Research Article

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Evaluation of the relationship between TNFα, sTNFR1, sTNFR2, sIL2R, IL6, neopterin with disease activity in ankylosing spondylitis

Ankilozan spondilitli hastalarda hastalık aktivitesi ile TNFα, sTNFR1, sTNFR2, sIL2R, IL6, neopterin arasındaki ilişkisinin değerlendirilmesi

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Abstract

Objective: We aimed to evaluate the relationship among TNFα, sTNFR1, sTNFR2, sIL2R, IL6, neopterin and disease activity in ankylosing spondylitis (AS).

Materials and methods: TNFα, sTNFR1, sTNFR2, sIL2R, IL6 and neopterin were measured in patients and controls. Patients were grouped according to disease activity and medication.

Results: Neopterin and sTNFR1 were not different while TNFα, sTNFR2, sIL2R and IL6 were high in patients than controls. There was no difference between active and inactive patients for TNFα, sIL2R and IL6. sTNFR2 was significantly lower in active patients. There was no relationship between CRP positivity and disease activity. AS patient groups are; 1: TNF blockers, 2: nonsteroidal anti-inflammatory drugs (NSAIDs), 3: disease modifying anti-rheumatic drugs (DMARDs), 4: TNF blockers and NSAIDs, 5: DMARDs and NSAIDs. sTNFR2 was significantly lower in active patients than in inactive, in Group 1. ESR levels were significantly lower in inactive patients than in active, in Group 1. ESR levels were significantly lower in inactive patients compared to active in group 3 and 4. There was no significant association between CRP positivity and disease activity.

Conclusion: According to our study, CRP is insufficient in evaluating AS disease activity. ESR can be useful in evaluating the disease activity. sTNFR2 might be useful as a biological indicator of disease activity in AS treated with TNF inhibitors alone.

Keywords: Ankylosing spondylitis; Neopterin; sTNFR; sIL2R; IL6.

Özet

Amaç: Ankilozan spondilitte (AS) TNFα, sTNFR1, sTNFR2, sIL2R, IL6, neopterin ve hastalık aktivitesi arasındaki ilişkisini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Hasta ve kontrol grubunda TNFα, sTNFR1, sTNFR2, sIL2R, IL6 ve neopterin düzeyleri ölçülü. AS’li hastalar hastalık aktivitesine ve kullanılsa ilaçları göre gruplandırıldı.

Bulgular: TNFα, sTNFR2, sIL2R ve IL6 düzeyleri hasta grubunda kontrol grubuna göre yüksek iken neopterin ve sTNFR1 düzeyleri farklı değildi. Aktif ve inaktif hastalar arasında TNFα, sIL2R ve IL6 düzeylerinde farklılık yoktu. Aktif hastalarda sTNFR2 anlamlı olarak düştü. CRP pozitifliği ve hastalık aktivitesi arasında ilişki yoktu. AS hasta grupları; 1: TNF blokerleri, 2: nonsteroidal anti-inflamatuvar ilaçlar (NSAID’ler), 3: hastalık modifye edici antiromatizmal ilaçlar (DMARD’ler), 4: TNF blokerleri ve NSAID’ler, 5: DMARD’ler ve NSAID’ler idi. Grup 1 hastalarda sTNFR2 düzeyi aktif hastalarda inaktif gruba göre anlamlı olarak düştü, ESR düzeyi grup 3 ve 4 hastalarındaki inaktif hastalarda aktif hastalara göre anlamlı olarak düştü. CRP pozitifliği ve hastalık aktivitesi arasında anlamlı ilişki yoktu.

Sonuç: Çalışmamızın verilerine göre AS hastalık aktivitesini değerlendirdiğinde CRP düzeyi yetersizdir. ESR, hastalık aktivitesini değerlendirdiğinde yararlı olabilir. sTNFR2 düzeyi tek başına TNF inhibitörleri ile tedavi edilen AS’li hastalarda hastalık aktivitesinin biyolojik bir belirteci olarak faydalı olabilir.
Anahtar Kelimeler: Ankilozan spondilit; Neopterin; sTNFR; sIL2R; IL6.

Introduction

Ankylosing spondylitis (AS) is a disabling form of seronegative spondyloarthritis that primarily affects axial skeleton and sacroiliac joint. The knowledge about AS pathogenesis is limited [1, 2]. Tumor necrosis factor (TNF) α plays a crucial role in several mechanisms causing inflammatory diseases like rheumatoid arthritis and other types of arthritis and also AS [3, 4]. TNFα is predominantly produced by active macrophages and T-lymphocytes and coordinates the pre-inflammatory signals [3, 5]. TNFα binds to two receptors; tumor necrosis factor receptor 1 (TNFR1) and tumor necrosis factor receptor 2 (TNFR2) [6, 7]. TNF receptors exist not only as bound but also as soluble molecules (sTNFR1 and sTNFR2). These soluble molecules float freely in serum and can bind to TNFα as natural TNFα antagonists. It is thought that sTNFR1 and sTNFR2 modulate the activity of TNFα in inflammatory reactions [8–10]. Interleukin (IL) 6 is a cytokine that plays role in the pathogenesis of arthritis [11]. IL6 is considered to be associated with the pathogenesis of AS [12–14]. However, the potential role of IL6 as a serum biomarker for disease activity and severity of AS have not been established [15]. IL2 is a potent immunomodulatory cytokine that activates cells of the immune system. Biological effects are mediated through cell surface receptors. There are studies showing that soluble interleukin 2 receptor (IL2R) increases in rheumatic diseases such as AS and RA [16, 17]. The elevation of serum levels of sIL2R in patients with AS compared to healthy individuals suggests that IL2 may affect various AS findings [18]. Neopterin is a marker associated with the activity of monocytes/macrophages. Neopterin level provides information about cell-mediated immune response status, as well as monitoring of rheumatologic disease progression and prognosis. Elevated levels of neopterin have been observed in rheumatoid arthritis [19, 20]. It has been shown that there is a relationship between disease activation and neopterin levels in systemic lupus erythematosus patients [20]. The use of new therapeutic treatments in AS has made disease activity monitoring even more important. The evaluation and follow-up of AS disease activity is commonly assessed according to Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). This index is obtained with the values of a visual analogue scale that evaluates fatigue, axial pain, peripheral pain, enthesitis, duration and intensity of morning stiffness. BASDAI does not use any marker of inflammatory activity in its calculation. Levels of CRP and ESH are less useful in monitoring ankylosing spondylitis disease activity than they are in other inflammatory conditions such as rheumatoid arthritis [21–23]. So, the disease activity is determined by the subjective evaluation of the patients [24, 25]. For this reason, we aimed to evaluate the association of TNFα, sTNFR1, sTNFR2, IL6, sIL2R and neopterin levels with disease activity in AS patients.

Materials and methods

Patient and control group

The number of patient and control group participants was defined with power analysis method. Acute or subacute viral/bacterial infection, autoimmune disease except for AS, vascular disease, thrombotic disease, malignity, diabetes mellitus, hypertension, chronic renal insufficiency, pregnancy and being on the age below 18 or above 65 years old were accepted as the exclusion criteria. We studied on a total of 160 patients between 18 and 65 ages whose 91 were male and 69 were female. They had a diagnosis of AS at Pamukkale University Medical Faculty Research and Application Hospital Rheumatology Department, according to Modified New York diagnosis criteria. The control group was comprised of 80 healthy persons with similar age and sex as the patient group, and having no clinical symptoms and findings.

Clinical and biochemical measurements

We drew 8–12 h fasting venous blood samples into a vacuum gelled and sedimentation tubes from all the participants between 8.30 and 10.30 in the morning. Serum levels of CRP were measured on a Cobas 8000 auto-analyzer (Roche-Hitachi Diagnostics, Japan) by immunoturbidimetric method (the minimum detectable concentration (MDC) and reference range were 0.1 mg/dL and <0.5 mg/dL, respectively). ESR levels were measured with Westergren method (reference range for ESR is <29 mm/h). Serum sIL2R levels were measured on Immulite 2000 automated analyzer (Diagnostic Products, Los Angeles, CA). Reportable range and analytical sensitivity sTNFR1 is 50–7500 U/mL and 5 U/mL respectively. Serum TNFα (Invitrogen, USA) levels were determined by sandwich ELISA method (MDC is <2 pg/mL).
sTNFR1 (Invitrogen, USA) and sTNFR2 (Invitrogen, USA) levels were determined by enzyme amplified sensitivity immunoassay method (MDC is 50 pg/mL and 0.1 ng/mL respectively). IL6 (Invitrogen, USA) (MDC is <2 pg/mL) and neopterin (DRG International, USA) (sensitivity is 0.7 nmol/L) levels were measured with ELISA method.

We applied the BASDAI questionnaire to the patient group in order to determine the disease activity. The patients whose BASDAI level was >4 were accepted as active AS [24].

AS patients were grouped according to the medication they used; Group 1: TNF blockers, Group 2: Nonsteroidal anti-inflammatory drugs (NSAIDs), Group 3: Disease Modifying Anti-Rheumatic Drugs (DMARDs), Group 4: TNF blockers and NSAIDs, Group 5: DMARDs and NSAIDs.

### Statistical analysis

All analyses were performed using SPSS software v17.0 (IBM, Chicago, USA). Normality of variables was assessed by Kolmogorov-Smirnov test. The differences between parameters which are not normally distributed were analyzed with Mann-Whitney U test, and were defined as median (1. and 3. quartiles). The difference between the variants which do not provide the parametric conditions among the active AS, inactive AS and control group was analyzed with Kruskal-Wallis test. In order to decide from which groups the difference was caused by, we applied Bonferroni corrected Mann-Whitney U test. Between-group comparison of nominal variables was done using chi-square analysis.

The power of the correlation among the variants was realized with Spearman correlation test as the distributions were non-parametric. In the correlation analysis, rho (correlation co-efficient) value between 0.000 and 0.49 was accepted as weak correlation, between 0.50 and 0.69 as medium correlation and ≥0.70 as strong correlation. Any p value <0.05 were considered to be significant.

### Results

The patient and control groups were similar for age and sex. Median (1.–3. quartiles) age in patient and control groups were 38 (30–45) and 39.5 (32.25–44.75), respectively. When the patients were grouped as active and inactive, median (1.–3. quartiles) age was 38 (32–44) and 38 (28–48), respectively.

TNFα, sTNFR1, sTNFR2, sIL2R, IL6, neopterin levels of patient and control groups were shown on Table 1. TNFα, sTNFR2, sIL2R, IL6 levels were found to be significantly higher in patient group than the control group (p = 0.0001, in all) (Figure 1). There was not a significant difference between groups in neopterin (p = 0.708) and sTNFR1 (p = 0.463) levels.

When the patients were grouped as active and inactive and compared to the control group, TNFα, sTNFR2, sIL2R and IL6 levels showed a significant difference among the three groups (Table 2).

When the active and inactive patients were compared, sTNFR2 (p = 0.042) levels were significantly low in active AS patients as compared to the inactive AS patients. TNFα (p = 0.095), sIL2R (p = 0.821) and IL6 (p = 0.846) levels were not observed significantly different (Figure 2). ESR (p = 0.0001) and CRP (p = 0.041) levels were measured to be significantly high in active patients compared to inactive ones. There was no relationship between CRP positivity and disease activity according to the reference range (Table 3).

In our study 155 of 160 AS patients were receiving treatments. When AS patients were grouped according to the medication they used; sTNFR2 (p = 0.021) level in Group 1 was significantly lower in active patients than in inactive patients. There was no significant difference in other

### Table 1: The levels of the parameters measured in AS patients and controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group (n=80)</th>
<th>AS patients (n=160)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>1. and 3. quartiles</td>
</tr>
<tr>
<td>TNFα (pg/mL)</td>
<td>13.11</td>
<td>11.98–16.28</td>
</tr>
<tr>
<td>sTNFR1 (ng/mL)</td>
<td>1.55</td>
<td>1.23–2.02</td>
</tr>
<tr>
<td>sTNFR2 (ng/mL)</td>
<td>3.75</td>
<td>3.01–4.78</td>
</tr>
<tr>
<td>sIL2R (U/mL)</td>
<td>309</td>
<td>245–387</td>
</tr>
<tr>
<td>IL6 (pg/mL)</td>
<td>2.3</td>
<td>1.64–2.74</td>
</tr>
<tr>
<td>Neopterin (ng/mL)</td>
<td>1.16</td>
<td>0.89–1.32</td>
</tr>
</tbody>
</table>

*p = 0.0001 against control group.
groups. TNFα, sIL2R, IL6 levels were not significantly different between active and inactive patients in all groups. ESR levels were significantly lower in inactive patients compared to active patients (p = 0.01 and p = 0.041, respectively) in group 3 and 4. In the other groups, there was no significant difference in ESR levels. There was no significant difference in CRP levels between active and inactive patients in all groups (Table 4). When evaluated according to the reference range, there was no significant association between CRP positivity and disease activity in all groups.

When the correlation between TNFα, sTNFR2, sIL2R, IL6 and CRP of the patients was evaluated, we found a weak correlation of IL6 (r = 0.451, p = 0.0001) and sIL2R (r = 0.173, p = 0.029) with CRP. We did not find a correlation between TNFα (p = 0.339) and sTNFR2 (p = 0.501) with CRP. When the correlation of TNFα, sTNFR2, sIL2R,
IL6 and ESR was evaluated, we observed a weak positive correlation between IL6 ($r = 0.297$, $p = 0.0001$) with ESR. We did not observe a correlation between TNFα ($p = 0.085$), sTNFR2 ($p = 0.716$) and sIL2R ($p = 0.748$) with ESR (Table 5).

### Discussion

Disease activity and outcome cannot be measured by a single variable in most rheumatologically disorders. The opinion about disease activity and outcome is based on different sources of information, such as patient complaints, clinical variables, laboratory variables (acute phase proteins) in clinical practice. There is no ‘gold standard’ to assess disease activity in patients with AS. It is known that patients and physicians have different opinions about disease activity [26]. The method commonly used to assess and monitor disease activity in AS patients is BASDAI [24]. Although they are inadequate to evaluate AS disease activity, the most commonly used acute phase proteins are ESR and CRP [22, 23]. There is no objective biomarker to assess disease activity. For this reason, we aimed to evaluate the association of TNFα, sTNFRI, sTNFR2, IL6, sIL2R and neopterin levels with disease activity determined according to BASDAI.

According to our study results, sIL2R and IL6 levels were significantly higher in AS patients than in the control group. No significant difference was observed in sIL2R and IL6 levels in active AS patients compared to the inactive ones. Bal et al. [17], Taylan et al. [27] and He et al. [15] reported similar results in their study.

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### Table 3: ESR and CRP levels in active and inactive AS patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inactive AS ($n = 91$)</th>
<th>Active AS ($n = 69$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median &amp; 1. and 3. quartiles</td>
<td>Median &amp; 1. and 3. quartiles</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>12 &amp; 7–25</td>
<td>22* &amp; 11–33</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.25 &amp; 0.08–0.96</td>
<td>0.62* &amp; 0.16–1.16</td>
</tr>
</tbody>
</table>

*p < 0.05 against inactive AS patients.
Cellular immune system plays an important role in the pathogenesis of the autoimmune diseases. In the conducted studies, it has been shown that neopterin levels increased in relation to the activity of the disease and it can be used as an activation determiner in RA and SLE patients [19, 28, 29]. There are limited data on controlled comparison of serum neopterin levels of AS patients and on clinical and laboratory findings in the literature. Zengin et al. [30] found the neopterin levels of AS patients significantly higher compared to the control group but neopterin, ESR and CRP levels of active and inactive patients showed no difference. It has been concluded that neopterin, just like ESR and CRP, do not predicts the AS clinic clearly. In our study, neopterin levels were not significantly different between AS patients and the control group. We think that this situation may be a consequence of the fact that the majority of our patient group is under medication. For this reason, we also conclude that neopterin levels are inadequate in determining disease activity in AS patients receiving treatment. ESR and CRP levels were significantly high in active patients compared the inactive ones. When CRP and ESR levels were evaluated according to the reference interval, there was no significant association between CRP positivity and disease activity. In routine practice, CRP and ESR levels are evaluated according to the reference interval. For this reason, CRP and ESR markers are insufficient to evaluate disease activity according to our data. Furthermore, there was no or weak correlation between ESR and CRP and inflammatory markers in our study (Table 5).

Table 4: TNFα, sTNFR1, sTNFR2, IL6, sIL2R, ESH and CRP levels of active and inactive AS patients grouped according to their medication.

<table>
<thead>
<tr>
<th>Drug groups</th>
<th>Disease activity</th>
<th>Median (25–75th percentile)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNFα (pg/mL)</td>
<td>sTNFR2 (ng/mL)</td>
</tr>
<tr>
<td>Anti TNFα</td>
<td>Inactive</td>
<td>20.5</td>
</tr>
<tr>
<td></td>
<td>Active</td>
<td>18.9</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Inactive</td>
<td>18.4</td>
</tr>
<tr>
<td></td>
<td>Active</td>
<td>13.6</td>
</tr>
<tr>
<td>DMARDs</td>
<td>Inactive</td>
<td>11.6–18.1</td>
</tr>
<tr>
<td></td>
<td>Active</td>
<td>14.2</td>
</tr>
<tr>
<td>Anti TNFα + NSAIDs</td>
<td>Inactive</td>
<td>11–16.4</td>
</tr>
<tr>
<td></td>
<td>Active</td>
<td>22.5</td>
</tr>
<tr>
<td>DMARDs + NSAIDs</td>
<td>Inactive</td>
<td>17–33</td>
</tr>
<tr>
<td></td>
<td>Active</td>
<td>31.4</td>
</tr>
</tbody>
</table>

*<p value< 0.05 against active patients group.

NSAIDs, nonsteroidal anti-inflammatory drugs; DMARDs, disease modifying anti-rheumatic drugs.

Table 5: The correlation between CRP and ESR with serum cytokine levels.

<table>
<thead>
<tr>
<th></th>
<th>TNFα</th>
<th>sTNFR2</th>
<th>sIL2R</th>
<th>IL6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESH (mm/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05.
sTNFR2 level was significantly lower in active patients than in inactive patients. TNFα level was not significantly different between active and inactive patients. Huang et al. [31] found the TNFα levels of AS patients significantly high compared to the controls. Lange et al. [32] and Bal et al. [17] also had similar results. Sveaas et al. [33] found that sTNFR1 and sTNFR2 levels were significantly higher in AS patients than control, but did not find significant differences between disease activity and sTNFR receptors.

The treatment of AS varies according to the clinical characteristics of the patients [34]. The presence of a BASDAI score ≥4 suggests that the control of the disease is suboptimal. In these situations, there are approaches such as changing the treatment or addition of new drugs. In the study of Schulz et al. [35] sTNFR1 levels were higher in AS patients than healthy controls. sTNFR2 levels were not significantly different between AS patients and the healthy controls. The levels of sTNFR1 were significantly lower in AS patients than baseline after treatment. There was no significant difference in sTNFR2 level after Infliximab treatment. In our study, we grouped AS patients according to the medication they used and found that sTNFR2 levels were significantly lower in active patients than in inactive patients in group 1. In the study of He et al. [15], the level of TNFα was not significantly different between AS patients before treatment and the control group. TNFα levels were found to be significantly high after treatment with TNFα blocker than baseline, and there was no significant relationship between BASDAI and TNFα and IL6. In another study of AS patients who received TNF blocker therapy, there was no correlation between disease activation parameters such as morning back stiffness, Bath Ankylosing Spondylitis Functional Index, Modified Enthesopathy Index, IL6 and TNFα. Similar to these studies, we also found that TNFα and IL6 levels in our study were not significantly different between active and inactive patients in all groups [36].

In our study, ESR levels were significantly higher in active AS patients than inactive patients in group 3 and 4. ESR levels were not significantly different between active and inactive patients in groups 1, 2 and 5. When we evaluated the CRP levels according to the reference interval, there was no significant association between CRP positivity and disease activity in all groups (p > 0.05 in all groups).

According to our study, CRP test used in routine practice is insufficient in evaluating AS disease activity. ESR test can be useful in evaluating the disease activity if drugs used by patients are taken into consideration. The measurement of sTNFR2 level might be useful as a biological indicator of disease activity in AS patients treated with TNF inhibitors alone.

References


