EFFECTS OF TACROLIMUS COMBINED WITH GLUCOCORTICOID ON REFRACTORY NEPHROPATHY SYNDROME AND THE INFLUENCES ON SERUM IL-6 AND TNF-A

Shi Su[1], Yanhai Song[1], Huanyu Song[1], Jie Guan[1], Yuling Zheng[1], Daibao Lv[2], Lianming Wang[1,*]
[1]Clinical Laboratory, The First Affiliated Hospital of Harbin Medical University, Harbin150001, Heilongjiang Province, China - [2]Internal Medicine-Cardiovascular Department, The First Affiliated Hospital of Harbin Medical University, Harbin150001, Heilongjiang Province, China

ABSTRACT

This research aimed at analyzing the effects of tacrolimus combined with glucocorticoid on refractory nephropathy syndrome (RNS) and its influencing factors. A total of 156 RNS patients admitted to our hospital were selected as research objects, patients treated with tacrolimus combined with glucocorticoid were taken as group A (79 cases), and patients treated with glucocorticoid were taken as group B (77 cases). Therapeutic effects and incidence rate of adverse reactions of both groups were assessed. Blood fat and renal function indexes were observed before and after treatment. Serum interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels before and after treatment were tested by enzyme-linked immunosorbent assay (ELISA). Compared with group B, the effective rate of group A was higher (P<0.001), and the incidence rate of adverse reactions was lower (P<0.05). After treatment, serum total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), urea nitrogen (BUN), IL-6, TNF-α, and 24-hour urine protein levels in group A were lower than those in group B (P<0.05), while those of high density lipoprotein (HDL) were higher (P<0.01). Multivariate Logistic regression analysis manifested that patients with IL-6 (<139.20 pg/ml), TNF-α level (<197.00 pg/ml) and tacrolimus combined with glucocorticoid before treatment had lower risks of ineffective treatments. In conclusion, tacrolimus combined with glucocorticoid has good clinical effects on RNS. Before treatment, risks of ineffective treatments for RNS patients with IL-6 (<139.20 pg/ml), TNF-α level (<197.00 pg/ml) and tacrolimus combined with glucocorticoid are reduced.

Keywords: *Tacrolimus, glucocorticoid, refractory nephropathy syndrome, IL-6, TNF-α.*

DOI: 10.19193/0393-6384_2021_4_389

Received *October15, 2020;* ***Accepted*** *March 2021*

Introduction

Nephrotic syndrome (NS) is a rare but serious renal disease that can affect adults and children all over the world. The main clinical manifestations include peripheral edema, massive proteinuria and hypoproteinemia, often accompanied by hyperlipidemia[(1)]. According to statistics, the morbidity of NS is 2-7 cases per 100,000 children and 3 cases per 10 adults[(2)]. The pathogenesis of NS includes primary glomerulonephritis, which is a secondary disease related to infection, drugs and tumors[(3)]. Glucocorticoid is the first-line therapy for NS. Most NS patients are sensitive to steroid drugs. Patients will develop steroid dependence after frequent relapse and repeated steroid use[(4)]. Refractory nephropathy syndrome (RNS) is a subset of NS patients, which has steroid resistance or steroid dependence, and often recurs. RNS accounts for 25-40% of NS cases in children and adolescents, and even as high as 70% in adults[(5)].

At present, there are still some difficulties in the medication of RNS, even after receiving treatment, the prognosis is still very poor[(6)].

Although combination therapy can reduce hormone dependence, immunosuppressive agents have high toxic and side effects, which may cause complications such as infection, hypertension, gastrointestinal hemorrhage, and so on, thus limiting their clinical application[7, 8]. Tacrolimus is a powerful new immunosuppressant, which can inhibit the immune system, especially T cells.

Its cytokine inhibitory properties are also different. It is mainly used to inhibit organ transplant rejection in the early stage. With the in-depth study of this drug, it is gradually used in a variety of immune-related diseases, including NS[9, 10]. Some studies have shown the relationship between various cytokines and proteinuria, and pointed out that glomerular permeability factors may be pathogenic factors of NS patients or animal models of proteinuria[11]. The increase of monocyte/macrophage cytokines, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and other inflammatory cytokines play a vital part in NS occurrence and recurrence[12].

Previous researches mainly focused on tacrolimus and glucocorticoid therapy for RNS[13, 14], but there are few researches on effects of tacrolimus combined with glucocorticoid therapy on serum IL-6 and TNF-α of RNS patients and influencing factors of therapeutic effects. This research applies tacrolimus combined with glucocorticoid treatment for RNS patients to observe changes and effects of IL-6 and TNF-α in therapy.

Data and methods

General data

Totally 156 RNS patients admitted to our hospital were selected as the research objects. Among them, those treated with tacrolimus combined with glucocorticoid were taken as group A (79 cases), and those treated with glucocorticoid were regarded as group B (77 cases).

ùThere were 45 males and 34 females in group A, aged 22-67 years, 42.6±5.8 years old on average. The course of disease was 4 months to 6 years, (2.7±1.1) years on average. There were 47 males and 30 females in group B, aged 23-65 years, (43.5±6.2) years old on average. The course of disease was 5 months to 5 years, (2.5±1.2) years on average.

The research was approved by the ethics committee of our hospital. Subjects and their guardians were informed and fully informed consent forms were signed.

Inclusion and exclusion criteria

Inclusion criteria:

• Patients met the diagnostic criteria for adult primary NS (15);

• Age ≥18 years old;

• 24-hour urine protein >3.5 g;

• Patients with hormone resistance, dependence or frequent relapse after regulating glucocorticoid treatment;

• Patients had relevant therapeutic indications.

Exclusion criteria:

• Patients complicated with severe liver dysfunction, hereditary nephropathy, diabetic nephropathy, hypertensive nephropathy, renal failure, severe infection, hematopoietic dysfunction, cardiovascular and cerebrovascular diseases, connective tissue diseases, lower limb venous angiitis obliterans, malignant tumors, heart diseases, infectious diseases, secondary NS;

• Cognitive dysfunction and mental diseases;

• Those allergic to the drugs used this time;

• Pregnant or lactating women.

Treatment methods

Group A was treated with tacrolimus (Hangzhou Sino-US East China Pharmaceutical Co., Ltd., China, batch number: H20084514) combined with glucocorticoid.

Medication mode

Tacrolimus capsule was given orally at 0.05-0.10 mg/(kg·d) and taken in the morning and evening twice/d. After one week of medication, the dosage was maintained at 5-10 mg/ml. On this basis, prednison (Tonghua Maoxiang Pharmaceutical Co., Ltd, China, batch number: H22026738) was given orally at 0.5-1.0 mg/(kg·d), twice/d. Group B was treated with cyclophosphamide (Baxter Oncology GmbH, batch number: H20160467) combined with glucocorticoid.

Administration mode

Cyclophosphamide was injected intravenously at 0.8-1.0 g/d, twice/d.

The oral dose of prednison was 0.5-1.0 mg/(kg·d), twice a day. The course of treatment in both groups was 6 months.

Efficacy evaluation and adverse reaction observation

After 6 months of treatment, therapeutic effects of both groups were evaluated.

Evaluation criteria

• Recovery: 24-hour urine protein level was <0.3g and edema and other clinical symptoms disappeared;

• Markedly effective: 24-hour urinary protein level was <1.5g and edema and other clinical symptoms basically disappeared;

• Effective: 24-hour urinary protein level was <1.5g and edema and other clinical symptoms relieved;

• Ineffective: clinical symptoms and signs did not improve or even deteriorate. (Healing+Markedly Effective+Effective)/Total Cases in Group×100%.

Main adverse reactions occurred during the treatment of both groups were observed, including gastrointestinal reactions, tachycardia, leukocyte inhibition and alopecia.

Detection methods

Totally 10 ml venous blood of patients with an empty stomach was taken one day before treatment and six months after treatment, and was placed in a vacuum tube without anticoagulant. Serum was centrifugally separated and placed in a low temperature refrigerator at -20°C for later use.

Urine of patients for 24-hour was collected and 3 ml was taken for follow-up examination. AU5800 automatic biochemical analyzer (Beckman Coupler Commercial Enterprise (China) Co., Ltd.) was used. Total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), urea nitrogen (BUN) and 24-hour urine protein levels (Beijing Century Ward Biotechnology Co., Ltd., China, 307, 306, 305, 304, 205, 209) in serum were detected. The detection process was strictly carried out in accordance with the instruction manual of instruments and kits. IL-6 and TNF-α in serum (KT-56428, IQP-163R, Xiamen Scientific Biotechnology Co., Ltd., China) levels were tested via enzyme-linked immunosorbent assay (ELISA) (16). Blank holes (no samples and enzyme-labeled reagents) and sample holes were set up to be tested. 50 μL sample diluent was supplemented to the enzyme-labeled coating plate, and then 10 μL samples to be tested was added (the final dilution of samples was 5 times). After sealing the plate and membrane, it was incubated for 30 min at 37°C, and liquid was discarded, washed 5 times repeatedly, and patted dry.

After that, 50 μL enzyme-labeled reagents was supplemented to each hole, except for blank holes. After the plate was sealed, it was incubated at 37°C for 30 min, the liquid was discarded, cleaned 5 times repeatedly, and dried. Then, 50 μL developer A was first added and then 50 μL developer B was added to each hole; they were mixed well with gentle shaking, and developed 15 min at 37°C under dark conditions; 50 μL stop solution was supplemented (blue turns yellow at this time).

Blank holes were zeroed, and the absorbance (OD value) was measured sequentially at 450 nm wavelength by BIOBASE1000 automatic enzyme immunoassay analyzer (Jinan Xinbesi Biotechnology Co., Ltd., China), and the IL-6 and TNF-α levels were counted.

Statistical methods

SPSS 21.0 (IBM Corp, Armonk, NY, U.S.) was applied to statistical analysis. The counting data were marked as number of cases/percentages (n/%) and compared under Chi-square test. When the theoretical frequency was less than 5, the continuity correction Chi-square test was used.

The measurement data were represented as mean±standard deviation (x±SD); the inter-group comparison was assessed via independent-samples T test, and comparison before and after treatment was evaluated via paired T test. The diagnostic value of serum IL-6 and TNF-α on therapeutic effects was evaluated by ROC curve. The risk factors affecting therapeutic effects were assessed under Logistic regression. P<0.05 denoted that differences were statistically marked.

Results

General data of groups A and B

There were no marked differences in gender, age, body mass index (BMI), course of disease, type of primary disease, urine volume, smoking history, drinking history, systolic blood pressure (SBP), diastolic blood pressure (DBP), white blood cell count (WBC), albumin (ALB) between groups A and B (P>0.05) (Table 1).

Results of effective rates in groups A and B

In group A, 36 cases were cured (45.57%), 22 were markedly effective (27.84%), 13 were effective (16.46%), 8 were ineffective (10.13%), and the effective rate was 89.87%.

In group B, 30 cases were cured (38.96%), 16 were markedly effective (20.78%), 7 were effective (9.09%), 24 were ineffective (31.17%), and the rate was 68.83%. The effective rate of group A was higher than that of group B (P<0.001) (Table 2).

Category	Group A (n=79)	Group B (n=77)	t/χ^2 value	P value
Gender			0.268	0.605
Male	45 (56.96)	47 (61.04)		
Female	34 (43.04)	30 (38.96)		
Age (years)	42.6±5.8	43.5±6.2	0.937	0.350
BMI (kg/m^2)	22.86±3.15	22.49±3.01	0.750	0.455
Course of disease (years)	2.7±1.1	2.5±1.2	1.622	0.107
Type of primary disease				
Hormonal resistance type	15 (18.98)	12 (15.58)		
Hormone dependent type	25 (31.65)	27 (35.07)		
Frequent recurrence type	39 (49.37)	38 (49.35)		
Pathological type			1.198	0.879
Minimally pathological nephropathy	10 (12.66)	9 (11.69)		
Membranous nephropathy	9 (11.39)	7 (9.09)		
Mesangial proliferativenephritis	42 (53.16)	44 (57.14)		
Mesangial capillary nephritis	10 (12.66)	12 (15.58)		
Focal segmental glomerulosclerosis	8 (10.13)	5 (6.49)		
Urine volume (ml/d)	758.26±151.07	776.48±162.53	0.726	0.469
Smoking history			0.087	0.768
Yes	31 (39.24)	32 (41.56)		
No	48 (60.76)	45 (58.44)		
Drinking history			0.128	0.720
Yes	33 (41.77)	30 (38.96)		
No	46 (58.23)	47 (61.04)		
Systolic pressure (mm Hg)	148.26±20.13	146.96±19.23	0.412	0.681
Diastolic pressure (mm Hg)	97.58±12.36	96.31±12.49	0.638	0.524
Place of Residence			0.007	0.931
City	58 (73.42)	57 (74.03)		
Countryside	21 (26.58)	20 (25.97)		
WBC (×10^9/L)	8.23±1.96	7.93±2.39	0.858	0.392
ALB (g/L)	25.41±6.74	23.55±6.71	1.727	0.086

Table 1: General data of groups A and B [n(%)]/($\bar{x}$±SD).

Group	n	Recovery	Markedly effective	Effective	Ineffective	Effective rate (%)
Group A	79	36 (45.57)	22 (27.84)	13 (16.46)	8 (10.13)	89.87
Group B	77	30 (38.96)	16 (20.78)	7 (9.09)	24 (31.17)	68.83
χ^2 value	-	-	-	-	-	10.590
P value	-	-	-	-	-	<0.001

Table 2: Comparison of results of effective rates between groups A and B [n(%)].

Incidence rates of adverse reactions in groups A and B

In group A, there were 3 gastrointestinal reaction cases (3.80%), 2 tachycardia (2.53%) and 2 alopecia (2.53%), and the incidence rate of adverse reactions was 8.86%. In group B, there were 5 gastrointestinal reaction cases (6.49%), 4 tachycardia (5.19%), 6 leukopenia (7.79%) and 7 alopecia (9.09%), and the rate was 28.57%. Compared with group B, the incidence rates of leukopenia and adverse reactions in group A were lower (both P<0.05) (Table 3).

Group	n	Gastrointestinal tract reaction	Tachycardia	Leukocyte inhibition	Alopecia	Total incidence rate (%)
Group A	79	3 (3.80)	2 (2.53)	0 (0.00)	2 (2.53)	8.86
Group B	77	5 (6.49)	4 (5.19)	6 (7.79)	7 (9.09)	28.57
χ^2 value	-	0.583	0.201	6.402	3.086	10.011
P value	-	0.445	0.654	0.011	0.079	0.002

Table 3: Comparison of results of incidence rates of adverse reactions between groups A and B [n(%)].

Results of blood fat level and renal function in groups A and B before and after treatment

Before treatment, there were no remarkable differences in serum TC, TG, LDL, HDL, BUN, and 24-hour urine protein level between groups A and B (P>0.05). After treatment, serum TC, TG, LDL, BUN and 24-hour urine protein levels in groups A and B decreased (P<0.05), while those of HDL increased (P<0.05). After treatment, serum TC, TG, LDL, BUN and 24-hour urine protein levels in group A were lower than those in group B (P<0.05), while those of HDL were higher (P<0.01) (Table 4).

Levels of serum IL-6 and TNF-α in groups A and B before and after treatment

Serum IL-6 and TNF-α levels in group A were (130.25±24.69) pg/ml, (191.13±29.45) pg/ml before treatment and (73.69±15.39) pg/ml and (85.63±21.71) pg/ml after treatment, respectively. Those of serum IL-6 and TNF-α in group B were (128.05±22.84) pg/ml, (189.96±28.17) pg/ml before treatment and (105.49±17.28) pg/ml and (107.15±21.48) pg/ml after treatment, respectively. There were no marked differences in serum IL-6 and TNF-α levels between groups A and B before treatment (P<0.001). After treatment, those of serum IL-6 and TNF-α in groups A and B were lower than those before treatment (P<0.001). After treatment, those of serum IL-6 and TNF-α in group A were lower than those in group B (P<0.001) (Figure 1).

Index	Group A (n=79)	Group B (n=77)	t value	P value
TC (mmol/L)				
Before treatment	11.85±4.63	11.24±5.36	0.761	0.448
After treatment	4.28±1.13	6.48±1.28	11.390	<0.001
t value	14.120	7.580	-	-
P value	<0.001	<0.001	-	-
TG (mmol/L)				
Before treatment	3.57±1.43	3.59±1.54	0.084	0.933
After treatment	1.23±0.37	2.48±0.62	15.340	<0.001
t value	14.080	5.867	-	-
P value	<0.001	<0.001	-	-
LDL (mmol/L)				
Before treatment	5.73±1.08	5.57±1.04	0.942	0.348
After treatment	2.49±0.67	3.86±0.71	12.400	<0.001
t value	22.660	11.920	-	-
P value	<0.001	<0.001	-	-
HDL (mmol/L)				
Before treatment	0.73±0.45	0.76±0.44	0.421	0.674
After treatment	1.31±0.71	0.99±0.65	2.934	0.004
t value	6.133	2.571	-	-
P value	<0.001	0.011	-	-
BUN (mmol/L)				
Before treatment	10.29±2.68	10.16±2.97	0.287	0.774
After treatment	6.18±1.47	8.74±1.52	10.690	<0.001
t value	11.950	3.735	-	-
P value	<0.001	<0.001	-	-
24-hour urine protein (g)				
Before treatment	6.33±2.47	6.29±2.28	0.105	0.917
After treatment	2.26±1.19	4.03±1.42	8.446	<0.001
t value	13.190	7.383	-	-
P value	<0.001	<0.001	-	-

Table 4: Comparison of results of blood fat and renal function between groups A and B before and after treatment ($\bar{x}$±SD).

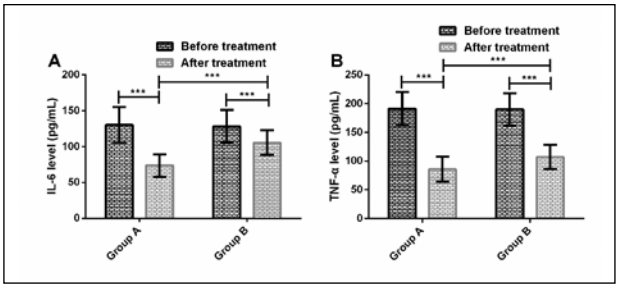

Figure 1: Comparison of results of serum IL-6 and TNF-α levels before and after treatment between groups A and B. *Before treatment, there were no significant differences in serum IL-6 levels between groups A and B (P<0.05). After treatment, serum IL-6 levels in groups A and B were lower than those before treatment (P<0.001). After treatment, serum IL-6 levels in group A were lower than those in group B (P<0.001) (A). There were no significant differences in serum TNF-α levels between groups A and B before treatment (P<0.05). After treatment, serum TNF-α levels in groups A and B were lower than those before treatment (P<0.001). After treatment, serum TNF-α levels in group A were lower than those in group B (P<0.001) (B). Note: ***P<0.001.*

Differences in clinical parameters and indicators between research group and control group

We considered 124 patients who were cured, markedly effective and effective as control group (CG) and 32 who were ineffective as research group (RG). There were no marked differences in gender, age, BMI, course of disease, type of primary disease, urine volume, history of smoking and drinking, SBP, DBP, place of residence, WBC, ALB, blood fat and renal function indexes between CG and RG (P>0.05), while there were marked differences in IL-6, TNF-α levels and treatment methods before treatment (P<0.05) (Table 5).

Multivariate logistic regression analysis affecting treatment effects

The ROC curve of diagnostic and therapeutic effects of IL-6 and TNF-α was drawn. AUC of diagnostic and therapeutic effects of serum IL-6 was 0.748 (95% CI: 0.645-0.851), diagnostic sensitivity was 65.63%, specificity was 78.23%, and optimal cut-off value was 139.20 pg/ml; AUC of diagnostic and therapeutic effects of TNF-α was 0.754 (95% CI: 0.643-0.866), diagnostic sensitivity was 68.75%, specificity was 79.84%, and optimal cut-off value was 197.00 pg/ml. Taking the cut-off value as the dividing point, IL-6 and TNF-α were subjected to binary classification conversion, and factors with statistical differences between RG and CG were introduced into binary Logistic regression equation. The results reveal that levels of IL-6, TNF-α and treatment methods before treatment were risk

factors affecting treatment effects (P<0.05). Risks of ineffective treatments for patients receiving tacrolimus combined with glucocorticoid before treatment were reduced (Table 6-8, Figure 2).

Category	Control group (n=124)	Research group (n=32)	t/χ^2 value	P value
Gender				
Male	75 (60.48)	17 (53.12)		
Female	49 (39.52)	15 (46.88)		
Age (years)	42.1±5.9	44.2±6.7	1.745	0.083
BMI (kg/m^2)	22.70±3.08	22.51±3.16	0.310	0.757
Course of disease (years)	2.5±1.2	2.8±1.1	1.282	0.202
Type of primary disease			0.234	0.890
Hormonal resistance type	22 (17.74)	5 (15.62)		
Hormone dependent type	42 (33.87)	10 (31.25)		
Frequent recurrence type	60 (48.39)	17 (53.13)		
Pathological type			2.824	0.588
Minimally pathological nephropathy	14 (11.29)	5 (15.62)		
Membranous nephropathy	13 (10.49)	3 (9.38)		
Mesangial proliferative nephritis	72 (58.06)	14 (43.75)		
Mesangial capillary nephritis	16 (12.90)	6 (18.75)		
Focal segmental glomerulosclerosis	9 (7.26)	4 (12.50)		
Urine volume (ml/d)	763.05±149.25	772.05±153.75	0.302	0.763
Smoking history			2.715	0.100
Yes	46 (37.10)	17 (53.12)		
No	78 (62.90)	15 (46.88)		
Drinking history			1.546	0.214
Yes	47 (37.90)	16 (50.00)		
No	77 (62.10)	16 (50.00)		
Systolic pressure (mmHg)	144.26±21.15	151.48±22.25	1.703	0.090
Diastolic pressure (mmHg)	95.14±12.03	97.25±12.57	0.876	0.382
Place of residence			0.513	0.474
City	93 (75.00)	22 (68.75)		
Countryside	31 (25.00)	10 (31.25)		
WBC (×10^9/L)	8.04±2.05	8.13±2.36	0.214	0.830
ALB (g/L)	24.36±6.78	24.62±6.83	0.193	0.847
TC (mmol/L) before treatment	11.47±4.52	11.60±4.45	0.146	0.884
TG (mmol/L) before treatment	3.53±1.34	3.64±1.57	0.399	0.690
LDL (mmol/L) before treatment	5.59±1.06	5.73±1.18	0.651	0.516
HDL (mmol/L) before treatment	0.72±0.43	0.79±0.47	0.805	0.422
BUN (mmol/L) before treatment	10.12±3.02	10.37±2.62	0.428	0.669
24-Hour urine protein before treatment (g)	6.24±2.49	6.38±2.56	0.282	0.778
IL-6 (pg/ml) before treatment	125.48±21.75	142.58±23.74	3.891	<0.001
TNF-α (pg/ml) before treatment	182.26±22.49	203.54±31.08	4.387	<0.001
Treatment method			10.591	0.001
Cyclophosphamide+ glucocorticoid	53 (42.74)	24 (75.00)		
Tacrolimus+glucocorticoid	71 (57.26)	8 (25.00)		

Table 5: Results of clinical parameters and pre-treatment indexes in RG and CG [n(%)]/(x±SD).

Index	AUC	95%CI	Std. Error	Cut-off (pg/ml)	Sensitivity (%)	Specificity (%)
IL-6	0.748	0.645-0.851	0.052	139.20	65.63	78.23
TNF-α	0.754	0.643-0.866	0.057	197.00	68.75	79.84

Table 6: Diagnostic value of serum IL-6 and TNF-α levels on therapeutic effects before treatment.

Factor	Variate	Assignment
IL-6 (pg/ml)	X1	≥139.20 pg/ml=1, <139.20 pg/ml=2
TNF-α (pg/ml)	X2	≥197.00 pg/ml=1, <197.00 pg/ml=2
Treatment method	X3	Cyclophosphamide+glucocorticoid =1 Tacrolimus+glucocorticoid =2

Table 7: Logistic multivariate regression analysis assignment.

Variate	B	Std. Error	Wals	P	OR	95% CI
IL-6 (pg/ml)	1.264	0.575	4.856	0.027	3.521	1.512-10.874
TNF-α (pg/ml)	0.813	0.352	5.334	0.022	2.256	1.132-4.495
Treatment method	0.952	0.328	8.446	0.004	2.594	1.365-4.927

Table 8: Multivariate logistic regression analysis affecting therapeutic effects.

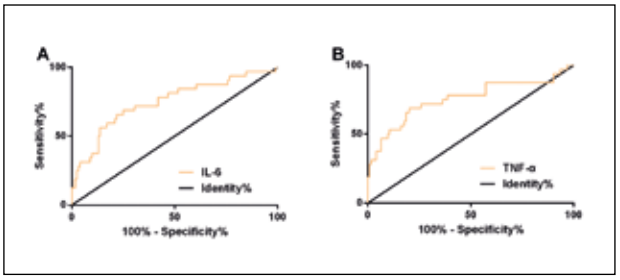

Figure 2: ROC curve of diagnosis and therapeutic effects of serum IL-6 and TNF-α levels before treatment. AUC of serum IL-6 diagnosis and therapeutic effects was 0.748 (95% CI: 0.645-0.851), diagnosis sensitivity was 65.63%, specificity was 78.23%, and the best cut-off value was 139.20 pg/ml (A). AUC of TNF-α diagnosis and therapeutic effects was 0.754 (95% CI: 0.643-0.866), diagnosis sensitivity was 68.75%, specificity was 79.84%, and best cut-off value was 197.00 pg/ml (B).

Discussion

RNS is a kind of NS that receives glucocorticoid treatment but its symptoms and signs are not relieved. Blood fat, renal function and urine protein levels reflect conditions of patients[17]. Delayed healing of RNS can cause thromboembolism, severe infection and other complications. Serious cases can cause renal failure, posing a great threat to the physical and mental health of patients[18]. Hormone resistance and hormone dependence will occur in NS patients after

long-term glucocorticoid treatment, which leads to poor therapeutic effects, increases the difficulty of treatments, and further affects the rehabilitation of patients[19].

Clinically, cyclophosphamide combined with glucocorticoid is the common way to treat RNS[20]. Cyclophosphamide is a kind of immunosuppressive agent, which can interfere with DNA synthesis and inhibit cell proliferation, but cannot directly act on effector cells, so its effects are relatively slow[21]. RNS patients are prone to adverse reactions such as hemorrhagic cystitis and leukocytosis when cyclophosphamide is used, and therapeutic effects are difficult to be improved[22]. Tacrolimus is a new type of immunosuppressant, which can inhibit the activation of T cells and further exert immunosuppressive effects[23]. In this research, compared with group B, the effective rate of group A is higher and the incidence rate of adverse reactions is lower, which indicates that tacrolimus combined with glucocorticoid has better clinical efficacy in RNS treatment. Li et al.[24] proved that use of tacrolimus in minimally pathological nephropathy had a better clinical remission rate than cyclophosphamide, and the remission time was shortened, which was similar to our research. Further studies show that tacrolimus can improve blood fat, renal function and 24-hour urine protein levels of RNS patients, and the incidence rate of leukopenia is lower. Ren et al.[25] claimed that the clinical remission rate of tacrolimus in patients with focal segmental glomerulosclerosis was higher than cyclophosphamide. Although there are no statistical differences, adverse reactions of tacrolimus are lower, which can reduce proteinuria and improve albumin level and renal function. This may be due to large number of cases included in this research, and only 33 patients were included in study of Ren et al. Therefore, therapeutic effects may differ statistically, but both drugs can improve blood fat level and renal function of patients.

Inflammatory factors can promote the aggregation of extracellular matrix and make mesangial cells of NS patients proliferate in large quantities. Inflammatory reactions mediated by inflammatory factors can promote NS progression. Inflammatory cytokines released by inflammatory reactions can cause kidney damage[26]. Yu et al.[27] found that after 8 weeks of prednisone treatment, the content of IL-6 in serum and urine of NS children was reduced, and changes of IL-6 might be an indicator for evaluating steroid sensitivity and prognosis of NS children. Other studies have shown that the TNF-α levels in blood and urine of NS children are increased, and those in peripheral blood mononuclear cells of NS children are also increased. TNF-α can induce experimental glomerular injury[28]. Therefore, inhibition of IL-6 and TNF-α levels may be the key to RNS therapy. In this research, levels of serum IL-6 and TNF-α in RNS patients decreased after treatment, while those treated with tacrolimus decreased more. This indicates that inhibiting IL-6 and TNF-α levels may be one of the therapeutic mechanisms of tacrolimus combined with glucocorticoid. This may be that prednisone can reduce the permeability of cell membrane and capillary wall and reduce the exudation of inflammatory factors[29]. Tacrolimus, as a powerful immunosuppressant, can organize the growth and proliferation of T-cell factors and further inhibit the level of inflammatory cells[30].

The combined use of the two may play a synergistic part, inhibit the synthesis and release of inflammatory cells, further relieve the renal function damage degree of RNS patients, and improve the hyperlipidemia state and urine protein. The use of RNS has always been a thorny problem in renal diseases, and its ultimate therapeutic effects have attracted much attention[31]. For this reason, we further conducted multivariate Logistic regression analysis on patients with ineffective treatment. The results manifest that risks of ineffective treatments for patients with IL-6 (<139.20 pg/ml), TNF-α level (<197.00 pg/ml) and tacrolimus combined with glucocorticoid before treatment are reduced, which indicates that the IL-6 and TNF-α levels have certain predictive value for RNS patients. However, there have been no previous studies on influencing factors of therapeutic effects of RNS patients, because the mechanism needs to be further observed in future studies by enlarging the sample size, prolonging the study time.

To sum up, tacrolimus combined with glucocorticoid has good clinical effects on RNS, which can reduce adverse reactions, reduce proteinuria, improve albumin level and renal function. Inhibiting IL-6 and TNF-α may be one of its therapeutic mechanisms. Before treatment, RNS patients with IL-6 (<139.20 pg/ml), TNF-α level (<197.00 pg/ml) and tacrolimus combined with glucocorticoid therapy have reduced risks of ineffective treatments.

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Corresponding Author:
Lianming Wang
Clinical Laboratory, The First Affiliated Hospital of Harbin Medical University, 23 Post Street, Nangang District, Harbin150001, Heilongjiang Province, China
Email: suziyi1023@126.com
(China)