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Serum Level of Interleukin-33 in Ankylosing Spondylitis Patients and Its Relation with Disease Parameters

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ABSTRACT

Background: Ankylosing spondylitis (AS) represents a chronic, progressively inflammatory condition characterized by persistent inflammation, bone erosion, and development of syndesmophytes. The mechanism behind AS development has been the subject of various investigations. However, the involvement of interleukin-33 (IL-33) in AS has not been fully addressed.

Objectives: The aim of this work was to assess serum IL-33 in AS patients versus controls, and elucidate its correlation with AS activity, function, sacroiliitis, enthesitis, and other AS disease indices.

Patients and Methods: Thirty patients diagnosed with AS had been included in this study, matched by age and sex to thirty healthy individuals. For all patients, assessment of sacroiliitis, peripheral arthritis, enthesitis, and assessment of different AS indices were done. IL-33 levels were measured in serum of both patients and controls.

Results: Serum IL-33 appeared to be significantly increased in AS patients (380.7 ± 296.8 pg/ml) compared to controls (45.2 ± 28.11 pg/ml) ($p<0.001$). Active AS patients exhibited markedly elevated IL-33 levels (678.1 ± 312.3 pg/ml) compared to their inactive counterparts (208.4 ± 49.3 pg/ml). IL-33 demonstrated a robust positive correlation with disease activity, yet showed no association with arthritis, sacroiliitis, or enthesitis.

Conclusions: Serum Il-33 was significantly higher in AS patients than control group. Serum IL-33 levels showed statistically significant correlations with AS disease activity, while displaying no discernible correlations with enthesitis, sacroiliitis, or peripheral arthritis. This could make it possible to include serum IL-33 as a part in the core set evaluation of AS disease activity.

Keywords: AS, Serum IL-33, Activity.

INTRODUCTION

Ankylosing spondylitis (AS) is an extensive inflammatory disorder mostly usually affect the axial spine and can present with a variety of clinical symptoms. Progressive spinal stiffness and persistent back pain are two of the condition's defining characteristics. AS is typified by involvement of different entheses, spine, sacroiliac and peripheral joints. AS can cause postural problems and frequently results in reduced spinal mobility [1-3]. Enthesitis as well as a higher chance of the sacroiliac joint and spine fusing together, are characteristics of active AS [4].

Rheumatic disorders, including AS, are influenced by interleukin-33 (IL-33) both during their onset and course [5]. Damage to the epithelium and endothelium causes the cytokine IL-33, an IL-1 family member to be exhibited, by attaching to its particular receptor, known as suppression of tumorigenicity 2 (ST2), IL-33 serves as an alert signal to preserve homeostasis [6,7].

As a result, IL-33 may operate as both a mediator and a marker in the development of AS. It also boosted the migration of neutrophils and improved the generation of tumor necrosis factor (TNF)-α caused by lipopolysaccharide [8].

The aim of this study was to contrast serum IL-33 levels between AS patients and healthy controls and delineate the relationship between IL-33 serum levels and AS activity, functional status, sacroiliitis, enthesitis, and other disease indices.

PATIENTS AND METHODS

Patients: This study enrolled thirty AS patients meeting the revised 1984 New York criteria [9], each paired with thirty healthy controls matched for age and sex. Participants were excluded if the disease onset was less than 18 years, had allergic disease like asthma, experienced a recent infection, had another autoimmune disease, malignancy, or receiving anti-TNF medication in the past ninety days.

Clinical evaluation: Each patient had a full history taking, thorough comprehensive clinical assessment with emphasis on articular involvement, clinical sacroilitis and clinical enthesitis.

Patients' assessment:

Disease activity was gauged through the application of the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [10] and the Ankylosing Spondylitis Disease Activity Score (ASDAS) [11]. Functional capacity was assessed using the Bath Ankylosing Spondylitis Functional Index (BASFI) [12]. The 3-point Bath AS Metrology Index (BASMI) scale was taken into consideration for measuring spinal mobility [13].

In addition, The AS Radiology index (BASRI-s) for the spine was assessed using lateral cervical and anteroposterior and lateral lumbar radiographs [14].

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Investigations: All patients underwent complete blood count (CBC), first-hour erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) testing. Additionally, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), blood urea nitrogen (BUN), and serum creatinine levels were measured. Plain X-rays of the cervical, lumbar spine, and sacroiliac joints were conducted. Serum IL-33 levels were quantified using the enzyme-linked immunosorbent assay (ELISA) method in all participants.

Ethical approval:

The study protocol was explained to all participants who offered an informed consent and was approved by Medical Ethical Committee of Minia Faculty of Medicine. The Helsinki Declaration was followed throughout the study`s conduct.

Statistical analysis:

Statistical analysis was meticulously executed utilizing the Statistical Package for the Social Sciences (SPSS) version 21. Quantitative data were presented as mean ± standard deviation (SD) and range, and were compared by independent–t test. Qualitative data were presented as frequency and percentage and Chi-square (χ^2) test was used to examine them. Spearman's correlation coefficient (r) was computed. The discernment of statistical significance was set at a threshold of < 0.05.

RESULTS

In the cohort of 30 AS patients, the demographic distribution included 20 males, accounting for 66.7%, and 10 females comprising 33.3%, yielding a male-to-female ratio of 2:1. The average age of AS patients was 29.4 ± 5.5 years. The mean age at disease onset was 24.5 ± 4.8 years and the mean duration of the disease was calculated at 4.86 ± 2.8 years. There was insignificant difference between the studied groups regarding age and sex (Table 1).

Table 1: Demographic data of AS patients and controls

		AS Patients (n=30)	Controls (n=30)	X^2/t	P value
Age (years)		29.4±5.5 (20-40)	28.2±4.5 (20-37)	0.8	0.4
Age at onset of disease (years)		24.5±4.8 (19-34)	-	-	-
Disease duration (years)		4.86±2.8 (1-10)	- -	-	-
Sex	**Male, n (%)**	20 (66.7%)	23 (76.7%)	0.7	0.4
	Female, n (%)	10 (33.3%)	7 (23.3%)		

Within AS group, mean Schober test was 13.53 ± 0.71 cm, mean right lateral flexion was 7.90 ± 2.26 cm, mean left lateral flexion was 7.90 ± 2.26 cm, mean tragus to wall was 16.46 ± 4.19 cm, mean cervical rotation was 66.16 ± 15.57 degree, mean intermalleolar distance was 89.33 ± 13.31 cm, mean chest expansion was 3.63 ± 0.67 cm, direct compression was positive in 96.7% of patients, Gaenslen's test was positive in 76.7%, Patrick's test in 70%, side compression test in 50%, pelvic compression test in 40%, and distraction test was positive in 20% (Table 2).

Table 2: Physical examination findings in patients with AS (n=30)

		AS Patients
Schober test (cm)	Range	12 – 15
	Mean±SD	13.53±0.71
Right lateral flexion test (cm)	Range	4 – 15
	Mean±SD	7.90±2.26
Left lateral flexion test (cm)	Range	4 – 15
	Mean±SD	7.90±2.26
Tragus to wall (cm)	Range	11 – 33
	Mean±SD	16.46±4.19
Cervical rotation (degree)	Range	30 – 85
	Mean±SD	66.16±15.57
Inter malleolar distance (cm)	Range	60 – 110
	Mean±SD	89.33±13.31
Chest expansion (cm)	Range	2 – 4
	Mean±SD	3.63±0.67
Direct compression	29 (96.7%)	
Gaenslen's test	23 (76.7%)	
Patrick's test	21 (70%)	
Side compression	15 (50%)	
Pelvic compression	12 (40%)	
Distraction test	6 (20%)	

At the time of the study, twenty patients (66.7%) were receiving sulfasalazine, two (6.6%) were receiving methotrexate while 8 patients (26.7%) were receiving both methotrexate and sulfasalazine.

BASDI mean was 3.63 ± 1.39 with 11 patients (36.7%) had BASDAI ≥ 4 (active). BASFI mean was 6.03 ±1.25, BASMI mean was 3.76 ± 1.33, BASRI mean was 4.88 ± 1.03, and ASDAS ESR mean was 2.63 ± 0.99. According to ASDAS ESR, 2 patients were inactive (6.6%), 11 patients (36.7%) had moderate activity, 11 patients (36.7%) had high activity while 6 patients (20%) had very high activity. The mean articular index was 3.83 ± 3.51, the mean number of swollen joints was 0.46 ± 0.97, while the mean number of tender joints was 2.96 ± 2.95 (Table 3).

Table 3: Assessment indices in patients with AS (n=30)

	AS patients	
	Range	Mean±SD
BASDI	2-7	3.63±1.39
BASFI	4-8	6.03±1.25
BASMI	0-6	3.76±1.33
BASRI	2.5-7	4.88±1.03
ASDAS ESR	1.2-5	2.63±0.99
RAI	0-10	3.83±3.51
S.J.C	0-4	0.46±0.97
T.J.C	0-10	2.96±2.95

BASDAI= Bath Ankylosing Spondylitis Disease Activity Index, BASFI= Bath Ankylosing Spondylitis Functional Index, BASMI= Bath Ankylosing Spondylitis Metrology Index, BASRI= Bath Ankylosing Spondylitis Radiographic Index, ASDAS ESR= Ankylosing spondylitis disease activity score Erythrocyte Sedimentation Rate, RAI=Ritchie articular index, S.J.C=swollen joint count, TJC=tender joint count.

Peripheral enthesitis was detected clinically in 25 patients, among them achilles tendinitis was the commonest being detected in 19 patients (63.3%) followed by planter fasciitis in 17 patients (56.7%), proximal patellar tendon in 5 patients (16.7%) while triceps tendon and quadriceps tendon were in one patient each (3.3%).

Regarding serum level of IL-33, it was appeared to be significantly increased in AS patients (380.7 ± 296.8 pg/ml) compared to controls (45.2 ± 28.11 pg/ml) ($p<0.001$). Based on BASDI score; active AS patients exhibited markedly elevated IL-33 levels (678.1 ± 312.3 pg/ml) compared to their inactive counterparts (208.4 ± 49.3 pg/ml) (Table 4).

Table 4: Serum level of IL-33 in AS patients and controls, active and inactive AS

	AS patients (n=30) mean±SD	Healthy control (n=30) mean±SD	t	p
Serum IL-33 (pg/ml)	380.7±26.8	45.2±8.11	8.8	0.0001**
	Active AS patients (n=11) mean±SD	**Inactive AS patients (n=19) mean±SD**	**t**	**p**
Serum IL-33 (pg/ml)	678.1±32.3	208.4±49.3	6.4	0.0001**

IL-33= Interleukin-33, **= Highly significant.

A statistically significant correlation between serum level of IL-33, ESR and CRP levels in patients with AS was found. A strong positive correlation between IL-33 serum and BASDAI and ASDASES were also noticed (Table 5).

Table 5: Correlation between serum levels of interleukin- 33 and different disease parameters

	Serum IL-33	
	r	p
Age (years)	0.08	0.43
Age at onset (years)	0.09	0.32
Disease duration (years)	0.24	0.42
Arthritis	0.1	0.36
Clinical enthesopathy	0.09	0.55
Sacroiliitis	0.07	0.44
BASDI	0.8	0.001**
BASFI	0.3	0.05
BASMI	0.1	0.40
ASDAS-ESR	0.6	0.001**
BASRI	0.1	0.40
ESR	0.8	0.001**
CRP	0.4	0.005**

IL-33= Interleukin-33, BASDAI= Bath Ankylosing Spondylitis Disease Activity Index, BASFI= Bath Ankylosing Spondylitis Functional Index, BASMI= Bath Ankylosing Spondylitis Metrology Index, ASDAS-ESR= Ankylosing spondylitis disease activity score Erythrocyte Sedimentation Rate, BASRI= Bath Ankylosing Spondylitis Radiographic Index, ESR=Erythrocyte Sedimentation Rate, CRP=C Reactive Protein, **= Highly significant.

DISCUSSION

AS represents a chronic, progressive inflammatory pathology predominantly affecting the axial skeleton, particularly the spine, along with other articulations. It causes severe disability and persistent pain, and it significantly impairs the social and economic well-being of its patients [15]. Despite a number of studies on the pathophysiology of AS, the precise cause and underlying processes remain unclear [16]. On the other hand, immunological deficiencies and genetic background are thought to be important disease-causing factors [17]. IL-33, a constituent of the IL-1 cytokine family, exhibits dual functionality, operating both as a cytokine and as a nuclear factor. IL-33 was predominantly exhibited by human monocytes, dendritic cells, astrocytes, keratinocytes, endothelial and epithelial cells. It has been reported that IL-33 modulates immune responses and inflammation, and this is linked to the development of T helper 2 immune responses. The creation of pathogenic autoantibody isoforms by B-cell hyperactivity has been linked to the cytokines that control T cell activation, and these isoforms can lead to tissue dysfunction in many autoimmune diseases [18].

Elevated concentrations of IL-33 have been implicated in the pathophysiology of numerous autoimmune pathologies, including rheumatoid arthritis and systemic lupus erythematosus. This association intimates a potential contributory role for IL-33 in the

initiation and exacerbation of inflammatory tissue damage and the dysregulation of immunological tolerance mechanisms. Cytokines may be an intriguing target for monitoring and treating many autoimmune illnesses [18]. But as far as we are aware, there has only been few studies that has examined the role of IL-33 in AS [8,19-22].

The primary objective of this work was to conduct a comprehensive analysis comparing serum IL-33 levels among AS patients and a demographically matched healthy control group. Furthermore, the study aimed to elucidate the intricate associations between IL-33 serum levels and various aspects of AS pathology, including disease activity, functional status, sacroiliitis, enthesitis, and other pertinent disease indices.

In our study, IL-33 serum level was significantly raised in AS patients than controls ($p<0.001$). In agreement with our findings, results of **Han *et al.*** [8], **Li *et al.*** [20], and **Gong *et al.*** [21], which came consistent with our findings. **Taylan *et al.*** documented a significant elevation in serum levels of IL-33 and other cytokines, as quantified by ELISA, in patients with AS relative to healthy controls, with statistical significance ($p < 0.05$) [22].

Hsu *et al.* [23] and **Smith** [24], concluded that high IL-33 may be caused by chronic inflammation, high TNF alpha concentration, and activated endothelium and epithelial cells; mast cells have also been identified as a source of IL-33 in rheumatic disease and inflammation.

Contrary to our results, **Madej *et al.***, who conducted research on the cytokine network and its part in the development of spondyloarthritis and reported that IL-33 serum level in spondyloarthropathy did not show any significant difference between patients and controls [25]. Furthermore, serum level of IL-33 levels was significantly elevated in our AS active patients than inactive AS patients ($p<0.01$). In accordance with that **Li *et al.*** [20], who studied 43 ankylosing spondylitis patients compared them with 27 healthy participants and found that IL-33 levels were significantly raised in active AS patients than inactive patients ($p < 0.05$). Serum levels of IL-33 might serve as a partial indicator of AS disease activity, implying that IL-33 could be integral to the pathogenic mechanisms underlying AS [20].

We used BASDAI to assess disease activity which ranged from 2 to 7 with a mean of (3.63 ± 1.39) and ASDAS ESR which ranged from 1.2 to 5.0 with a mean of (2.63 ± 0.99). In agreement with our study, **van der Heijde *et al.***, 2009 [11] who agreed that the conventional way to assess the activity of axSpA is to use composite indices such as ASDAS and BASDAI. A separate investigation by **Sveaas *et al.***, encompassed a cohort of 143 individuals diagnosed with AS and contrasted their data with that of 124 control subjects from the general population. The study elucidated that there was no significant correlation between the ASDAS or the BASDAI and the plasma levels of cytokines and cytokine receptors in AS patients [26]. It is possible that clinical indices of disease activity would have been more efficiently correlated with local cytokine levels than circulating ones, which is why cytokine investigations in synovial fluid or inflammatory tissue were not conducted.

In the present work, serum level of IL-33 shows a significant correlation with ESR and CRP levels. Consistent with our results, **Mok *et al.***, [27] and **Beltrán *et al.*** [28], who reported that serum level of IL-33 was strongly correlated with acute phase reactants. An opposing view reported by **Kim *et al.*** [29] who did not show any significant relation between IL-33 and acute phase reactants.

Limitations of this current study is the limited sample size. This could result in failure of some associations between IL-33 and AS manifestations to achieve statistical significance even when the relation was strong (type II statistical error). However, AS is a rare disease and recruitment of a sample large would have needed years of work. Another restriction was the lack of IL-33 testing in tissues, which could provide valuable data in terms of inflammatory-sites.

CONCULSIONS

Conclusively, serum IL-33 levels were notably elevated in patients with AS compared to those in healthy control subjects. Serum IL-33 demonstrated a statistically significant association with disease activity indices specific to AS, while showing no discernible correlations with indicators of enthesitis, sacroiliitis, or peripheral arthritis. This may enable the use of IL-33 as a part of an assessment criteria of AS disease activity.

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