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Original Article

Correlation of miRNA-155 and IL-17 mRNA expression in peripheral blood of female patients with oral lichen planus

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Abstract: Oral lichen planus (OLP) is a chronic disease that commonly affects the middle-aged women. Nowadays, the cause of OLP is still obscure. Here, we aimed to investigate the expression and correlation of miR-155 and IL-17 mRNA in peripheral blood of women OLP patients. Finally, 261 women OLP patients (138 non-erosive and 123 erosive OLP patients) and 254 women healthy controls were recruited. Real time RT-PCR was used to detect the relative expression level of miR-155 and IL-17 mRNA in peripheral blood. Receiver-operator characteristic (ROC) analysis was used to assess the accuracy of miRNA-155 and IL-17 in predicting the degree of lesion. Compared to healthy controls, both the relative expression levels of miR-155 and IL-17 mRNA were significantly higher in OLP patients. Compared to non-erosive OLP patients, their relative expression levels were significantly higher in erosive OLP patients. The ROC analysis showed that they could effectively distinguish erosive OLP patients from non-erosive OLP patients. These results indicated that both miR-155 and IL-17 mRNA might have an important role in the pathogenesis of OLP, and they could be used as potential biomarkers for predicting the degree of lesion.

Keywords: Oral lichen planus, OLP, miRNA-155, IL-17, peripheral blood

Introduction

Oral lichen planus (OLP) is a common multifactorial disease that could occur in combination with or without lichen planus of the skin. It is reported to affect about 1% of the world population [1]. Generally speaking, OLP is a disease of adulthood that rarely affects children, and typically affects women with middle-age [2]. This disease has kinds of clinical symptoms, such as erosive, erythematous forms and reticular. In most cases, OLP is not very serious. However, it has an important feature, which is the risk of developing mouth cancer; for example, patients with OLP for ten years might have 1% chance of developing mouth cancer [3].

Currently, the true cause of OLP is still unclear, but it seems to have relationship with the immune system in our body. Sometimes, it could be resulted from some certain materials or medicines using in dental fillings. Nowadays, many researchers reported that immune factors had an important role in the pathogenesis of OLP, and thought it was a T cell-mediated chronic inflammatory disease [4, 5]. The Th17 cell is a recently discovered new subset of CD4+ helper T cells [6]. It could secrete interleukin 17 (IL-17). IL-17 is a powerful inflammatory cytokines, and associated with many autoimmune diseases and inflammatory related diseases [7]. Additionally, micro RNA (miRNA) is a small non-coding RNA that widely exists in the organism, which is involved in the regulation of many biological functions [8]. Some studies showed that the abnormal expression of miRNA was closely associated with the inflammatory diseases and autoimmune diseases [9, 10]. As an important type of miRNA, miR-155 was reported that its expression level was significantly increased in patients with rheumatoid arthritis (an autoimmune disease), and had a closely relationship with the disease activity [11].

Therefore, in this study, we recruited 261 women patients with OLP and 254 healthy controls (women) to detect the expression levels of miRNA-155 and IL-17 mRNA in their peripheral blood. The relationship between OLP and these two factors was analyzed to provide insight into the underlying pathogenesis of OLP.

Materials and methods

Subjects recruited

Women patients with OLP were recruited from Department of Implantology, Shandong University School of Stomatology between January 2010 and January 2016. Our work was approved by the Ethical Committee of Shandong University, and the procedure was conducted according to the approved regulations and guidelines. All patients were correctly diagnosed by pathological examination, and divided into two major groups: non-erosive OLP and erosive OLP group. At the same time, the age- and gender-matched healthy controls (women) were recruited from the Medical examination center of Shandong University School of Stomatology. Candidates that met the following criteria were included in this study: no history of systemic disease or immune system disease; no smoking; not taking any antibiotics, non-steroidal anti-inflammatory drugs, immunomodulatory drugs that could affect the immune function in the last three months; not receiving any surgery or medications for oral-related disease in the last year; without other oral infectious diseases; not in the period of menstrual, pregnancy and lactation. All the included subjects provided the written informed consent.

Total RNA extraction

Elbow venous blood samples were taken from both the patients with OLP and healthy controls, and used to obtain the plasma. Total RNA from plasma was extracted using RNeasy Mini kit (Qiagen, Mississauga, ON, Canada) according to the manufacturer's instructions. Then, the NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE) was used to measure the concentration and purity of the total RNA. And, the A260/A280 ratio of 1.8-2.0 suggested that the purity of the total RNA was good. The cDNA was synthesized from the total RNA using the Prime Script RT Reagent kit (Takara Bio Inc., Otsu, Japan).

MiR-155 detection

Real time RT-PCR was performed using primer set for miR-155 and U6 gene. Gene sequences of forward primers were as follows: miR-155 (5'-CGTTAATGCTAATCGTGATAG-3'), U6 (5'-CTCGCTTCGGCAGCACA-3'); reverse primers adopted the kit universal primers. Each real-time PCR reaction was amplified with SYBR Premix ExTaqTM II (Takara Bio Inc., Otsu, Japan) using a Bio-Rad CFX96 Real Time System (Bio-Rad, Hercules, CA). The reaction mixtures were heated at 94°C for 10 min, then 40 cycles of 14 seconds at 95°C and 20 seconds at 60°C. Each sample was repeated three times, and the mean was considered as the final result. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ equation.

IL-17 detection

Real time RT-PCR was performed using primer set for IL-17 and β-actin. Gene sequences of primers were as follows: IL-17 (forward primer: 5'-CCACCTCACCTTGGAATCTC-3'; reverse primer: 5'-CAGGATCTCTTGCTGGATGG-3'), β-actin (forward primer: 5'-TGTACGTGGACATCCGCAAAG-3'; reverse primer: 5'-CTGGAAGGTGGACAGCGAGG-3'). Reaction mixture was prepared on the ice: SYBR Premix Ex Taq (Tli RNaseH Plus), 12.5 μl; forward primer (10 μmol/L), 0.5 μl; reverse primer (10 μmol/L), 0.5 μl; cDNA, 1 μl; and nuclease-free water, 10.5 μl. These mixtures were heated at 95°C for 10 min, then 40 cycles of 15 second at 95°C, 30 second at 56°C and 30 second at 72°C. Each sample was repeated three times, and the mean was considered as the final result. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ equation.

Statistical analyses

Mean and standard deviation (SD) were used to represent the continuous data; number and percentage were used to represent dichotomous data. The one-way analysis of variance (one-way ANOVA), student's t-test and Chi-square test were performed when appropriate. The Bonferroni or Tamhane's T2 post-hoc test was performed to determine which two groups

Table 1. Demographic of included patients

Variable	HC	non-erosive OLP	erosive OLP	*p*-value
Number	254	138	123	-
Age (year)	46.6 (7.8)	47.2 (8.0)	46.5 (7.5)	0.671
Duration (m)	-	16.8 (8.8)	18.6 (9.4)	0.114
Married (Y/N)	184/70	96/42	87/36	0.826
BMI (kg/m^2)	22.1 (3.1)	22.8 (3.5)	21.9 (3.4)	0.587
Education (year)	11.5 (2.8)	12.3 (3.2)	12.0 (3.0)	0.457

HC: healthy controls; m: month; Y: yes; N: no; BMI: body mass index.

significantly differed, if a significant difference was found [12]. The binary logical regression analysis and receiver-operator characteristic (ROC) analysis were conducted with the relative expression level of miRNA-155 and IL-17 mRNA. The area under the curve (AUC) was used to assess the accuracy of miRNA-155 and IL-17 mRNA in predicting the degree of lesion of OLP. SPSS 19.0 was used to do data analysis and *p*-value <0.05 was considered statistically significant.

Results

Demographic data

Among the recruited patients with OLP, there were 138 non-erosive and 123 erosive OLP patients with average age of 47.2 ± 8.0 and 46.5 ± 7.5 years, respectively. The age of OLP patients ranged from 22 to 68 years. The average age of health controls was 46.6 ± 7.8 years, aged from 21 to 67 years. The duration of OLP was 16.8 ± 8.8 years in non-erosive group, which was similar to the 18.6 ± 9.4 years in erosive group. The difference between the groups on the demographic data was not statistically significant. The detailed information was shown in **Table 1**.

MiR-155 relative expression level

The relative expression level of MiR-155 was 1.27 ± 0.66 in the healthy controls, 3.39 ± 1.44 in the non-erosive OLP patients and 5.71 ± 2.34 in the erosive OLP patients (**Figure 1**). The one-way ANOVA analysis showed that the relative expression level of MiR-155 was significantly different among the three groups (P<0.00001). The Bonferroni post-hoc test showed that compared to the healthy controls, both the non-erosive and erosive OLP patients had significantly higher relative expression level of MiR-155 (P<0.00001, P<0.00001). And, compared to the non-erosive OLP patients, the erosive OLP patients had significantly higher relative expression level of MiR-155 (P<0.00001).

IL-17 mRNA relative expression level

The relative expression level of IL-17 mRNA was 0.40 ± 0.21 in the healthy controls, 0.85 ± 0.39 in the non-erosive OLP patients and 1.60 ± 0.55 in the erosive OLP patients (**Figure 1**). The one-way ANOVA analysis showed that the relative expression level of IL-17 mRNA was significantly different among the three groups (P<0.00001). The Bonferroni post-hoc test showed that compared to the healthy controls, both the non-erosive and erosive OLP patients had significantly higher relative expression level of IL-17 mRNA (P<0.00001, P<0.00001). And, compared to the non-erosive OLP patients, the erosive OLP patients had significantly higher relative expression level of IL-17 mRNA (P<0.00001).

ROC analysis

In order to evaluate whether the relative expression level of MiR-155 and IL-17 mRNA could effectively distinguish erosive OLP patients from non-erosive OLP patients, we conducted ROC analysis (**Figure 2**). Firstly, we separately evaluate the discrimination accuracy of these two indexes. The AUC of MiR-155 and IL-17 mRNA was 0.805 (95% CI=0.751-0.859) and 0.864 (95% CI=0.818-0.909), respectively. Secondly, we used these two indexes to conduct binary logical regression analysis, and obtained a formula: $P(Y=1)=1/(1+e^{-X})$, X=0.530*(MiR-155)+2.678*(IL-17 mRNA)-5.623. Then, this formula was used to conduct ROC analysis, and an AUC of 0.901 (95% CI=0.863-0.940) was obtained.

Discussion

As far as we know, this was the first study that investigated the expression level of MiRNA-155 and IL-17 mRNA in Chinese women patients with OLP. By analyzing the data from 261 women patients, we found that compared to the healthy controls (women), both the relative

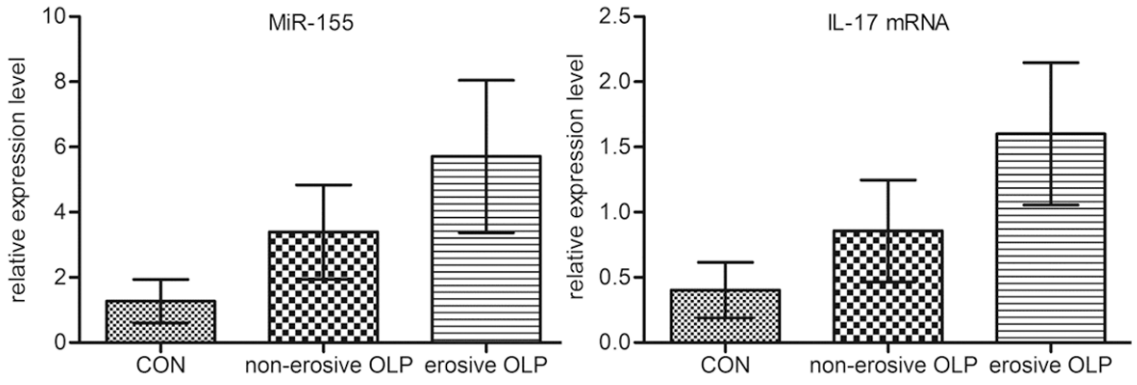

Figure 1. Relative expression level of MiR-155 and IL-17 mRNA.

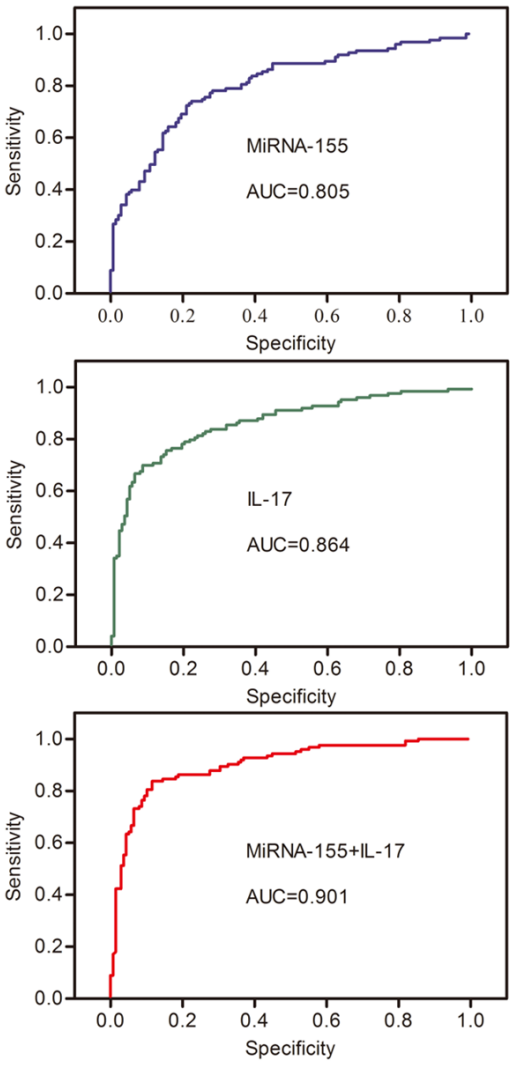

Figure 2. ROC analyses of MiR-155 and IL-17 mR.

expression level of MiRNA-155 and IL-17 mRNA were significantly higher in both non-erosive and erosive OLP patients. Moreover, these two indexes could effectively distinguish erosive OLP patients from non-erosive OLP patients. These results showed that both MiRNA-155 and IL-17 mRNA might have an important role in the occurrence and development of OLP in women, and could become potential predictors for the degree of lesion of OLP.

Many researches showed that OLP was closely associated with the abnormal immune function, and the T cell-mediated immune reactions played an important role in its onset [13, 14]. IL-17 could enhance the differentiation of T cells to produce various cytokines, such as IL-1, tumor necrosis factor TNF-α, metalloproteinases and various chemokines. Meanwhile, IL-17 could induce local inflammatory mediator or chemokines infiltration and cause tissue damage, which at last resulted in inflammatory diseases [15]. Some researchers reported that compared to the normal group, the OLP group had the significantly higher expression level of IL-17 mRNA in both epithelium and lamina propria [16]. Previous studies found that the relative expression level of IL-17 mRNA was significantly higher in patients with autoimmune disease, such as rheumatoid arthritis [17] and systemic lupus erythematosus [18]. These results showed that IL-17 mRNA might be correlated with the pathogenesis of OLP.

Previous studies indicated that both TNF-α and IL-1 could induce the over-expression level of miRNA-155 in immune cells in vitro [19, 20]. MiRNA-155 has an important role in the immune system of mammalian, especially in regulating T helper cell differentiation [21].

Researchers also reported that the over-expression level of miRNA-155 in activated CD4+ T cells could promote Th1 differentiation, and the down-expression level could promote Th2 differentiation [22]. Cell experiment showed that miRNA-155 could inhibit the release of inflammatory mediators, such as IL-8, IL-6 and TNF-α, via exerting control over myeloid differentiation primary response gene 88 (MyD88) [23]. Here, we found that the relative expression level of miRNA-155 was significantly increased in OLP patients, which indicated that the miRNA-155 might have a vital role in the genesis and development of OLP.

In addition, in this study, we found that compared to the non-erosive OLP patients, both the relative expression level of IL-17 mRNA and miRNA-155 were significantly higher in the erosive OLP patients. The ROC analysis showed that both IL-17 mRNA and miRNA-155 could be used as an index to predict the degree of lesion of OLP. Moreover, the combined application of IL-17 mRNA and miRNA-155 could provide a higher accuracy for predicting the degree of lesion than relying on any single index in isolation. These results also indicated that these two indexes had an important role in the progression of OLP.

Limitations of this study should be noticed. First, the number of the recruited OLP patients was relatively small. Then larger samples are needed to verify and support our conclusion. Second, all subjects were from the same ethnicity and site, which might limited the applicability of our findings [24]. Third, we only recruited the women OLP patients. Therefore, it is unknown whether or not our findings could be appropriated for men OLP patients.

However, our study found that both the relative expression level of IL-17 mRNA and miRNA-155 were significantly higher in OLP patients than in health controls. Moreover, we found that they could be used as potential objective laboratory-based indexes for predicting the degree of lesion of OLP. Our findings could be helpful for the investigation of the pathogenesis of OLP.

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Disclosure of conflict of interest

None.

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