

## Original Article

# Effects of low molecular weight heparin combined with prednisone on coagulation and kidney function of pediatric with nephrotic syndrome

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**Abstract:** Objective: The goal of this study was to explore the efficacy of low molecular weight heparin (LMWH) combined with prednisone and its influence on coagulation and kidney function of pediatric with nephrotic syndrome. Methods: A total of 94 pediatric cases of nephrotic syndrome were divided into an experimental group (n=47) and a control group (n=47) according to different treatments. Pediatric in the control group merely received prednisone (2 mg/(kg·d) 3 times/day, with an adjustment of the dose after 2 weeks) treatment, while LMWH (150 IU/(kg·d) once/day) was added on the treatment plan of the experimental group. The two groups' clinical efficacies were compared, including coagulation function (prothrombin time (PT), activated partial thromboplastin time (APTT)), kidney function (blood urinary nitrogen (BUN), creatinine clearance rate (Ccr), serum creatinine (SCr)), bone metabolism (blood GLA protein (BGP), bone alkaline phosphatase (BALP)), and other blood biochemistry criterion (serum albumen (Alb), including total cholesterol (TC)). Adverse events were monitored in both groups and compared. Results: The clinical effective rate of the experimental group was significantly higher than that of the control group (P=0.036). After treatment, both groups showed significantly-elevated Ccr levels (both P<0.001) with a more distinct elevation in the experimental group (P<0.01). The BUN and SCr levels were all significantly decreased (both P<0.001), especially in the experimental group (both P<0.01) compared with the control group. Comparison of coagulation function among paired before-treatment/after-treatment and control/experimental groups resulted in no significant difference (all P>0.05). For bone metabolism indexes, both groups showed significantly elevated BGP levels (both P<0.001) and decreased BALP levels (both P<0.001) and the experimental group had higher BGP level (P<0.01) and lower BALP level (P<0.01) compared with those of control group respectively. Alb level was elevated significantly in both groups (both P<0.001) and was higher in experimental group (P<0.01) while TC level was decreased significantly (both P<0.001) and lower in experimental group (P<0.01). Comparison between incidences of adverse events between groups showed no difference (P=0.168). Conclusion: For pediatric patients with nephrotic syndrome, LMWH combined with prednisone could significantly improve their clinical symptoms with improved kidney function, coagulation function and bone metabolism.

**Keywords:** Nephrotic syndrome, low molecular weight heparin, prednisone, coagulation function, kidney function, bone metabolism

## Introduction

Nephrotic syndrome (NS), a common urinary system disease in pediatrics, refers to multiple clinical symptoms induced by the abnormally increased permeability of glomerular filtration membrane which causes critical loss of plasma proteins through kidney [1-3]. Currently, the exact mechanism of pediatric NS remains unclear and may be related with coagulation dysfunction which leads to hypercoagulability and high

viscosity in blood. Due to the refractory nature, NS has a big impact on children's physical and mental health.

Previous clinical experience suggested prednisone therapy. However, some pediatric patients were resistant to the sole prednisone treatment. Moreover, prolonged use of prednisone would cause various adverse reactions including peptic ulcer and hypertension which further impaired physical growth and psychological

well-being. Clinical studies have discovered that pediatric NS was related with intrarenal coagulation disorder and glomerular capillary thrombosis caused by hyper-coagulation status [4-6]. Studies have showed that low molecular weight heparin (LMWH), developed from heparin, was effective in anti-thrombin and might relieve the hypercoagulability in glomerulus and achieve remarkable effect on treating kidney failure with hemorrhage [7, 8]. There are limited reports on treating pediatric NS with LMWH combined with prednisone and none reporting its influence on bone metabolism. This study explored the clinical efficacy of LMWH combined with prednisone and its influence on coagulation function, kidney function, and bone metabolism of pediatric patients with NS.

### Materials and methods

#### *Patients' recruitment*

A total of 94 pediatric cases of NS in Yichang Central People Hospital, The First Clinical Medical College, Three Gorges University clinic from June 2015 to January 2017 were included as study subjects and their clinical data were collected. All patients were divided into 2 groups according to different treatments: experimental group (n=47) and control group (n=47). The study was reviewed and approved by the Ethics Committee of Yichang Central People Hospital, The First Clinical Medical College, Three Gorges University and has gained informed consent of the included pediatric and their guardians.

NS was diagnosed using the criteria made by kidney disease group of national pediatric association in 2001: patient with edema, hyperlipemia and other clinical presentation, have a >7 mmol/L cholesterol level [9]. The urine protein, exceeding 50 mg/(kg·d), lasts over 14 days, and albumins is less than 30 g/L.

The eligible case were required to meet all the following inclusion criteria: Without anaphylactic purpura nephritis; without lupus nephritis; normal immune function; no contradiction to the study; without malignant tumor; with complete clinical data; less than 8 years' old; with primary NS.

Exclusion criteria: With heart, liver, lung or some other organ disorder; with family history;

poor compliance; abnormal coagulation function.

#### *Methods*

Routine treatment like anti-infection, diuresis, detumescence, balancing electrolyte and nutrition supplement were given to all the included patients.

The pediatric control group received prednisone acetate tablets (Xi'an DiSai biological pharmaceutical Co., Ltd.) at the dose of 2 mg/(kg·d) for 3 times a day (no more than 60 mg/d). Two weeks after the urine protein concentration was significantly decreased, the dose was reduced to 2.0 mg/(kg·d) for once every two days and 4 weeks later, the dose would be continued by 3.0-4.0 mg per 2 weeks until the suspension.

Patients in the experimental group would accept LMWH (Shenzhen Saibaoer biological medicine Co., Ltd.) on the basis of control group's plan, at the dose of 150 IU/(kg·d) for twice a day subcutaneously.

Both groups received drug treatment for 5 weeks and examination in clinic after the therapy and were reminded of that daily diet should contain low salt and adequate amount of high quality protein.

#### *Clinical efficacy*

Clinical efficacy was categorized into 3 levels: excellent, effective, and invalid. Excellent referred to that clinical symptoms and signs of the patient disappeared and that all the blood indexes returned to normal level (albumins was above 30 g/L). Effective referred to that the clinical symptoms and signs were mostly relieved and blood indexes were basically normal (urine protein was mildly positive). Invalid meant that all the clinical symptoms and signs remained or even exacerbated along with the existence of abnormal blood indexes. The effective rate (ER) = number of cases evaluated as excellent or valid/total number of cases \*100%.

#### *Outcome measures*

Fasting venous blood 3 mL was collected from elbow veins before and after the treatment in both groups in the morning. Serum was separated from the blood samples by centrifugation

## Effects of LMWH combined with prednisone on pediatric with NS

**Table 1.** Comparison of clinical results between the two groups

Group	Gender (n)		Age (year)	Average course of disease
	Male	Female		
Experimental group (n=47)	28	19	6.03±1.96	4.88±1.95
Control group (n=47)	27	20	6.02±1.95	4.73±1.93
t/χ <sup>2</sup>	0.044		0.025	0.375
P	0.834		0.980	0.709

**Table 2.** Comparison of clinical efficacy between the two groups

Group	Excellent (n)	Valid (n)	Invalid (n)	Clinical efficacy rate (n, %)
Experimental group (n=47)	20	24	3	44 (93.62)
Control group (n=47)	15	22	10	37 (78.72)
χ <sup>2</sup>	-	-	-	4.374
P	-	-	-	0.036

**Table 3.** Comparison of kidney function between the two groups ( $\bar{x} \pm sd$ )

Group	BUN (mmol/L)	Ccr (mL/min)	SCr (μmol/L)
Experimental group (n=47)			
Before treatment	8.91±0.70	52.20±4.39	92.17±10.14
After treatment	7.03±0.48**###	63.11±4.55**###	75.44±7.20**###
Control group (n=47)			
Before treatment	8.90±0.68	52.19±4.57	92.15±10.09
After treatment	8.47±0.52###	59.02±4.93###	85.83±7.21###

Note: \*\*, compared with the control group, P<0.01; ###, compared with the same group before treatment, P≤0.001; BUN, blood urea nitrogen; Ccr, creatinine clearance rate; SCr, serum creatinine.

at 3,500 rpm for 5 minutes and stored at -20°C for further examination.

Major outcome measures included kidney function indicators: blood urinary nitrogen (BUN), creatinine clearance rate (Ccr) and serum creatinine (SCr). Coagulation function indicators: Prothrombin time (PT) and activated partial thromboplastin time (APTT). Bone metabolism indicators: blood GLA protein (BGP) and bone alkaline phosphatase (BALP).

Secondary outcome measures included: other blood biochemical indexes: Serum albumen (Alb) and blood total cholesterol (TC); and the incidence of all kinds of adverse events.

### Statistical analysis

SPSS 20.0 software was used for data analysis. Quantitative data is expressed as mean ± sd ( $\bar{x} \pm sd$ ). Paired t test was performed within

group before-after comparison and independent t test was performed between groups. Categorical data are expressed as percentage (%) and analyzed with χ<sup>2</sup> test. P<0.05 indicates statistically significant differences.

## Results

### Baseline characteristics

Clinical data of the two groups included gender, age and average course of disease. Statistical analysis showed no difference (all P>0.05) among the two groups in clinical data, proving the viability of between-group comparison analysis (**Table 1**).

### Comparison of clinical efficacy

The ER of experimental group was significantly higher than that of the control group (P<0.05; **Table 2**).

### Comparison of kidney function

Statistical analysis showed no difference between the two groups in kidney function before treatment (P>0.05). After the treatment, both groups showed significantly-elevated Ccr levels (both P≤0.001) with a more distinct elevation in experimental group (P≤0.01). BUN and SCr levels were all significantly decreased (both P≤0.001), especially in the experimental group (P≤0.01) compared with those of control group (**Table 3**).

### Comparison of coagulation function

Comparison of coagulation function among paired before-treatment/after-treatment and control/experimental groups resulted in no significant difference (all P>0.05) (**Table 4**).

## Effects of LMWH combined with prednisone on pediatric with NS

**Table 4.** Comparison of coagulation function between the two groups ( $\bar{x} \pm sd$ )

Group	PT (s)	APTT (s)
Experimental group (n=47)		
Before treatment	12.81±1.34	28.47±8.59
After treatment	13.11±1.29	30.11±7.42
Control group (n=47)		
Before treatment	12.80±1.42	28.46±8.60
After treatment	13.24±1.30	30.14±7.51

Note: PT, prothrombin time; APTT, activated partial thromboplastin time.

**Table 5.** Comparison of bone metabolism indexes between the two groups ( $\bar{x} \pm sd$ )

Group	BGP (mg/L)	BALP ( $\mu$ g/L)
Experimental group (n=47)		
Before treatment	14.96±4.88	176.47±9.01
After treatment	21.09±5.31 <sup>**,###</sup>	150.02±6.83 <sup>**,###</sup>
Control group (n=47)		
Before treatment	14.97±4.75	176.50±8.45
After treatment	17.22±5.42 <sup>###</sup>	164.22±7.12 <sup>###</sup>

Note: \*\*, compared with the control group,  $P < 0.01$ ; ###, compared with the same group before treatment,  $P \leq 0.001$ ; BGP, bone GLA protein; BALP, bone alkaline phosphatase.

### Comparison of bone metabolism indexes

Before treatment no significant difference was shown between the two groups in bone metabolism indexes (both  $P > 0.05$ ). After treatment, both groups showed significantly elevated BGP levels (both  $P \leq 0.001$ ) and decreased BALP levels (both  $P \leq 0.001$ ), when the experimental group had even higher BGP level ( $P < 0.01$ ) and lower BALP level ( $P < 0.01$ ) compared with control group respectively (**Table 5** and **Figure 1**).

### Comparison of Alb and TC levels

Before treatment no significant difference was shown between the two groups in Alb and TC levels (both  $P > 0.05$ ). After treatment Alb level was elevated significantly in both groups (both  $P \leq 0.001$ ) and even higher in experimental group ( $P < 0.01$ ) while TC level was decreased significantly (both  $P \leq 0.001$ ) and even lower in experimental group ( $P < 0.01$ ; **Table 6**).

### Comparison of adverse reactions

One case of subcutaneous ecchymosis was recorded in the experimental group and the incidence was 2.13% (1/47). 4 cases of dizzi-

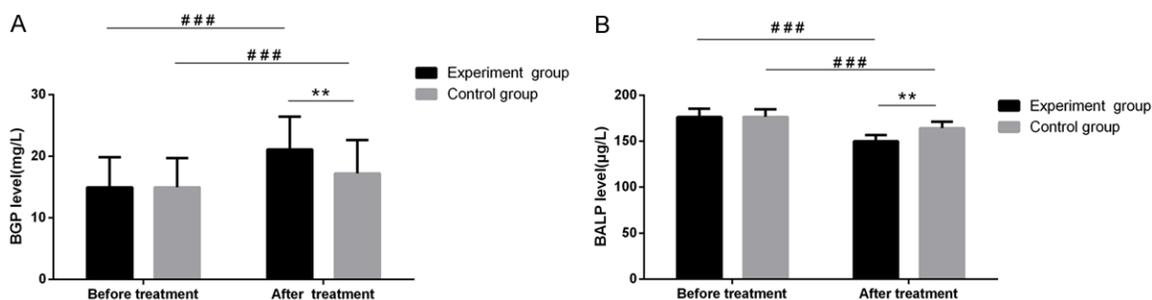
ness were recorded in the control group and the incidence was 8.51% (4/47). No liver/kidney injury, organ disorder, gastrointestinal hemorrhage or other severe adverse reactions was recorded and all the mild sickness was cured after treatment. There was no significant difference in adverse reaction occurrence between the two groups ( $P = 0.168$ ,  $\chi^2 = 1.901$ ).

## Discussion

Pediatric NS is a disease related to progressive renal fibrosis induced by various factors [10-12]. Clinical statistics have shown that the disease occurs mostly among pre-school children, especially those between 3-5 years' old. Currently, major principles of pediatric NS are alleviation of clinical symptoms, elimination of disease oncology and inducement [13]. This study focuses on the treatment plan of LMWH combined with prednisone on 47 cases of pediatric NS. The results show that the clinical ER of experimental group was significantly higher than prednisone alone, indicating that the combination would effectively alleviate the clinical symptoms and improve detumescence and blood pressure control. This result was similar to reports from some related studies [14, 15]. The significant efficacy was assumed to be partly due to the rapid effect of prednisone which could help to regulate secretion of aldosterone and antidiuretic hormone (ADH) to control the inflammation and repair the permeability of glomerular basement membrane (GBM), and partly due to the effect of LMWH on assisting repair of injured vascular endothelium cell for further clinical improvement.

Clinicians believe that the hypercoagulability and high viscosity of blood play key roles in the development of NS, for which effective anticoagulation therapy would be helpful [16]. Research indicates that LMWH not only presents a relatively long biological half-life period and high bioavailability, but also is bound majorly with anti-thrombin and rarely to plasma and platelets, which guaranteed a good anticoagulation effect and prevented the spontaneous hemorrhage [17, 18]. Additionally, LMWH was

## Effects of LMWH combined with prednisone on pediatric with NS



**Figure 1.** Comparison of bone metabolism indexes between the two groups. A: Comparison of BGP level between the two groups; B: Comparison of BALP level between the two groups; \*\*, comparing the two,  $P < 0.01$ ; ###, comparing the two,  $P \leq 0.001$ ; BGP, bone GLA protein; BALP, bone alkaline phosphatase.

**Table 6.** Comparison of Alb and TC levels between the two groups

Group	Alb (g/L)	TC (mmol/L)
Experimental group (n=47)		
Before treatment	18.19±4.39	12.17±2.89
After treatment	33.30±5.91 <sup>**###</sup>	5.09±2.58 <sup>**###</sup>
Control group (n=47)		
Before treatment	18.20±4.28	12.15±2.76
After treatment	25.44±5.09 <sup>###</sup>	8.32±2.87 <sup>###</sup>

Note: \*\*, compared with the control group,  $P < 0.01$ ; ###, compared with the same group before treatment,  $P \leq 0.001$ ; Alb, albumin; TC, total cholesterol.

effective in improving negative charge on GBM and later inhibiting the occurrence of proteinuria. This study showed that, after treatment, Ccr and Alb levels were significantly increased in both groups, especially for experimental group. BUN, Scr, and TC levels were decreased when experimental group presented even lower levels. Coagulation function analysis ended up with no significant difference between groups. All these data helped make a conclusion that the use of LMWH combined with prednisone would help to improve kidney function and hyperlipemia of pediatric NS patients without causing hemorrhage by maintaining normal AP-TT and PT levels.

There is still lack of clinical confirmation of the influence on bone metabolism on NS children using this combination therapy. But some study proved that NS, though majorly influencing kidney, had impact on all body systems. It could cause bone cell apoptosis and accelerate osteoclast recruitment to suppress bone absorption and reduce formation of new bone during the treatment [19]. Some studies showed that bone metabolism level abnormalities occurred

even in primary visit among some NS children, probably because of blood calcium loss induced by large amount of urine protein [20, 21]. This study shows that after treatment, both groups had higher levels of BGP and lower of BALP, when the experimental group presented even higher BGP level and lower BALP level compared with the control group. The combination could significantly improve the bone metabolism but the exact mechanism remains to be discovered by multi-center control study based on a large sample size.

In summary, for NS children, LMWH combined with prednisone can significantly alleviate clinical symptoms and edema, and improve kidney function, coagulation function and bone metabolism, for which it deserves wider clinical application.

### Disclosure of conflict of interest

None.

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## Effects of LMWH combined with prednisone on pediatric with NS

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