Original Paper

CYCLOSPORINE & MYCOPHENOLATE MOFETIL IN THE TREATMENT OF CYCLOPHOSPHAMIDE REFRACTORY CLASS-IV LUPUS NEPHRITIS

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Abstract

Class IV Lupus Nephritis is a difficult medical situation requiring aggressive management. Many patients do not respond to conventional cyclophosphamide (CPM) therapy. The aim of this study was to evaluate the effect of cyclosporine (CsA) and mycophenolate mofetil (MMF) in the treatment of CPM refractory class IV Lupus nephritis. The study was conducted at Combined Military Hospital (CMH) and Cantonment General Hospital (CGH) of Dhaka over a period of 8 years (from January 2000 till December 2008). CPM refractory Class IV Lupus nephritis patients were randomly assigned into 2 groups cyclosporine (4mg/kg/day) and mycophenolate mofetil (1000-2000mg/day). Thirty one patients completed at least one year follow up and were included in the study. Sixteen patients were included in cyclosporine group and 15 patients in mycophenolate mofetil group. CsA treated patients had a remission rate of 87.5% which was 80% in MMF group. The average remission time was 16.21 weeks in CsA and 20.91 weeks in MMF group. The urinary total protein(UTP) and creatinine clearance (CCr) values were similar in both groups, 0.54 gm vs 0.66 gm & 81 vs 86 ml/min. The systemic lupus erythematosus disease activity index (SLEDAI) was 9.56 and 9.2 in CsA and MMF group which came down to 1.92 and 1.83 in the same groups after remission. In this study It was found that both cyclosporine and mycophenolate mofetil were very effective in the treatment of CPM refractory class IV Lupus nephritis with slight better response with cyclosporine.

Key words: Lupus Nephritis, mycophenolate mofetil, cyclosporine.

Introduction

Lupus nephritis is an inflammation of kidney caused by Systemic lupus erythematosus (SLE) which is an autoimmune disease. According to World Health Organization (WHO), Lupus nephritis can be classified into 5 classes (Class I - Class V) on the basis of renal biopsy. Class IV lupus nephritis include Diffuse Progressive Glomerulonephritis (DPGN) which involves >50% of glomeruli. Standard treatment protocols for lupus nephritis involve intravenous pulses of cyclophosphamide (IV CPM) & corticosteroids. The traditional CPM regimen for DPGN is divided into a 6 month induction phase and a 2 year maintenance phase. The induction phase consists of monthly pulses for 6 month CPM with steroids. The maintenance regimen is quarterly pulse CPM for 2 years or 1 year beyond remission[1,2,3]. Although pulsed IV CPM is effective in improving renal survival, but therapeutic inefficacy of standard regimen is due to side effects, inability to induce remission or relapse of disease during therapy or following withdrawal of therapy[4].

Cyclosporine (CsA) & mycophenolate mofetil (MMF) are newer immunosuppressive agents that are used in organ transplantation, has been now used in patients with refractory DPGN i.e in patients who are intolerant of or resistant to conventional immunosuppressive agents.

The aim of this study, was to evaluate the efficacy of CsA & MMF in the treatment of CPM refractory Class - IV Lupus Nephritis.

Materials and Methods

Place and duration of study. The study was carried out in the Nephrology center of Combined Military Hospital (CMH) and Cantonment General Hospital (CGH) of Dhaka from January 2000 to December 2008.

Patient selection. All patients were biopsy proven class-IV lupus nephritis. Patients who failed to attain a remission after pulse CPM therapy and patients who relapsed after CPM therapy were randomly assigned into CsA and MMF groups.

Distribution of patient. Total 40 patients of CPM refractory class-IV lupus nephritis were randomly divided into 2 groups with 20 in each. During this study period, 4 patients of CsA group and 5 from MMF group were not available for follow up. So, results of 16 patients from CsA & 15 from MMF group are presented here.

Dose of drug. CsA group patients were given cyclosporine orally at the dose of 4mg/kg/day divided

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into two daily dosages after meal and MMF group patients were given mycophenolate mofetil at the dose of 1000-2000 mg/day depending on the body surface area in divided doses before meal. MMF was initiated at a dose of 1 gm/day in patient weighing <50kg; 1.5 gm/day where body weight was 50-70 kg & 2.0 gm/day in those over 70kg. Patients of both groups also received concomitant corticosteroids administered at a maximum dose of 60 mg/day & tapered every 2 weeks.

Follow up. The patients were followed up weekly for 3 months, fortnightly for 3 months, monthly for 6 months. After 1 year, patients were evaluated at every 3 months. During each visit the patients were examined and investigated to assess:

☐ Disease activities whether patient develop new onset of seizure, psychosis, organic brain syndrome, lupus headache, cranial nerve disorder, visual disturbance, vasculitis, arthritis, myositis, cerebrovascular accident, haematuria, proteinuria, pyuria, urinary cast, new rash, oral ulcer, alopecia, pleurisy, pericarditis, fever, leucopenia, thrombocytopenia, low complement, increase DNA binding.

☐ Complications / side effects of drugs.

Investigation. During each follow up visit urine and blood samples were collected to determine following routine parameters:

- Urine - routine , culture and sensitivity
- Urine - Albumin-Creatinine Ratio.
- Complements (C_3 C_4)
- Complete blood count and ESR
- Serum Urea, Creatinine
- Creatinine clearance rate and UTP were measured at 6 months interval.
- X-ray chest, Ultrasonogram and other relevant investigations were also done depending upon the clinical situation.

The remission was declared if there was no haematuria or cast in urine; ESR and blood counts were normal; urinary total protein (UTP) and creatinine clearance rate(CCr) improved; C_3 & C_4 values were normal; patients were symptom free.

Statistical analysis. Mean and standard deviation of values were calculated. Unpaired students' t test was done where applicable.

Results

Over a period of 9 years, 31 patients refractory to pulse cyclophosphamide were available for the study. Sixteen patients were enrolled in CsA group and 15 in MMF group. The baseline characteristics of both groups are summarized in table-I. There was no significant difference for any item between 2 groups ($p>0.10$). Data were collected at entry to the study. Measurements included C_3, antinuclear antibody (ANA), anti-ds DNA, ESR, serum creatinine, urinary total protein (UTP) and creatinine clearance rate (CCr). Before treatment, all the ESR values were very high in both the groups: 92.44 and 91.73 mm at the end of 1st hour. The mean ESR significantly fell down ($p<0.01$ or 0.001) in both groups to 29.94 and 26.13 mm at the end of 1st hour (Table-II).

In both groups, creatinine clearance rate increased at the end of study ($p<0.01$ or 0.001). The mean value increased from 74.94 ml/min to 86 ml/min in CsA group and 73.87ml/min to 86.27 ml/min in MMF group (Table-III). A significant reduction of proteinurea was noted with both drugs (Table-IV). In CsA group after treatment mean value was 0.55gm/day & in MMF group it was 0.66 gm/day which is almost similar ($p>0.10$).

Initially mean SLE disease activity index was 9.63 & 9.2 in CsA & MMF group respectively which came down to 2.44 in CsA group & 2.73 in MMF group (Table-V).

Table-I: The baseline characteristics of both groups.

Parameter	CsA group	MMF group
Age of patient (range)	10-50 years	10-45 years
Female	14 (87.5%)	13 (86.7%)
Male	02 (12.5%)	02 (13.3%)
UTP (range)	2.2-8.6 gm/day	2.4-8.2 gm/day
CCr (range)	66-90 ml/min	60-87 ml/min
C_3	Low (100%)	Low (100%)
ESR (range)	80-100 mm at 1st hour	80-120 mm at 1st hour
ANA	Positive (100%)	Positive (100%)
Anti ds DNA	Positive (100%)	Positive (100%)
Serum Creatinine (range)	150-290 mg/dl	150-300 mg/dl

Table-II: ESR values of CsA & MMF group before and after treatment.

Patient	CsA group		MMF group	
	Before (mm in 1st hour)	After (mm in 1st hour)	Before (mm in 1st hour)	After (mm in 1st hour)
1	110	28	82	18
2	82	40	87	24
3	86	36	89	16
4	84	28	109	18
5	92	37	102	44
6	96	42	120	38
7	106	38	80	22
8	100	29	82	28
9	92	30	90	30
10	88	36	86	26
11	84	28	92	16
12	80	28	100	28
13	89	20	90	24
14	104	10	87	25
15	100	22	80	35
16	86	27	-	-
Mean ± SD	92.44 ± 9.22	29.94 ± 8.24	91.73 ± 11.42	26.13 ± 8.13
Statistical analysis				
Cyclosporine before vs after			p < 0.001	
Mycophenolate mofetil before vs after			p < 0.001	
Cyclosporine before vs Mycophenolate mofetil before			p > 0.10	
Cyclosporine after vs Mycophenolate mofetil after			p > 0.10	

There was no significant difference in SLEDAI scores between remission group and the patients who are not in remission.

Finally, CsA group remission achieved in 14 patients out of 16 with a mean time duration of 16.21 weeks and in MMF group remission occur in 12 patients out of 15 with a remission time of 28.91 weeks (Table-VI).

No patient had to stop treatment temporarily or permanently due to side effects. However in CsA group 8 out of 16 patients suffered from hypertension which was

Table-III: CCr of CsA and MMF groups before and after treatment.

Patient	CsA group		MMF group	
	Before (ml/min)	After (ml/min)	Before (ml/min)	After (ml/min)
1	80	86	60	76
2	68	96	62	86
3	68	76	68	72
4	90	110	70	82
5	86	92	66	92
6	88	102	86	96
7	68	78	87	106
8	70	88	80	84
9	88	96	62	76
10	60	66	80	86
11	80	89	70	88
12	70	82	82	90
13	80	81	71	82
14	66	70	83	86
15	68	80	81	92
16	69	84	-	-
Mean ± SD	74.94 ± 9.48	86 ± 11.49	73.87 ± 9.27	86.27 ± 8.55
Statistical analysis				
Cyclosporine before vs after			p < 0.01	
Mycophenolate mofetil before vs after			p < 0.001	
Cyclosporine before vs Mycophenolate mofetil before			p > 0.10	
Cyclosporine after vs Mycophenolate mofetil after			p > 0.10	

Table-IV: UTP values in CsA & MMF group before and after.

Patient	CsA group		MMF group	
	Before (gm/day)	After (gm/day)	Before (gm/day)	After (gm/day)
1	8.0	0.42	2.4	0.30
2	7.2	0.36	2.7	0.25
3	2.2	0.74	6.2	1.40
4	2.3	0.82	7.5	0.85
5	2.9	0.28	7.0	0.86
6	3.2	0.48	3.1	0.45
7	3.5	0.36	4.0	0.82
8	4.1	0.86	2.4	0.66
9	2.5	0.22	3.2	0.92
10	5.2	0.92	4.0	0.68
11	6.2	0.55	7.7	0.26
12	2.4	0.56	8.2	0.52
13	8.6	0.52	2.8	0.50
14	2.9	0.77	8.0	0.64
15	3.5	0.44	2.5	0.78
16	4.7	0.52	-	-
Mean ± SD	4.34 ± 2.11	0.55 ± 0.21	4.78 ± 2.33	0.66 ± 0.30
Statistical analysis				
Cyclosporine before vs after			$p < 0.001$	
Mycophenolate mofetil before vs after			$p < 0.001$	
Cyclosporine before vs Mycophenolate mofetil before			$p > 0.10$	
Cyclosporine after vs Mycophenolate mofetil after			$p > 0.10$	

Table-V: Systemic lupus erythematosus disease activity index (SLE DAI) in CsA and MMF group before and after.

Patient	CsA group		MMF group	
	Before	After	Before	After
1	09	2	16	2
2	16	1	09	2
3	08	2	08	1
4	08	2	08	7
5	08	2	09	2
6	08	2	08	2
7	06	2	08	2
8	09	2	08	2
9	15	2	09	6
10	10	2	10	6
11	10	2	10	2
12	09	6	09	2
13	08	6	10	1
14	10	2	08	2
15	10	2	08	2
16	10	2	-	-
Mean ± SD	9.63 ± 2.55	2.44 ± 1.42	9.2 ± 2.04	2.73 ± 1.91
Statistical analysis				
Cyclosporine before vs after			$p < 0.001$	
Mycophenolate mofetil before vs after			$p < 0.001$	
Cyclosporine before vs Mycophenolate mofetil before			$p > 0.10$	
Cyclosporine after vs Mycophenolate mofetil after			$p > 0.10$	

Table-VI: Remission time (in weeks).

Patient	CsA group	MMF group
1	12	19
2	Not in remission	18
3	16	22
4	15	23
5	17	No remission
6	Not in remission	No remission
7	18	23
8	19	18
9	20	22
10	13	19
11	18	22
12	13	19
13	17	23
14	18	No remission
15	15	23
16	16	-
Mean ± SD	16. 21 ± 2.39	20. 92 ± 2.11
	$p < 0.001$	

Table-VII: Summary of results.

Parameter	CsA group	MMF group
Number of patient	16	15
Remission rate	87.5%	80%
Remission time (weeks)	16.21 ± 2.39	20.91 ± 2.11
Urinary total protein (gm)	0.55 ± 0.21	0.66 ± 0.30
Creatinine clearance rate (ml/min)	86 ± 11.49	86.27 ± 8.55
ESR (mm at 1st hour)	29.94 ± 8.24	26.13 ± 8.13
SLE DAI	2.44 ± 1.42	2.73 ± 1.91

Table-VIII: Side effects of both groups.

Symptoms	CsA group	MMF group
GI symptoms	10 (62.50%)	10 (66.66%)
Hypertension	08 (50.00%)	00
Hypertrichosis	09 (56.25%)	00

treated with anti hypertensive drugs. Recommended anti-hypertensives were ACE inhibitor, Ca channel blocker, beta blocker. In CsA group 9 out of 16 patients reported with hypertrichosis. Gastro-intestinal symtoms were common in both groups, but did not require drug discontinuation.

Discussion

It is well known that remission rates following induction therapy for lupus nephritis is closely related to long term renal survival. The classical induction protocol recommended by National Institute of Health (USA)-prednisolone with monthly pulses of intravenous Cyclophosphamide (IV CPM 0.75-1.0 g/m^2) for 6 month followed by quarterly pulses for an additional 2 year or for at least 1 year after renal remission. It is widely used because it produces high remission rate and therefore offers good long term prospects for preserving kidney function. Unfortunately, several patients do not achieve renal remission after 4th cycle of therapy with intermittent boluses of IV CPM and become refractory to CPM and need to be treated with other immunosuppressive agents like cyclosporine (CsA), mycophenolate mofetil (MMF), azathioprine. Cyclosporine and mycophenolate mofetil, are newer immunosuppressive drugs initially was used in organ transplantation, has been now used increasingly in autoimmune diseases and immune related disease of kidney like lupus nephritis. Cyclosporine reversibly inhibit T-helper cell function by blocking the intracellular signaling cascade of T cell activation & transcription of T-cell specific cytokines, such as IL-2[5].

Tokuda el al[6] reported that low dose CsA (3.5mg/kg/day) with concomitant use of corticosteroids could reduce disease activity index along with a reduction in lupus serologies. They also did not observe any nephrotxicity at this dose consistent with the results of present series. Transient nephrotoxicity was observed by Deteix & Feutran et al[7,8] in 7 out of 16 patients and hypertension in 7 out of 12 patients treated with a dose up to 10mg/kg.

Miescher published their results for the longer time use of CsA in 14 SLE patients with nephritis. Kidney biopsies were performed after 17 months of therapy and no significant acute or chronic CsA toxicity was seen[9]. This was confirmed in a recent study of Dostal et al[10]. Eleven patients with biopsy proven lupus nephritis were treated for 1 year with a starting dose of 5mg/kg/day of cyclosporine. On rebiopsy, 3 patients had their WHO Class altered from IV to III & 5 patients changed their status from high severity grade to low severity. No significant CsA related changes were seen on renal biopsy. There was no significant increase in patients' baseline serum creatinine value, 45% of patient did experience hypertension but this responded to antihypertensive and allowed the continuation of CsA.

An advantage of CsA is that, it does not influence polymorphonuclear leucocytes or macrophages and therefore carries a low risk of infection[11]. So, no severe infection was observed in the present study during CsA therapy. Inspite of its immunosuppressive effects CsA does not increase the incidence of solid neoplasm like CPM[12]. Total 87.5% of reported patients achieved a long term control of the disease with low dose CsA and additional low dose steroid treatment without major side effects that necessitated interruption of reported treatment protocol.

MMF is quite effective and generally well tolerated, safe & successful in clinical trial, especially in Class IV lupus nephritis refractory to traditional therapies[13,14]. MMF inhibits inosine monophosphate dehydrogenase,

restricting B & T lymphocyte proliferation, antibody production & expression of proinflammatory adhesion molecules on lymphocytes[15].

In the study of Li et al[16], 23 patients with diffuse proliferative glomerulonephritis refractory to treatment with steroid & CPM were treated with MMF 1.0-1.5 g/day. Over a follow up period of 9 months, the 24 hr urinary protein fell from 3.88 to 0.75 gm & the serum creatinine from 178.4 to 94.5 μmol/L. In another study of Chen et al, 6 patients with lupus nephritis (WHO Class-IV) were treated with prednisolone and MMF 1-1.5 gm/day for 6 months and then 0.75-1.0 gm/day till remission. Five out of 6 patients responded with a significant improvement in renal function & proteinuria[17]. Hu & colleagues conducted a 6 month comparative trial in 46 patients with Class-IV lupus nephritis, concluding that MMF is more effective than IV CPM, in reducing proteinuria, haematuria, anti-ds DNA antibody titre and improving renal histology in renal biopsy[18]. Fu et al, describe 2 Chinese children with lupus nephritis refractory to CsA and CPM who responded to MMF treatment. All clinical symptoms disappeared, even serum auto antibody become negative after 12 months of treatment[19]. Although MMF is a promising agent as induction & maintenance therapy of lupus nephritis with less side effects on fertility than CPM, there are several cases in which MMF could not prevent renal lupus flare[20]. Dooley et al, reported 12 patients with relapsing or resistant nephritis previously treated with CPM therapy[21]. There was reduction in urinary protein creatinine ratio. Chan et al,[22] reported comparable results when studying the use of MMF compared with oral CPM for induction of remission in Class-IV lupus nephritis. 81% of patients treated with MMF achieving complete remission which is similar to results of present study compared with 76% in CPM group with a significant reduction of 24 hours proteinuria in both groups. Ginzler has compared MMF with the NIH regimen of pulsed IV CPM for induction therapy of active lupus nephritis (Class-III,IV,V) with increased remission rate (complete & partial) in the MMF group. The authors concluded that MMF was as effective as their standard regimen for induction therapy in proliferative lupus nephritis[23].

In this study, 15 patients of Class-IV lupus nephritis had been treated with MMF. It was found that MMF was effective in reducing SLE DAI score, ESR, anti-ds DNA, 24 hours total urinary protein & increase complements levels over the course of follow up. It is evident from this study that both the drugs can reduce disease activities & improve immunological parameters. So, both cyclosporine & mycophenolate mofetil are effective in the treatment of cyclophosphamide refractory Class-IV Lupus Nephritis. The study also shows a better remission rate with cyclosporine where the incidence of minor side effects are also higher. So, a close drug safety monitoring is always required.

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