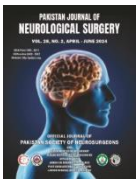

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Original Research

Azathioprine vs. Methotrexate Effectiveness in the Treatment of Generalized Myasthenia Gravis (MG)

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ABSTRACT

Objective: The current study investigated the effectiveness of Azathioprine versus Methotrexate in the treatment of generalized MG.

Materials & Methods: An observational, open-label retrospective study was conducted in the Department of Neurology, Mayo Hospital, Lahore, Pakistan. All generalized MG patients with positive acetylcholine receptor antibodies (MGFA class II, III, or IV) aged ≥16 years, were included. Group 1 (n=31) was taking the combination of oral Prednisolone and Azathioprine (AZA) and group 2 (n=31) was taking the combination of Prednisolone and Methotrexate (MTX). The clinical response was assessed by Myasthenia gravis activities of daily living (MG-ADL) score at the 3rd, 6th, 9th, 12th, & 18th months.

Results: At 3rd-month follow-up, the mean MG-ADL score was 2.97 (AZA) vs. 3.39 (MTX), after the 6th month, the score was 0.48(AZA) vs. 1.13 (MTX) (p-value=0.009)**,** after the 9th month: the score was 0.26 (AZA), vs. 0.97(MTX) (p-value=0.002), after the 12th month, the score was 0.29 (AZA) vs. 0.74 (MTX) (p-value=0.079), after 15th month, the score was 0.16(AZA) vs. 0.65(MTX) (p-value=0.009) and after 18th month, the score was 0.42(AZA) vs. 0.52(MTX) (p-value=0.703).

Conclusion: Azathioprine is significantly more efficacious from the 6th, 9th, 12th and after the 15th-month follow-up as compared to Methotrexate in the treatment of MG; however, on the 18th-month follow-up, both steroid-sparing drugs were equally effective. There appears to be no difference in the effectiveness of Azathioprine versus Methotrexate in the treatment of generalized MG.

Keywords: Azathioprine, Methotrexate, Generalized Myasthenia Gravis, Myasthenia Gravis Foundation of America (MGFA).

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INTRODUCTION

Myasthenia gravis (MG) is the most prevalent neuromuscular junction disorder. The illness is characterized by a varied involvement of the ocular, bulbar, limb, and respiratory muscles. The peak age of onset in females is 20-30 years, while in men it is 50- 60 years. The global prevalence of MG is around 20.6 instances per million people, with an annual incidence rate of 1 per 300,000 people.[1-2] The general in-hospital mortality rate is 2.2%, with the MG crisis having a higher rate (4.47%). Death was predicted by age and respiratory failure.[3-4] If properly treated, the prognosis of MG patients is typically positive, with increased quality of life. The condition -is caused by helper T-cell-induced antibody production, which targets the nicotinic acetylcholine receptor (AchR), fixing complement and decreasing the amount of AChR at the postsynaptic membrane over time.[5] The use of corticosteroids has transformed the treatment of MG. Because steroids have dangerous adverse effects when used for an extended length of time, steroid-sparing drugs must be used to reduce the dose-related side effects of corticosteroids. Azathioprine is being utilized as a first-line immunosuppressant in MG patients.[6] Because of the lack of cost-effectiveness and multiple side effects, its use has been limited, so other immunosuppressants, such as Methotrexate, can be used as steroid-sparing agents, as multiple trials have shown similar efficacy and reduced side effects as compared to Azathioprine in generalized MG. The benefits of Methotrexate include a once-weekly oral dose, ease of availability, and the capacity to be used for extended periods.

MG is an acquired autoimmune condition characterized by an antibody-mediated blockage of neuromuscular transmission, which results in skeletal muscle weakening. When autoantibodies develop against the nicotinic acetylcholine postsynaptic receptors in the neuromuscular junction of skeletal muscles, an autoimmune assault occurs.[6] During acute flares of the illness, plasmapheresis and high-dose intravenous immunoglobulin may be employed. Mechanical ventilation may be necessary if the breathing muscles become severely weak. Acetylcholinesterase inhibitors may be briefly retained once intubated to minimize airway secretions.[7] Most cases of MG are idiopathic. Although the exact reason for its emergence is unknown, the final effect is a disruption in immune system control. IgG to AChR is found in up to 90% of generalized cases. Anti-AChR antibodies can sometimes be found in persons who do not develop clinical myasthenia [8]. Several medicines, including the following, can cause or worsen MG symptoms. Penicillamine can cause myasthenia, with raised anti-AChR antibody titers reported in 90% of instances; however, the weakening is moderate, and full recovery is reached weeks to months after discontinuation of the medication.[9,3] Symptomatic treatment includes acetylcholinesterase inhibitors, rapid immunomodulating (short-term) treatment with plasmapheresis & intravenous immunoglobulin, immunomodulating treatment (long-term) with glucocorticoids and other immunosuppressive drugs.[10] Acetylcholinesterase inhibitors are the first-line therapy for MG patients. Acetylcholinesterase inhibitors are used to treat symptoms by increasing the quantity of accessible acetylcholine at the neuromuscular junction. They do not affect illness development or prognosis. The most widely used medication is Pyridostigmine. It has a quick beginning effect and reaches maximal activity in around two hours. The impact lasts around three to four hours. The cholinergic characteristics of Pyridostigmine cause the majority of the drug's adverse side effects, which include stomach cramps, bronchial secretions, nausea, and bradycardia. Plasma Exchange and Intravenous Immunoglobulin have a rapid onset of action and result in improvement within days, however, this is only temporary.[11]

Intravenous Immunoglobulins (IVIg) involves separating immunoglobulins from pooled human

plasma using ethanol cryoprecipitation and is delivered for 5 days at a dosage of 0.4 g/kg/day. IVIg's mode of action is complicated. Suppression of cytokine competition with autoantibodies and inhibition of complement deposition are two factors. Other ways include interfering with the binding of the Fc receptor on macrophages, the Ig receptor on B cells, and antigen detection by sensitized T cells. More precise immunoadsorption strategies for removing pathogenic anti-AChR antibodies have recently been discovered, allowing for a more tailored approach to MG therapy. In patients treated with immunoadsorption methods, clinical studies revealed a substantial reduction in blocking antibodies as well as clinical improvement.[12-13] IVIg is regarded safe, however, problems such as thrombosis owing to increased blood viscosity and other issues associated with high amounts of the infused preparation do occur in rare circumstances. Corticosteroids are the first and most widely used immunosuppressive drugs in MG. Prednisone is often used when cholinesterase inhibitors alone do not properly treat MG symptoms. An exacerbation can occur within the first 7-10 days after commencing large doses of prednisone and can linger for several days. Cholinesterase inhibitors are commonly used to treat this deterioration in moderate cases.[14-15]

Azathioprine (non-steroidal immunosuppressive drug), a purine analog, inhibits T- and B-cell proliferation by reducing nucleic acid synthesis. It has been used as an immunosuppressive medication in MG patients and is effective in 70%-90% of MG patients. The clinical response might take up to 15 months to notice. When used with Prednisone, it may be more effective and tolerable than Prednisone alone. Hepatotoxicity and leukopenia are two of the negative side effects.[16] Mycophenolate Mofetil inhibits purine production selectively, decreasing both T-cell and B-cell growth.[17] The typical dose for MG is 1000 mg twice a day, however, doses up to 3000 mg daily are possible. Higher dosages have been linked to myelosuppression. The medicine is not recommended during pregnancy and should be taken with caution in individuals with renal illness, gastrointestinal disease, and bone marrow suppression.[18] Cyclophosphamide, both intravenously and orally given, is an effective therapy for MG.[19] Cyclosporine inhibits the creation of IL-2 cytokine receptors and other proteins required for CD4+ T cell activity. Cyclosporin is mostly prescribed for people who do not tolerate or react to azathioprine. Its utility as a steroid-sparing drug has been substantiated by large retrospective investigations.[20]

Methotrexate is used to treat leukemia as well as some forms of breast, cutaneous, head & neck, lung, and uterine cancer. Methotrexate is also used to treat people with severe psoriasis and rheumatoid arthritis. It is also used to treat children with active polyarticular-course juvenile rheumatoid arthritis. Methotrexate is a low-cost immunosuppressant. Azathioprine, on the other hand, is regarded as a costly treatment in poor countries. Despite the need for less expensive MG medicines in impoverished nations, industrialized countries are looking into more expensive solutions.[21] Methotrexate is an effective immunosuppressant in target-organ autoimmune diseases including Crohn's disease and psoriasis, and it is the preferred disease-modifying medication in rheumatoid arthritis (RA). Methotrexate is a structural analog of folic acid that inhibits cell proliferation by interfering with DNA synthesis through metabolic interference [22]. Corticosteroids are the mainstay of treatment, along with steroid-sparing medications such as methotrexate (MTX), azathioprine (AZA), cyclosporine, mycophenolate mofetil, and cyclophosphamide. The conflicting data and inconsistent evidence of various oral immunomodulating drugs have motivated us to compare the effectiveness of AZA versus MTX in the treatment of generalized MG.

MATERIAL AND METHODS

Study Design & Setting

An observational, open-label retrospective study of a cohort of patients with generalized MG treated with the two most commonly used oral immunomodulating drugs i.e. Azathioprine and Methotrexate, was conducted in the Department of Neurology, King Edward Medical University (KEMU), Mayo Hospital, Lahore. The data was acquired from patient files in the Neurology department's medical record between mid-February 2019 and mid-July 2020, with the patient's permission. The study conformed to the institutional ethical standards and was approved by the Institutional Review Board of KEMU, Lahore.

Inclusion Criteria

This retrospective study included a sequential series of all patients of generalized MG over the study duration fulfilling retrospectively chosen inclusion criteria, which were ascertained by the reference to the patient notes. These criteria selected patients between 18 and 60 years of age with positive acetylcholine receptor antibodies (MGFA class II, III, or IV).

Exclusion Criteria

The following cases were excluded: myasthenic crisis, congenital myasthenic syndrome, inflammatory/non-inflammatory myopathies, thyrotoxicosis, spinal muscular atrophy, chronic liver disease, chronic kidney disease, interstitial lung disease, malignancies, pregnant and breastfeeding women.

Treatment Protocol

The Hospital's Ethics Committee agreed to the adopted therapy regimen. Clinical response after the treatment was assessed according to MG-ADL. Sixty-two generalized MG patients (31 in each group) of myasthenia gravis Foundation of America (MGFA) Class II, III, and IV, were included.

Group 1 (AZA) was treated with a combination of oral Prednisolone and Azathioprine. Azathioprine was started as follows: 50 mg daily for 2 weeks, 100 mg daily for 2 weeks, and then 150 mg daily afterward till the end of the study. Prednisolone was started at a dose of 1 mg per kg body weight (not more than 60 mg maximum) and continued for the first 12 weeks. At the beginning of the 13th week, gradual taper i.e., 5 mg per week was started and eventually tapering stopped at a 10 mg dose at the end of the 22nd week and this dose was continued till the end of the study. Clinical response was assessed by MG-ADL every tri-monthly.

Group 2 (MTX) was treated with a combination of oral Prednisolone and Methotrexate. Methotrexate was started as follows: 7.5 mg once a week for 4 weeks, 10 mg once a week for the next 4 weeks, and then 15 mg once a week afterward till the end of the study. The same protocol of initiation and tapering was followed for Prednisolone as described for group 1. Clinical response was assessed by the Myasthenia Gravis-Activities of Daily Living (MG-ADL) scores at the 3rd, 6th, 9th, 12th, 15th, & 18th months. The Activities of Daily Living (ADL) profile is a patient-reported eight-item questionnaire (see supplementary information) developed to measure MG symptoms and their influence on everyday activities (talking, chewing, swallowing, breathing, brushing, arising from a chair, & double vision, eyelid droop).[22]

Data Collection

On a designed proforma (see supplementary information), clinical response was evaluated using Myasthenia Gravis-Activities of Daily Living (MG-ADL) scores at the third, sixth, ninth, twelfth, fifteenth, and eighteenth months. MG-ADL scale is a patient-reported eight-item scoring system that was developed to measure MG symptoms and their influence on everyday activities (talking, chewing, swallowing, breathing, brushing, and arising from a chair, double vision and eyelid droop).

RESULTS

Background Information

The mean age of the patients was 29.90±11.46 years with minimum and maximum ages of 16 & 62 years, respectively. In AZA group-1, the mean age of the patients was 32.40±13.05 years while in MTX group-2, it was 27.81±9.32 years.

Among 62 patients, 32(51.61%) patients were male and 30(48.39%) were female. In the Azathioprine group, 19(61.3%) patients were male and in the Methotrexate group, 13(41.9%) patients were male. In Azathioprine group, 12(38.7%) patients were female and in Methotrexate group, 18(58.1%) patients were female **(Table 1)**.

Table 1: Distribution of gender between study groups.

		Study Groups		
		Azathioprine n=31	**Methotrexate n=31**	**Total**
Gender	Male	19	13	32
		61.3%	41.9%	51.6%
	Female	12	18	30
		38.7%	58.1%	48.4%
Total		31	31	61
		100.0%	100.0%	100.0%

Scores from Scale of MG-ADL

Tables 2-7 describe the overall mean, maximum, & minimum scores of MG-ADL after the 3rd, 6th, 9th, 12th, and 18th months.

Table 2: MG-ADL (Myasthenia Gravis - activities of daily living) score after 3rd month

MG-ADL Score After The 3rd Month	
n	62
Mean	3.18
SD	1.03
Minimum	2
Maximum	7

Table 3: MG-ADL score after the 6th month.

MG-ADL Score After The 6th Month	
n	62
Mean	0.81
SD	0.98
Minimum	0
Maximum	5

Table 4: MG-ADL score after the 9th month

MG-ADL Score After 9th Month	
n	62
Mean	0.61
SD	0.930
Minimum	0
Maximum	5

Table 5: MG-ADL score after the 12th month.

MG-ADL Score After 12th Month	
n	62
Mean	0.52
SD	1.004
Minimum	0
Maximum	6

Table 6: MG-ADL score after the 15th month.

MG-ADL Score After 15th Month	
n	62
Mean	0.40
SD	0.73
Minimum	0
Maximum	4

Table 7: MG-ADL score after the 18th month.

MG-ADL Score After 18th Month	
n	62
Mean	0.47
SD	0.998
Minimum	0
Maximum	6

Comparison of MG-ADL Score After 3rd Month Between Study Groups

Tables 8-13 mention the mean MG-ADL scores reported in both patient groups (Azathioprine & Methotrexate) along with the p values generated from the chi-square test for comparisons. There existed a significant difference (p-value=0.009) between the mean MG-ADL values in the 6th month in patient groups (Azathioprine vs. Methotrexate). There also existed a significant difference (p-value=0.002) between the mean MG-ADL values at the 9th month in patient groups (Azathioprine vs. Methotrexate). Similarly, there existed a significant difference (p-value=0.009) between the mean MG-ADL values at the 15th month in patient groups (Azathioprine vs. Methotrexate).

DISCUSSION

Azathioprine, Cyclophosphamide, Cyclosporine, Mycophenolate Mofetil, and Intravenous Immunoglobulin (IVIg) have all been tested in MG patients with varied degrees of.[23-28] Heckmann et al, (2011)[21] conducted a trial of MTX vs. AZA as steroid-sparing agents in generalized MG. According to this study, in the Azathioprine group, the mean age of the patients was 42.7 years while in the Methotrexate group, the mean age of the patients was 48 years. In the Azathioprine group out of 15 patients, 9 were females and 6 were males, similarly, in the Methotrexate group out of 16 patients 10 were females and 06 were males. In the current study, In AZA group-1, the mean age of the patients was 32 years while in MTX group-2, it was 27 years. In the Azathioprine group, 19 patients were male and in the Methotrexate group, 13 patients were male. In the Azathioprine group, 12 patients were female and in the Methotrexate group, 18 patients were female. Pasnoor et al, (2016)[29] mentioned that in the Methotrexate

Table 8: Comparison of MG-ADL scores after the 3rd month between study groups.

MG-ADL score		Study Groups		p-value
		Azathioprine	Methotrexate	
After 3rd Month	n	31	31	
	Mean	2.97	3.39	0.112
	SD	0.65	1.283	

Table 9: Comparison of MG-ADL score after the 6th month between study groups.

MG-ADL Score		Study Groups		p-value
		Azathioprine	Methotrexate	
After 6th Month	N	31	31	
	Mean	0.48	1.13	0.009*
	SD	0.51	1.231	

*Significant difference

Table 10: Comparison of MG-ADL score after the 9th month between study groups.

MG-ADL Score		Study Groups		p-value
		Azathioprine	Methotrexate	
After 9th month	n	31	31	
	Mean	0.26	0.97	0.002*
	SD	0.44	1.140	

*Significant difference

Table 11: Comparison of MG-ADL score after the 12th month between study groups.

MG-ADL Score		Study Groups		p-value
		Azathioprine	Methotrexate	
After 12th Month	n	31	31	
	Mean	0.29	0.74	0.079
	SD	0.74	1.316	

Table 12: Comparison of MG-ADL score after the 15th month between study groups.

MG-ADL Score		Study Groups		p-value
		Azathioprine	Methotrexate	
After 15th Month	n	31	31	
	Mean	0.16	0.65	0.009*
	SD	0.37	0.915	

*Significant difference

group, the median age of the patients was 66.5 years while in the placebo group, the median age of the patients was 68.6 years. In the Methotrexate group, 76% of patients were male and in the placebo group, 64% of patients were male.

In our study, in 3rd month both, groups showed insignificant differences between the study groups and MG-ADL scores. Significant differences exist in MG-ADL scores between groups at 6 months (AZA: 0.48 vs. MTX: 1.13), 9 months (AZA: 0.26 vs. MTX: 0.97), and 15 (AZA: 0.16 vs. MTX: 0.65) months. After the third month, the mean MG-ADL score was 2.97 (AZA) vs. 3.39 (MTX), after the sixth month, it was 0.48 (AZA) vs. 1.13 (MTX), after the ninth month, it was 0.26 (AZA) vs. 0.97 (MTX), after the 12th month, it was 0.29 (AZA) vs. 0.74 (MTX), after the fifteenth month, it was 0.16 (AZA (MTX). The current study compared the effectiveness of Azathioprine versus Methotrexate in the treatment of generalized MG. Azathioprine is considerably more successful than Methotrexate in the treatment of generalized MG at the 6th, 9th, 12th, and 15th month follow-ups; but, at the 18th month follow-up, both steroid-sparing medications were similarly beneficial. There is little difference between Azathioprine and Methotrexate in the treatment of generalized MG.[30-32] This drug is often used in combination with Prednisone. Although formal scientific evidence for its efficacy in MG is sparse, a controlled trial demonstrating the superiority of the combination of prednisone—azathioprine over Prednisone alone is often referenced. Methotrexate should be administered only when first-line immunosuppressive medications are ineffective.[33] Methotrexate is beneficial in other autoimmune illnesses, but it has not been fully studied for MG. Yet, because it is typically well tolerated, it should be attempted in selected MG patients with a significant functional loss. The majority of MG sufferers requires long-term treatment. A careful approach to complete medication removal is suggested for individuals in stable remission while on immunosuppressants. Low-dose prednisone, Azathioprine, or other immunomodulating drug may be adequate to maintain stable conditions in such individuals, but they are also required to avoid future exacerbations.[30] Recently, Menon & Bril (2022)[34] mentioned that Azathioprine is long-term safe and well tolerated, with a low incidence of adverse effects such as hematological, gastrointestinal, dermatological, and infectious. Beginning at 50 mg/kg/day for two weeks, followed by a progressive escalation to maximal dosages of 2-3 mg/kg/day, seldom leads to idiosyncratic responses, but periodic monitoring of peripheral blood counts and liver enzymes is essential.

Di et al, (2022)[35] conducted an open-labeled study of prednisone combination with MTX 10 mg orally once a week against prednisone alone in 40 newly diagnosed MG patients from MGFA Classes II and III. The results, showed that MTX has steroid-sparing potential in individuals with widespread MG who have MGFA Class II and Class III. Methotrexate, with its advantages of once-weekly dose, modest side effects, and low cost, is projected to be a steroid-sparing treatment for patients with mild to moderate generalized MG, particularly in financially challenged health systems. Pasnoor et al, (2016)[29] performed a randomized, placebo-controlled study of 50 generalized MG patients on steady prednisone dosages averaging 20 mg per day. In contrast to Heckmann et al, (2011),[21] discovered that adding MTX for 12 months against placebo resulted in no difference in the average daily prednisone dosage between the two groups from month 4 to month

Table 13: Comparison of MG-ADL score after the 18th month between study groups.

MG-ADL Score		Study Groups		p-value
		Azathioprine	Methotrexate	
After 18th Month	n	31	31	
	Mean	0.42	0.52	0.703
	SD	1.12	0.851	

12. Nevertheless, the research's selection of patients with modest severity (MGFA II and III) and short study period may have restricted its findings.

The advantage of Azathioprine was not shown until month 12 in the positive Azathioprine trial, which used the Prednisone dosage as the major endpoint. One of the mycophenolate study's post-hoc complaints was that it was merely a three-month trial. As a result, we have designed a 12-month study for this MTX. The favorable benefits of Azathioprine were not evident until 12 months in the azathioprine study in MG, thus we suggest that subsequent studies should be at least as long [33, 20, 28]. Based on serum antibodies and clinical features, subgroups should include early-onset, late-onset thymoma, MUSK LRP4 antibody-negative, and ocular Myasthenia Gravis. Pyridostigmine is the best symptomatic treatment, whereas Corticosteroids, Azathioprine, and thymectomy are first-line immunosuppressive treatments for people who do not respond well to symptomatic therapy. Additional immunomodulatory drugs are being developed; however therapeutic decisions are hampered by a dearth of controlled studies. The majority of patients require long-term drug treatment, which must be tailored to their form of Myasthenia Gravis.[30] Myasthenia Gravis Foundation of America (2013) provided new guidelines for the use of Rituximab, Eculizumab, and Methotrexate, as well as guidelines for early immunosuppression in ocular MG and MG associated with immune checkpoint inhibitor treatment. Based on fresh research, this revised formal consensus advice of international MG specialists gives recommendations to doctors worldwide caring for patients with MG.[36]

CONCLUSION & RECOMMENDATIONS

Azathioprine was significantly more efficacious from the 6th, 9th, 12th, and 15th-month follow-up as compared to Methotrexate in the treatment of MG, however, on the 18th month follow up both drugs were equally effective. There is a demand for tailored immunomodulatory therapy, leading to a continuing drive to discover safer and more efficacious treatments for generalized Myasthenia Gravis. The recent discovery of biologicals, which have a more specific mode of action and better side effect profiles, may revolutionize the MG treatment algorithm in the future. It is recommended that in the future further studies should be done with larger sample sizes and at different facilities to evaluate the findings of our study.

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AUTHORS CONTRIBUTION

Sr.#	Author's Full Name	Intellectual Contribution to Paper in Terms of:
1.	Sadaf Iftikhar & Asfand Kousar	1. Study design, paper writing and methodology.
2.	Asfand Kousar & Saman Shahid	3. Data collection, analysis and calculations.
3.	Aysha Ghayyur & Adina Qaiser	5. Literature review and referencing.
4.	Sadaf Iftikhar & Saman Shahid	6. Editing and quality insurer.