European Review for Medical and Pharmacological Sciences 2024; 28: 80-89

Correlation of serum IL-6, TNF-α levels and disease activity in patients with ankylosing spondylitis

W.-B. ZHAO[1], K.-R. LIN[2], Q.-F. XU[1]

[1]Orthopedic Ward Five, The First People's Hospital of Pingdingshan City, Pingdingshan, China
[2]Orthopedic Ward Three, The First People's Hospital of Pingdingshan City, Pingdingshan, China

Abstract. – OBJECTIVE: The aim of this study was to investigate the serum interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) levels in patients with ankylosing spondylitis (AS), and the correlation between serum levels and disease activity.

PATIENTS AND METHODS: ELISA was used to detect the serum TNF-α and IL-6 levels of AS patients (n=40) and normal controls (n=40) who were hospitalized or outpatient-diagnosed from June 2021 to May 2023. C-reactive protein (CRP) was detected by immune-enhanced turbidimetry. The erythrocyte sedimentation rate was determined by Wei's manual method. The correlation was analyzed by Pearson's correlation analysis.

RESULTS: The levels of TNF-α and IL-6 in the AS group were significantly higher than those in the control group, and the levels of TNF-α and IL-6 in AS patients in the active phase were higher than those in the stable phase ($p<0.05$). CRP level was positively correlated with TNF-α, and IL-6 (r=0.02886 and 0.0273, $p<0.05$). Erythrocyte sedimentation rate (ESR) level was also positively correlated with TNF-α, and IL-6 (r=0.07568 and 0.0613, $p<0.05$).

CONCLUSIONS: Serum TNF-α and IL-6 levels are correlated with AS disease activity, suggesting that they may be involved in the inflammatory response of AS.

Key Words:
IL-6, TNF-α, Disease activity, Ankylosing spondylitis.

Introduction

Ankylosing spondylitis (AS) is a chronic systemic inflammatory joint disease. It is the prototype of spondyloarthritis (SpA). It primarily affects the axial bones, including the spinal and sacroiliac joints, as well as seronegative spondyloarthritis. Inflammation affects synovial and cartilaginous joints and the sites where tendons and ligaments attach to bone (tendon ends)[1].

AS commonly leads to fibrous and bony ankylosis. The most common symptoms include inflammatory low-back pain, stiffness, and limited motion. Furthermore, some patients may experience peripheral arthritis, tendinopathy, ophthalmia, and other extra-articular manifestations. Patients may, therefore, have spinal fusion and hip involvement or extraspinal manifestations that lead to severe dysfunction, resulting in difficulties with living behaviors[2].

AS usually begins in adolescence or early adulthood with a male-to-female ratio of 2-3:1. There is a high incidence in young males aged 20-30; the main pathological changes are inflammation, bone destruction, osteophyte formation, and finally, bone fusion, ankylosing deformity. The late stage of the disease seriously affects the quality of life of patients[3].

Inflammation is a crucial factor in the pathogenesis of AS, and some studies[4,5] have recently provided important insights into its genetic structure. In addition to *HLA-B27*, the discovery of many novel gene associations points to known and other inflammatory cytokine pathways that are involved in the pathogenesis of AS. Single nucleotide polymorphisms in cytokines and their receptors and intracellular signaling molecules identify tumor necrosis factor (TNF), IL-1, IL-6, and IL-23/IL-17 as major cytokine pathways[6].

The exact pathogenesis of AS has not yet been elucidated, and genetic, immune, and environmental factors all play a role. Among them, *human leukocyte antigen* (*HLA*)-*B27* is currently the most well-known pathogenic gene. *HLA-B27* may be the main gene involved in AS susceptibility. However,

Corresponding Author: Qifei Xu, MD; e-mail: Zhaowubin123456@163.com; 196091215@mail.sit.edu.cn

it acts together with other genes and has little effect on determining the severity of the disease. About 90% of AS patients are positive for *HLA-B27*[7].

The study of animal models and human tissue samples has found[8,9] that TNF-α plays a crucial role in the pathogenesis of AS. Transgenic mice overexpressing TNF-α can develop axial and tendon-end lesions similar to human AS. The levels of inflammatory markers, TNF-a, and IL-6 in the serum of AS patients were higher than those of other non-inflammatory low-back pain patients and healthy controls[10]. However, cytokine concentrations were not correlated with laboratory and clinical parameters reflecting disease activity. There are a large number of TNF-α T cells and macrophages in the sacroiliac joint inflammation site, and a large amount of TNF-α mRNA and protein expression was also found in the sacroiliac joint biopsy tissue. TNF is considered to be a potent pro-inflammatory molecule. The expression of TNF in AS patients increases the importance of TNF in this disease. This has been confirmed[11] by successful treatments using anti-TNF drugs. Despite the success of anti-TNF agents in remission of disease activity, to date, radiographic evidence of anti-TNF therapy in AS suggests[12] no change in radiographic progression.

TNF-α plays a role in mediating inflammation and immune regulation in the immune response. Highly expressed in blood and tissues in many autoimmune diseases, the importance of TNF-α has received extensive attention. In recent years, anti-TNF-α monoclonal antibody has been used[13] to treat a variety of autoimmune diseases, including rheumatoid arthritis, psoriatic arthritis, and so on. There are a variety of anti-tumor necrosis factor inhibitors used clinically at home and abroad that have achieved good curative effects. This study[14] was subsequently used in many countries (infliximab) for the treatment of AS. These open studies[15,16] have shown that TNF-α inhibitors have an evident curative effect, indicating that TNF-α may be a pathogenic factor, which is related to the activity of the disease. Compared with healthy subjects, very high concentrations of TNF-α mRNA and protein expression levels were found[17] in AS serum and sacroiliac joint biopsy tissue. Therefore, detecting the expression level of TNF-α in the body is expected to become a new auxiliary index for AS disease diagnosis[18].

In recent years, it has been found[19] that interleukin-6 (IL-6) also plays a crucial part in the pathogenesis of AS. Targeted therapeutic drugs against IL-6 have been proven effective in various autoimmune diseases[20]. Tocilizumab has been approved for the treatment of rheumatoid arthritis. An important feature of AS pathology is the abnormal regulation of the cytokine network. Several studies[21-24] have shown that AS patients have higher serum IL-6 levels than healthy controls. Moreover, immunohistochemical staining found[25] that the level of IL-6 in the sacroiliac joints of AS patients was also overexpressed. This suggested that IL-6 was related to disease activity and could be used as an index to evaluate disease activity.

Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) is currently the most widely used tool for evaluating the disease activity of patients with ankylosing spondylitis, and the evaluation of BASDAI can effectively reflect the disease activity of patients[26]. CRP is a non-specific marker that reflects systemic inflammation. In acute inflammation and infection, CRP has a good correlation with disease activity[27]. Erythrocyte sedimentation rate (ESR) indicates the sedimentation velocity of red blood cells, reflecting the activity of spondylitis rheumatic fever[28]. Many studies[29,30] found that ESR and CRP are increased in AS patients. Therefore, BASDAI, ESR, and CRP are currently recognized as indicators for judging AS disease activity.

Therefore, the purpose of this study is to analyze the levels of TNF-α and IL-6 in the peripheral blood of patients with ankylosing spondylitis, and their correlation with the disease. The study was designed to provide a solid foundation for clinical assessment and treatment.

Patients and Methods

Patients who were in our hospital's rheumatology department from June 2021 to May 2023 were selected. Patients were diagnosed with ankylosing spondylitis according to the 1984 revised New York criteria[31]. Among them, 40 patients were included in the AS group, and 40 healthy blood donors were in the control group. At the same time, we selected healthy subjects in our hospital's physical examination center as the normal control group. A total of 120 patients had no symptoms or signs of angina; no diabetes mellitus (DM), high blood pressure, liver, kidney, or other diseases.

Inclusion Criteria

(1) Patients who met the diagnostic criteria of the Chinese Medical Association Rheumatology

Branch[32]. (2) complete examination and medical history data. (3) Informed consent of the patient's family members.

Exclusion Criteria

(1) No mental or intellectual developmental disorders. (2) Severe liver and kidney insufficiency or heart failure. (3) Acute myocarditis. (4) Severe infection. (5) Autoimmune or connective tissue disease. (6) Acute phase of cerebral infarction. (7) Malignant tumors.

Methods

A total of 2 ml of peripheral blood was drawn in the active phase and remission phase, respectively, and 2 ml of peripheral blood was drawn in the control group at the same time. The serum was separated and stored at -20°C for testing. IL-6 and TNF-α were determined by the conventional ELISA method. ESR and CRP levels were measured in both groups at the same time.

The BASDAI was used to judge the period of disease activity. There are 6 questions in the BASDAI questionnaire, which are assessed using a 10 cm visual analog scale method, and using mm to record. An X is marked at the corresponding position on each 10 cm visual analog scale, with 0 indicating no effect and 10 indicating extremely severe.

Determination of Serum Factor Concentrations

The kits were all provided by IND Company (USA). The methods used to perform the examinations were enzyme-linked immunosorbent assay (ELISA) and scattering rate turbidimetry. The instrument was a Deling BEP2000 automatic microplate reader (Deling Co., Ltd., London, OH, USA). The operation was performed strictly according to the manufacturer's instructions. IL-6 and TNF-α were detected by ELISA (Shenzhen Jingmei Bioengineering Co., Ltd., Shenzhen, China), by inspection professionals in strict accordance with the test instructions. CRP was detected by the immune-enhanced turbidimetric method (Shenzhen Guosai Biotechnology Co., Ltd., Shenzhen, China). ESR was determined by the Wester-gren manual method[33].

Statistical Analysis

The data were analyzed using SPSS 21.0 (IBM Corp., Armonk, NY, USA). The data were expressed as (±s), Pairwise comparison was carried out by the least significant digit (LSD) method, and the correlation coefficient was obtained by linear regression analysis. $p<0.05$ was considered statistically different.

Results

Comparison of Clinical Data

We compared the general clinical data of selected patients between the two groups (Table I). The results showed that the age and sex between the two groups were comparable ($p>0.05$). Among them, the minimum age of onset in the AS group was 13 years old. Peripheral joint involvement accounted for 75% of patients, of whom 70% were treated with biologics, and 47.5% had a family history.

Comparison of Expression Levels of TNF-α and IL-6 in AS Patients and Normal Controls

We measured TNF-α, IL-6, CRP, and ESR levels between the two groups. The results showed (Table II, Figure 1) that the four indicators of AS were higher than those of the control group, with $p<0.05$. This shows that the levels of these cytokines in AS patients are higher than those in normal individuals.

Comparison of the Expression Levels of TNF-α and IL-6 in the Active and Inactive Phases of the AS Group

We also compared the expressions of TNF-α, IL-6, CRP, and ESR in the active and inactive phases of the AS group. The results showed

Table I. Comparison of general clinical data of patients.

Indexes	Control group (n = 40)	AS group (n = 40)	t/χ^2	p
Age	29.48 ± 6.05	27.80 ± .57	1.190	0.238
Gender (Male/Female)	25/15	17/23	3.208	0.073
Disease course/month	-	34.85 ± 14.18	-	-
Peripheral joint involvement	-	30	-	-
Used biological agents	-	28	-	-
Family history	-	19	-	-

Table II. Comparison of each index among 2 groups ($\bar{x} \pm s$).

Indexes	Control group (n = 40)	AS group (n = 40)	t/χ^2	p
TNF-α (pg/ml)	6.20 ± 1.32	66.18 ± 21.81	-17.361	0.000
IL-6 (pg/ml)	2.07 ± 0.64	76.17 ± 15.78	-28.675	0.000
CRP (mg/dL)	1.77 ± 0.51	33.13 ± 5.02	-39.307	0.000
ESR (mm/h)	8.72 ± 1.24	59.76 ± 13.46	-23.881	0.000

Erythrocyte sedimentation rate (ESR), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, C-reactive protein (CRP).

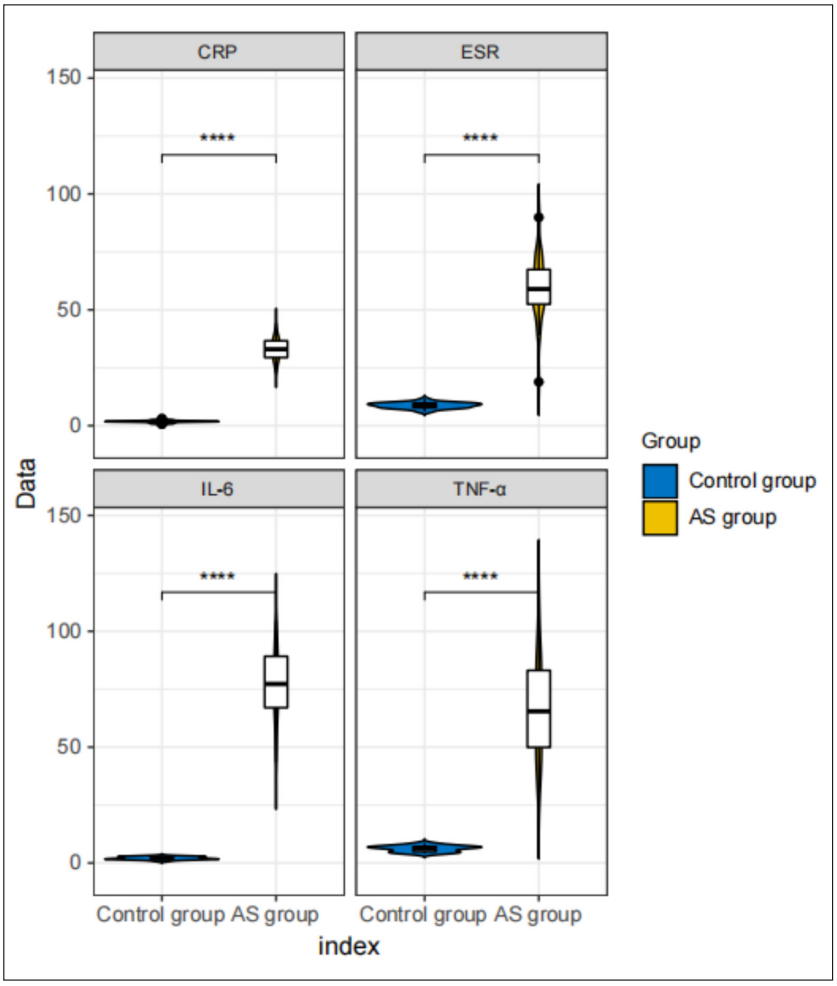

Figure 1. Violin plot for comparison of each index between the two groups.

(Table III, Figure 2) that in the active phase, the expression of these four factors was relatively higher in AS patients. In the stable period, the expressions were reduced, and $p<0.05$.

Correlation Between TNF-α and Other Clinical Disease Activity Indicators in AS Patients

We performed a correlation analysis on the indicators in the AS group (Table IV). We used Pearson's correlation analysis and the result showed that CRP level was positively correlated with TNF-α, and IL-6 (r=0.02886 and 0.0273, $p<0.05$). ESR level was also positively correlated with TNF-α, and IL-6 (r=0.07568 and 0.0613, $p<0.05$).

Discussion

AS is a chronic inflammatory disease. Inflammation and bone destruction are typical patho-

Table III. Comparison of the expression levels of TNF-α and IL-6 in the active and inactive phases of the AS group.

Indexes	AS group active phase (n=40)	AS group inactive phase (n=40)	t/χ^2	p
TNF-α (pg/ml)	76.03 ± 27.35	40.32 ± 14.53	7.293	0.000
IL-6 (pg/ml)	31.43 ± 13.85	4.65 ± 1.85	12.121	0.000
CRP (mg/dL)	56.86 ± 20.27	5.76 ± 2.16	15.854	0.000
ESR (mm/h)	83.11 ± 34.65	26.24 ± 14.61	9.565	0.000

Erythrocyte sedimentation rate (ESR), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, C-reactive protein (CRP).

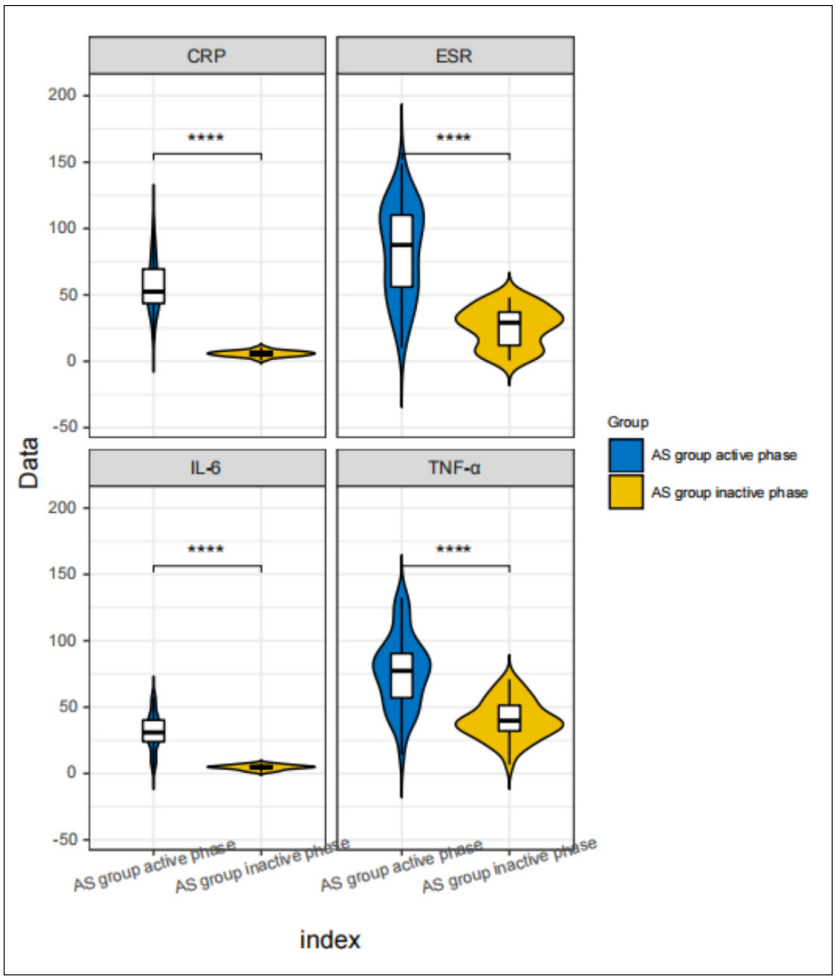

Figure 2. Violin plot for comparison of the expression levels of TNF-α and IL-6 in the active and inactive phases of the AS group.

Table IV. Correlation between TNF-α and other clinical disease activity indicators in AS patients.

Index	TNF-α (pg/ml)		IL-6 (pg/ml)	
	r	p	r	p
CRP (mg/dL)	0.02886	0.027	0.0273	0.045
ESR (mm/h)	0.07568	0.017	0.0613	0.025

Erythrocyte sedimentation rate (ESR), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, C-reactive protein (CRP).

logical changes during its development. As the disease progresses, inflammation affects synovial joints, cartilaginous joints, and sites where tendons and ligaments attach to bone, causing fibrous and bony ankylosis. In the later stage, the spine shows a "bamboo-like" change, resulting in disability or even loss of the labor force of the patient, which brings a heavy burden to the family and society[34]. Epidemiological surveys[35] show that its prevalence is higher in men than in women, and the age of onset is between 15 and 30 years old, which is strongly correlated with the expression of *HLA-B27*. Inflammation is an important part of AS disability, and controlling inflammation is the key to delaying disease progression[36]. The disorder of the body's immune function is a critical factor in the occurrence and development of AS inflammation, and the change of T lymphocyte subsets plays a crucial part in causing this disorder.

TNF-α is mainly secreted by monocytes and phagocytes. It exerts biological effects by binding to corresponding receptors and is an important link between specific immune responses and acute inflammation. The biological effect of TNF-α depends on its concentration. Within the normal concentration range, TNF-α can regulate adaptive immunity, kill target cells, and induce apoptosis, thereby protecting the body, while overexpression can lead to systemic toxicity. TNF-α can activate polymorphonuclear cells, stimulate prostaglandin production, inhibit alkaline phosphatase and collagen synthesis, and cause bone and cartilage destruction. TNF-α can promote chondrocytes to secrete plasmin activators and promote the transformation of plasminogen into plasmin, thereby accelerating the process of arthritis damage. In addition, it can induce macrophages, synoviocytes, fibroblasts, and chondrocytes to produce IL-1, IL-8, and other cytokines to aggravate tissue damage[37]. The application of TNF-α antagonists can relieve the degree and duration of morning stiffness, chest expansion, and inflammatory back pain to a certain extent.

CRP and ESR are two typical acute-phase reactants used to assess inflammation in patients. However, they are nonspecific and have low sensitivity[38], especially in patients with axial SpA without radiologic changes, and the acute phase reactants are mostly within the normal range[39]. Many studies aim to identify biomarkers to predict the clinical treatment effect or disease activity of AS due to its efficient and useful detection method. After discovering and understanding new effective pathological ways, IL-6 has been the focus of public attention in recent years. However, although some studies[40,41] have shown the association between IL-6 and disease activity, others have found[42,43] the opposite.

IL-6 is a multifunctional cytokine in the interleukin family. IL-6 in the blood is mainly produced by activated monocyte-macrophages, and they are the first to produce IL-6 when the body has an inflammatory response[44]. IL-6 is a pleiotropic cytokine involved in pro-metabolism, bone metabolism, regeneration, and nerve conduction, and plays a crucial part in the regulation and growth of many tumors. In addition to these biological activities, IL-6 exhibits autoregulatory homeostatic and anti-inflammatory properties in obesity-associated inflammation and exercise[45-47]. IL-6 is secreted by a variety of cells, including monocytes, fibroblasts, and endothelial cells. T cells, B cells, osteoblasts and adipocytes play a crucial role in stimulating the production of IL-6. Importantly, overexpression and aberrant expression of the IL-6 signaling pathway are hallmarks of aggressive disease development in autoimmune diseases and cancer. Under normal circumstances, the level of IL-6 in serum is relatively low, but it will increase rapidly in disease states or some special cases[48]. This shows that IL-6 is closely related to the pathogenesis of autoimmune diseases, infections, and tumors, including AS, and IL-6 is more sensitive than CRP in evaluating disease activity[49-51]. IL-6/IL-6R may participate in the whole AS process. However, the specific mechanism of *IL-6* gene polymorphism in AS remains to be further explored[44].

In AS patients, the factors at the core are TNF-α and IL-6. TNF-α and IL-6 are important mediators in AS joint inflammation. IL-6 can induce the production of other cytokines, such as IL-1, IL-2, and TNF-α, to play a pathogenic role, so inhibiting abnormally elevated serum inflammatory cytokines is an important means of treating both diseases. The observations of this study showed that in the active stage of the disease, the serum TNF-α and IL-6 levels of the patients were higher than those in the inactive stage and the healthy control group. The patients in the active stage are mostly male patients with early disease and relatively young age. This shows that cytokines TNF-α and IL-6 are closely related to the disease activity of AS. It also showed that TNF-α and IL-6, as inflammatory factors, directly participated in the process of the acute active phase.

The results of this study are roughly the same as those reported in the literature above, suggesting that IL-6 and TNF-α have a significant synergistic effect and regulate each other to promote the occurrence and development of diseases. In addition, our study found that TNF-α and IL-6 were positively correlated with clinical disease activity indicators, which suggested that TNF-α and IL-6 may be involved in the development of active AS disease. TNF-α is a cytokine upstream of IL-6 and is a pro-inflammatory cytokine. It sits at the apex of a network of interconnected pro-inflammatory cytokines. IL-6 can induce the proliferation and differentiation of B cells, secrete a variety of antibodies, and induce the production of acute phase reaction proteins. It cooperates with various cytokines to increase the production of cytokines by synoviocytes. It can promote bone destruction and bone resorption, and thus participate in the occurrence and development of AS patients[52,53].

Limitations

This study also has some limitations. First, the sample size was small. Secondly, this study was a single-center study. Finally, the correlation between other inflammatory indicators and disease activity in patients with ankylosing spondylitis was not explored. In the future, we will perform a multicenter study with a larger sample size to explore the correlation between inflammatory markers and disease activity in patients with mandatory spondylitis.

Overall, in AS, inflammation plays a crucial part in its pathogenesis. Therefore, controlling inflammation is the primary goal of treating AS patients. Cytokines have been recognized as important mediators in the pathogenesis of AS, and many *in vivo* and *in vitro* research results[40,41] have shown that cytokines may mediate the interaction between cells and cause the release of tissue enzymes to cause joint damage. We can also observe that cytokines play a crucial part in the process of cell growth, and their pathological effects are caused by excessive production or insufficient inhibitors. During the course of infectious diseases, the release of cytokines in the body increases, and the body conducts an anti-infection immune response, followed by complex immune function changes. Although good progress has been made in the diagnosis and treatment of AS in recent years, the exact etiology of the disease has not yet been confirmed, nor can a radical cure be found.

Conclusions

Serum TNF-α and IL-6 levels are correlated with AS disease activity, suggesting that they may be involved in the inflammatory response of AS.

Conflict of Interest

The authors declare that they have no conflict of interests.

Acknowledgements

We thank the members of the First People's Hospital of Pingdingshan City, Medical University for helpful discussions. We apologize to the scientists whose work could not be cited due to space limitations.

Authors' Contribution

Wubin Zhao conceived the structure of the manuscript. Kuiran Lin did the experiments and made the figures. Qifei Xu reviewed and edited the manuscript. All authors read and approved the final manuscript.

Funding

None.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval

This study was approved by the Ethics Committee of the First People's Hospital of Pingdingshan [Pingyi Medical Ethics (2023) No. 6)]. The study was conducted following the ethical regulations of Helsinki Declaration.

Informed Consent

All patients signed the informed consent form.

Authors' Contribution

Wubin Zhao: 0009-0009-0444-3494

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