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Original Article

Effects of methotrexate combined with leflunomide on efficacy, joint function and inflammatory factors in patients with rheumatoid arthritis

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Abstract: Objective: To investigate the effect of methotrexate (MTX) combined with leflunomide (LEF) in the treatment of rheumatoid arthritis (RA). Methods: A total of 100 patients with RA who were admitted to our hospital from December 2016 to December 2019 were selected, among which 55 patients received MTX combined with LEF (research group, RG), and 45 patients were treated with MTX alone (control group, CG). The efficacy, incidence of adverse reactions, joint function (joint swelling index, joint tenderness index, duration time of morning stiffness), disease activity score and pain score (DAS-28, VAS), inflammation indexes (ESR, CRP, TNF-α, IL-1β), blood lipid indexes (TG, TC, LDL-C, HDL-C) and quality of life (HAQ score) were observed and compared in the two groups. Results: The total effective rate in RG was significantly higher than that in CG. The incidence of adverse reactions, joint function related indicators, DAS-28, VAS, various inflammatory indicators, TC, LDL-C and HAQ scores were significantly lower than those in CG. TG and HDL-C had no significant difference with the CG. Conclusion: Compared with MTX alone, MTX combined with LEF has higher efficacy, higher recovery degree of joint function and lower levels of inflammatory factors in the treatment of patients with RA.

Keywords: Methotrexate, leflunomide, rheumatoid arthritis, efficacy

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease affected by many factors, such as self or environment, which brings different degrees of joint function damage (joint swelling and tenderness, etc.) to patients and has negative impact on the quality of life of patients [1, 2]. Risk factors for RA include age and long course of disease, which may lead to increased risk of patients complicated with cardiovascular diseases, brittle fractures and malignant tumors. In addition, women are of greater potential risk for RA compared to men [3, 4]. The pathological mechanism of RA involves articular cartilage destruction and inflammation. If patients are not treated promptly and effectively, the risk of disability will be as high as 70% [5, 6]. Pharmacological intervention is a common therapeutic option for RA. Conventional drugs are non-steroidal anti-inflammatory drugs (e.g., diclofenac). However, patients will still suffer from chronic pain and the efficacy is not ideal because of its potential side effects and drug resistance. In addition, there is no cure method for RA currently [7-9]. Therefore, it is still an urgent matter to explore reliable pharmacological treatment strategies for RA, which is of great significance for improving the quality of life of RA patients.

Methotrexate (MTX) is a disease-modifying anti-rheumatic drug commonly used in the treatment of RA, which can alleviate inflammatory diseases. Its mechanism of action is related to the activation and inhibition of inflammation-related pathways and the maintenance of inflammatory microenvironment [10, 11]. Animal studies have shown that MTX can also be carried on the composite nano-carriers to directly target the drug locally to the diseased site. By effectively blocking NF-kB signal pathway and the release of proinflammatory factors, the drug efficacy can be fully exerted, and the toxicity of MTX can be avoided [12]. Although MTX has a strong efficacy in most patients, it

has potential side effects such as gastrointestinal toxicity. The combination of drugs is the way to solve this problem [13]. Leflunomide (LEF) is an immunosuppressive agent that can inhibit bone erosion in pathological development of RA by participating in HIF1α-LEF-AHR-CRP signal transduction pathway [14]. Some studies have shown that intra-articular injection of LEF implant may be an effective local treatment scheme, which is helpful to treat synovitis caused by RA [15]. In addition, LEF is also a substitute drug scheme for patients with insufficient response to the efficacy of MTX, which can significantly relieve clinical symptoms by improving inflammatory indicators in patients [16].

At present, there is little research on the combination of MTX and LEF in the treatment of RA. This research was mainly designed to compare the effects of MTX combined with LEF and MTX alone on the efficacy, joint function and inflammatory factors of RA, which may provide new insights for the treatment of RA.

Materials and methods

Baseline data

From December 2016 to December 2019, 100 patients with RA who were admitted to Ningbo First Hospital were selected. Fifty-five patients received MTX combined with LEF (RG), including 16 males and 39 females, with an average age of 60.13±5.77 years old. Forty-five patients were treated with MTX alone (CG), including 12 males and 33 females, with an average age of 58.84±5.26 years old. This study has been approved by the ethics committee of the hospital. All subjects signed full informed consent form.

Inclusion criteria: The patient met the standards of the American college of rheumatology in association with the European league against rheumatism in 2010 [17]; the patient had high treatment compliance; the patient was an adult; the patient had taken a drug that had a potential impact on the outcome of the study within the last six months.

Exclusion criteria were as follows: comorbid with malignant tumor or severe systemic dysfunction; mental illness or communication disorder; comorbid with infectious diseases; orthopedic diseases caused by other diseases. The inclusion criteria were applicable to patients in both groups.

Treatment methods

CG: Patients received MTX (15 mg) orally (Xinyi Pharmaceutical Co., LTD., Shanghai, China, H31020644) once a week for 6 months.

RG: Patients received MTX (10 mg, once a week) and LEF (10 mg, once a night) orally (Changzheng-Xinkai Pharmaceutical Co., Ltd., Suzhou, China, H20000550) for 6 months.

Therapeutic evaluation

After treatment, the improvement degree was more than 75%, which was regarded as markedly effective. The improvement degree was 30-75%, which was regarded as effective. Other conditions, including inconspicuous improvement or deterioration, were considered to be ineffective. Total effective rate = (number of cases with markedly effective + number of cases with effective)/total number of cases × 100%.

Observation indexes

Incidence of adverse reactions: The cases of adverse reactions (including rash, pruritus, elevated alanine aminotransferase (ALT), decreased leukocyte count (WBC) and gastrointestinal discomfort) were mainly observed during treatment and after treatment for 1 month.

The joint function was mainly evaluated from joint swelling index, joint tenderness index and duration time of morning stiffness before treatment and after treatment for 1 month. The number of joint swelling and pain was recorded by pressing the patient's elbow joint, knee joint and other 28 joint parts. Joint swelling indexes: There was no swelling (0 points); If the swelling range did not exceed the protruding part of joint bone, it was recorded as slight swelling (1 point); If the swelling was consistent with the protruding part of bone, it was recorded as moderate swelling (2 points); If the swelling range was higher than the protruding part of bone or there was joint effusion, it was recorded as severe swelling (3 points). Joint tenderness indexes were as follows: no pain at all (0 points); mild tenderness, free movement (1 point); moderate tenderness, mild limitation of movement (2 points); severe pain, extreme limi-

tation of movement (3 points). The total score was used as the final result for patients' joint swelling index and joint tenderness index.

The disease activity and pain score were evaluated by disease activity score (DAS-28) [18] and visual analogue scale (VAS) [19] respectively before treatment and after treatment for 1 month. Among them, the higher DAS-28 score, the higher the disease activity. A level higher than 5.1 indicated high activity, while a level lower than 3.2 indicated low activity. VAS score was proportional to pain sensation, with 0, 1-3, 4-6 and 7-10 corresponding to painless, mild and tolerable pain, moderate pain that was barely tolerable and unbearable severe pain, respectively.

Inflammation indexes: The serum erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were mainly tested in fasting venous blood before treatment and after treatment for 1 month, and measuring method was enzyme-linked immunosorbent assay (ELISA).

Blood lipid indexes: The levels of serum triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) were mainly detected by PUZS-300 full-automatic biochemical analyzer (Shanghai Dibosi Biological Technology Co. Ltd., China).

The quality of life was assessed by Patient Health Assessment Scale (HAQ) [20] before treatment and after treatment for 1 month. The assessment contents involved overall health, disability index, mental health, etc. The score was inversely proportional to quality of life, and 0.22 was the critical point of joint disability.

Statistical analysis

The GraphPad Prism 6 (GraphPad Software, San Diego, USA) was used to analyze data and generate pictures. The counting data were expressed as the number/percentage (n/%) of cases. The measurement data were expressed as mean ± SEM. The comparison of counting data in the two groups was conducted by Chi-square test. When the theoretical frequency in Chi-square test was less than 5, the continuity correction Chi-square test was used. The comparison of measuring data in the two groups was conducted by independent sample t-test. The comparison within groups before and after treatment was conducted by paired t-test. The difference was statistically significant with $P < 0.05$.

Results

Baseline data

There were no significant differences between the two groups in gender, age, average age, course of disease, family history, number of painful joints, diabetes history, drinking history, smoking history, place of residence and other aspects ($P < 0.05$) (**Table 1**).

The efficacy of MTX combined with LEF was better than MTX in the treatment of RA

After treatment, the total effective rate in RG was 90.91%, and that in CG was 75.56%. The total effective rate in RG was significantly higher than that of CG. The difference was statistically significant ($P < 0.05$) (**Table 2**).

There was no significant difference in the incidence of adverse reactions between the two groups

There was no significant difference between the two groups in the incidence of rash, pruritus, ALT elevation, WBC reduction, gastrointestinal discomfort and other adverse reactions ($P > 0.05$) (**Table 3**).

MTX combined with LEF could improve the recovery speed of joint function in the treatment of RA

There was no significant difference between the two groups in joint swelling index (**Figure 1A**), joint tenderness index (**Figure 1B**) and duration time of morning stiffness (**Figure 1C**) before treatment ($P > 0.05$). After treatment, the above indice of patients were significantly reduced in the two groups, and the joint swelling index and joint tenderness index of RG were significantly lower than those of CG (**Figure 1A**, **1B**), and the duration time of morning stiffness was significantly shorter than that of CG (**Figure 1C**), with statistically significant differences ($P < 0.05$).

MTX combined with LEF could reduce disease activity and pain in the treatment of RA

There was no significant difference in DAS-28 (**Figure 2A**) and VAS scores (**Figure 2B**)

Table 1. Baseline data of patients in the two groups [n (%), mean ± SD]

Factors	n	CG (n = 45)	RG (n = 55)	χ^2/t	P
Gender				0.072	0.788
Male	28	12 (26.67)	16 (29.09)		
Female	72	33 (73.33)	39 (70.91)		
Age/year (s) old				0.449	0.503
< 60	43	21 (46.67)	22 (40.00)		
≥ 60	57	24 (53.33)	33 (60.00)		
Average age/year (s) old	100	58.84±5.26	60.13±5.77	1.157	0.250
Course	100	4.29±1.04	4.48±1.20	0.836	0.405
Family history				0.718	0.397
No	69	33 (73.33)	36 (65.45)		
Yes	31	12 (26.67)	19 (34.55)		
Number of painful joints (pieces)	100	4.62±0.75	4.50±0.89	0.719	0.474
Diabetes history				0.100	0.752
No	65	30 (66.67)	35 (63.64)		
Yes	35	15 (33.33)	20 (36.36)		
Drinking history				2.309	0.129
No	63	32 (71.11)	31 (56.36)		
Yes	37	13 (28.89)	24 (43.64)		
Smoking history				0.103	0.748
No	74	34 (75.56)	40 (72.73)		
Yes	26	11 (24.44)	15 (27.27)		
Place of residence				0.051	0.821
Rural	39	17 (37.78)	22 (40.00)		
City	61	28 (63.22)	33 (60.00)		

Table 2. Clinical efficacy of patients in the two groups [n (%)]

Grouping	n	Marked effect	Effective	Ineffective	Total effective rate (%)
CG	45	16 (35.56)	18 (40.00)	11 (24.44)	75.56
RG	55	29 (52.73)	21 (38.18)	5 (9.09)	90.91
χ^2 value	-	-	-	-	4.341
P value	-	-	-	-	0.037

Table 3. The incidence of adverse reactions of patients in the two groups [n (%)]

Category	CG (n = 45)	RG (n = 55)	χ^2 value	*P* value
Rash	1 (2.22)	2 (3.64)	-	-
Pruritus	1 (2.22)	2 (3.64)	-	-
ALT elevation	1 (2.22)	2 (3.64)	-	-
WBC reduction	0 (0.00)	0 (0.00)	-	-
Gastrointestinal discomfort	1 (2.22)	1 (1.82)	-	-
Total	4 (8.89)	7 (12.73)	0.372	0.542

between the two groups before treatment (P > 0.05). After treatment, the aforementioned two indexes of patients were significantly reduced in the two groups, and the DAS-28 and VAS scores in RG were significantly lower than those in CG (**Figure 2A**, **2B**), with statistically significant differences (P < 0.05).

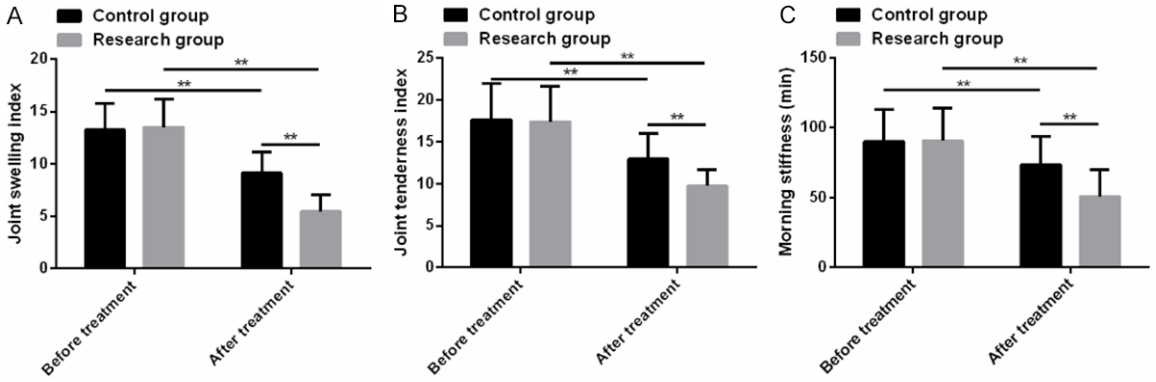

Figure 1. Joint function of patients in the two groups. A. After treatment, the joint swelling index in RG was significantly reduced and lower than that in CG. B. After treatment, the joint tenderness index in RG was significantly reduced and lower than that in CG. C. After treatment, the duration time of morning stiffness in RG was significantly reduced and shorter than that in CG. Note: **P < 0.01.

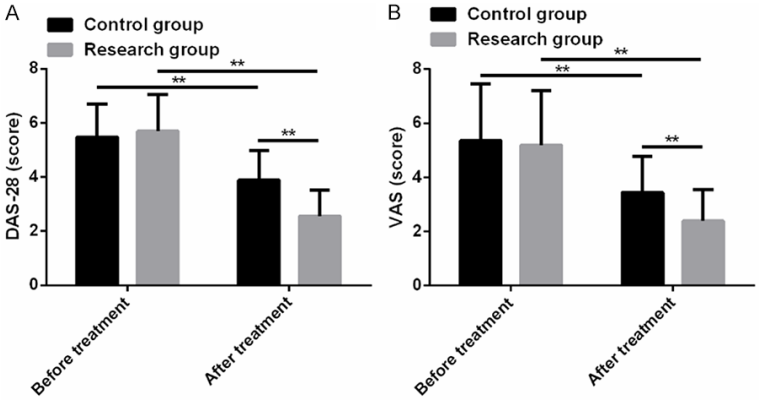

Figure 2. Disease activity and pain of patients in the two groups. A. After treatment, the DAS-28 score in RG was significantly decreased and lower than that in CG. B. After treatment, the VAS score in RG was significantly decreased and lower than that in CG. Note: **P < 0.01.

MTX combined with LEF could inhibit inflammatory state in the treatment of RA

There was no significant difference in the inflammatory indicators ESR (**Figure 3A**), CRP (**Figure 3B**), TNF-α (**Figure 3C**), and IL-1β (**Figure 3D**) between the two groups before treatment (P > 0.05). After treatment, the four indexes of patients were significantly reduced in the two groups, and the above indexes in RG were significantly lower than those in CG (**Figure 3A-D**), with statistically significant differences (P < 0.05).

MTX combined with LEF could improve blood lipid indexes in the treatment of RA

There was no significant difference in TG (**Figure 4A**), TC (**Figure 4B**), LDL-C (**Figure 4C**), HDL-C (**Figure 4D**) between the two groups before treatment (P > 0.05). After treatment, TG, TC (RG, P < 0.05) and LDL-C (P < 0.05) of patients decreased to different degrees in the two groups (**Figure 4A-C**), while HDL-C (**Figure 4D**) increased to different degrees (RG, P<0.05), and TC and LDL-C in RG were significantly lower than those in CG (**Figure 4B**, **4C**).

MTX combined with LEF could improve the quality of life in the treatment of RA

There was no significant difference in HAQ scores between the two groups before treatment (**Figure 5**). After treatment, the score of patients was significantly reduced in the two groups, and the HAQ score in RG was significantly lower than that in CG (**Figure 5**), and the difference was statistically significant (P < 0.05).

Discussion

RA, as a chronic inflammatory joint disease, has an impact on approximate 1% of the world's population, and its destructive force can make 50% of adults unable to work normally during the onset [21]. MTX has a variety of clinical applications, including the treatment of acute lymphoblastic leukemia, gestational trophoblastic tumor and other medical situations. It is also a first-line treatment for RA, and its appli-

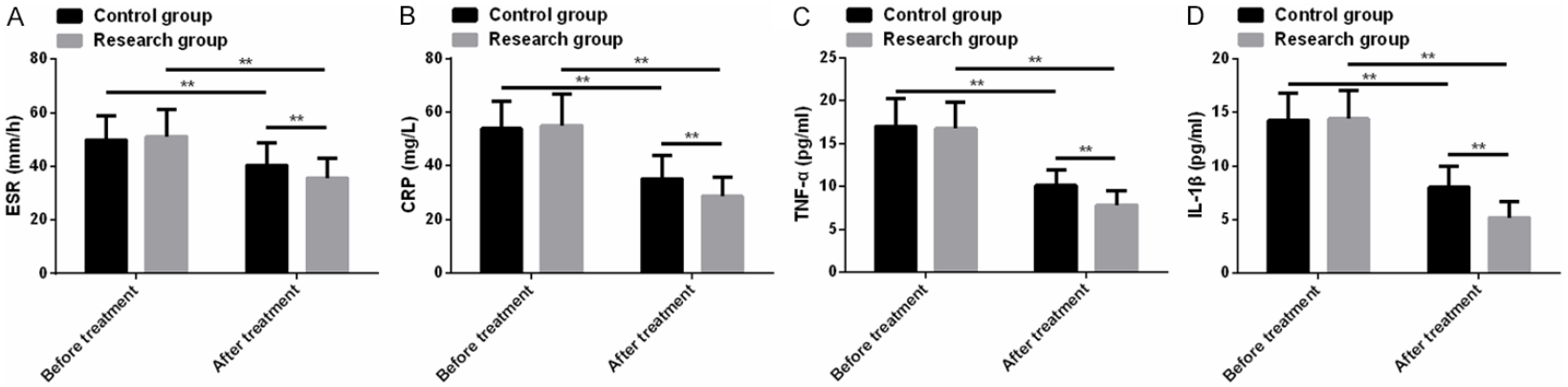

Figure 3. Inflammatory state of patients in the two groups. A. After treatment, the ESR in RG was significantly decreased and lower than that in CG. B. After treatment, the CRP in RG was significantly decreased and lower than that in CG. C. After treatment, the TNF-α in RG was significantly decreased and lower than that in CG. D. After treatment, the IL-1β in RG was significantly decreased and lower than that in CG. Note: **P < 0.01.

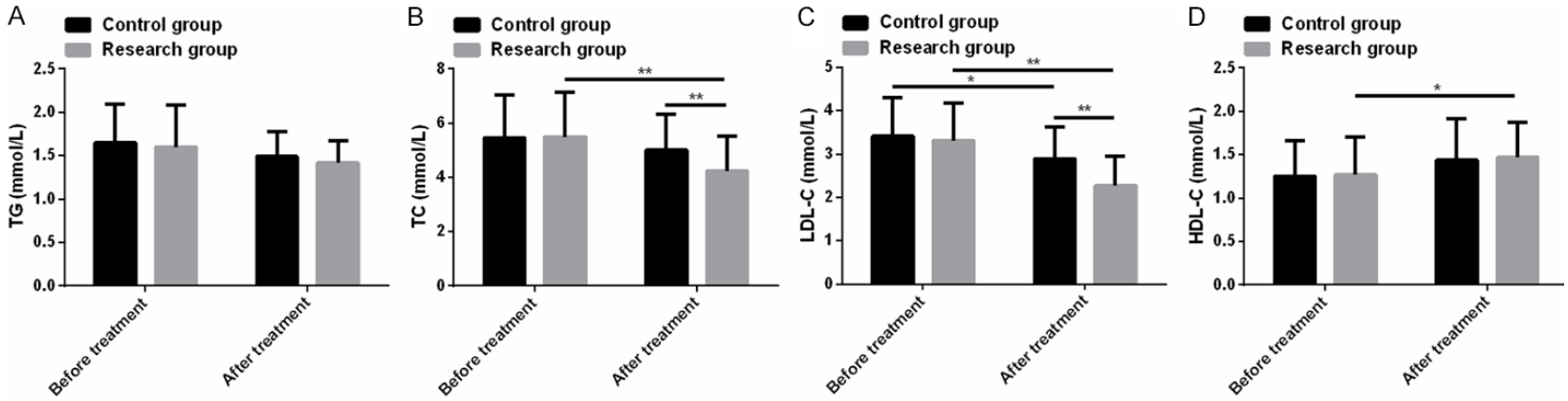

Figure 4. Blood lipid indexes of patients in the two groups. A. After treatment, the TG was slightly decreased in RG, but there was no significant difference with the CG. B. After treatment, the TC in RG was significantly decreased and lower than that in CG. C. After treatment, the LDL-C in RG was significantly decreased and lower than that in CG. D. After treatment, the HDL-C was significantly increased in RG, but there was no significant difference with the CG. Note: *P < 0.05, **P < 0.01.

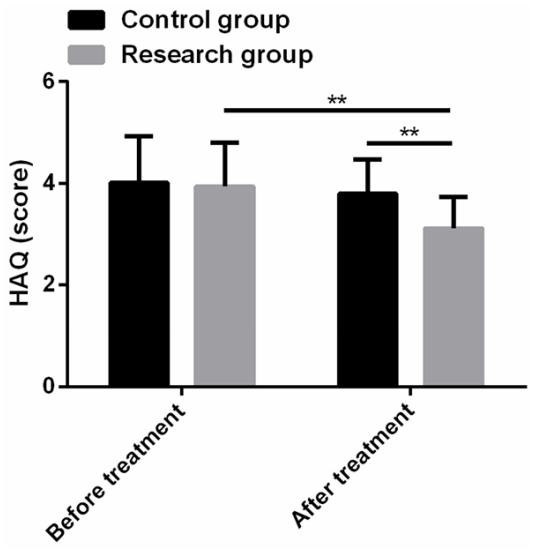

Figure 5. HAQ score of patients in the two groups.

cation in intra-articular injection is also helpful to inhibit the severity of synovitis in RA patients [22-25]. In the report of Zhong et al. [26], the combination of MTX with LEF can improve immune function and clinical symptoms by targeting TH22 cells and inhibiting IL-22 secretion in the treatment of RA. We discussed the therapeutic effects of these two drugs in RA, which was of great value to the treatment and normal work of RA patients.

In this study, patients in RG received the combined treatment of MTX with LEF, while patients in CG were treated with MTX alone. The results showed that the total effective rate in RG was significantly higher than that in CG (90.91% VS 75.56%), which indicated that MTX combined with LEF was helpful to improve the efficacy in the treatment of RA and the patients benefit more with the combined treatment. Then, the security research results showed that the incidence rate of adverse reactions in RG was not significantly different from that in CG, indicating that the pharmacological strategies of RG did not increase the incidence of adverse reactions and would be considered safe. In this study, we observed adverse reactions including rash, pruritus, gastrointestinal discomfort, etc. These are common adverse events of patients with RA [27]. In the research of Wang et al. [28], when MTX and LEF act jointly on RA, LEF can enhance the blood drug concentration of MTX and major toxic metabolites of MTX, and make them aggregate in liver and kidney by preventing their bile excretion, which may be helpful to explain the adverse reactions caused by the two combination drugs. In addition, the studies by Bird [29] et al. have shown that MTX combined with LEF has better tolerance for the treatment of RA, and its safety is equivalent to MTX or LEF monotherapy, which is similar to our research results. We evaluated the joint function of patients in the two groups and found that the joint swelling index, tenderness index and morning stiffness time in RG were significantly lower or shorter than those in CG, suggesting that the combination of MTX and LEF was more conducive to the recovery of joint function of RA patients. In addition, the results of DAS-28 and VAS scoring showed that the two scoring results of RG were significantly lower than those of CG, which indicated that MTX combined with LEF was helpful to reduce disease activity and pain in the treatment of patients with RA.

The pathological process of RA is complicated, but it is a known phenomenon for the inflammatory microenvironment created by excessive release of inflammatory related factors in the joint microenvironment. The dynamic changes of inflammatory ecology can help us to measure the efficacy of RA [30, 31]. Our data showed that ESR, CRP, TNF-α, IL-1β of patients were significantly decreased in the two groups after treatment, and the above inflammatory indexes in RG were significantly lower than those in CG, suggesting that the combined action of MTX and LEF on RA had more significant inhibitory effect on inflammatory microenvironment. Studies have shown that ESR is related to disease activity in RA patients, while CRP is related to synovial CD19 infiltration [32, 33]. It has previously bee shown that TNF-α and IL-1β participate in the destruction of articular cartilage caused by excessive release of inflammatory factors in RA microenvironment, which can induce adaptive immunity of synovial fibroblasts [34]. Studies have also reported that RA is a potential risk factor for cardiovascular diseases and may cause abnormal blood lipid in patients [35]. Therefore, we also explored the influence of two medication strategies on blood lipid index of patients. The results showed that TC and LDL-C of patients in RG were significantly decreased and lower than those in CG after treatment, while TG and HDL-C did not show significant advantages, suggesting that MTX combined with LEF was helpful to obviously

improve blood lipid index to some extent in the treatment of RA patients. Finally, we also studied the quality of life of patients in the two groups. The results showed that the HAQ score of the patients in RG was significantly lower than that in CG, which indicated that MTX combined with LEF could improve the quality of life in RA patients.

There is still room for improvement in this study. First of all, we may supplement the pharmacological mechanism of MTX and LEF combination to further explore the regulatory network at the molecular level. Secondly, we can increase the long-term efficacy of the two medication strategies to further demonstrate the potential long-term advantages of combined medication.

In short, MTX combined with LEF has the advantages of high efficacy, remarkable recovery of joint function and significant inhibition of inflammatory microenvironment in the treatment of patients with RA, which is worthy of clinical promotion.

Disclosure of conflict of interest

None.

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