Azathioprine in the treatment of children with steroid dependent nephrotic syndrome

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Abstract:

Introduction: In steroid dependent nephrotic syndrome (SDNS), another agent is required to spare the use of steroids and avoid the side effects that result from long term steroid therapy. Several immunosuppressives are used in SDNS. Azathioprine had been tried successfully in adults with MCNS, but few studies were done in children and on small number of patients.

The aim of this study was to assess the role of azathioprine in treatment of SDNS and comparing it with other agents commonly used for SDNS, namely levamizole and cyclophosphamide.

Materials and Methods: We retrospectively reviewed the files of 40 patients with SDNS, chosen randomly from cases following in the nephrology clinic, Children Hospital, Cairo University.

The following data were recorded: demographic data (age of onset, duration of the disease, height and weight and their percentiles), result of renal biopsy (if done), side effects of steroid therapy and treatment regimens used (for each: order of use, duration, side effects and response assessed by mean prednisone dose {mg/kg/day} and rate of relapse/year before and after the treatment).

Results: In the SDNS patients, the mean steroid dose at which relapses occur before the use of any immunosuppressive was 0.34 ± 0.23 mg/kg/day (range, 0.05 -1) and the mean rate of relapse/year was 1.40 ± 0.55.

Sixteen patients with SDNS received azathioprine, their ages ranged from 8 to 18 years with a mean of 12.38±2.57 years. The mean age of onset and mean duration of the disease were 6.50±2.92 and 5.88 ± 2.06 years respectively. The duration of use of azathioprine in patients ranged from 5 to 56 months with a mean of 25.06±16.86 months. No patients experienced side effects of azathioprine during the course of treatment. When comparing azathioprine with the other lines of therapy used in SDNS (levamizole and cyclophosphamide), the mean prednisone dose (mg/kg/day) was significantly reduced from 0.26 ± 0.14 to 0.13 ± 0.17 after use of azathioprine (p=0.03), and from 0.32 ± 0.23 to 0.15 ± 0.21 after use of levamizole (p=0.01) and from 0.36 ± 0.26 to 0.09 ± 0.11 after use of cyclophosphamide (p=0.00).

Conclusion: Azathioprine has equivalent results to levamizole and both have minimal side effects, but levamizole can be used as first line due to its lower cost and more safety. Azathioprine can be used in patients who failed to stop prednisone after cyclophosphamide or in those who failed to show any response to levamizole.
Key words: Azathioprine, steroid dependent nephrotic syndrome.

Introduction:

Steroid dependent nephrotic syndrome (SDNS) is defined as two consecutive relapses, which are not associated with concurrent infections while tapering steroids or within 14 days of stopping therapy (1). Another agent is required to spare the use of steroids and avoid the side effects that result from long term steroid therapy. Several immunosuppressives are used in SDNS. Cyclophosphamide is a well-known alternative agent to spare the use of steroids and avoid the side-effects that result from long-term steroid therapy (2-4). Most children may continue to have SDNS despite receiving cyclophosphamide; additional alternative drugs may be needed (4). Levamisole appears to be effective in prolonging the duration of remission and decreasing the steroid dose in children with SDNS (5).

A proportion of patients with SDNS still experience frequent relapses despite long-term treatment with steroids, levamisole, or/and cyclophosphamide. Therapy with mycophenolate moeftil (MMF) and tapering doses of prednisolone appears to be a promising intervention in children with SDNS (6). Long-term remission can be achieved with cyclosporine A (CSA), after alternative treatment with cytotoxic drugs or levamisole has failed. Nevertheless, severe SDNS recurs in some patients despite CSA maintenance therapy (7). Azathioprine had been tried successfully in adults with MCNS (8), but few studies were done in children and on small number of patients (9,10).

However, each of these agents has its limitations. Cyclosporine and MMF are expensive agents in addition to the potential nephrotoxic effect of the former (11), and cyclophosphamide has a maximum cumulative dose so it can not be used for extended period or given in repeated courses (12,13). Azathioprine and levamizole are cheap agents with few side effects and can be used for prolonged period of time (14,15).

Objectives:

The aim of this study was to assess the role of azathioprine in treatment of SDNS and comparing it with other agents commonly used for SDNS, namely levamizole and cyclophosphamide.

Subjects and methods:

We retrospectively reviewed the files of 40 patients with SDNS, chosen randomly from cases following in the nephrology clinic, Children Hospital, Cairo University. The patients' files numbers are arranged serially, files were randomly selected electronically using Microsoft office (Excel 2007). Steroid dependent NS is defined as patients responding to initial corticosteroid treatment by entering complete remission but develop a relapse either while still receiving steroids or within 2 weeks of discontinuation of treatment following a steroid taper (1).

The following data were recorded: demographic data (age of onset, duration of the disease, height and weight and their percentiles), result of renal biopsy (if done), side effects of steroid therapy and treatment regimens used (for each: order of use, duration, side effects and response assessed by mean prednisone dose {mg/kg/day} and rate of relapse/year before and after the treatment).

In our unit, the three most commonly used immunosuppressives in SDNS are levamizole, azathioprine and cyclophosphamide. Cyclosporine was not used as the first line of treatment of SDNS in our unit due to the higher cost and the need for longer duration of therapy. In our study group, it was only used in 3 patients as a last option and leaded to further decrease of the steroid dose but with inability to stop the drug completely.

Statistical analysis:

Data were collected, checked, revised and entered the computer. Data analyzed by SPSS statistical package version 19. Excel computer program was used to tabulate the results, and represent it graphically. Quantitative variables were expressed as mean and standard error. Qualitative variables were expressed as count and percent. One Way ANOVA used to declare the significant difference between groups at $p<0.05$. Duncan multiple comparison test at $p<0.05$ was used to declare the significant between each two groups.

Chi square test used to declare the significant difference in the distribution between groups at $p<0.05$.

Results:

Table (1) shows the characteristics of SDNS patients in comparison to patients with steroid sensitive nephrotic syndrome (SSNS) with infrequent relapses.

Table (1): Comparison between the characteristics of SDNS and SSNS patients:

	Total (n=70)	**SSNS (n=30)**	**SDNS (n=40)**	**P-value**
Age (yrs) mean ± SD median (range)	 9.79 ± 3.45 10 (3 – 18)	 9.06 ± 3.98 8 (3 – 18)	 10.34 ± 2.91 10.50 (5-18)	0.12
Age of onset (yrs) mean±SD median (range)	 4.77 ± 2.73 4 (1.5 – 12)	 4.42 ± 2.80 4 (1 – 12)	 5.04 ± 2.69 5 (1.5 – 12)	0.35
Duration of follow up (yrs) mean ± SD median (range)	 5.01 ± 2.23 5 (0.5-9)	 4.63 ± 2.72 4.5 (0.5-9)	 5.30 ± 1.75 5 (3 – 9)	0.22
Male/Female	53/17	24/6	29/11	0.58
Consanguinity, n (%)	6 (8.57%)	3 (10%)	3 (7.5%)	1
Positive family history, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
Weight (kg) mean ± SD median (range)	 35.24 ± 14.61 31.5 (15-86)	 31.87 ± 13.66 28.5 (15-70)	 37.78 ± 14.95 33 (20 – 86)	0.09
Weight percentile <5th, n (%) >95th, n (%)	 3 (4.92%) 3 (4.29%)	 1 (3.33%) 0 (0%)	 2 (5%) 3 (7.5%)	 1 0.25
Height (cm) mean ± SD	 132.53±16.09	 128.17±20.44	 135.8 ± 11.03	0.04
Height percentile <5th, n (%) >95th, n (%)	 9 (12.86) 0 (0%)	 2 (6.67%) 0 (0%)	 7 (17.5%) 0 (0%)	 0.29 -
BMI (kg/m^2) mean ± SD	 19.63 ± 4.76	 18.79 ± 3.66	 20.26 ± 5.40	0.20
CRI, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-

*P< 0.05 is significant

SSNS: Steroid-sensitive nephrotic syndrome BMI: Body mass index

SDNS: Steroid-dependent nephrotic syndrome CRI: Chronic renal insufficiency

Only five of the SDNS patients had performed a renal biopsy, 2 showed MCNS and 3 showed mesangioproliferative glomerulonephritis. Due to the low number of biopsies, results were not correlated to them.

In the SDNS patients, the mean±SD steroid dose at which relapses occur before the use of any immunosuppressive was 0.34 ± 0.23 mg/kg/day (range, 0.05 -1) and the mean ± SD rate of relapse/year was 1.40 ± 0.55.

Sixteen patients with SDNS received azathioprine, their ages ranged from 8 to 18 years with a mean±SD of 12.38±2.57 years. The mean age of onset and mean duration of the disease were 6.50±2.92 (range 2 - 12) years and 5.88 ± 2.06 (range, 3–9) years respectively. Levamizole was used in 27 patients and cyclophosphamide in 17 patients (several patients used more than one drug).

The duration of use of azathioprine in patients ranged from 5 to 56 months with a mean ± SD of 25.06±16.86 months and a median of 21 months, while levamizole use ranged from 3 to 36 months with a mean ± SD of 16.52 ± 9.80 months and a median of 16 months. No patients experienced side effects of azathioprine or levamizole during the course of treatment.

All patients treated with cyclophosphamide received it as oral therapy at a dose of 2 mg/kg/day for 3 months except one patient who received it as monthly IV therapy for 6 months. Three patients experienced transient leucopenia for which the drug was stopped temporarily and 2 patients suffered from moderate alopecia.

When comparing azathioprine with the other lines of therapy used in SDNS (levamizole and cyclophosphamide), the mean prednisone dose (mg/kg/day) was significantly reduced from 0.26 ± 0.14 (median, 0.25 – range, 0.004 – 0.5) to 0.13 ± 0.17 (median, 0.08 – range, 0.00 – 0.5) after use of azathioprine (p=0.03), and from 0.32 ± 0.23 (median, 0.3 – range, 0.05 – 1.0) to 0.15 ± 0.21 (median 0.1 – range, 0.00 – 0.9) after use of levamizole (p=0.01) and from 0.36 ± 0.26 (median 0.3 – range, 0.07 – 0.9) to 0.09 ± 0.11 (median, 0.05 – range, 0.00 – 0.3) after use of cyclophosphamide (p=0.00) (Figure 1 and Table 2). Figure (2) shows reduction of mean relapse rate/year before and after treatment with azathioprine, levamezole and cyclophosphamide.

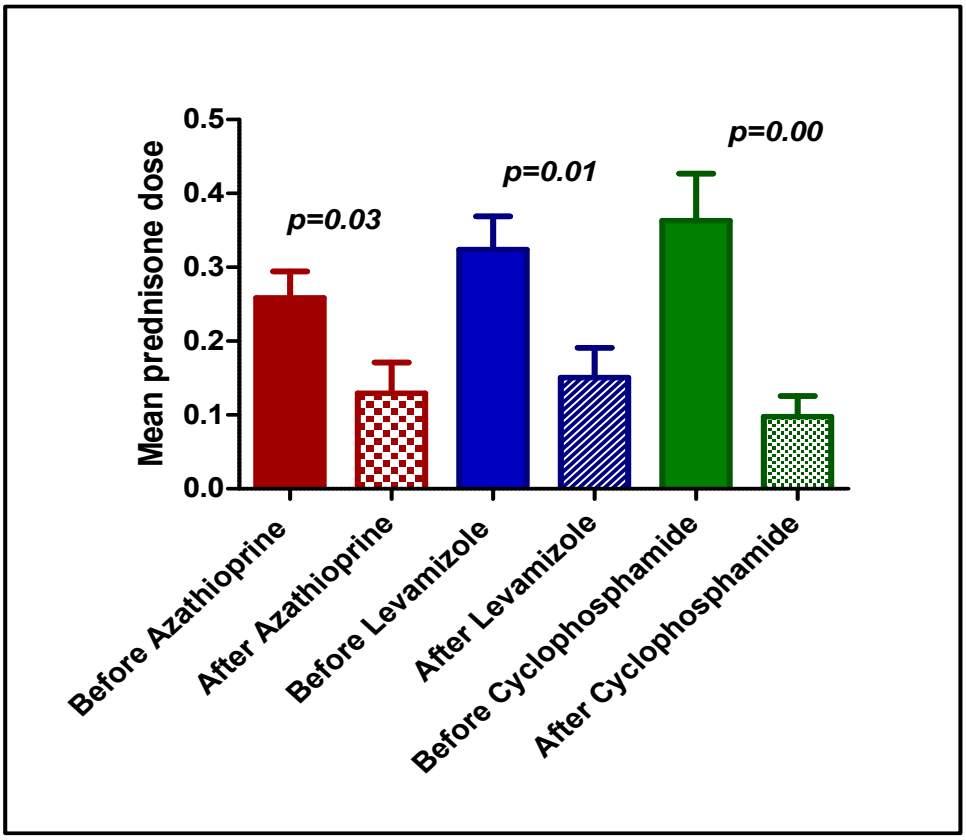

Figure (1): Reduction of mean prednisone (± SD) dose before and after treatment

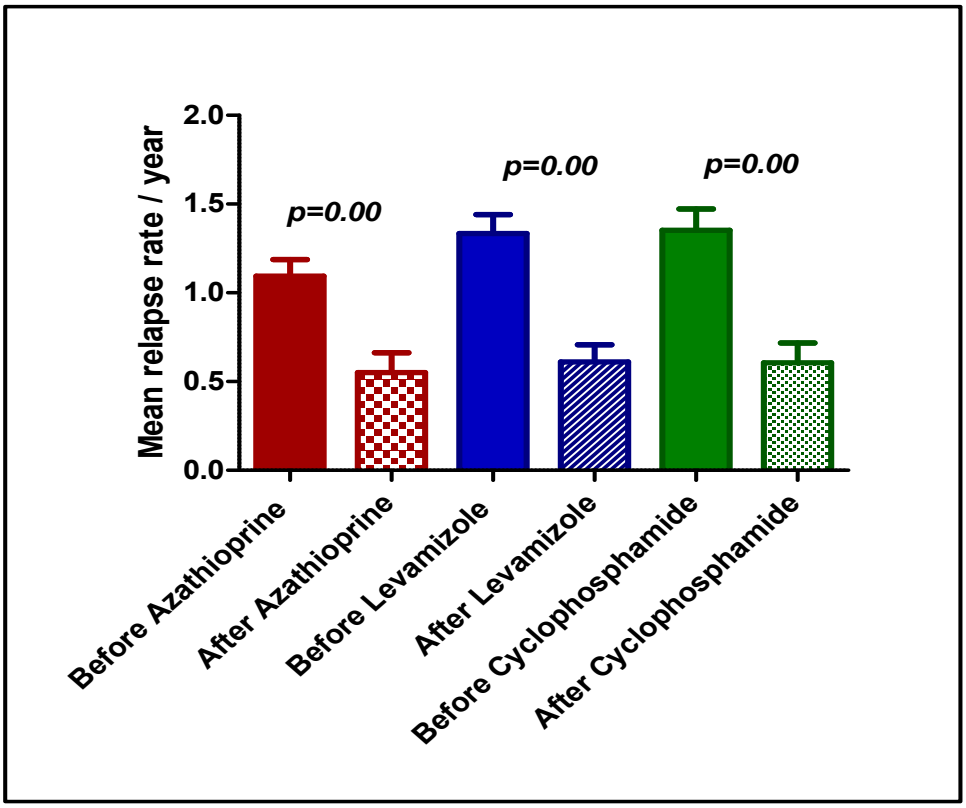

Figure (2): Reduction of mean relapse rate / year dose before and after treatment

Table (2): Mean prednisone dose (mg/kg/day) and mean relapse/year before and after use of azathioprine, levamizole and cyclophosphamide

	Azathioprine N=16	Levamizole n=27	Cyclo-phosphamide n=17	P-value			
				1 vs 2	1 vs 3	2 vs 3	All
Prednisone dose (mg/kg/day) at which relapse occur				0.37	0.17	0.56	0.41
Mean±SD	0.26 ± 0.14	0.32 ± 0.23	0.36 ± 0.26				
Median (range)	0.3 (0.05-1.0)	0.3 (0.05-1.0)	0.3 (0.07-0.9)				
Prednisone dose (mg/kg/day) after treatment				0.73	0.53	0.35	0.63
Mean±SD	0.13 ± 0.17	0.15 ± 0.21	0.09 ± 0.11				
Median (range)	0.1 (0.0-0.9)	0.1 (0.0-0.9)	0.05 (0.0-0.3)				
Percent of reduction of mean prednisone dose	50%	53.13%	75%	-	-	-	-
Mean relapse/year before treatment	1.09 ± 0.38 (0.5 – 2)	1.33 ± 0.55 (1 – 3)	1.35 ± 0.49 (1 – 2)	0.13	0.10	0.91	0.24
Mean relapse/year after treatment	0.55 ± 0.45 (0.0 – 1)	0.61 ± 0.50 (0 - 2)	0.61 ± 0.46 (0 – 1)	0.69	0.73	0.97	0.91

*P< 0.05 is significant

The pattern of response with different modalities of therapy is shown in table (3).

Table (3): Pattern of response to azathioprine, levamizole and cyclophosphamide

	Azathioprine n=16	Levamizole n=27	Cyclo-phosphamide n=17	P-value			
				1 vs 2	1 vs 3	2 vs 3	All
Stopped prednisone	7 (43.75%)	9 (33.33%)	7 (41.18%)	0.53	1	0.75	0.76
Decreased prednisone dose	6 (37.5%)	13 (48.15%)	10 (58.82%)	0.54	0.31	0.55	0.47
No effect	3 (18.75%)	5 (18.5%)	0 (0%)	1	0.53	0.56	0.16

*P< 0.05 is significant

Out of the 40 SDNS patients, 24 patients became infrequent relapsers (60%), 13 patients (32.5%) became dependent on a lower prednisone dose and 3 patients (7.5%) showed no improvement. No statistically significant difference was detected between patients who became infrequent relapsers and those who became SDNS on a lower dose or showed no improvement regarding the mean prednisone dose at which relapse occurs (before starting any immuosuppressants) *(P=0.2)*.

Table (4) shows mean prednisone dose at which relapse occurs as a factor predicting no improvement on immunosuppressive therapy.

Table 4: Mean prednisone dose at which relapse occurs as a factor predicting no improvement on immunosuppressive therapy

Item	Infrequent relapsers (n=24)	SDNS on lower dose or no improvement (n=16)	P – value
Mean prednisone dose at which relapse occurs (before starting any immuosuppressants), mean ± SD (range)	0.31 ± 0.20	0.40 ± 0.27	0.20

*P< 0.05 is significant

SDNS: steroid dependent nephrotic syndrome

Discussion:

In SDNS patients, repeated and prolonged high dosage of prednisone might lead to severe side effects. In order to avoid it, different non-steroid treatment have been used such as alkylating agents (cyclophosphamide, chlorambucil), levamisole, MMF or calcineurin inhibitors (16).

In our study, children with SDNS have been treated with various medications, including levamisole, azathioprine, cyclophosphamide and cyclosporine, the later was used in only 3 patients, results not shown. The dependence on cyclosporine to maintain remission in addition to its high cost and potential nephrotoxicity limits its use for SDNS. Cyclophosphamide is a potent agent with proved efficacy (2-4) but its use in peri-pubertal patients with its potential gonadotoxicity, in addition to possibility of hemorrhagic cystitis and induction of leucopenia makes its use limited to more

severe or resistant cases. Levamizole and azathioprine are relatively safe and cheap agents; the effect of the later is not adequately studied in children (9).

Out of 40 patients of our study, 24 patients became infrequent relapsers (60%), 13 patients (32.5%) became dependent on a lower prednisone dose and 3 patients (7.5%) showed no improvement.

All the three drugs (levamizole, azathioprine and cyclophosphamide) resulted in significant reduction of prednisone dose and significant reduction of mean relapse rate per year but the degree of reduction was not statistically significant between the 3 drugs. Although azathioprine led to stoppage of prednisone in higher percent of our patients compared to levamizole and cyclophosphamide (43.75% versus 33.33% and 41.8% respectively), this difference was not statistically significant.

Madani et al. (2010) (5), who retrospectively studied effect of levamisole on treatment of SDNS, found a significant reduction of steroid dose after treatment with levamisole. The number of relapses was also significantly decreased. Also in another study done by ***Sumegi et al (2004)*** (17) in Hungary that evaluated the effects of levamisole treatment on prolonged outcomes in 34 children with NS concluded that levamisole could significantly reduce both relapse rate and the cumulative steroid dose in SDNS and frequent relapsers NS patients.

Regarding azathioprine, some studies showed results similar to our study. In a retrospective study done in Japan (10) that studied efficacy of long term azathioprine for relapsing NS on 7 patients who had an initial attack of nephrotic syndrome (NS) in childhood and had frequent relapses even after cyclophosphamide therapy were given a 2-year course of azathioprine. They found that the mean annual relapse rates had decreased from 2.4±0.5 in the year preceding azathioprine to 0.4±0.8 in the 1st and 2nd years after its initiation. Six of their patients were observed for more than 6 months after discontinuation of the therapy and they were relapse free during this period. Average doses of prednisone also decreased in the 2nd and subsequent years after the therapy.

Tanaka et al. (1999) (9) used azathioprine in 2 SDNS patients who were previously treated by other immunosuppressives; azathioprine resulted in maintaining sustained remission and reducing steroid dose in both patients. Both previous studies were done on Japanese children.

Another study done by ***Cade et al. (1986)*** (8), showed progressive improvement in all 13 patients of the study so that they were in complete remission after one to three years, but this study was done in adult patients. Contradicting to our results, however, other old studies (18,19) showed inadequate therapeutic effect of azathioprine in SDNS results. Ethnic background may play a role in the response to azathioprine.

In our study group, 6 of the patients in which cyclophosphamide lead to decrease of the prednisone dose used azathioprine after that, 3 of them stopped prednisone and 3 of them further decreased its dose, so azathioprine is a useful agent to be used following cyclophosphamide to further decrease steroids or stop it completely.

Three of the patients in which levamizole did not lead to any improvement used azathioprine, it led to decrease of the dose of steroids.

Conclusion: Azathioprine has equivalent results to levamizole and both have minimal side effects, but levamizole can be used as first line due to its lower cost and more safety. Azathioprine can be used in patients who failed to stop prednisone after cyclophosphamide or in those who failed to show any response to levamizole. Cyclophosphamide is better to be preserved to patients relapsing on higher dose steroids.

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