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Original Article

Effects of methotrexate combined with tocilizumab on growth and bone metabolism in children with juvenile idiopathic arthritis

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Abstract: Objective: This study was designed to determine the effects of methotrexate combined with tocilizumab on growth and bone metabolism in children with juvenile idiopathic arthritis (JIA). Methods: The medical records of 112 children with JIA treated in the First Affiliated Hospital of Hunan University of Traditional Chinese Medicine from March 2019 to June 2021 were collected and analyzed retrospectively. There were 51 patients treated with methotrexate alone who were assigned to the control group. The remaining 61 patients treated with methotrexate combined with tocilizumab were assigned to the observation group. The efficacy, adverse reactions, and growth after the treatment were compared between the two groups. A multiple variable logistic regression analysis was performed to analyze the independent risk factors affecting the efficacy on children. Results: The observation group had significantly better improvement rates of Pediatric American College of Rheumatology Criteria (ACR) Ped 50 and ACR Ped 70 than the control group ($P<0.05$). The incidence of adverse reactions in the two groups was not significantly different ($P>0.05$). After therapy, the observation group showed significantly lower C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) levels than the control group ($P<0.001$). Significantly higher Z values of the height and weight was shown in the observation group compared to the control group ($P<0.01$). The observation group showed significantly lower levels of receptor activator of nuclear factor κB ligand (RANKL) and β-collagen degradation products (β-CTX) than the control group. A significantly lower osteoprotegerin (OPG) level was seen in the observation group when compared to the control group ($P<0.001$). A multivariate logistic regression analysis showed that a longer course of disease, disease type, and treatment with methotrexate alone were the independent risk factors for the failure to improve the efficacy on patients ($P<0.05$). Conclusion: Methotrexate combined with tocilizumab can deliver good efficacy on children with JIA, quickly alleviate their clinical symptoms and laboratory indicators, and control the disease progress. It is safe because it will not increase the incidence of adverse reactions.

Keywords: Methotrexate, tocilizumab, juvenile idiopathic arthritis, growth, bone metabolism

Introduction

Juvenile idiopathic arthritis (JIA) refers to arthritis with unexplained joint pain and swelling before the age of 16 that lasts over 6 weeks and is confirmed to be no other diseases [1, 2]. It is one common rheumatic disease in children, with an incidence of (0.8-22.6)/100,000 people and a prevalence of (7-401)/100,000 people [3]. With features of recurrent acute inflammation and swelling of one or more joints, JIA is often accompanied by pain, sleep problems, fatigue, morning stiffness, and difficulty in doing activities at home and participating in school and social activities [4]. The purpose of intervention on JIA is to prevent joint injury, relieve symptoms, and prolong the remission period, to maintain or improve the activity and social participation of children with JIA. After the onset of JIA, arthritis will plague patients for a long time. With the gradual accumulation of lesions, the disability rate is high. This seriously disrupts the patients' later life [5]. The optimization of management and standardized treat-

ment to improve the treatment effect on JIA in adulthood is the focus of current treatment. There are studies showing that JIA patients in China tend to be older and more severely ill Early administration of biologics often leads to better outcomes [6].

The treatment of JIA is classified into traditional drug therapy and biological agent therapy [7]. Traditional therapeutic drugs include non-steroidal anti-inflammatory drugs, anti-rheumatic drugs, glucocorticoids, immunosuppressants [8]. Biological drugs include tumour necrosis factor-α antagonist, interleukin-6 (IL-6) receptor antagonist, and IL-1 receptor antagonist [9]. Methotrexate, a typical drug for the treatment of JIA, is increasingly applied to the treatment of JIA, with immunosuppressive effect. This can inhibit the immune function of the body and relieve the symptoms of JIA [10]. The drug takes effect slowly, and long-term use of it triggers a high incidence of adverse reactions [11]. Many studies have shown that methotrexate has a relatively high risk of myelosuppression and hepatotoxicity during treatment. Better treatments are entailed to reduce the side effects of drugs and improve their efficacy [12]. IL-6 is a pro-inflammatory cytokine, with a crucial function in the pathogenesis of JIA [13]. Tocilizumab is a fully humanized monoclonal antibody against IL-6 receptor. It can block IL-6 signal transmission by competitively binding to IL-6 receptor, reducing inflammation and joint injury [14]. The effects of methotrexate combined with tocilizumab on the growth and development and bone metabolism of children with JIA are rarely studied.

This study retrospectively analysed 112 children with JIA treated in The First Affiliated Hospital of Hunan University of Traditional Chinese Medicine to discuss and explore the efficacy and safety of different treatments, with the purpose of providing reference for clinical treatment selection of JIA.

Methods and data

Inclusion and exclusion criteria

Inclusion criteria: Children who met the JIA diagnostic criteria issued by the International Federation of Rheumatology Associations [15] and diagnosed with JIA; children <16 years old and those with detailed clinical data; patients who received monotherapy with methotrexate alone or combined treatment of methotrexate and Tozumab; and patients whose C-reactive protein (CRP) and receptor activator of nuclear factor κB ligand (RANKL), osteoprotegerin (OPG) and β-collagen degradation products (β-CTx), and erythrocyte sedimentation rate (ESR) were determined and completely recorded.

Exclusion criteria: Children with serious organic diseases of bodily systems, primary immunodeficiency disease, tuberculosis, viral hepatitis, or infectious diseases; children with other connective tissue diseases; children who had allergic reactions to drugs involved in the treatment; children who had received treatment with growth hormone or gonadotropin-releasing hormone analogues; and children with poor compliance with treatment.

Patient data

Medical records of 154 children with JIA treated from March 2019 to June 2021 were collected and screened according to the inclusion and exclusion criteria. There were 112 cases that met the criteria. There were 51 patients treated with methotrexate alone assigned to the control group. The remaining 61 patients treated with methotrexate combined with tocilizumab were assigned to the observation group. The research was carried out with the permission of the First Affiliated Hospital of Hunan University of Traditional Chinese Medicine Medical Ethics Committee (approval number: 20190218).

Therapeutic regimen

The control group was treated with methotrexate tablets (Shanghai Pharmaceuticals Sine) 10 mg/m^2 each time, once a week, for 2 consecutive weeks. Based on treatment to the control group, the observation group was treated with Tocilizumab injection (Roche Holding AG) with the dosage of 12 mg/kg for children with body weight <30 kg, and 8 mg/kg for children with body weight ≥30 kg. The injection was dissolved in 100 mL 0.9% sodium chloride injection, and was administered through intravenous drip, once every two weeks for 12 consecutive weeks. Children with joint pain symptoms were provided with non-steroidal anti-inflammatory drugs (naproxen). Glucocorticoid (pred-

nisone) was applied locally when the joint cavity had a distinct effusion.

Evaluation of efficacy

The efficacy on the two groups was evaluated after 12 weeks of treatment according to the Pediatric American College of Rheumatology Criteria (ACR Ped) 30/50/70/90. It covered 6 core items: (1) Evaluation of the overall disease activity of children by doctors; (2) Evaluation of current health status by parents/children; (3) Functional evaluation of children; (4) Count of active arthritis; (5) Count of restricted joints; and (6) Laboratory indexes: ESR and CRP. The ACR Ped 30 improvement was judged to be achieved if at least three of the six core indicators were improved by more than 30%, and no one was deteriorated by more than 30%. The ACR Ped 50/70 improvement was judged to be achieved if there were at least three improvements by more than 50% or 70%.

Growth condition

After one year of treatment, the growth and development of patients were evaluated. The Z values of height and weight were calculated (Z value = measured value-average/standard deviation of the same sex and age). Both short stature and low weight were defined as Z value <-2, where -3< Z value <-2 indicated moderate short stature. Z value <-3 indicated severe short stature.

Collection of detection indicators

Through the First Affiliated Hospital of Hunan University of Traditional Chinese Medicine HIS system, the electronic medical records of patients were inquired. The clinicopathological data of all children were recorded in detail. Indicators were collected through the LIS system, including treatment efficacy, adverse reactions, CRP, RANKL, OPG, β-CTx, ESR, height, and weight Z-values.

Outcome measures

Primary outcome measures: The efficacy on the two groups was evaluated.

Secondary outcome measures: The incidence of adverse reactions in the two groups was collected. CRP and RANKL, OPG and β-CTx in the children before and after therapy were quantified by enzyme-linked immuno-sorbent assay (ELISA). The data of ESR before and after treatment were collected. Z values of height and weight of the two groups before and after therapy were calculated.

Statistical analyses

This study adopted SPSS 25.0 software for statistical analysis. Measured data in normal distribution were described as mean ± SD. Inter-group comparison was conducted using the independent-samples T test. Intro-group comparison at different time points was made using the Paired t test. Counting data were described as a percentage. Their inter-group comparison and intro-group comparison were conducted using the X^2 test and Fisher exact probability test, respectively. A multiple variable logistic regression analysis was performed to analyze the independent risk factors affecting the efficacy on children. P<0.05 suggests a significant difference.

Results

Baseline data

Comparison of baseline data between the two groups revealed no significant difference between them in age, sex, course, type, family history of rheumatic diseases, existence of brother/sister, gestational age, and place of residence (all P>0.05, **Table 1**).

Comparison of efficacy

According to comparison of clinical efficacy between the two groups, the observation group was not significantly different from the control group in the improvement rate of ACR Ped 30 (P=0.155). The observation group showed significantly higher improvement rates of ACR Ped 50 and ACR Ped 70 than the control group (P=0.044, P=0.034, **Table 2**).

Comparison of incidence of adverse reactions after treatment

According to a comparison of the incidence of adverse reactions between the two groups, the two groups were not significantly different in the total incidence of adverse reactions (P= 0.430, **Table 3**).

Table 1. Baseline data

	Observation group (n=61)	Control group (n=51)	X^2/t	*P* value
Age (years)	9.79±2.12	10.39±2.00	1.530	0.129
Gender			0.118	0.732
Male	22 (36.07)	20 (39.22)		
Female	39 (63.93)	31 (60.78)		
Course of disease (months)	7.80±3.60	8.73±3.12	1.446	0.151
Type			1.440	0.487
Oligoarticular type	12 (19.67)	15 (29.41)		
Multi-joint type	30 (49.18)	22 (43.14)		
Whole body joint type	19 (31.15)	14 (27.45)		
Family history of rheumatic diseases			0.542	0.462
Yes	12 (19.67)	13 (25.49)		
No	49 (80.33)	38 (74.51)		
Is the child the only child?			1.951	0.163
Yes	34 (55.74)	35 (68.63)		
No	27 (44.26)	16 (31.37)		
Gestational age			0.224	0.636
Premature delivery	5 (8.20)	3 (5.88)		
Mature	56 (91.80)	48 (94.12)		
Place of residence			0.184	0.668
Urban area	47 (77.05)	41 (80.39)		
Rural area	14 (22.95)	10 (19.61)		

Table 2. Efficacy

	ACR Ped 30	ACR Ped 50	ACR Ped 70
Observation group (n=61)	59 (96.72)	56 (91.80)	53 (86.89)
Control group (n=51)	46 (90.20)	40 (78.43)	36 (70.59)
X^2	2.018	4.056	4.521
P	0.155	0.044	0.034

Table 3. Adverse reactions

	Observation group (n=61)	Control group (n=51)	X^2	P
Rash	3 (4.92)	1 (1.96)	0.705	0.401
Infection	1 (1.64)	0 (0.00)	0.844	0.358
Elevated transaminase	3 (4.92)	2 (3.92)	0.065	0.799
Neutropenia	2 (3.28)	2 (3.92)	0.033	0.855
Total adverse reactions	9 (14.75)	5 (9.80)	0.622	0.430

Changes of CRP and ESR in the two groups after treatment

According to statistics about the changes of CRP and ESR in the two groups before and after treatment, the two groups were not significantly different in CRP and ESR levels (P>0.05) before the treatment. After therapy, the observation group showed significantly lower levels of CRP and ESR than the control group (P< 0.001, **Figure 1**).

Comparison of the growth and development between the two groups

According to a comparison of the Z values of height and weight between the two groups before and after treatment, the two groups showed no significant difference in the Z values before the treatment (P=0.735, P=0.536). After the treatment, the observation group showed significantly higher Z values of height and weight than the control group (both P<0.001, **Table 4**).

Comparison of bone metabolism indexes between the two groups

According to a comparison of bone metabolism indexes between the two groups before and

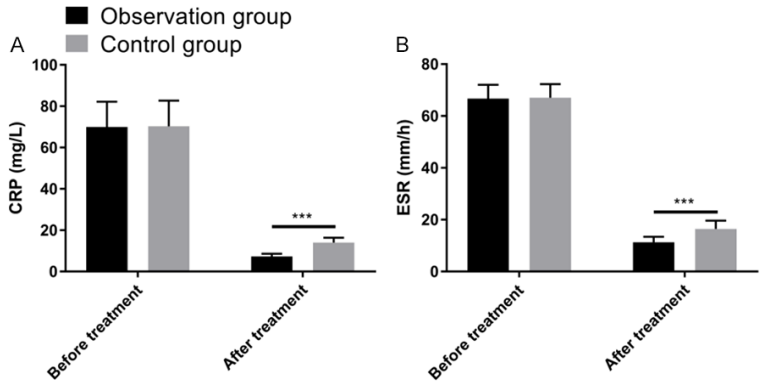

Figure 1. Changes of CRP and ESR in the two groups after the treatment. A. After the treatment, the observation group showed a significantly lower CRP level than the control group (P<0.001). B. After the treatment, the observation group showed a significantly lower ESR level than the control group (P<0.001). CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate.

after the treatment, the two groups were not significantly different in RANKL, OPG, and β-CTx levels (all P>0.05). After the treatment, the observation group showed significantly lower levels of RANKL and β-CTx than the control group (both P<0.001) and a significantly higher OPG level than the control group (P<0.001, **Figure 2**).

Univariate analysis of risk factors influencing efficacy

According to the ACR Ped 70 of efficacy in the children, the children were reassigned to an improved group and an unimproved group. According to a comparison of the two groups, the two groups were significantly different in the course of disease, disease type, and therapeutic regimen (all P<0.05, **Table 5**).

Multivariate analysis

A multivariate logistic regression analysis was conducted on indexes with significant difference in univariate analysis, a longer course of disease, disease type, and treatment with methotrexate alone were the independent risk factors for the failure to improve the efficacy on patients (all P<0.05, **Table 6**).

Discussion

JIA often compromises the normal growth and development of children because of its long course and protracted illness. Children often have decreased bone mineral content, abnormal bone metabolism, increased bone fragility, and significantly increased risk of fracture because of the disease and treatment. This causes them to suffer extreme compromised quality of life and prognosis [16, 17]. The pathogenesis of JIA remains unclear. A study has pointed out that innate immune system disorder, NK cell dysfunction, genetic susceptibility, gene polymorphism, and environmental factors are the reasons for the development of JIA [18]. Over the past few years, with the deepening of research, over-activation of innate immune system in JIA cases was found to have a strong association with JIA. IL-6 was found to have a wide involvement in the development of this disease. IL-6 has become a novel therapeutic target [19].

ESR and CRP are effective indexes to evaluate the disease activity of JIA [20]. In this study, after 12 weeks of therapy, the observation group showed significantly lower ESR and CRP levels than the control group and significantly higher improvement rates of ACR Ped 50 and ACR Ped 70 than the control group. The results suggested that the combined application of methotrexate and tocilizumab can quickly improve the laboratory indexes of children, control the symptoms of diseases, and quickly relieve the illness. The possible reasons are as follows: The combination of drugs plays a synergistic role. Based on the immunosuppressive mechanism of methotrexate, tocilizumab blocks IL-6 to inhibit plasma cell differentiation and immunoglobulin secretion, inhibiting the humoral immunity of children. After antagonizing the IL-6 receptor, it can inhibit T cell differentiation, down-regulate T cell expression, and lower the cellular immunity of children [21]. Methotrexate is a folic acid antagonist. It can inhibit the proliferation of T cells by competitively inhibiting the activities of dihydrofolate reductase and formyltransferase, exerting immunosuppressive and anti-inflammatory effects [22]. Tocilizumab can specifically bind to IL-6 receptor, block the formation of IL-6 and IL-6 receptor complex, and inhibit the activity of

Table 4. Growth and development status

	Z value of height		Z value of weight	
	Before treatment	After treatment	Before treatment	After treatment
Observation group (n=61)	-1.62±0.64	-0.94±0.38*	-0.57±0.33	-0.27±0.18*
Control group (n=51)	-1.58±0.60	-1.21±0.45*	-0.61±0.35	-0.41±0.26*
t	0.339	3.443	0.621	3.354
P	0.735	<0.001	0.536	0.001

*P<0.05 *vs.* the situation before treatment.

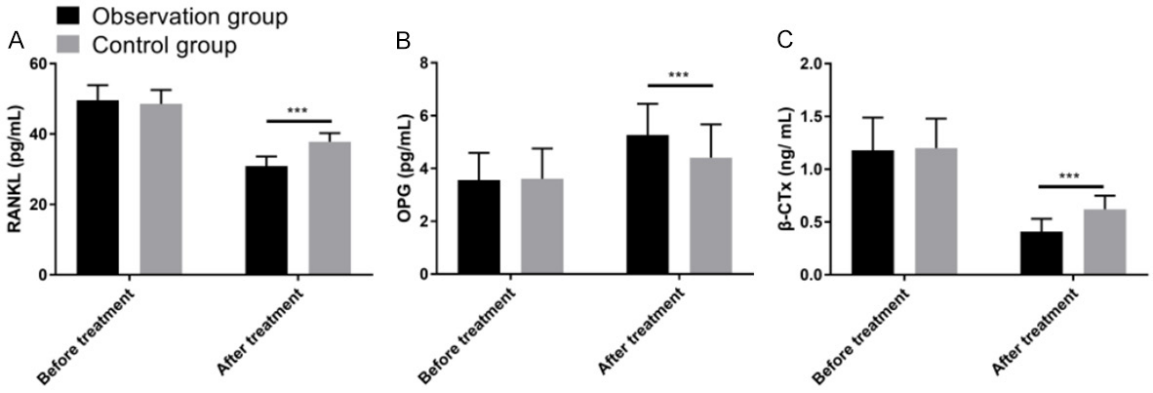

Figure 2. Comparison of bone metabolism indexes between the two groups. A. After the treatment, the observation group showed a significantly lower RANKL level than the control group (P<0.001). B. After the treatment, the observation group showed a significantly higher OPG level than the control group (P<0.001). C. After the treatment, the observation group showed a significantly lower β-CTx level than the control group (P<0.001). RANKL: Receptor Activator of Nuclear factor κB Ligand; OPG: Osteoprotegerin; β-CTx: β-Collagen degradation products.

IL-6, to suppress the differentiation of Th17 cells and relieve joint symptoms and inflammatory reaction [23]. The synergistic effect of the two drugs can exert a better function. This study compared the Z values of weight and height between the two groups after the treatment to detect the growth and development of the two groups. After the treatment, the observation group showed significantly higher Z values of height and weight than the control group. This indicated that the combined treatment can contribute to better growth and development for the children.

Prior research has revealed the involvement of osteoblasts and osteoclasts in the progression of JIA, and revealed a higher osteoclasts level in children with JIA than that in healthy children [24]. Tocilizumab can inhibit osteoclast differentiation and alleviate bone destruction and osteoporosis around the joints [25]. The bone metabolism was compared between the two groups. According to the results, after the treatment, the observation group showed significantly lower RANKL and β-CTx levels than the control group, and a significantly higher OPG level than the control group. RANKL is expressed on the surface of osteoclasts. OPG can inhibit osteoclasts. These results suggested that the therapeutic effect of tocilizumab can be enhanced by inhibiting osteoclasts. According to a multivariate logistic regression analysis, a longer course of disease, disease type, and treatment with methotrexate alone were the independent risk factors for the failure to improve the efficacy on patients. Doctors need to pay more attention to children with these risk factors.

This study had some limitations. The specific influence mechanism of methotrexate combined with tocilizumab needs more exploration by follow-up basic experiments. This study was only a report of one time point. It indicated the current situation of a growing point for the growth and development of children with JIA, but did not explain the growth and development trend of children. The treatment of JIA

Table 5. Univariate analysis

	Improved group (n=89)	Non-improved group (n=23)	X^2/t	*P*
Age (years)	10.00±2.03	10.30±2.30		
Gender			1.609	0.205
Male	36 (40.45)	6 (26.09)		
Female	53 (59.55)	17 (73.91)		
Course of disease (months)	7.47±3.09	11.13±3.02		
Type			8.250	0.016
Oligoarticular type	25 (28.09)	2 (8.70)		
Multi-joint type	43 (48.31)	9 (39.13)		
Whole body joint type	21 (23.60)	12 (52.17)		
Family history of rheumatic diseases			0.347	0.556
Yes	21 (23.08)	4 (17.39)		
No	70 (76.92)	19 (82.61)		
Is the child the only child?			1.089	0.297
Yes	57 (64.04)	12 (52.17)		
No	32 (35.96)	11 (47.83)		
Gestational age			1.519	0.218
Premature delivery	5 (5.62)	3 (13.04)		
Mature	84 (94.38)	20 (86.96)		
Place of residence			1.394	0.238
Urban area	72 (80.90)	16 (69.57)		
Rural area	17 (19.10)	7 (30.43)		
Therapeutic regimen			4.521	0.034
Monotherapy with methotrexate	36 (40.45)	15 (65.22)		
Combined treatment with methotrexate and tuzumab	53 (59.55)	8 (34.78)		

Table 6. Multivariate analysis table

	B	S.E.	Wals	Sig.	Exp (B)	95% C.I of EXP (B)	
						Lower limit	Upper limit
Course of disease	0.387	0.095	16.514	0.001	1.473	1.222	1.775
Disease Type	1.299	0.455	8.151	0.004	3.665	1.503	8.94
Therapeutic regimen	-1.273	0.600	4.504	0.034	0.280	0.086	0.907

with tocilizumab is controversy. Large-dose and multi-data studies are required.

In concluion, methotrexate combined with tocilizumab can deliver higher efficacy on children with JIA, quickly alleviate their clinical symptoms and laboratory indicators, and control the disease progress. It is safe because it will not increase the incidence of adverse reactions.

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Disclosure of conflict of interest

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