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Original Article

Efficacy of cyclophosphamide plus prednisone for patients with systemic lupus erythematosus and the effects on immune function

Shengli Zhang

Dermatosis Prevention and Treatment Station of Wenshang County, Jining 272500, Shandong Province, China

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Abstract: Objective: This paper aimed to exploring the efficacy of cyclophosphamide (CTX) plus prednisone (PDN) on patients with systemic lupus erythematosus (SLE), and investigating the effects of this drug combination on patients' immune function, so as to provide reference for the clinical treatment of SLE. Methods: Admitted to our hospital from August 2017 to December 2019, 124 patients with SLE were selected as the research subjects. Among them, 74 cases were treated with CTX plus PDN in the research group, and 50 cases were treated with PDN in the control group. The two groups were compared in terms of efficacy, adverse reactions, levels of immunoglobulins, serum complements, IL-6 and IL-10, and scores of the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Results: The overall response rate (ORR) of treatment was better in the research group compared with the control group ($P<0.05$), and the incidence of adverse reactions was lower in the research group ($P<0.05$). Before treatment, the differences between the two groups were not significant in the levels of serum IgA, IgG, IgM, IL-6 and IL-10 and in the SLEDAI scores ($P>0.05$). After treatment, these measures were reduced remarkably in both groups, but the reduction was more significant in the research group ($P<0.05$). Before treatment, the differences in the levels of serum C3 and C4 were not significant between the two groups ($P>0.05$). After treatment, the levels rose remarkably in both groups, and the increase was more significant in the research group ($P<0.05$). Conclusion: For patients with SLE, CTX plus PDN has a high ORR with few adverse reactions, and it can obviously improve the immune function of patients, so it is worthy of further clinical application.

Keywords: Cyclophosphamide, prednisone, systemic lupus erythematosus, immune functions

Introduction

As a systemic autoimmune disease that has unknown causes, systemic lupus erythematosus (SLE) is characterized by autoimmune inflammation, and its major clinical features are multiple autoantibodies (represented by antinuclear antibodies in serum) and multiple organ damage [1-3]. According to statistics, in China its incidence has risen in recent years, and that among young women is relatively high [4]. In clinical practice, most patients with SLE suffer from myasthenia, abnormal body temperature and joint pain, and untimely or incorrect treatment will damage patient's nervous system and renal function, thus greatly affecting quality of life and even threatening life itself in severe cases [5, 6].

Prednisone (PDN) is a synthetic glucocorticoid drug that has a satisfactory anti-allergic and anti-inflammatory effect [7]. It can remarkably inhibit the proliferation of connective tissues, improve the permeability of cell membranes and the levels of inflammatory cytokines, and inhibit the production of toxic cytokines, so this drug has better effects on treating SLE; however, more experiments have revealed that the effects of such drugs alone have not been completely effective in treatment [8-10]. As a clinically common anti-tumor drug, cyclophosphamide (CTX) acts on the S-phase and G2-terminal cells, and has satisfactory effects in treating many autoimmune diseases [11-13]. CTX has a certain immunosuppressive effect, and its combination with glucocorticoids has satisfactory efficacy in alleviating the severity

of illness [14]. However, there are relatively few studies about the efficacy of this drug combination on treating SLE and about its effects on the immune function of patients with this disease.

Therefore, the efficacy and the effects were analyzed in this study, in order to explore effective treatment methods for the patients and provide reference for clinical treatment.

Materials and methods

General information

Treated in the Dermatosis prevention and treatment station of Wenshang County from August 2017 to December 2019, 124 patients with SLE were selected as the research subjects. All patients signed sn informed consent form. This study was approved after review by the Hospital Ethics Committee. The patients were divided into the control group (n=50) and the research group (n=74) based on therapeutic schemes. Inclusion criteria: All patients met the diagnostic criteria of SLE, which were published by the American College of Rheumatology in 1997 [15]; patients suffered from active SLE when admitted to our hospital; patients had normal thinking and consciousness and could communicate with medical personnel; patients had not received systemic or drug treatment before admission, and no hormone drugs or immunosuppressants were used for control; patients had stable indicators of vital signs. Exclusion criteria: Those who were aged ≥80; those with serious immune diseases; those complicated with severe organ diseases such as heart and lung; those with confused thinking and consciousness and who were unable to communicate; and pregnant or lactating women.

Therapeutic methods

Patients in the control group were administrated PDN (0.8 mg/kg; Tianjin Tianyao Pharmaceuticals Co., Ltd., H20203400) every morning for 6 weeks. The dosage of this drug was adjusted based on individual differences and maintained at 5-10 mg/d. Those in the research group were administrated CTX (Tonghua Maoxiang Pharmaceutical Co., Ltd., H22022988) and PDN. CTX (500 mg) and normal saline (250 mL) were mixed for intravenous drip, once every 2 weeks. After 3 rounds of treatment, the administration was changed to once every 4 weeks for 3 months. During the treatment, the patients' medication was closely observed, and their discomfort was treated in real time according to specific situations to avoid the progression of SLE.

Outcome measures

Judgment criteria for clinical efficacy: (1) Markedly effective: after treatment, the patients' clinical symptoms completely disappeared and their clinical examination results were improved remarkably. (2) Effective: The symptoms and the results were slightly improved after treatment. (3) Ineffective: The symptoms and the results were not changed before and after treatment.

Clinical overall response rate (ORR) = (total number of cases - ineffective cases)/total number of cases × 100%.

Before and after treatment, venous blood from the elbow (3 mL) in a fasted state was collected from the patients and then centrifuged, to obtain the upper serum for determination. Enzyme-linked immunosorbent assay (ELISA) was conducted to test the levels of serum immunoglobulins (IgA, IgG, IgM; Wuhan Yipu Biotechnology Co., Ltd., CK-E11520, CK-E11521, CK-E11522) and inflammatory cytokines (IL-6, IL-10; Beijing Solarbio Science & Technology Co., Ltd., SEKH-0013, SEKH-0018). Immunoturbidimetry was carried out to detect the levels of complements (C3, C4; Xiamen Huijia Biotechnology Co., Ltd., GMS70135, GMS70069).

During the medication, the adverse reactions of the patients in both groups were counted.

Before treatment and at 24 weeks after treatment, the disease activity of the patients in both groups was scored with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [16]. No disease activity was scored as 0-4 points; mild disease activity was scored as 5-9 points; moderate disease activity was scored as 10-14 points; severe disease activity was scored as ≥15 points.

Statistical analysis

In this experiment, the SPSS 22.0 statistical software was used to statistically analyze the data. Graph pad was used to illustrate figures.

Table 1. Comparison of general information

Categories	Research group (n=74)	Control group (n=50)	t value	*P* value
Gender			0.804	0.370
Male	21 (28.38)	18 (36)		
Female	53 (71.62)	32 (64)		
Age	33.62±7.44	33.52±4.29	0.086	0.932
Course of disease	1.58±0.43	1.72±0.35	1.913	0.058
Systolic blood pressure	117.42±15.62	117.18±16.74	0.083	0.935
Diastolic blood pressure	73.47±12.46	73.96±13.09	0.211	0.834
SLEDAI scores	25.60±7.70	25.89±7.95	0.203	0.839
Severity			0.116	0.733
Moderate	51 (68.92)	33 (66)		
Severe	23 (31.08)	17 (34)		

Note: SLEDAI scores: the scores of the Systemic Lupus Erythematosus Disease Activity Index.

Table 2. Comparison of clinical efficacy

Categories	Markedly effective (/%)	Effective (/%)	Ineffective (/%)	ORR (/%)
Research group (n=74)	53 (71.62)	16 (21.62)	5 (6.76)	69 (93.24)
Control group (n=50)	29 (58)	11 (22)	10 (20)	40 (80)
χ^2 value				4.921
P value				0.027

Measurement data were expressed as ($\bar{x}$ ± sd) and compared by a *t* test. Count data were expressed as [*n* (%)] and compared by a χ^2 test. When P<0.05, the difference was statistically significant.

Results

Comparison of general information

In the research group, there were 13 males and 32 females who were aged 22-61 years, with an average age of (33.62±7.44) years, a course of disease of 1-4 years and an average course of disease of (1.58±0.43) years. In the control group, there were 14 males and 36 females who were aged 21-58 years, with an average age of (33.52±4.29) years, a course of disease of 1-3 years and an average course of disease of (1.72±0.35) years. The differences in the general information were not statistically significant between the two groups (P>0.05) (**Table 1**).

Comparison of clinical efficacy

The research group consisted of 53 markedly effective patients (71.62%), 16 effective patients (21.62%) and 5 ineffective patients (6.76%), with the ORR of 93.24%. The control group consisted of 29 markedly effective patients (58%), 11 effective patients (22%) and 10 ineffective patients (20%), with the ORR of 80%. The ORR was remarkably higher in the research group compared with the control group (P<0.05) (**Table 2**).

Comparison of incidence of adverse reactions

In the research group, only 1 case developed mouth ulcers, and the incidence of adverse reactions was 5.0%. In the control group, 3 cases that developed mouth ulcers, 2 cases that developed dyspepsia, 1 case that developed abnormal menstruation and 1 case that developed hypertension, with the incidence of adverse reactions of 40.0%. The difference in the occurrence of adverse reactions was large between the two groups, and the incidence was remarkably lower in the research group (P<0.05) (**Table 3**).

Comparison of immunoglobulin levels

Before treatment, the levels of serum IgA, IgG and IgM were not significantly different between the research and control groups (P> 0.05). After treatment, the levels reduced in both groups (P<0.05), but they were lower in the research group (P<0.05) (**Figure 1**).

Comparison of serum inflammatory cytokines

Before treatment, the levels of serum IL-6 and IL-10 were not significantly different between the research and control groups (P>0.05). After treatment, the levels were reduced in both groups (P<0.05), but they were lower in the research group (P<0.05) (**Table 4**).

Comparison of complement levels before and after treatment

Before treatment, the levels of C3 and C4 were not significantly different between the research

Table 3. Comparison of incidence of adverse reactions

Categories	Mouth ulcer	Dyspepsia	Abnormal menstruation	Hypertension	Incidence of adverse reactions
Research group (n=74)	1 (1.35)	0 (0)	0 (0)	0 (0)	1 (1.35)
Control group (n=50)	3 (6)	2 (4)	1 (2)	1 (2)	7 (14)
χ^2 value					7.910
P value					0.005

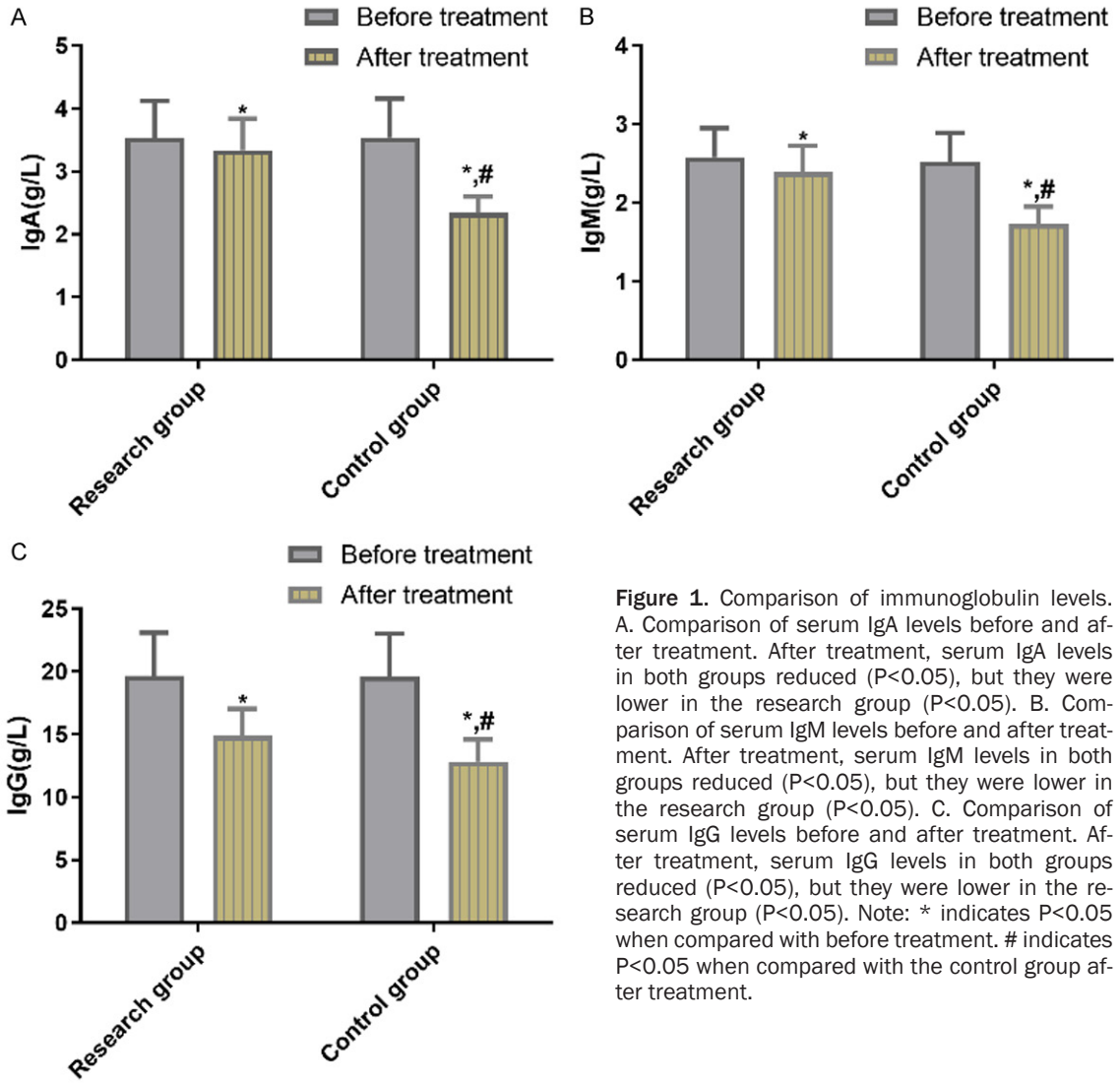

Figure 1. Comparison of immunoglobulin levels. A. Comparison of serum IgA levels before and after treatment. After treatment, serum IgA levels in both groups reduced (P<0.05), but they were lower in the research group (P<0.05). B. Comparison of serum IgM levels before and after treatment. After treatment, serum IgM levels in both groups reduced (P<0.05), but they were lower in the research group (P<0.05). C. Comparison of serum IgG levels before and after treatment. After treatment, serum IgG levels in both groups reduced (P<0.05), but they were lower in the research group (P<0.05). Note: * indicates P<0.05 when compared with before treatment. # indicates P<0.05 when compared with the control group after treatment.

and control groups (P>0.05). After treatment, the levels reduced in both groups (P<0.05), but they were remarkably lower in the research group (P<0.05) (**Table 5**).

Comparison of SLEDAI scores before and after treatment

Before treatment, the SLEDAI scores were not significantly different between the research and control groups (P>0.05). After treatment, the scores were reduced in both groups (P< 0.05), but they were remarkably lower in the research group (P<0.05) (**Figure 2**).

Discussion

SLE has unclear pathogenic factors, which are generally considered to have a relationship with genetic factors, environmental factors and the

Table 4. Comparison of serum inflammatory cytokines

Groups	IL-6 (p/ng.L)		IL-10 (p/ng.L)	
	Before treatment	After treatment	Before treatment	After treatment
Research group (n=74)	41.83±9.46	22.48±7.64	70.69±2.94	55.53±1.84
Control group (n=50)	41.91±9.57	14.29±6.44	70.77±2.68	39.56±1.95
t value	0.046	6.229	0.154	46.280
P value	0.963	<0.01	0.878	<0.01

Table 5. Comparison of complement levels before and after treatment

Groups	C3 (mg/L)		C4 (mg/L)	
	Before treatment	After treatment	Before treatment	After treatment
Research group (n=74)	0.45±0.06	0.93±0.04	0.14±0.06	0.32±0.07
Control group (n=50)	0.47±0.08	0.67±0.08	0.16±0.08	0.21±0.05
t value	1.473	23.910	0.115	9.577
P value	0.143	<0.01	1.589	<0.01

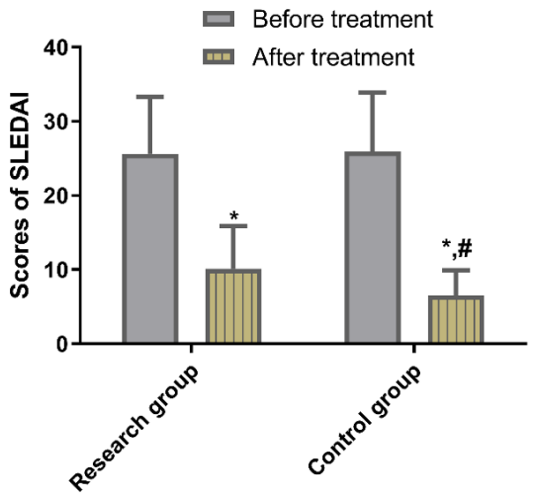

Figure 2. Comparison of SLEDAI scores before and after treatment. Before treatment, the SLEDAI scores were not significantly different between the two groups (P>0.05). After treatment, the scores in both groups reduced (P<0.05), but they were remarkably lower in the research group (P<0.05). Note: * indicates P<0.05 when compared with before treatment. # indicates P<0.05 when compared with the control group after treatment.

endocrine system [17]. With the development of medical science, current treatment methods can better control the conditions of most patients, and the 10-year survival rate of disease has been greatly increased, although many diseases are not completely cured [18, 19]. Since SLE is prone to recurrent attacks and has suddenly mild or severe conditions, patients with the disease generally need lifelong medication to control the deterioration. This means that the effects of medication and the adverse reactions of drugs are essential for patients, so unsatisfactory effects and severe adverse reactions are very unfavorable to their health [20, 21].

Glucocorticoids are widely used for treating patients with SLE, and a commonly used drug among them is PDN. This drug inhibits connective tissues from proliferation, thus controlling the permeability of cell membranes and inhibiting inflammatory exudation; besides, it inhibits the release of toxic substances and histamine, thereby relieving inflammatory responses, inhibiting immune responses, and preventing allergies [22-24]. As a specific alkylating agent, CTX enhances anti-tumor activity *in vivo*, inhibits lymphocytes and antibodies from proliferation, and prevents allergy, as well as reduces the levels of immunoglobulins [25]. In our study, the ORR was remarkably higher in the research group compared with the control group (P<0.05), while the incidence of adverse reactions was remarkably lower in this group (P<0.05). This suggests that the combination medication has relatively high effectiveness and safety. Mainly existing in fresh serum and tissue fluid, complements are a group of glycoproteins, which have enzyme activity and can be activated by antigen-antibody complexes or other stress reactions; they are widely involved in the immune response and regulation of the body, mediating the traumatic responses of immunopathogenesis [26, 27]. In relevant clinical reports, according to the examinations of related items, patients with SLE have rising expression levels of serum immunoglobulins and reducing levels of C3 and C4, so the effectiveness of clinical treatment can be judged through detecting the above indicators and observing the patients' clinical symptoms and manifestations [28]. In our study, after treatment, the expression levels of IgM, IgA and IgG

were lower in the research group compared with the control group (P<0.05), but those of C3 and C4 were higher (P<0.05). This indicates that compared with PDN plus methotrexate, the combination of PDN with CTX can remarkably reduce the levels of the three immunoglobulins, and increase those of the two complements. This is possibly because CTX as a clinical cell cycle non-specific agent that can block and eliminate proliferating B lymphocytes and inactive T lymphocytes, thereby lowering the expression levels of the immunoglobulins, reducing the levels of serum immune complexes, and promoting the activation of C3 and C4. In this study, after treatment, IL-6 and IL-10 levels were lower in the research group compared with the control group (P<0.05). This suggests that PDN plus CTX can remarkably reduce the expression levels of serum inflammatory cytokines, which demonstrates that the combined medication is helpful to reduce inflammatory responses and improve therapeutic effects. In this study, the improvement of the SLEDAI scores was better in the research group compared with the control group (P<0.05). It can be seen that the efficacy of the combined medication is more advantageous, and that the effects on improving health in patients with severe conditions are more exact.

This study has confirmed the definite effects of CTX plus PDN on the treatment of SLE, but it still has certain shortcomings. On one hand, patients with SLE still suffer from some adverse reactions after the treatment with the two drugs. On the other hand, the therapeutic mechanism on the disease has not been explored. These shortcomings need to be further remedied in future studies.

In summary, for patients with SLE, CTX plus PDN has a high ORR and few adverse reactions, and it can obviously improve the immune functions of the patients, so it is worthy of further clinical application.

Disclosure of conflict of interest

None.

Address correspondence to: Shengli Zhang, Dermatosis Prevention and Treatment Station of Wenshang County, Zhongdu Street, Wenshang County, Jining 272500, Shandong Province, China. Tel: +86-13853769991; E-mail: zhangshengli234@163.com

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