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Tacrolimus Can Induce Remission in Cyclosporine (CsA) and Mycophenolate Mofetil (MMF) Resistant Pediatric Onset Nephrotic Syndrome

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Introduction. Nephrotic syndrome (NS) is a common pediatric renal disorder. Most of these patients are steroid responsive. 10%–20% of children with new onset NS are resistant to steroid therapy. Patients who are resistant to steroids have limited treatment options such as calcineurin inhibitors (CNIs), mycophenolate mofetil (MMF), and rituximab. Despite several studies had documented that tacrolimus is superior to cyclosporine A (CsA) and MMF in treating SRNS but no study was conducted to prove the efficacy of tacrolimus in treating CsA and MMF resistant NS in pediatric populations. The study objective was to evaluate the role of tacrolimus in treating refractory idiopathic nephrotic syndrome.

Methods. One hundred and twenty patients with idiopathic nephrotic syndrome were included in the study. Patients with steroid resistant NS were given cyclosporine (CsA) (first step protocol). In patients with cyclosporine resistant NS a combination of CsA+ MMF was given as a second step protocol. Unresponsive patients received tacrolimus as a third step treatment protocol. Tacrolimus was given at a starting dose of 0.1 mg/kg/d then the dose was modified according to serum trough levels and patients were followed up for 12 months to evaluate the outcome.

Results. Out of 120 patients, 15 cases were both cyclosporine and MMF resistant and received tacrolimus. Tacrolimus had induced remission in 11 (73.3%) patients during the first 6 months of therapy. Eight patients achieved complete remission and three patients reached partial remission.

Conclusions. Tacrolimus is effective in treating refractory multi-drug resistant NS with favorable outcomes in childhood onset NS.

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INTRODUCTION

Nephrotic syndrome (NS) characterized by the presence of proteinuria, hypoalbuminemia, hyperlipidemia, and edema is one of the most common pediatric renal disorders.[1] Most of NS patients are steroid responsive, about 40%–60% of them exhibit frequent relapses or become steroid-dependent. About 10%–20% of pediatric patients with idiopathic nephrotic syndrome are resistant to steroids and cyclophosphamide (CYC) is found to induce remission in about 25%–30% of these patients.[2] Other steroid-sparing agents as calcineurin inhibitors (CNIs), mycophenolate mofetil (MMF) and rituximab can induce remission in about 20% to 70% of patients with steroid-resistant nephrotic syndrome.[3-5] On the other hand, serious side effects of these medications; for example, nephrotoxicity, life-threatening

infections, hypertension, and hirsutism remain a major problem.[4,6] The treatment options for patients with SRNS who are resistant to other immune suppressive as CNIs, MMF and alkylating agents or different combinations of these agents are very limited and inducing remission in these patients is challenging.[7] Rituximab, new anti-CD20 monoclonal antibody which, acts through depleting B-lymphocytes was found to induce remission in refractory pediatric-onset nephrotic syndrome. Several studies had reported that rituximab is more effective in inducing remission in steroid-dependent (SDNS) and frequent-relapsing nephrotic syndrome rather than in SRNS.[8,9] Some studies have compared the efficacy and safety of cyclosporine A and tacrolimus[10,11] but there is no study regarding the efficacy of tacrolimus in treating CsA or MMF resistant NS except for a single case report in children[12] and few adult studies.[13,14] In this study, we aimed to estimate the role of tacrolimus in inducing remission in refractory idiopathic pediatric-onset nephrotic syndrome as a step before rituximab which is an expensive drug and mostly unavailable especially in developing countries.

MATERIALS AND METHODS

One hundred and twenty patients admitted to our hospital between Jan 2011 and March 2016 fulfilling the criteria of INS (proteinuria > 2mg/mg creatinine, serum albumin < 2.5 g/dL, total cholesterol > 250 g/dL, and edema) included in this study while 15 patients were excluded (Figure). Patients with INS, aging between 1-14 years which went into remission with prednisolone (2 mg/kg/d) by 6 weeks, considered as SSNS and did not undergo renal biopsy. Patients who did not achieve remission after 8 weeks of prednisolone were considered steroid resistant nephrotic syndrome (SRNS) and underwent renal biopsy. First-step protocol for SRNS was as following: Cs A (150 mg/m^2/d) and prednisolone (1 mg/kg/d) every other day for 6 months. If remission was obtained 3 months after complete remission, prednisolone was withdrawn. Second-step protocol for patients who did not enter remission on the first step

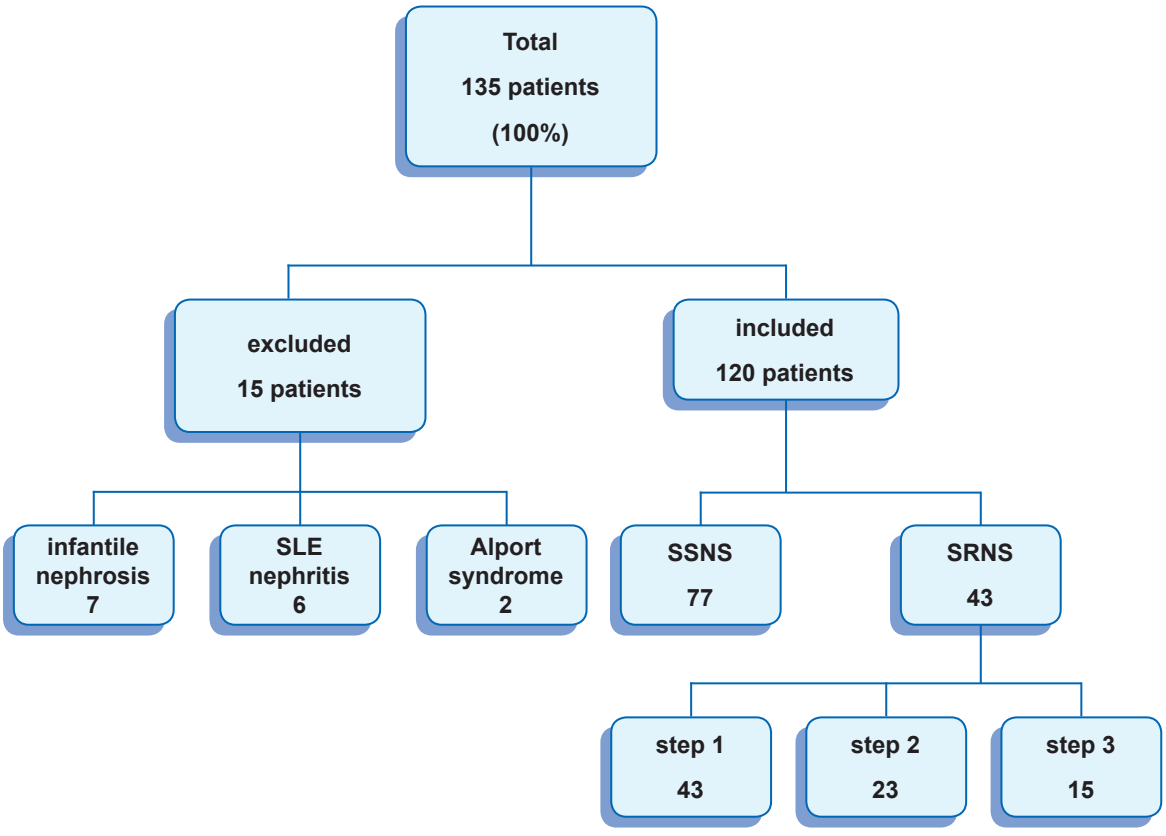

Figure. It shows the patients recruitment in the study.

treatment was: Cs A 150 mg/m^2/d and MMF 1200 mg/m^2/d given for 6 months with withdrawing prednisolone to a dose of 0.5 mg/kg every other day. If complete remission was obtained, 3 months later CsA was tapered. With any relapse during tapering phase, we returned to previous dose of cyclosporine. Third-step protocol for patients who did not enter into remission on the second step protocol was: Tacrolimus (Tac) 1mg/kg/d then the dose was adjusted to maintain serum trough level between 3-10 ng/dL and prednisolone 0.5 mg/kg every other day was given for 6 months. Patients who did not achieve remission were considered tacrolimus resistant while responders had continued the treatment for another 6 months. Exclusion criteria were: 1) estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 at the onset or at any time during the study period, 2) secondary NS, 3) patients with abnormal serum complement (C3 and C4) levels, and 4) familial nephrotic syndrome.

End of the study: either deterioration of eGFR < 60 mL/min/m^2 or completion of the course. All patients were followed up monthly by blood pressure measurement, urinary protein to creatinine ratio (uPCR), serum albumin, kidney function tests, eGFR, complete blood count, random blood sugar and serum trough levels of CsA/Tac.

Ethics Approval

The study was permitted by the Faculty of Medicine, Beni-Suef university research ethics committee and in accordance with Helsinki declaration of Bioethics and its later amendments (approval number: FMBSUREC/05032014 /HEBA).

Informed Consent

It was obtained from all participants' caregivers.

Study Definitions

- Complete remission: uPCR < 0.2 mg/mg creatinine
- Partial remission: uPCR > 0.2 mg/mg creatinine but less than 2 mg/mg creatinine
- Relapse: uPCR > 2 mg/mg creatinine
- Resistance to Tac therapy was defined as persistence of nephrotic proteinuria after 6 months of Tac treatment with effective plasma concentration

Statistical Analysis

Statistical analysis was done using statistical package for social sciences (SPSS), computer software (version22), IBM software, USA. Data were described in the form of median (IQR) for quantitative data, and frequency and proportions for qualitative data. A *P* value < .05 was considered statistically significant. Differences were analyzed between the groups by Student t test as regards normally distributed data; otherwise, Mann–Whitney U test was used.

RESULTS

Out of 120 patients, 77 (64%) were steroid responsive (42 males, 35 females) while 43 (35.8%, [25 males, 18 females]) were steroid resistant (Table 1). Out of 43 SRNS patients, 20 (46.5%) patients responded to CsA therapy. Other 23 patients who entered the second step protocol, eight (34.7%) patients responded to CsA and MMF combination therapy while the remaining 15 (65.3%) patients were resistant and entered the third step treatment protocol (Table 2). The demographic and laboratory data of patients at each step are shown in Table 3. The results of renal biopsy of patients received Tac were as following: eight patients had minimal change NS (MCNS), five had focal segmental glomerular sclerosis (FSGS) and two patients with mesangio- proliferative glomerulonephritis (MesPGN). All patients had received tacrolimus on a dose of 0.1-0.25 mg/kg/d and maintained serum trough levels between 3-10 ng/dL. Four patients (26.6%) were tacrolimus resistant (all of them had FSGS) so, treatment was

Table 1. Sex Demography of the Study Population

	Male	Female
SSNS	42	35
SRNS	25	18
CsA Resistant NS	15	8
CsA + MMF Resistant NS	11	4

SSNS, steroid sensitive nephrotic syndrome; SRNS, steroid resistant nephrotic syndrome; CsA, cyclosporine A; MMF, mycophenolate mofetil

Table 2. Response to Therapy in the Three Study Phases

	Complete Remission	Partial Remission	Resistant
Step 1 Protocol	18	2	23
Step 2 Protocol	6	2	15
Step 3 Protocol	8	3	4

Table 3. Demographic and Laboratory Data of Patients at the Three Study Phases

	Phase 1	Phase 2	Phase 3	*P*
Age, years	8 (3-12)	8 (4-11)	7 (4-13)	> .05
Weight, kg	28 (16-45)	32 (17-45)	24.5 (16-47)	> .05
Height, cm	121 (88-145)	123 (98-145)	110 (92-152)	> .05
Albumin, g/dL	2.6 (1.2-4.7)	2.1 (1.2-3.9)	3.7 (1.5-4.7)	< .001
Cholesterol, mg/dL	244 (98-530)	368 (198-530)	188 (102-356)	< .001
uPCR, mg/mg creat	3.3 (0-9)	5 (0.1-8)	185 (0-5)	< .05
Creatinine, mg/dL	0.5 (0.4-1.1)	0.5 (0.4-1.1)	0.5 (0.3-9)	< .05
eGFR, mL/min/1.73m^2	119 (63-210)	110 (63-140)	119 (75-180)	> .05

uPCR = Urine Protein (g/dL), Creatinine (mg/dL), eGFR = estimated glomerular filtration rate (0.5 * height (cm)/s Creat)

discontinued and three patients (20%) were partial responders (2 MCD and 1 MesPGN). The remaining eight patients (53.3%) achieved complete remission within the first four months of treatment (6 MCD, 1 FSGS, and 1 MesPGN; Table 4).

The median follow up duration was 3 (2.5-5) years and all the participants had completed the one-year duration of tacrolimus treatment without major events (acute kidney injury, severe infection, or hyperglycemia). The most frequent adverse effects of tacrolimus therapy were diarrhea (5 patients, 33.3%), abdominal pain (4 patients, 26.6%), and simple infections in three (20%) patients.

DISCUSSION

Several studies had documented that tacrolimus is more efficient than CsA[10,11] and MMF[15] in treating steroid-resistant NS but there is no study on the efficacy of tacrolimus in treating CsA or MMF resistant NS apart from a single case report in treating a child with refractory NS who was resistant to CYC, CsA and MMF with tacrolimus.[12] There are scanty adult studies that reported the efficacy of tacrolimus in treating CsA resistant NS in both MCNS and FSGS[13,14] and they reported that tacrolimus might be promising in treating those patients. All our patients were adherent to follow up visits and treatment. In our study, despite all patients entered the third-step treatment were refractory to all immune-suppressors even to combination therapy they had a stunning response to tacrolimus therapy. Four patients out of 15 were tacrolimus resistant and 11 patients had achieved remission (complete remission in 8 patients and partial remission in 3 patients) with cessation of proteinuria during the 1st 6 months of treatment. The four tacrolimus resistant patients had passed into ESRD and maintenance dialysis later on. Only a few mild side effects of tacrolimus were reported in our patients mainly gastrointestinal tract symptoms without any major side effects as AKI, serious infections, hypertension or hyperglycemia. The frequency of infections as (urinary tract, respiratory tract or gastrointestinal tract infections) was much lower than while on CsA treatment denoting the safety of Tac as well. Despite that CsA and tacrolimus are both calcineurin inhibitors but the clinical profile of tacrolimus differs in several points from that of cyclosporine. It has been demonstrated that tacrolimus has higher immune-suppressive effects than cyclosporine, mostly because of the greater affinity of it's complex with FK binding protein to calcineurin.[17] Tacrolimus, however, is considerably more powerful as an anti lymphocytic agent than CsA, as evidenced by the superior potency of the former drug in inhibiting antigen-driven T cell activation, cytokine production and lymphocyte proliferation in vitro.[18] Tacrolimus not only has an immune-suppressor effect but also ameliorates

Table 4. Frequency of response to treatment in the three study stages according to renal pathology.

	MCD		FSGS		MesPGN	
	Responding	Resistant	Responding	Resistant	Responding	Resistant
Step 1 protocol	8 (47%)	9 (53%)	8 (42%)	11 (58%)	4 (57%)	3 (43%)
Step 2 protocol	1 (11%)	8 (89%)	6 (54.5%)	5 (45.5%)	1 (33.3%)	2 (66.7%)
Step three protocol	8 (100%)	0	1 (20%)	4 (80%)	2 (2 100%)	0

MCD; minimal change disease, FSGS ; focal segmental glomerular sclerosis, MesPGN; mesangial proliferation glomerulo-nephritis

proteinuria by inhibiting the redistribution of CN-A and nephrin in the slit diaphragm restoring its integrity.[19] Previous studies had shown that about 70% of patients with SRNS would respond to one of the alternative immune-suppressors.[5] In our study, the remission rate after second step protocol was 65% while the total remission rate (sum of remission after the three-step protocol) was 93%. In the study done by Nikibakhsh *et al.* [20] they reported that 77.7% (seven out of nine patients) of FSGS patients were resistant to combination CsA and MMF therapy. In our study, only 21% of FSGS (4 out of 19) nephrotic patients were resistant to all lines of therapy. The highest rate of response in our patients was found in MCD patients (88% complete remission rate and 12% partial remission) at the end of the study. Although rituximab had shown promising outcomes in patients with refractory INS, its very high cost and the difficulties in getting it especially in poor countries makes tacrolimus a new hope for these patients and their treating doctors.

CONCLUSION

Our study had shown that tacrolimus was very effective in inducing remission in refractory multidrug resistant NS.

COMPLIANCE WITH ETHICAL STANDARDS

- The authors declare that there is no conflict of interest.
- An informed consent was obtained from patients' caregivers.
- University hospital ethical committee approved the research protocol.

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