

# Efficacy and Safety of Rituximab in Children With Steroid- and Cyclosporine-resistant and Steroid- and Cyclosporine-dependent Nephrotic Syndrome

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**Introduction.** There is evidence of the effectiveness of rituximab in treatment of nephrotic syndrome in children. The present study aimed to assess safety and the therapeutic effectiveness of rituximab in steroid- and cyclosporine-resistant pediatric nephrotic syndrome. **Materials and Methods.** Forty-three children with steroid- and cyclosporine-resistant or steroid- and cyclosporine-dependent noncongenital nephrotic syndrome were included in the study to receive intravenous rituximab, 375 mg/m<sup>2</sup>/wk, for 4 weeks. The children were followed up for 2 years. Effectiveness was defined as remission of proteinuria in response to rituximab. Side effects of rituximab were monitored.

**Results.** Overall, 23 (57.1%) of the children had steroid- and cyclosporine-resistant nephrotic syndrome, of whom 8 (34.8%) revealed complete response and 3 (13%) revealed partial response. Seven children (16.7%) had late-resistant nephrotic syndrome, of whom 6 (85.7%) revealed complete response and none revealed partial response. Ten children (26.2%) had steroid- and cyclosporine-dependence all of whom revealed complete response to rituximab. Complete response rate was significantly higher in those with drug-dependent pattern than the other groups ( $P = .002$ ). There was no association between response to rituximab and pathological basis of disease. Side effects were found in 4 patients as leukopenia in 2, alopecia in 1, and eosinophilia in 1.

**Conclusions.** Rituximab is effective for children with nephrotic syndrome with high efficacy and well tolerability, especially in those with steroid- and cyclosporine-dependent nephrotic syndrome.

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## INTRODUCTION

Idiopathic nephrotic syndrome, with an overall incidence of 2 or more children out of 100000, is the most prevalent chronic glomerular disease in childhood.<sup>1</sup> Although many affected children suffer from pathological minimal changes and

most of them respond well to steroids, a notable number of children may face with relapsing patterns.<sup>2</sup> About 10% to 20% of the patients develop resistance to steroid therapy. In such patients, the treatment protocol is based on administrating immunosuppressive agents, such as cyclosporine,<sup>3,4</sup>

which can be associated with some complications such as nephrotoxicity and hematotoxicity.<sup>5,6</sup> Unfortunately, we have demonstrated in a previous study that about 29.4% of children with primary resistant nephrotic syndrome show resistance for cyclosporine.<sup>7</sup> Thus, development of new treatment options for treating nephrotic syndrome resistant to both steroids and immunosuppressive agents is urgently required.

Rituximab is a chimeric anti-CD20 monoclonal antibody, which inhibits CD20-mediated B-cell proliferation and differentiation, resulting in depletion of peripheral blood B lymphocytes. During the recent decade, anecdotal evidence has been reported on efficacy of rituximab for treating nephrotic syndrome. In some patients with nephrotic syndrome, the use of rituximab has resulted in long-term remission of disease.<sup>8-10</sup> However, some other studies showed no significant treatment response to this drug regarding long-term remission.<sup>11,12</sup> Some case reports and case series also reported high effectiveness of rituximab in patients with resistance to both steroids and immunosuppressive drugs.<sup>13-18</sup> The present study aimed to assess safety and therapeutic effectiveness of rituximab in steroid- and cyclosporine-resistant and steroid- and cyclosporine-dependent pediatric nephrotic syndrome and compare them with each other.

## MATERIALS AND METHODS

This study was performed on children aged lower than 18 years with steroid- and cyclosporine-resistant or steroid- and cyclosporine-dependent nephrotic syndrome who were candidates for administrating rituximab at Ali Asghar Children's Hospital in Tehran, between 2014 and 2015. Children with congenital nephrotic syndrome were not included. Steroid- and cyclosporine-resistant nephrotic syndrome was defined as no response to steroid after 1 month and also no response to cyclosporine after 6 months.<sup>3,7</sup> Steroid- and cyclosporine-dependent nephrotic syndrome was defined as steroid-dependence with frequent relapses while the dose of cyclosporine is tapered.<sup>4,5,8</sup> In addition, late drug-resistant nephrotic syndrome was defined as recurrence in whom remission was not gained in spite of receiving steroid for 4 weeks.<sup>15</sup>

The interval between diagnosis of nephrotic

syndrome and prescription of rituximab was different in patients but at least 7 months after diagnosis. Treatment with rituximab was explained to all patients and their parents and written consent for using this treatment method was taken from them. All of the patients received intravenous rituximab (Zytux; 375 mg/m<sup>2</sup> per week) for 4 weeks. Patients would be excluded from the study in case of infusion reactions to rituximab or lack of cooperation of patients.

Collection of 24-hour urine and visits for monitoring side effects were scheduled weekly for up to 4 weeks, once per month up to 3 months thereafter, and then every 3 months. After discontinuing rituximab, steroid and cyclosporine were tapered gradually and mycophenolate mofetil (1200 mg/m<sup>2</sup>/d) was administered for 2 years after rituximab infusion. The blood gamma globulin was not assessed after the infusion of rituximab.

The primary study endpoint was rituximab treatment response that was defined as no proteinuria for complete response and reduction of more than 50% protein for partial response in 24-hour urine sample since the start of treatment until 3 months after the last dose.<sup>12</sup> Protein and creatinine concentrations were measured in 24-hour urine. The secondary endpoint was to determine proteinuria recurrence (proteinuria  $\geq$  40 mg/h/m<sup>2</sup>) in those who revealed remission after treatment and assessing safety of drug by monitoring side effects. Leukopenia, serum sickness, leukoencephalopathy, and related infections were side effects monitored in this study.<sup>19</sup> The patients were followed up for 2 years.

Results were presented as mean  $\pm$  standard deviation for quantitative variables and were summarized as absolute frequencies and percentages for categorical variables. Normality of data was examined using the Kolmogorov-Smirnoff test. Categorical variables were compared using the chi-square test or the Fisher exact test when more than 20% of cells with expected counts of less than 5 were observed. Quantitative variables were also compared with the *t* test, the 1-way analysis of variance test, or the nonparametric Wilcoxon, Mann-Whitney U, or Kruskal-Wallis tests. The SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, IL, USA) was used for the statistical analysis. *P* values less than .05 were considered significant.

## RESULTS

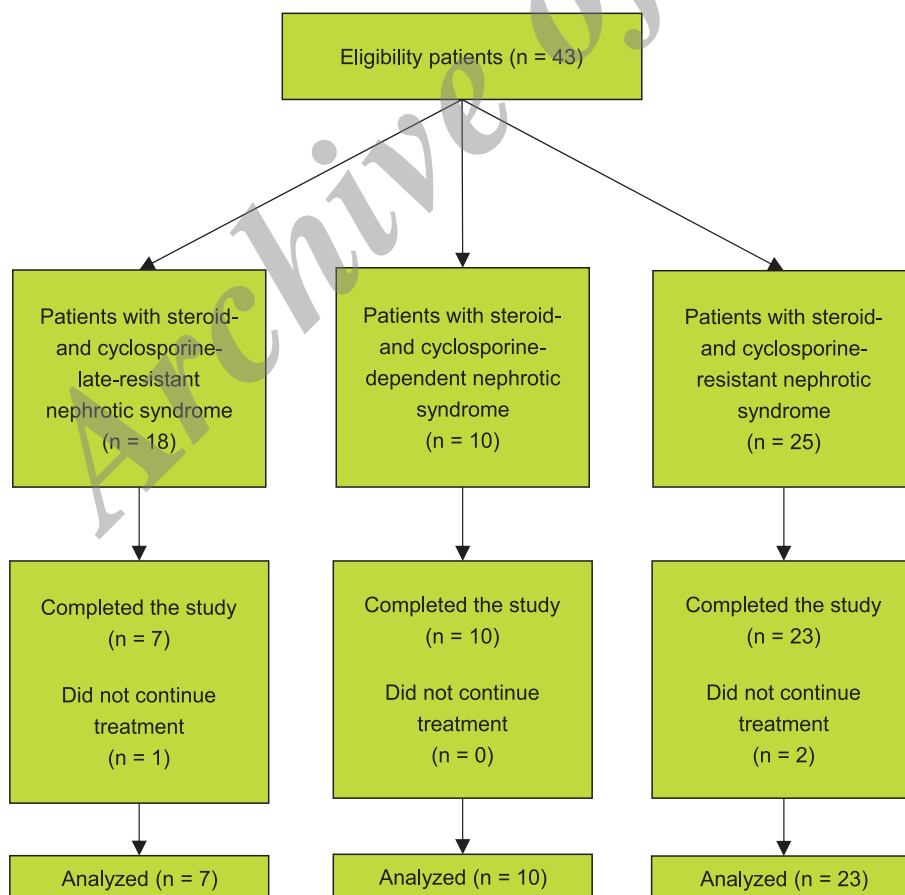
In total, among 43 children with nephrotic syndrome, 3 patients did not continue the treatment and 40 patients who continued the study were followed up for 2 years (Figure). The average age of the participants was  $8.35 \pm 4.48$  years (range, 2 to 17 years) and 22 (54.8%) were males. Regarding underlying pathological patterns, 29 (73.8%) had focal segmental glomerulosclerosis, 9 (21.4%) had minimal change disease, and 2 (4.8%) suffered from membranous glomerulonephritis.

Overall, 23 (57.1%) of the children suffered from steroid- and cyclosporine-resistant nephrotic syndrome, 7 (16.7%) had late drug resistant nephrotic syndrome, and 10 (26.2%) had steroid- and cyclosporine-dependent pattern of the disease. Characteristics of the patients who continued therapy is shown in the Table. The median serum creatinine level was 0.6 mg/dL (range, 0.5 mg/dL to 5.0 mg/dL) before treatment, and serum creatinine level after treatment was 0.6 mg/dL (range, 0.4 mg/dL to 5.00 mg/dL;  $P = .89$ ). Regarding response

to treatment, 24 (60.0%) responded completely to treatment with rituximab, 3 (7.5%) had partial response, and others remained with no significant response to this medication.

Among the children with steroid- and cyclosporine-resistant disease, 8 (34.8%) revealed complete response and 3 (13%) revealed partial response, while in the group with late resistant pattern, 6 (85.7%) revealed complete response and none of them revealed partial response. In those with steroid- and cyclosporine-dependent pediatric nephrotic syndrome, all of the patients revealed complete response to rituximab. As a result, complete response was significantly higher in those with drug-dependent pattern than the other groups ( $P = .002$ ).

In patients with steroid- and cyclosporine-dependent nephrotic syndrome, within 6 months before treatment with rituximab, several recurrences happened (mean episodes,  $3.25 \pm 0.88$ ), while during 6 months after treatment with rituximab, no recurrences were observed ( $P = .01$ ). Also, the



Flowchart of the study.

Characteristics of 43 Children With Nephrotic Syndrome\*

Characteristics	Steroid- and Cyclosporine-dependent Group (n = 10)	Steroid- and Cyclosporine-resistant Group (n = 23)	Steroid- and Cyclosporine-late-resistant Group (n = 7)
Age at starting treatment, y	6.80 ± 2.78	7.60 ± 4.43	13.00 ± 3.93
Sex			
Male	8 (80.0)	11 (47.8)	3 (42.9)
Female	2 (20.0)	12 (52.2)	4 (57.1)
Baseline pathology			
FSGS	5 (50.0)	19 (82.6)	5 (71.4)
MGN	0	2 (8.7)	0
MCD	5 (50.0)	2 (8.7)	2 (28.6)
Pretreatment hypertension	1 (10.0)	11 (47.8)	2 (28.6)

\*Values are mean ± standard deviation for age and frequency (percentage) for others. FSGS indicates focal segmental glomerulosclerosis; MGN, membranous glomerulonephritis; and MCD, minimal change disease.

patients with steroid- and cyclosporine-resistant and late resistant nephrotic syndrome did not have remission before treatment with rituximab; therefore, they did not experience any recurrence. However, after treatment, recurrence occurred in 6 (54.6%) of the patients who responded to rituximab and suffered from steroid- and cyclosporine-resistant nephrotic syndrome, and in patients with late resistant, recurrence occurred in 2 (33.4%).

Among the patients with complete and partial response, recurrence of nephrotic syndrome occurred in 8 (29.6%) after rituximab treatment. The duration of remission in the patients who had recurrence of nephrotic syndrome was 9 months in 1 (12.5%), 12 months in 6 (75%), and 24 months in 1 (12.5%).

The response rate to rituximab did not differ between the boys and girls as complete response was observed in 14 (63.6%) and 10 (55.6%); partial response, in 1 (4.5%) and 2 (11.1%); and no response, in 7 (31.8%) and 6 (33.4%); respectively ( $P = .94$ ). The response was also independent of the patients' mean age ( $8.07 \pm 4.22$  years in complete response,  $8.67 \pm 4.93$  years in partial response, and  $8.45 \pm 4.70$  years in no response status;  $P = .61$ ).

There was no association between response to rituximab and pathological basis of disease; complete and partial response to treatment was 16 (55.2%) and 3 (10.3%) in focal segmental glomerulosclerosis, 7 (77.8%) and zero, in minimal change disease, and 1 (50%) and zero in membranous glomerulonephritis, respectively ( $P = .54$ ).

Drug-related side effects were found in 4 patients as leukopenia in 2 children, alopecia in 1, and eosinophilia in 1.

## DISCUSSION

This study showed that treatment of children with nephrotic syndrome using rituximab led to a better response rate, especially in those with steroid- and cyclosporine-dependent pattern. In other words, children with cyclosporine-dependent pattern showed more complete treatment response than other groups of the study. We found drug-related side effects only in 9.6% of patients that were not life-threatening. Treatment of those children with rituximab using standard dosage ( $375 \text{ mg/m}^2/\text{wk}$ , 4 doses) resulted in a high complete response rate as well as low recurrence rate, particularly in those with steroid- and cyclosporine-dependent nephrotic syndrome. Another important point in our study was that among all baseline variables, only dependence on steroids and immunosuppressive agents could affect the response to rituximab, and other variables including sex, age, and the underlying renal pathology had no effects on response to treatment.

In a systematic review by Otukesh and coworkers,<sup>20</sup> 13 studies on the efficacy of rituximab treatment in patient with nephrotic syndrome and cyclosporine-dependent pattern were reviewed; all of those studies, similar to our study, have shown that rituximab is an effective drug in nephrotic syndrome and can reduce the chance of relapse. In addition, 7 studies in this review have shown that rituximab is not effective for cyclosporine-resistance pattern. Side effects of rituximab that occurred in this study were alopecia, leukopenia, and eosinophilia. In a similar study, Fujinaga and colleagues<sup>21</sup> found other adverse effects of rituximab, including severe neutropenia and hypogammaglobulinemia identified in 5% and 15%

of children treated with rituximab, respectively. Similar to this study, they also showed no life-threatening infections in their cohort. In another study by Webb and coworkers,<sup>22</sup> the median time to first relapse was 14 months after rituximab that was slightly higher than that reported in this study. In contrast with our study, allergic reaction to infusion was observed in 2 patients and it was the only side effect of using rituximab in their study.<sup>22</sup> Ravani and colleagues<sup>23</sup> also showed median relapse times of 18 months and 6 months in the patients who received rituximab and other drugs, respectively, indicating more prolonged relapse time after receiving rituximab. Also, in their study, in the rituximab group, nausea and skin rash during infusion were common; transient acute arthritis occurred in 1 child.

Sinha and colleagues<sup>24</sup> designed a study to assess the efficacy and safety of rituximab for children with steroid-dependent and steroid-resistant nephrotic syndrome. They administered rituximab in 193 patients with calcineurin inhibitor resistant (CNI) and CNI-dependent steroid-resistant and steroid-dependent nephrotic syndrome. Results indicated that rituximab decreased relapse rate by 81.8% and 71.0% in patients with steroid-dependent and CNI-dependent steroid-resistant pattern ( $P < .001$ ). Moreover, rituximab made a longer recuperation in patient with steroid dependence compare with CNI dependence ( $P < .001$ ). Result of using rituximab in steroid- and CNI-resistant nephrotic syndrome was unfavorable, with remission in 29.3%; generally they concluded that the use of rituximab was superior to the pointed drugs in reducing relapse rates and need for immunosuppressive medications in patients with steroid-dependent and CNI-dependent steroid-resistant nephrotic syndrome.<sup>24</sup>

In a cohort study, Gulati and colleagues followed 57 patients (33 with steroid-resistant and 24 with steroid-dependent nephrotic syndrome) for 12 months or longer.<sup>15</sup> After 6 months with rituximab therapy, 21.7% of patient with steroid resistance showed complete respond and 51.5% of them had no respond. Similar to our findings, rituximab revealed a higher efficacy in patients with steroid-dependent nephrotic syndrome. Also, among patients with the steroid-dependent pattern, relapse rate was reduced by 95% approximately. This was similar to our results for patients with

steroid- and cyclosporine-dependent nephrotic syndrome; they did not experience recurrence after treatment with rituximab. Kemper and coworkers<sup>25</sup> retrospectively followed up 37 patients with steroid-dependent nephrotic syndrome who used rituximab for treatment. In 26 patients, relapse did not occur 12 months after treatment, and 24 patients experienced relapse in range of 5.2 to 64.1 months. They considered about the initial dose of rituximab in their study but our initial dose of rituximab in all of the patients was similar. Their assessing the initial dose of rituximab revealed that the time of first relapse in patients who received 1 or 2 initial infusions was significantly shorter than patients who received 3 or 4 initial infusions of rituximab.

All of the patients were followed up in each group for 2 years, which is a strength point of this study. Our limitations were lack of cooperation of patients due to high price for drug and fear of side effects of rituximab.

## CONCLUSIONS

Rituximab, as a chimeric anti-CD20 monoclonal antibody, was effective for patients with complicated and refractory nephrotic syndrome with high efficacy and well tolerability. In fact, this medication can be administered with full confidence because of its high clinical efficacy, minimized drug-related side effects, and also reduced recurrence, especially in those children with steroid- and cyclosporine-dependent nephrotic syndrome.

## CONFLICT OF INTEREST

None declared.

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