions. The FCAP Array (version 3.0) software was used to plot the standard curve and analyse the data.

2.4.1. Detection of salivary HBD-2, hBD-3 and cytokines

The expression of salivary HBD-2, HBD-3 and cytokines was determined using ELISA kits according to the manufacturer's instructions (Cusabio Biotech, Wuhan, China). Optical density was measured on a MultiSkan FC microplate photometer (Thermo Fisher Scientific Corporation, Finland). All standards (Cusabio Biotech, Wuhan, China) and samples were tested in duplicate.

2.5. Statistical analyses

The Graphpad prism SPSS Statistics (version 19.0) software was used for statistical analysis. Data were expressed as the mean ± SD or the median and interquartile range depending on the type of data. The two-sample *t*-test for independent samples and chi-square test were used to estimate differences in age and sex between patients with EOLP and healthy individuals, respectively. The Mann–Whitney *U* test was used to compare the expression of T cell-related cytokines and beta defensins and oral Candida load between patients with EOLP and healthy individuals. The Wilcoxon rank-sum test was used to compare the expression of T cell-related cytokines and beta defensins, oral Candida load and clinical sign and symptom scores in patients with EOLP before and after treatment. The correlation between each clinical index (clinical sign and symptom scores and oral Candida load) and immune index (expression of T cell-related cytokines, hBD-2 and hBD-3 in saliva) was examined via Spearman rank correlation analysis and logistic regression analysis. *P*-values of <0.05 were considered significant.

Table 1
NRS pain score scale and criteria of NRS score.

3. Results

3.1. Clinical data of patients

Of the 30 patients with erosive OLP, 7 were men and 23 were women aged 22–69 years, with an average age of 47.8 ± 12.3 years. Of the 20 healthy individuals, 5 were men and 15 were women aged 24–56 years, with an average age of 42.2 ± 9.5 years. Lesions were found at the following sites: buccal cavity (90.00 %), gingiva (36.67 %), tongue (26.67 %) and lips (6.67 %). The buccal cavity appeared to be the most common site (Fig. 1). For example, EOLP patient appeared ulceration, erythema, and keratotic white striae on the tongue back, tongue margin and cheek mucosa; (Fig. 2(A-D)) and the lesion of EOLP patient improved significantly or healed after treatment with Prednisone acetate. (Fig. 3(A-D))

4. Levels of salivary Th1, Th2, and Th17 related cytokines, HBD-2 and HBD-3 in patients with EOLP

4.1. Before and after treatment with prednisone acetate

The levels of salivary IL-1β, IL-6, hBD-2 and hBD-3 were significantly higher in patients with EOLP than in healthy individuals ($P < 0.05$). The levels of salivary TNF-α, IFN-γ, IL -4 and IL-10 were higher in patients with EOLP than in healthy individuals; however, the difference was not significant ($P > 0.05$) (Table 2). The salivary IFN-γ/IL-4 and IL-1/IL-6 ratios were significantly less in EOLP patients than in healthy individuals ($P < 0.05$). (Fig. 4A and B).

After oral prednisone treatment for 1 week, the levels of salivary IL-1β, IFN-γ, TNF-α, IL-6, hBD-2 and hBD-3 significantly decreased in patients with EOLP ($P < 0.05$). However, the levels of salivary IL-6, hBD-2 and hBD-3 were higher and those of TNF-α were lower in EOLP patients than in healthy individuals ($P < 0.05$) (Table 2). The IFN-γ/IL-4 ratio decreased in patients treated with prednisone acetate and was lower than that in healthy individuals ($P < 0.05$) (Fig. 4A). Although the IL-1/IL-6 ratio increased in patients treated with prínisone acetate ($P > 0.05$), it was lower than that in healthy individuals ($P < 0.05$). (Fig. 4B).

5. Numerical rating scale (NRS) and clinical sign scores of patients with EOLP

Significant differences were observed in NRS scores before and after treatment with prednisone acetate. The NRS scores of patients with EOLP before treatment were 4.10 ± 1.79, whereas those after treatment were 1.93 ± 1.72 ($Z = -4.046$, $P = 0.001$) (Fig. 5A). The Sign scores of patients with EOLP before treatment were 4.53 ± 0.51, whereas those after treatment were 2.77 ± 1.36 ($Z = -4.356$, $P = 0.001$) (Fig. 5B).

6. Effects of prednisone treatment on patients with ELOP

Prednisone acetate treatment was effective in 80 % patients with EOLP. Besides, salivary TNF-α levels were higher in the treatment-ineffective group than in the treatment-effective group (6.19 [13.74] versus 32.04 [46.09]; $P < 0.05$). Differences in salivary IL-6 levels before and after treatment were significantly higher in the treatment-effective group than in the treatment-ineffective group ($P < 0.05$).

7. Oral *candida* load

Before prednisone treatment, oral *Candida* load was significantly higher in patients with EOLP than in healthy individuals ($Z = -2.840$, $P = 0.005$). Although oral *Candida* load increased in patients with EOLP after treatment with prednisone acetate for 1 week, it was not significantly different between patients and healthy individuals ($Z = -1.867$, $P = 0.062$). (Table 3).

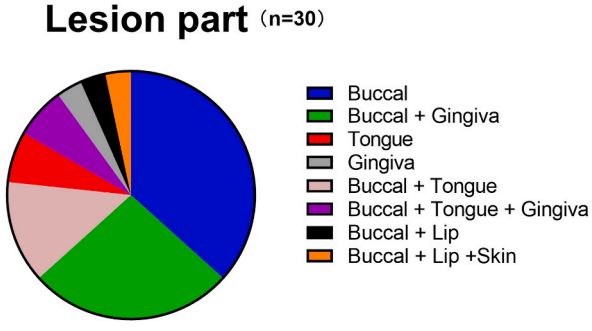

Fig. 1. Distribution of lesion parts in the EOLP patient group.
Fig. 1 legend: Distribution of lesion sites in the EOLP patient group showed in Fig. 1, different colors represent different lesion parts.

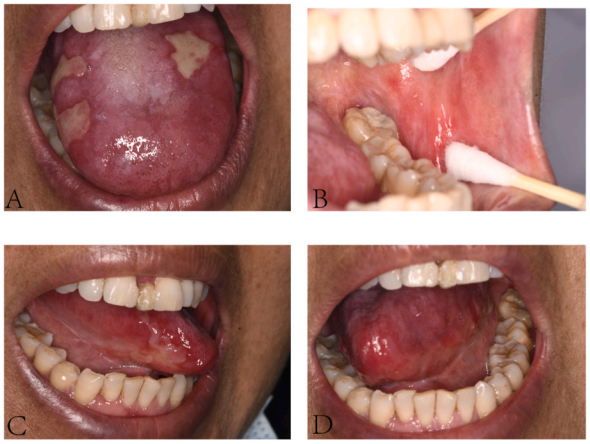

Fig. 2. The clinical manifestations of EOLP patients before treatment with Prednisone acetate.
Fig. 2 legend: Fig. 2A–D showed that EOLP patient appeared ulceration, erythema, and keratotic white striae on the tongue back, tongue margin and cheek mucosa before treatment with Prednisone acetate.

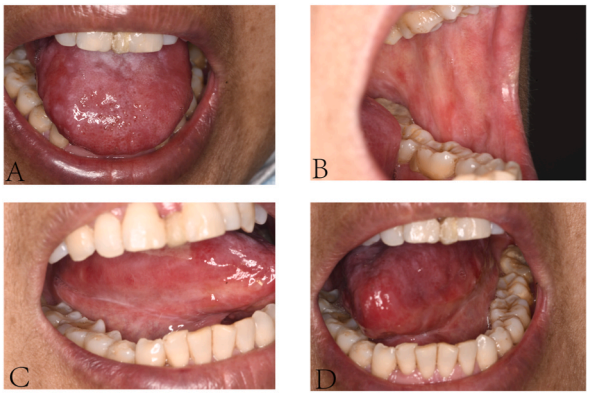

Fig. 3. The clinical manifestations of EOLP patients after treatment with Prednisone acetate.
Fig. 3 legend: Fig. 3A–D showed that the lesion of EOLP patient improved significantly or healed after treatment with Prednisone acetate, the of area of ulceration, erythema, and keratotic white striae on the tongue back, tongue margin and cheek mucosa reduced.

Table 2
The difference of T cell-related cytokines and hBDs levels between EOLP patients and Healthy controls.

	Pre-treatment vs. HC		Pre-treatment vs. post-treatment		Post-treatment vs. HC	
	Z Value	*P* value#	Z value	*P Value**	Z value	*P value#*
IL-1β	−2.654	0.008	−3.075	0.002	−0.495	0.621
TNF-α	−1.584	0.113	−4.350	0.001	−2.120	0.034
IFN-γ	−0.761	0.446	−2.524	0.012	−0.532	0.596
IL-4	−1.905	0.057	−0.905	0.365	−1.807	0.071
IL-6	−4.911	0.001	−3.425	0.001	−2.733	0.006
IL-10	−1.720	0.085	−0.043	0.966	−1.870	0.062
IL-17	−0.323	0.747	−1.507	0.132	−0.094	0.925
hBD-2	−3.713	0.001	−2.026	0.043	−2.812	0.005
hBD-3	−3.891	0.001	−2.540	0.011	−3.198	0.001

8. Correlation between the levels of Th1-, Th2- and Th17-related cytokines and β-defensins in saliva and the scores of clinical symptoms and signs of patients with EOLP

NRS scores were positively correlated with IL-1β levels before treatment with prednisone acetate ($r = 0.371$, $P = 0.043$). Clinical sign scores were positively correlated with IL-6 levels before ($r = 0.533$, $P = 0.002$) and after ($r = 0.508$, $P = 0.004$) treatment. In addition, NRS scores and TNF-α levels decreased after treatment ($r = 0.412$, $P = 0.024$).

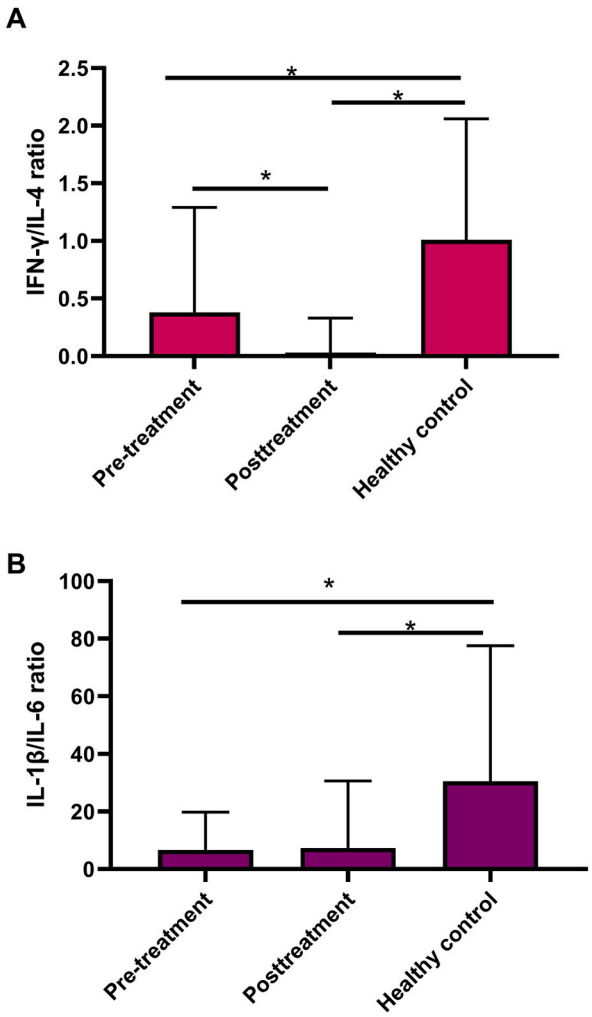

Fig. 4. Th1/Th2 cytokine ratio in saliva between EOLP patients and controls.
Fig. 4 Legned: Fig. 4A showed the change of IFN-γ/IL-4 ratio in patients treated with prednisone acetate; Fig. 4B showed the change of IL-1/IL-6 ratio in patients treated with prínisone acetate; *, Compared with and pre-treatment $p < 0.05$, using Wilcoxon paired symbol rank sum test; #, compared with healthy control group $p < 0.05$, using Mann-Whitney *U* test.

No significant correlation was observed between oral Candida load (CFU) and various immune indices in patients with EOLP before treatment; however, CFU was negatively correlated with TNF-α levels ($r = -0.489$, $P = 0.006$) after 1 week of treatment with prednisone acetate. CFU was negatively correlated with the IFN-γ/IL-4 ratio in healthy individuals ($r = -0.469$, $P = 0.037$). Before treatment, hBD-2 expression was positively correlated with IFN-γ levels ($r = 0.491$, $P = 0.006$) and negatively correlated with IL-17 levels ($r = -0.459$, $P = 0.011$) (Table 4); and hBD-3 expression was negatively correlated with IL-17 ($r = -0.453$, $P = 0.012$) and IL-10 ($r = -0.396$, $P = 0.045$) levels (Table 5).

9. Logistic regression analysis of salivary T cell-related cytokines of patients with ELOP

To examine the salivary immune features of patients with EOLP, the abovementioned factors related to the development of EOLP were included in the logistic regression model (patients with EOLP = 1, healthy individuals = 0), and the retrograde LR method was used for analysis.

The severity of EOLP was positively correlated with salivary IL-6 levels and negatively correlated with the IFN-γ/IL-4 ratio, indicating that IL-6 may be a risk factor and the IFN-γ/IL-4 ratio may be a protective factor for the pathogenesis of EOLP (Table 6).

10. Discussion

OLP is a chronic inflammatory disorder characterised by T-cell-mediated immune responses against epithelial cells. Although the pathogenesis of OLP remains unclear, several pathogenic mechanisms have been proposed. In particular, the balance between Th1 and

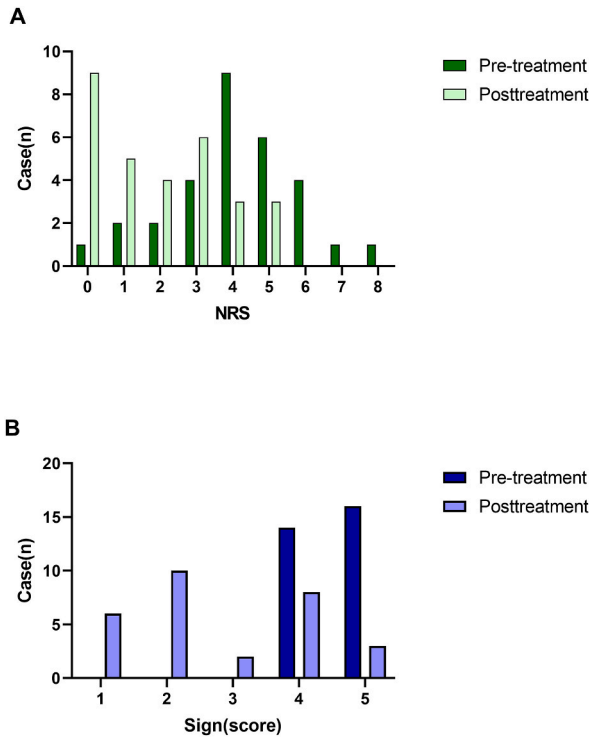

Fig. 5. NRS and Signs scores of patients with ELOP.
Fig. 5 legend: Fig. 5A showed NRS scores distribution of EOLP patients before and after treatment; Fig. 5B showed Sign scores distribution of EOLP patients before and after treatment.

Table 3
The oral CFU of ELOP patients and healthy control (median (IQR))

Group	The number of positive OC	The rate of positive OC (%)	The load of OC (CFU/ml)
Pre-treatment (n = 30)	16	53.3	362 (580)
Post-treatment (n = 30)	20	66.7	443 (625)
Healthy control (n = 20)	4	20.0	19 (80)

Table 4
Correlation analysis of hBD-2 expression level with IFN-γ and IL-17 expression level in saliva of EOLP patients before treatment.

	IFN-γ expression level	IL-17 expression level
r-value	0.491	0.006
p-value	−0.459	0.011

Table 5
Correlation analysis of hBD-3 expression level with IL-17 and IL-10 expression level in saliva of EOLP patients before treatment.

	IL-17 expression level	IL-10 expression level
r-value	−0.453	0.012
p-value	−0.396	0.045

Th2 cells and their cytokines plays an important role in the pathogenesis of OLP. In order to explore the roles of T helper 1 (Th1)/Th2/Th17- related cytokines and human beta defensins 2 and 3 (hBD-2 and -3) in the pathogenesis of EOLP and the effects of glucocorticoids on EOLP, patients with EOLP were included in this study to treated with prednisone and examined before and after treatment, and the levels of IL-1β, TNF-α, IFN-γ, IL-6, hBD-2 and hBD-3 decreased after treatment with prednisone for 1 week. This study also

Table 6
Logistic regression analysis of EOLP and salivary T cell-related inflammatory cytokine.

Y	X	B	SE	Wals	Sig.	Exp (B)	95%CI
EOLP	IL-6	1.336	0.656	4.153	0.042	3.803	1.052–13.744
	IFN-γ/IL-4	−14.382	7.250	3.935	0.047	0.001	0.001–0.842

demonstrated that the salivary IFN-γ/IL-4 ratio was significantly lower in patients with EOLP than in healthy controls. This result is consistent with the results of other studies. [8,25], indicating that Th1/Th2 imbalance exists in the local immune response to EOLP. However, the role of the salivary IFN-γ/IL-4 ratio in OLP remains controversial. Some studies have reported that the salivary IFN-γ/IL-4 ratio is significantly higher in patients with EOLP than in healthy individuals [26,27].

In this study, the levels of salivary IL-1β and IL-6 were significantly higher in patients with EOLP than in healthy individuals, which is consistent with the result of a study by Rhodus et al. [28]. IL-1β rapidly activates various cells such as T cells, neutrophils and macrophages and stimulates its own expression and that of TNF-α, IL-6 and IL-8, which activate cells by binding to the corresponding receptors. The internal signalling pathway that triggers the cascade reaction by cytokines may be the primary cause of chronic OLP lesions [29]. Previous studies have revealed that IL-1β is expressed in the lesion tissues of patients with OLP, and IL-1 levels are significantly high in non-irritating total saliva, isotonic saline-containing sputum and lesion tissue exudates of patients [7,29,30]. These results indicate that IL-1β plays a role in the pathogenesis of EOLP. During chronic inflammation, elevated IL-6 levels can induce the production of IL-1, TNF-α and other proinflammatory factors to maintain the local inflammatory response to OLP lesions. Yamamoto [31] found that keratinocytes in OLP lesions produced more IL-6 than normal tissues and keratinocytes in inflammatory gingiva in vitro. Upregulation of salivary IL-6 in patients with EOLP may be attributed to the production of larger amounts of IL-6 by monocytes, T cells, macrophages and keratinocytes in the local tissue. IL-6 concentration in saliva is considered an effective indicator for monitoring the progression and treatment of OLP and reflecting the malignant potential of OLP to some extent [27,28,32].

HBDs have chemotactic and immunostimulatory effects and broad-spectrum antibacterial activity, which may be involved in the development of various oral mucosal diseases. In this study, the levels of salivary hBD-2 and hBD-3 were low in healthy individuals but significantly high in patients with EOLP. Furthermore, hBD-2 levels were positively correlated with IFN-γ levels and negatively correlated with IL-17 levels, whereas hBD-3 levels were negatively correlated with IL-17 and IL-10 levels. hBD-2 is highly induced in OLP lesions and may serve as an index for assessing active inflammation. In addition, it may be associated with the presence of typical band-like $CD8^+$ infiltrates in OLP [17,33]. Kanda et al. [34] reported that serum hBD-2 levels were elevated in patients with psoriasis and were positively correlated with IFN-γ and IL-10 levels but negatively correlated with IL-17 levels in vitro. IL-1β, IFN-γ and IL-17 can upregulate the expression of hBD-2, whereas IL-4 and IL-10 can inhibit hBD-2 expression in keratinocytes. hBD-2 can promote the secretion of IL-1β, IFN-γ, IL-10 and other cytokines and inhibit the secretion of IL-17. Although the functions of hBD-2 and hBD-3 may be different, they synergistically work with cytokines to influence the progression of OLP.

Owing to the anti-inflammatory and immunosuppressive effects of glucocorticoids (GCs), they can be used for the systemic treatment of severe atrophy, erosive OLP and a wide range of lesions [35]. Prednisone can effectively alleviate the clinical symptoms of OLP and reduce the severity of lesions. It is a commonly used systemic hormonal medication in patients with OLP owing to its strong anti-inflammatory effects and fewer side effects [36]. In this study, salivary TNF-α levels were higher in the treatment-ineffective group than in the treatment-effective group before treatment with prednisone, indicating that the clinical efficacy of prednisone may be related to TNF-α expression. Studies have demonstrated that pro-inflammatory cytokines such as IL-1β and TNF-α can activate the transcription factor NF-κB, which activates the transcription of inflammatory genes and induces the production and release of more inflammatory mediators [37]. GRα expression is downregulated in the lesions of patients with OLP and is negatively correlated with NF-κB expression, suggesting that some factors inhibit GRα expression in mucosal lesions. Activation of NF-κB can downregulate the expression of GRα and lead to GC resistance [38,39].

In this study, the levels of salivary IL-1β, TNF-α, IFN-γ, IL-6, hBD-2 and hBD-3 were significantly lower after 1 week of treatment with prednisone, indicating that prednisone can simultaneously inhibit the expression of Th1- and Th2-related cytokines and beta defensins in patients with EOLP. Anderson et al. reported that DEX treatment significantly reduced the expression of IL-4, IFN-γ, IL-6, IL-10, IL-17 A and TNF in the PBMCs of healthy volunteers [40]. After 1 week of treatment with prednisone, the reduction in salivary IL-6 and IL-1β levels in patients in the treatment-effective group was higher than that in the treatment-ineffective group, and IL-1β levels could be restored. These findings indicate that short-term treatment with GCs may influence the clinical outcomes of EOLP by decreasing the expression of IL-1β and IL-6. Ghalla et al. [41] reported that the concentration of salivary IFN-γ, TNF-α and TNFR-2 was significantly low in patients with EOLP after prednisone treatment. In addition, Rhodus et al. [28] demonstrated that dexamethasone significantly reduced the concentration of inflammatory cytokines such as IL-1, TNF-α, IL-6 and IL-8 in the saliva of patients with EOLP; in particular, the concentration of IL-1 and IL-8 decreased to normal levels after 6 weeks of treatment. The hBD-2 can induce direct anti-microbial immune response and mediate immune cell chemotaxis and enhance their cytokine production [42]. Synthesis and production of hBD-2 by epithelial cells are significantly induced by inflammatory mediators including TNF-α, IFN-γ, and IL-1β. Meanwhile, expression of hBD-2 in OLP and tongue cancer lesions was higher than in normal oral epithelium [17]. Importantly, tumor-derived cells had markedly low transcriptional levels of hBD-2. This suggests a potential association between hBD-2 and the chronic inflammatory (and tumorigenic?) process in OLP [17]. Besides, localization of human beta-defensin 3 mRNA in normal oral epithelium, leukoplakia, and lichen planus: an in situ hybridization study [18].

However, the degree of inhibition of Th1/Th2 cytokines by prednisone acetate in patients with EOLP is inconsistent, and that of IFN-γ may be more pronounced. In this study, salivary IFN-γ levels significantly decreased after treatment, and the IFN-γ/IL-4 ratio was

further reduced. GCs can reduce IFN-γ levels in autoimmune diseases [43,44]. Agarwal [45] suggested that GCs cause a shift in the Th1/Th2 balance dominated by Th2 cytokines by reducing the expression of Th1-type cytokines such as IL-12 and IFN-γ. Youngnak et al. [46] reported that the expression of IFN-γ decreased in patients with OLP after treatment with 0.1 % fluocinolone acetate.

The findings of this study indicate that patients with EOLP are more likely to be associated with *Candida* infection than healthy individuals. The IFN-γ/IL-4 balance is associated with *Candida* infection. IFN-γ is thought to prevent *Candida* infection [47]. Subcutaneous injection of IFN-γ in patients with HIV with oropharyngeal candidiasis with azole-resistant disease can significantly improve their clinical symptoms [48]. GC treatment may increase the risk of oral candidiasis in patients with ELOP, and Candida counts -bacterial counts (CFUs) are negatively correlated with TNF-α levels. Edens et al. reported that the incidence of oral fungal infections (OFIs) increased after steroid treatment in patients with OLP with low stimulated salivary flow [49]. In addition, a study demonstrated that mice infected with *Candida albicans* had decreased *Candida* counts in their tongue after oral administration of TNF-α, indicating that TNF-α exerts protective effects against oral candidiasis. TNF-α can induce the production of cytokines such as IFN-γ, IL-1 and IL-6 to enhance the immune response to *Candida* infection [50]. A recent study showed that *anti*-TNF treatment of inflammatory bowel disease resulted in a 2-fold increase in the risk of opportunistic infections [51]. Therefore, TNF-α may play a role in alleviating *Candida* infection.

The expression levels of the above inflammatory factors in saliva play a certain role in monitoring the occurrence and development of OLP, which provides a scientific basis and reference for studying the pathogenesis of OLP and exploring OLP immunotherapy. However, the role of IL-17 in the pathogenesis and defense of EOLP remains to be further confirmed. IL-17. Besides, the treatment of immunotherapy in EOLP need to be further studied.

11. Conclusions

Abnormal expression of IL-1β and IL-6 and imbalance of Th1/Th2 in local tissues may be associated with EOLP. High IL-1β levels may serve as a risk factor and IFN-γ/IL-4 balance may serve as a protective factor for EOLP. GC treatment inhibits Th1/Th2 cytokines, with the inhibitory effects on Th1-related cytokines being more significant. Salivary IL-6 levels may be associated with the treatment efficacy of GCs in patients with EOLP. Therefore, salivary IL-6 can be considered a reference indicator for monitoring the progression of EOLP and assessing treatment efficacy and prognosis in patients with EOLP.

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CRediT authorship contribution statement

Lanlan Jiang: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Yuxiao Huang:** Writing – review & editing, Methodology, Investigation. **Meifei Fang:** Methodology, Formal analysis. **Xinyu Chen:** Methodology. **Doudou Feng:** Formal analysis. **Jiaxuan Liu:** Data curation. **Qiaozhi Jiang:** Writing – review & editing. **Renchuan Tao:** Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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