ORIGINAL RESEARCH ARTICLE

Effect of tacrolimus plus mycophenolate mofetil in the therapy of children with steroid-resistant nephrotic syndrome

DOI: 10.29063/ajrh2025/v29i5s.3

*Lili Zhao, Jia Hao and Lei Zhang**

Department of Pediatrics, Shengjing Hospital of China Medical University, Shenyang, Liaoning, 110004, China

***For Correspondence:** Email: *zhanglei_edu@hotmail.com*

Abstract

This study mainly explored the influence of tacrolimus in association with mycophenolate mofetil (MMF, CellCept) in treating steroid-resistant nephrotic syndrome (SRNS) children. A total of 124 SRNS children were chosen and separated into observation group and control group. The control group received basic treatment and tacrolimus treatment. In contrast, the observation group received MMF in addition to basic and tacrolimus treatments. The results showed that the observation group presented higher total effective rate, better improvements of 24 h urinary protein quantification, than the control group. Albumin and cholesterol levels, lower fibrinogen level, lower blood urea nitrogen, serum creatinine, along with cystatin C levels, better improvements of interferon-γ, interleukin-2 and interleukin -13 levels, and lower total occurrence of adverse reactions. We conclude that Tacrolimus combined with MMF can effectively reduce inflammation along with improve the renal function of SRNS children. *(Afr J Reprod Health 2025; 29 [5s]: 20-26).*

Keywords: Steroid-resistant nephrotic syndrome; Tacrolimus; Mycophenolate mofetil; Renal function

Résumé

Cette étude a principalement exploré l'influence du tacrolimus en association avec le mycophénolate mofétil (MMF, CellCept) dans le traitement des enfants atteints du syndrome néphrotique cortico-résistant (SNCR). Au total, 124 enfants atteints du SNCR ont été sélectionnés et répartis en un groupe d'observation et un groupe témoin. Le groupe témoin a reçu un traitement de base et un traitement au tacrolimus. En revanche, le groupe d'observation a reçu du MMF en plus des traitements de base et du tacrolimus. Les résultats ont montré que le groupe d'observation présentait un taux d'efficacité total plus élevé et une meilleure amélioration de la quantification des protéines urinaires sur 24 heures que le groupe témoin. Les taux d'albumine et de cholestérol, le taux de fibrinogène et d'urée sanguine, la créatinine sérique et les taux de cystatine C étaient plus faibles, de même que les taux d'interféron-γ, d'interleukine-2 et d'interleukine-13, et la fréquence totale des effets indésirables était plus faible. Nous concluons que le tacrolimus associé au MMF peut réduire efficacement l'inflammation et améliorer la fonction rénale des enfants atteints du SNCR. *(Afr J Reprod Health 2025; 29 [5s]: 20-26).*

Mots-clés: Syndrome néphrotique corticorésistant ; Tacrolimus ; Mycophénolate mofétil ; Fonction rénale

Introduction

Nephrotic syndrome belongs to a common glomerular disease in children of all ages, which is characterized by massive proteinuria, hypoalbuminemia, peripheral edema along with hyperlipidemia.[1] Based on the initial response to corticosteroid therapy, pediatric nephrotic syndrome is usually divided into steroid-sensitive nephrotic syndrome and steroid-resistant nephrotic syndrome (SRNS).[2] In children with SRNS, a large amount of protein flows to the mesangium, resulting in glomerulosclerosis, which can cause chronic renal failure.[3] Some children with SRNS may develop into end-stage renal disease.[4] In order to avoid this progression, several different treatment regimens (cyclophosphamide, cyclosporine, tacrolimus, and glucocorticoids) have been adopted clinically, but the treatment results are unsatisfactory, and often need to be combined with immunosuppressive therapy,[5] because the pathogenesis of SRNS is closely related to the abnormal immune system, and immunosuppressants can reduce kidney damage by regulating the immune response.[6]

Tacrolimus, as a new immunosuppressant, can inhibit the stimulation of T cells along with T

helper cell-dependent B cells proliferation by inhibiting calmodulin, and inhibit the expression of lymphokines and receptors, thereby achieving immunosuppression.[7] Mycophenolate mofetil (MMF) belongs to a new immunosuppressive agent, and has the advantages of not affecting carbohydrate and lipid metabolism, no nephrotoxicity, and few adverse reactions.[8] Both of them have been extensively applied in treating children with SRNS,[9, 10] but the combination of them has not been reported. Here, we aimed to investigate the effectiveness of tacrolimus combined with MMF in treating SRNS children.

Methods

Materials

One hundred and twenty-four children with SRNS who accepted therapy in Shengjing Hospital of China Medical University, Shenyang, China from May 2019 to May 2022 were chosen as the research participants. Utilizing the computer random grouping method, the children were separated into the observation group (OG, n=62) and the control group (CG, n=62). No difference was observed in the sociodemographic variables of children in both groups (P>0.05, Table 1). The inclusion criteria were: (1) Children who met the diagnostic criteria of SRNS, and confirmed by pathological examination. (2) At the time of new treatment, serum creatinine was ≤133 μmol/L and 24 h urinary protein was ≥4 g/24 h. (3) Biopsy was performed within one month with pathological findings of stage I or II membranous nephropathy. (4) No immunosuppressant or hormone drugs had been used recently. (5) Albumin and plasma were not infused.

The exclusion criteria: (1) Children had severe infection; (2) SRNS caused by secondary glomerular diseases, containing lupus nephritis, hepatitis B virus-associated nephritis, lgA nephropathy, atypical streptococcal nephritis, drug-induced nephritis, and purpura nephritis; (3) patients with incomplete; and clinical data (4) recent use of any immunosuppressants.

Treatments

Basic treatment: Both groups were given low-protein, high-calorie diet, controlled protein intake 25-30 g/d, high-calorie food intake 130 kJ/(kg·d), oral 0.2-0.3 g/(kg·d) amino acid and high-vitamin diet. Meanwhile, patients received angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors and low molecular weight heparin and other conventional treatment. Both groups accepted prednisone acetate tablets based on the above treatment. The dosage was 1 mg/(kg·times), and the maximum dose was 60 mg/d. After 3 days of continuous use, the medication time was changed to take it in the morning every other day for 4 to 6 weeks, and then the dosage was gradually reduced.

Based on the above basic treatment, children in the CG received tacrolimus. The initial dose of tacrolimus was 0.10-0.15 mg/(kg·d), and the blood concentration was kept at 5-10 μg/ L. The dose was gradually adjusted after one week of medication.

Based on the basic treatment and tacrolimus, children in the OG were given MMF (CellCept) 20-30 $mg \cdot kg^{-1} \cdot d^{-1}$, divided into two oral doses. Children in both groups accepted therapy for 1 year.

Observed indices

Clinical efficacy criteria: After treatment, the children's urine routine indicators and hematuria biochemical indicators returned to normal, and their clinical signs and symptoms completely disappeared, and they were identified as complete remission. After treatment, the children's urine protein positive test results were less than 3+, and the clinical presentations were improved relative to those before treatment, which was identified as partial remission. After treatment, the children's clinical presentations did not improve, and the urine protein test results were more than 3+, which was considered as inefficiency. The total treatment response rate =1 -inefficiency. 24 h urinary protein quantification and biochemical indexes: Fasting venous blood of children were collected in the morning, and urinary protein, serum albumin (Alb) and serum cholesterol (Chol) were measured by Olympus automatic biochemical analyzer.

Determination of coagulation and fibrinolysis levels: Fasting venous blood was obtained from children, and the serum was separated after centrifugation. Sodium citrate was used to anticoagulant blood, and the blood was centrifuged, and the plasma was separated and tested on the machine. Prothrombin time (PT) as well as activated partial thromboplastin time (APTT) was measured

Table 1: General data of children in both groups

Items		Control group (n=62)	Observation group (n=62)	P
Gender (male/female)		33/29	34/28	0.9
Average age (years)		5.2±1.5	5.3±1.6	0.7
Average course of disease (months)		25.5±5.1	25.6±5.2	0.9
Average BMI (kg/m^2)		22.7±2.4	22.6±2.5	0.8
Pathological staging of	Stage I	31	32	
membranous nephropathy	Stage II	31	30	0.9

by magnetic bead coagulation method. Fibrinogen (FIb) as well as D-dimer (D-D) levels was determined by immunoturbidimetry. The recurrence frequency and hormone dosage in both groups were compared.

The levels of blood urea nitrogen (BUN), serum creatinine (Scr), along with cystatin C (CysC) were tested by nephelometry. Fasting venous blood was gathered, centrifuged, and serum was obtained for the testing of for interferon (INF)-γ, interleukin (IL)-2 and IL-13. Incidence of adverse reactions containing gastrointestinal reaction, myelosuppression, and transaminase elevation in both groups during treatment was compared.

Statistical analysis

Data were analyzed by SPSS 26.0 statistical software. The count data were exhibited as n/% and compared using χ^2 test. The measurement data were exhibited as (x±s) and compared using t test. P<0.05 meant the difference was significant.

Ethical considerations

This study was consistent with the ethical standards of the 1964 Declaration of Helsinki and its later amendments, and was approved by the Ethics Committee of Shengjing Hospital of China Medical University. All the families of the children gave informed consent for the study.

Results

Clinical efficacy in both groups

The total effective rate in the OG was 96.77% (60/62), and that in the CG was 82.25% (51/62). Relative to the CG, the OG presented better total effective rate (χ^2=6.96, P<0.05, Figure 1).

24 h urinary protein quantification and biochemical index levels in both groups

Before treatment, there were no significant differences in 24 h urinary protein quantification, Alb along with Chol levels between the two groups (P>0.05). Followed by therapy, 24 h urinary protein quantification, Alb along with Chol levels in both groups were improved, and those in the OG were better relative to the CG (P<0.05, Figure 1).

Coagulation and fibrinolysis index levels in both groups

Before treatment, there were no significant differences in PT, APTT, D-D and Fib levels between the two groups (P>0.05). Followed by treatment, PT level was increased, D-D and FIb levels were declined in both groups, and relative to the CG, the OG had lower FIb level (P<0.05). However, no difference was seen in PT, APTT along with D-D levels between both groups followed by treatment (P>0.05, Figure 2).

Recurrence frequency and hormone dosage in both groups

Before treatment, there were no significant differences in the recurrence frequency and the dose of hormone between the two groups (P>0.05). Followed by treatment, the recurrence frequency and the dose of hormone in both groups were reduced (P<0.05). However, no difference was discovered between both groups (P>0.05, Figure 3).

Renal function index levels in both groups

Before treatment, there were no significant differences in BUN, Scr, along with CysC levels between the two groups (P>0.05). Followed by treatment, BUN, Scr, along with CysC levels in both

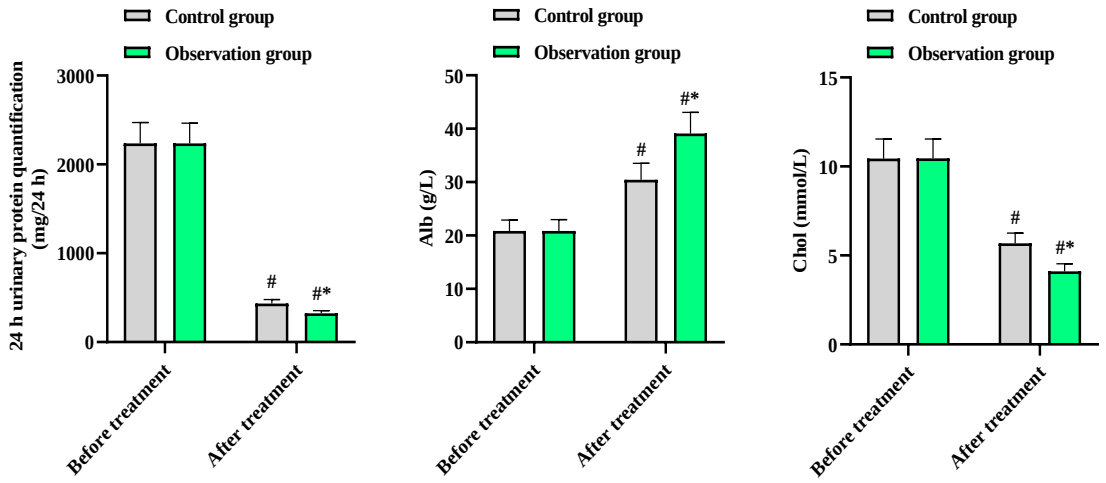

Figure 1: 24 h urinary protein quantification and biochemical index levels in both groups. #P<0.05, compared to before treatment. *P<0.05, compared to the control group

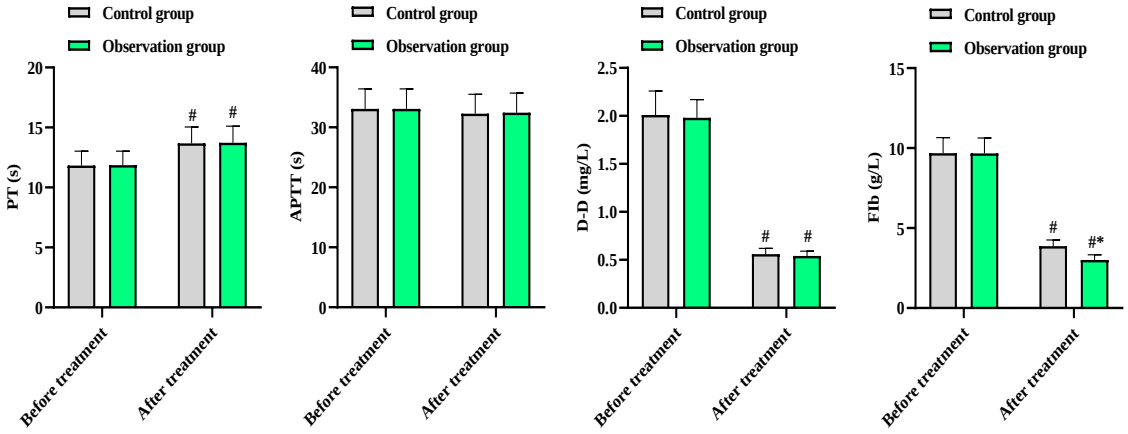

Figure 2: Coagulation and fibrinolysis index levels in both groups. #P<0.05, compared to before treatment. *P<0.05, compared to the control group

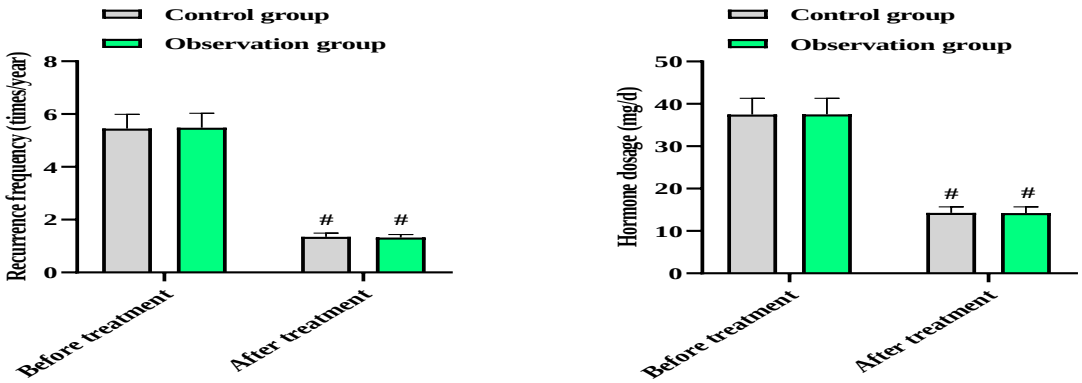

Figure 3: Recurrence frequency and hormone dosage in both groups. #P<0.05, compared to before treatment

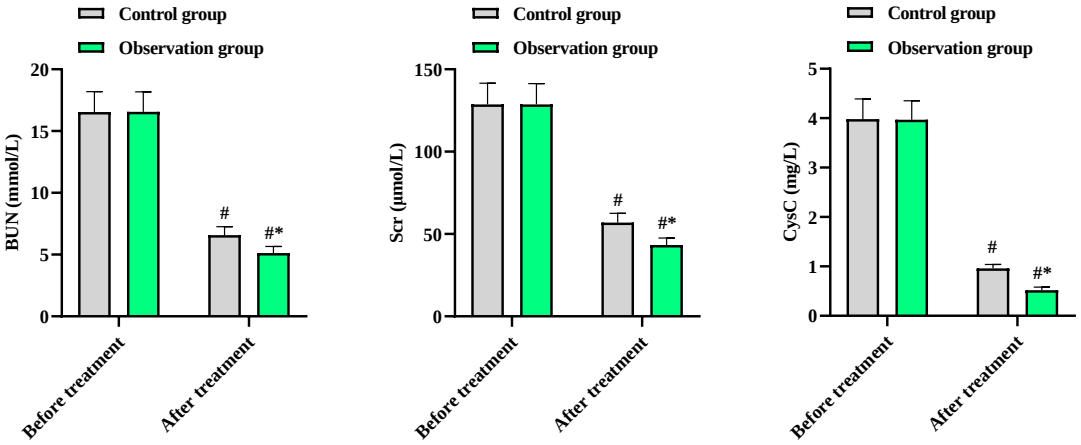

Figure 4: Renal function index levels in both groups. #P<0.05, compared to before treatment. *P<0.05, compared to the control group

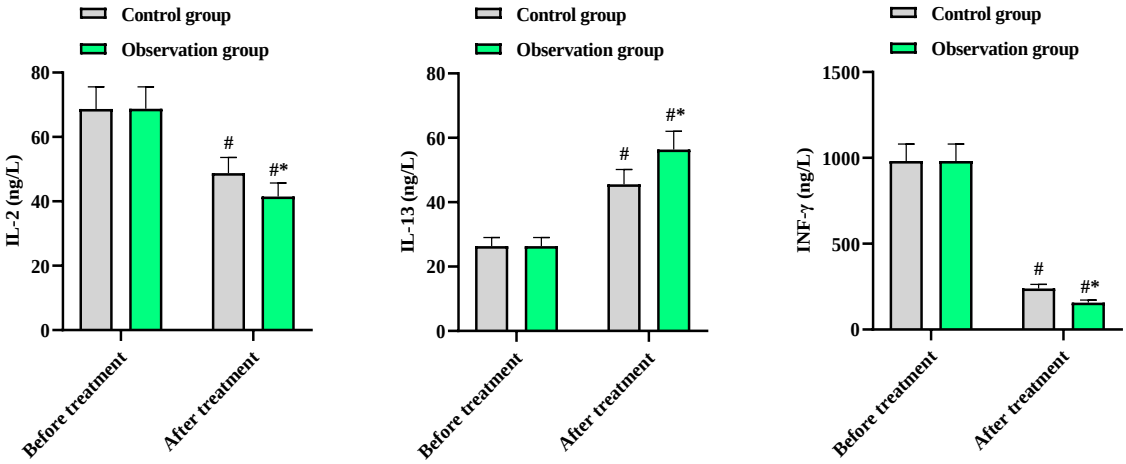

Figure 5: Levels of inflammatory factors in both groups. #P<0.05, compared to before treatment. *P<0.05, compared to the control group

Table 2: Incidence of adverse reactions in both groups (n, %)

Groups	N	Gastrointestinal reaction	Myelosuppression	Transaminase elevation	Incidence rate
Control group	62	6 (9.7)	1 (1.6)	1 (1.6)	8 (12.9)
Observation group	62	1 (1.6)	0 (0.0)	0 (0.0)	1 (1.6)
χ^2		5.9			
P		<0.05			

groups were declined, and those in the OG were lower compared to the CG (P<0.05, Figure 4).

Levels of inflammatory markers in both groups

Before treatment, there were no significant differences in the levels of IL-2, IL-13 and INF-γ between the two groups (P>0.05). Followed by treatment, the levels of IL-2, and INF-γ were declined, and the level of IL-13 was elevated in both groups (P<0.05). Relative to the CG, the OG had better improvements of theses inflammatory markers (P<0.05, Figure 5).

Incidence of adverse reactions in both groups

The total incidence of adverse reactions in the CG was 12.9%, and that in the OG was 1.6%. Relative to the CG, the OG had lower total occurrence of adverse reactions ($P<0.05$, Table 2).

Discussion

SRNS belongs to a group of common diseases of the urinary system, and its incidence has been increasing over the years.[11] The pathogenesis of SRNS has not been elucidated. In the process of hormone therapy, the newly treated children are sensitive to hormones, and prednisone tablets are the conventional drug for treating SRNS children, which has the advantages of strong immune suppression.[12] It mainly inhibits the immune response mediated by human B lymphocytes and T lymphocytes to inhibit the local cytokine release activated by inflammatory response complement, so as to achieve the treatment of kidney diseases, and has significant efficacy.[13] Nevertheless, it has adverse reactions such as gonadal toxicity, which limits its clinical application. 50%-60% of children with hormone sensitivity will have hormone dependence and frequent recurrence.[14] The use of large doses and the recurrence of disease cause serious harm to children and their families. In order to avoid drug toxicity and side effects resulted by blindly prolonging the utilization of sufficient amount of hormones, the therapy of hormones combined with immunosuppressants has been a clinical consensus.[15]

As a new calcium regulated neurophosphatase inhibitor, tacrolimus has many characteristics, such as high selectivity and strong effect. It mainly combines with human cytoplasmic binding protein 12 to form a complex, which can block phosphatase phosphorylation, selectively inactivate T cells, and inhibit the secretion of IL-2 cytokines.[16, 17] Thus, it can reduce the immune mediated inflammatory response of the body, promote the kidney function of children, as well as also have immune suppression related effects on human body autoantibodies.[18]

MMF is a new type of immunosuppressants, which is a selective cytotoxic drug, and has been extensively used to prevent the rejection of allogeneic kidney transplantation, and it is also used for treating autoimmune kidney diseases.[19] It is rapidly hydrolyzed to mycophenolic acid (MPA) with immunosuppressive activity in vivo. MPA can inhibit the inosine monophosphine dehydrogenase, block the classical synthesis pathway of guanine nucleotide in T and B lymphocytes, reduce the concentration of circulating immune complexes and correspondingly reduce the deposition of immune complexes in the kidney, and delay kidney damage.[20]

In this study, the outcomes displayed that relative to the CG, the OG had better total effective rate, better improvement of 24 h urinary protein quantification, Alb along with Chol levels, lower levels of BUN, Scr, and CysC Fib, better improvements of INF-γ, IL-2 and IL-13 levels, and lower total occurrence of adverse reactions. All above data reflected that tacrolimus combined with MMF in treating SRNS children could protect the kidney, effectively alleviate the symptoms of proteinuria and reduce glomerular damage by regulating immune effects and inhibiting the activity of T cells, which was similar to previous studies.[21]

Strengths and limitations

Strengths include the novelty of the subject and the affordability and availability of Tacrolimus and MMF. Our study may provide a treatment option for SRNS children. This study was conducted with a small sample size and short follow-up time.

Conclusion

Tacrolimus combined with MMF can reduce inflammation along with improve the renal function of SRNS children, which has significant clinical therapeutic effect.

Competing interests

The authors report no actual or potential conflicts of interest.

Contribution of authors

Zhao LL and Hao J: conceived and designed the study, as well as collected and analysed the data. Zhao LL and Zhang L: prepared the manuscript. All authors mentioned in the article approved the manuscript.

References

1. Wang CS and Greenbaum LA. Nephrotic Syndrome. Pediatr Clin North Am. 2019; 66(1): 73-85.
2. Rodriguez-Ballestas E and Reid-Adam J. Nephrotic Syndrome. Pediatr Rev. 2022; 43(2): 87-99.
3. Trautmann A, Schnaidt S, Lipska-Ziętkiewicz BS, Bodria M, Ozaltin F, Emma F, Anarat A, Melk A, Azocar M, Oh J, Saeed B, Gheisari A, Caliskan S, Gellermann J, Higuita LMS, Jankauskiene A, Drozdz D, Mir S, Balat A, Szczepanska M, Paripovic D, Zurowska A, Bogdanovic R, Yilmaz A, Ranchin B, Baskin E, Erdogan O, Remuzzi G, Firszt-Adamczyk A, Kuzma-Mroczkowska E, Litwin M, Murer L, Tkaczyk M, Jardim H, Wasilewska A, Printza N, Fidan K, Simkova E, Borzecka H, Staude H, Hees K and Schaefer F. Long-Term Outcome of Steroid-Resistant Nephrotic Syndrome in Children. J Am Soc Nephrol. 2017; 28(10): 3055-3065.
4. Warejko JK, Tan W, Daga A, Schapiro D, Lawson JA, Shril S, Lovric S, Ashraf S, Rao J, Hermle T, Jobst-Schwan T, Widmeier E, Majmundar AJ, Schneider R, Gee HY, Schmidt JM, Vivante A, van der Ven AT, Ityel H, Chen J, Sadowski CE, Kohl S, Pabst WL, Nakayama M, Somers MJG, Rodig NM, Daouk G, Baum M, Stein DR, Ferguson MA, Traum AZ, Soliman NA, Kari JA, El Desoky S, Fathy H, Zenker M, Bakkaloglu SA, Müller D, Noyan A, Ozaltin F, Cadnapaphornchai MA, Hashmi S, Hopcian J, Kopp JB, Benador N, Bockenhauer D, Bogdanovic R, Stajić N, Chernin G, Ettenger R, Fehrenbach H, Kemper M, Munarriz RL, Podracka L, Büscher R, Serdaroglu E, Tasic V, Mane S, Lifton RP, Braun DA and Hildebrandt F. Whole Exome Sequencing of Patients with Steroid-Resistant Nephrotic Syndrome. Clin J Am Soc Nephrol. 2018; 13(1): 53-62.
5. Shin JI, Kronbichler A, Oh J and Meijers B. Nephrotic Syndrome: Genetics, Mechanism, and Therapies. Biomed Res Int. 2018; 20186215946.
6. Campbell RE and Thurman JM. The Immune System and Idiopathic Nephrotic Syndrome. Clin J Am Soc Nephrol. 2022; 17(12): 1823-1834.
7. Schutte-Nutgen K, Tholking G, Suwelack B and Reuter S. Tacrolimus - Pharmacokinetic Considerations for Clinicians. Curr Drug Metab. 2018; 19(4): 342-350.
8. Chakrabarti K, Frame D, Al Abbas M and McCune WJ. The use of mycophenolate mofetil area under the curve. Curr Opin Rheumatol. 2021; 33(3): 221-232.
9. Iijima K, Sako M, Oba M, Tanaka S, Hamada R, Sakai T, Ohwada Y, Ninchoji T, Yamamura T, Machida H, Shima Y, Tanaka R, Kaito H, Araki Y, Morohashi T, Kumagai N, Gotoh Y, Ikezumi Y, Kubota T, Kamei K, Fujita N, Ohtsuka Y, Okamoto T, Yamada T, Tanaka E, Shimizu M, Horinouchi T, Konishi A, Omori T, Nakanishi K, Ishikura K, Ito S, Nakamura H and Nozu K. Mycophenolate Mofetil after Rituximab for Childhood-Onset Complicated Frequently-Relapsing or Steroid-Dependent Nephrotic Syndrome. J Am Soc Nephrol. 2022; 33(2): 401-419.
10. Tullus K, Webb H and Bagga A. Management of steroid-resistant nephrotic syndrome in children and adolescents. Lancet Child Adolesc Health. 2018; 2(12): 880-890.
11. Lee JM, Kronbichler A, Shin JI and Oh J. Current understandings in treating children with steroid-resistant nephrotic syndrome. Pediatr Nephrol. 2021; 36(4): 747-761.
12. Noone DG, Iijima K and Parekh R. Idiopathic nephrotic syndrome in children. Lancet. 2018; 392(10141): 61-74.
13. Hejazian SM, Zununi Vahed S, Moghaddas Sani H, Nariman-Saleh-Fam Z, Bastami M, Hosseiniyan Khatibi SM, Ardalan M and Samadi N. Steroid-resistant nephrotic syndrome: pharmacogenetics and epigenetic points and views. Expert Rev Clin Pharmacol. 2020; 13(2): 147-156.
14. Kamei K, Ishikura K, Sako M, Ito S, Nozu K and Iijima K. Rituximab therapy for refractory steroid-resistant nephrotic syndrome in children. Pediatr Nephrol. 2020; 35(1): 17-24.
15. Zhao QC, Li WN, Li ZJ, Li Y and Sun DH. Effect of voriconazole combined with glucocorticoid on nephrotic syndrome in children. Eur Rev Med Pharmacol Sci. 2021; 25(15): 5018-5025.
16. Basu B, Sander A, Roy B, Preussler S, Barua S, Mahapatra TKS and Schaefer F. Efficacy of Rituximab vs Tacrolimus in Pediatric Corticosteroid-Dependent Nephrotic Syndrome: A Randomized Clinical Trial. JAMA Pediatr. 2018; 172(8): 757-764.
17. Shumei Y, Yi L, Huanyu M, Zhibin L, Wanlin J, Liqun X and Huan Y. IL-2 gene polymorphisms affect tacrolimus response in myasthenia gravis. Eur J Clin Pharmacol. 2019; 75(6): 795-800.
18. Chen HX, Cheng Q, Li F, He QN, Cao Y, Yi ZW and Wu XC. Efficacy and safety of tacrolimus and low-dose prednisone in Chinese children with steroid-resistant nephrotic syndrome. World J Pediatr. 2020; 16(2): 159-167.
19. Maripuri S and Kasiske BL. The role of mycophenolate mofetil in kidney transplantation revisited. Transplant Rev (Orlando). 2014; 28(1): 26-31.
20. Moreno-Arquieta IA, Cardenas-de la Garza JA, Esquivel-Valerio JA, Riega-Torres J, Cuellar-Barboza A, Herz-Ruelas ME, Ocampo-Candiani J and Galarza-Delgado DA. Mycophenolate mofetil and mycophenolic acid for the treatment of eosinophilic fasciitis: report of two cases and literature review. J Dermatolog Treat. 2022; 33(3): 1765-1768.
21. Roberti I and Vyas S. Long-term outcome of children with steroid-resistant nephrotic syndrome treated with tacrolimus. Pediatr Nephrol. 2010; 25(6): 1117-1124.