ORIGINAL ARTICLE

The Effect of Interleukin 23 Levels on Clinical and Laboratory Parameters of Patients with Ankylosing Spondylitis

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ABSTRACT

Objective: *In the present study, we aimed to investigate the relationship between interleukin 23 (IL-23) and the clinical and laboratory parameters in patients with ankylosing spondylitis (AS). Ankylosing spondylitis causes structural and functional inability, particularly in the axial skeleton, and results in the inflammatory lower back pain. At the same time, we aimed to investigate the relationship between IL-23 levels and disease related variables in patients with AS.*

Methods: *A total of 38 patients with AS (33 males and 5 females) and 42 healthy controls (32 males and 10 female) were enrolled in the study. The demographic characteristics of the participants were recorded. As laboratory findings, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and IL-23 values were noted. Bath AS Disease Activity Index, Bath AS Functional Index, Visual Analogue Scale, and AS Quality of Life scales of the patients were measured.*

Results: *The mean age of the AS group and the control subjects was 32.4 ± 7.06 and 30.0 ± 6.24 years, respectively. The ESR, CRP and IL-23 levels were significantly higher in the AS group compared to those of the healthy controls (*$p < 0.001$, $p < 0.013$, $p < 0.012$, *respectively). There was a significant correlation between ESR, CRP, and IL-23 levels in patients with AS (*$r = 0.328$, $p = 0.030$ *and* $r = 0.392$, $p = 0.008$, *respectively). While 12 subjects (31.5%) were positive for peripheral arthritis, 26 patients were negative (68.4%). The IL-23 levels were significantly higher in the group that was positive for peripheral arthritis (*$p < 0.05$*).*

Conclusion: *Interleukin 23 may play a role in the progression and/or pathogenesis of AS and is most likely involved in the joint problems independent of the classic inflammatory response measures.*

Keywords: Ankylosing spondylitis, C-reactive protein, interleukin 23, disease activity.

INTRODUCTION

Spondyloarthropathy (SpA) is a common name given for chronic rheumatologic diseases that have common physiopathological, clinical, radiological, and genetic features. Characteristic symptoms and findings of SpA include chronic inflammatory lumbar pain, peripheral joint arthritis and extra-skeletal involvement (1). The most important component of the disease is ankylosing spondylitis (AS). Ankylosing spondylitis is a chronic, inflammatory rheumatic disease that affects main sacroiliac joints and spine (2, 3). Nowadays, research on the pathogenesis of AS has been focused on defining the main factors and events that develop in the disease as well as inflammatory mediators and regulators of the course of disease.

The Bath AS Disease Activity Index (BASDAI), Bath Ankylosing Functional Index (BASFI) and visual analogue scale (VAS) are commonly used and are valid

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and reliable result scales based on patients' own report. These scales are developed for evaluating a patient's situation in clinical studies and daily clinic practice. Ankylosing spondylitis is a common disease that affects axial skeleton and causes a decrease in the quality of life along with the inadequacy in function structure and low back pain and inflammation.

Interleukin 23 (IL-23) is a heterodimer cytokine member of the IL-12 family. It arises only from its sub-group P19 and IL-12 and their common sub-group P40. Interleukin 23 is released from dendritic cells, B cells and antigen producing cells like macrophages and activated monocytes (4).

Both IL-23 and IL-17 have been documented for their critical roles in the development and maintenance of autoimmune inflammation. For example, they are involved in maintenance and expansion of TH17 cells, which is responsible for many autoimmune diseases and releasing IL-17. The mechanisms whereby IL-23 induces autoimmunity are not completely understood. Interleukin 23 not only synergizes with IL-6 and IL-1 to promote Th17 development, but also stimulates Th17 expansion and prolongs IL-17 production. A growing body of evidence has revealed the possible role of the IL-23/IL-17 axis in the pathogenesis of AS (5–7). Very few studies show that the axis of IL-23/Th17/IL-17 is included in SpA pathogenesis (8, 9). It has been documented that IL-23 levels are increased in the sera of the patients with AS. However, to the best of our knowledge no report has been published that investigated the correlation between IL-23 levels and AS activation (7, 10). The relationship between IL-23 with disease activity and functional status is well known. Thus, we aimed to investigate the relationship between IL-23 and clinical and laboratory parameters in patients with AS.

SUBJECTS AND METHODS

Thirty-eight consecutive patients with the diagnosis of AS were referred to the Medical Faculty, Department of Physical Rehabilitation, Dicle University, between December 15, 2013 and March 30, 2014 and were evaluated. All patients were suffering from chronic low back pain. The diagnosis of AS was confirmed according to the modified New York Criteria (11). As a control group, 42 healthy individuals that accepted the study protocols were included. In all patients and control subjects, informed consent was obtained prior to examinations. Venous blood samples were taken from antecubital veins of the participants for laboratory tests. The study was approved by the local ethic committee with a number 2013/203 and conducted according to the tenets of the Declaration of Helsinki. We also divided the patients into three groups based on drug administration: group 1 = usage of non-steroidal anti-inflammatory drugs (NSAID); group 2 = usage of disease-modifying anti-rheumatic drug (DMARD) + NSAID; and group 3 = usage of anti-tumour necrosis factor + DMARD.

The demographic characteristics such as age, gender, height, weight, educational level and family history of all participants and the duration of the disease, medication used before admission, and extra-articular manifestations of patients with AS were recorded.

Patients who do not meet these criteria were accepted as mechanical back pain patients and excluded from the study. Patients with any kind of collagen tissue disorders or any other inflammatory articular diseases, malignancies, diseases of the central nervous system, chronic kidney disease, chronic liver disease, and thyroid diseases besides the AS, and those who were pregnant were excluded from the study.

Assessment of disease activity

Bath AS Disease Activity Index consisted of a 10-cm horizontal VAS used to answer six questions pertaining to the five major symptoms of AS. The symptoms assessed included fatigue, spinal pain, peripheral joint pain or swelling, tenderness, and morning stiffness. The questions were answered on a VAS, anchored with labels 'none' and 'very severe' at either end of the first five items and '0 hour' and '2 or more hours' for the duration of morning stiffness. The mean score of the two items on morning stiffness was considered as one variable. The resulting score was then divided by 5 to give the final BASDAI score (0–10, 12).

Assessment of functional status

Bath AS Functional Index consisted of eight questions on daily activities and two additional questions that assessed a patient's ability to cope with everyday life. Each question was answered on a 10-cm horizontal VAS. Scores on each item ranged from 0 (easy) to 10 (impossible). An average of the 10 items was calculated to obtain the final score, with higher scores indicating greater disability (13, 14).

To define the patient's degree of comfort during the last 48 hours, we used the VAS pain score (VAS; 0 = no pain, 10 = worst possible pain). Patients were asked to answer the question of 'During the past month, how long has your morning stiffness usually lasted from the time you wake up?' Their answers were recorded as minutes.

In the assessment of quality of life, the Turkish version of AS Quality of Life (ASQoL) was used, which was previously modified by Duruöz *et al* (15). AS Quality of Life is composed of 18 yes/no questions. The answer of 'yes' was considered as two points, whereas the answer of 'no' was considered as one point. The sum of the test result was calculated by adding each point gathered from 18 questions together. A high score referred to 'good quality of life'. Complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), IL-23, HLA-B27, and routine biochemistry parameters were studied from the blood taken from antecubital veins of the participants.

Statistical analysis

Statistical analyses were performed using the Statistics Package for Social Sciences software version 18.0 (SPSS Inc., Chicago, IL, USA). The distribution of the data was assessed using the Kolmogorov–Smirnov test. Independent samples *t*-test was used for comparisons demographical and clinical data of patients and control groups. While the data were not normally distributed, a Mann–Whitney *U* test was used for binary comparisons of the groups and a Kruskal–Wallis test was used to test differences between the three sub-groups. Categorical data were analysed with the Chi-squared test. Spearman's correlation was used to determine the strength of the relationship among the laboratory and clinical parameters of the patients.

RESULTS

A total of 38 patients with AS and 42 healthy individuals as a control group were included in the study. The mean age of the AS group and the control subjects was 32.4 ± 7.06 and 30.0 ± 6.24 years, respectively. There was no significant difference in any of the demographic parameters between the groups ($p > 0.05$) (Table 1). The comparison of the laboratory parameters of the patients with AS and healthy controls is indicated in Table 1. The ESR, CRP and IL-23 levels were significantly higher in the AS group compared to those of the control group ($p < 0.001$, $p < 0.013$, $p < 0.012$, respectively).

There was neither a significant difference in the demographic parameters nor laboratory parameters among the three patient sub-groups ($p > 0.05$) (Table 2).

All clinical parameters among the three patient subgroups were significantly different than the duration of morning stiffness (Table 3).

In the correlation analysis, there was a significant correlation between ESR, CRP and IL-23 levels in patients with AS ($r = 0.328$, $p = 0.030$ and $r = 0.392$, $p = 0.008$, respectively). In the AS group, there was no significant correlation between the IL-23 levels and the laboratory or clinical scores other than ESR and CRP. Also, BASDAI scores were significantly correlated with ASQoL, BASFI and VAS pain scores.

Furthermore, patients with AS were divided into two groups according to the presence of peripheral arthritis. While 12 subjects (31.5%) were positive for peripheral

Table 1: Patient and control groups demographics data

	Group	n	Mean ± SD	*p*
Age	Patients	38	32.42 ± 7.06	0.13
	Controls	42	30.02 ± 6.24	
Length	Patients	38	173.0 ± 7.5	0.42
	Controls	42	173.9 ± 6.98	
Weight	Patients	38	73.84 ± 14.07	0.69
	Controls	42	72.16 ± 11.70	
ESR	Patients	38	13.68 ± 1.83	≤ 0.001
	Controls	42	4.12 ± 1.34	
CRP	Patients	38	2.07 ± 3.23	0.013
	Controls	42	0.47 ± 0.14	
IL23	Patients	38	31.08 ± 17.49	0.012
	Controls	42	22.03 ± 17.75	

SD = standard deviation, SEM = standard error of the mean, *p* = probability, ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, IL23 = interleukin 23.

Table 2: Patient demographics acute phase reactant levels in the three different drug groups

	Group	n	Mean ± SD	*p*
Age	1st drug group	8	32.1 ± 7.56	0.99
	2nd drug group	9	32.3 ± 7.92	
	3rd drug group	21	32.5 ± 6.86	
Length	1st drug group	8	168 ± 9.47	0.19
	2nd drug group	9	175 ± 7.49	
	3rd drug group	21	173 ± 7.5	
Weight	1st drug group	8	71.1 ± 11.0	0.77
	2nd drug group	9	75.3 ± 22.0	
	3rd drug group	21	76.4 ± 12.2	
ESR	1st drug group	8	18.5 ± 14.28	0.30
	2nd drug group	9	12.4 ± 10.02	
	3rd drug group	21	12.3 ± 11.65	
CRP	1st drug group	8	3.3 ± 5.46	0.83
	2nd drug group	9	1.22 ± 1.27	
	3rd drug group	21	1.94 ± 2.68	
IL 23	1st drug group	8	19.67 ± 13.88	0.13
	2nd drug group	9	33.30 ± 15.72	
	3rd drug group	21	34.47 ± 18.26	

SD = standard deviation, SEM = standard error of the mean, *p* = probability, ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, IL23 = interleukin 23.

Table 3: Comparison of the clinical parameters among the three patient sub-groups

	Group	n	Mean ± SD	*p*	*p**
Visual analogue scale	1st drug group	8	6.56 ± 1.91	< 0.001	0.74[a]
	2nd drug group	9	6.83 ± 2.17		0.000[b]
	3rd drug group	21	2.90 ± 2.16		0.000[c]
ASQoL	1st drug group	8	25.50 ± 7.32	0.026	0.815[a]
	2nd drug group	9	24.22 ± 4.91		0.139[b]
	3rd drug group	21	30.33 ± 5.74		0.007[c]
BASDAI	1st drug group	8	5.53 ± 2.44	0.003	0.815[a]
	2nd drug group	9	5.4 ± 2.07		0.004[b]
	3rd drug group	21	2.74 ± 2.16		0.005[c]
BASFI	1st drug group	8	3.25 ± 2.56	0.044	1.0[a]
	2nd drug group	9	3.46 ± 2.56		0.032[b]
	3rd drug group	21	1.56 ± 1.85		0.063[c]
Duration of MS	1st drug group	8	35.62 ± 32.00	0.33	NA
	2nd drug group	9	55.55 ± 45.51		NA
	3rd drug group	21	31.66 ± 37.09		NA
Intensity of MS	1st drug group	8	5.25 ± 3.95	0.029	0.606[a]
	2nd drug group	9	6.33 ± 3.46		0.093[b]
	3rd drug group	21	2.66 ± 2.83		0.014[c]

'*p*' probability (Kruskal Wallis), *p** Probability (Mann–Whitney *U* test).
[a] *p*-value of the binary comparison between the 1st drug group and the 2nd drug group.
[b] *p*-value of the binary comparison between the 1st drug group and the 3rd drug group.
[c] *p*-value of the binary comparison between the 2nd drug group and the 3rd drug group.

SD = standard deviation, SEM = standard error of the mean, *p* = probability, ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, IL23 = interleukin 23, VAS = visual analogue scale, ASQoL = Ankylosing Spondylitis Quality of Life, BASDAI = Bath Ankylosing Spondylitis Disease Activity Index, BASFI = Bath Ankylosing Spondylitis Functional Index, duration of MS = morning stiffness, intensity of MS = intensity of morning stiffness.

arthritis, 26 patients were negative (68.4%). The IL-23 levels were significantly higher in the group that was positive for peripheral arthritis ($p < 0.05$).

DISCUSSION

Th17 $CD4^+$ T-cells and their products have been recognized with increasing frequency in association with several human autoimmune or immune-mediated inflammatory diseases like multiple sclerosis, inflammatory bowel disease, psoriasis, and rheumatoid arthritis (RA) (16). The discovery of CD4 Th17 T-cells and the IL-23/IL-17 axis has challenged the existing paradigm and the role of Th1 T-cells in many autoimmune diseases (17).

lthough previous studies have shown conflicting results, a relationship and possible role between IL-23 and AS pathogenesis has been reported (5, 7, 18, 19). Previous studies demonstrated that the receptor of IL-23 is one of the main genetic factors in AS susceptibility and the levels of IL-23 are higher in patients with AS (5, 18). Similarly, Taylan *et al* (19) demonstrated that the levels of IL-23 and IL-17 are significantly increased in patients with AS compared to the control group. In contrast, it was reported that IL-23 serum levels were not found different in patients with AS and control groups (9, 20). In the present study, we found that the IL-23 levels were significantly higher in patients with AS compared to the control group. This finding suggests that IL-23 may be significant in the occurrence and the development of AS.

In the diagnosis and monitoring of AS, new publications about the elevated ESR or CRP level were shown to be present in only 30%–40% of patients with AS, and normal values did not rule out the presence of inflammation (7). When compared with other inflammatory rheumatic diseases like rheumatoid arthritis, it was stated that acute phase reactants are less useful in monitoring patients with AS. In our study, ESR and CRP levels were increased in the AS group compared to controls, and it suggests that ESR and CRP increased with the inflammatory changes that appear during the course of AS. Also, the IL-23 levels were significantly higher in the group that was positive for peripheral arthritis in the present study.

The effect of peripheral arthritis on acute phase reactants still remains controversial. It was proposed that higher CRP and ESR levels were observed more frequently in patients with AS with the presence of arthritis (21). In another study, Ozgocmen and Khan (6) revealed that acute phase reactants were higher in patients with AS who had peripheral joint involvement than in those who do not. On the other hand, a study conducted by Liu *et al* showed no significant correlations between ESR and CRP levels, and perceptions of peripheral arthritis were found. Also, they concluded that neither ESR nor CRP was superior for assessing disease activity in patients with AS (22). Similar in some aspects with these results, Poddubnyy *et al* found no significant differences in the level of CRP between patients with and without joint involvement (23). The levels of CRP and IL-23 can be used in the clinical follow-up of AS patients.

In current clinical practice, one of the main aims of the treatment of chronic diseases is to increase the quality of life. Therefore, we used the ASQoL index to measure patient's level of life quality, different from the many other studies. Barkham *et al* found that patients with AS with BASDAI greater than or equal to 4 have worse functional situation and life quality level than those with BASDAI less than 4. Also, when correlation analyses were performed, they observed that the

BASDAI score was positively correlated with functional impairment but negatively correlated with the quality of life (24). Bostan *et al* (25) stated that female patients with AS whose functional index showed low levels of impairment have a low level of life quality. In our study, the patients with high ASQoL scores had significantly higher BASDAI, BASFI and VAS scores. We believe that low life quality is an indicator of high disease activity for our patient group, which is consistent with the literature. However, the disease activity and the physical limitation may have individual effects on life quality and functionality based on the patients with AS that we evaluated (26).

This study had a few limitations. First, it had a cross-sectional design, and prospective studies are needed in order to fully reveal the relationship between the clinical findings of AS and IL-23 levels. Second, our sample size was even lower in patients with AS. Such limitations prevented us from reaching definitive and clear conclusions about the influence of IL-23 on AS. Ankylosing spondylitis is a progressive disease that should be monitored routinely throughout a patient's life.

Interleukin 23 should be used as a clinical marker and findings can be strengthened by the combined information obtained from BASDAI, BASFI, VAS, and ASQoL analyses. Also, it should be investigated whether the IL-23 repressive effect has a protective role in the development and intensification of the disease.

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