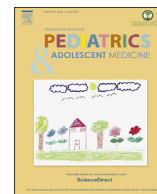


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# International Journal of Pediatrics and Adolescent Medicine

journal homepage: <http://www.elsevier.com/locate/ijpam>

Original research article

## Experience with second line drugs in frequently relapsing and steroid dependent childhood nephrotic syndrome in a large Saudi center



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### ARTICLE INFO

#### Article history:

Received 17 December 2016

Received in revised form

27 February 2017

Accepted 5 March 2017

Available online 22 March 2017

#### Keywords:

Levamisole

Cyclophosphamide

Mycophenolate Mofetil

Cyclosporine

Steroid dependant

Frequently relapsing

Prednisolone

### ABSTRACT

**Background and objectives:** To assess the efficacy and safety of second line drugs used at our center in frequently relapsing and steroid dependant (FR/SD) childhood nephrotic syndrome.

**Patients and methods:** This was a retrospective study over a period of 3 years (July 2012 to July 2015) on the use of 4 second line drugs in FR/SD nephrotic syndrome in children treated at our center. These drugs were Levamisole, Mycophenolate Mofetil (MMF), Cyclophosphamide, and Cyclosporine. We studied the relapse rate per year, cumulative dose of steroids, success, failure, and side effects of these drugs. Statistical analyses were done with the help of a statistician using the T-test and the "N-1" Chi-Square test.

**Results:** We reviewed the charts of 60 children. All had FR/SD nephrotic syndrome and received a 3 month protocol of prednisolone. 20 received Levamisole (33%), 12 received Cyclophosphamide (20%), 20 received MMF (25%), and 13 received Cyclosporine (22%).

All the four drugs significantly reduced the relapse rate and the cumulative dose of steroids ( $P < .0001$ ). Treatment success was best with Cyclosporine (69.2%), and treatment failure was the least with Cyclosporine (7.6%). However, treatment success and failure with Cyclosporine when compared to other three drugs was not statistically significant. No dangerous side effects were seen with any of the 4 drugs in the observation period.

**Conclusion:** All the second line drugs in our study were equally effective. However, we recommend that the initial treatment of FR/SD nephrotic syndrome should be chosen with the least toxic yet equally efficacious drug Levamisole.

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### 1. Introduction

Idiopathic nephrotic syndrome (INS) occurs commonly between the ages of 1–6 years. The sex ratio is usually 2:1. The annual incidence of INS in children in USA and Europe has been estimated to 1–3 per 100,000 children below the age of 16 with a cumulative prevalence of 16 per 100,000 children [1]. Histopathologically, it could be minimal change, diffuse mesangial proliferation, or focal and segmental glomerulosclerosis. The International Study of

Kidney Disease in Children (ISKDC) found minimal change in 76.6% of children with primary nephrotic syndrome [1].

90% of INS are steroid responsive and 10% are steroid resistant. Among the steroid responsive cases, 30% are cured after the initial episode, 10% are infrequent relapsers, and 60% are either frequent relapsers or steroid dependant or both [2]. This group of frequent relapsers and steroid dependant idiopathic nephrotic syndrome required repeated courses of corticosteroids which predisposes them to side effects of steroids such as stunted growth, hypertension, hyperglycemia, gastric hyperacidity and ulceration, osteoporosis, and metabolic bone disease.

In order to avoid steroid toxicity, certain second line drugs have been used. These are Levamisole, Cyclophosphamide, Mycophenolate mofetil, Cyclosporine, Tacrolimus, and Rituximab.

Levamisole is an immunostimulant and immunomodulator. It is

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Peer review under responsibility of King Faisal Specialist Hospital & Research Centre (General Organization), Saudi Arabia.

quite effective in reducing the relapse rate but after stopping it the relapses start occurring again. The most common side effect is reversible neutropenia but, its long-term safety is not well established. Oral cyclophosphamide is an alkylating agent which effectively suppresses the T-cells. Because of its serious side effects like bone marrow suppression, gonadal toxicity, and cancer, it is used with caution (not exceeding the cumulative dose). Mycophenolate mofetil is a T-cell immunosuppressant; its efficacy is dose dependant. A higher than the recommended dose is more effective in reducing the relapse rate but, its main drawback is that the relapses start occurring again as soon as it is stopped. As for its main side effect, it is diarrhea.

Calcineurin inhibitors (cyclosporine and tacrolimus) are effective T-cell immunosuppressants. They have a higher efficacy than the other previously mentioned drugs, but they carry serious side effects like nephrotoxicity, neurotoxicity, hypertension, hyperglycemia, and diabetes mellitus. Also, the patient relapses as soon as these drugs are stopped.

Recently, several reports described the efficacy and safety of rituximab in FR/SD INS. Rituximab is an anti-CD20 chimeric monoclonal antibody which effectively suppresses the B-cells leading ultimately to the suppression of T-cells.

Since it carries the risk of serious infection, it is currently reserved for difficult cases of FR/SD nephrotic syndrome.

Our study was an observational retrospective study on 4 commonly used second line drugs in FR/SD INS in children. The main aim was to compare the efficacy and safety of these 4 second line drugs so as to plan future prospective studies on second line drugs in FR/SD INS. The second aim was to develop a systematic approach and protocol of the use of these second line drugs in FR/SD INS.

## 2. Patients and methods

We reviewed the charts of children with FR/SD idiopathic nephrotic syndrome presenting to our center over a period of 3 years (July 2012 to July 2015).

### 2.1. Definitions

The definitions of nephrotic syndrome, remission, relapse, frequent relapse, and steroid dependant were as per the International Study of Kidney Disease in Children (ISKDC) (Table 1).

### 2.2. Treatment of first episode of nephrotic syndrome

All the patients received a 3-month protocol of corticosteroids [3], as follows:

#### 2.2.1. 3 Month protocol

Prednisolone 60 mg/m<sup>2</sup> [2] once daily (OD) x 6 weeks, followed by, 40 mg/m<sup>2</sup> [2] every other day (EOD) x 4 weeks, then, 20 mg/m<sup>2</sup> [2] EOD x 1 week, finally, 10 mg/m<sup>2</sup> [2] EOD x 1 week.

#### 2.2.2. The treatment of relapses was as follows [3].

Prednisolone 60mg/m<sup>2</sup> [2] OD till urine protein was negative for 5 days, followed by, 40 mg/m<sup>2</sup> [2] EOD x 4weeks, then, 30mg/m<sup>2</sup> [2] EOD x 4 weeks, finally, 20mg/m<sup>2</sup> [2] EOD x 4 weeks.

#### 2.2.3. Measurements

We recorded the following parameters weight, mean age at first episode, mean age at entry into study, serum creatinine, serum albumin, lipid profile, urine protein/creatinine ratio, at the first episode, at entry into the study, and at the end of the study. We also recorded the serum drug levels (Cyclosporine), number of relapses per year, and the cumulative dose of steroids before and after second line therapy. Calculation of cumulative dose of prednisolone was as follows: it was the total steroid dose over 1 year (in mg) adjusted to a surface area of 1 m<sup>2</sup>.

#### 2.2.4. The patients received the following second line drugs in the following doses

Levamisole 2.5 mg/kg EOD for 1 year, oral Cyclophosphamide 2 mg/kg x 12 weeks, Mycophenolate Mofetil (MMF) 1200 mg/m<sup>2</sup>/day in 2 divided doses for one year, and Cyclosporine 5 mg/kg/day in 2 divided doses for one year (Serum drug level 80–100 mg/ml). All these second line drugs were accompanied by low dose alternate day prednisolone.

#### 2.2.5. Inclusion and exclusion criteria

##### 2.2.5.1. Inclusion criteria

- All patients who had FR/SD idiopathic nephrotic syndrome.
- All patients who had received low dose prednisolone less than 0.75 mg/kg EOD initially for at least 1 year.

##### 2.2.5.2. Exclusion criteria

- All patients of FR/SD who had received previous immunosuppressive drugs.
- All patients of steroid resistant syndrome.
- All patients of genetic nephrotic syndrome (e.g NPSH<sub>2</sub>, NPSH<sub>1</sub>).
- All patients of congenital or infantile nephrotic syndrome.

The following parameters were recorded and analyzed:

1. Mean relapse rate per year.
2. Mean cumulative dose of corticosteroids before, during, and after stopping second line drugs per year.
3. Treatment success was presented as the number and percentage of patients with complete absence of proteinuria with only low dose prednisolone, without second line drugs in the third year.

**Table 1**

Definitions.

Nephrotic syndrome: proteinuria >40 mg/h/m <sup>2</sup> , >50 mg/kg/day, protein/creatinine ratio >0.2 g/mmol (>2 g/g), and hypoalbuminemia <25 g/L with or without edema
Remission: proteinuria < 4 mg/h/m <sup>2</sup> or 0-trace on Albustix for 3 consecutive days
Steroid responsive: complete remission achieved with steroid therapy
Steroid resistant: failure to achieve remission following 4 week prednisolone 60 mg/m <sup>2</sup> , followed by 3 methylprednisolone pulses
Relapse: proteinuria > 40 mg/h/m <sup>2</sup> , > 50 mg/kg/day, Albustix+++ for 3 consecutive days after having been in remission
Frequent relapser: 2 or more relapses within 6 months of initial response for 4 or more relapses within a period of 1 year
Steroid dependence: 2 consecutive relapses during corticosteroid therapy or within 14 days after cessation of therapy
Early nonresponder: steroid resistance during the first episode
Late nonresponder: steroid resistance in a patient who had previously responded to-corticosteroids therapy

4. Treatment failure was presented as the and percentage of cases with recurrence of FR/SD in the third year who required other second line drugs to maintain remission.

Drugs were considered effective if they were able to reduce the relapse rate, cumulative dose of steroids, and those having a higher percentage of treatment success, and a lower percentage of treatment failure. The comparison was done before and after giving second line drugs.

### 2.2.6. Statistical analyses

Comparison of means was done using the T-test ( $P < .05$  was taken significant result), and comparison of percentages was done using the “N-1” Chi square test with 95% confidence intervals [4,5].

## 3. Results

We reviewed the charts of 60 children with both frequent relapses and steroid dependency over a 3-year period. 20 (33.3%) cases received Levamisole for 1 year, 12 (20%) cases received oral Cyclophosphamide for three months, 15 (25%) cases received Mycophenolate Mofetil (MMF) for 1 year, and 13 (21.6%) cases received Cyclosporine for 1 year. For the first year, they received only low dose prednisolone. As for the second year, second line and low dose prednisolone, and only low dose prednisolone was given during the third year. Tables 2 and 3 show the baseline data upon initial episode of INS and at the end of the third year. There was a significant change ( $P < .0001$ ) in the urine protein/creatinine ratio, the mean serum albumin levels, and the mean cholesterol levels upon the initial episode of INS and at the end of the third year (post second line drugs). However, no significant change was seen in the serum creatinine or triglyceride levels (NS). All the 4 drugs reduced the relapse rate and cumulative dose of steroids equally during therapy for 1 year and post therapy for another year ( $P < 0.0001$ ). (Table 4).

### 3.1. Side effects

With Levamisole, 4 cases had transient neutropenia for which it was discontinued. When neutropenia resolved, it was restarted. With Cyclophosphamide, 3 cases had transient neutropenia and 1 hemorrhagic cystitis; the drug was restarted after they resolved. With cyclosporine, all cases had mild hirsutism and gum hyperplasia, 4 cases had mild hypertension, no cases had impaired renal function. With MMF, 3 cases had diarrhea. MMF dose was reduced and the diarrhea resolved. After that, MMF was resumed as before in half the dose initially and after 1–2 weeks, full dose was resumed. All the 4 drugs reduced the relapse rate and cumulative dose of steroids equally during therapy for 1 year and post therapy for another year ( $P < .0001$ ).

## 4. Discussion

Various second line drugs have been used in children to treat FR/SD nephrotic syndrome. They include Levamisole,

**Table 3**

The baseline data at the end of the third year.

Mean protein/creatinine ratio	500 mg/mol $\pm$ 80
Mean serum creatinine	32 $\mu$ mol/L $\pm$ 2
Serum albumin	35 g/L $\pm$ 3
Mean cholesterol $\pm$ SD	4.0 $\pm$ 1 mmol/L
Mean triglyceride $\pm$ SD	1.8 $\pm$ 1 mmol/L

Cyclophosphamide, Mycophenolate Mofetil, Cyclosporine, Tacrolimus, and Rituximab. Several retrospective as well as prospective studies have been conducted on the use of these second line drugs [6–12]. On review of literature, there was no study so far on the comparison of the 4 second line drugs used in our study with each other.

In our study of 60 children, there were significant reductions in both the mean relapse rate and the cumulative dose of steroids with all 4 second line drugs ( $P < .0001$ ). Treatment success was best with Cyclosporine (69.2%) and treatment failure was the least (7.6%). When compared to the other 3 drugs, these results were not significant. No major side effects were seen with any drug.

Sudha Ekambaram et al reported a retrospective study of 97 children with SDNS or FRNS. Levamisole was found to be effective in majority (77.3%), with better efficacy in children with FRNS as compared to those with SDNS. The mean cumulative steroid dose 1 year before therapy was 4109 (1154) mg/m<sup>2</sup>, and 1 year post therapy was 661 (11) mg/m<sup>2</sup>. The relapses were also less during the period of post Levamisole therapy. They did not observe any side effects even in those who completed 2 years of daily Levamisole therapy [6].

In our own prospective and controlled study on Levamisole versus low dose prednisolone, the relapse rate was reduced more significantly in the Levamisole group. It was reduced by 0.29 in the Levamisole group versus 0.11 relapses per patient per month in the control group. The mean cumulative dose of steroids was also reduced more significantly in the Levamisole group. It was reduced by 293 vs 102 mg/m<sup>2</sup>/month in the control group. Therapy failure was seen in 3/32 (9.4%) in Levamisole group versus 12/54 (50%) in the control group. No side effects of Levamisole were seen [7].

In a study on Levamisole, in FR/SD nephrotic syndrome by Madani et al, the steroid dose was significantly decreased (Mean reduction of 0.39  $\pm$  0.46 g + 0.33 $\pm$ 0.38 g) after treatment with Levamisole. The number of relapses also significantly decreased with a mean reduction of 0.92  $\pm$  0.98 episodes to 1.07  $\pm$  1.20 relapses per year. Their conclusion was that Levamisole appears to be effective in prolonging the duration of remission and decreasing the steroid dose in children with FR/SD nephrotic syndrome [8].

In a retrospective analysis of Levamisole vs. Cyclophosphamide by Alsaran K, no significant difference was seen in the efficacy of these 2 drugs in therapy of FR/SD nephrotic syndrome. Their recommendation was that Levamisole could replace Cyclophosphamide in this group of patients [9].

In a randomized control trial on MMF versus Levamisole by Basu B et al, MMF was associated with a higher rate of 12 month relapse

**Table 2**

The baseline data of all 60 patients upon initial episode.

Mean Age $\pm$ SD upon initial episode	3.75 years $\pm$ 1.1 yrs.
Mean Age $\pm$ SD at the start of second line drugs	4.8 years $\pm$ 1.0 yrs.
Sex: Male/female ratio	1.9:1
Mean $\pm$ SD urine protein/creatinine ratio (mg/mmol)	1500 $\pm$ 160
Mean Serum Creatinine ( $\mu$ mol/L)	30 $\pm$ 5
Mean Albumin $\pm$ SD (g/L)	18 g/L $\pm$ 5
Mean cholesterol $\pm$ SD (mmol/L)	5.8 $\pm$ 1.2
Mean triglyceride $\pm$ SD (mmol/L)	2.0 $\pm$ 1

**Table 4**  
The relapse rate, cumulative dose, treatment success, treatment failure, and side effects of drugs of relapse of the 4 second line drugs.

Observations	Levamisole			Cyclophosphamide			MMF			Cyclosporine		
	1yr Pre	1 yr During	1 yr Post	1yr Pre	1 yr During	Post	1yr Pre	1 yr During	Post	1yr Pre	1 yr During	Post
Relapse rate (Relapses/per 1 yr)	3.6 (0.5)	1.6 (0.6)	1.0 (0.9)	4.0 (0.6)	1.8 (0.7)	1.3 (0.8)	3.9 (0.6)	1.5 (0.7)	0.9 (0.8)	4.1 (0.4)	1.5 (0.5)	0.8 (0.7)
Cumulative dose of steroids Mg/m <sup>2</sup> /year	4100 (1005)	1700 (650)	900 (15)	3900 (950)	1800 (600)	920 (18)	4300 (1010)	1610 (595)	850 (20)	4350 (1100)	1510 (600)	705 (25)
Treatment success	12/20 (60%)			8/12 (66.6%)			9/15 (60%)			9/13 (69.2%)		
Treatment failure	2/20 (10%)			1/12 (8.33%)			2/15 (13.3%)			1/13 (7.6%)		
Side effects of drugs	4 patients reversible Neuropenia			3 patients transient neutropenia 1 hemorrhagic cystitis both improved after stopping drug			3 patients diarrhea Improved upon stopping MMF			Mild hirsutism gum-hyperplasia 4 patients mild hypertension		

free survival than Levamisole [(38% vs 16%)  $P = .003$ ] and 33% lower cumulative prednisolone than Levamisole [ $45.7 \pm 29.7$  vs  $67.8 \pm 35.8$  mg/kg per year]. 64% of patients in the MMF arm vs 46% in the levamisole arm were off steroid therapy at 12 months. With Levamisole, no side effects were seen. With MMF, diarrhea was seen in 7% which resolved after stopping drug temporarily [10].

In a prospective study on MMF in FR/SD childhood nephrotic syndrome by Hogg et al, 24 (75%) of 32 patients stayed in remission throughout the 6 months of MMF therapy. The relapse rate in these patients improved from one episode every 2 months before MMF to one every 14.7 months after MMF. It was concluded that MMF is effective for maintaining remission in patients who have FRNS, received a MMF treatment for at least 6 months, and was associated with a low incidence of adverse events [11].

In a randomized prospective open label and crossover trial of MMF vs Cyclosporine in FR/SD nephrotic syndrome, patients were divided into 2 groups A and B. Group A received MMF initially for 1 year and cyclosporine for the second year. Group B received cyclosporine for first year and MMF for second year. Results were significantly better in group B. They recommended to use group B protocol in FR/SD nephrotic syndrome in children [12].

In summary, several studies showed the efficacy and safety of Levamisole, cyclophosphamide, MMF, and cyclosporine in FR/SD childhood nephrotic syndrome. This study helped us to develop the following systematic approach in FR/SD nephrotic syndrome for the use of second line drugs at our center. We start with low-dose prednisolone (0.75 mg/kg on alternate days) and if still relapsing we go on to Levamisole 2.5 mg/kg on alternate days. If still relapsing, we go on to oral cyclophosphamide 2 mg/kg for 12 weeks and if still no response we start MMF (1200 mg/day in 2 divided doses). If still relapsing, we use Cyclosporine 5 mg/kg in 2 divided doses to maintain blood level (80–100 ng/ml) for 1 year.

## 5. Conclusion

All the 4 drugs reduced the relapse rate and cumulative dose of corticosteroids significantly and did not show any serious side effects during the observation period. We conclude that the use of second line drugs in FR/SD nephrotic syndrome should start with the least toxic but equally effective drug levamisole and proceed systematically to more toxic drugs like cyclosporine.

## Conflict of interest

All authors indicate they have no potential conflicts of interest to disclose.

## Acknowledgment

We are thankful to all consultants and specialists in Pediatric Nephrology Department for their cooperation and advice. We are grateful to the statistician who provided statistical analyses. We are extremely thankful to Miss Husna and Miss Karen Reario for their secretarial help.

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