Outcome of Myasthenia gravis treated with high-dose prednisolone and azathioprine: A single centre ambispective study from India

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Abstract

Purpose: Myasthenia gravis (MG) is treated with many disease modifying therapies, namely corticosteroids, thymectomy and immunosuppressants, alone or in various combinations. But still, till today no consensus over the optimum therapy for MG has been made.

Methods: Out of total 101 patients with MG, 37 patients fulfilled the inclusion criteria and in them we ambispectively studied factors affecting the outcome in MG treated, to induce leukopenia, with prednisolone (PSL) plus azathioprine(AZA), from January 1993 through July 2014. Patients were grouped according to the outcome: pharmacological remission (PR), complete stable remission (CSR), non-remitter and remitters with or without relapse. Their demographic characteristics, MGFA Class, dose of PSL and AZA, time to achieve remission, duration of remission, leukocyte counts, thymus status, follow-up duration, results of repetitive nerve stimulation, and side effects profile were compared.

Results: Total 81% patients remitted; PR (83%) was commoner than CSR (p=0.003). Factors favoring remission were early onset disease, therapeutic leukopenia (p=0.003) and longer follow-up (OR5, p=0.08); those associated with relapse were abnormal thymus (CI-1.1-3.4; p=0.09), MGFA class IIb (CI 0.9-3; p=0.09) and male gender. Side effects occurred in 48%. Conclusion: Aggressive therapy with prednisolone plus azathioprine induces remission in a high percentage of patients with generalized MG.

Keywords: Myaesthenia Gravis; Remission; Relapse; Immunosupression; Prednisolone; Azathioprine; Pyridostigmine

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INTRODUCTION

Like many other diseases of autoimmune etiology, acquired MG is a potentially treatable disease[1]. During last four decades the quality of life and survival of these patients has showed significant betterment with availability of symptomatic treatment (pyridostigmine, PyD) and disease modifying interventions (prednisolone{PSL}, thymectomy, and immunosuppressants)[2]. But even after several decades of research the optimum treatment

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for MG still remains unsettled. Answers are still being explored for whether PSL and immunosuppressants should be initiated simultaneously or later should be offered to only those who fail to respond to lone PSL treatment and whether thymectomy should be restricted to only those with thymoma or offered to all surgically fit patients of MG[3-6]. Further, the researchers have applied different clinical outcomes measures for evaluating above mentioned therapies. Recent researchers comparing PSL and Azathioprine (AZA) or PSL and thymectomy have targeted a 'minimal manifestation status' (MM) wherein the patients are permitted to use PyD for symptom control as against earlier ones who strived to achieve a PyD free stage with immunomodulators[6,7].

Underlying the clinical symptoms of MG is poor transmission of impulses across the neuromuscular

Table 1. Patient characteristics

Variables		Study population (n=37)	p value
Gender (number/percentage)			
Male		20/54.05	0.4 (ns)*1
Female		17/45.94	
Age			
Mean age at onset (years±SD)		43±19.03	
Early onset MG (number/ percentage)		22/59.4	0.1 (ns)*
Late onset MG (number/ percentage)		15/40.6	
Mean duration of disease prior to start of therapy (months±SD)		16.46±30.05	
AchRAb Status(number/percentage)			
Positive		16/43.24	
Negative		3/8.1	
Not known		18/48.64	
Thymus gland(number/percentage)			
Normal		28/75.67	0.006 (s)*
Thymoma and Thymic Hyperplasia		9/24.33	
MGFA classification (number/percentage)			
IIa		28/ 75.67	
IIb		7/18.91	
IIIa		1/2.70	
V		1/2.70	
Preexisting T2DM (number/percentage)		6/16.2	
Decrement upon Repetitive Nerve Stimulation			
Mean RNS decrement (±SD)		21.25±13	
Borderline decrement (number/percentage)		4/10.8	
Intermediate decrement (number/percentage)		23/62	
Gross decrement (number/percentage)		10/27	
Mean Follow up (months± SD)		67.3± 56	
Mean prednisolone dose (mg±SD)		19.25±12.35	
Mean azathioprine dose (mg±SD)		119.6±38.24	
MG Crisis (number of patients/percentage)		5/13.5	
Deaths (number of patients/percentage)		3/8	
Untoward effects 0f therapy (number/percentage)	New onset hypertension	7/18.9	
	New onset hyperglycemia	1/2.7	
	Depression	13/35	
	Cataract	9/24	
	Secondary infections	12/32.4	
	Weight gain	3/8	
	Glaucoma	2/5.4	
	Acne	5/13.5	

±SD; ns= not significant; s= significant; *Comparison of proportions (chi square)

Table 2. Determinants of Remission

Variables Study population (n=37)	Remitters n=30 (81%)	Non remitters n=7 (18.9%)	Significance
Gender(number/percentage)			
Male	17/56.66%	3/42.85%	RR= 0.63 p= 0.50 (ns)[1]
Female	13/43.33%	4/57.1%	
Age			
Mean Age at onset (years ± SD)	42.33±19.14	45.85±19.89	p=0.66(ns)*
Early onset MG (number/percentage)	20/66.6%	2/28.57%	OR 5; CI 0.8-30.4; p=0.08 (ms)[2]
Late onset MG(number/percentage)	10/33.4%	5/71.42%	
Mean Duration of disease prior to start of therapy (months± SD)	16.93±40.95	16.58±35.86	p= 0.67 (ns)*
Thymus gland(number/percentage)			
Normal	23/63.3%	5/71.42%	OR 1.31; CI 0.2-8.3 p=0.77
Thymoma and Thymic Hyperplasia	7/23.3%	2/28.57%	
MGFA class(number/percentage)			
IIa	23/76.66%	5/71.43%	RR= 1.6, CI 0.3-6.5 p=0.51 (ns)
Iib	5/16.66%	2/28.57%	
IIIa	1/3.33%	0	
V	1/3.33%	0	
RNS Decrement			
Mean Decrement (± SD)	20.94±13.28	22.57±13.56	p= 0.77 (ns)*
Borderline RNS-Dc (number of patients)	4	0	
Intermediate RNS-Dc (number of patient)	19	4	
Gross RNS-Dc (number of patients)	7	3	
Mean WBC Counts/ mm3(mean± SD)			
Baseline	10334±3142.3	----------	p=0.003(s)3*
Lowest during follow up	7994±2863.2		
Baseline	-----	11200±3596	p=0.19(ns)*
Lowest during follow up		8183±4605.5	
Mean Follow up (months ± SD)	76.25±60.25	34.03±26.38	p= 0.08 (ms)*
Mean prednisolone dose (mg± SD)	17.5±11.8	25±15.5	p=0.15 (ns)*
Mean azathioprine dose (mg± SD)	120±39.06	117.85±37.40	p=0.89(ns)*
Mean Time to TOPs (months ± SD)	4.67±3.43	NA	
Median Duration of Remission (months)	24	NA	

[1]Non significant; *proportion of means; [2]marginal significant; [3]significant

junction (NMJ) due to quantitative or qualitative changes in acetylcholine receptors (AchR) located on its postsynaptic membrane. Antibodies directed against these AchR (AchR-Ab) are implicated in the pathophysiology of acquired MG. These AchR-Ab bind and cross link the AchRs leading to compliment mediated breakdown of postsynaptic membrane and a marked reduction in number of AchRs available to respond to the quanta of acetylcholine released from nerve terminals. This leads to reduction in safety factor of NMJ, defined as ratio of number of acetylcholine quanta released to the number of quanta required to initiate an action potential. PyD, a reversible acetylecholinesterase inhibitor, slows down the hydrolysis of acetylcholine in synaptic cleft, resultant accumulation of acetylcholine in synaptic fold increases the safety factor thereby facilitating generation of an action potential[8].

PSL and AZA are commonly used agents for treating autoimmune disorders, and have different mode of action. PSL inhibits the antigen processing thus impairs the

Table 3. RNS decrement and remission

n= 30	Borderline RNS decrement (n=4)	Intermediate RNS decrement (n=19)	Gross RNS decrement (n=7)
Mean maintenance dose of PSL (± SD)	27.5±16.5	17.64±11.071	16.07±11.51
Mean dose of AZA (± SD)	137± 63	115± 34.52	121±392

[1]compared to borderline group the difference was insignificant (p=0.15 & 0.2); [2]compared with borderline group the difference was not significant (p0.3 & 0.6)

Table 4. TOPs in remitters (n=30)

	TOPs (mean, months ± SD)	p value
Gender (number)		
Male (n=17)	4.89±3.5	p= 0.6 (ns)
Female (n=13)	4.22±3.31	
Age (number)		
EO (n=20)	4.84±3.95	p=0.62(ns)
LO (n=30)	4.19±1.96	
MGFA Class (number)		
IIa (n=23)	4.83±3.62	p=0.3(ns)
IIb (n=5)	4.03±2.86	
Thymus status(number)		
Normal (n=23)	4.03±2.86	p=0.09 (ms)≠
Abnormal (n=7)	6.46±4.53	
RNS-Dc (number)		
Borderline (n=4)	3.69±1.65	
Intermediate (n=19)	4.64±4.33	p=0.4 (ns)¶
Gross (n=7)	2.47±2.84	p=0.9(ns) ¶

non-significant;≠mildlysignificant¶ compared with borderline

functions of monocyte-macrophage lineage. In addition it also inhibits activation of T-cells. AZA interferes with the cell cycle such that T-cell and B-cell proliferation gets inhibited[9]. While treating auto-immune diseases immunosuppressants are used, in dosage sufficient to control the clinical symptoms and underlying immuno-pathology. The effective dosages of immunosuppressants are guided by either blood levels of their metabolites and/or by monitoring haematological changes[10] produced by these agents. WBC counts and red blood cell (RBC) volume has been used as surrogate marker of adequate immunosuppression and have been found to correlate with the blood levels of AZA metabolites[11,12]. AZA has been used for treatment of MG but haematological parameters are rarely the target. We are aware of only one study utilizing blood parameters to achieve effective immunosuppression[13]. Thus responsiveness to PyD, in context of MG under treatment, means targeting of suboptimal control over autoimmune mechanisms responsible for reduced safety factor and paucity of AchR at NMJs. Even then MM status, where patients still require PyD for symptom relief, is being targeted probably as a trade-off between quality of life and complications of immunosuppressants[6,7]. This essentially means acceptance of inadequate control over the pathophysiological mechanisms and could be one of the

Table 5. Characteristics of PR and CSR

Remitters	PR n=25 (83%)	CSR n=5 (17%)	p= value 0.003(s)
Gender (number/percentage)			
Male	13/52%	4/80%	
Female	12/48%	1/20%	
Age			
Mean age at onset, (years± SD)	44.48±18.18	31.6±20.42	
Early onset MG (number/percentage)	16/64%	4/80%	
Late onset MG(number/percentage)	9/36%	1/20%	
Mean duration of disease prior to start of therapy (months± SD),	18.69±43.20	13.47±19.91	0.79 (ns)
Thymus Status (number/percentage)			
Normal	16/64%	3/60%	
Abnormal	5/20%	2/40%	
MGFA classification (number/percentage)			
IIa	20/80%	3/60%	
IIb	4/16%	1/20%	
IIIa	0	1/20%	
V	1/4%	0	
Mean RNS decrement (± SD)	20.68±13.83	22.14±8.27	0.82(ns)
Median follow up duration(months)	56	46	
Number of patients who relapsed	12	4	
Median duration of remission (months)	23.8	28.1	

reasons for reported lower rates of remission in patients treated with immunosuppressants.

Given that our understanding of prevention and management of infections has bettered over time and both PSL and AZA are readily available agents for immunomodulation in MG, we sought to investigate the outcome of our patients who were treated with a combination of these agents. We aimed at obtaining PyD free control of symptoms by using AZA in dosage leading to a reduction in WBC counts. We were particularly interested in identifying the factors affecting remission, relapse, and untoward effects.

MATERIAL AND METHODS

The study was conducted between April 2014 and December 2015 at Sri Aurobindo Institute of Medical Sciences and Post graduate Institute, Indore, India, a tertiary care teaching hospital in the rural setting, established in the year 2005. We retrospectively studied the case records of patients who were diagnosed with MG from January 1993 through July 2014 and who did not receive disease modifying therapy before visiting us. Total 101 such patients could be identified. These patients were treated consecutively with oral PSL and AZA and were followed up prospectively by one of the authors (AKS) initially at his private practice and eventually in this department after the year 2005.

MG was suspected on presence of exertion-induced muscle weakness improving with rest[14]. MG was diagnosed if clinical suspicion was supported by at least one of the following: a) decrement of 10% or more on repetitive nerve stimulation (RNS-Dc), b) sustained improvement in muscle weakness after administration of intramuscular neostigmine or c) high titres of AchR-Ab.[15-17] RNS was performed using five stimuli of supramaximal constant current at 3 Hz, responses were recorded using surface EMG electrodes. Decrement in CMAP amplitude of first and third, fourth or fifth response was noted[16]. In case of decrement less than 10%, after one minute exercise of the target muscle the stimulation was repeated immediate, one and three minutes post exercise. Procedure

Table 6. Determinants of Relapse

Parameters	Remitter (n=30) Type I Remission n=14 (46%)	Type II Remission n=16 (54%)	Significance
Gender (number/percentage)			
Male	6/42.9	12/75	OR 4, CI 0.8-18; p=0.07 (ms)1
Female	8/57.1	4/25	
Age			
Mean age of onset, (years± SD)	45.21±19.08	39.81±19.44	0.45(ns)
Early onset(number/percentage)	8/57.1	12/75	OR 2.2, CI 0.4-10.5, p=0.3 (ns)2
Late onset(number/percentage)	6/42.9	4/25	
Thymus gland status (number/percentage)			
Abnormal	1/7.1	6/37.5	RR 1.97, CI 1.1-3.4; p=0.01(s)
Normal	13/92.9	10/62.5	
MGFA class (number/percentage)			
IIa	12/85.71	11/68.7	RR=1.6, CI 0.9-3; p=0.0.09 (ms)
IIb	1/7.14	4/25	
IIIa&V	1/7.14	1/6.25	
Mean RNS decrement, (± SD)	18.80±15.90	22.81±10.67	0.41(ns)
Mean duration of disease prior to start of therapy, (months± SD)	7.3±11.3	26.79±52.97	0.19(ns)
Mean PSL dose (mg± SD)	17.14±12.62	17.65±11.56	0.9(ns)
Mean AZA dose (mg± SD)	125±31.62	114.28±46.73	0.46(ns)
Mean time TOPs (months± SD)	3.91±2.16	5.2±4.18	0.30 (ns)
Median duration of remission before relapse (months)	Not applicable	19	

± SD; [1]ms=mild significant; [2]non-significant;*Total 25 events of relapse

was done on Nicolet, Viking Quest, USA, version 12.0.0

Following information was extracted from the case records –

a) age at onset of the symptoms, b) gender, c) initial symptoms and signs and severity thereof, d) delay between the first symptoms and initiation of therapy, e) time lapse between initiation of therapy and withdrawal of PyD, f) time to achieve remission, g) untoward effects, h) status of thymus gland, i) Presence or absence of AchR antibodies, j) Values of repetitive nerve stimulation (RNS), k) Hemogram and biochemistry including liver function tests, renal profile and electrolyte reports during follow-up.

Patients with pure ocular MG (MGFA Class I), follow-up of fewer than 12 months, revision of diagnosis during follow-up and those who refused therapy, were not included for analysis.

2.1 THERAPY PROTOCOL

All patients received PyD, the dose of which was titrated so as to achieve maximum possible symptomatic relief. Oral PSL and AZA (hereafter referred as IS) were introduced simultaneously, former at a dose of 1mg/kg/day with breakfast daily and later at dose of one mg/kg/day, divided and given twice a day with meals. Dose of AZA was escalated on monthly basis till patient entered in remission or limits of dose (3mg/kg) reached or haematological changes precluded further increase of doses. WBC reduction and increase in RBC volume was desirable, former was kept above 3000 per mm^3 and later

between 98 to 100 fl.11 In case of counts falling below or RBC volume reaching the intended targets, AZA was withheld till improvement of the blood picture.

High dose of daily steroid was continued till the PyD could be withdrawn without any symptom relapse. On achieving this status attempts were made to switch to alternate day regimen, for which the ongoing daily dose of steroid was doubled and given on alternate days. Subject to stabilization of clinical symptoms for six months a slow taper (5 mg every 6-8 weeks) of PSL was attempted, till it could be stopped or the symptoms relapsed. In case of relapse the steroid was increased to the last effective dose. Another attempt to taper was made after several months. If the patient continued to do well without PSL, slow taper of AZA was initiated after 12 months of steroid withdrawal. AZA was tapered at the rate of 50 mg every 3 months. In case of deterioration, PSL was reintroduced to the smallest possible dose and slowly escalated till the remission was achieved; AZA was also readjusted to achieve the haematological criteria as mentioned above.

In patients who failed to remit, the dose of IS was adjusted to the minimum possible so as to control MG symptoms with 240 mg or less of PyD. Patients with diabetes were treated on similar lines. Blood glucose was tightly controlled with help of an endocrinologist.

Secondary infections were treated aggressively with appropriate antimicrobials and temporary reduction of PSL by fifty percent of current dose and withdrawal of AZA. IS therapy was reinstituted in full dose after the control of infection.

Patients with MG crisis were given standard treatment comprising of respiratory support, immuno-globulins and/ or plasmapheresis.

All patients were advised to take potassium rich diet for initial four weeks, oral potassium supplements were prescribed only to those with documented hypokalemia. Intramuscular injections of Vitamin D3 (6 lac units per week for four weeks every 6 months). Calcium supplements were given daily while bisphosphonate agents were prescribed yearly. In addition, they were advised to engage in weight bearing activities[18,19].

Patients younger than 45 years were given an option to consider thymus gland removal, a procedure compulsorily performed in patients with thymoma. Post thymectomy the remission was achieved using IS, as per the procedure detailed above.

The study was approved by the institutional scientific and research committee. Consent for retrospective analysis was not required, but a written consent was obtained from new patients registered after April 2014. Patients were counseled about maintaining hygiene and precautions for protection from cross infections and the importance of strict follow-up and adherence to the prescribed treatment.

2.2 DATA MANAGEMENT

2.2.1. Patients were grouped as per outcome into remitters and non-remitters. The remission was divided into pharmacological remission and complete stable remission[20]. Remitters were also sub grouped into Type I remitters and Type II remitters.

2.2.2. Following data was obtained from the clinical records: gender, age at onset of symptoms, duration of disease prior to initiation of therapy, thymus status (normal or abnormal thymus), MGFA classification at the time of initiation of treatment, AchR-Ab status (positive/negative/ not known), time to achieve off PyD status (TOPs) and follow up duration[21-23].

2.2.3. WBC counts at initiation of therapy and lowest count at the time of PyD withdrawal were noted in remitters. In non-remitters, the lowest WBC count after 6 months (or later) of treatment initiation was noted. Difference between two counts was calculated for both groups[24,25].

2.2.4. Doses of PSL and AZA required for maintaining remission were noted. In non-remitters, lowest dose of both the agents needed to keep requirement of PyD at or below 240 mg/day was noted.

2.2.5. Untoward effects of therapy (new onset hypertension, new-onset hyperglycemia, cataract formation, glaucoma, weight gain, secondary infections, depression, acne) myasthenia crisis and death were noted.

2.2.6. Values of RNS-Dc were noted. Patients were arbitrarily classified according to the percentage of decrement into borderline (less than 10%), intermediate (11-30%) and gross (more than 30%).

2.2.7. Parameters definitions 21

1. Early onset myasthenia gravis (EOMG): onset at age younger than 50 years.

2. Late onset myasthenia gravis (LOMG): onset after 50 years of age.
3. Thymus status: as judged on posteroanterior and lateral X-ray chest prior to the availability of CT scan facility in town till the year 2000, and/or HRCT chest post availability of CT imaging[26].
4. Maintenance dose of immunomodulators (MD-IS): Lowest dose of PSL+AZA required on which patients remains asymptomatic, with or without PyD.
5. TOPs: Time elapsed between initiation of IS and ability to withdraw PyD, without symptom relapse.
6. Complete stable remission (CSR): When patient is devoid of any symptoms or signs (exercise-induced muscle weakness) of MG, for 12 months or more, after withdrawal of IS and PyD.
7. Pharmacological remission (PR): When patients on MD-IS, has no symptom or sign (exercise induced muscle weakness) of MG even after six months from withdrawal of PyD. But the symptoms relapse on withdrawing IS.
8. Type I remission: Patients who had no relapse of symptoms after having achieved remission (CSR or PR).
9. Type II remission: Patients who had relapses after achieving remission (PR or CSR).
10. Non remission (NR): When patient requires PyD, despite MD-IS as per the outlined protocol.
11. Relapse: Resurgence of PyD responsive muscle fatigability, after stable PR for six months (on MD-IS) or CSR for one year. Number of relapses and cause thereof were noted. Treatment protocol required induction of a relapse during the process of finding minimum effective dose of steroid (iatrogenic), therefore this episode was not incorporated while counting relapses.
12. Weight Gain: Weight gain of ≥5% at the end of follow up from the initiation of therapy.

2.3 DATA ANALYSIS

Effect of age, gender, MGFA class, RNS-Dc, duration of MG prior to starting therapy, status of thymus gland, duration of follow-up, mean dose of PSL, maximum dose of AZA, difference between initial and later WBC counts and TOPs on outcome were analyzed using MedCalc (version 16.8), an online statistical software[27].

Chi-square test was applied for categorical variables and Univariate odds ratio was calculated to assess the determinants of remission and relapse, amongst the study subjects. A p-value <0.05 was taken as statistically significant. p values up to 0.09 were considered to be a trend towards significance. The odds ratio was calculated with 95% confidence interval. To assess the positive and negative outcomes with respect to risk factors, the relative risk was calculated.

RESULTS

Out of 101 cases, (61 males, 40 females, mean age 44.5 years), 64(40 males, 24 females, mean age 47.7 years)were excluded for following reasons -follow-up of less than 12 months (n=31), intolerance to the medication or refusal of treatment (n=9), pure ocular (n=20), revision of diagnosis (n=4, had mitochondrial cytopathies).[28] Remaining (n=37) fulfilled the inclusion criteria and formed the study group, characteristics of them are given in table 1.

Males and females were almost equal in numbers (p=0.4). Mean age at onset of MG was 43 (±19) years. Patients with early onset of disease marginally outnumbered those with late onset, the difference was not significant (proportion of means, p=0.1).

Number of patients with radiologically normal thymus was significantly more than those with abnormal gland (28 v/s 9, the proportion of means, p=0.006). The abnormal thymus was surgically removed in eight of them, one patient, with small thymoma, refused surgery. None of the patients with radiologically normal thymus agreed for surgery.

Patients with moderately severe disease dominated the study population, at the initiation of therapy 80% patients belonged to MGFA class II (IIa-28 & IIb-7).

The median follow-up of the study population was 54 months (mean 67.3 ± 56, range 12.1 to 267 months).

Only 4 of 37 (11%) patients showed a RNS-Dc of

10 percent or less, remaining had decrement of more than 10 percent. In former, the clinical diagnosis of MG was supported by positive AchR-Ab (n=3) and positive neostigmine test.

The median duration of MG at the time of initiation of therapy for the study population was three months (mean 18.35 ±38.54 months).

Fifty-two events of untoward effects of therapy were recorded in 18 (48%) patients, 19 (52%) remained free of IS related side effects. Infections and depression were the most often encountered side effects.

Infections were mostly non-life threatening (respiratory tract, skin, and gastroenteritis) only a few with lower respiratory tract infection (n=3) were hospitalized for aggressive antimicrobial therapy.

Weight gain was fairly common during the initial phase of treatment (when patients received a high dose of PSL) but on MD-IS improvement was seen in almost all the patients except three of them (8%).

Five patients had MG crisis during the course of follow-up. The crisis was precipitated either due to infection or stoppage of IS despite repeated counselling sessions.

Total three patients died during the study period, only one of it was related to MG (crisis) other two died of cardiac causes.

The mean of lowest WBC counts in patients who suffered infection and those who didn't, was not significant (7316 mm^3 ±SD=3235 and 8150±3317; p=0.48). The average dose of AZA in patients with infection was 133± SD 34 mg, that in patients without infection was 113± 38.94, this difference was not significant (p=0.13). None of the patients with infection had pre-existing diabetes.

3.1 Remission:

Thirty patients (81%) achieved remission while 7 (19%) failed to remit (Table 2). Risk of non-remission did not differ between the genders (p= 0.5, RR 0.63).

The difference in the mean age at onset, between remitters and non-remitters, was not significant. However, significantly higher number of patients from early onset group had remission than those from late onset group (OR 5, p=0.08).

Thymus status did not affect the outcome significantly. There were nine patients with radiologically abnormal thymus, eight of them underwent thymectomy, one with small thymoma did not agree for surgery. Seven out of nine patients with abnormal thymus obtained remission.

Weak positive correlation between class IIa and remission was seen but this was not statistically significant.

The follow-up duration was significantly longer in remitters as compared to those who failed to remit (p= 0.08). Review of records revealed that non-remitters failed to comply with the suggested treatment regimen.

The difference between mean RNS-Dc in remitters and non-remitter was not significant (p= 0.77). Out of 30 remitters, 63.33% (n=19) had intermediate decrement while 23.34% (n=7) had gross decrement. The dose of MD-IS did not differ significantly between three groups of RNS-Dc (Table 3).

Eighty percent of remitters were off PyD within a median time of 3 months. There was no significant difference in TOPs between genders, EOMG and LOMG, MGFA class and RNS-Dc groups. However, there was a mildly significant trend suggesting subjects with abnormal thymus take a longer time to go off PyD (Table 4).

The mean dose of oral PSL in remitters and non-remitters was 17.5±11.8mg (median 15 mg) and 25±15.5 mg (median 20 mg). The difference was not significant (p=0.15) and that of AZA was 120±39 mg per day (mode and median 100).

Attainment of the targeted leukopenia was significantly associated with induction of remission. Leukopenia was seen in remitters as well as non-remitters. Comparison of mean of initial and lowest WBC count during follow-up in non-remitters was not significant (p=0.23), which was highly significant in remitters (comparison of mean p=0.003)

PR was the most common outcome (PR 83%, CSR 17%). The dose of IS, duration of follow-up, delay in initiation of therapy and RNS-Dc were not significantly different between them (Table 5). Out of surgically treated patients with abnormal thymus two had CSR, four had PR. One who declined thymus removal achieved PR.

3.2 Relapse:

Type I remission was seen in 46%, while 54% had Type II remission. (Table 4)

Relapses were fairly common in both, PR and CSR.

Type II remitters relapsed after remaining in remission for a median period of 24 months (Table 5). Patients who entered CSR relapsed after spending 28 months (median time) in remission. Twelve of 25 PR relapsed after remaining stable on MD-IS for a median period of 23.68 months (mean 29.2 ± 18.36 months).

Males, patients with abnormal thymus and those with bulbar symptoms (MGFA IIb) were at higher risk of relapse (CI 0.9-3; p=0.09). There was no association of TOPs, RNS-Dc, and age of onset and duration of disease prior to the start of therapy with the risk of relapse.

Some patients had recurrent relapses. There were total 25 events of relapses in sixteen Type II remitters with twelve of them experiencing more the one relapse. Causes of relapse were tapering of steroids (56%), spontaneous (28%), self-cessation of medications and secondary infection (8% each). Males were at higher risk of recurrent relapses. Out of total relapses, 19 (76%) occurred in males and remaining six (24%) in females (p= 0.057). Thymus status was not linked with recurrent relapses.

DISCUSSION

In 1993, Gajdos et al, raised a possibility that severe forms of MG may respond better to treatment with PSL plus AZA rather than mono-therapy with one of them[3]. Since then one of us (AKS) started treating severe MG with combination of above mentioned pharmacological agents and aimed at achieving leukopenia, a parameter linked with better chances of remission in autoimmune disorders treated with AZA[25]. Presented is the outcome of the patients, seen between January 1993 through July 2014.

Our study shows that 81 percent of moderately severe MG patients remit on IS at last follow-up. These rates are much higher than previously reported rates of 15 to 43%. Previous researchers have consistently noted low rates of remission in patients treated with either PSL or some other cytotoxic agent.

As the facilities of antibody analysis was not readily available till recent past the AchR-Ab status could be tested in 51.35 % of the patients. We believe that this would not affect the results of our study because neither the presence nor the absolute concentration of antibodies precisely predicts disease class in all MG patients, nor does it accurately predict clinical disease course or therapeutic response in individual patients[29]. Also Gajdos et al in a randomized trial comparing PSL and AZA reported that around 70% of patients in either group obtain remission in the first year of therapy but 48% of them relapse later on[3]. Palace et al found that levels of AChR-Ab rise sharply in patients treated with PSL only at 2 years while the titres in PSL+AZA group fell consistently[4], Tindall et al in a randomized trial of cyclosporine with placebo also noted that decline in the titre of this antibody[30]. Thus in those treated with PSL and AZA from beginning high rates of remission are not unexpected .

It is known that immunosuppressive effects of AZA are progressively bettered over time. Heckmann et al evaluated high-dose PSL+AZA therapy, targeted to maintain blood counts at minimum safe levels, and at 24 months they found that only fifty percent patients remain in remission[13]. Median follow-up of our study population, remitters and non-remitters were 54, 56 and 40 months respectively and we found that higher chances of remission are linked with longer duration of follow-up.

The majority had PR (83%) while 17% obtained CSR (p=0.003), this observation agrees with that of previous researchers[7,31,32]. There was no difference in risk of non-remission between the gender. Also, mean duration of disease prior to initiation of IS did not predict chances of remission.

Fifty-nine percent of our patients had EOMG. We found that these patients have better odds of remitting, a finding in concordance with earlier reports[33-36].

Reduction in WBC count was significantly linked with chances of remission (p=.003, Table 2). It is known that steroid therapy initially causes an increase in WBC count and it gets stabilized[37]. As the effect of AZA takes over, there is proportional leukopenia suggestive of AZA effect in adequate dosage[11].

While Symonette et al found that RNS-Dc has a direct correlation with severity of MG[38], others did not find any correlation between it and clinical outcome[39]. We looked into the correlation of RNS-Dc with remission, relapse and mean MD-IS, but found no significant correlation (Table 2 & 3). Symonette et al has reported exclusively on RNS-Dc using proximal nerves in AChR-Ab positive subjects[38]. We, as Roy et al[39], included patients with RNS-Dc in distal and cranial muscles as well. Further, AChR-Ab

status was not known in many of our patients. Thus, relationship between RNS-Dc and response to therapy needs further evaluation.

We did not find linkage between thymus pathology and chances of remission. Abnormal thymus and longer duration of disease prior to initiation of therapy has been linked with poorer chances of remission by some[31,33,34] while others did not find any correlation between thymus pathology and outcome. Then in surgically oriented trials why thymectomized patients tend to have a better outcome than patients treated with IS alone, remains an enigma? Researchers comparing thymectomy and medical treatment have used IS therapy differently. Such that in patients failing after thymectomy end up receiving both PSL and AZA, and in non-responders to medical treatment the IS therapy does not get optimized[6,32,40]. Wolfe G et al targeted to achieve MM status while treating MG with thymectomy + PSL or PSL alone; AZA was used selectively in both the groups. They could meet their targeted clinical status in 67% of former and 47% in later group, based on which superiority of surgical treatment was claimed[6]. Kawaguchi et al looked into the outcome of patients treated with thymectomy and PSL. They also used IS on case to case basis and found that 34% thymectomized patients remitted as against 21% in medical group[40]. It is noteworthy that their 73% and 12% of the thymectomized received PSL and IS respectively while only 44% of medically treated patients received PSL. Palace J et al have demonstrated that MG treated with PSL alone tend to have lower remission (20%) as against 75% in PSL plus AZA group[4]. Gajdos P et al in 1993 has shown that PSL and AZA used as mono-therapy fail frequently in achieving remission, while combining the two improves the outcome favourably[3].

Mean TOPs in remitters was 4.67 ± 3.43 months and 36 % of them could be withdrawn off PyD in three months or less (range 0.7 to 15 months). We argued above TOPs could be a surrogate marker for the minimum time the IS agents require to assert their control over underlying immuno-pathology. As effects of AZA take long time to begin[11], thus TOPs, in effect, appears to be a measure of anti inflammatory[37] and lymphokine function alteration effects of PSL. Immunosuppresion of T-cells, located in thymus, by PSL therefore appears to be responsible for TOPs. It is further supported by our findings that patients with abnormal thymus had longer TOPs (Table 4).

As noted by Khadilkar et al[7], we also found that relapses are fairly common in MG. We found that after remaining in remission for a fairly long period (median 24 months) 53 % of them relapsed. Male gender, abnormal thymus status and patients with oropharyngeal symptoms had mild to moderate increased risk of recurrent relapse. Four of five CSR (80%) and 12 of 25 PR (48%) relapsed after remaining in remission for a median time of 28.16 months and 23.86 months respectively. Relapses were precipitated by infections (n=3) and self-cessation of treatment (n=3). MG crisis precipitated in one patient due to infection, proved fatal.

In our study, IS induced new onset hypertension and steroid induced diabetes incidence was 18.9% and 2.7% respectively. Our results were similar with those of Diaz et al[41] while the rates of diabetes in other studies varied from 9% to 33%[34,39,41]. The low rates of our study for new onset diabetes could be due to our alternate day regimen as well as our target to taper steroids to the lowest dose possible[42] and to the life style modification measures which we adhere to.

The main limitation of our study is its ambispective nature. The outcome was not assessed using any clinical score which might have resulted in higher assessment of remission rates. Non-availability of facility to test AchR-Ab and CT scanners during the initial phase of study period might have affected the quality of our study negatively.

CONCLUSION

Our study raises a possibility that moderately severe MG patients treated with PSL+AZA so as to achieve leukopenia have high rates of remission (81%), particularly if they are followed-up longer. Patients with early-onset disease have higher chances of achieving remission (OR 5, p= .08). PR is more frequently achieved with this regimen as compared with CSR (p= .003), but 54% of them relapse. Male gender, abnormal thymus status, MGFA class IIb are associated with higher risk of relapse. No life-threatening infections were encountered in our group of patients treated with high dose of immunosuppressants. Relation of RNS-Dc and that of TOPs needs further evaluation.

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Abbreviations:
AchR : Acetylcholine Receptor
AchR-Ab : Acetylcholine Receptor Antibody
AZA : Azathioprine
CSR : Complete Stable Remission
EOMG : Early Onset Myasthenia Gravis
EMG : Electromyography
HRCT : High Resolution Computerised Tomography
IS : Prednisolone + Azathioprine
LOMG : Late Onset Myasthenia Gravis
MD-IS : Maintainance Dose Immunosuppression
MG : Myasthenia Gravis
MGFA : Myasthenia Gravis Foundation of America
MM : Minimum Manifestation status
NMJ: NeuroMuscular Junction
NR : Non Remission
PR : Pharmacological Remission
PSL : Prednisolone
PyD : Pyridostigmine
RBC : Red Blood Cell
RNS :Repetitive Nerve Stimulation
RNS-Dc : Repetitive Nerve Stimulation Decrement
TOPs : Time to achieve Off Pyridostigmine status
WBC : White Blood Cell

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