A study on correlation of serum TNF-α, IL-2, 6 concentration and immune function in patients with recurrent oral ulcers

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Objective: To investigate the correlation of tumor necrosis factor α (TNF-α), interleukin-2, 6 (IL-2, 6) and immune function in patients with recurrent oral ulcers (ROU). Methods: 68 patients with recurrent oral ulcers and 60 healthy controls were recruited from January 2014 to December 2014. The levels of TNF-α, IL-2, IL-6 were measured in both serum and gingival cervical fluid of patients with ROU and control subjects by ELISA assay. The levels of immune cells (CD3+, CD4+, CD4+/CD8-) of two groups were detected using flow cytometry. The correlation of TNF-α, IL-2, IL-6 concentration and immune function were measured with Pearson correlation factor analysis. Results: The levels of TNF-α, IL-2, IL-6 were significantly higher in patients with ROU than those in control groups (P<0.05). And the levels of CD4+ and CD4+/CD8- were significantly lower in patients with ROU than those in the control group, while the levels of CD8+ were higher (P<0.05). According to Pearson correlation factor analysis, there was positive correlation between serum TNF-α concentration and IL-2, IL-6 and CD4+/CD8- (P<0.05); while there was no correlation between serum TNF-α concentration and CD3+, CD4+ (P>0.05). Conclusion: ROU may be associated with immune dysfunction in patients. TNF-α concentration measurement may reflect immune function, which helps determine the prognosis.

1. Introduction

Recurrent oral ulcers (ROU) is one of the most frequently occurring dental diseases, of which pathogenesis is associated with many factors, including bacterial infections, oral mucosal viral and immune imbalances, among which the immune imbalance plays a vital role in ROU[1]. Th1-type cells are T-lymphocyte subsets and capable of differentiating IL-2 and IL-6 cytokine, whose immune activity is regulated by numerous cytokines[2]. To some extent, TNF-α is responsible for adjusting the functions of immune cells and endothelial cell and regulating body immune function[3]. Recent studies have found that, TNF-α abnormal release is a specific process in ROU, in which immune cells inflammatory response can lead to TNF-α abnormal release, thus epithelial injury and the ulcers occur[4]. The study will investigate the correlation of TNF-α, IL-2, 6 concentrations and immune function in patients with recurrent oral ulcers, aiming to provide guidance on clinical prevention and treatment for ROU patients.

2. Materials and methods

2.1. Clinical information

68 Patients with ROU admitted to our hospital were enrolled in the study from January 2013 to December 2014, who relapsed at least once a month within the past six months and were at ulceration without receiving any medication or systematic treatment in the past nearly one month. ROU group and control group have same exclusion criteria: (1) oral mucosa disease; (2) patients with severe oral ulcer; (3) patients with significant organic disease presenting in liver, kidney and lung; (4) autoimmune diseases; (5) systemic inflammatory responses. The ROU group included 32 males and 36
females, aged 26-60 years mean age (42.8±3.9); while the control group included 30 males and 30 females, aged 27-62 years (mean age 43.5±4.2). No statistical differences were observed in the two groups in terms of sex and age (P>0.05), and they are comparable.

2.2. Treatment method

(1) Cytokines determination: 3 mL blood sample were drawn on the day of the visit In the ROU group and on the day of physical examination in the control group. After collection, the blood was centrifuged with supernate remained. In strict accordance with the operating instructions, serum TNF-α, IL-2 and IL-6 levels were determined using ELISA assay (the TNF-α kit was supplied by Zhengzhou Ansai Biotechnology Co., Ltd., the IL-2 kit by Shanghai Jimi Biotechnology Co., Ltd. And the IL-6 kit by Jingmei Bioengineering Co., Ltd.).

(2) Immune cells determination: 5 mL fasting venous blood was collected in the morning before anesthesia induction, at the end of surgery and on day 1, 3 and 7 after operative for both two groups, respectively. After collection, heparin anticoagulant was added, and the resulting mixture was centrifuged at 1 000 r/min for 10 min. After centrifugation, supernate was discard; the cell ratio of CD3+, CD4+, CD8+ and CD4+/CD8+ in peripheral blood were determined using with Beckm Epics flow cytometry, which is supplied by American Beckman Company.

2.3. Statistical analysis

Measurement data represents in (Mean±SD). SPSS17.0 software was employed for data processing, t test for inter-groups measurement data, and Pearson correlation analysis for one–factor analysis. P<0.05 was considered statistically significant.

3. Results

3.1. The comparison of the concentration of TNF-α, IL-2, IL-6 between two groups

The levels of TNF-α, IL-2, IL-6 were significantly higher in patients with ROU than those in control groups (P<0.05), and the difference is significantly statistical. Seen in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>TNF-α(mean±SD)</th>
<th>IL-2(mean±SD)</th>
<th>IL-6(mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROU</td>
<td>68</td>
<td>15.63±3.85</td>
<td>39.52±3.56</td>
<td>2.85±0.48</td>
</tr>
<tr>
<td>Control</td>
<td>60</td>
<td>10.12±1.22</td>
<td>22.78±10.25</td>
<td>0.68±0.13</td>
</tr>
</tbody>
</table>

3.2. Immunity Level Comparison

The levels of CD4+ and CD4+/CD8+ were significantly lower in patients with ROU than those in the control group, while the levels of CD8+ were higher than control groups (P<0.05), and the difference was statistically significant (P<0.05). Seen in Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>CD3+(%)</th>
<th>CD4+(%)</th>
<th>CD8+(%)</th>
<th>CD4+/CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROU</td>
<td>68</td>
<td>72.92±3.84</td>
<td>34.98±4.22</td>
<td>33.25±5.36</td>
<td>1.25±0.58</td>
</tr>
<tr>
<td>Control</td>
<td>60</td>
<td>71.29±2.94</td>
<td>31.89±3.84</td>
<td>22.15±4.02</td>
<td>1.85±0.92</td>
</tr>
</tbody>
</table>

3.3. Correlation of TNF-α and IL-2, 6 concentration with immune function

According to Pearson correlation factor analysis, there was positive correlation between serum TNF-α concentration and IL-2, IL-6 and CD4+/CD8+ (P<0.05); while there was no correlation between serum TNF-α concentration and CD3+, CD4+ (P>0.05) . Seen in Table 3.

<table>
<thead>
<tr>
<th>Correlation</th>
<th>IL-2</th>
<th>IL-6</th>
<th>CD3+</th>
<th>CD4+</th>
<th>CD4+/CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>0.325</td>
<td>0.369</td>
<td>0.112</td>
<td>0.102</td>
<td>0.345</td>
</tr>
<tr>
<td>P</td>
<td>0.025</td>
<td>0.010</td>
<td>0.725</td>
<td>0.698</td>
<td>0.015</td>
</tr>
</tbody>
</table>

4. Discussion

The immune function maintenance mainly depends on the balance among immune cell subsets, immune cells and a variety of immune factors. T-lymphocyte subsets comprises CD3+, CD4+ and CD8+ cells, among which the CD4+, CD8+ and CD4+/CD8+ cells were body's important immune regulatory cells and can be as a significant parameter of body immune function[5]. The CD4+ cells and CD8+ cells normally are in balance, and the CD4+ cells as supporting roles and the CD8+ cells’ immune inhibition are of vital importance for body immune function maintaining and health keeping. If CD4+/CD8+ cells decrease, the body immune function will be interrupted and various diseases will be liable to appear[6].

Ma Xiaozhe et al[7] has made some investigations on immunology for patients with ROU, and the results show, Patients with ROU all have T lymphocyte subsets imbalance and immune function decrease issues. It’s also noted that, the CD4+, CD4+/CD8+ levels is significantly lower in ROU group than in control group, while CD8+ levels higher, which suggest that T-cell subsets imbalance is prevalent in patients with ROU. We believe that the immune imbalance of patients with ROU may be associated with decreased CD4+ and increased CD8+, which suggests that Patients with ROU...
present two-way immune dysfunction.

Immune imbalance is an important cause of ROU. And related scholars think[8] TNF-α excessive release is a specific process that can lead to ulcer occurring through toxic effects of immune cells. The study shows that, serum TNF-α level is significantly higher in ROU group than in control group (P<0.05), which suggests that TNF-α may be involved in the pathogenesis and progression of ROU. TNF-α plays an important role in regulating the functions of immune cells and endothelial cells and enhancing the body immune function, but ROU may caused by TNF-α-mediated inflammation acting on the oral mucosa, which can lead tissues to dissolution, edema and ulceration[9].

Various cytokines interact and affect with each other in the body rather than exist independently, by which synthesis and secretion and gene expression of each other to form a complex network of cellular factors[10]. Cytokine network balance comprises cell antagonistic and synergistic balance. For ROU patients, elevated levels of TNF-α can stimulate the function and number variation of IL-2 and IL-6 cytokines appear, thus cytokine network balance is interrupted and a variety of inflammatory damage factors give cascade reaction, which is characterized by release of pro- as IL-2 and IL-6 and anti-inflammatory cytokines as IL-10, resulting in inflammatory response and further aggravating and prompting ulcer worsening[11]. Chemotactic effect of TNF-α can strengthen the phagocytosis of the monocyte and neutrophil, which drives a variety of chemokines releasing as cytokines, oxygen free radicals and elastins. As a result, issue factor was destroyed and the oral mucosal injury occurred[12].

Using correlation analysis to further investigate the effects of TNF-α on immune function, the study results showed that serum TNF-α and IL-2, IL-6 and CD4+/CD8+ was positively correlated (P<0.05), suggesting that TNF-α and ROU immune imbalance are closely related, by which acting on inflammatory cells, cytokines and adhesion molecules to exacerbate the body's inflammatory response and to aggravate ulcerative diseases. However, TNF-α and IL-2, IL-6 and other pro-inflammatory factors have similar biological profile and can construct into complex cytokine network each other, thus the only blocking of TNF-α can not improve the body immune response. As a result, more investigations should be made on overall cytokine network so as to effectively inhibit the effects of inflammatory cytokines on the body immune response.

In summary, the ROU may be associated with patients’ immune dysfunction. And TNF-α determination can reflect body immune functions, which is beneficial for prognosis confirmation.

References


