Detection and clinical significance of rheumatoid factor, anti–CCP antibody, immunoglobulin and cytokines in serum of patients with rheumatoid arthritis

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Abstract

Objective: To detect the content of rheumatoid factor, ring citrulline peptide antibody (CCP), immunoglobulin (Ig) and cytokines in serum of patients with rheumatoid arthritis (RA) and explore the clinical application value of serum indexes for RA diagnosis, treatment and prognostic.

Methods: A total of 98 patients with RA were selected, of which 52 cases in the activity and 46 patients in remission, 65 healthy people as controls. Contents of serum RF and Ig (IgM, IgG, IgA) in every group were detected by methods of immune scattering turbidimetry respectively; the content of serum anti-CCP antibody and cytokines IL-1β, TNF-α, IFN-γ and IL-4 were measured by methods of enzyme-linked immunosorbent respectively. Change of detection index levels between groups were compared and the correlation between cytokines and RF, anti CCP, Ig and DAS28 score in patients with RA were analyzed.

Results: The contents of serum RF, CCP antibody, Ig (IgM and IgG, IgA) of RA group (activity and remission) were respectively significantly higher than that of the control group (P<0.05, or P<0.01). The contents of serum levels of IL-1β, TNF-α, IFN-γ and IL-4 of RA group (activity and remission) were respectively significantly higher than that of the control group (P<0.05, or P<0.01). The intra-group comparison between activity group and remission group in RA showed no significant difference in IL-4 level (P>0.05). The levels of cytokine IL-1β, TNF-α, IFN-γ were positively correlated with RF, CCP antibody, Ig and DAS28 scores in RA activity while which were only positively correlated with DAS28 scores of RA in remission, and the IL-4 level had no significant correlation with other indicators.

Conclusions: The detection of levels of RF, CCP antibody, Ig and cytokine for the diagnosis, treatment and prognosis of RA has important reference value.

1. Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease, the incidence is 0.5%-1%[1]. The commonly used laboratory examination index of RA is mainly RF and its role in the diagnosis of RA was limited by the low sensitivity and specificity[2]. Foreign research shows that the sensitivity and specificity of anti CCP antibody and Ig in diagnosing RA is relatively high[3,4]. This study detected the serum levels of RF, anti- CCP antibody, Ig and cytokines in patients with RA to analysis the relation between indexes and their relevance with RA disease activity and further discussed the function of the index levels in RA diagnosis, disease progression monitoring and prognosis.

2. Materials and methods

2.1. General information

All 98 RA patients (38 male, 60 female) were admitted in our hospital from May 2013 to February 2015 with the average age of (43.6±9.7) years, the condition judgment indicators were as follows:
Swollen joint count $\geq 6$; Tender joint count $\geq 6$; Morning stiffness duration $\geq 45$ min; ESR (westergren method) $\geq 28$ mm/h, and the diagnostic criteria were according to the RA classification standards of the American Rheumatism Association in 1987\(^5\), excluding diabetes, high blood pressure, cancer, kidney disease, acute infections and other autoimmune diseases, etc. The DAS28 score was used as the assessment of disease activity in patients with RA: 
\[
\text{DAS28} = 0.56 \times \text{SQRTr} (T28) + 0.28 \times \text{SQRTr} (SW28) + 0.70 \times \ln (\text{ESR}) + 1.08 + 0.16.
\]
T28 represented double proximal interphalangeal joints, interphalangeal joint, wrist, elbow, shoulder joint and knee joint for a total of 28 joint tenderness or passive activity of tenderness count; SW28 said the number of 28 of swollen joints above, DAS28 ranged from 0-10, the higher the score, the higher the prompt disease activity. According to the standard, there were 52 RA patients in active stage while 46 patients with RA in remission. An alternate 65 healthy eligible passed the physical examination were selected as the control group (21 male and 43 female), average age (39.8±10.5) years. There was no significant difference among the general information including gender and age of the research subjects, and the dates was comparable ($P>0.05$).

2.2. Sample preparation

Limosis vein blood of subjects of groups were extracted and placed for 2 h at room temperature, centrifugated at 1 000 \( \times \) g for 20 min, serum was stored at -20 \( ^\circ \)C for later use.

2.3. Observation indexes and test methods

RF, anti-CCP antibody, Ig (IgM and IgG, IgA), cytokines, joint swelling and joint tenderness were detected. Contents of serum RF and Ig (IgM, IgG, IgA) in every group were detected by methods of immune scattering turbidimetry respectively, and the contents of serum anti-CCP antibody and cytokines IL-1\( \beta \), TNF-\( \alpha \), IFN-\( \gamma \), IL-4 by methods of enzyme-linked immunosorbent respectively.

2.4. Statistical analysis

In the data analysis process, SPSS17.0 statistical software was used for the statistical analysis, and data were presented as mean±standard deviation, while the comparison between the two groups were performed by using $t$-tests and correlation analysis by using Pearson analysis, $P<0.05$ showed significant difference.

3. results

3.1. Comparison of levels of serum RF and anti CCP antibody of each group

Compared with control group, the serum levels of RF and anti CCP antibody at different condition time in RA patients were significantly elevated ($P<0.05$, or $P<0.01$), and the increase in RA patients in activity were more significant, as shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>RF</th>
<th>Anti-CCP antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA in the activity</td>
<td>52</td>
<td>33.39±13.12*</td>
<td>29.40±6.24*</td>
</tr>
<tr>
<td>RA in remission</td>
<td>46</td>
<td>13.01±5.14*</td>
<td>17.68±1.68*</td>
</tr>
<tr>
<td>The control group</td>
<td>65</td>
<td>7.49±3.52</td>
<td>4.99±1.30</td>
</tr>
</tbody>
</table>

Note: compared with the control group, *$P<0.05$, **$P<0.01$.

3.2. Comparison of levels of Ig of each group

Compared with the control group, the levels of three types of serum Ig in the different periods of the illness in RA patients were significantly elevated ($P<0.05$, or $P<0.01$). The change of IgG levels between RA group and the control group, RA activity and remission group were most significant. And the IgG level was the highest in serum immunoglobulin, as shown in Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>IgM</th>
<th>IgG</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA in the activity</td>
<td>52</td>
<td>18.82±3.70</td>
<td>25.72±1.20*</td>
<td>5.87±0.88*</td>
</tr>
<tr>
<td>RA in remission</td>
<td>46</td>
<td>12.49±4.80*</td>
<td>17.73±1.41*</td>
<td>2.05±1.04*</td>
</tr>
<tr>
<td>The control group</td>
<td>65</td>
<td>8.90±3.51</td>
<td>11.93±4.38</td>
<td>1.31±0.81</td>
</tr>
</tbody>
</table>

Note: compared with the control group, *$P<0.05$, **$P<0.01$.

3.3. Comparison of levels of serum cytokine of each group

The levels of serum cytokine IL-1\( \beta \), TNF-\( \alpha \), IFN-\( \gamma \) and IL-4 in RA patients (activity and remission) were significantly higher than that of the control group ($P<0.05$, or $P<0.01$). The levels of cytokine IL-1\( \beta \), TNF-\( \alpha \) and IFN-\( \gamma \) were increased more significantly in the
activity (P<0.05), while change in IL-4 level within the group was not significant (P>0.05) (Table 3).

3.4. Relativity analysis of cytokines and the rest of the indicators

The analysis showed that the levels of cytokines IL-1β, TNF-α, IFN-γ of RA patients in the activity were positively correlated with RF, anti CCP antibody, Ig and DAS28 score (P<0.05, or P<0.01). While they were only positively correlated with DAS28 score in remission (P<0.05, or P<0.01), and IL-4 levels had no significant correlation with other indicators (P>0.05), as shown in Table 4.

4. Discussion

RA is a chronic, progressive and autoimmune disease mostly affects the joints, the main pathological characteristics of which is continuous and progressive synovitis, and the disease can in turn lead to cartilage injury and joint bone erosion, the clinical manifestations are joint swelling, tenderness and stiff in the activity, and the jaw joints can also be affected, the disease could lead to joint rigidity, deformity and dysfunction by severe bone destruction and absorption in the late period[6,7].

The early diagnosis and standard treatment of RA is very important to slow disease progression and improve prognosis. The current generic RA clinical diagnostic criteria is the 1987 years ACR revision standard mainly based on the clinical manifestations, X-ray plain film and serum index RF[8]. But due to the fact that RF not only exists in RA patients, but also expresses positively in the serum of other autoimmune diseases patients except RA, so the RF negative doesn’t rule out RA, and the separate RF positive was not enough to diagnosis of RA, and the high volatility and unpredictability course of RA condition makes the sensitivity and specificity of the disease indexes higher[9,10]. An anti-CCP antibody is an early diagnostic marker with high specificity of RA, which have been classified into 2010 ACR - EULAR RA diagnosis standard. Research shows that the sensitivity of anti -CCP antibody was 65%–80%, and the specificity is up to 90%[11-13]. Ig (IgM and IgG, IgA) is the main antibody involved in humoral immunity, and the humoral immune disorder is one of the important links result in the pathogenesis of RA. Elevated levels of serum Ig has important reference value in the clinical diagnosis of RA, and the rise level of which is closely related to the degree of progression of RA[14,15]. According to the results of this study, compared with the healthy people, the levels of serum RF, anti-CCP antibody and Ig in patients with RA increased significantly, the elevated degree of each index levels of RA patients in the activity is more obvious than that of RA patients in remission, which showed that the RF, anti-CCP antibody and Ig are all involved in the pathogenesis process of RA.

Cytokines are extracellular glycoprotein molecules produced or secreted to the outside of the cell by the body's immune cells and non-immune cells, which play an important regulating role in RA disease process[16,17]. This study compared the levels of serum cytokines of patients with RA and healthy crowd, the result showed that the levels of serum IL-1β, TNF-α, IFN-γ and IL-4 of RA patients were higher than that of the control group obviously, explains that cytokines IL-1β, TNF-α, IFN-γ and IL-4 are generally involved in pathological process of RA, the cytokines levels were also different at different condition time of RA. The levels of IL-1β, TNF-α, IFN-γ of patients in the activity were significantly higher than that of patients in remission, and IL-4 levels rising in remission with no significant difference. This may be due to the fact that Th1/Th2 cytokine imbalance is the main pathogenesis of RA patients, Th1 cells and their secretion of cytokines IFN-γ is given priority to cause a series of inflammatory reaction and the increase of the secretion of inflammatory cytokines IL-1β and TNF-α, while Th2 mainly secreted IL-4 and played an anti-inflammatory role in inhibiting the release of inflammatory cytokines IL-1β, TNF-α[18,19]. There exists a large number of IFN-γ in active RA patients body, and the rise of the level of anti-inflammatory mechanism IL-4 is not enough to fully control the progress of the inflammation, which result in a obvious rise ratio of the Th1/Th2 and promote the deterioration and persistent of the inflammation[20]. The results further suggest that cytokines may play an important role in the pathogenesis of RA.

RF, anti-CCP antibody and Ig are not only the indexes of early diagnosis of RA, but also are closely associated with RA illness development[21]. The evaluation of RA condition is usually given priority to DAS28 standards, and through analyzing the correlation between cytokines and RF, anti-CCP antibodies, Ig and the DAS28 score, it shows that cytokines IL-1β, TNF-α, IFN-γ were positively correlated with RA, anti-CCP antibody, Ig and DAS scores, while they were only positively correlated with the DAS28 scores in the remission, and the IL-4 level had no significant correlation with the rest of the indicators. Therefore, considering the change of RF level, the rise of levels of IL-1β, TNF-α and IFN-γ could be evaluated as reference index of RA illness development.

To sum up, the joint detection of serum index RF, anti-CCP antibody, Ig and cytokine IL-1β, TNF-α, IFN-γ has important reference value on the early diagnosis of RA, evaluation of illness development degree and prognostic evaluation.

References


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