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Original Article

Thalidomide plus leflunomide for rheumatoid arthritis and its impacts on D-dimer expression

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Abstract: Objective: To elucidate the clinical efficacy of thalidomide plus leflunomide in the management of rheumatoid arthritis (RA) and its impacts on D-dimer expression. Methods: One hundred and fifty RA patients admitted to Weifang People's Hospital were randomly subdivided into the observation group (n=75) and the control group (n=75). The patients in the control group were treated with thalidomide, and those in the observation group received additional leflunomide based on the regimen of the control group. The clinical response of the two groups was compared, and D-dimer expression in each group was examined. Results: The total response rate was 90.67% in the observation group and 78.67% in the control group. The clinical response to treatment was worse in the control group than that in the observation group ($P<0.05$). Twelve weeks after treatment, the score for disease activity in 28 joints (DAS28), erythrocyte sedimentation rates (ESR), C-reactive protein (CRP), joint function scores, the number of swollen joints, the number of tender joints, and the duration of morning stiffness of patients were improved in both groups compared to those before treatment, with greater improvements in the observation group (all $P<0.05$). However, no difference was seen in the total incidence of adverse events ($P>0.05$). Detection of D-dimer expression indicated the D-dimer expression in the observation group was considerably decreased when compared with the control group ($P<0.05$). Conclusion: Thalidomide-leflunomide medication contributes to improvements in disease, joint functions, and the microcirculation, and a decrease in D-dimer expression in RA patients.

Keywords: Thalidomide, leflunomide, rheumatoid arthritis, D-dimer, clinical response

Introduction

Rheumatoid arthritis (RA) is common in both the young and middle-aged populations, and is more prevalent in females than in males. It is an autoimmune disease present with joint synovial inflammation and extra-articular lesions [1]. RA is an important factor leading to the loss of labor force or even disability of young and middle-aged patients. It severely affects the lives of patients and imposes huge economic pressure on both families and the society. It is one of the dominant disability-induced diseases in humans, hence effective treatment regimens are urgent [2-4]. Therefore, developing better treatment protocols to reduce the incidence of RA is a major issue faced by clinicians.

Currently, RA is primarily treated by glucocorticoids, non-steroidal anti-inflammatory drugs, and anti-rheumatic drugs [5]. Thalidomide is a newly-developed glutamate derivative that acts as an anti-inflammation and anti-angiogenesis agent by inhibiting secretion of inflammatory cytokines (such as IL-6, vascular endothelial growth factor (VEGF), and TNF-α) [6]. Leflunomide is an isoxazole derivative that exerts an anti-inflammatory effect by inhibiting expression of cytokines (nuclear factor-κB) and adhesion molecules, and suppressing the proliferation of lymphocytes and B-cells [7]. Both thalidomide and leflunomide are used for the treatment of RA, but few studies involve in the combination of the two agents [8, 9]. Efficacy and safety of thalidomide-leflunomide medication in treating RA warrant further validation. In recent decades, some studies have reported the hypercoagulable state is present in RA patients, and that the concentration of D-dimer is closely related to the disease activity of RA patients [10]. However, few studies are involved in exploring the impacts of thalidomide and leflunomide on the D-dimer expression in the treatment of RA.

Therefore, this study aimed to investigate the efficacy and safety of the combination of thalidomide with leflunomide in treatment of RA, and the impact of the combined regimen on D-dimer expression, in hope of providing reference for clinical treatment of RA.

Materials and methods

Study participants

From January 2013 to January 2016, 150 RA patients were randomly arranged to receive thalidomide (control group, n=75) or the combination of leflunomide and thalidomide (observation group, n=75). There were 58 male patients and 92 female patients, with a mean age of 42.7±6.4 years (range, 18-70 years). Patients were eligible for enrolment if they had confirmed symptoms of RA meeting the diagnostic criteria for RA which were specified in the 1987 revised criteria of the American Rheumatism Association. This included affected knees that had not been treated with thalidomide and leflunomide within the previous three months or they had voluntarily participated in the experiment in the study [11]. Patients were excluded if they had other systemic inflammatory response syndrome (SIRS); if they were pregnant; it they had cardiovascular or hepatorenal disorder, a history of mental and neurological illness, or a chronic pain syndrome. If they were difficult to communicate with verbally and did not comply with the treatment, if they were allergic to thalidomide and leflunomide, if they had joint bone tumors, had bone metastases from various cancers or if they had recent acute stroke were further exclusion criteria. This study was approved from the Medical Ethics Committee of Weifang People's Hospital and patients or their families submitted written informed consent.

Treatment protocols

The patients in the control group were administered with thalidomide alone at a dose of 50 mg per day for 12 weeks. Thalidomide (National drug code No., H20103705) was purchased from Shanghai Guang Rui Biological Technology, China. In contrast, those in the observation group were treated with thalidomide plus leflunomide 20 mg twice daily for 12 weeks. Teflunomide (National drug code No., H2000-0550) was purchased from Shanghai Shifeng Biotechnology, China.

Outcome measures

After completion of treatment, the efficacy (covering the score for disease activity in 28 joints (DAS28), erythrocyte sedimentation rates (ESR), C-reactive protein (CRP)), joint functions (including joint function score, the number of swollen joints, the number of tender joints, the duration of morning stiffness, D-dimer levels and the incidence of adverse events were observed among patients in the two groups. Joint function scores were evaluated in reference to the revised criteria for the classification of global functional status in rheumatoid arthritis released by the American College of Rheumatology in 1991 [12]. ESR was detected by Wei's method, and the levels of D-dimer and CRP were examined by enzyme-linked immunosorbent assay (ELISA). The ELISA kits were bought from Shanghai Jingkang Biological Engineering, China.

Clinical response evaluation

The clinical response to medication was evaluated by the following three diagnostic criteria: good response, moderate response, and non-response [13].

The patient was classified as a good responder if a difference in the DAS28 was less than 1.2 than that of a non-responder or the DAS28 was less than 3.2, the ESR was less than or equal to 15 mm/h in male, less than or equal to 20 mm/h in female, or the CRP was less than or equal to 8 mg/L.

The patient was classified as a moderate responder if improvement in DAS28 was between 0.6 and 1.2 than before treatment, or the DAS28 was greater than or equal to 3.2 but less than 5.1.

The patient was classified as a non-responder if improvement in DAS28 was less than 0.6 than before treatment or the DAS28 was greater than or equal to 5.1.

Statistical analysis

Statistical analyses were done using SPSS software, version 22.0 (Asia Analytics Formerly, SPSS, China). Count data were presented as percentages (n, %) and processed by the Chi-square tests. Measurement data are described as mean ± sd; data without normal distribution

Table 1. Baseline patient data

	Control group (n=75)	Observation group (n=75)	Statistics	*P* value
Sex (n, %)			0.167	0.867
Male	30 (40.00)	29 (38.67)		
Female	45 (60.00)	46 (61.33)		
Mean age (year)	42.5±6.1	42.9±6.7	0.382	0.703
Course of disease (year)	6.77±3.59	7.12±3.48	0.606	0.545
RA affected site (n, %)			0.091	0.955
Upper extremity	35 (46.67)	34 (45.33)		
Lower extremity	34 (45.33)	34 (45.33)		
Other	6 (8)	7 (9.34)		
Smoking (n, %)			0.568	0.570
Yes	55 (78.57)	58 (77.33)		
No	20 (21.43)	17 (22.67)		
Anti-CCP antibody (n, %)			0.272	0.786
Negative	68 (90.67)	67 (89.33)		
Positive	7 (9.33)	8 (10.67)		
Rheumatoid factor (U/mL)	217.36±18.44	222.88±17.28	1.892	0.060

Table 2. Analysis of clinical response to the treatment at 12 weeks (n, %)

	Control group (n=75)	Observation group (n=75)	Statistics	*P* value
Total response rate	59 (78.67)	68 (90.67)	2.039	0.041
Good response	17 (22.67)	29 (38.67)	2.125	0.034
Moderate response	42 (56.00)	39 (52.00)	0.492	0.623
Non-response	16 (21.33)	7 (9.33)	2.039	0.041

Table 3. Comparison of clinical response evaluation indexes of patients

		Control group	Observation group	t	P
DAS28	Pre-treatment	3.6±0.4	3.7±0.5	1.353	0.178
	Post-treatment	3.4±0.3	3.0±0.6	5.164	<0.001
	t	3.464	7.762		
	P	0.001	<0.001		
ESR (mm/h)	Pre-treatment	21.9±8.6	21.4±8.4	0.360	0.719
	Post-treatment	17.1±4.8	14.2±4.1	3.978	<0.001
	t	4.221	6.671		
	P	<0.001	<0.001		
CRP (mg/L)	Pre-treatment	58.33±12.46	59.42±13.17	0.521	0.603
	Post-treatment	16.72±4.58	10.69±3.77	8.803	<0.001
	t	27.15	30.81		
	P	<0.001	<0.001		

were analyzed by means of the non-parametric KS test; for data that were normally distributed, inter-group comparisons at the same time point was conducted by the independent samples t-tests whereas intra-group comparisons before and after treatment were performed with the paired t-tests. Significance was set at *P* values <0.05.

Results

Baseline data

A total of 150 RA patients were enrolled in this study, with an average age of 42.7±6.4 years. Of them, 75 were assigned to the control group, including 30 male patients and 45 female patients, with an average age of 42.5±6.1 years. The observation group included 75, with 29 males and 46 females, and a mean age of (42.9±6.7) years. The two groups were well-matched in basic data (average age and sex ratio) (P>0.05, **Table 1**).

Clinical response of patients 12 weeks after treatment

Twelve weeks after treatment, the total response rate was 90.67% in the observation group and 78.67% in the control group. The total response rate was lower in the control group than in the observation group (P<0.05, **Table 2**).

Comparison of clinical response evaluation indexes of patients between the two groups

No differences were noted in the DAS28, the ESR, and the CRP levels before treatment be-

Table 4. Evaluation of joint functions of patients before and after treatment

		Control group	Observation group	t	P
Joint function score	Pre-treatment	2.9±0.4	3.0±0.6	1.201	0.232
	Post-treatment	1.8±0.3	1.1±0.1	19.170	<0.001
	t	19.05	27.05		
	P	<0.001	<0.001		
Number of swollen joints	Pre-treatment	7.6±5.6	7.5±5.7	0.108	0.914
	Post-treatment	3.6±1.4	3.1±1.7	2.037	0.044
	t	6.001	6.406		
	P	<0.001	<0.001		
Number of tender joints	Pre-treatment	13.5±5.8	13.2±5.3	0.331	0.741
	Post-treatment	9.6±4.5	8.1±4.7	1.996	0.048
	t	4.601	6.235		
	P	<0.001	<0.001		
Duration of morning stiffness	Pre-treatment	131.56±33.47	135.02±34.22	0.626	0.532
	Post-treatment	77.03±18.06	53.56±14.27	8.840	<0.001
	t	12.42	19.03		
	P	<0.001	<0.001		

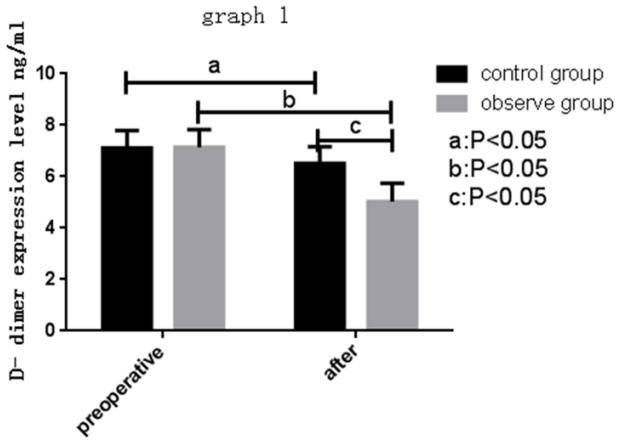

Figure 1. Serum D-dimer levels before and after treatment in both groups. The D-dimer expression was detected by the ELISA, and the D-dimer levels in the control group and the treatment group were not different from those before treatment (P>0.05; 7.11±0.68 ng/mL vs 7.14±0.69 ng/mL). Twelve weeks after treatment, the D-dimer expression in both groups was significantly lower than those before treatment (P<0.05). The D-dimer level in the observation group was lower than that in the control group (P<0.05) (5.03±0.71 ng/mL vs 6.51±0.66 ng/mL).

tween the observation group and the control group (all P>0.05). Twelve weeks after treatment, the DAS28, the ESR, and the CRP levels lowered versus before treatment in both groups (all P<0.05), with significant reductions in the observation group versus the control group (all P<0.05, **Table 3**).

Evaluation of joint functions of patients before and after treatment

Before treatment, the joint function scores, the number of swollen joints, the number of tender joints, and the durations of morning stiffness of patients varied insignificantly between the two study groups (all P>0.05). Twelve weeks after treatment, great improvements in all the above-mentioned variables were noted among patients of both groups (all P<0.05), with lower joint function scores, fewer swollen joints and tender joints, and a shorter duration of morning stiffness of patients in the observation group (all P<0.05, **Table 4**).

Changes in the D-dimer levels of patients before and after treatment

Before treatment, there was no disparity in the D-dimer levels of patients between the control group and the treatment group (P>0.05; 7.11±0.68 ng/mL vs 7.14±0.69 ng/mL). Twelve weeks after treatment, the patients in both

Table 5. Adverse events of patients 12 weeks after treatment (n, %)

	Control group (n=75)	Observation group (n=75)	Statistics	P
Incidence of adverse events	28 (34.67)	17 (25.33)	1.960	0.050
Rash	6 (8.00)	4 (5.33)	0.655	0.513
Respiratory tract infection	5 (6.67)	3 (4.00)	0.727	0.467
Lethargy	3 (4.00)	1 (1.33)	1.014	0.311
Gastrointestinal discomfort	5 (6.67)	3 (4.00)	0.727	0.467
Elevated alanine aminotransferase level	4 (5.33)	2 (2.67)	0.833	0.405
Lower white blood cell count	0 (0.00)	2 (2.67)	1.424	0.155
Menstrual disorders	3 (4.00)	1 (1.33)	1.014	0.311
Others	2 (2.67)	1 (1.33)	0.583	0.560

groups had significantly lower D-dimer levels than before treatment (P<0.05). The D-dimer level was lower in the observation group than in the control group (P<0.05; 5.03±0.71 ng/mL vs 6.51±0.66 ng/mL, as shown in **Figure 1**.

Adverse events of patients 12 weeks after treatment

Twelve weeks after treatment, the rate of adverse events of patients was lower, though insignificantly than that of the control group (P>0.05) and differences in the incidences of rash, lethargy, gastrointestinal discomfort, and menstrual disorders were insignificant between the two groups (all P>0.05). Elevated alanine aminotransferase expression was observed in patients of both groups, so measures were taken for liver protection in the course of treatment. Reduced white blood cell counts and respiratory tract infection were also noted in patients of both groups, and anti-infective treatment was performed during the treatment accordingly (**Table 5**).

Discussion

RA has a high incidence of morbidity and disability, and the duration of treatment is also relatively long [14]. At present, drugs for RA are not very specific in clinical practice, and their efficacy is not satisfactory [15]. Currently, the combined medication to relieve RA at the early stage is internationally recognized as a better treatment method [16]. However, the types of combined drugs still need improving. Therefore, this study was designed to provide more evidence for the clinical treatment of RA by examining the efficacy and safety of thalidomide-leflunomide in the management of RA. RA patients are often accompanied by microthrombosis affecting the organs, and D-dimer is a newly discovered coagulation factor closely associated with RA [17]. D-dimer closely correlates with the disease activity of RA, with higher D-dimer levels indicating more severe joint pain [18]. As a result, we also explored the effects of thalidomide-leflunomide on the microcirculation of RA patients in the current study, with an aim to bring some insight into developing effective protocols for the management of RA.

The current study demonstrated that the total response rate was 90.67% in RA patients with thalidomide-leflunomide medication, significantly higher than 78.67% in those with thalidomide alone. Nevertheless, few reports of studies have been involved in the use of thalidomide for treatment of RA. The leflunomide-methotrexate combination improves the clinical response to the treatment for RA, but it increases the incidence of adverse events and adversely affects the hepatic function and bone marrow function in patients [19]. In the current study, a larger proportion of patients with elevated alanine aminotransferase levels were noted in RA patients with thalidomide plus leflunomide than those with thalidomide alone. Hence, we speculated that leflunomide might be associated with liver injury and bone marrow suppression. Thus, clinicians should prescribe leflunomide to patients with caution. However, we did not find a statistical difference in the proportion of patients with injured liver and suppressed bone marrow between the two groups, which might be attributed to our relatively smaller sample size. The data with a larger sample size are required for future analysis. Moreover, the results of the current study indi-

cated no difference in the proportion of patients with respiratory tract infections between the two groups. Conway et al. also stated in a study that leflunomide did not contribute to more severe respiratory tract infection, which was well aligned with our results [20]. Therefore, thalidomide-leflunomide medication results in better efficacy for RA, but its safety still needs further confirmation.

Further, in the current study, the clinical response to medication was evaluated among RA patients. A good response rate was remarkably increased in RA patients with thalidomide plus leflunomide than those with thalidomide alone. The indicators for clinical response evaluation reveals that the DAS28, the ESR, and the CRP levels of patients after treatment were lower in the observation group versus the control group. A greater improvement in joint function was observed in the observation group than in the control group and this difference was statistically significant, which further convinces us that thalidomide-leflunomide are effective in treatment of RA.

The two groups of patients were measured for the D-dimer levels of patients before and after treatment in the current study. No difference was found in the D-dimer levels before treatment between the two groups. However, 12 weeks after treatment, the D-dimer level was lower in the observation group than in the control group, suggesting that thalidomide-leflunomide might be conductive to the improvement of microcirculation in RA patients, which is of great significance in reducing micro-thrombosis and improving organ functions in RA patients. The D-dimer levels can be used to assess disease activity of RA, and elevated D-dimer levels can be applied for dynamic observation of the fibrinolysis levels in patients. It is of great value in timely clinical treatment of RA patients for the purpose of improving their microcirculatory disorders, and guiding early prediction and treatment of cardiovascular diseases [21]. However, due to limited conditions, we did not dynamically observe the therapeutic effects of thalidomide-leflunomide medication, and failed to elucidate the association between D-dimer and thalidomide-leflunomide medication in the treatment of RA in the current study. Therefore, targeted analyses are required in future research.

In conclusion, thalidomide-leflunomide medication effectively improves the symptoms of RA, joint functions, and microcirculation, but reduces the D-dimer levels in RA patients. As a result, it is worth clinically extensive use.

Disclosure of conflict of interest

None.

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