

**Effect of Simvastatin and Relationship between Bilirubin and Blood Lipid Level in Patients with  
Glucocorticoid-Resistant Nephrotic Syndrome**

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**ABSTRACT**

**Objective:** The aim of this study was to investigate the relationship between bilirubin levels and hyperlipidemia in patients with glucocorticoid-resistant nephrotic syndrome (NS).

**Methods:** This study was a double-blind randomized controlled trial. A total of 172 patients with glucocorticoid resistance met the inclusion criteria and were divided into 2 groups (the treatment and control groups), each with 86 patients. Because of worsening renal function and discharge from the hospital, eventually 68 patients from the treatment group were administered simvastatin (10 mg/d) for 4 weeks and 78 patients from the control group were treated without lipid-lowering drugs for the same duration. Triglyceride (TG), total cholesterol (TCHO), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), serum total protein, serum albumin, total bilirubin, direct bilirubin, and 24-h urinary protein (UPr) levels were determined before and after treatment.

**Results:** TCHO, TG, and LDL-C levels significantly decreased, but the bilirubin level significantly increased in the treatment group compared to the levels in the control group and those before treatment ( $P < 0.01$ ). Additionally, HDL-C and 24-h UPr levels were not significantly different between the treatment and control groups ( $P > 0.05$ ).

**Conclusion:** Primary NS patients with glucocorticoid resistance have low bilirubin levels. After treatment with simvastatin, total bilirubin levels increased and blood lipid levels reduced, but urinary albumin levels did not improve, indicating that bilirubin levels might be related with blood lipid levels in these patients.

**Keywords:** Bilirubin, blood lipid, glucocorticoid resistance, nephrotic syndrome, statins

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## INTRODUCTION

Hyperlipidemia is one of the most important clinical manifestations of nephrotic syndrome (NS), and involves high total cholesterol (TCHO), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels. These high levels not only accelerate the development of glomerular lesions (1) but also induce cardiovascular diseases (2). Generally, total antioxidant capacity is reduced in patients with NS and glomerular lesions (3), resulting in the presence of excess oxygen free radicals (4, 5). Additionally, LDL-C is easily deposited on the vascular walls and is oxidized to OX-LDL that induces cardiovascular diseases, such as atherogenesis. Furthermore, bilirubin is not only the yellow breakdown product of normal heme catabolism, but also a potentially powerful antioxidant that acts by inhibiting NADPH oxidase (6, 7). High bilirubin levels reduce oxidative lesions (8) and prevent the oxidation of LDL by 2, 2'-azobis (2-methylpropanimidamide) dihydrochloride at 37°C (9). Moreover, these high levels prevent peroxidative lesions induced by LDL (10). Therefore, the relationship between blood lipid and bilirubin levels is important in NS patients with dyslipidemia.

Bilirubin levels are closely associated with a number of diseases. Many studies (11-13) have confirmed that bilirubin plays a critical role in chronic diseases, but few studies have demonstrated the role of bilirubin in NS patients. Only our previous study showed that low bilirubin levels were present in NS patients who were sensitive to glucocorticoids and not effectively treated with statins (14). However, statins were effective for patients with hyperlipidemia. Olbricht et al. (15) used simvastatin to treat NS patients with hyperlipidemia, and the results indicate that simvastatin helps reduce blood lipid levels. Furthermore,

Aguilar-Salinas et al. showed that lovastatin significantly decreases the production of LDL-apoB in NS patients with hyperlipidemia (16). Although statins reduce blood lipid levels, our previous studies showed that steroids combined with statins treat NS mainly through the ability of steroids to reduce blood lipid levels without a superimposed effect. The present study aimed to further confirm this opinion in NS patients who were resistant to glucocorticoids and confirm the beneficial effects of statins in these patients.

## **MATERIALS AND METHODS**

### **Subjects**

A total of 475 patients (252 male and 223 female patients) with primary NS who were diagnosed by kidney biopsy at our hospital were enrolled in this study, and all patients received glucocorticoid therapy for 8 weeks without remission. The mean age of the patients was  $48.2 \pm 15.8$  years. The pathological types included mesangial proliferative glomerulonephritis, mesangial glomerulonephritis, focal sectional glomerular sclerosis, minimal change NS, and membranoproliferative glomerulonephritis. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Beidaihe Sanatorium. Written informed consent was obtained from all participants.

### **Diagnostic criteria of glucocorticoid resistance**

Positive urokinase protein for 4–8 weeks after administration of a routine dose of glucocorticoid (1.5-2.0 mg/kg/d, maximum dose: 60 mg/d) to treat primary NS.

### **Exclusion criteria**

All patients did not use any lipid-lowering drug before the start of this study or antioxidant drug during this study, such as vitamin E and Vitamin C. Additionally, patients with hepatobiliary diseases, blood diseases, hepatitis B and C, severe infections, family hypercholesterolemia, and increased blood urea and plasma creatinine levels were excluded.

### **Groups**

This study was a double-blind randomized controlled trial (Figure 1). A total of 172 patients with glucocorticoid resistance met the inclusion criteria and were divided into 2 groups (the treatment and control groups), each with 86 patients. However, 5 patients left the hospital and 7 had worsening renal function in the treatment group, and one patient left the hospital, one was transferred to another department, and 4 had worsening renal function in the control group. Finally, the treatment group included 68 patients (39 male and 29 female patients; mean age,  $47.1 \pm 13.7$  years) who were administered simvastatin, and the control group included 78 patients (46 male and 32 female patients; mean age,  $50.6 \pm 14.2$  years) who were treated without lipid-lowering drugs.

### **Specimen collection**

All patients maintained a low fat diet 3 days before treatment with statins and after treatment. After fasting for more than 12 h, venous blood samples were collected before and after treatment.

## **Treatment**

All patients were initially treated with glucocorticoids for 8 weeks. Patients in the treatment group were then administered simvastatin (10 mg/d) after dinner for 4 weeks, and patients in the control group were treated without lipid-lowering drugs for the same duration. The administration of cytotoxic drugs was stopped in both groups. Moreover, if renal function worsened (blood creatinine level,  $>300 \mu\text{mol/L}$ ), the treatment was stopped and the patient was excluded.

## **Detection assay**

All specimens were analyzed using an automatic biochemical analyzer (Olympus AU640). The reagents were obtained from Randox Company, and the controls were obtained from Roche. All analyses were repeated thrice.

## **Statistical analysis**

Statistical analysis was performed using the SPSS 13.0 software. Data are presented as mean  $\pm$  SD. The Student's t-test was used to compare data between the groups, and the paired t-test was used to compare data between before and after treatment. Count data were compared using the chi-square test. A *P*-value  $<0.05$  was considered to indicate a significant difference.

## **RESULTS**

### **Comparison at baseline**

There were no significant differences in age, sex, and pathological types ( $P>0.05$ ) using the

chi-square test between the groups (Table 1).

### **Comparison of related biochemical indexes**

There were significant differences in TCHO, TG, LDL-C, and TBIL levels between the groups; furthermore, these levels were significantly different between before and after treatment in the treatment group. TCHO, TG, and LDL-C levels reduced and bilirubin levels increased in the treatment group compared to the levels in the control group and those before treatment. However, there were no significant differences in HDL-C and 24-h UPr levels between the groups ( $P>0.05$ , Table 2).

### **DISCUSSION**

Lipid dysmetabolism in NS is complex with many abnormal components, and the pathogenesis of hyperlipidemia is still not clear (17). Based on the findings of our previous study, we chose NS patients with steroid-resistance in the present study. In this study, statins improved blood lipid metabolism, which confirmed the ability of statins to reduce lipids in NS patients. Furthermore, we explored the pathogenesis of hyperlipidemia and the possible involvement of bilirubin by analyzing the changes in bilirubin and blood lipid levels before and after treatment.

In the present study, TCHO, TG, and LDL-C levels reduced in patients with glucocorticoid resistance after treatment with statins compared to the levels in the control group and those before treatment. Simvastatin is a HMG CoA reductase inhibitor and a classic and effective lipid-lowering agent. It reduces cholesterol synthesis in hepatocytes by

inhibiting HMG CoA reductase and then increases LDL receptors to reduce the serum cholesterol level (18). Recent studies found that statin therapy upregulates LDL receptors in nephritic animal models, such as rats (19, 20), and the upregulation of LDL receptors correlates with the reduction in TCHO, TG, and LDL-C levels. Aguilar-Salinas et al. found that lovastatin significantly decreases TG, TCHO, and LDL-C levels except bilirubin and 24-h UPr levels in NS patients with treatment periods of 6 weeks separated by a 2-week washout period. These results are in line with our results. Furthermore, blood lipid levels did not reduce significantly in the control group receiving glucocorticoid therapy; thus, glucocorticoids are not effective in reducing blood lipid levels, but statins are effective.

We found that bilirubin levels increased after treatment with statins compared to the levels in the control group and those before treatment, but there were no significant differences in HDL-C and 24-h UPr levels between before and after treatment. Valdivielso et al. (21) performed a prospective, open, 6-month study in NS patients and found that atorvastatin administered daily for 6 months increases HDL-C levels and reduces 24-h UPr levels. These findings differ from ours, and we believe that the treatment duration may have caused this difference, as our duration was shorter than that in their study. This result should be confirmed in future studies. The combination of bilirubin and albumin is non-specific and non-covalent reversible, and the binding rate between bilirubin and albumin is over 99% (22). Additionally, the molecular barrier of the glomerular filtration membrane surface and the charge barrier are damaged in NS patients, resulting in the excretion of most bilirubin-albumin complexes in the urine through the glomerular filtration membrane. In the present study, NS patients with lipid dysmetabolism and glucocorticoid resistance were

treated with statins in the treatment group and without statins in the control group. We found that blood and urinary protein levels did not improve in the control group, indicating that the bilirubin-albumin complexes were being excreted in the urine. Therefore, there was no significant difference in bilirubin levels between before and after treatment in the control group. In the treatment group, although proteinuria did not change, TCHO, TG, and LDL-C levels reduced and bilirubin levels increased compared to levels in the control group and those before treatment. The antioxidant effect of bilirubin helped avoid peroxidative lesions induced by LDL-C. Moreover, this study confirmed that bilirubin is involved in lipid metabolism in NS patients with lipid dysmetabolism.

In conclusion, statins directly reduced lipid levels but did not change urinary protein levels in NS patients with lipid dysmetabolism who were resistant to glucocorticoids. Furthermore, bilirubin levels increased after treatment with statins compared to the levels before treatment, indicating that bilirubin levels may be directly or indirectly related with lipid levels.

### **Conflicts of interest**

All of the authors declare that they have no conflicts of interest regarding this paper.



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Table 1: Comparisons of demographic and clinical characteristics

Count data	n	Treatment group (n=68)	Control (n=78)	group	$\chi^2$	<i>P value</i>
Sex (%)					0.073	0.459
Male	85	39 (57.4)	43 (55.1)			
Female	61	29 (42.6)	35 (44.9)			
Age (%)					0.112	0.998
20~	16	7 (10.3)	9 (11.5)			
35~	31	14 (20.6)	17 (21.8)			
45~	44	21 (30.9)	23 (29.5)			
55~	34	16 (23.5)	18 (23.1)			
>65	21	10 (14.7)	11 (14.1)			
Pathological types (%)					0.503	0.973
MsPGN	58	28 (41.2)	30 (38.5)			
MGN	47	22 (32.4)	25 (32.1)			
FSGS	23	10 (14.7)	12 (15.4)			
MCNS	13	6 (8.8)	7 (9.0)			
MPGN	5	2 (3.0)	4 (5.1)			

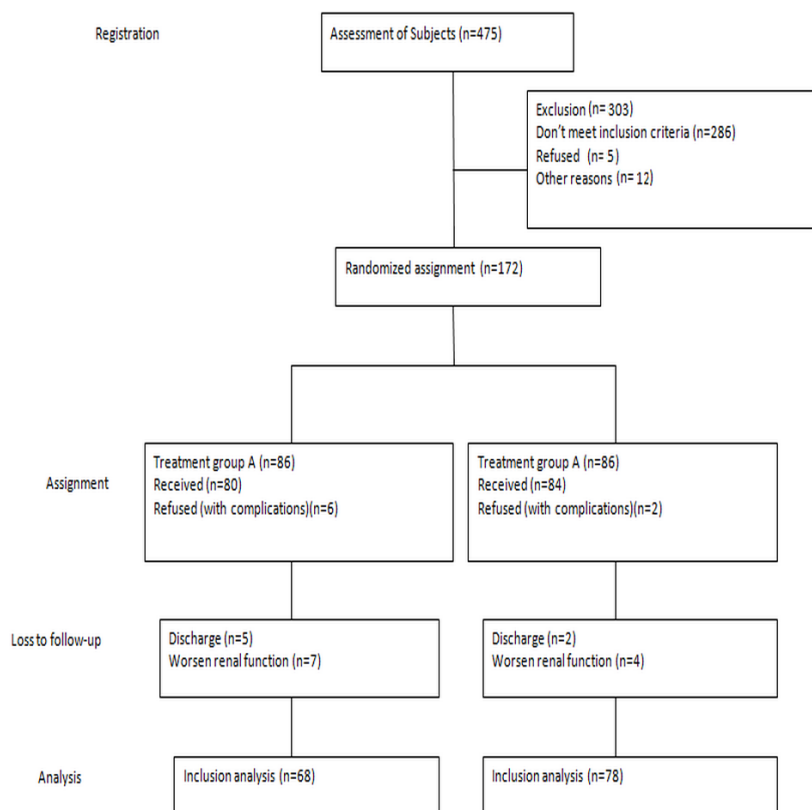


Fig. 1: Design of clinical trial.

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**Running title:** Glucocorticoid-resistant Nephrotic Syndrome

**Brief synopsis:** This study further confirmed steroids combined with statins were treated NS mainly due to steroids effect to reduce blood lipid without superimposed effect and chose nephrotic patients who were resistant to glucocorticoid so as to ensure the effect of statins.