Interferon-gamma and interleukin-10 levels in serum and saliva are related to different types of oral lichen planus

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Abstract

BACKGROUND: Many cytokines can be detected in saliva and serum, and have more clinical significance in the diagnosis, prognosis and treatment of oral mucosa disease.

OBJECTIVE: To compare the interferon-γ and interleukin-10 levels in serum and saliva of patients with different types of oral lichen planus and to explore the feasibility of saliva samples as a substitute of blood samples to study the interferon-γ and interleukin-10 levels in serum and saliva.

METHODS: Totally 45 patients with oral lichen planus admitted at the Department of Periodontology, the Stomatological Hospital of Jiamusi University from January to July 2014 were enrolled, including 15 cases of erosion type (erosion group), 15 cases of congestive erythema (congestive erythema group) and 15 cases of reticulate type (reticulate group). Another 15 healthy controls admitted for physical examination at the Department of Physical Examination, the Stomatological Hospital of Jiamusi University were enrolled as controls. ELISA method was used to detect the interferon-γ and interleukin-10 levels in serum and saliva in the four groups.

RESULTS AND CONCLUSION: Compared with the control group, the interferon-γ levels in serum and saliva were lower in the other three groups ($P < 0.01$), while there were significant differences in the interferon-γ level among the patients with different types of oral lichen planus ($P < 0.01$). The interleukin-10 levels in serum and saliva were significantly higher in the erosion group and congestive erythema group than those in the control group ($P < 0.01$ or $P < 0.05$) and reticulate group $P < 0.01$ or $P < 0.05$). Experimental findings suggest that the levels of interferon-γ and interleukin-10 in serum and saliva are highly correlated in patients with different types of oral lichen planus, and saliva samples can be instead of blood samples to detect the levels of interferon-γ and interleukin-10 in patients with oral lichen planus.

Subject headings: Interferon-gamma; Interleukin-10; Serum; Saliva
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INTRODUCTION

Oral lichen planus is one of the most common chronic superficial inflammatory oral mucosal diseases, with the prevalence rate of about 0.51% and the malignant transformation rate of 0.4%–3.2%[1], and its pathogen is unclear. The main symptoms of patients with oral lichen planus include white stripes of the oral mucosa, congestive erythema, erosion, ulcers or blisters, and most damages occur symmetrically and the minority of lesions has malignant transformation. Oral lichen planus can be delayed to life. Most studies have suggest that oral lichen planus is a variation in epithelial cell antigenicity induced by various pathogenic factors, and the altered antigens are incepted by Langerhans cells and keratinocytes to activate and release various activating factors that are supported for T lymphocytes to start a series of immune responses[2]. Moreover, oral lichen planus may be a T cell-mediated inflammatory disease that is possibly related to the body's own immune mechanisms, and thus its pathogenesis presumably involves both specific and non-specific immunity mechanisms[3]. Pathological results show that a large number of lymphocytes present with a dense strip-shaped infiltration in the epithelial lamina propria, which is one of the typical pathological manifestations of oral lichen planus. Therefore, oral lichen planus is considered to be related to immune factors[4].

Interferon-γ is a typical Th1-type cytokine that can upregulate the expression of major histocompatibility complex II (MHC II)

molecules on the cell surface, and it is indispensable in Th1 cell differentiation process. Interferon-γ can activate macrophages, enhance expression of endothelial cell adhesion molecules, and have important pro-inflammatory effects. Interleukin-10 is a class of anti-inflammatory and anti-allergic cytokines, with a role in the regulation of cytokines, and it can inhibit the release of pro-inflammatory cytokines and delivery of antigens that has an important role in immune regulation[5]. Interleukin-10 is produced mainly by Th2 cells that can be derived from mononuclear-phagocytic cells, B lymphocytes, and mast cells. Interleukin-10 can play a role in antigen-presenting cells or T cells to induce allogeneic or autologous antigen immune tolerance[6], which can reverse the allergen hyperresponsiveness to immune tolerance at different stages of the body's immune response[7]. Studies have shown that changes in the serum interleukin-10 level may be one of reasons for the rest and recurrence of oral lichen planus. Serum levels of interferon-γ and interleukin-10 are reported to be changed in patients with oral lichen planus, and have effects on oral lichen planus[8]. The main components of the saliva and blood are similar, both of which are important body fluids. Many cytokines in saliva and serum can be detected, and the saliva testing has already begun in-depth research or been used in clinical monitoring and treatment of certain diseases, such as rheumatoid arthritis, Sjogren's syndrome and other autoimmune diseases[9].

Therefore, we planned to analyze the correlation between interferon-γ and interleukin-10 levels in saliva and serum of patients with oral lichen planus, attempting to use the saliva as a biological sample to provide a faster and more convenient detection.

SUBJECTS AND METHODS

Design

Clinical non-randomized controlled observation.

Time and setting

The experiment was completed in the Laboratory of Life Science of Jiamusi University from January to July 2014.

Subjects

Totally 45 patients admitted for oral lichen planus admitted at the Department of Periodontology, the Stomatological Hospital of Jiamusi University from January to July 2014 were enrolled, including 12 males and 33 females, aged 18–55 years with an average of 42 years. There were 15 cases of erosion type, 15 cases of congestive erythema type and 15 cases of reticulate type.

Another 15 healthy controls, 18–55 years old, admitted for physical examination at the Department of Physical Examination, the Stomatological Hospital of Jiamusi University, were enrolled as controls.

Diagnostic criteria

Patients were clinically and pathologically diagnosed to have oral lichen planus.

Inclusive criteria

Patients were eligible if they (1) met the diagnostic criteria; (2) had no laser, hormones, immune modulation therapy and drugs for external use within recently 3 months; (3) had normal dental and periodontal tissues; (4) patients and their families gave informed consents.

Exclusive criteria

Patients were not eligible if they (1) had systemic diseases and (2) other oral infectious diseases.

Main reagents and instruments for detection of interferon-γ and interleukin-10:

Reagent and equipment	Source
High-speed centrifuge	Four-Ring Science Instrument Plant Beijing Co., Ltd., China
Microplate reader	BIO-RAD, USA
ELISA plates of interferon-γ and interleukin-10	BOSTER, China
Cryogenic refrigerator	SANYO, Japan
Pipettor	Shanghai Cheer Instrument Co., Ltd., China
EP tube and filter paper, disposable empty needle	Haimen Wishful Experimental Equipment Factory, China

Methods

Saliva sample collection

At 8–10 a.m., patients were asked to be in sitting position with head upright and do not speak without swallowing. Patients rinsed the mouth repeatedly with 100mL distilled water, so the saliva was naturally accumulated in the floor of the mouth, with no sputum contamination. A disposable empty needle was placed in the saliva pool in the mouth floor, did not touch the mouth mucosa, and then extracted 1 mL saliva sample immediately (preferably within 5 minutes) into the sterilized EP tube that was immediately placed in a −70 ℃ refrigerator.

Blood sample collection

At 8–10 a.m., routine fasting blood samples (2 mL) were collected from the venous blood vessel of the middle of the elbow, and the samples were placed in water bath for 30 minutes, centrifuged at 3 000 r/min for 5 minutes, and then, the 1 mL supernatant was encased into the sterilized EP tube that was placed in the −70 ℃refrigerator.

Sandwich ELISA test

Using the ELISA method, standard interferon-γ and interleukin-10 as well as interferon-γ and interleukin-10 samples with unknown mass concentration showed specific binding to avidin-biotin and avidin of anti-human interferon-γ and interleukin-10, respectively; then, enzyme substrate was added and the color reaction occurred. The absorbance values at 450 nm in all specimens were measured, and interferon-γ and interleukin-10 with the standard concentration and their absorbance values were used to draw a standard curve, by which, the

concentration of interferon-γ and interleukin-10 in all specimens were measured.

Main outcome measures

The levels of interferon-γ and interleukin-10 in saliva and serum.

Statistical analysis

Data were expressed as mean±SD and analyzed using SPSS 13.0. A value of a=0.05 was considered significance. Independent-sample t-test was used for intergroup comparison.

RESULTS

Quantitative analysis of participants and clinical data

All the participants were enrolled in result analysis, and their clinical data are shown in **Table 1**.

Interferon-γ levels in saliva and serum of patients with different types of oral lichen planus

Compared with the control group, the interferon-γ levels in serum and saliva were significantly lower in the other three groups (P < 0.01), while there were significant differences in the interferon-γ level among the patients with different types of oral lichen planus (P < 0.01; **Table 2**).

Interleukin-10 levels in saliva and serum of patients with different types of oral lichen planus

The interleukin-10 levels in serum and saliva were significantly higher in the erosion group and congestive erythema group than those in the control group (P < 0.01 or P < 0.05) and reticulate group (P < 0.01 or P < 0.05; **Table 3**).

Table 1 Clinical data of participants

Item	Oral lichen planus			Control group
	Erosion group	Congestive erythema group	Reticulate group	
n	15	15	15	15
Sex (*n*, male/female)	6/9	4/11	2/13	3/12
Age ($\bar{x}$±*s*, year)	47.8±8.61	53.1±8.30	53.0±7.09	46.1±15.7
Lesion site	Buccal mucosa, tongue	Buccal mucosa, tongue	Buccal mucosa, tongue	–
Other oral infectious diseases	None	None	None	None

Table 2 Interferon-γ levels in saliva and serum of patients with different types of oral lichen planus ($\bar{x}$±*s*, *n* =15, ng/L)

Group	Serum	Saliva
Erosion	17.37±1.13[a]	6.36±1.56[a]
Congestive erythema	20.88±3.19[ab]	8.57±0.11[ab]
Reticulate	26.85±1.78[abc]	11.49±0.89[abc]
Control	35.46±0.98	18.73±1.42

Note: [a]P < 0.01, *vs.* control group; [b]P < 0.01, *vs.* erosion group; [c]P < 0.01, *vs.* congestive erythema group.

Table 3 Interleukin-10 levels in saliva and serum of patients with different types of oral lichen planus ($\bar{x}$±*s*, *n*=15, ng/L)

Group	Serum	Saliva
Erosion	33.11±1.61[a]	16.66±1.48[a]
Congestive erythema	32.03±0.81[b]	12.47±0.7[4b]
Reticulate	26.93±1.61[de]	6.30±1.23[ce]
Control	25.85±2.95	4.57±0.85

Note: [a]P < 0.01, [b]P < 0.05, *vs.* control group; [c]P < 0.01, [d]P < 0.05, *vs.* erosion group; [e]P < 0.05, *vs.* congestive erythema group.

DISCUSSION

Oral lichen planus is a T lymphocyte-mediated inflammatory disease, and the strip-shaped infiltration in a large number of lymphocytes in the epithelial lamina propria is one of its typical pathological manifestations. Increasing evidences have proved that the variation of T cell-mediated immune function is closely related to the pathogenesis of oral lichen planus, which is presented with the Th1/Th2 imbalance in lesions, saliva and tissue exudates[10-12]. Infiltrated T lymphocytes can secrete a variety of cytokines. Studies have shown that Th1 and Th2 dominant reactions exist in patients with oral lichen planus. Th1 cells produce interferon-γ, interleukin-2, and tumor necrosis factor-α, while Th2 cells produce interleukin-4, interleukin-5, interleukin-6, and interleukin-10. When a class of cytokines occurs, the activities of another type of cytokines may be increased accordingly in order to effectively prevent immune imbalance. Therefore, the balance of Th1 and Th2 cytokines is very important for the maintenance of normal immune function. The reactions between the two types of cytokines can be cross-regulated to achieve a balance. For example, interleukin-4 and interleukin-10 can inhibit Th1 response to prevent the infinite value of Th1 cell and its cytokines. Interferon-γ can develop an inhibitor effect on Th2 activity[13]. Different cytokines have different biological roles in promoting or inhibiting the development of inflammation, and thus, they show different roles in the development of the disease. In the treatment of oral lichen planus, local application of corticosteroids is mainly preferred, and new factors and new technologies have been applied constantly[14-15], such as, the subcutaneous injection of epidermal specific transfer factor in patients with oral lichen planus[16].

Interferon-γ is a typical Th1-type cytokine that can upregulate the expression of major histocompatibility complex II molecules on cell surface, and it is indispensable in Th1 cell differentiation. Interferon-γ can activate macrophages, and enhance expression of endothelial cell adhesion molecules, exerting an important pro-inflammatory role. Interferon-γ contributes to the removal of lymphocytes from the blood vessels, and then the lymphocytes enter into the infiltration zone to play a role. Interleukin-10 is mainly produced by Th2 cells, which are derived from mononuclear macrophages, B lymphocytes, and mast cells, and interleukin-10 inhibits the release of pro-inflammatory cytokines and delivery of antigens, which is a pleiotropic cytokine and has anti-inflammatory and anti-allergic effects, thus playing an important role in regulating the immune response[17-22]. Deng *et al* [23] found that the interleukin-10

level in the peripheral blood was significantly higher in the patients with oral lichen planus than healthy controls.

Saliva is the most accessible human biological fluid containing a series of proteins, NDA, RNA that can be used for early diagnosis and therapeutic monitoring of diseases[24]. Saliva as a diagnostic specimen is easy and inexpensive to be obtained as a result of painless, and has greater advantages than serum. Saliva and serum detection results are highly comparable[25]. As a result of local production, saliva also has a greater value than serum in disease diagnosis. Influencing factors that exist in the saliva test are that overflow of carbon dioxide during the collection, changes in pH, easy to produce bacteria and precipitation. If the saliva specimen cannot be saved in time, the experimental results will be impacted. As above, the collection and storage of saliva is crucial for ensuring the objective results. Therefore, after collecting the saliva specimens should be centrifuged to take the supernatant within 5 minutes that is then placed at a −70 ℃ refrigerator. Repeated freezing and thawing is the most taboo. In addition, the immune status of the patient is changing at the different stages of oral lichen planus, and the level of serum cytokines may also vary. Therefore, different proportions of enrolled patients at different stages of oral lichen planus can result in different testing results.

In this study, the levels of interferon-γ and interleukin-10 in saliva and serum of patients with three types of oral lichen planus were detected and compared with normal controls. The levels of interferon-γ in saliva and serum of patients with three types of oral lichen planus were significantly lower than those in the control group ($P < 0.05$ or $P < 0.01$), indicating that interferon-γ exerts an important role in the development of oral lichen planus. The interleukin-10 levels in serum and saliva were significantly higher in the erosion group and congestive erythema group than those in the control group and reticulate group ($P < 0.01$ or $P < 0.05$), suggesting that interleukin-10 is of great significance in the development of oral lichen planus. Experimental findings from the present study demonstrate that the levels of interferon-γ and interleukin-10 in serum and saliva are highly correlated in patients with different types of oral lichen planus.

REFERENCES

[1] Lodi G, Scully C, Carrozzo M, et al. Current controversies in oral lichen planus: report of an international consensus meeting. Part 2. Clinical management and malignant transformation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;100(2):164-178.

[2] Femiano F, Cozzolino F, Gaeta GM, et al. Recent advances on the pathogenesis of oral lichen planus (OLP). The adhesion molecules. Minerva Stomatol. 1999;48(4): 151-159.

[3] Sugerman PB, Savage NW, Walsh LJ, et al. The pathogenesis of oral lichen planus. Crit Rev Oral Biol Med. 2002;13(4):350-365.

[4] Billiau A, Heremans H, Vermeire K, et al. Immunomodulatory properties of interferon-gamma. An update. Ann N Y Acad Sci. 1998;856:22-32.

[5] Chen QM, Zhou ZT. Oral Mucosal Diseases. Beijing: People's Medical Publishing House, 2012:103-110.

[6] Moore KW, de Waal Malefyt R, Coffman RL, et al. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001;19:683-765.

[7] Han YP. Investigation of the pathogenesis of oral lichen planus: role of Th1/Th2 cell and its cytokines. Fuzhou: Fujian Medical University, 2011.

[8] Garza KM, Chan SM, Suri R, et al. Role of antigen-presenting cells in mediating tolerance and autoimmunity. J Exp Med. 2000;191(11):2021-2027.

[9] Zhu JH, Yan XL, Gao CY, et al. Expression and correlation of IL-10 in serum and saliva of patients with oral lichen planus. Linchuang Kouqiang Yixue Zazhi. 2014, 30(4): 221-223.

[10] Iijima W, Ohtani H, Nakayama T, et al. Infiltrating CD8+ T cells in oral lichen planus predominantly express CCR5 and CXCR3 and carry respective chemokine ligands RANTES/CCL5 and IP-10/CXCL10 in their cytolytic granules: a potential self-recruiting mechanism. Am J Pathol. 2003;163(1):261-268.

[11] Zhou G, Fan MW, Liu JY. Effects of glucocorticoids on T helper cells balance in oral lichen planus. Zhonghua Kouqiang Yixue Zazhi. 2005;40(2):98-101.

[12] Liu W, Dan H, Wang Z, et al. IFN-gamma and IL-4 in saliva of patients with oral lichen planus: a study in an ethnic Chinese population. Inflammation. 2009;32(3):176-181.

[13] Kelso A. Th1 and Th2 subsets: paradigms lost? Immunol Today. 1995;16(8):374-379.

[14] Scully C, Carrozzo M. Oral mucosal disease: Lichen planus. Br J Oral Maxillofac Surg. 2008;46(1):15-21.

[15] Jungell P, Konttinen YT, Nortamo P, et al. Immunoelectron microscopic study of distribution of T cell subsets in oral lichen planus. Scand J Dent Res. 1989;97(4):361-367.

[16] Wang XJ, Liu Q, Gu Y. Treatment of oral lichen planus with epidermal transfer factor. Shiyong Kouqiang Yixue Zazhi. 2008;24(5):717.

[17] Gong FL. Medical Immunology. Beijing: Science Press. 2008.

[18] Fromowitz FB, Viola MV, Chao S, et al. ras p21 expression in the progression of breast cancer. Hum Pathol. 1987; 18(12):1268-1275.

[19] Li BQ. Oral Mucosal Diseases. Beijing: People's Medical Publishing House, 2006:83-87.

[20] Lai HC, Cao HK. Mechanism and progress of lichen planus cell infiltration. Shanghai Kouqiang Yixue. 1995;4(1):46-48,50.

[21] Deng GH, Chen ZL, Chen HB, et al. Significance of cell immunoreactions and cell apoptosis in oral lichen planus. Huaxi Kouqiang Yixue Zazhi. 2009;27(3):256-259.

[22] Zhou G. CD4+T Lymphocyte Subsets and Th1/Th2 Polarization in Oral Lichen Planus. Wuhan: Wuhan University. 2004.

[23] Deng GH, Han YP, Wang HR, et al. The role of IL-2/IL-10 cell in the pathogenesis of oral lichen planus. Linchuang Kouqiang Yixue Zazhi. 2012;28(12):715-718.

[24] Edgar WM. Saliva: its secretion, composition and functions. Br Dent J. 1992;172(8):305-312.

[25] Nagler RM, Hershkovich O, Lischinsky S, et al. Saliva analysis in the clinical setting: revisiting an underused diagnostic tool. J Investig Med. 2002;50(3):214-225.

血清及唾液中干扰素γ和白细胞介素10水平与不同类型的口腔扁平苔藓

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文章亮点：

实验通过比较不同类型口腔扁平苔藓患者唾液及血清中干扰素γ和白细胞介素10的表达情况以及其相关性，以进一步了解口腔扁平苔藓患者唾液取代血液作为生物学样本来研究口腔扁平苔藓的中干扰素γ和白细胞介素10的可行性。

关键词：

组织构建；组织工程；口腔扁平苔藓；干扰素γ；白细胞介素10；血清；唾液；细胞因子

主题词：

干扰素γ；白细胞介素10；血清；唾液

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摘要

背景：许多细胞因子在唾液和血清中都能检测出，并且在口腔黏膜疾病的诊断，预后的监控和治疗方面更具有临床意义。

目的：比较不同类型口腔扁平苔藓患者血清及唾液中的白细胞介素10和干扰素γ的水平，探索唾液作为生物学样本代替血液来研究口腔扁平苔藓中干扰素γ和白细胞介素10的可行性。

方法：收集2014年1至7月在佳木斯大学附属口腔医院牙周黏膜病科就诊的口腔扁平苔藓患者45例，根据患者疾病类型分为糜烂型(糜烂组)15例，充血红斑型(充血红斑组)15例，网纹型(网纹组)15例。另取15名就诊于佳木斯大学附属口腔医院体检科身体健康者作为对照组。采用ELISA法分别检测4组对象血清和唾液中干扰素γ和白细胞介素10的表达水平。

结果与结论：与正常组相比，口腔扁平苔藓患者血清和唾液中干扰素γ水平较低($P < 0.01$)，且糜烂组、充血红斑组和网纹组患者血清和唾液中干扰素γ水平差异有显著性意义。与正常组相比，糜烂组和充血红斑组患者血清和唾液中白细胞介素10水平较高($P < 0.01$或$P < 0.05$)；与网纹组相比，糜烂组和充血红斑组患者血清和唾液中白细胞介素10水平较高。提示口腔扁平苔藓患者血清和唾液中干扰素γ和白细胞介素10水平高度相关，可以通过检查唾液替代血液来研究口腔扁平苔藓中干扰素γ和白细胞介素10的水平。

作者贡献：朱建华进行实验设计，实验实施为刘娜，实验评估为赵长荣、刘继光，资料收集为刘娜、赵长荣，朱建华成文，刘继光审校，朱建华对文章负责。

利益冲突：文章及内容不涉及相关利益冲突。

伦理要求：参与实验的患病个体及其家属自愿参加，在充分了解本治疗方案的前提下签署“知情同意书”；干预及治疗方案获医院伦理委员会批准。

学术术语：口腔扁平苔藓--一种常见的慢性口腔黏膜皮肤疾病，一般不具有传染性，其发病与精神因素(如疲劳、焦虑、紧张)、免疫因素、内分泌因素、感染因素、微循环障碍因素、微量元素缺乏以及某些全身疾病(糖尿病、感染、高血压、消化道功能紊乱)有关。

作者声明：文章为原创作品，无抄袭剽窃，无泄密及署名和专利争议，内容及数据真实，文责自负。

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