Elevated serum interleukin-23 levels in ankylosing spondylitis patients and the relationship with disease activity

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ABSTRACT

This study was aimed to evaluate the relationship between serum interleukin-23 (IL-23) levels and ankylosing spondylitis (AS). Twenty male patients diagnosed with ankylosing spondylitis according to the 1984 modified New York criteria for AS and twenty male healthy controls were included in this study. The demographic characteristics, clinical and laboratory findings of the patients were recorded. Serum IL-23 levels, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured in both the AS and control groups. The Bath ankylosing spondylitis disease activity index (BASDAI), the Bath ankylosing spondylitis functional index (BASFI), and the Bath ankylosing spondylitis metrology index (BASMI) were evaluated as disease activity parameters. The AS patients were divided into two subgroups as active and inactive in respect of CRP, ESR levels and BASDAI scores. The mean serum IL-23 levels of the AS and control groups were $334.45 \pm 176.54$ pg/ml and $166.49 \pm 177.50$ pg/ml respectively, and there was a significant difference between the groups. Correlation analysis of serum IL-23 levels with clinical and laboratory parameters showed that there were positive correlations between serum IL-23 levels and the BASDAI, BASFI scores in total, active and inactive patients and the BASMI scores in total and inactive patients and negative correlations between serum IL-23 levels and ESR in inactive patients. It was shown that altered serum IL-23 levels were related to AS disease activity. Further studies in large patient series are necessary to investigate the role of IL-23 protein in etiopathogenesis of AS.

Key Words: ankylosing spondylitis, disease activity, interleukin-23

INTRODUCTION

Ankylosing spondylitis (AS) is a subcategory and the most notable part of spondyloarthritis (SpA) and is an auto-immune-related chronic inflammatory disease which is characterized by a diverse spectrum of clinical manifestations, including axial skeletal ankylosis, inflammation of the sacroiliac joints, and peripheral inflammatory arthropathy. The exact pathogenesis and the etiology of AS is not fully understood. Complex interactions of environmental factors and immune responses are thought to play a role in the development of disease. Supported by studies on families and twins, it has been known for a long time that AS has a strong genetic component and genetic factors influence the immune responses and the progression of AS. The most

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important evidence of the genetic component of AS is the presence of strong HLA-B27 antigen association with the pathogenesis of the disease. However, since HLA-B27 contribution to AS genetic risk is approximately 16%, other genes apart from HLA-B27 are thought to be involved in the pathogenesis of the disease.\(^5\)

IL-23 is indicated as the necessary mediator for organ-specific autoimmune diseases. It is a heterodimeric cytokine with a specific p19 subunit and a p40 subunit shared with IL-12 which has additional inflammatory effects apart of IL-12.\(^5\) It has been demonstrated that IL-23 can increase and stabilize the Th17 cells in disease models and humans.\(^6\)

Previous studies have suggested that dysregulation of the IL-23/IL-17 axis plays a dominant role in the progression of chronic autoimmune inflammation involving the central nervous system and joints.\(^7\) Since IL-23-deficient rats have been shown to be resistant to experimental autoimmune encephalomyelitis and collagen induced arthritis, the importance of this cytokine in the autoimmune pathogenesis is considerable. Therefore, the IL-23 pathway is thought to be involved in the pathogenesis of AS. The aim of this study was to describe the relationship between IL-23 and AS.

**MATERIALS AND METHODS**

Twenty Caucasian male AS patients who were admitted to our outpatient clinic and met the modified New York criteria\(^8\) for the diagnosis of AS were enrolled in the study. A detailed general health and medication history of patients was recorded to include appropriate subjects and exclude irrelevant subjects. Exclusion criteria were chronic systemic disease, other rheumatic diseases, infections or malignant tumors, usage of steroids, cytotoxic drugs and immunosuppressive agents.

The control group was composed of twenty age-matched healthy male who had normal physical examination and routine test results and had no chronic or endocrinological diseases. The disease activity of the AS patients was evaluated using the Bath ankylosing spondylitis disease activity index (BASDAI)\(^9\), the Bath ankylosing spondylitis functional index (BASFI)\(^10\) and the Bath ankylosing spondylitis metrology index (BASMI).\(^11\) Sacroiliac joint radiographs of all patients were taken and sacroiliitis was confirmed by a qualified radiologist. HLA-B27- carriage was identified by flow cytometry.

The demographic characteristics, clinical and laboratory findings were recorded by the same researcher. Serum IL-23 levels were measured in the AS patients and healthy controls using human IL-23 enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, Vienna). Disease activity was assessed by laboratory parameters including ESR and CRP. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured and evaluated as inflammatory markers of AS. Serum CRP levels was measured by the immunonephelometric method (Siemens, Munich, Germany) according to the manufacturer’s instructions and results were expressed as mg/L. ESR was measured by capillary photometry and results were expressed as mm/hour.

The AS patients were divided into two subgroups in respect of CRP, ESR, and BASDAI. Patients with a CRP level >8 mg/L and ESR >20 mm/h and patients with a BASDAI score ≥4 were accepted as active patients, while the others were accepted as inactive patients.\(^12\)

Ethics approval was obtained for this study, informed consent was obtained from each participant and patient anonymity was preserved. The procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.
Statistical analysis

Data were analyzed using the SPSS/PC statistical software package (SPSS, v.20.0 for Windows, SPSS Inc. Chicago). All results were expressed as mean±standard deviation and percentage. The Student’s t test was used to evaluate the significance of differences between groups. Pearson correlation analysis was used to determine the correlations between findings. Values of p<0.05 were considered significant, at 95% confidence interval.

RESULTS

The baseline characteristics of the patients are shown in Table 1. There was no significant difference between age and gender distribution of AS patients and healthy controls (p>0.05) and also between the AS subgroups (p>0.05). The mean age of the patients was 42 years (range, 22–64 years) and the mean disease duration was 8 years (range, 1–36 years). 90% of patients were positive for HLA-B27 and 100% of patients had sacroiliits.

The mean serum IL-23 levels in the patient and control groups were 334.45±176.54 pg/ml and 166.49±177.50 pg/ml, respectively, and there was a significant difference between the groups (p=0.021). Serum CRP levels were significantly higher in the patient group (p<0.01) but the elevated ESR in the patient group was not significant. When active and inactive AS groups were compared, it was observed that there was no significant difference in terms of IL-23 levels but there was a significant difference between CRP levels (p<0.05) and ESR (p<0.01) (Table 1).

| Table 1 Demographic and Clinical Data of the Groups |
|-----------------------------------------------------|
| Controls (n=20) | Total (n=20) | Patients Active (n=6) | Inactive (n=14) |
|-----------------|-------------|-----------------------|-----------------|
| Age, years, mean±SD | 40.2±10.2 | 42.6±10.4 | 44.2±9.8 | 41.9±10.6 |
| Disease duration, years, mean±SD | – | 8.6±6.2 | 8.8±6.5 | 8.5±6.0 |
| No of men/no of women | 20/0 | 20/0 | 6/0 | 14/0 |
| BASDAI score, mean±SD | – | 3.90±1.91 | 4.67±2.06 | 3.57±1.82 |
| BASFI, mean±SD | – | 4.20±1.85 | 4.83±1.72 | 3.86±1.95 |
| BASMI, mean±SD | – | 4.00±1.58 | 4.67±1.50 | 3.64±1.64 |
| HLA-B27 positivity | – | 18 (90%) | 5 (83.33%) | 13 (92.85%) |
| Sacroiliits positivity | – | 20 (100%) | 6 (100%) | 14 (100%) |
| IL-23 (pg/ml), mean±SD | 166.49±177.50 | 334.45±176.54** | 383.24±218.48 | 315.09±158.21 |
| CRP (mg/L), mean±SD | 3.62±2.23 | 21.36±38.35*** | 56.13±58.34 | 6.46±6.60** |
| ESR (mm/hour), mean±SD | 13.5±10.2 | 16.25±20.66 | 37.66±26.07 | 7.07±5.22 *** |

SD, standard deviation; BASDAI, Bath ankylosing spondylitis disease activity index; BASFI, Bath ankylosing spondylitis functional index; BASMI, Bath ankylosing spondylitis metrology index; IL-23, interleukin-23; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Comparisons: *controls vs total patients, **active patients vs inactive patients (Student’s t test); *, p<0.05; **, p<0.01

When correlation analysis was applied to the AS and subgroups, it was seen that the serum IL-23 levels were positively correlated with the BASDAI and BASFI scores of total, active and inactive patients (p<0.01) and the BASMI scores of total and inactive patients (p<0.01).
Correlation analysis of IL-23 between CRP and ESR revealed that IL-23 was only negatively correlated with ESR in inactive patients (p<0.05) (Table 2).

Table 2  Correlation Coefficients (r) of Serum IL-23 Levels with Clinical and Laboratory Findings of Ankylosing Spondylitis in the Patient Group

|          | Patients |
|----------|----------|
|          | Total (n=20) | Active (n=6) | Inactive (n=14) |
|          | r         | r          | r             |
| BASDAI   | 0.954**   | 0.957**    | 0.963**       |
| BASFI    | 0.849**   | 0.835**    | 0.892**       |
| BASMI    | 0.822**   | 0.764      | 0.886**       |
| CRP      | 0.311     | 0.470      | –0.236        |
| ESR      | 0.293     | 0.600      | –0.536**      |

BASDAI, Bath ankylosing spondylitis disease activity index; BASFI, Bath ankylosing spondylitis functional index; BASMI, Bath ankylosing spondylitis metrology index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; *, p<0.05; **, p<0.01

**DISCUSSION**

Ankylosing spondylitis (AS) is a progressive chronic inflammatory disease with unclear pathogenesis, which affects the sacroiliac joints and the spine. Complex interactions of genetic and environmental factors have been indicated to have role in pathogenesis.2) Although the etiopathogenesis of AS is not clearly understood, accumulating data have suggested that elevated peripheral blood levels of some pro-inflammatory cytokines (IL-1β, IFN-γ, TNF-α, IL-6, IL-17 and IL-23) might play a role in AS pathogenesis.13-16) These theses are supported by the evidence that disease symptoms and activity could be released by blocking the aforementioned cytokines.17,18) There have been significant studies conducted on proinflammatory cytokines in AS, which have demonstrated the role of IL-23 in the pathogenesis of the disease. Experimental animal model studies have shown the relationship of IL-23 and SpA progression.19) This relationship has been confirmed by clinical studies reporting elevated serum20), and synovial fluid21) levels of IL-23 in AS patients. These findings have been supported by genetic studies demonstrating that interleukin-23 receptor (IL-23R) has a key role in various chronic inflammatory diseases including AS.22,23) Considering the results of these genetic studies, it has been speculated that IL-23R gene is responsible for genetic predisposition to AS24) and it has been reported to be related with disease symptoms25) in different populations. As a consequence of all these studies, IL-23 is noted as a central cytokine in the pathogenesis of spondylarthritis.26) Previous studies have suggested that dysregulation of the IL-23/Th17 axis plays the main role in some inflammatory and autoimmune diseases including AS.27-29)

Therefore, in this study, the role of IL-23 was investigated in the etiopathogenesis of AS. The mean serum IL-23 levels in the patient and control groups were 334.45±176.54 pg/ml and 166.49±177.50 pg/ml, respectively, and there was a significant difference between the groups (p=0.021). This elevation was significantly related with BASDAI, BASFI, BASMI (p<0.01) suggesting that IL-23 production is stimulated by inflammatory response. BASDAI and BASFI are questionnaires which are widely used to evaluate the effects of AS
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on the quality of life of AS patients. BASDAI reflects the intensity of disease activity and BASFI reflects physical function. BASMI is a metrological index that is used to measure spinal mobility, and higher BASMI scores reflect the severity of the patient’s limitation. Identifying sensitive and specific biomarkers in spondyloarthropathy is a significant field of interest. HLA-B27, which has high sensitivity but low specificity, is currently used for the diagnosis of spondyloarthropathies.

ESR and CRP are the most prominent indicators of inflammation and they have been reported to be increased in patients with AS as well as in patients with active AS. There are also studies underlining that ESR and CRP are not fully sufficient to reflect disease activity and their value in AS clinical trials is limited. The predictive value of CRP and ESR for reflecting disease activity is low due to their low sensitivity and specificity.

In the current study, CRP was significantly higher in AS patients compared to the control subjects but there was no significant difference between the ESR levels of the patients and control group. Spearman correlation analysis showed no significant correlation of IL-23 with CRP and ESR in the patient and control groups (p>0.05). As the patients were divided into active and inactive, only ESR was significantly and negatively related with IL-23, confirming the data that the value of ESR and CRP in AS clinical trials is questionable.

The results of this study revealed that serum IL-23 levels were significantly higher in AS patients compared to the healthy control group and the IL-23 levels were related to disease activity. However, the means by which the dysregulation of the IL-23 pathway plays a role in the inflammatory regulation of AS remains unclear. Future studies to clarify this point may lead to further research considering IL-23 as a potential treatment target, in addition to TNF-α, to reduce peripheral inflammation in AS therapy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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