Research article

Serum levels of CXCL-8, IL-10, and TNF-alpha in ankylosing spondylitis patients

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(Received: July 2021

Revised: July 2021

Accepted: August 2021)

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ABSTRACT

Introduction and Aim: Ankylosing spondylitis (AS) is the least common joint pain and physical disability in all parts of the world, including Iraq. However, AS is also one of the rheumatological disorders characterized by chronic arthritis, where cytokines can play a role in the pathogenesis of the disease. Therefore, the current study aims to determine the serum levels of three cytokines, which are CXCL-8, IL-10, and TNF- α , in an Iraqi sample of AS patients.

Materials and Methods: The enzyme-linked immunosorbent assay (ELISA) was employed to measure the concentrations of the three cytokines in the serum samples of AS patients.

Results: The percentage of male patients was higher than that of females, but there was no significant difference ($P > 0.05$) between patients and controls in the distribution of males and females. The results revealed a significant increase in the level of TNF- α (36.1 ± 18.3 vs. 20.3 ± 10.6 pg/ml; $P < 0.001$), a non-significant increase in the level of CXCL-8 (20.5 ± 19.6 vs. 14.7 ± 9.1 pg/ml; $P = 0.057$), and a significant decrease in the level of IL-10 (45.5 ± 22.9 vs. 65.2 ± 13.6 pg/ml; $P < 0.001$) in the sera of AS patients compared with the control. The patients also demonstrated a significant increase in erythrocyte sedimentation rate (ESR: 32.5 ± 25.4 vs. 5.4 ± 4.8 mm/h; $P \leq 0.001$). Receiver operating characteristic (ROC) curve analysis revealed that ESR was the most significant factor followed by IL-10, and then TNF- α , while there was no diagnostic significance for CXCL-8.

Conclusion: The current study is the first of its kind in evaluating the levels of CXCL-8, IL-10, and TNF- α in the serum of patients with AS in Iraq. The results showed a significant increase in TNF- α , a non-significant increase in CXCL-8, and a significant decrease in IL-10 levels.

Keywords: Ankylosing spondylitis; autoimmune disease; IL-10; CXCL-8; TNF-alpha

INTRODUCTION

Spondylitis belongs to a group of rheumatic diseases known as spondyloarthropathies (SpA) (1). It is believed that the primary mechanism of its occurrence is autoimmunity or autoinflammation (2); that is, it is a chronic inflammatory autoimmune disease that primarily affects the axial spine, with the hallmark of sacroiliitis, involving the adjacent joints, tendons, and ligaments. Prevalence estimates range from 0.1 to 2% in different populations. AS prevalence has been reported to range from 1 to 2 per 1000 in North America, while the prevalence is 0.9% worldwide (3). Men have a higher incidence than females, with a ratio of 2 - 3: 1. (4). A male-to-female ratio of 5:1 was recorded in Iraqi adult patients (5), while the results for another group of patients enrolled in the rheumatology unit at Baghdad Teaching Hospital showed that 90.6% were males, with a male-to-female ratio of 9:1 (6).

A study of samples in the Kurdistan region of Iraq showed that the ratio of males to females was 5.8:1 (7). It was also reported that the peak age range for onset is 15-35 years (5). AS is an arthritis of unknown etiology. However, it is suggested that the cause is complex interactions between genetic susceptibility and environmental factors to stimulate immune

reactions that initiate the auto-inflammatory immune response and the development of AS. In addition, the disease is positively associated with the human leukocyte antigen-27 (HLA-B27) (8). Since the disease is associated with the development of inflammatory responses, it has been suggested that cellular kinetics play a role in its pathogenesis and activity. This role is maintained by the observations that the use of cytokine inhibitors may influence the symptoms and activity of AS (9).

Cytokines are important mediators of inflammation and are associated with the pathogenesis of many inflammatory diseases. One of the chemokines involved is chemokine 8, recently called CXCL8, which is a member of the CXC chemokine subfamily (10). It is produced by blood cells and many types of tissues (11). Neutrophils are a specific major target of CXCL8 action. Many of the pathophysiological actions of CXCL-8 depend on the activation of neutrophils (11). Interleukin-10 (IL-10), an anti-inflammatory cytokine during infection, inactivates Th1 T helper cells, natural killer (NK) cells, and macrophages, all of which are required for optimal elimination of pathogens, but also contribute to tissue damage. As a result, IL-10 can impair pathogen clearance and improve immunopathology (12). The inflammatory cytokine tumor necrosis factor alpha

(TNF α) is a cell signaling protein implicated in systemic inflammation and is one of the cytokines that constitute the acute phase reaction. It is produced mainly by activated macrophages but can also be produced by many other cell types, such as T helper cells, NK cells, neutrophils, mast cells, eosinophils, and neurons. TNF is a member of the TNF superfamily, which consists of different membrane proteins with a homologous TNF domain (13). The present study is the first of its kind that aims to assess the serum levels of the cytokines CXCL8, IL-10, and TNF- α in Iraqi patients with AS. The association of these cellular kinetics with age, sex, disease duration, disease activity score (DAS), and their relationships with the patient's family history of HLA-B27 positivity were also investigated. The study also assessed the levels of liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), along with the parameters of ESR, Hb, and WBC count in AS patients.

MATERIALS AND METHODS

A case-control study was conducted on 51 patients with AS and 51 healthy controls (blood donors) during the period September 1, 2020 - December 31, 2021. The patients were admitted to the Arthritis Units at Baghdad Teaching Hospital and Al-Imamin Al-Kadhimain Teaching Hospital. The diagnosis of AS was based on the criteria of the Assessment of Spondylo-Arthritis International Society (ASAS) (14). Data on age, sex, and disease duration were recorded. Besides, the AS disease activity score was also recorded and based on assessment of erythrocyte sedimentation rate (ASDAS-ESR). In addition to patients, healthy subjects were included as control.

A sample of venous blood (10 ml) was collected from each participant and distributed as follows; 3ml in an EDTA tube for assessment of Hb, WBC, ESR, and liver function enzymes; 2ml was used for HLA-B27 test; and the remaining 5ml was used for the ELISA assay.

Serum levels of cytokines

The levels of three cytokines (CXCL8, IL-10 and TNF- α) were measured in the sera of participants using sandwich enzyme-linked immunosorbent assay kits, and manufacturer's instructions were followed (PeproTech Company, UK).

Statistical analysis

Categorical variables were given as number and percentage, and significant differences were evaluated by Fisher exact test or Pearson Chi-square test. Continuous variables were given as mean $\pm$ standard deviation (SD), and statistically significant differences between the means were evaluated by analysis of variance (ANOVA) test followed by the least significant difference (LSD) test. Receiver operating

characteristic (ROC) curve analysis was used to estimate the area under the curve (AUC), cut-off value, sensitivity, and specificity. A probability (P) $\leq$ 0.05 were considered significant. Data were statistically analyzed using IBM SPSS Statistics for Windows, version 19 (IBM Corp., Armonk, N.Y., USA).

RESULTS

Basic characteristics of patients with spondylitis and control

The mean age of AS patients was 35.0 ± 9.8 years, while it was lower in the control (32.1 ± 10.7 years), with no significant differences being observed ($P > 0.05$). When the patients and control were divided into different age groups (>40 , $30-40$, <30 years), no significant differences were observed ($P > 0.05$). The percentage of males in the patients' group was higher than that of females (88.2% vs. 11.8%). A similar observation was made in the control group (74.5% vs. 25.5%).

The results of the distribution of patients according to the duration of the disease also showed that most patients were within a duration of 5 years or higher (80.4%, $n=41$), whereas a lower percentage was noticed for those of less than 5 years (19.6%, $n=10$). When the patients were distributed according to the severity of the disease, most patients were within a severity level of ≤ 2.1 (88.2%, $n=45$), whereas the percentage of those with a severity level of ≤ 2.1 was lower (11.8%, $n=6$). According to the degree of functional disability, most patients were within grade I, II (58.8%, $n=30$), whereas a lower proportion was recorded for those within grade III, IV (41.2%, $n=21$).

The current study also showed that the proportion of HLA-B27 positive AS patients (45.1%, $n=23$) was lower than that of HLA-B27 negative patients (54.9%, $n=28$). There was also a significant decrease ($P < 0.001$) in the level of Hb in the serum of patients (13.2 ± 1.6 g/dL) compared with the control group (14.6 ± 2.3 g/dL). The results also revealed a significant increase ($P < 0.001$) in the WBC count in the blood of AS patients (8.7 ± 2.5 cells $\times 10^9$ /L) compared with the control group (6.6 ± 1.4 cells $\times 10^9$ /L), along with a significant increase ($P < 0.001$) in ESR value in the patients (32.5 ± 25.4 mm/hour) as compared to the control (5.4 ± 4.8 mm/hour).

The results also demonstrated a significant decrease ($P \leq 0.05$) in the level of ALT in the serum of patients with AS (19.3 ± 11.1 units/liter) compared with the control group (23.5 ± 9.5 units / liter). However, a significant increase ($P \leq 0.05$) was recorded in the level of AST in the serum of the patients (22.4 ± 17.7 units / liter) compared with the control (15.8 ± 4.8 units / liter), as shown in (Table 1).

Table 1: Baseline characteristics of ankylosing spondylitis patients and controls

Characteristics		Patients (n = 51)	Controls (n = 51)	p-value
Mean age $\pm$ SD; year		35.0 $\pm$ 9.8	32.1 $\pm$ 10.7	0.756
Age groups; N (%)	< 30	18(35.3)	24(47.1)	0.343
	30 – 40	16(31.4)	16(31.4)	0.343
	> 40	17(33.3)	11(21.6)	0.343
Gender; N (%)	Male	45(88.2)	38(74.5)	0.075
	Female	6(11.8)	13(25.5)	0.075
ASDAS; N (%)	< 2.1	6(11.8)	NA	-
	$\geq$ 2.1	45(88.2)	NA	-
Disease duration; N (%)	<5	10(19.6)	NA	-
	$\geq$ 5	41(80.4)	NA	-
Functional Scale; N (%)	I,II	30(58.8)	NA	-
	III, IV	21(41.2)	NA	-
HLA-B27; N (%)	Positive	23(45.1)	NA	-
	Negative	28(54.9)	NA	-
Hb; mean $\pm$ SD; g/dL		13.2 $\pm$ 1.6	14.6 $\pm$ 2.3	<0.001
WBC $\times 10^9$ /L; mean $\pm$ SD		8.7 $\pm$ 2.5	6.6 $\pm$ 1.4	<0.001
ESR; mean $\pm$ SD; mm/hour		32.5 $\pm$ 25.4	5.4 $\pm$ 4.8	<0.001
ALT; mean $\pm$ SD; U/L		19.3 $\pm$ 11.1	23.5 $\pm$ 9.5	0.044
AST; mean $\pm$ SD; U/L		22.4 $\pm$ 17.7	15.8 $\pm$ 4.8	0.012

SD: Standard deviation; ASDAS: Ankylosing spondylitis disease activity score; Hb: Hemoglobin; WBC: White blood cell count; ESR: Erythrocyte sedimentation rate; Not applicable; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Serum levels of CXCL-8, IL-10, and TNF- α

The results of the current study showed a non-significant increase in the level of CXCL-8 in the serum of patients (20.5 $\pm$ 19.6 pg/ml) as compared to the control group (14.7 $\pm$ 9.1pg/ml). However, a significant decrease ($P < 0.001$) was recorded in the level of IL-10 in the serum of the patients (45.5 $\pm$ 22.9 pg/ml) compared with the control group (65.2 $\pm$ 13.6 pg/ml). Moreover, a significant increase ($P < 0.001$) was observed in the level of TNF- α in the serum of the patients (36.1 $\pm$ 18.3pg / ml) compared with the control (20.3 $\pm$ 10.6 pg/ml), as shown in Table 2.

Table 2: Serum levels of CXCL8, IL-10 and TNF- α in ankylosing spondylitis patients and controls.

Cytokine	Mean $\pm$ SD (pg/mL)		p-value
	Patients (n= 51)	Controls (n = 51)	
CXCL8	20.5 $\pm$ 19.6	14.7 $\pm$ 9.1	0.057
IL-10	45.5 $\pm$ 22.9	65.2 $\pm$ 13.6	<0.001
TNF- α	36.1 $\pm$ 18.3	20.3 $\pm$ 10.6	<0.001

SD: Standard deviation.

Association of serum levels of CXCL8, IL-10, and TNF- α with SA patient characteristics

The results of the current study showed no significant differences between males (20.7 $\pm$ 19.7pg/ml) and females (18.4 $\pm$ 20.2pg/ml) in the serum level of CXCL-8 in SA patients. The results also revealed a non-significant difference in the serum level of CXCL-8 in the patients between the studied age groups (<30 years=18.9 $\pm$ 19.0, 30-40 years=18.8 $\pm$ 13.6, >40 years=14.1 $\pm$ 10.9pg/ml). The results also showed non-significant differences ($P \leq 0.05$) in the levels of CXCL-8 in AS patients within different

duration of the disease, reaching the highest level in the group of <5 years duration (31.2 $\pm$ 28.2pg/ml) and the lowest level in the group of ≤ 5 years duration (17.8 $\pm$ 16.3pg/ml). In addition, no significant differences were found in the levels of CXCL-8 in the patients of different disease severity ranks, which reached values of (21.3 $\pm$ 19.1pg/ml) in the group of < 2.1 severity and (20.4 $\pm$ 19.9pg/ml) in the group of ≥ 2.1 severity. Also, no significant differences were recorded in the level of CXCL-8 in the patients with different degrees of functional disability, reaching values of (20.3 $\pm$ 19.5pg / ml) in the group of I, II degree of functional disability and (20.7 $\pm$ 20.1pg / ml) in the group of III, IV degree. As for HLA-B27 antigen positivity, HLA-B27-positive patients had no significant differences in the level of CXCL-8 (18.9 $\pm$ 16.6pg/ml) as compared to HLA-B27-negative patients (21.7 $\pm$ 21.9pg/ml; Table 3).

The results of the current study also showed no significant differences in the serum levels of IL-10 between male (46.2 $\pm$ 23.3pg/ml) and female (40.2 $\pm$ 19.8pg/ml) SA patients. There were also no significant differences in the serum levels of IL-10 in AS patients of the age groups of <30 years (47.1 $\pm$ 23.4pg/ml), 30 -40 years (43.8 $\pm$ 18.2pg/ml), and >40 years (44.7 $\pm$ 21.3pg/ml). In addition, no significant differences were recorded in the serum levels of IL-10 in AS patients with different durations of the disease, reaching its higher level in the duration group of <5 years (47.6 $\pm$ 25.4pg/ ml) and lower level in the group of ≤ 5 years (45.0 $\pm$ 22.5pg / ml). No significant differences were also recorded in the serum levels of IL-10 in SA patients with different ranks of disease severity, reaching values of (46.5 $\pm$ 24.0pg / ml) in the severity group of < 2.1 and (45.4 $\pm$ 23.0pg/ml) in the

severity group of ≥ 2.1 . Also, no significant differences were found in the serum levels of IL-10 in SA patients with different functional disability grades, reaching values of (46.0 ± 23.0 pg / ml) in patients with I, II, degree of functional disability and (44.8 ± 23.3 pg / ml) in the group with III, IV degree. As for the results of HLA-B27 antigen positivity in AS patients, there was no significant differences in the level of IL-10 between HLA-B27-positive (45.5 ± 22.2 pg/ml) and negative patients (45.5 ± 23.7 pg/ml) (Table 3).

The results of the current study showed a significant difference ($P \leq 0.05$) in the level of TNF- α between males (34.3 ± 15.3 pg/ml) and females (48.9 ± 32.5 pg/ml) in the serum of patients with AS. However, no significant differences were recorded in the serum levels TNF- α in the patients of different age groups

(<30 years = 26.8 ± 17.5 , 30-40 years = 28.7 ± 18.5 , >40 years = 29.7 ± 13.9 pg/ml). Also, no significant differences were found in TNF- α levels in the patients with different durations of the disease (<5 years= 33.7 ± 17.1 , ≤ 5 years = 36.6 ± 18.7 pg/ml). No significant differences were found in the level of TNF- α in patients with different ranks of disease severity, reaching (37.6 ± 12.6 pg/ml) in the severity group of < 2.1 and (35.9 ± 19.0 pg/ml) in the severity group of ≥ 2.1 . However, a significant difference was recorded in the level of TNF- α in patients with I, II grade of functional disability (40.4 ± 20.0 pg/ml) As compared to those with grade III, IV (30.0 ± 13.8 pg /ml). As for HLA-B27 antigen positivity, there was no significant difference in the level of TNF- α between HLA-B27-positive (36.1 ± 22.0 pg/ml) and negative to (36.0 ± 15.0 pg/ml) patients (Table 3).

Table 3: Serum levels of CXCL8, IL-10 and TNF- α stratified by characteristics of ankylosing spondylitis patients.

Characteristics		Mean $\pm$ SD (pg/mL)		
		CXCL8	IL-10	TNF- α
Gender	Male	20.7 ± 19.7	46.2 ± 23.3	34.3 ± 15.3
	Female	18.4 ± 20.2	40.2 ± 19.8	48.9 ± 32.5
	p-value	0.728	0.466	0.024
Age groups	< 30	18.9 ± 19.0	47.1 ± 23.4	26.8 ± 17.5
	30 - 40	18.8 ± 13.6	43.8 ± 18.2	28.7 ± 18.5
	> 40	14.1 ± 10.9	44.7 ± 21.3	29.7 ± 13.9
	p-value	0.399	0.887	0.676
Disease duration (year)	< 5	31.2 ± 28.2	47.6 ± 25.4	33.7 ± 17.1
	≥ 5	17.8 ± 16.3	45.0 ± 22.5	36.6 ± 18.7
	p-value	0.052	0.757	0.657
ASDAS	< 2.1	21.3 ± 19.1	46.5 ± 24.0	37.6 ± 12.6
	≥ 2.1	20.4 ± 19.9	45.4 ± 23.0	35.9 ± 19.0
	p-value	0.911	0.916	0.833
Functional scale	I,II	20.3 ± 19.5	46.0 ± 23.0	40.4 ± 20.0
	III,IV	20.7 ± 20.1	44.8 ± 23.3	30.0 ± 13.8
	p-value	0.943	0.848	0.041
HLA-B27	Positive	18.9 ± 16.6	45.5 ± 22.2	36.1 ± 22.0
	Negative	21.7 ± 21.9	45.5 ± 23.7	36.0 ± 15.0
	p-value	0.611	1	0.978

ASDAS: Ankylosing spondylitis disease activity score; SD: Standard deviation.

Receiver Operating Characteristic (ROC) curve analysis

The area under the curve was determined for the parameters of ESR, IL-10, TNF- α , and CXCL-8. The most significant factor was found to be the ESR, which

occupied an area of 0.938, followed by IL-10 (0.826), and TNF- α (0.787), while CXCL-8 was not of diagnostic importance, as its ROC value reached only 0.549 (Table 4).

Table 4: Receiver operating characteristic (ROC) curve analysis of ESR, CXCL8, IL-10 and TNF- α in AS patients.

Parameter	AUC	95% CI	p-value	Cut-off value	Sensitivity (%)	Specificity (%)
ESR	0.938	0.892-0.983	< 0.001	9.0 mm/h	88.2	89.8
CXCL8	0.549	0.436-0.662	0.397	12.3 pg/mL	52.9	51.0
IL-10	0.826	0.732-0.920	< 0.001	52.8 pg/mL	80.4	80.4
TNF- α	0.787	0.701-0.874	< 0.001	25.7 pg/m	66.7	64.7

The current study showed that, for the ROC analysis of the parameter of ESR, the recorded values were as follows: cut-off value 9.0 hours/mm, sensitivity 88.2, and specificity 89.8, and confidence interval 0.892-0.983, under probability level of $P < 0.001$. The erythrocyte sedimentation rate (ESR) is the most

important diagnostic test studied because it occupied the largest area under the curve (0.938), as shown in (Figure 1).

Fig. 1: Receiver operating characteristic (ROC) curve analysis of ESR for predicting ankylosing spondylitis

The present study showed, based on the ROC curve analysis of IL-10, a cut-off value of 52.8 mm/pg, sensitivity of 80.4, specificity of 80.4, and confidence interval of 0.732-0.920, under a probability level of $p < 0.001$. IL-10 is the second significant diagnostic tests studied, after ESR, for the diagnosis of SA (Figure 2).

Fig. 2: Receiver operating characteristic (ROC) curve analysis of IL-10 for predicting ankylosing spondylitis.

The ROC analysis of TNF- α showed a cut-off value of 25.7 mm/pg, sensitivity of 66.7, specificity of 64.7, and confidence interval of 0.701-0.874 ($p < 0.001$). TNF- α is considered as the third significant diagnostic parameter of SA, after ESR and IL-10 (Fig. 3).

Fig. 3: Receiver operating characteristic (ROC) curve analysis of TNF- α for predicting ankylosing spondylitis.

The ROC curve analysis of IL-8 showed a cut-off value of 12.3 mm/pg, sensitivity of 52.9, specificity of 51.0, and confidence interval of 0.436-0.662 ($p < 0.001$). IL-8 is considered as having no diagnostic significance for SA, as found by the present results (Figure 4).

Fig. 4: Receiver operating characteristic (ROC) curve analysis of IL-8 for predicting ankylosing spondylitis.

DISCUSSION

The results of the present study showed no significant difference between the mean age of SA patients and control. These results are consistent with those of an earlier study (16), where the mean age of patients was found to be 38.0 ± 9.0 years, which had no significant differences to that of the control. When the patients and control were divided into different age groups (>40 , $30-40$, <30 years), no significant difference was observed in the numbers of patients. These results differed from those of a previous study (6), which reported that the percentage of SA patients with age of <30 years was 50.0%, while that for $30-40$ years was

42.5%, and for >40 years was 7.5%, indicating that the age of 30 constituted half of the cases, while only 7.5% were over the age of 40 years. In addition, the results of the present study are relatively consistent with those of a previous investigation (16), which demonstrated that the number of males was 78 (78.0%) in the patients' group and 99 (67.8%) in the control, with no statistically significant differences. Another study (6) reported that the prevalence of AS in the studied sample was 0.9%, with males constituting 90.6% and the ratio of males to females was 9:1. Autoimmune diseases are known to generally affect women more than men, except in the case of AS where the case is the opposite, which may be due to late diagnosis in women. Studies that have attempted to explain sex bias in AS have shown no evidence of sex-linked genetic factors or hormonal factor (e.g., androgen levels). However, the reason why males are affected more than females is not known (17). The results of the distribution of patients according to the duration of the disease revealed that most of the patients were within the duration group of ≤ 5 years. Distribution of patients according to the degree of functional impairment showed that most of them were within grade I, II. AS is the first disease that drew attention to the role of the human HLA-B27, where its frequency increases in patients as compared to control subjects. The current study showed that HLA-B27 positive SA patients represent a lower percentage than that of HLA-B27 negative ones, which may be due to the small size of the studied sample. HLA-B27 is used occasionally as a diagnostic parameter for AS, but the diagnosis varies from one population group to another, which suggests that antigen prevalence is affected by race. These same divisions were observed in an earlier study (6) which was conducted on a sample of Iraqi adult patients enrolled in the rheumatoid arthritis unit at Baghdad Teaching Hospital. The percentage of HLA-B27 positive patients was 55%, with a significant association between peripheral arthritis, high employment ranking, and severe disease activity. The reason behind these results may be the difference in the number of patients and the duration of the disease. Also, another study (18) showed that the rate of HLA-B27 antigen appearance is low among Syrian patients with SA as compared to other populations. The results of the current study also showed a significant decrease ($p < 0.001$) in the level of hemoglobin in the serum of patients with AS. These results matched those of a previous one (19), where Hb level in the patients (13.4 ± 1.8 pg/dl) was lower than that in the control. The results of the current study showed a significant increase in the WBC count ($P \leq 0.05$) in the serum of patients with AS, yet the number of cells was within normal limits. This study agrees with an earlier one (20) where WBC number was shown to be increased in AS patients. The results also showed a significant increase in the ESR. The value of ESR is correlated with disease activity,

as applied in this study, but it could also be measured based on C-reactive protein level (20).

There was also a significant decrease in the level of ALT and a significant increase in the level of AST in the serum of SA patients. These results matched those of a previous investigation (20), where the level of AST was significantly higher in AS patients (21.60 ± 6.07 U/L) as compared to the normal level (20.33 ± 5.36 U/L), but the values were still within the normal limits. The results of the current study showed a non-significant elevation in the level of CXCL-8 in the serum of patients with AS. CXCL-8 is a pro-inflammatory chemokine, originally identified as a neutrophil chemoattractant, which makes an important contribution in stimulating innate immunity through its effect on neutrophil chemotaxis and activation (21). These results are in accordance with those of an earlier work (22) which showed, using several methods of analysis, that higher disease activity and worse function in patients with confirmed AS are associated with increased levels of cytokines/chemicals (CXCL8) along with neutrophil activation and / or vascular formation. IL-8 levels appeared to be elevated in SA patients when compared to the control group subjects (23). Also, a significant decrease was observed in the level of IL-10 ($P \leq 0.05$) in the serum of patients with AS spondylitis. This implies an altered immune regulation because IL-10 regulates the immune system through the suppression of the cellular immune responses. In a previous study (17), patients with AS were found to have higher production of IL-10 by CD8+ T cells compared with any of the control groups. IL-10 do not have a significant effect on the susceptibility to AS, but may play a secondary role in determining age of disease onset and disease severity. The results also indicated a significant increase in the level of TNF-alpha ($P \leq 0.05$) in the serum of patients with AS. TNF-alpha is a biological factor that is mainly produced by active macrophages and monocytes during inflammatory responses. It can also stimulate the production of other proinflammatory cytokines. Apart from this, TNF- α stimulates endothelial cells to express adhesion molecules so that leukocytes can be attracted to inflammatory joints. TNF- α can also enhance the synthesis of metalloproteins and inhibit the synthesis of proteoglycans in cartilage (24). In a previous study (17), TNF- α level was found to be lower in the peripheral blood of AS patients.

The results of the current study showed no significant difference in the level of CXCL-8 ($P \leq 0.05$) in relation to sex, age, disease severity, and functional impairment degree. Also, there was no significant difference in the level of CXCL-8 between HLA-B27-positive and HLA-B27-negative patients. However, the difference in the level of CXCL-8 in AS patients approached a significant value ($P = 0.052$) when different durations of the disease were compared. The

results also showed no significant difference in the level of IL-10 ($P \leq 0.05$) in relation to all these parameters.

The results of the current study showed a significant difference in the serum level of TNF- α ($P \leq 0.05$) between males and females with AS. However, no significant differences in the level of TNF- α were found in relation to age, severity of the disease, HLA-B27 positivity, and duration of the disease. In addition, a significant difference was recorded based on the degree of functional disability, where the level of TNF- α was high in the patients with the I, II degree of functional disability and then decreased when the disease settled to the third and fourth stages.

Based on the ROC analysis, the area under the curve was determined for the different parameters covered by the present study. The most significant factors were found to be, respectively, the ESR, IL-10, and TNF- α , whereas CXCL-8 had no diagnostic significance. These results are in accordance with those of a previous work (25), which reported that ESR has the most significant area under the curve, with sensitivity of 70.0% and specificity of 71.8%.

CONCLUSION

In conclusion, a significant decrease was observed in the serum level of IL-10 in AS patients. There was also a significant decrease in the levels of ALT with a significant increase in the level of AST in the serum of patients. The most significant factor was found to be ESR, followed by IL-10, and then TNF- α , with the presence of significant differences, while there was no diagnostic significance for CXCL-8 which showed no significant difference.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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