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

B Elbey[1], O Ayyıldız[2], ÜC Yazgan[3], R Çevik [4], MA Sarıyıldız[4], İ Kaplan[5]

ABSTRACT

Objectives: 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). AS 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: Thirty eight (33 male/5 female) AS patients and 42 healthy controls (32 male 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 Interleukin 23 (IL-23) values were noted. Bath AS disease activity index (BASDAI), Bath AS functional index (BASFI), Visual Analog scale (VAS), and quality of life in patients with AS (ASQoL) scales of the patients were measured.

Results: The mean age of the AS group and the control subjects were 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, CRP, IL-23, disease activity

[1]Department of Immunology, Faculty of Medicine, Dicle Univercity, Diyarbakır, [2]Department of Haematology, [3]Department of Physiology, Faculty of Medicine, Dicle Univercity, Diyarbakır Dicle, [4]Department of Physical medicine and rehabilitation and [5]Department of Biochemistry, Diyarbakır Diyarbakır, Dicle Univercity, Faculty of Medicine, Diyarbakır, Turkey.

Correspondence: Dr B Elbey, Department of Immunology, Faculty of Medicine, Dicle Univercity, Diyarbakır, Turkey. Tel: 0 412 2488001-4058, Gsm: 0 5324824677. Fax: 04122488660. E-mail: drbilalelbey@gmail.com

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 (ILP), 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 (SIJ) and spine (2, 3). Nowadays, research into the pathogenesis of AS is 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 Analog Scales (VAS) are commonly used and are valid 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 subgroup P19 and IL-12 and their common subgroup P40. Interleukin- 23 is released from dendritic cells, B cells, and antigen producing cells like macrophages and activated monocytes (4).

Interleukin- 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 AS patients. 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 not well known .Thus we aimed to investigate the relationship between IL-23 and clinical and laboratory parameters in patients with AS.

Patients and methods

Thirty eight consecutive patients with the diagnosis of AS were referred to the Dicle University, Medical Faculty, Department of Physical Rehabilitation between December 15, 2013 and March 30, 2014 and were evaluated. All patients were suffering from chronic low back pain. The diagnosed 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 NSAID; group 2 = usage of DMARD + NSAID; and also group 3 = usage of Anti-TNF + 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 AS patients 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 (BASDAI), 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 h' 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 five to give the final BASDAI score (0–10, 12).

Assessment of functional status: Bath AS functional index (BASFI), consisted of eight questions on daily activities and two additional questions that assessed a patients' 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 the10 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 visual analog scale 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). ASQoL 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, ESR, 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 subgroups. 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

Thirty eight 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 were 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 AS patients and healthy controls is indicated in Table 1. 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).

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 Rektant Protein, IL23: Interleukin 23

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

Table 2. Patient demographics acute phase reactant levels in the tree 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 Rektant Protein, IL23: Interleukin 23

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

Table 3. Comparison of the clinical parameters among the tree patient subgroups

	Group	**n**	**Mean ± SD**	***p***	***p****
	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]
BASF I	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 (Kruscal wallis), ***p**** Probability (mann whitney u testi)

"a" *p*-value of the binary comparison between the 1st drug group and the 2nd drug group, p "b" *p*-value of the binary comparison between the 1st drug group and the 3rd drug group, **p** "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 Rektant Protein, **IL23:** Interleukin 23, **VAS:** Visual Analog Acale, **ASQoL:** quality of life in patients with Ankylosing Spondylitis, **BASDAI:** Bath Ankylosing Spondylitis disease activity index, **BASFI:** Bath Ankylosing Spondylitis functional index, Duration of MS:Morning Stifness, **Intensity of MS:** Intensity of Morning Stifness.

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 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 MS, IBD, psoriasis and RA (16). The discovery of CD4 Th17 T-cells and the interleukin-23 (IL-23) / IL-17 axis has challenged the existing paradigm and the role of Th1 T-cells in many autoimmune diseases (17).

Although 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 level of IL-23 and IL-17 are significantly increased in AS patients compared to the control group. Contrary, 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 AS patients compared to the control group. This finding suggest 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 was 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 AS patients. In our study, ESR and CRP levels were increased in the AS group compared to controls, and suggests that ESR and CRP are 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, Ozgoçmen *et al* (6) revealed that acute phase reactants were higher in AS patients who had peripheral joint involvement than patients who do not. On the other hand, a study conducted by Liu *et al* showed no significant correlations between ESR-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 life quality. 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 AS patients with BASDAI greater than or equal to four have worse functional situation and life quality level than patients with BASDAI less than four. 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 AS patients 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. AS 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 strengthen by the combined information obtained from BASDAI, BASFI, VAS and ASQoL analyses. Also, it should be investigated whether the IL-23repressive effects has a protective role in the development and intensification of the disease.

REFERENCES

1. Ozgocmen S, Khan MA. Current concept of spondyloarthritis: special emphasis on early referral and diagnosis. Current rheumatology reports. 2012; **14:** 409–14.

2. Van Der Linden S, Van Der Heijde D. Ankylosing spondylitis: clinical features. Rheumatic Disease Clinics of North America. 1998; **24:** 663–76.

3. Khan M, Van der Linden S. Ankylosing spondylitis: clinical aspects. The spondylarthritides Oxford University Press, Oxford. 1998: 27–40.

4. McKenzie BS, Kastelein RA, Cua DJ. Understanding the IL-23–IL-17 immune pathway. Trends in immunology. 2006; **27:** 17–23.

5. Rueda B, Orozco G, Raya E, Fernandez-Sueiro JL, Mulero J, Blanco FJ, et al. The IL23R Arg381Gln non-synonymous polymorphism confers susceptibility to ankylosing spondylitis. Annals of the rheumatic diseases. 2008; **67:** 1451–4.

6. Ozgocmen S, Godekmerdan A, Ozkurt-Zengin F. Acute-phase response, clinical measures and disease activity in ankylosing spondylitis. Joint Bone Spine. 2007; **74:** 249–53.

7. Chen W-S, Chang Y-S, Lin K-C, Lai C-C, Wang S-H, Hsiao K-H, et al. Association of serum interleukin-17 and interleukin-23 levels with disease activity in Chinese patients with ankylosing spondylitis. Journal of the Chinese Medical Association. 2012; **75:** 303–8.

8. Colbert RA, Deodhar AA, Fox D, Gravallese EM, Khan MA, McGONAGLE D, et al. Entheses and bones in spondyloarthritis: 2008 Annual research and education meeting of the Spondyloarthritis Research and Therapy Network (SPARTAN). The Journal of rheumatology. 2009; **36:** 1527–31.

9. Wang X, Lin Z, Wei Q, Jiang Y, Gu J. Expression of IL-23 and IL-17 and effect of IL-23 on IL-17 production in ankylosing spondylitis. Rheumatology international. 2009; **29:** 1343–7.

10. MeiY PF. Increased serumIL-17andIL-23in thepatientwith ankylosingspondylitis. Clin Rheumatol. 2011; **30:** 269–73.

11. Linden SVD, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. Arthritis & Rheumatism. 1984; **27:** 361–8.

12. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. The Journal of Rheumatology. 1994; **21:** 2286–91.

13. Akkoc Y, Karatepe AG, Akar S, Kirazli Y, Akkoc N. A Turkish version of the bath ankylosing spondylitis disease activity index: reliability and validity. Rheumatology international. 2005; **25:** 280–4.

14. Calin A, Garrett S, Whitelock H, Kennedy L, O'hea J, Mallorie P, et al. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. The Journal of rheumatology. 1994; **21:** 2281–5.

15. Duruöz M, Doward L, Turan Y, Cerrahoglu L, Yurtkuran M, Calis M, et al. Translation and validation of the Turkish version of the ankylosing spondylitis quality of life (ASQoL) Questionnaire. Rheumatology international. 2013; **33:** 2717–22.

16. Layh-Schmitt G, Colbert RA. The interleukin-23/interleukin-17 axis in spondyloarthritis. Current opinion in rheumatology. 2008; **20:** 392–7.

17. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. New England Journal of Medicine. 2009; **361:** 888–98.

18. Karaderi T, Harvey D, Farrar C, Appleton LH, Stone MA, Sturrock RD, et al. Association between the interleukin 23 receptor and ankylosing spondylitis is confirmed by a new UK case–control study and meta-analysis of published series. Rheumatology. 2009; **48:** 386–9.

19. Taylan A, Sari I, Kozaci DL, Yuksel A, Bilge S, Yildiz Y, et al. Evaluation of the T helper 17 axis in ankylosing spondylitis. Rheumatology international. 2012; **32:** 2511–5.

20. Wendling D, Cedoz J-P, Racadot E. Serum and synovial fluid levels of p40 IL12/23 in spondyloarthropathy patients. Clinical rheumatology. 2009; **28:** 187–90.

21. Laurent M, Panayi G. Acute-phase proteins and serum immunoglobulins in ankylosing spondylitis. Annals of the rheumatic diseases. 1983; **42:** 524–8.

22. Liu B, Guo C, Liu W, Wu N, Xing Q. [The value of erythrocyte sedimentation rate and C-reactive protein in evaluating disease activity in ankylosing spondylitis]. Zhonghua nei ke za zhi [Chinese journal of internal medicine]. 2005; **44:** 566–9.

23. Poddubnyy D, Rudwaleit M, Listing J, Braun J, Sieper J. Comparison of a high sensitivity and standard C reactive protein measurement in patients with ankylosing spondylitis and non-radiographic axial spondyloarthritis. Annals of the rheumatic diseases. 2010; **69:** 1338–41.

24. Barkham N, Kong K, Tennant A, Fraser A, Hensor E, Keenan A, et al. The unmet need for anti-tumour necrosis factor (anti-TNF) therapy in ankylosing spondylitis. Rheumatology. 2005; **44:** 1277–81.

25. Bostan EE, Borman P, Bodur H, Barca N. Functional disability and quality of life in patients with ankylosing spondylitis. Rheumatology international. 2003; **23:** 121–6.

26. Peker O. Osteoartrozda ayırıcı tanı. In: Kutsal YG (ed). Osteoartroz (modern tıp seminerleri-7). Ankara: Günes kitabevi; 2000. 94–108.