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Original Article

Effects of glucocorticoids on the levels of serum tumor necrosis factor alpha and interleukin 6 in patients with rheumatoid arthritis

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Abstract: Objective: The study was designed to explore the effects of glucocorticoid therapy on the levels of serum interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) in patients with rheumatoid arthritis (RA). Methods: Clinical information of 100 patients with RA who were admitted to our hospital from 2015 to 2018 were retrospectively collected and divided into two groups according to the random number table method. Patients receiving routine treatment were classified as the control group (n = 50) and those receiving glucocorticoid therapy based on routine treatment were classified as the observation group (n = 50). Pre- and post-treatment clinical effects, tender joint counts, swollen joint counts; periods of morning stiffness, visual analog scale (VAS) scores, Disease Activity Score-28 (DAS28), erythrocyte sedimentation rate (ESR), and rheumatoid factor (RF), IL-6, and TNF-α levels were compared between the two groups. Results: Compared with the control group, the observation group had a higher total effective rate. The observation group exhibited lower tender and swollen joint counts and shorter morning stiffness periods than the control group ($P < 0.05$). The VAS scores and DAS28 in the observation group were significantly lower than those in the control group ($P < 0.05$). The ESRs and RF levels as well as the post-treatment IL-6 and TNF-α levels were lower in the observation group than in the control group ($P < 0.05$). Conclusion: Glucocorticoids show beneficial effects on alleviating RA symptoms. Due to the limited sample size in the study, future studies with a larger cohort and over a longer investigation period are warranted to provide comprehensive results.

Keywords: Rheumatoid arthritis, conventional therapy, glucocorticoids, IL-6, TNF-α

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease with a high clinical incidence [1]. Synovial tissue hyperplasia is one of the principal symptoms of RA, which can lead to severe damage to the articular cartilage and bones with disease progression. Patients may lose their ability to work and face life-threatening levels of damage to their bodies [1]. Clinically, the pathogenesis of RA has not yet been entirely elucidated. High incidence and mortality rates of RA render the development of effective treatments for this disease critical [2].

Multiple drugs have been developed in an attempt to cure RA, including commonly used herbal preparations, glucocorticoids, biological agents, and nonsteroidal anti-inflammatory drugs [3, 4]. Glucocorticoids were particularly found to exhibit noticeable anti-inflammatory functions and offer several benefits to patients diagnosed with certain complex heterogeneous disorders [5]. Glucocorticoids work by inhibiting the transcription of proinflammatory factors [6], which are closely associated with the occurrence and progression of RA [7]. This study was designed to investigate the efficacy of glucocorticoid application in RA treatment and its effects on interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α), which are important proinflammatory factors [8].

Materials and methods

Clinical data

The clinical data of 100 patients with RA were admitted to our hospital from 2015 to 2018 were divided into two groups according to the

random number table method. Patients (n = 50) receiving routine treatment were classified as the control group and those (n = 50) receiving glucocorticoid therapy based on routine treatment were classified as the observation group. The control group included 28 males and 22 females (age, 22-70 years), and the observation group included 30 males and 20 females (age, 23-68 years). Inclusion criteria: patients who provided the signed written informed consent, who presented no contraindications to the use of drugs prescribed, and who had not received any slow-acting antirheumatic drugs before participating in this study were included. Exclusion criteria: patients with allergies, hematopoietic system diseases, abnormal heart, liver, or kidney functions, severe joint deformities, or any endocrine system disease were excluded. This study was agreed and approved by the Medical Ethics Committee of The First People's Hospital of Fuyang Hangzhou.

Therapies

Patients in the control group received routine treatment, including 5.0-12.5 mg methotrexate tablets (SFDA Approval No. H31020644; 100 tablets of 2.5 mg each; Shanghai Pharmaceuticals Sine) once a week and 5 mg leflunomide tablets (SFDA Approval No. H20050175; 30 tablets of 10 mg each; Fujian Huitian Biopharma Co., Ltd.) twice per day (in the morning and evening) orally. The treatment lasted for 6 months.

Patients in the observation group received glucocorticoid therapy in addition to the routine treatment, including 10 mg prednisone tablets (SFDA Approval No. H33021207; 5 mg per pill; Zhejiang Xianju Pharmaceutical Co., Ltd.) twice per day (in the morning and evening) orally. The treatment lasted for 6 months.

Patients in both groups were instructed to maintain adequate exercise and rest levels while using the study medication.

Observation indices

Curative effects: The curative effects were evaluated as follows [9]: ineffective: when joint and clinical symptoms were not improved and/or conditions were further aggravated; effective: when the joint motion was increased, most clinical symptoms were notably improved, and X-ray examination revealed no additional joint swelling; cured: when the joint motion was normal, no clinical symptoms were observed, and X-ray examination revealed healthy joints. The combination of effective and cured categories was used to calculate the total effective rate.

Symptoms and signs: Tender joint count, swollen joint count, and morning stiffness periods were measured pre- and post-treatment for intergroup comparisons.

Visual analog scale (VAS) and disease activity score-28 (DAS28)

The pain intensity of patients was evaluated through VAS pre- and post-treatment. The intensity was expressed on a scale of 0-10 points, with a score of 0 point indicating no pain and 10 points indicating the highest level of pain. The score ranges were described as follows: 1-3, slight pain that could be completely tolerable; 4-6 points, pain that affected sleep quality but remained tolerable; and 7-10, increasing pain that was intolerable and required treatment for suppression. Patients were instructed to select the number that best represented the pain they experienced [10, 11]. In addition, a DAS28 of ≤2.6 points indicated an alleviation of disease activity, that of ≥3.2 points indicated active disease, and that of ≥5.1 points indicated highly active disease [12, 13].

Erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF)

Pre- and post-treatment, 2 mL of fasting venous blood was collected in the morning from patients in both groups and centrifuged at 3,000 rpm to separate the serum. The separated serum was used to determine RF levels using enzyme-linked immunosorbent assay (ELISA) kit (Dalian Nanmei Pharmaceutical Co., Ltd., Dalian, China) following the manufacturer's protocol. To measure ESR, 1.28 mL of fasting venous blood was collected in the morning from patients in both groups and subjected to anticoagulation management and intensive mixing. ESR was measured using a fully automatic dynamic ESR analyzer (Shenyang Baokang Biotechnology Co., Ltd., Shenyang, China).

Table 1. Comparison of general information of both groups [n (%)]/ ($\bar{x}$ ± s)

Information		Observation group (n = 50)	Control group (n = 50)	t/X^2	*P*
Sex (% of group)	Male	30 (60.00)	28 (56.00)	0.164	0.685
	Female	20 (40.00)	22 (44.00)		
Age (years)		45.26 ± 2.28	45.96 ± 2.16	1.576	0.118
Course of disease (years)		4.96 ± 0.58	5.02 ± 0.38	0.612	0.542
Pathogenic site					
Articulatio humeris		18 (36.00)	16 (32.00)	0.178	0.673
Articulatio carpi		10 (20.00)	13 (26.00)	0.508	0.476
Knee joints		13 (26.00)	11 (22.00)	0.219	0.640
Others		9 (18.00)	10 (20.00)	0.065	0.799

TNF-α and IL-6

Pre- and post-treatment, 2 mL fasting venous blood was collected in the morning from patients in both groups and centrifuged at 3,000 rpm to separate the serum. TNF-α and IL-6 levels were determined using an ELISA kit (Hebei Changtian Pharmaceutical Co., Ltd., Baoding, China).

Instruments and reagents

Label indicator, microplate washer, enzyme-linked strip, micropipettor, 0.05 M pH 9.6 carbonate buffer solution, 0.15 M pH 7.4 PBST, diluent, blocking solution, 0.2 M Na_2HPO_4, 0.1 M citric acid, 0.1 M EDTA, substrate reaction solution A, substrate reaction solution B, and stop buffer (2 M H_2SO_4) were used.

Specific operation process of ELISA

The recombinant protein was diluted with buffer solution at 1:100, 1:200, 1:400, 1:800, and 1:1600, and 100 μL was added to each well. Two rows of parallel and negative control wells were set. The recombinant protein was coated overnight at 4°C (12-18 h) or at 37°C for 5 h. The next day, it was sealed with block solution at 37°C for 1 h and washed with PBST three times for 2 min each time. After that, 100 μL of the primary antibody diluted 1:1000 with the diluent was added, incubated at 37°C for 30 min, and then washed with PBST three times for 2 min each time. Next, 100 μL 1:1000 diluted HRP-labeled secondary antibody (rabbit anti-horse IgG) was incubated at 37°C for 15 min. After washing, ELISA color development A solution and B solution, 50 μL each, were added respectively, and 50 μL stop buffer was added after 5 min. Finally, the A450 nm was determined, and it was determined as positive when the ratio of absorbance value of test well and negative control well was > 2.1 at A450 nm.

Statistical analysis

SPSS version 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. All measurements were expressed as mean and standard deviation. Normally distributed data were calculated using independent samples t-test, and non-normally distributed data were calculated using the Mann-Whitney U test. Paired t-tests were used for intergroup comparisons. Enumeration data were expressed as [n (%)], and intergroup enumeration data were assessed using the chi-square test. Statistical graphics were drawn by Graphpad Prism 8. $P < 0.05$ indicated significant differences.

Results

Comparison of general information between the two groups

The observation group included 30 males (60.00%) and 20 females (40.00%), ranged 23-68 years, with a mean age of (45.26 ± 2.28) years. The disease duration ranged from 1 to 8 years, with a mean duration of (4.96 ± 0.58) years. The pathogenic site was the articulatio humeri in 18 (36%), the articulatio carpi in 10 (20%), the knee joints in 13 (26%), and other sites in 9 (18%) patients. The control group included 28 males (56.00%) and 22 females (44.00%), ranged 22-70 years, with a mean age of (45.96 ± 2.16) years. The disease duration ranged from 2 to 9 years, with a mean duration of (5.02 ± 0.38) years. The pathogenic site was the articulatio humeri in 16 (32%), the articulatio carpi in 13 (26%), the knee joints in 11 (22%), and other sites in 10 (20%) patients. No statistically notable distinctions in sex, average age, average disease duration, and pathogenic site were noted between the two groups ($P > 0.05$) (**Table 1**).

Table 2. Comparison of clinical effects of treatment groups [n (%)]

Group	Cases	Cured	Effective	Ineffective	Total effective rate
Control group	50	23 (46.00)	15 (30.00)	12 (24.00)	38 (76.00)
Observation group	50	35 (70.00)	13 (26.00)	2 (4.00)	48 (96.00)
X^2					8.305
P					0.004

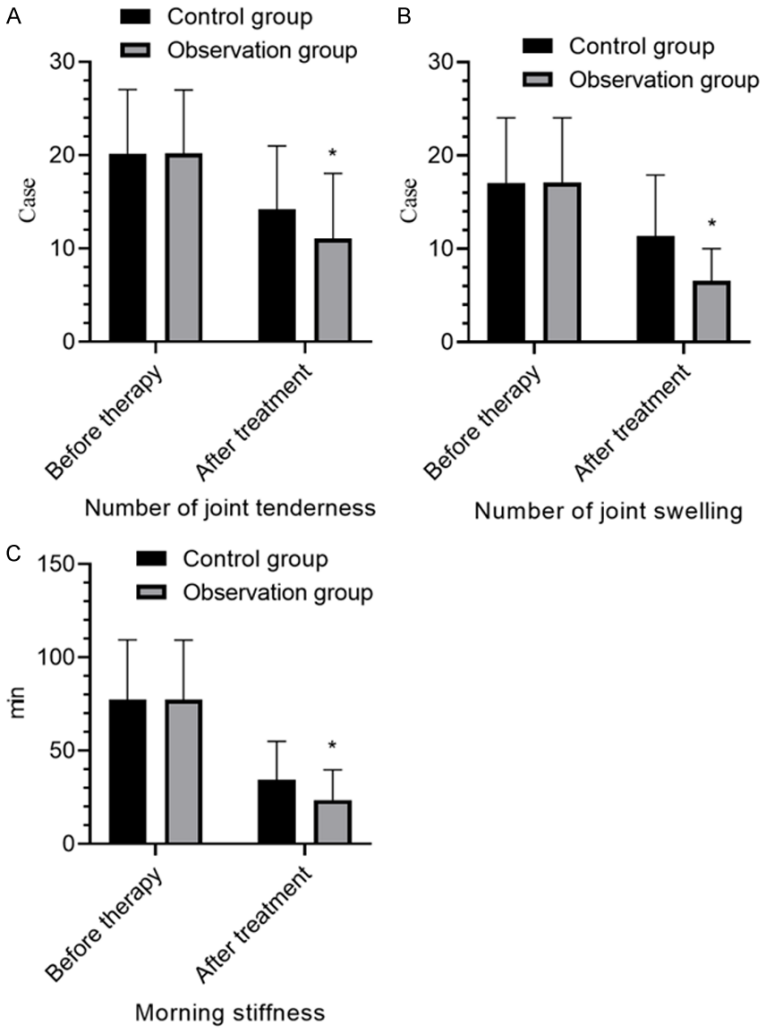

Figure 1. Comparison of the symptoms and signs among treatment groups. A: Compared with tender joint counts between the two groups before treatment, $P > 0.05$; after treatment, the observation group had less tender joint counts than the control group, $P < 0.05$. B: Compared with swollen joint counts between the two groups before treatment, $P > 0.05$; after treatment, the observation group had less swollen joint counts than the control group, $P < 0.05$. C: Compared with periods of morning stiffness between the two groups before treatment, $P > 0.05$; after treatment, the observation group had shorter periods of morning stiffness than the control group, $P < 0.05$. *Compared with the control group, $P < 0.05$.

Comparison of clinical effects between the two groups

In the observation group, there were 23 (70%) cured cases, 13 (26%) effective cases, and 2 (4%) ineffective cases, with the total effective rate of 96%. In the control group, there were 23 (46%) cured cases, 15 (30%) effective cases, and 12 (24%) ineffective cases, with the total effective rate of 76% ($P < 0.05$) (**Table 2**).

Comparison of symptoms and signs between the two groups

The post-treatment tender joint and swollen joint counts were lower and morning stiffness periods were shorter than the pre-treatment values in both groups ($P < 0.05$). In addition, the observation group exhibited lower tender joint and swollen joint counts post-treatment but shorter morning stiffness periods ($P < 0.05$, all) than the control group (**Figure 1**).

Comparison of VAS scores and DAS28 between the two groups

Although the pre-treatment VAS scores and DAS28 of the two groups presented no significant differences ($P > 0.05$), these scores were decreased post-treatment ($P < 0.05$). The post-treatment VAS scores and DAS28 in the observation group were lower than those in the control group ($P < 0.05$) (**Figure 2**).

Comparison of ESR and RF levels and TNF-α and IL-6 levels between the two groups

The post-treatment ESR and RF levels in the two groups decreased relative to the respective pretreatment values ($P < 0.05$). The ESR

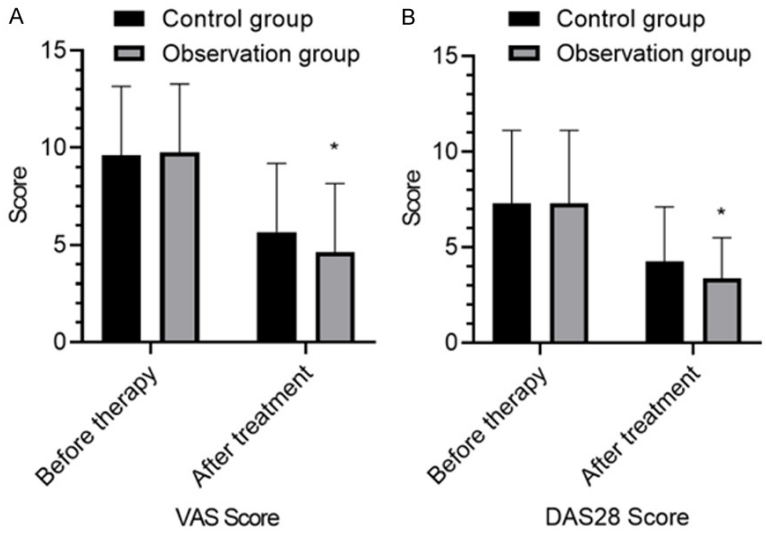

Figure 2. Comparison of VAS scores and DAS28 of treatment groups. A: Compared with VAS scores between the two groups before treatment, $P > 0.05$; after treatment, the observation group had lower VAS scores than the control group, $P < 0.05$. B: Compared with DAS28 scores between the two groups before treatment, $P > 0.05$; after treatment, the observation group had lower DAS28 scores than the control group, $P < 0.05$. *Compared with the control group, $P < 0.05$.

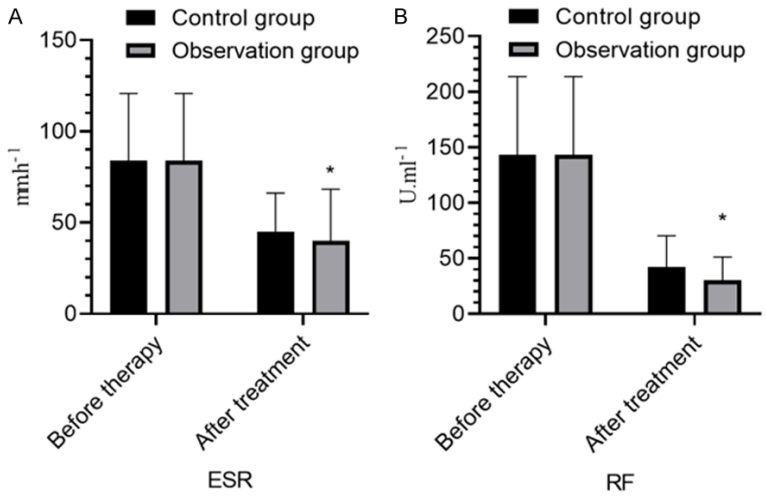

Figure 3. Comparison of the ESR values and RF levels of treatment groups. A: Compared with ESR values between the two groups before treatment, $P > 0.05$; after treatment, the observation group had lower ESR values than the control group, $P < 0.05$. B: Compared with RF levels between the two groups before treatment, $P > 0.05$; after treatment, the observation group had lower RF levels than the control group, $P < 0.05$. *Compared with the control group, $P < 0.05$.

and RF levels in the observation group were lower than those in the control group ($P < 0.05$) (**Figure 3**). The post-treatment TNF-α and IL-6 levels were lower ($P < 0.05$) than pre-treatment levels in both the groups. Specifically, the observation group exhibited higher post-treatment levels than the control group ($P < 0.05$) (**Figure 4**).

Discussion

At present, there are many patients with RA in China. The primary symptoms of RA include cartilage damage and synovitis, and the pathogenic factors that commonly cause RA include infections, immediate environment, and genetic factors [14]. Synovitis is a basic pathological change associated with RA that can be classified into multiple stages such as an inflammatory stage and a stage of pannus formation and fibrosis. If not treated timely, synovitis can ultimately cause damage to the bones and articular cartilage, potentially leading to joint deformities or life-threatening disabilities [15, 16]. At present, various drugs, including nonsteroidal anti-inflammatory drugs, slow-acting antirheumatic drugs, glucocorticoids, biological agents, adhesion molecules inhibitors, and drugs that induce synovial cell apoptosis, are used to treat RA clinically. In addition, stem cell transplantation, immune purification therapy, gene therapy, and surgical therapy have also been widely used in the treatment of this disease. In clinical practice, appropriate treatment methods are usually selected based on the severity of the patient's condition.

Methotrexate is the drug of choice for treating RA due to its mode of action, through which it inhibits the activity of dihydrofolate reductase and promotes the synthesis of pyrimidine and purine nucleotides while suppressing thymidyl-

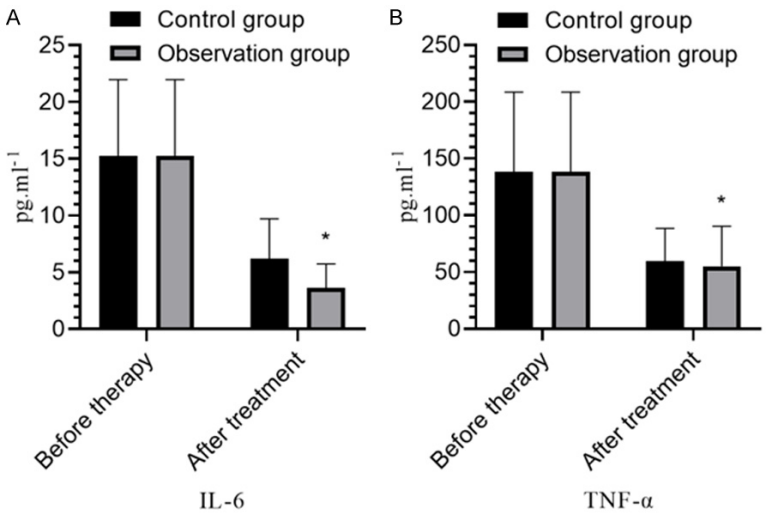

Figure 4. Comparison of IL-6 and TNF-α of treatment groups. A: Compared with IL-6 between the two groups before treatment, $P > 0.05$; after treatment, the observation group had lower IL-6 than the control group, $P < 0.05$. B: Compared with TNF-α between the two groups before treatment, $P > 0.05$; after treatment, the observation group had lower TNF-α than the control group, $P < 0.05$. *Compared with the control group, $P < 0.05$.

ic acid levels [17, 18]. Leflunomide, a type of isoxazole derivative, affects cell proliferation signaling by blocking deoxyribonucleic and ribonucleic acid synthesis and prompts the recovery of patients with RA through the inhibition of T-lymphocyte protein tyrosine kinase activity [19]. In the present study, patients with RA were given conventional treatment in combination with or without glucocorticoids to test for potential resulting improvements. Glucocorticoids help regulate lipid levels and have an anti-inflammatory action. During early stages of inflammation, glucocorticoids can inhibit angiotelectasis, relieve tissue edema and exudation, and suppress leukocytic phagocytosis, reducing inflammatory reactions. In the late stages of inflammation, glucocorticoids can inhibit capillary and fibroblast hyperplasia and granulation tissue formation, lowering the incidence of various inflammatory complications [20].

The findings in the study indicated that the total effective rates were 96% and 76% in the observation group and control group, respectively. The post-treatment tender joint counts, swollen joint counts, morning stiffness periods, VAS scores, DAS28, and ESR and RF levels in the observation group were lower than those in the control group, suggesting that the combination of routine treatment with glucocorticoids was advantageous. The observational group may have benefited from the use of prednisone, a glucocorticoid that has been widely clinically used to date. Prednisone shows significant anti-inflammatory and antiallergic effects and reduces inflammatory liquid exudation by lowering the permeability of capillary walls and cytomembranes. Prednisone suppresses the production and release of toxins, alleviates damage to cartilaginous joints, and improves other RA symptoms. Bakker et al. [21] treated patients with RA with methotrexate combined with placebo and methotrexate combined with prednisone respectively. The results showed that the clinical remission rate of patients in the methotrexate combined with prednisone group was 82%, significantly higher than that of 70% in the methotrexate combined with placebo group, suggesting that prednisone in addition to conventional treatment can play a synergistic role in the treatment of RA, which is also highly consistent with the results of this study. To explore its action mechanism, the prednisone is a glucocorticoid with high clinical utility ratio and significant anti-inflammatory and anti-allergic effect, which can significantly reduce the permeability of capillary wall and cell membrane, prompt the decrease in inflammatory fluid seepage, inhibit the release and generation of toxic substances in the body, reduce the articular cartilage injury, and alleviate various symptoms. In a previous study [22], 210 patients with RA were randomly classified into glucocorticoid and nonglucocorticoid treatment groups. Both groups were administered disease-modifying antirheumatic drugs, and the results indicated that the clinical remission rates during the first and second years in the glucocorticoid treatment group were higher than those in the nonglucocorticoid treatment group, indicating that administration of small doses of glucocorticoids for treating RA contributes to disease control and clinical symptom alleviation, consistent with the findings of the present study.

RA is an inflammatory process characterized by synovial hyperplasia and inflammatory cell

infiltration. Its occurrence and development may be closely related to immune responses. IL-6, an important proinflammatory factor, is synthesized by endothelial cells and lymphocytes and participates in the synthesis of acute reactive proteins that can damage articular cartilage [23]. High IL-6 levels promote B lymphocyte autoantibody production and RF release. RFs in turn aggravate the inflammatory reactions, leading to increased degree of injury to the articular cartilage. TNF-α, another proinflammatory factor, is primarily secreted by mononuclear macrophages and promotes chemotactic factor production by synovial endothelial cells, release of proinflammatory factors, and reactions occurring in the synovium of joints [24]. In addition, TNF-α destroys the articular cartilage by facilitating the proliferation and differentiation of fibroblasts and synovial cells and leads to pannus formation in the synovial tissues through the activation of vascular endothelial cells. In this study, the observation group exhibited lower TNF-α and IL-6 levels than the control group, indicating that glucocorticoids combined with conventional methods in RA treatment can help reduce inflammation. A possible mechanism underlying this is the enhancement of body's endogenous defenses by glucocorticoids, further triggering anti-inflammatory activity.

Briefly, the use of glucocorticoids in RA treatment can help ensure shorter periods of morning stiffness, reduce physical pain, improve clinical symptoms and joint motion, and lower the IL-6 and TNF-α levels. These effects help speed up the elimination of inflammatory conditions.

This study has some limitations. Due to the small sample size included in this study, the results are not representative enough. More attention should be paid to this aspect in future studies with a larger sample size, longer time, and more comprehensive study analysis, so as to further explore the clinical efficacy of glucocorticoid therapy for RA and its impact on IL-6 and TNF-α.

Disclosure of conflict of interest

None.

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