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PERFIL DE FALA E COGNITIVO DE PACIENTES COM MIASTENIA GRAVIS

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Tese submetida ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Doutor

Orientador: Dr. Geraldo Pereira Jotz
Coorientadora: Dra. Maira Rozenfeld Olchik

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Porto Alegre, 31 de março de 2020.

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Prof ºDrº Jonas Alex Morales Saute – UFRGS

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Profº Drº Artur Francisco Schumacher Schuh – UFRGS

Dedico esse trabalho ao meu pai (*in memorian*), que onde quer que esteja, está muito feliz por ver onde eu cheguei.

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"Mucha gente pequeña, en lugares
pequeños, haciendo cosas pequeñas,
puede cambiar el mundo".
(Eduardo Galeano)

RESUMO

A miastenia gravis (MG) é uma doença autoimune, caracterizada por fraqueza e fadiga muscular flutuante dos músculos esqueléticos. Alterações no padrão vocal podem ser o sintoma inicial em até 15% dos pacientes. Além disso, déficits cognitivos vêm sendo descritos, com queixa de até 60% dos pacientes. O objetivo deste estudo foi descrever o perfil de fala e cognitivo de pacientes com MG. Realizou-se um estudo transversal, com 39 sujeitos com diagnóstico de MG a partir de eletromiografia e anticorpos, fora da crise e 18 controles, pareados por idade e sexo. Excluíram-se indivíduos com história de outros eventos neurológicos prévios e qualquer distúrbio sensorial ou motor que impossibilitassem a realização dos testes. Avaliação da fala compreendeu gravação de tarefas de fala, avaliação perceptiva-auditiva e análise acústica. Para avaliação cognitiva utilizou-se uma bateria de testes cognitivos (testes de rastreio, memória, linguagem e planejamento). Além disso, os pacientes com MG responderam questionários de auto percepção sobre qualidade de vida, sono, depressão e fala**.** No que diz respeito a fala, encontrou-se na análise perceptiva-auditiva uma alta porcentagem de alteração nas bases motoras fonação (95,2%) e respiração (52,63%) nos pacientes com MG, sendo que na respiração houve diferença significativa entre os grupos. Na análise acústica encontrou-se alteração nas bases motoras de fonação, com valores de shimmer significativamente maiores no grupo MG em comparação ao grupo controle e articulação com diferença significativa entre os grupos para o primeiro formante do “iu”. Não encontramos correlação entre os aspectos motores, clínicos e os questionários de autopercepção com a análise acústica. Com relação a cognição, verificou-se a presença de déficits cognitivos no teste de rastreio MOCA (66.7%) e nos testes de memória imediata (59.0%) e memória recente (56.4%). Após a análise de regressão de Poisson, com variância robusta, verificou-se que pacientes com diagnóstico de depressão apresentaram razão de prevalência (RP)=1.887 (IC:1.166-3.054) para escores alterados no MOCA, RP=9.533 (IC:1.600 - 56.788) para déficit em fluência verbal fonológica (FVF) e RP=12.426 (IC:2.177-70.931) fluência verbal semântica (FVS). Além disso, indivíduos que faziam uso de glucocorticosteróides e com escore no BDI indicativo de depressão apresentaram, respectivamente, RP=11.227 (IC:1,736 -

72.604) e RP=0.351 (IC:0.13- 0.904) para alteração de memória de retenção de curto prazo (A6). Dessa forma, conclui-se que nesta amostra os pacientes com MG apresentaram disartria, com alteração nas bases motoras fonação e respiração. As alterações não mostraram correlação com tempo e estadiamento da doença e questionários de autopercepção. Um pior padrão de fonação foi correlacionado com o uso de glucocorticoides. Com relação a cognição, houve a presença de déficits de memória e funções executivas indivíduos com MG. Além disso, encontrou-se uma associação de depressão e uso de glucocorticosteróides com prejuízo nas tarefas de memória.

Palavras-chaves: Miastenia Gravis. Fonoaudiologia. Fala. Voz. Disartria. Cognição. Memória.

ABSTRACT

Myasthenia gravis (MG) is an autoimmune disease, characterized by fluctuating muscle weakness and fatigue in skeletal muscles. Changes in the vocal pattern can be the initial symptom in up to 15% of patients. In addition, cognitive deficits have been described, with complaints from up to 60% of patients. The aim of this study was to describe the speech and cognitive profile of patients with MG. A cross-sectional study was carried out, with 39 subjects diagnosed with MG from electromyography and antibodies, out of the crisis and 18 controls, matched for age and sex. Individuals with a history of other previous neurological events and any sensory or motor disturbances that made it impossible to perform the tests were excluded. Speech assessment comprised recording of speech tasks, auditory-perceptual assessment and acoustic analysis. For cognitive evaluation, a battery of cognitive tests (screening, memory, language and planning tests) was used. In addition, patients with MG answered self-perception questionnaires about quality of life, sleep, depression and speech. With regard to speech, in the auditory-perceptual analysis a high percentage of alterations in the motor bases were found: phonation (95.2%) and breathing (52.63%) in patients with MG, and in breathing there was a significant difference between groups. In the acoustic analysis, alterations in the motor bases of phonation were found, with shimmer values significantly higher in the MG group compared to the control and articulation group with a significant difference between the groups for the first formant of the “iu”. We did not find any correlation between motor, clinical and self-perception questionnaires with acoustic analysis. With regard to cognition, cognitive deficits were found in the MOCA screening test (66.7%) and in the tests of immediate memory (59.0%) and recent memory (56.4%). After Poisson regression analysis, with robust variance, it was found that patients diagnosed with depression had a prevalence ratio (PR) = 1,887 (CI: 1,166-3,054) for altered MOCA scores, PR = 9,533 (CI: 1,600 - 56,788) for deficit in phonological verbal fluency (FVF) and PR = 12,426 (CI: 2,177-70,931) semantic verbal fluency (FVS). In addition, individuals who used glucocorticosteroids and with a BDI score indicating depression had, respectively, PR = 11.227 (CI: 1.736 - 72.604) and PR = 0.351 (CI: 0.13-0.904) for alteration of short retention memory. term (A6). Thus, it is concluded that in

this sample, patients with MG presented dysarthria, with changes in the motor bases, phonation and breathing. The changes did not show correlation with time and stage of the disease and self-perception questionnaires. A worse phonation pattern was correlated with the use of glucocorticoids. With regard to cognition, there was the presence of deficits in memory and executive functions in individuals with MG. In addition, an association of depression and the use of glucocorticosteroids with impaired memory tasks was found.

Keywords: Myasthenia Gravis. Speech therapy. Speech. Voice. Dysarthria. Cognition. Memory.

LISTA DE ABREVIATURAS

AChR	Receptor da acetilcolina do músculo esquelético
BRISA	Base Regional de Informações de Avaliação de Tecnologias de Saúde das Américas
Central	The Cochrane Central Register of Controlled Trials The Cochrane Library
EGG	Eletroglotografia
JNM	Junção neuromuscular
MG	Miastenia Gravis
MuSK	*Muscle-specific tyrosine kinase*
nAchR	Receptor nicotinico de acetilcolina
PPE	Potencial da placa terminal
PICO	Acrônimo para: paciente ou problema, intervenção, controle ou comparação e desfecho
SNC	Sistema Nervoso Central

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1 INTRODUÇÃO

A miastenia gravis (MG) é uma doença autoimune, causada pela presença de anticorpos patogênicos na junção neuromuscular (JNM). Isso ocasiona uma falha na transmissão neuromuscular, resultando em fraqueza e fadiga muscular flutuante dos músculos esqueléticos (JUEL et al.,2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012). Os músculos mais afetados são dos olhos, rosto, pescoço, braços e tronco (MONTERO-ODASSO, 2005; MERIGGIOLI et al., 2009; PAUL et al., 2000; TROUTH et al., 2012).

A MG pode ser subdividida com base em anticorpos séricos e características clínicas. Os subtipos clínicos incluem MG ocular, MG generalizada de início precoce e MG de início tardio. Os subtipos de anticorpos incluem MG com anticorpos AChR (80%), MG com anticorpos anti-MuSK (4%), MG com anticorpos anti-LRP4 (2%), miastenia soronegativa e miastenia com doenças auto-imunes coexistentes (MURTHY, 2020).

Embora a MG seja uma doença incomum, a taxa de prevalência vem aumentado ao longo do tempo, com estimativas recentes aproximando-se de 20 por 100.000 na população dos EUA. Esse aumento da prevalência deve-se possivelmente ao aprimoramento do diagnóstico e tratamento da MG, e à crescente longevidade da população em geral (JUEL et al.,2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).

Além da fraqueza ocular e em membros, a fraqueza bulbar, caracterizada por disfagia, disartria ou dificuldades na mastigação, tem se mostrado sintoma inicial em até 15% dos pacientes (JUEL et al.,2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012). Dessa forma, se faz importante compreender melhor como esses sintomas se manifestam na MG e quais as sequelas que permanecem após às crises.

Além disso, alterações cognitivas em pacientes com MG vêm sendo descritas na literatura desde a década de 80 (TUCKER et al., 1988). Estudos (IWASAKI et al., 1993; HAMED et al., 2014; KALTSATOU et al., 2015) apontam uma queixa de memória em até 60% dos pacientes. Os estudos na literatura até o momento não conseguiram esclarecer se há um comprometimento em SNC devido a fisiopatologia da MG ou se esses déficits estão relacionados a fatores

associados a doença, como depressão, tratamento medicamentoso, insuficiência respiratória e/ou fadiga mental.

Tendo em vista essas lacunas na literatura faz-se importante pesquisas sobre essa temática a fim de se caracterizar o padrão de fala e o perfil cognitivo de pacientes com MG, auxiliando no diagnóstico diferencial e tratamento dos pacientes.

2 REVISÃO DA LITERATURA

2. 1 Estratégias de busca

Tendo em vista que este estudo avaliou dois aspectos distintos da MG, realizou-se duas revisões sistematizadas da literatura em separado, a fim de obtermos o maior número de estudos sobre as temáticas. Em ambas às revisões, para a busca dos artigos consultou-se às bases de dados: PubMed, The Cochrane Central Register of Controlled Trials The Cochrane Library (Central), LILACS, PAHO, Base Regional de Informações de Avaliação de Tecnologias de Saúde das Américas (BRISA) e BIREME.

A revisão sobre a temática de fala foi realizada em agosto de 2019, sem limite de tempo. Utilizou-se os descritores de população, doença e desfecho de interesse, cruzados entre sim, conforme o sugerido pelo acrônimo PICO (paciente ou problema, intervenção, controle ou comparação e desfecho):

- **População:** “Adult” [Mesh] OR Adults OR “Aged” [Mesh] OR “Aged, 80 and over” [Mesh] OR “Middle Aged” [Mesh] OR “Young Adult” [Mesh] OR Adult, Young OR Adults, Young OR Young Adults OR Elderly
- **Doença:** “Myasthenia Gravis” [Mesh] OR “Myasthenia Gravis, Autoimmune, Experimental” [Mesh] OR “Myasthenic Syndromes, Congenital” [Mesh] OR “Myasthenia Gravis with Thymus Hyperplasia” [Supplementary Concept] OR “Myasthenia, Limb-Girdle, Autoimmune” [Supplementary Concept] OR “myasthenia gravis anti-skeletal muscle antibody” [Supplementary Concept] OR (Myasthenia AND Gravis AND Ocular) OR (Myasthenia AND Gravis AND Generalized) OR (Passive AND Transfer AND Experimental AND Autoimmune AND Myasthenia AND Gravis) OR (Myasthenic AND Syndromes AND Congenital AND Slow AND Channel) OR (Congenital AND Myasthenic AND Syndromes AND Presynaptic) OR (Congenital AND Myasthenic AND Syndromes AND Postsynaptic)
- **Desfecho de fala:** "Speech"[Mesh] OR "Speech Sound Disorder"[Mesh] OR "Speech Production Measurement"[Mesh] OR "Speech Perception"[Mesh] OR "Speech Intelligibility"[Mesh] OR "Speech Disorders"[Mesh] OR "Speech Articulation Tests"[Mesh] OR "Speech Acoustics"[Mesh] OR "Articulation Disorders"[Mesh] OR "Sound

Spectrography"[Mesh] OR "Voice"[Mesh] OR "Voice Quality"[Mesh] OR "Voice Disorders"[Mesh] OR "Dysarthria"[Mesh]

Foram encontrados 67 artigos, foram incluídos 2 estudos encontrados fora da revisão. No total foram selecionados 18 artigos relacionados a avaliação de fala em pacientes com MG.

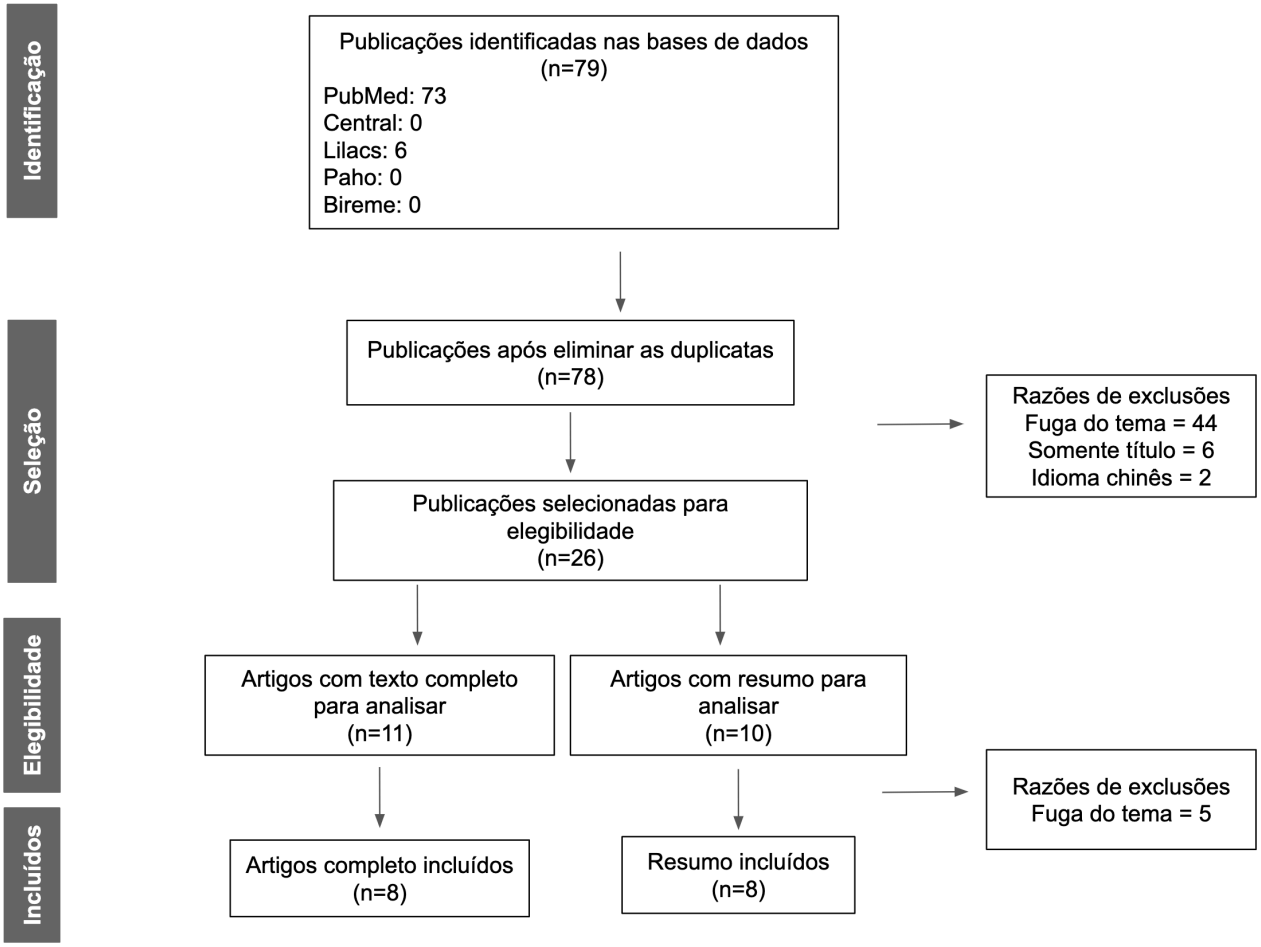

Figura 1: Fluxograma de fala
Desenvolvida pela autora.

A revisão sobre a temática de cognição foi realizada nos meses de maio de 2019, sem limite de tempo. Utilizou-se os descritores de população, doença e desfecho de interesse, cruzados entre sim, conforme o sugerido pelo acrônimo PICO (paciente ou problema, intervenção, controle ou comparação e desfecho):

- **População:** “Adult” [Mesh] OR Adults OR “Aged” [Mesh] OR “Aged, 80 and over” [Mesh] OR “Middle Aged” [Mesh] OR “Young Adult” [Mesh] OR Adult, Young OR Adults, Young OR Young Adults OR Elderly

- **Doença:** “Myasthenia Gravis” [Mesh] OR “Myasthenia Gravis, Autoimmune, Experimental” [Mesh] OR “Myasthenic Syndromes, Congenital” [Mesh] OR “Myasthenia Gravis with Thymus Hyperplasia” [Supplementary Concept] OR “Myasthenia, Limb-Girdle, Autoimmune” [Supplementary Concept] OR “myasthenia gravis anti-skeletal muscle antibody” [Supplementary Concept] OR (Myasthenia AND Gravis AND Ocular) OR (Myasthenia AND Gravis AND Generalized) OR (Passive AND Transfer AND Experimental AND Autoimmune AND Myasthenia AND Gravis) OR (Myasthenic AND Syndromes AND Congenital AND Slow AND Channel) OR (Congenital AND Myasthenic AND Syndromes AND Presynaptic) OR (Congenital AND Myasthenic AND Syndromes AND Postsynaptic)
- **Desfecho de cognição:** “Cognition” [Mesh] OR “Cognition Disorders” [Mesh] OR “Cognitive Dysfunction” [Mesh] OR “Mental Status and Dementia Tests” [Mesh] OR “Memory” [Mesh] OR “Spatial memory” [Mesh] OR “Memory, episodic” [Mesh] OR “Memory, Long-Term” [Mesh] OR “Memory, Short-Term” [Mesh] OR “Memory Disorders” [Mesh] OR “Wechsler Memory Scale” [Mesh] OR “Memory and Learning Tests” OR “Neuropsychological Tests” [Mesh] OR “Language” [Mesh] OR “Language Tests” [Mesh] OR “Language Disorders” [Mesh] OR “Attention” [Mesh] OR “Executive Function” [Mesh] OR (Cognitive AND Impairments) OR (Cognitive AND Impairment AND Mild) OR (Mild AND Neurocognitive AND Disorder) OR (Cognitive AND Decline) OR (Mental AND Deterioration) OR (Mental AND Status AND Tests) OR (Neurocognitive AND Test) OR (Mini AND Mental AND State AND Examination) OR (Cognitive AND Assessment AND Screening AND Instrument) OR (Working AND Memory) OR (Memory AND Immediate) OR (Immediate AND Recall) OR (Cognitive AND Retention AND Disorder) OR (Memory AND Loss) OR (Semantic AND Memory AND Disorders) OR (Memory AND Deficits) OR (Brief AND Cognitive AND Status AND Exam) OR (Rey AND Auditory AND Verbal AND Learning AND Test) OR (Test AND of AND Memory AND and AND Learning) OR (Test AND of AND Memory AND Malingering) OR (Rivermead AND Behavioural AND Memory AND Test) OR (California AND Verbal AND Learning AND Test) OR (TOMAL) OR

(Hooper AND Visual AND Organization AND Test) OR (Controlled AND Oral AND Word AND Association AND Test) OR (Cognitive AND Function AND Scanner) OR (Continuous AND Performance AND Task) OR (AX-CPT) OR (Rey-Osterrieth AND Complex AND Figure) OR (Clock AND Test) OR (NEPSY) OR (Symbol AND Digit AND Modalities AND Test) OR (Paced AND Auditory AND Serial AND Addition AND Test) OR (Test AND of AND Everyday AND Attention) OR (Tower AND of AND London AND Test) OR (Cambridge AND Neuropsychological AND Test AND Automated AND Battery) OR (CANTAB) OR (Aphasia AND Tests) OR (Memory AND for AND Designs AND Test) OR (Repeatable AND Battery AND for AND the AND Assessment AND of AND Neuropsychological AND Status) OR (Delis-Kaplan AND Executive AND Function AND System) OR (Boston AND Diagnostic AND Aphasia AND Examination) OR (Comprehensive AND Aphasia AND Test) OR (Vocabulary AND Tests) OR (Language AND Comprehension AND Tests).

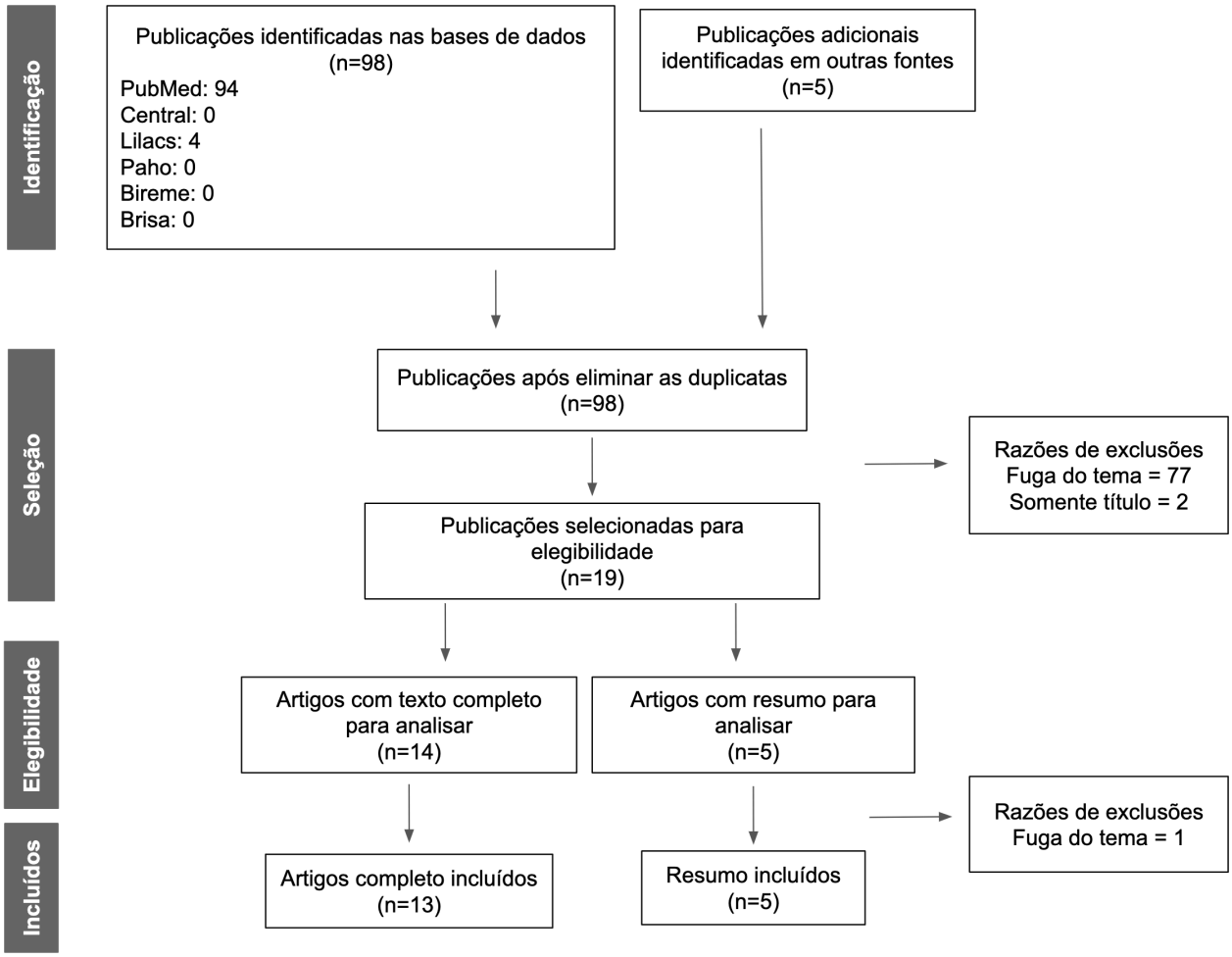

Figura 2: Fluxograma de cognição
Desenvolvida pela autora.

Foram encontrados 98 artigos, foram incluídos 5 artigos encontrados na bibliografia dos estudo analisados. No total foram selecionados 19 artigos relacionados ao perfil cognitivo de pacientes com MG.

Contudo, antes de detalharmos os aspectos relacionados às questões da pesquisa encontrados na busca sistematizada à literatura, realizaremos uma breve revisão sobre a Miastenia Gravis, sua fisiopatologia, sintomas e principais estratégias de tratamento.

2. 2 Miastenia Gravis

A miastenia gravis adquirida (MG) é um distúrbio autoimune mediado por anticorpos da junção neuromuscular (JNM). Na maioria dos casos, é causada por anticorpos patogênicos direcionados ao receptor da acetilcolina do músculo esquelético (AChR). Em outros, componentes da placa terminal do músculo pós-

sináptico, como o receptor tirosina-quinase específico do músculo (MUSK) podem servir como alvos para o ataque auto-imune (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).

Em MG, a perda de AChRs funcionais resulta em uma diminuição da amplitude do potencial da placa terminal (PPE). Desta forma, o PPE fica abaixo do limiar necessário para a geração do potencial de ação das fibras musculares durante a despolarização repetitiva dos nervos. Isso resulta em uma falha na transmissão neuromuscular (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).

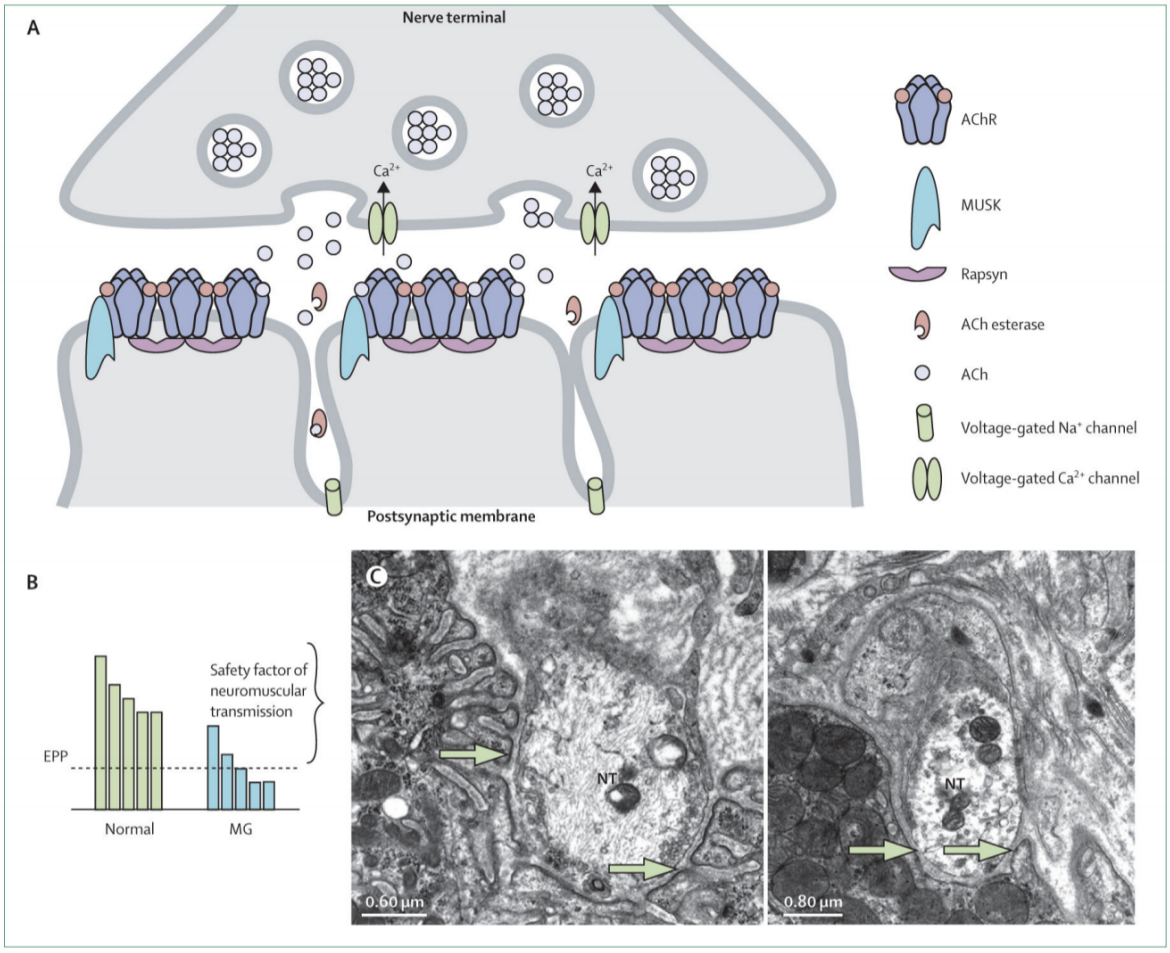

Figura 3: Junção neuromuscular normal (JNN) e fisiopatologia dos componentes MG. Extraído do estudo de Meriggioli et al. (2009). Neuromuscular. ACh = acetilcolina. AChR = receptor ACh. EPP = potencial da placa final. MG = miastenia gravis. MUSK = receptor tirosina-quinase específico do músculo. NMJ = junção neuromuscular. NT = terminal nervoso.

A causa precisa da resposta auto-imune em MG é desconhecida, até o momento, mas anormalidades no timo (hiperplasia e neoplasia) quase certamente desempenha um papel em pacientes com anticorpos anti-AChR, e é

provável que a predisposição genética também influencie em quais pacientes desenvolvem o distúrbio (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).

Antes do uso generalizado de imunomoduladores, o prognóstico para pacientes com MG era reservado, com cerca de 30% de mortalidade. Juntamente com os avanços da ventilação mecânica e nos cuidados intensivos, a imunoterapia tem sido um dos principais fatores que contribuem para melhorar os resultados em MG, e a mortalidade específica por doença atualmente é inferior a 5% (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).

2. 2. 1 Epidemiologia

A MG é uma doença relativamente incomum, embora a prevalência tenha aumentado ao longo do tempo, com estimativas recentes aproximando-se de 20 por 100.000 na população dos EUA. Esse aumento da prevalência provavelmente se deve ao aprimoramento do diagnóstico e tratamento da MG, e à crescente longevidade da população em geral (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012). Estimativas recentes apontam para uma prevalência em 131-145 por milhão de habitantes, com taxas de incidência de 6,7 por milhões em menores de 50 anos e 34 por milhão em aqueles com mais de 50 anos de idade (MERIGGIOLI et al., 2009). Até o momento, não foram encontrados dados de prevalência na população brasileira.

A ocorrência de MG é influenciada por sexo e idade: as mulheres são afetadas quase três vezes mais que os homens no início da idade adulta (idade <40 anos), enquanto a incidência é aproximadamente igual durante a puberdade e após os 40 anos. Após os 50 anos, a incidência é maior nos homens (MONTERO-ODASSO, 2005; JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).

Em um levantamento de dados no qual objetivou-se determinar o perfil clínico de pacientes com MG atendidos em dois hospitais universitários em Minas Gerais/Brasil, no período de 1981 a 2009, verificou-se que dos 69% dos pacientes eram do sexo feminino, com uma proporção de 2,3:1 para homens. No grupo com mais de 50 anos, houve uma proporção maior de homens (1,2:1), sendo mais evidente em 60 anos, com uma proporção de 1,5:1. Nas pessoas acima de 50 anos, a disfonia era tão frequente quanto a diplopia. (AGUIAR et al.,

2010). Em um estudo mais recente brasileiro (MOURÃO et al., 2014) no qual foram avaliados 69 pacientes de um hospital universitário com MG verificou-se que a maioria dos pacientes era do sexo feminino (91%) com uma proporção feminino: masculino de 4:1.

Tabela 1: Dados epidemiológicos Brasil

Estudo	N	Sexo		Tipo de estudo	Local
		Masculino	Feminino		
Cunha et al. (1999)	153	48 (32%)	104 (68%)	Retrospectivo	Curitiba /PR
Assis et al. (1999)	41	24 (58,5%)	17 (41,5%)	Retrospectivo	São Paulo/ SP
Aguiar et al., (2010)	122	37 (30,3%)	85 (69,7%)	Retrospectivo	Belo Horizonte, MG
Estudo	**N**	**Razão feminino/masculino**		**Tipo de estudo**	
Saraiva et al. (1996)	324	6,4:1		Transversal	Belo Horizonte, MG
Mourão et al. (2014)	69	4:1		Retrospectivo	Belo Horizonte, MG

Elaborado pela autora, a partir dos estudos de Aguiar et al. (2010) e Mourão et al. (2014).

2. 2. 2 Etiologia

A MG auto-imune resulta de ataque imunológico mediado por anticorpos e dependente de células T na membrana pós-sináptica da junção neuromuscular. A transmissão neuromuscular anormal e a fraqueza clínica em MG resultam dos efeitos de anticorpos que se ligam a vários epítopos da região da placa terminal do músculo esquelético. Na maioria dos casos, os anticorpos se ligam à principal região imunogênica da subunidade α do AChR, embora pacientes com MG com anticorpos para MuSK exibam fraqueza clínica e achados fisiológicos bastante semelhantes aos pacientes com MG com anticorpos AChR. O MuSK inicia a agregação de AChRs na placa muscular durante o desenvolvimento, mas a função do MuSK no músculo esquelético maduro e a fisiopatologia do MG relacionada aos anticorpos MuSK são atualmente desconhecidas. A perda funcional dos AChRs reduz a probabilidade de transmissão neuromuscular bem-sucedida após a liberação de acetilcolina pelo terminal do nervo motor, resultando em fraqueza clínica nos músculos estriados (JUEL et al., 2007; TROUTH et al., 2012).

2. 2. 3 Subtipos

A MG pode ser subdividida com base em anticorpos séricos e características clínicas. Os subtipos clínicos incluem MG ocular, MG

generalizada de início precoce e MG de início tardio. Os subtipos de anticorpos incluem MG com anticorpos AChR (80%), MG com anticorpos anti-MuSK (4%), MG com anticorpos anti-LRP4 (2%), miastenia soronegativa e miastenia com doenças auto-imunes coexistentes (KONECZNY et al., 2019; MURTHY, 2020).

Na MG ocular, a fraqueza é limitada aos músculos oculares. Esse subtipo compreende 17% de todos os MG em populações brancas. Até 50% desses pacientes possuem anticorpos anti-AChR. Não a uma relação direta a anormalidade no timo. Paciente scom MG generalizada de início precoce (início antes dos 50 anos) são mais frequentemente do sexo feminino (3:1), possuem anticorpos anti-AChR e hiperplasia do timo. Já os de início tardio (início após os 50 anos) são mais frequentemente do sexo masculino (1,5:1), geralmente apresentam histologia tímica normal ou atrofia tímica relacionada a idade, com presença dos anticorpos anti-AChR e/ou anticorpos para proteínas musculares estriadas, como a titina e o receptor de rianodina. MG associada ao timoma também conhecida como MG paraneoplásico, possuem anticorpos não patogênicos contra musculo entriado, titina, receptor de rianodina e anticorpos anti-AChR (KONECZNY et al., 2019; MURTHY, 2020).

A MG com anticorpos anti-MuSK apresenta um fenótipo grave, com fraqueza respiratória e da musculatura bulbar, mais frequente no sexo feminino (9:1), sem alterações no timo. Na MG com anticorpos anti-LRP4 há uma predominância em sexo feminino (2,5:1), o fenótipo é leve, podem apresentar hiperplasia ou timo normal. Já ma MG com anticorpos anti-Agrin é observada fraqueza. Uscular generalizada e sem tmimoma (KONECZNY et al., 2019; MURTHY, 2020).

2.2. 4 Sintomas

A principal manifestação da MG é fraqueza e fadiga muscular flutuante dos músculos esqueléticos, que se agrava com o esforço e melhora com o repouso. Os músculos mais afetados são dos olhos, rosto, pescoço, braços e tronco. Além disso, pode-se observar sintomas oculares (diplopia e ptose) e sintomas bulbares (disartria, disfagia, fraqueza mastigatória, fraqueza na musculatura facial e dificuldades respiratórias) (MONTERO-ODASSO, 2005; MERIGGIOLI et al., 2009; PAUL et al., 2000; TROUTH et al., 2012)

A fraqueza ocular, apresentando-se como ptose flutuante e/ou diplopia, é a apresentação inicial mais comum de MG, ocorrendo em aproximadamente 85% dos pacientes. A fraqueza bulbar, com disfagia, disartria ou dificuldades na mastigação, é o sintoma inicial em até 15% dos pacientes (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).

Apesar de ter um amplo espectro de gravidade, podemos subdividir a MG em duas formas clínicas: a ocular, na qual a doença ocorre somente nos músculos oculares (cerca de 10% dos casos) e a generalizada, na qual existe envolvimento de vários grupos musculares (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).

2. 2. 5 Diagnóstico

O diagnóstico de MG pode ser realizado por meio de diferentes testes:

- Teste de cloreto de edrofônio (Tensilon test): é um inibidor da acetilcolinesterase de ação curta que prolonga a duração da ação da acetilcolina na JNM aumentando a amplitude e duração do PPE. O cloreto de edrofônio melhora temporariamente o fator de segurança da transmissão neuromuscular e pode provocar uma melhora na força em pacientes com transmissão neuromuscular anormal. Consiste na administração de edrofônio por via intravenosa e na observação do paciente para uma melhora na força muscular. A sensibilidade no diagnóstico de MG é de 71,5-95% para doença generalizada.
- Teste da bolsa de gelo: é um teste não farmacológico, no qual é colocada uma bolsa de gelo sobre o olho de 2 a 5 minutos e avalia-se a melhora da ptose.
- Eletroneuroestimulação: consiste na estimulação repetitiva do nervo. É o teste eletrofisiológico mais comumente usado na transmissão neuromuscular. O resultado do teste repetitivo de estimulação nervosa é anormal em aproximadamente 75% dos pacientes com MG generalizada (<50% da MG ocular) e é mais provável que seja anormal em um músculo proximal ou facial.
- Eletromiografia de fibra única: é realizada usando um eletrodo de agulha concêntrico especialmente construído que permite a identificação de potenciais de ação de fibras musculares individuais. Esse teste revela

instabilidade anormal em 95-99% dos pacientes com MG, se os músculos apropriados forem examinados.

- Testes sorológicos:
    - Anticorpos de AChR: consiste na medição da quantidade de anticorpo sérico que precipita a AChR muscular, detectada pela ligação com o antagonista colinérgico radiomarcado α-bungarotoxina. A sensibilidade deste teste é de aproximadamente 85% para MG generalizada e 50% para MG ocular.
    - Anticorpos dos musculos anti-estriados: esses anticorpos reagem com elementos contráteis do músculo esquelético. Eles são encontrados em 30% dos pacientes com MG no início do adulto e parecem ser mais comuns em pacientes com início tardio da doença.
    - Anticorpos anti-MUSK: o MuSK parece facilitar o agrupamento de AChR na região da placa terminal na junção neuromuscular em desenvolvimento. Foram encontrados em cerca de um terço dos pacientes com MG generalizada.
- Exames de imagem: tomografia computadorizada ou ressonância magnética de tórax devem ser realizadas em todos os pacientes com MG confirmada para excluir a presença de timoma (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).

2. 2. 6 Estratégias de tratamento

A seguir serão descritas as principais estratégias de tratamento:

- Inibidores da colinesterase: são medicamentos administrados por via oral, que aumentam a quantidade de acetilcolina disponível para ligação na junção neuromuscular e são o tratamento de primeira linha em pacientes com MG (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).
- Azatioprina: é convertida hepaticamente em 6-mercaptopurina, um anti-metabolito ativo que bloqueia a síntese de nucleotídeos e a proliferação de linfócitos T. A azatioprina é um agente eficaz para a modulação imune a longo prazo em MG como uma droga poupadora de esteróides ou como

imunoterapia inicial (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).

- Ciclosporina: exerce um efeito imunomodulador ao bloquear a produção de interleucina-2 e a proliferação de linfócitos T. Embora eficaz, o uso da ciclosporina em MG tem sido limitado por sua nefrotoxicidade e inúmeras interações medicamentosas (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).
- Corticosteróides: são medicamentos administrados por via oral. Foram os primeiros medicamentos imunossupressores a serem usados em MG e continuam sendo a terapia imunológica direcionada mais comumente usada (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).
- Micofenolato de mofetil: é um modulador imune relativamente novo que inibe seletivamente a proliferação de linfócitos T e B, bloqueando a síntese de purina exclusivamente nos linfócitos. É usado em MG tanto como agente poupador de esteróides quanto como imunoterapia inicial em pacientes com risco de complicações com corticosteróides (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).
- Troca plasmática e imunoglobulina intravenosa: são usadas para o tratamento a curto prazo das exacerbações de MG e quando é desejável obter uma resposta clínica rápida. A troca plasmática reduz temporariamente as concentrações de anticorpos anti-AChR circulantes e produz melhora em questão de dias na maioria dos pacientes com MG adquirida (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).
- Timectomia: é um tratamento cirúrgico que consiste na retirada do timo. É altamente recomendado para pacientes com timoma (JUEL et al., 2007; MERIGGIOLI et al., 2009; TROUTH et al., 2012).
- Reabilitação: em combinação com outras formas de tratamento médico pode ajudar a aliviar os sintomas e melhorar a função em MG. O objetivo principal é aumentar a força do indivíduo para facilitar o retorno ao trabalho e às atividades da vida diária. Recomenda-se uma abordagem interdisciplinar, incluindo fisioterapia, terapia ocupacional, psicologia e fonoaudiologia (TROUTH et al., 2012).

2. 3 Miastenia Gravis e Fala

Disartria é o nome dado às alterações de fala resultantes de distúrbios no controle neuromuscular dos mecanismos da fala, que podem comprometer as funções de respiração, fonação, ressonância, articulação e prosódia, de forma isolada ou associada (DARLEY et al., 1969; ORTIZ et al., 2008). É causada devido a danos no SNC e/ou SNP, resultando em problemas na comunicação oral como paralisia, fraqueza ou incoordenação da musculatura da fala (ORTIZ et al., 2008).

A classificação da Disartria da Mayo Clinic é composta por seis categorias: (1) fácida, (2) espástica e Neurônio motor superior unilateral (UMN), (3) atáxica, (4) hipocinética, (5) hipercinética e (6) mista.

A avaliação das disartrias baseia-se no tripé: avaliação perceptiva-auditiva, avaliação acústica da voz e fala e auto avaliação do paciente (PADOVANI, 2011). A compreensão de atributos capazes de identificar a presença desses transtornos da fala e distingui-los tem sido interesse de pesquisadores, tendo em vista a possibilidade de auxiliar no diagnóstico diferencial e precoce entre diferentes doenças neurológicas e neuromuscular (ORTIZ et al., 2008; PADOVANI, 2011).

Embora pouco frequente, a disartria pode ser o primeiro e único sintoma da MG em alguns casos. Além disso, em alguns casos pode demorar anos para evoluir a outros sintomas, o que em muitos momentos dificulta o diagnóstico, sendo comumente confundida com Acidente Vascular Encefálico (LIU et al., 2007; MONTERO-ODASSO, 2005). Dessa forma, saber às características acústicas e auditivas desse sintoma pode auxiliar no diagnóstico diferencial e monitoramento clínico da doença (FERREIRA et al., 2007; KONSTANTOPOULS et al., 2017).

No estudo de Mao et al. (2001), disfonia foi o primeiro sintoma em 6% dos pacientes avaliados, sendo que, em 60% dos casos, esse sintoma apareceu com a progressão da doença. Além disso, em um estudo anterior verificou-se que dificuldades de fala e deglutição aparecem como sintomas iniciais em mais de 27% dos casos (NEAL et al., 1987). Um estudo brasileiro verificou que as dificuldades de deglutição e alterações vocais estavam presentes mais comumente na faixa etária de 12 a 50 anos, sendo que na faixa etária acima dos

50 anos, a presença de disfonia foi tão frequente quanto a de diplopia (AGUIAR et al., 2010).

A queixa dos pacientes com MG é de uma voz ofegante e nasal e dificuldade em falar alta intensidade. Essas dificuldades podem aparecer em qualquer estágio da doença, frequentemente associado a disfagia (MAO et al., 2001). No estudo de Konstantnopuls et al. (2017), a disartria foi mais proeminente em pacientes com anticorpos para quinase específica do músculo (MuSK), 40% dos quais exibem envolvimento bulbar no início da doença, incluindo fraqueza na face, faringe e língua.

A disartria na MG é categorizada como do tipo flácida. As características proeminentes da disartria flácida na MG consistem em hiponasalidade, articulação imprecisa e soprosidade continua com progressão e aumento da gravidade com a fala prolongada. Na literatura, as alterações de voz e fala frequentemente encontradas em pacientes com MG são: hipernasalidade, dificuldade em sustentar o pitch, fadiga vocal, afonia intermitente, estridor, soprosidade ou aspereza de voz, incompetência glótica, mudanças na qualidade vocal, imprecisão articulatória e alteração da fluência verbal (MONTERO-ODASSO, 2005; MAO et al., 2001; LIU et al., 2007).

Segundo a literatura, a disartria encontradas na MG está relacionados à fadiga e fraqueza dos músculos adutores e tensor das pregas vocais, fraqueza dos músculos da língua, palato e constritores, incompetência glótica para adução de pregas vocais e insuficiência velofaríngea (MONTERO-ODASSO, 2005; MAO et al., 2001).

Na literatura há poucos estudos que realizaram análise acústica da fala e voz de pacientes com MG. Os dados encontrados demonstram instabilidade no traçado espectrográfico com ausência de harmônicos nas frequências altas devido à fraqueza e lentidão ou falta de coordenação dos músculos laríngeos, além de distribuição irregular de energia no trato vocal (ORTIZ et al., 2008). Em pacientes com miastenia laríngea tiveram valores menores na faixa de frequência da fonação e tempo máximo de fonação e taxa de fluxo máximo maior do que valores normativos para sua e populações de controle pareadas por sexo (MAO et al., 2001). Além disso, verificou-se lentidão na produção diadococinesia, aumento de jitter, aumento frequência fundamental média e intervalo de pitch reduzido (KONSTANTOPOULS et al., 2017).

A fraqueza miastênica dos músculos da laringe ocasiona um fechamento glótico incompleto durante a deglutição que pode ser associado as alterações vocais. Além disso, na videoendoscopia laríngea tem-se observado elevação palatina reduzida ou ausente (JUEL et al., 2007).

Em um estudo no qual comparou-se variáveis acústicas da voz e fala com dados quantitavos da eletroglotografia (EGG) observou-se aumento do desvio padrão da frequência fundamental (fonação sustentada), bem como aumento da média fundamental frequência e faixa de frequência fundamental (leitura). Na diadococinesia, a fraqueza e a fadiga muscular podem explicar a maior duração média em silêncio antes da produção da sílaba / pa / e as durações médias mais baixas para as consoantes / p / e / t /. Um achado interessante desse estudo é que a fadiga na fala é expressa como maior duração do silêncio entre sílabas e menor duração das consoantes, mas não como maior ou menor duração da vogal. Assim, parece que a fadiga força o paciente a descansar mais durante a leitura, expresso em intervalos de silêncio aumentados, como observado na prática neurológica diária. Os autores apontam que o que melhor diferenciou os pacientes com MG dos controles foi frequência fundamental média durante a fonação sustentada, o desvio padrão da frequência fundamental, a frequência fundamental média e a faixa de frequência fundamental durante a leitura, conforme bem como as durações silenciosas entre as sílabas durante o DDK (KONSTANTOPOULS et al., 2017).

A partir do exposto, será apresentado nas tabelas 2 e 3 os principais achados dos estudos encontrados nesta revisão literatura. Observa-se uma escassez de estudo sobre essa temática, tendo em vista que apenas quatro estudos apresentaram dados da análise acústica da voz e fala e apenas um estudo aplicou protocolos de autopercepção da fala em pacientes com MG. Não foram encontrados dados sobre análise perceptiva auditiva na MG. Dessa forma, reforça-se a importância de mais estudos sobre essa temática.

Tabela 2: Resumo dos resultados dos relatos de caso de disartria em MG.

Autores	Amostra	Queixas	Avaliações
Neiman (1975)	Sexo feminino 20 anos	Presença de disfonia respiratória intermitente e afonia como única manifestação da MG	As cordas vocais eram flácidas e aduzidas à posição paramediana para fonação. A melhora da função laríngea foi demonstrada com edrofônio e brometo de neostigmina. A terapia com brometo de piridostigmina (Mestinon) manteve a fonação normal.

Michalska (1996)	11 casos	A disfonia era o único sintoma da doença durante um período de vários meses a vários anos.	Os pacientes apresentaram os critérios adequados para o diagnóstico de MG, nos quais são utilizados o teste repetitivo de eletroestimulação e o método SFEMG de alta sensibilidade.
Montero-Odasso (2005)	Sexo masculino 77 anos	Início agudo de alterações no tom da voz e fala arrastada.	A única anormalidade encontrada foi nas pregas vocais levemente edemaciadas e a avaliação do fonoaudiológica com resultado normal. Após o paciente apresentou piora, com perda peso e engasgos.
Ferreira et al. (2007)	Sexo masculino 72 anos	Queixa de dificuldades para engolir sólidos e líquidos, nos últimos 6 meses com piora gradual e perda de peso. Negava disfonia.	Avaliação neurológica verificou presença de disartria e voz anasalada, com discurso arrastado. Avaliação otorrinolaringologia, constatou lentidão dos movimentos de adução e abdução das cordas vocais, bem como diminuição muito marcada dos movimentos da base da língua à deglutição.
Kanemaru et al. (2007)	Sexo masculino 76 anos	Queixa de episódios de engasgo e dispneia.	No exame de endoscopia com fibroscópio observou-se voz fraca, com adequado espaço glótico para respiração, sem alteração na voz e paralisia de pregas vocais com melhora após injeção intravenosa de edrofônico.
Tremolizzo (2015)	4 casos	Disartria súbita como síntoma inicial.	Exame médico (neurologista) hipótese diagnóstica inicial de acidente vascular encefálico. Após piora dos sintomas iniciais foi realizada eletroneuromiografia, na qual confirmou-se o diagnóstico de MG.
Tsunoda et al. (2017)	Sexo feminino 65 anos	Voz rouca e ofegante.	Exame estroboscópico da laringe revelou uma fenda glótica e atrofia das pregas vocais, levando à incompetência glótica durante a fonação. O tempo máximo de fonação era de apenas 7 segundos.
Chandra e Pant (2019)	Sexo feminino 51 anos	Súbita diminuição na textura e tom da voz nos últimos 15 dias, com melhora ao acordar e piora ao final do dia.	No exame médico (neurologista) observou-se que a articulação da fala estava intacta, mas a intensidade vocal estava reduzida e lenta.

Tabela 3: Resumo dos estudos encontrados sobre avaliação de fala em MG.

Autores	Amostra	Testes	Resultados	Conclusão
Tóth et al. (1999)	MG Controles	Análise computadorizada dos seguintes parâmetros vocais: faixa vocal, dinâmica da voz, tempo máximo de fonação, frequência fundamental e intensidade média durante a fala. As investigações foram realizadas antes e após a ingestão de Mestinon.	Observou-se nós pacientes com MG, que o alcance e a dinâmica da voz estavam bastante prejudicados, que o tempo máximo de fonação estava reduzido e, a frequência fundamental média durante a	Os autores enfatizam o papel do otorrinolaringologista e da avaliação objetiva da voz no diagnóstico de MG e de outras doenças neurológicas semelhantes.

			fala aumentou, enquanto a intensidade vocal diminui. O Mestinon resultou em uma melhora em todos esses parâmetros, no entanto, eles ainda estavam alterados em comparação com os indivíduos controle.	
Mao et al. (2001)	40 casos de miastenia gravis nos quais a disfonia foi o sintoma inicial e predominante.	- Estrobovideolaringoscopia. - Eletroglotografia. - Análise acústica da voz falada e cantada. - Teste de função pulmonar.	Queixas mais comuns foram rouquidão, fadiga vocal e dificuldades com o pitch. O achado mais comum foi déficit na mobilidade das pregas vocais, unilateral ou bilateral. Os pacientes com MG apresentaram diminuição nos valores de frequência fundamental, da faixa de frequência da fonação e do tempo máximo de fonação. Os valores para vazão máxima foram mais que o dobro dos valores normais. Esses resultados foram significativamente piores do que os valores normativos para a população e com controles. Todos os pacientes, exceto três, nessa série, apresentaram evidências de comprometimento da mobilidade	Não houve diferenças estatisticamente significantes nas medidas objetivas identificadas que possam servir para distinguir a miastenia gravis de outras causas de disfonia. No entanto, houve uma tendência em pacientes com miastenia com um valor mais alto de shimmer.

			das pregas vocais no exame da laringe. Disfonia de tensão muscular foi observada em 77,5% dos pacientes, um achado que atribuímos a esforços compensatórios.	
Sun et al. (2005)	83 MG 40 controles	Medidas acústicas foram realizadas antes e depois do teste e tratamento da neostigmina.	Os parâmetros básicos da análise de acústica da voz incluíram F0, shimmer, jitter e NNE. O valor de F0 e NNE estavam significativamente aumentados no grupo de MG, quando comparado aos controles saudáveis. A neostigmina resultou em uma melhoria nos parâmetros de F0 e NNE.	
Hou et al. (2007)	30 MG	- Eletromiografia laríngea.	Cerca de 36,7% dos pacientes com MG apresentavam sintomas de rouquidão, fadiga vocal, disfonia e disfagia. Os movimentos das pregas vocais de 16,7% dos pacientes com MG eram mais fracos do que o normal e não realizavam fechamento glótico completo. Na eletromiografia laríngea a amplitude vocal e o tempo máximo de fonação foram muito menores que o normal, todos os músculos laríngeos estavam afetados.	
Liu et al. (2007)	7 casos de pacientes com queixas exclusivas laríngeas com diagnóstico incorreto, oriundo de uma revisão de prontuários de 15 anos, com uma amostra de 1520 pacientes com MG.	Análise da acústica voz antes e após o teste de neostigmina e exame fibrolaringoscópico.	Todos os pacientes responderam ao teste de neostigmina positivamente, com melhora nos parâmetros vocais o que confirmou o diagnóstico de MG. Ao exame observou-se déficit na mobilidade das pregas vocais, disfonia por tensão muscular e fechamento glótico incompleto. A porcentagem de pacientes com MG com queixas laríngeas exclusivas foi de 0,46%. Os sintomas comuns e únicos aos pacientes com MG na laringe foram: disfonia, disartria e disfagia flutuante devido a fadiga.	
Xu et.al. (2009)	32 MG	Eletromiografia dos músculos laríngeos, com agulha concêntrica bipolar.	Dos 32 pacientes com MG, os achados do estudo da estimulação repetitiva dos nervos (MRN) foram positivos para os músculos laríngeos em 28 pacientes (87,5%) e negativos em 4 pacientes (12,5%). Os oito pacientes (25%) apresentaram sintomas laríngeos leve rouquidão,	

			fraqueza vocal e fadiga vocal. Entre esses 8 pacientes, 4 apresentaram sinais de alteração laríngea como como comprometimento da mobilidade das pregas vocais, incompetência glótica durante a fonação ou tosse e diminuição discreta da onda mucosa das pregas vocais. Não houve anormalidade laríngea evidente nos outros 28 pacientes que apresentavam movimento de prega vocal e fechamento glótico normal durante a fonação e tosse. Na estroboscopia observou-se comprometimento da mobilidade das pregas vocais e incompetência glótica durante a fonação ou tosse estavam presentes apenas em 4 pacientes (12,5%). Os autores concluem que o teste de MRN para os músculos laríngeos é muito sensível (87,5%) para MG, e é um indicador útil para o diagnóstico da doença.
Konstantopoulos et al. (2017)	12 MG 24 controles	- Electroglottography (EGG). - The Frenchay Dysarthria Assessment (FDA). - The voice handicap index (VHI). - Gravação da fala (fonação sustentada, diadococinesia e leitura de um texto).	Todos os pacientes com MG relataram voz ofegante e afonia, independentemente do status do anticorpo, 5 paciente relataram dificuldade articulatória associado à fadiga durante o dia, metade dos pacientes relatou dificuldades de deglutição e apenas 2 relataram hipernasalidade. No FDA verificou-se que a maioria dos os participantes do grupo MG (7/12, 58%) exibiram tempo máximo de fonação reduzido, alteração no controle do volume da voz e soprosidade. Além disso, participantes com MG exibiram lentidão na elevação da língua (6/12, 50%), no movimento lateral da língua (5/12, 42%), na protrusão da língua (5 / 12, 42%) e no movimento alternado da língua (6/12, 50%). O escore médio do VHI mostrou que os participantes do MG perceberam sua disfonia como afetando sua qualidade de vida em comparação aos controles saudáveis. Na fonação e leitura sustentadas, o grupo MG apresentou maior variabilidade fonatória (valores mais altos para frequência fundamental média e desvio padrão da frequência fundamental), além de mais variação nas pregas vocais vibratórias (valores mais altos para frequência fundamental média e faixa de frequência fundamental) em comparação ao grupo controle. Na diadococinesia o grupo MG mostrou

			intervalos de silêncio mais longos durante uma série de produções de sílabas / pa / e / ka / e uma maior duração do som / t / quando produziu a sílaba / ta /.
Yang et al. (2019)	30 MG		A disartria foi o sintoma primário mais frequente (14/30), seguido pela disfagia (11/30), fala arrastada (4/30) e disfonia (1/30).

2. 4 Miastenia Gravis e Cognição

Alterações cognitivas em pacientes com MG vem sendo descritas na literatura desde a década de 80. O primeiro estudo que aponta déficits cognitivos em pacientes com MG foi de Tucker et al. (1988) no qual os autores investigam os possíveis efeitos colinérgicos centrais da MG medidos pela disfunção cognitiva. Foi aplicada uma bateria de tarefas cognitivas em 12 indivíduos com MG comparando a controles saudáveis e controles com doença crônica de natureza não neurológica. Os resultados apontaram que o grupo miastênico foi significativamente prejudicado em relação aos grupos de controle médico e saudável para desempenho dos testes de memória. Dessa forma, os autores sustentaram sua hipótese de que a MG tem efeitos colinérgicos centrais manifestados por disfunção cognitiva. Desde então diversos estudos investigaram a presença de déficits cognitivos em pacientes com MG, bem como quais os possíveis agentes causadores, como por exemplo, efeitos colinérgicos, medicamentoso, ansiedade e depressão. Até o momento foram encontradas três revisões de literatura (KESSEY, 1999; PAUL et al., 2001; MAO et al., 2015) sobre essa temática.

Kessey (1999) encontrou diversos estudos que alegavam que os efeitos colinérgicos centrais em MG seriam causados pelo uso de anticolinesterases no tratamento de MG ou pela presença de anticorpos dos receptores de acetilcolina no sérum e no fluido cérebro espinhal em pacientes com MG. As diferenças antigênicas entre AchR muscular e AchRs neuronais, juntamente com as concentrações muito baixas de anticorpos AchR muscular no líquido cefalorraquidiano, segundo o autor, tornam altamente improváveis as alegações de que os sistemas colinérgicos do SNC sejam afetados por esses anticorpos musculares em pacientes com MG. Além disso, o autor acrescenta que os

déficits cognitivos se realmente presentes em pacientes com MG são mais provavelmente causados por mecanismos periféricos do que centrais, como fraqueza na respiração, deglutição, fala, movimento dos membros e dos olhos, desaturação de oxigênio durante o sono, efeito adverso de altas doses de medicamentos como a prednisona e depressão. Sendo a conclusão dessa revisão de que os estudos eram inconsistentes, e que aqueles estudos que identificaram déficits cognitivos, fatores como depressão ou uso de medicamentos (especialmente prednisona) geralmente não foram levados em consideração.

Na revisão de Paul et al. (2001) manteve-se a mesma discussão sobre os possíveis efeitos dos receptores de nicotina na cognição dos pacientes com MG. Esses receptores são também localizados em regiões subcorticais e corticais no cérebro, onde eles estão envolvidos com processos não cognitivos e cognitivos específicos. Porém os autores destacam que não pode ser excluído a possibilidade de que a função cognitiva na MG pode ser afetada por processos alternativos diretamente (por exemplo, citocinas, apnéia) ou indiretamente (depressão, medicamentos) associados à doença. Além disso, destacam que quase 60% dos indivíduos com MG queixam-se de dificuldades de memória.

Corroborando com esses estudos, em uma revisão sistemática com meta-análise recente (MAO et al., 2015) foram encontrados oito estudos, todos utilizaram controles saudáveis, pareados por sexo, idade e educação. Os critérios de inclusão não estavam bem descritos e todos foram transversais. As avaliações cognitivas destes estudos foram compostas por 16 tarefas, sendo que nove revelaram tamanhos de efeito moderados. O aprendizado verbal e o domínio da memória (memória lógico-imediata, memória com atraso lógico e memória de memória imediata) pareceram ser os mais afetados em pacientes com MG. Em contraste, não houve evidência significativamente de que os pacientes com MG foram piores em termos de atenção, fluência de resposta e aprendizagem visual e memória. Desta forma, a conclusão desta revisão foi que os pacientes com MG (1) podem ter um desempenho pior do que os controles saudáveis em uma variedade de domínios cognitivos; (2) alterações de memória de evocação tardia parecem estar associadas a pacientes com MG após a remoção dos idosos usando análises de sensibilidade, porém, a capacidade de recordação imediata apresentou-se preservada em pacientes com MG; (3)

aprendizagem verbal e domínio da memória parece ser o mais significativamente afetados de acordo com as categorias cognitivas.

Contudo, os autores (MAO et al., 2015) esclarecem que esses achados devem ser interpretados com cautela devido à heterogeneidade metodológica dos estudos incluídos, pois há vários potenciais vieses como diferenças metodológicas, diferentes critérios de inclusão, estudos com resultados não significativos não publicados, diferentes tipos de parâmetros neuropsicológicos. Além disso, os autores sugerem o uso de dados normativos de validação dos testes cognitivos ao invés de comparação com grupo controle, por serem dados oriundo de uma população mais abrangente e com maior controle metodológico. Devido ao pequeno número de estudos, não foi possível realizar modelos de meta-regressão com múltiplos preditores e interações.

Além do exposto, a seguir serão apresentados os principais achados dos estudos encontrados em nossa revisão de literatura (tabelas 4 e 5).

Tabela 4: Resumos dos artigos completos analisados sobre perfil cognitivo em MG.

Autores	Amostra	Testes	Resultados	Conclusão
Lewis et al. (1989)	5 MG 8 controles	- Teste de atenção seletiva, com as tarefas de processamento usada por Wesnes e Warburton(1984) . - Inventário de depressão de Beck. - National Adult Reading Test (mensuração de QI).	Pacientes com miastenia gravis não obtiveram nenhum déficit absoluto de desempenho, nem declínio do desempenho ao longo do tempo, quando comparado com seu próprio desempenho após o tratamento (plasmaferase) ou com os controles saudáveis.	Este estudo não encontrou evidências para apoiar o envolvimento colinérgico do SNC na MG.
Iwasaki et al. (1993)	5 MG 5 controles	- Mini Exame do Estado Mental. - Zung auto classificação de depressão. - Teste de memória lógica.	A média dos escores do MEEM dos pacientes com MG foi significativamente menor do que os controles. O escore médio no questionário de depressão dos pacientes com MG foi significativamente maior do que o normal controles. Nos testes de memória lógica, os indivíduos com MG foram significativamente inferiores os controles normais.	Os resultados sustentam a hipótese de que há efeitos colinérgicos centrais na MG, manifestada por disfunção cognitiva.
Paul et al. (2000)	28 MG 18 controles	- Tarefa span de dígitos. - Teste de fluência verbal categórica e semântica.	57% dos pacientes com MG apresentavam queixas cognitivas. Não foi encontrada diferença estatística no escore da escala de depressão entre	Pacientes com MG apresentaram déficit cognitivo leve e significativamente menor que o grupo controle nas tarefas de

		- The symbol digit modalites test (SDMT). - Califórnia Teste de Aprendizado Verbal. - subteste Visual Reproduction (Reprodução Visual I e II) da Escala de Memória de Wechsler. - Figura complexa de Rey. - Inventário de depressão de Chiago.	os grupos, porém o grupo MG apresentou escores maiores. O grupo de pacientes com MG apresentou escores significativamente piores nos testes de fluência verbal, SDMT, CVLT quando comparado aos controles saudáveis.	fluência de resposta, processamento de informação e aprendizado verbal e visual. Os autores acreditam que essas alterações podem estar relacionadas a fadiga, apneia e processos autoimunes.
Paul et al. (2002)	28 MG 18 controles	- The Multicomponent Fatigue Inventory (MFI). - Chicago Multiscale Depression Inventory (CMDI). - The Digit Span subtest and Symbol Digit Modalities from the Wechsler Adult Intelligence Scale. - Teste de fluência verbal categórica e semântica. - The symbol digit modalities test (SDMT). - The California Verbal Learning Test (CVLT). - Visual Reproduction test (VR). - The Rey Complex Figure Test.	Pacientes com MG apresentaram um desempenho pior do que os controles no SDMT, testes de fluência, tarefa de aprendizado do CVLT e o teste imediato do teste de VR. Depois de concluir a bateria cognitiva, os pacientes com MG relataram aumentos significativos na fadiga mental e física em comparação com os controles. O escore de humor (CDMI) correlacionou-se com apenas uma medida cognitiva (subtarefa de memória).	Os resultados revelaram uma relação entre os níveis percebidos de aumento da fadiga e desempenho cognitivo. Sendo assim, o comprometimento cognitivos pode estar associado à fadiga percebida pelos pacientes com MG.
Marra et al. (2009)	100 MG maiores de 60 anos 31 controles	- Mini Exame do Estado Mental (MEEM) - Versão estendida da Mental Deterioration Battery (MDB): memória, atenção, praxia construtiva, reconhecimento de expressão facial, linguagem, tarefa de inteligência e funções executivas.	Não verificou diferenças significativas entre os escores do grupo MG e controles. Os pacientes tratados com prednisona obtiveram melhores escores nos testes de memória verbal, de atenção, praxia e funções executivas.	MG por si só não parece aumentar o risco de demência. Resultados não apoiam a hipótese de envolvimento colinérgico do SNC em MG, mas confirmam a presença de déficits de atenção, memória e funções executivas, provavelmente relacionados à idade.
Sitek et al. (2009)	33 MG 30 controles	- Mini Exame do Estado Mental (MEEM).	Os pacientes com MG tiveram um desempenho significativamente pior do	Os resultados não confirmaram o comprometimento do

		- Auditory Verbal Learning Test (AVLT). - Extensão de Dígitos e Semelhanças da Escala Wechsler de Inteligência Adulta Revisada (WAIS-R) - Teste de fluência verbal. - Trail Making Test (TMT). - Finger Tapping Test (FTT). - Inventário de Depressão de Beck. - MG Disability Scale.	que os controle no FTT e no TMT. A análise de correlação para o escore do BDI, o tempo desde o início da MG, o tempo desde o diagnóstico de MG, a idade, o escore da MGFA Clinical Classification e o escore de incapacidade do GM foram realizados exclusivamente para o grupo MG. Não foram detectadas correlações estatisticamente significantes.	SNC em pacientes com MG.
Hamed et al. (2014)	20 MG	- Registro encefalográfico (P300) - Mini Exame do Estado MEntal (MEEM). - Stanford - Binet Escala de Inteligência 4ª edição (SBIS) - Wechsler Escala de Memória - Revisada (WMS-R). - Inventário de Depressão de Beck.	Na amostra todos os pacientes tinham sintomas depressivos de leve a moderado. Todos tiveram testes cognitivos alterados e anormalidade na latência e amplitude do P300. Encontrou-se déficits cognitivos nas tarefas de relações verbais, compreensão, raciocínio visual, análise de padrões, quantificação, memória de contas e de curto prazo, span de dígito para frente e para trás, controle mental, memória lógica e aprendizagem associada.	Os resultados deste estudo indicam que pacientes com MG podem apresentar baixo desempenho em diferentes tarefas cognitivas indicando envolvimento do SNC na MG.
Kaltsatou et al. (2015)	32 MG 33 controles	- Wechsler Memory Scale (WMS) - Hamilton Depression Rating Scale (HAM-D) - Pupilometria.	Pacientes com MG apresentaram função pupilar anormal em comparação com indivíduos saudáveis. Além disso, pacientes com MG apresentaram escores significativamente diminuídos no teste de memória em comparação ao grupo controle.	Foi encontrada disfunção cognitiva em pacientes com MG. Os autores sugerem o uso da pupilometria com testes de memória para elucidação do comprometimento cognitivo na MG.
Eizaguirre et al. (2017)	24 MG 24 controles	- Mini Exame do Estado Mental - Teste seletivo memória - The Brief Repeatable Battery of Neuropsychology Tests (BRB-N), The Paced Auditory Serial Addition Task (PASAT, versão de 2 e 3 segundos).	O escore dos pacientes com MG comparado a dados normativos mostrou que 37,5% dos pacientes apresentaram déficit de atenção, 33% prejuízo em memória e 29,2% funções executivas. Além disso, quando comparado aos controles verificou-se pior desempenho estatisticamente	Presença de deterioração cognitiva em pacientes com MG.

		- sub-teste de Analogias e Sequência Número-letra da Wechsler Adult Intelligence Scale Third Edition (WAIS III). - Inventário de Depressão de Beck.	significativo dos pacientes com MG em armazenamento, recuperação e memória diferida, nas tarefas do PASAT e em analogias. Contudo, os pacientes com MG eram significativamente mais depressivos do que os controles. Além disso, encontrou-se relação entre depressão e déficit cognitivo em 58,3% da amostra de MG.	
Jordan et al. (2017)	33 MG 17 controles	- *The d2-R Attention and Concentration Test.* - *The Timetap-Task.* - Teste de Fluência Verbal de Regensburger (RWT). - *The Paced Auditory Serial Addition Test* (PASAT). - *The Fatigue Scale for Motor and Cognitive Function* (FSMC). - *The Myasthenia Gravis Fatigue Scale* (MGFS). - *The Center for Epidemiological Studies Depression Scale- Short Form* (CES-D). - *The Pittsburgh Sleep Quality Index* (PSQI). - Escala de Atividades de Vida Diária em MG (ADL). - Questionário de qualidade de vida em MG (MG-QoL15). - Escal visual analógica (EVA).	O escore no teste PASAT foi estatisticamente diferente, indicando menor nível de desempenho no grupo com MG. A escala de fadiga, bem como escore total do MGFS foram significativamente maiores nos pacientes com MG do que nos controles.	Presença de fatigabilidade cognitiva em pacientes com MG. Não foi encontrado indicativo de influência dos anticorpos a nível de SNC.

Tabela 5: Síntese dos resumos de estudos analisados sobre perfil cognitivo em MG.

Autores	Amostra	Testes	Resultados	Conclusão
Tucker et al. (1988)	12 MG Controles	- The Boston Naming Test. - Rey Auditory Verbal Learning Test (AVLT). - The Logical Memory and Design Reproduction portions of	O grupo de MG apresentou escore significativamente pior em relação aos grupos de controle médico e saudável no desempenho dos Boston Naming Test	Os resultados sustentam a hipótese de que a MG tem efeitos colinérgicos centrais manifestados por disfunção cognitiva.

		the Wechsler Memory Scale (WMS).	e WMS. Além disso, apresentou escore significativamente pior em relação ao grupos de controle saudável no AVLT.	
Iwasaki et al. (1990)	27 MG 27 controles	Bateria de testes para avaliar as funções cognitivas.	O grupo MG apresentou pontuações significativamente mais baixas no MEEM e no teste de memória.	Os resultados indicaram que os pacientes com MG apresentaram comprometimento cognitivo, demonstrando um envolvimento das vias colinérgicas no SNC.
Bartel e Lotz (1995)	16 MG Controles	- Eletroencefalogramas clínicos (EEGs). - The Randt Memory Test. - Tarefas da computerized Neurobehavioral Evaluation Battery. - Questionário de ansiedade. - The Profile of Mood States (POMS).	Não houve diferenças significativas entre os grupos no desempenho da memória e apenas um achado significativo isolado em uma medida cronometrada na comparação símbolo-dígito. Encontrou-se níveis significativamente mais altos de ansiedade, tensão, raiva, fadiga e confusão associados ao grupo MG. EEGs anormais ocorreram em 35% dos pacientes com MG, principalmente com retardo difuso médio-moderado.	As alterações de memória encontradas não relacionaram-se à idade ou ao tipo de MG. A medicação foi sugerida como um possível fator de influência nos déficits de memória..
Glennester et al. (1996)	11 MG	Avaliou os pacientes com MG sintomática no antes e após o tratamento com imunossupressor (prednisolona mais azatioprina ou placebo) - Subtarefas de memória, lógica e reprodução do Projeto da Wechsler Memory Scale. - Teste de Aprendizagem Verbal Auditiva de Rey. - Tarefa Peterson-Peterson. - Tarefa de vigilância auditiva.	A força muscular melhorou significativamente durante o período de tratamento, mas o desempenho geral nos testes de memória ou atenção não.	Esses resultados falham em substanciar relatos de déficits centrais funcionalmente significativos na MG.
Stepansky et al. (1997)	19 MG 10 controles	Polissonografia do sono e testes neuropsicológicos.	Observou-se que 60% das apneias e hipopneias no grupo MG era do tipo central, com dessaturação de oxigênio	Em comparação com pacientes sem apneia do sono, os miastênicos com apneias do sono

			resultante durante o sono REM, mas nenhuma diminuição do sono REM. Em testes neuropsicológicos, encontrou-se desempenho normal de vigilância, mas diminuição da função de memória na MG.	tiveram uma função de memória prejudicada.

A partir dos dados encontrados, observa-se que há limitações nos estudos anteriores que examinaram a cognição em MG e que até o momento nenhum estudo conseguiu verificar as reais causas de déficits cognitivos em pacientes com MG. O fato de mais da metade dos pacientes com MG queixar-se de problemas de memória ressalta a importância de testar empiricamente a cognição nesta população. As principais possíveis causas dos déficits cognitivos descritas pelos estudos foram: (1) distúrbios respiratórios noturnos, que podem reduzir a oxigenação cerebral causando danos ao SNC; (2) aumento da fadiga mental; (3) deficiência colinérgica central devido ao envolvimento dos nAChRs centrais e vias colinérgicas centrais pelo processo da doença de MG; (4) alterações de humor como depressão e ansiedade (EIZAGUIRRE et al.,2017; Paul et al.,2000).

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3 JUSTIFICATIVA

Este estudo justifica-se por haver poucos dados sobre as alterações de fala e cognitivas em pacientes com MG. No que diz respeito a fala, percebe-se uma ausência de dados sobre a correlação das características da disartria na MG com aspectos clínicos da doença (tempo de doença, uso de medicamentos, idade, distúrbios no sono e depressão), bem como uma comparação entre a análise acústica e perceptiva-auditiva com os protocolos de autopercepção. Dessa forma, há somente dados específicos da fala sem uma análise de quais pacientes apresentam pior padrão de fala e quais fatores podem prejudicar. Com relação a cognição, alguns estudos mostraram uma tendência ao declínio cognitivo em pacientes com MG, contudo resultados conflitantes também foram publicados. Além disso, não foram encontradas pesquisas que avaliem esses aspectos em pacientes com MG na população brasileira.

4 OBJETIVOS

4. 1 Objetivo geral

Descrever o perfil de fala e cognitivo de pacientes com MG.

4. 2 Objetivos específicos

- Caracterizar o padrão de fala de pacientes com MG, descrevendo as características da disartria nessa população, bem como o grau.
- Correlacionar o grau da disartria com idade, sexo, escolaridade, tempo de doença, idade de início dos sintomas, gravidade motora, medicações utilizadas para o tratamento da MG, classificação clínica de MG e auto percepção das dificuldades de fala.
- Correlacionar às alterações de fala encontradas em duas formas de avaliação da fala.
- Correlacionar os achados cognitivos com idade, sexo, escolaridade, tempo de doença, idade de início dos sintomas, gravidade motora, medicações utilizadas para o tratamento da MG e classificação clínica de MG.
- Correlacionar os achados cognitivos com auto percepção de depressão, alterações no sono e qualidade de vida.

5 ARTIGO CIENTÍFICOS

5. 1 Artigo 1

Submetido a revista Folia Phoniatrica & Logopaedica

Speech characteristics in individuals with Myasthenia Gravis: a case control study

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Short title: Speech characteristics in individuals with Myasthenia Gravis

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Keywords: speech; dysarthria; speech acoustics; myasthenia gravis

ABSTRACT

Purpose: describe the speech pattern of patients with Myasthenia Gravis (MG), comparing with control groups.

Methods: Case-control study. Participants were divided in case group (GMG) with 39 patients diagnosed with MG and control group (CG) with 18 individuals matched for age and sex. GMG was evaluated with clinical and motor scales and answered self-perceived quality of life, sleep, depression and speech questionnaires. Speech assessment of both groups included: recording of speech tasks, acoustic and auditory-perceptual analysis.

Results: In the GMG, 68.24% of the patients were female, with average age of 50.21 years old (±16.47), 14.18 years (±9.52) of disease duration and a motor scale of 11.19 points (±8.79). The auditory-perceptual analysis verified that the GMG presented a high percentage of alterations in the motor bases phonation (95.2%) and breathing (52.63%), whereas in breathing there was a significant difference between the groups. The acoustic analysis verified a change in the motor base of phonation, with significantly higher shimmer values in the GMG compared to the CG, and articulation with a significant difference between the groups for the first formant of the “iu” ($p = <0.001$). No correlation

was found between motor, clinical and self-perception questionnaires with acoustic analysis.

Conclusion: It was observed the presence of dysarthria in the GMG, with changes in the motor bases phonation and breathing. The changes did not show correlation with time and stage of the disease and self-perception questionnaires. A worse phonation pattern was correlated with the use of glucocorticoids.

INTRODUCTION

Myasthenia Gravis (MG) is an autoimmune disease. The characteristic symptoms of the disease are muscle weakness and fatigue, with worsening on exercise. Although infrequent, dysarthria can be the first and only symptom of MG in 6 to 27% of cases (Mao et al., 2001; Neal & Clarke, 1987). In addition, in some cases it may take years to evolve to other symptoms, which in many cases makes the diagnosis difficult, being commonly confused with stroke (Liu et al., 2007; Montero-Odasso, 2006).

Dysarthria in MG is categorized as flaccid, with the presence of the following changes: hypernasality, difficulty in sustaining the frequency, vocal fatigue, intermittent aphonia, stridor, roughness, glottal incompetence, breathy or rough vocal quality, articulatory imprecision and verbal fluency change (Mao et al., 2001; Liu et al., 2007; Montero-Odasso, 2006; Ortiz & Carrillo, 2008; Konstantopoulos et al., 2017). The prevalence of dysarthria in MG varies from 6 to 27% as an initial symptom, affecting about 60% of patients with disease progression (Mao et al., 2001; Neal & Clarke, 1987). A Brazilian study found that the presence of dysphonia in the age group above 50 years old was as frequent as that of diplopia (Aguiar et al., 2010). Patients complain of a breathy, nasal

voice and difficulty in speaking with high intensity. These symptoms can appear at any stage of the disease, and are often associated with dysphagia (Mao et al., 2001; Liu et al., 2007).

Few studies to date have evaluated and described the speech pattern of patients with MG. In the acoustic analysis, the studies found (Mao et al., 2001; 5, Ortiz & Carrillo, 2008; Konstantopoulos et al., 2017; Padovani, 2011; Sun et al., 2005; Tóth et al., 1999) impaired range and vocal dynamics, reduced maximum phonation time, increased average fundamental frequency during speech, increased standard deviation of fundamental frequency, decreased vocal intensity and longer silence intervals during diadochokinesis. Furthermore, when compared to healthy control patients, these results were significantly worse. Of these, only one study presented data on auditory-perceptual analysis (Konstantopoulos et al., 2017). This study found maximum phonation time, reduced control of sound and voice and slow tongue movements (Konstantopoulos et al., 2017). With regard to the clinical aspects of MG, only one study correlated speech findings with antibodies, verifying that the subgroup with the presence of acetylcholine antibodies (AchR) had more frequent phonation problems (time for sustained phonation and breathing) while participants with anti-MuSK more often had articulatory difficulties and hypernasality (Konstantopoulos et al., 2017).

From the above, there is an absence of data on the correlation of the characteristics of dysarthria in MG with clinical aspects of the disease (duration of illness, use of medications, age, sleep disorders and depression), as well as a comparison between auditory-perceptual and acoustic analysis with self-perception protocols. Thus, there is only data for isolated speech parameters

without an analysis of what factors can impair speech in MG. Therefore, the purpose of this study was to describe the auditory-perceptual and acoustic pattern of speech in patients with MG, correlating with clinical aspects of the disease and comparing with healthy control patients, in order to identify which variable characteristics are associated with dysarthria in this population, as well as the speech characteristics associated with the disease.

MATERIALS AND METHODS

Study design

We opted for a case control study.

Subjects

The study included patients diagnosed with MG, recruited from an Outpatient Clinic for Neuromuscular Diseases from Hospital de Clínicas de Porto Alegre, Brazil. The diagnosis of MG was confirmed by electromyography and / or the presence of anti-striated acetylcholine / Musk / anti-striated muscle.

The control group was composed of individuals matched for age and sex, with no diagnosis of neurological diseases. A questionnaire was applied with questions about diagnoses, medication use, previous surgeries and other comorbidities in order to rule out the presence of any disease in the control group that could interfere with the speech pattern. The CG was composed of individuals from the community who do physical activities in community centers.

The exclusion criteria for both groups were individuals with a history of other previous neurological events, any sensory or motor disorders that

prevented the tests from being performed, systemic diseases and / or structural changes that affected the voice and / or speech were excluded.

Procedures

All person was assessed individually, in a room at the hospital's Research Center. The recording of the speech and application of the questionnaires lasted an average of 20 minutes. Data collection was always performed by the same previously trained researcher. The collection period occurred between February 2017 and December 2018.

Questionnaires and scales for MG group:

- Socio-demographic questionnaire: structured questionnaire containing questions regarding general patient data, such as age; sex; schooling; initial symptom.

- Medical record data: data were collected from the patients' medical record, according to the last consultation carried out before the evaluation: history of diseases; illness time; diagnosis age; medications in use; surgical history; disease staging; motor symptoms.

- MG quality of life scale (MG-QOL 15): self-reported questionnaire, specific to assess quality of life in patients with MG. The questionnaire has 15 items, ranging from 0 to 60 points, the higher the score, the worse the patient’s quality of life is considered (Sadjadi et al., 2012; Burns et al., 2010).

- Questionnaires Radbould Oral Inventory Motor for Parkinson's disease (ROMP): consists of a questionnaire for self-perception of speech, swallowing and saliva changes. Only the speech subitem composed of seven items was applied, each item has a scale from one to five points (one normal and five being

the worst score) in which the patient must mark how he feels (Kalf et al., 2011; Presotto et al., 2018).

- Beck Depression Inventory (BDI): self-assessment tool for surveying the intensity of depressive symptoms (Beck & Ward, 1961). For the score, the values proposed by Gorenstein and Andrade (1998) were used: less than 10 points – without depression or minimal depression; 10 to 18 points – mild to moderate depression; 19 to 29 points – moderate to severe depression; 30 to 63 points – severe depression.

- Epworth sleepiness scale: self-reported questionnaire involving eight questions that assesses the probability of falling asleep in eight situations involving daily activities. The global score ranges from 0 to 24, with scores above 10 suggesting the diagnosis of excessive daytime sleepiness (EDS) (Bertolazi et al., 2009).

- The Quantitative Myasthenia Gravis Score (QMGS): clinical scale used as an outcome measure in MG. It consists of 13 items, with a maximum score of 39 points, where a higher score indicates more serious disease (Burns et al., 2010; Benatar et al., 2012; Oliveira et al., 2016).

- The Myasthenia Gravis Foundation of America Classification (MGFA classification): it is a clinical classification, which distinguishes patients into 5 classes, which range from Class I characterized by any ocular muscle weakness, with all other normal muscle strengths to Class V defined as intubation, with or without mechanical ventilation, except when used during routine postoperative management (Jaretzki et al., 1988). The patients were classified after QMGS findings.

Speech assessment for both groups

- Speech collection: Speech and voice were recorded directly on the Acer Aspire One 725-0899 Windows 7 computer, using the Audacity software, the PureAudio Andrea sound card, USB Audio*Software*, version 6.0.0, and headset microphone (Karsect HT2). Emissions of the extended vowel “a”, diphthong “iu” were repeatedly recorded, diadocokinesia (repetition of syllables /pataka/ in sequence as many times as the patient can achieve in a single breath) and counting numbers 20 to 30, with a model offered by the researcher, in usual frequency and intensity, self-selected by the individual.

- Perceptual-auditory speech analysis: all collected tasks were analyzed. The analysis was carried out by 5 blind judges simultaneously entering consensus after all the audio broadcasts of each participant. The judges classified each of the 5 speech motor bases in: normal or altered (mild, moderate or severe).

- Speech acoustic analysis: two programs were used *Voxmetria* software, CTS Informática, *Voice Analysis* module and PRAAT open software. All collected tasks were analyzed in the open software PRAAT, used in speech analysis and synthesis (Boersma & Weenick, 2006), as shown in chart 1. The program was developed by linguists Paul Boersma & David Weenink and its focus is sound analysis, through parameters such as frequency, wavelength, decibels, among others. Besides that, the *Voxmetry* software, from CTS Informática, was used to extract the F0 variability values, in semitones. Two clients were created for the acoustic analysis performed in this software – one to evaluate the female voices and the other, the male voices, in the Vocal Analysis module. The same vowel / a / analyzed in the PRAAT program was imported and the sampling range was

automatically converted from 44100Hz to 22050Hz. The sample was analyzed, and the results presented in a statistical report.

Chart 1. Motor bases, tasks performed and variables resulting from the acoustic evaluation of speech.

Motor Base	Assignment	Resulting variable
Phonation	Vowel A sustained in a single breath and a single repetition. Only one production of the individual will be requested.	**Fundamental frequency (Fo):** number of cycles that the vocal folds perform per second. For Brazilian Portuguese speakers, the frequency range of normality for females is 150-250 Hz and 80-150 Hz for males (Behlau, 2001). **Jitter:** it is a measure of disturbance of the fundamental frequency characterized by the irregularity of the vibration of the vocal fold mucosa correlating with the biomechanical characteristics of the vocal folds and with the variation of neuromuscular control. The normative values of PRAAT is 0.680% as a threshold for pathology for *jitter rap* (Boersma & Weenick, 2006). **Shimmer:** it is a measure of disturbance in the amplitude of the sound wave and offers an indirect perception of noise in vocal production; their values increase the greater the amount of noise in an emission. The normative values of PRAAT is 3.810% as a threshold for pathology for *shimmer local* (Boersma & Weenick, 2006). **Fundamental frequency variability:** value of the maximum fundamental frequency (Fo max) – Minimum fundamental frequency (Fo min). The value obtained in the program is converted to semitones. So far, there is no standard of normality for Brazilian Portuguese speakers.
Resonance	Count the numbers from 20 to 30 Repetition diphtong /iu/ sounds alternately, the greatest number of times in a single breath	**Through connected speech**, the evaluators observed whether there was a resonance deviation during chained speech, namely excess or lack of nasality, pharyngeal focus, laryngeal**.** **Repetition of two combined vowels:** extraction of the third and fourth formants of vocal production. The / i / and / u / are the most oral vowels in Brazilian Portuguese, which allows to perceive changes in nasality control, when altered in this test (aurally). So far, there is no standard of normality for Brazilian Portuguese speakers.
Prosody	Count the numbers from 20 to 30	**Fundamental frequency variability:** value of the maximum fundamental frequency (Fo max) – Minimum fundamental frequency (Fo min). The value obtained in the program is converted to semitones. There is a Brazilian study how who found the following values for the control group, based medium intensity: vocal extension weak 8.49 semitones, medium 9.08 and strong 10.45 and these values made it possible to differentiate between dysphonic and healthy voices (Moraes & Behlau, 2010)
Breathing	Vowel A sustained in a single breath and a single repetition. Only one production of the individual will be requested.	Indicates the subject's ability to control the aerodynamic forces of the pulmonary current and the myoelastic forces of the larynx. It is related to the subject's vital capacity. For Brazilian Portuguese speakers, the standard of normality for females is 14 if, for males, 20 seconds (Behlau, 2001).

Articulation	Alternate repetition of /pataka/ as fast as the subject can achieve in a single breath. The evaluator will give the models before production by the subject.	**Diadochokinesis:** ability to perform alternating movements quickly and repeatedly, assesses the neuromuscular integrity of the speech-language organs. This index is usually determined by the number of syllables per second, with normal values varying from four to six syllables, with a certain reduction in the number with age (Behlau, 2001).Youngs adults 6.58 syllables per second and Elderly people 6.13 syllables per second (Padovani, Gielow & Behlau, 2009)
	Repetition diphtong /iu/ sounds alternately, the greatest number of times in a single breath	**Repetition of two combined vowels:** extraction of the first two formants of vocal production. It assesses the ability of rapid and rhythmic transition of articulation points, thus evaluating articulatory mobility. The analysis of the second formant has shown a high correlation with the perception of intelligibility of vocalizations and, therefore, can be a predictive of speech intelligibility. The normative values in the Motor Speech Profile of Key Elemetrics was F2 in women 609,580Hz and F2 in men 548,260Hz.

Statistical analysis

The software was used Statistical Package for the Social Sciences (SPSS). The level of statistical significance adopted was $p < 0.05$. The statistical tests were selected according to the distribution of data provided by the Shapiro-Wilk test and histograms. Continuous variables were described using the terms minimum, maximum, average and standard deviation. Categorical variables were described by percentage and N. The comparison between the case and control groups was performed using the T test for independent samples for variables with normal distribution. The non-parametric T test for independent samples was used for variables with non-normal distribution. Fisher's exact test was used to compare the auditory-perceptual analysis between the groups. Pearson and Spearman's Correlation was used to verify whether there was a correlation between clinical variables and self-perception questionnaires with acoustic analysis. The T test was used in the comparison between acoustic analysis and medication use and diagnosis of depression.

RESULTS

Initially, 88 patients with MG were included in the study. Of those, 50 patients excluded for the following reasons: 39 (44.31%) did not attend the scheduled date for evaluation, eight (9.09%) did not accept to participate in the study, one (1.13%) patient was hospitalized, one (1.13%) was under 18 years old and one (1.13%) patient had Spanish as her native language. The final sample of this study was composed of 38 individuals with generalized MG and 18 control patients matched for age and sex.

According to the MGFA classification, there was a similar percentage between classes I, II and III, respectively 26.3% (n = 10), 34.2% (n = 13), 31.6% (n = 12) and a small number of class IV patients 7.9% (n = 3).

The results on the clinical, sociodemographic and self-perception questionnaires are described in table 1. There was no statistical difference in the ages of both groups (p = 0.087).

Table 1: Description of sociodemographic and clinical variables and self-perception questionnaires (speech, quality of life, depression and sleep).

Variables		GMG		GC	
		Average	DP	average	DP
Age in years		50.21	16.47	51.22	16.24
Education in years		9.13	4.28	-	-
Illness time in years		14.18	9.52	-	-
ROMP		12.82	5.59	-	-
MGQOL		16.13	15.41	-	-
QMGS		11.19	8.74	-	-
BDI		12.59	11.70	-	-
Variables		**%**	**N**	**%**	**N**
Sex	Female	68.42	26	55.5	10
	Male	31.57	12	44.4	8
Immunomodulators	Uses	50	19	-	-
	Does not use	50	19	-	-
Acetylcholinesterase inhibitors	Uses	92.1	35	-	-
	Does not use	7.9	3	-	-
Glucocorticoids	Uses	57.9	22	-	-
	Does not use	42.1	16	-	-
Associated diseases	Yes	73.7	28	-	-
	No	26.3	10	-	-
Diagnosis of depression	Yes	18.4	7	-	-
	No	81.6	31	-	-

BDI classification	No depression	47.4	18	-	-
	Mild depression	13.2	5	-	-
	Moderate depression	21.1	8	-	-
	Severe depression	7.9	3	-	-
	Did not answer	10.5	4	-	-

GMG = myasthenia gravis group; CG = control group; SD = standard deviation; N = number of individuals; % = percentage; ROMP = Radbould Oral Inventory Motor Questionnaires for Parkinson's disease; MGQOL = MG quality of life scale; QMGS = The Quantitative Myasthenia Gravis Score; BDI = Beck Depression Inventory

Regarding the auditory-perceptual analysis, there was a high percentage of patients in the MG group with changes in motor bases, phonation and breathing. The CG presented only one subject with slight alteration in the motor bases of phonation and breathing (table 2). In addition, when comparing the normal and altered numbers for each group, it was found that the distribution tends to show that the case group is significantly altered when compared to the control group on the motor base of breathing. Due to the shape of the phonation and articulation variables, it was not possible to perform association analysis (table 3).

Table 2: Result of the auditory-perceptual analysis by percentage of normal and altered.

Motor Bases	Classification	GMG% (n)	GC% (n)
Phonation	Normal	28.9 (11)	93.8 (15)
	Slight change	68.4 (26)	6.3 (1)
	Moderate change	2.6 (1)	-
Breathing	Normal	47.4 (18)	93.8 (15)
	Slight change	44.7 (17)	6.3 (1)
	Moderate change	7.9 (3)	-
Resonance	Normal	84.2 (32)	100 (16)
	Slight change	10.5 (4)	-
	Moderate change	2.6 (1)	-
	Severe change	2.6 (1)	-
Prosody	Normal	92.1 (35)	100 (16)
	Slight change	5.3 (2)	-
	Moderate change	2.6 (1)	-
Articulation	Normal	63.2 (24)	100 (16)
	Slight change	26.3 (10)	-
	Moderate change	10.5 (4)	-

Note: the audio of two individuals was not analyzed in the CG.

Table 3: Cross between the results of the auditory-perceptual analysis between the groups.

Motor Bases	GMG		GC		p
	normal	changed	Normal	changed	
Breathing	18 (54.5%)	20 (95.2%)	15 (45.5%)	1 (4.8%)	0.001
Resonance	32 (66.7%)	6 (100%)	16 (33.3%)	0 (0.0%)	0.092

Prosody	35 (68.8%)	3 (100.0%)	16 (31.4%)	0 (0.0%)	0.247

Fisher's exact test; p = p value.

In the acoustic analysis, a significant difference was observed between the groups for *shimmer local*. Thus, the GMG presented greater noise in the vocal emission than the CG. Moreover, the formant 1 and 2 of the syllable repetition / iu / showed a significantly lower value in the GMG when compared to the CG. The other variables analyzed did not show statistical difference between the groups (tables 4 and 5)

Table 4: Comparison of parameters of acoustic analysis: parametric analysis.

Motor Base	Parameters of acoustic analysis	GMG		GC		Difference from averages	IC (95%)	p
		average	DP	average	DP			
Phonation	Shimmer local	11.30%	4.43%	57.03%	3.23	54.27%	1.92: 6.62	0.001
	FF average vowel	176.39Hz	43.78 Hz	169.20 Hz	44.18 Hz	7.19 Hz	-18.00: 32.38	0.570
Ressonance	IU F3	2986.58Hz	193.13Hz	3049.33Hz	215.52Hz	-62.75Hz	-177.74: 52.23	0.279
Articulation	PATAKA[a]	5.44	1.63	5.22	1.87	0.22	-0.76: 1.20	0.654
	IU[a]	1.27	0.38	1.20	0.35	0.07	-0.14: 0.28	0.517
	IU F2	1708.64Hz	188.91Hz	1822.04Hz	176.41Hz	-113.40Hz	-219.57: -7.23	0.037
Prosody	FF average count	174.22 Hz	37.49 Hz	158.16 Hz	40.12 Hz	16.05 Hz	-6.05: 38.16	0.151
	FF semitone variability count	17,46	5,79	15,18	4,8	2.28	-0.95: -0.76	0.676

T test; GMG = myasthenia gravis group; CG = control group; SD = standard deviation; CI = confidence interval; p = p-value; [a] syllables per second; FF = fundamental frequency; F = formant

Table 5:Comparison of the parameters of the acoustic analysis: non-parametric analyzes

Motor Base	Parameters of acoustic analysis	GMG					GC					
		average	DP	median	Interquartile range		average	DP	median	Interquartile range		
					25	75				25	75	
Phonation	Jitter rap	0.59%	0.46%	0.50%	0.22	0.91	0.29%	0.23%	0.19%	0.12	0.53	4
	FF minimal vowel	139.19 Hz	52.18 Hz	121.38 Hz	96.20 Hz	184.53 Hz	130.05 Hz	47.27 Hz	119.85 Hz	86.38	179.03	77
	FF maximum vowel	301.03 Hz	129.81 Hz	266.23 Hz	180.04 Hz	424.87 Hz	258.90 Hz	131.51 Hz	222.85 Hz	148.16	397.08	39
	FF DP vowel	36.29 Hz	33.06 Hz	21.65 Hz	6.02 Hz	62.66 Hz	267.25 Hz	32.41 Hz	14.18 Hz	1.72	47.56	77
	FF Variability vowel in Hz	118.66	87.018	97.81	30.95	184.92	126.64	101.76	103.38	31.96	214.91	92
	FF Variability vowel in semitone	10.02	7.026	9.5	4.00	15.25	11.00	7.91	10.00	4.00	18.50	99
Breathing	TMF vowel	5.41	3.00	5.24	2.61	7.49	9.36	4.70	8.19	5.47	12.92	20
Prosody	FF minimal count	94.96 Hz	31.09 Hz	85.06 Hz	76.60	101.13	89.84 Hz	23.27 Hz	81.47 Hz	77.85	94.33	84
	FF maximum count	420.03 Hz	86.77 Hz	454.39 Hz	361.06	487.44	409.52 Hz	108.42 Hz	473.24 Hz	343.31	490.53	70
	FF DP count	59.41 Hz	32.53 Hz	57.12 Hz	37.29	71.94	71.50 Hz	108.79 Hz	37.15 Hz	25.01	85.05	17
Articulation	IU F1	442.45 Hz	107.37 Hz	421.15 Hz	358.54	502.87	542.39 Hz	79.51 Hz	523.41 Hz	502.57	607.16	<0
Ressonance	IU F4	4940.26 Hz	5306.08 Hz	4117.90 Hz	3966.67	4235.86	4047.43 Hz	227.97 Hz	4032.84 Hz	3962.45	4233.73	39

Nonparametric test for independent samples; GMG = myasthenia gravis group; CG = control group; [a] syllables / second; FF = fundamental frequency; SD = standard deviation; F = formant; MPT = maximum phonation time p = p-value.

Table 6 shows the correlation analysis between acoustic analysis and self-perception questionnaires, motor scale and clinical variables. A significant difference was observed between the use of glucocorticoids with the acoustic analysis, thus, patients using glucocorticoids had a higher percentage of irregularity in the vocal fold vibration during phonation (Table 7).

Table 6: Correlation analysis between parameters of the acoustic analysis with sociodemographic, clinical and self-perception questionnaires (speech, quality of life, depression and sleep).

	Jitter rap		Shimmer local		FF Variability	
	p	R	p	R	p	R
Age[a]	0.352	0.155	0.249	0.192	0.194	-0.215
Education[a]	0.107	-0.266	0.021	-0.347	0.198	-0.214
Disease duration[a]	0.733	0.057	0.517	0.109	0.719	-0.060
QMGS[a]	0.427	-0.137	0.836	0.036	0.598	-0.091
MG QOL[º]	0.409	-0.138	0.940	0.013	0.228	-0,200
ROMP[º]	0.725	0.059	0.192	0.216	0.296	-0.175
Total BDI[º]	0.134	-0.263	0.459	0.131	0.708	-0.067

[a]Pearson correlation; º Spearman correlation; p = P value; R = correlation coefficient; FF = fundamental frequency; ROMP = Radbould Oral Inventory Motor Questionnaires for Parkinson's disease; MG QOL = MG quality of life scale; QMGS = The Quantitative Myasthenia Gravis Score; BDI = Beck Depression Inventory

Table 7: Comparison between acoustic analysis and medication use and diagnosis of depression.

			average	DP	p	IC 95%
Jitter rap	Depression	Yes	0.46%	0.33%	0.406	-0.55: 0.23
		No	0.62%	0.48%		
Shimmer local		Yes	12.03%	3.94%	0.630	-2.91: 4.69
		No	11.14%	4.58%		
FF Semitone Variability		Yes	9.42	5.71	0.807	-6.77: 5.30
		No	10.16	7.36		
Jitter rap	Immunomodulators	Uses	0.46%	0.33%	0.080	-0.58: 0.03
		Does not use	0.72%	0.54%		
Shimmer local		Uses	10.04%	3.87%	0.078	-5.36: 0.29
		Does not use	12.57%	4.69%		
FF Semitone Variability		Uses	12.21	5.88	0.054	-0.08: 8.81
		Does not use	7.84	7.53		
Jitter rap	Acetylcholinesterase inhibitors	Uses	0.58%	0.40%	0.493	0.76: 0.37
		Does not use	0.77%	1.07%		
Shimmer local		Uses	11.33%	4.18%	0.890	-5.10: 5.86
		Does not use	10.96%	8.09%		
FF Semitone Variability		Uses	9.85	7.2	0.619	-10.80: 6.51

		Does not use	12.00	3.60		
Jitter rap	Glucocorticoids	Uses	0.71%	0.52%	0.046	-0.01: 0.57
		Does not use	0.43%	0.30%		
Shimmer local		Uses	12.20%	4.78%	0.147	-0.78: 5.03
		Does not use	10.07%	3.69%		
FF Semitone Variability		Uses	8.50	6.20	0.118	-8.21: 0.96
		Does not use	12.12	7.72		

T test; SD = standard deviation; CI = confidence interval; p = p-value; FF = fundamental frequency

DISCUSSION

From the results presented above, it was found in this sample that patients with MG presented altered motor bases of phonation and breathing, predominantly classified as mild, in the auditory-perceptual analysis. In addition, when comparing the normal and altered numbers for each group, the motor base of breathing presented significantly altered in the MG patients. The main impairment in respiratory subsystem may be related to the general characteristic of the disease, muscle weakness and fatigue (Mao et al., 2001; Neal & Clarke, 1987; Liu et al., 2007; Montero-Odasso, 2006).

The acoustic analysis observed changes in the motor bases of phonation and articulation, corroborating what was found in the auditory-perceptual analysis. However, it is proven important to carry out the two analysis, considering that only the auditory-perceptual analysis was sensitive to detect the change in the motor base of breathing. The hypothesis is that the number of respiratory cycles per second is not significantly reduced in the GMG, but the pneumophonoarticulatory coordination is, showing the alteration in the functionality of patients with MG perceived in the auditory-perceptual analysis.

Regarding the motor base of breathing, measured in the acoustic analysis by the TMF, it was observed that the patients with MG presented values well below the

standard of normality and lower than the CG, but without statistical difference (Behlau, 2001). This may be because only a single measurement of the sustained vowel “a” was made and the participants did not correctly understand the requested task. The change in breathing showed difficulty in patients with MG to maintain miolaringeal balance for longer. This finding may be related to the muscle fatigue found in MG.

For phonation, in the acoustic analysis, it was found that patients with MG had significantly higher values for *shimmer* than the CG. The shimmer is a measure of disturbance in the amplitude of the sound wave and offers an indirect perception of noise in vocal production. Their values increase the greater the amount of noise in an emission (Padovani, 2011). In addition, the values found for measuring the disturbance of the amplitude of the sound wave (*shimmer local*) in the GMG were higher than the standard of normality of PRAAT (3.810%) and literature (up to 3%) (Behlau, 2001; Figueiredo et al., 2003) and corroborated with that found in the study of Padovani (2011) for patients with MG. Clinically, the *shimmer* demonstrates the patient's difficulty in maintaining airflow in the voice, due to reduced glottic resistance and mass lesions in the vocal folds, being correlated with the presence of noise at emission and with breathiness (Behlau, 2001; Figueiredo et al., 2003).

In the acoustic analysis regarding the articulation, a significant difference was observed between the groups only for the first formant of the “iu”, which was significantly lower in the GMG. The rapid fatigue in MG could bring less amplitude in the alternation of movements for speech, leading in its extreme difficulty to articulatory imprecision or progressive loss of speech. The extraction of the first and second formants from the repetition of two alternating vowels assesses the ability of rapid and rhythmic transition of the two opposites articulatory points, thus evaluating the articulatory mobility. There is a description in the literature that the degree of opening

of a vowel has a direct relationship with the first formant and the degree of anteriorization of the vowel, or how free the pharynx is or is not, due to the displacement of the tongue, a direct relationship with the second formant (Padovani, 2011). So far, there is no standard of normality for Brazilian Portuguese speakers.

Literature (Padovani, 2011; Yang et al., 2011; Kumar et al., 2018; Padovani et al., 2009) presents a prevalence of the use of diadochokinesis to measure the articulatory quality of patients with neurological diseases. In this study, it can be seen that this was not a sensitive measure to verify acoustically the articulation deficits in our population. However, in the auditory-perceptual analysis, it is possible to see the deficit of functionality of these patients, considering that in this analysis not only the number of syllables per second is evaluated, but also the quality in terms of articulation accuracy and rhythm maintenance. We emphasize that the fact of using pataka and alternating movements can take more time for the manifestation of fatigue when compared to the repeated emission of the same syllable, such as / pa / / ta / or / ka /, as in the latter there is overload due to rapid repetition of the same motor action. It may be a suggestion for future work, include these measures too.

We did not find any correlation between the scores on the motor scale and duration of illness with the variables of the acoustic analysis. Thus, it appears that in this sample, patients with MG presented dysarthria regardless of the severity and duration of the disease. Regarding the use of medications, worse phonation production was observed in patients using glucocorticoids. Thus, it is suggested that these patients be referred early for speech assessment.

The studies found that acoustically analyzed the speech of patients with MG described that the maximum phonation time and vocal intensity was reduced and the average fundamental frequency during speech increased, when compared to healthy

control patients (Mao et al., Konstantopoulos et al., 2017; Sun et al., 2005; Tóth et al., 1999). Our study found such changes; however, only vocal intensity was significantly reduced when compared to control patients.

The data from the acoustic analysis are in agreement with that described on imaging exams that show deficit in vocal fold mobility, unilateral or bilateral, incomplete glottic closure (Mao et al., 2001; Liu et al., 2007; Xu et al., 2009) and with laryngeal electromyography in which it are observed vocal fold movements weaker than normal and not performing complete glottic closure in 16.7% of patients with MG (Xu et al., 2009; Hou et al., 2007).

When correlating data from the acoustic analysis with the self-perception questionnaire of speech, it was found that the questionnaire was not sensitive to differentiate patients with greater and lesser speech impairment. These data corroborate the findings of other neurological diseases (Pawlukowska et al., 2018; Miller et al., 2011), such as Parkinson's disease. The degree and type of speech disorders found in the objective assessment do not necessarily reflect their subjective perception in the functioning of articulatory organs. Therefore, it is important that the multiprofessional team that accompanies these patients have proactive approach, referring them to speech therapy clinical evaluation independently of patients' complaints, based not only on self-perception questionnaires.

The data found in our study reinforced the importance of assessing dysarthria using auditory-perceptual analysis, acoustic assessment of voice and speech and self-assessment of the patient. The clinical assessment with auditory-perceptual analysis direct the altered subsistence and acoustic assessment of voice explain how this is happening. This would promote a more adequate understanding of which parameters are able to identify the presence of these speech disorders in patients with MG. It would

also make it possible to assist in the differential and early diagnosis between different neurological and neuromuscular diseases and to enable a comprehensive treatment of these patients (Ortiz & Carrillo, 2008; Padovani, 2011; Pawlukowska et al., 2018). Moreover, based on the findings we suggest that for the acoustic analysis of dysarthria in MG the speech therapist is more attentive to the values of IU shimmer and formants. Future studies should investigate a specific speech analysis protocol for this disease.

Furthermore, it was found that studying the speech of patients with neurological disease without associating it with the clinical aspects of the disease generates a risk of characterizing dysarthria inappropriately. This study found changes mainly in the motor bases of phonation and breathing, which is not consistent with the simplistic characterization of the literature that patients with MG have flaccid dysarthria (Mao et al., 2001; Liu et al., 2007; Montero-Odasso, 2006; Konstantopoulos et al., 2017).

Study limitations

The fact of we just collected one production of vowel A can be interfered in the data of breathing. Although, the changes in speech remain even after the crisis, we found a difficult in these patients come to evaluation and therapy. It is probable because they are young and functional.

Another limitation of the study is that it was carried out in a public hospital, a reference in the treatment of patients with MG. This may have taken more serious patients to our recruitment universe, as they require more specialized care. As such, the representative power of the sample may have been reduced. Furthermore, we cannot be analyzed for the correlation between antibodies and cognitive performance because the test was not available in the public health system.

CONCLUSION

The perceptual-auditory and acoustic pattern of speech of patients with MG was dysarthria, with changes in the motor bases phonation, breathing and articulation. Phonation was the motor basis with the highest percentage of alteration and breathing with the greatest perceptual-auditory differentiation between individuals with MG and control patients. Furtermore, we observed ther importance of speech assessment regardless of the time and severity of the disease and the patient's complaints, considering that in this sample we found that patients with MG presented dysarthria regardless of the severity and duration of the disease. In addition, it was found that the speech self-perception questionnaire was not sensitive to differentiate patients with greater and lesser speech impairment. Regarding the use of medications, worse phonation production was observed in patients using glucocorticoids.

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5. 2 Artigo 2

Submetido a revista Neuromuscular Disorders

Title Page

Cognitive performance in patients with Myasthenia Gravis: an association with glucocorticosteroid use and depression

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RSR: analysis and interpretation of data; writing of the article.

GPJ: conception and study design; final approval of the version to be submitted.

MRO: conception and study design; data analysis and interpretation; final approval of the version to be submitted.

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Highlights

1. Results suggest cognitive impairment in patients with MG.

2. In this sample, memory and verbal fluency impairments were associated with depression.

3. In this sample, the use of glucocorticosteroids was associated with a decline in memory.

Abstract

We investigated the cognitive performance of patients with Myasthenia Gravis (MG). It was performed a cross-sectional study. A battery of cognitive assessment was applied and self-report questionnaires regarding quality of life (QoL), sleep and depression. The sample was composed by 39 patients diagnosed with MG. The scores showed a predominance of cognitive impairment on the Montreal Cognitive Assessment screening test (MOCA) (66.7%) and on the immediate (59.0%) and recent memory (56.4%) tests. However, after the Poisson regression analysis with robust variance, it was found that patients diagnosed with depression had a prevalence ratio (PR)=1,887(CI:1,166-3,054) for lower MOCA scores, PR=9,533(CI:1,600-56,788) for poorer Phonemic verbal fluency scores and PR=12,426(IC:2,177-70,931) on the Semantic Verbal Fluency test. Moreover, concerning a decline in short-term memory retention, patients using glucocorticosteroids (GC) and with Beck Depression Inventory scores indicating depression showed PR=11,227(CI: 1.736-72.604) and PR=0.35(CI:0.13-0.904), respectively. No correlation was found between QoL questionnaire and performance on cognitive tests. We found worst performance in tasks of memory and executive functions in MG patients. These are not associated with the time and severity of the disease. However, a significant prevalence ratio was found for poorer memory performance in patients diagnosed with depression and in patients using GC.

Keywords: Myasthenia Gravis; Cognition; Cognitive assessment; Glucocorticoids; Depression.

1. Introduction

Myasthenia Gravis (MG) is an autoimmune disease caused by the destruction of nicotinic acetylcholine receptors (nAChRs) at the motor end plates found in striated muscles. Its incidence ranges from 1 to 9 cases per million of the general population. The prevalence rate of MG ranges from 15 to 179 cases per million of the worldwide population. In the Brazilian population, there are no data regarding the national prevalence of the disease [1, 2].

Although it is a predominantly muscular disease, cognitive impairment in patients with MG has been discussed in literature. Some studies found cognitive decline on memory [3, 4, 5, 6, 7, 8, 9], attention, executive functioning [8, 10], verbal fluency [8,9] and planning tasks [6, 11]. However, there are other studies [12, 13, 14, 15] that found no difference in the cognitive performance of MG patients when compared to healthy controls.

In a recent systematic review and meta-analyses [16], the authors described four main explanations for the cognitive deficits found among many MG patients: (1) central pathogenic antibody effect (Abs) against acetylcholine receptors (AChRs); (2) for some patients, a lack of certain protective factors such as age, disease severity, and type of treatment; (3) mood disturbances; and (4) the possible effect of nonspecific immunological processes.

Based on the above, it appears that, while some studies have shown a tendency toward cognitive decline in patients with MG, conflicting results have also been published. Thus, considering this lack of clarity in the literature, along with the research gap regarding the affected Brazilian population, the aim of this study was to investigate the cognitive performance of patients with MG. Besides that, it is known that depression, worsening quality of life, sleep problems and medications can negatively influence in cognitive performance. Therefore, we verified the association between cognitive performance with clinical aspects and quality of life in patients with MG.

2. Materials and Methods

2.1 Study Design

This was a cross-sectional, exploratory study.

2. 2 Subjects

We recruited patients from a neuromuscular diseases outpatient clinic at a hospital in Porto Alegre, Brazil, which which is a reference in the treatment for patients diagnosed with MG in the state. Diagnosis of the disease was confirmed by electromyography and / or the presence of AChR / Musk / Striated Muscle antibodies. Patients were evaluated outside of crisis episodes and with medication stabilized for at least 6 months. We excluded patients with a history of other primary neurological (e.g. transient ischemic attacks, cerebrovascular stroke or epilepsy), psychiatric (e.g. major depression), previous serious head injury or any sensory or motor disorder that would preclude psychological testing (as blindness or deafness).

The collection period took place between February 2017 and December 2018. All subjects were informed about our research objectives and signed a consent form. This study was approved by the Central Research Ethics Committee of the hospital, certificate of approval number 120399.

2. 3 Procedures

All patients were assessed in a room at the hospital's research center individually. The duration of a complete test per patient lasted an average of 40 minutes. This evaluation was always performed by the same previously trained researcher. All instruments and questionnaires used have been translated and validated to suit the Brazilian population.

2. 4 Measurements

2. 4. 1 Questionnaires

- Socio-demographic questionnaire: a structured questionnaire used to gather general patient data, such as age, gender, education, length of illness, age of diagnosis, initial symptoms and marital status.

- MG quality of life scale (MG-QOL 15): a self-report questionnaire specifically designed to assess the quality of life of patients with MG. It has 15 items, with a score range from 0 to 60 points. The higher the score, the worse the patient's perception of quality of life is [17].

- Beck Depression Inventory (BDI): a self - assessment tool used to survey the intensity of depressive symptoms [18]. To determine each score, the values proposed by Gorenstein and Andrade (1998) [19] were used: less than 10 points - no depression or minimal depression; 10 to 18 points - mild to moderate depression; 19 to 29 points - moderate to severe depression; 30 to 63 points - severe depression.

- Epworth Sleepiness Scale: an 8-item self-report questionnaire which assesses the likelihood of falling asleep in eight situations involving daily activities. The overall score ranges from 0 to 24; scores above 10 suggest excessive daytime sleepiness (EDS) [20].

2. 4. 2 Motor Scales

- The Quantitative Myasthenia Gravis Score (QMGS): a clinical scale used as an outcome measure for MG. It consists of 13 items, with a maximum score of 39 points. The higher score, the more serious the disease [21, 22, 23].

- The Myasthenia Gravis Foundation of America clinical classification (MGFA clinical classification): Patients are organized into 5 classes, ranging from Class I – which is characterized by any ocular muscle weakness, but no other muscle strength issues - to Class V. This last category defines patients who have undergone intubation, (with or without mechanical ventilation) under circumstances that do not include routine postoperative management. To classify the patients we used the data obtained using the QMCS [21].

2. 4. 3 Cognitive Tests

- Mini Mental State Examination (MMSE): the cutoff points for the Brazilian population is for formal education for over 8 years: 28 points; 5 and 8 years: 26 points; 1 and 4 years: 25 points and illiterate patients: 20 points [24].

- Montreal Cognitive Assessment (MoCA): The cutoff point for the Brazilian population is 26 points, with an extra point for individuals with 12 years or less of formal education [26].

- Semantic Verbal Fluency (SVF): There are specific normative values for Brazilian Portuguese speakers: a score of $\geq$ 9 named animals for subjects with up to 8 years of formal education and $\geq$ 13 named animals for those with over 9 years of formal education [27].

- Phonemic verbal fluency (PVF): Normative standard scores for the Brazilian population are classified by age and stratified into different periods of formal education [28].

- Rey Auditory Verbal Learning Test (RAVLT): Normative standard scores for Brazilian Portuguese speakers are classified by age (20-59 years and over 60 years) and sex (female and male) [29].

2. 4. 4 Medication

The drugs used for the treatment of MG were gathered from patient clinical records and divided into three classes:

1) Acetylcholinesterase inhibitors: pyridostigmine;

2) Immunomodulators: Azathioprine, methotrexate, cyclophosphamide, mycophenolate;

3) Glucocorticosteroids (CG): prednisone.

In addition, patients who were prescribed antidepressants and/or had reports of depression in their medical records were quantified.

2. 5 Statistical Analysis

Statistical tests were selected according to the distribution of data provided by the Shapiro- Wilk test and histograms. Continuous variables were described using the terms minimum, maximum, mean and standard deviation. The scores found on the cognitive tests were described as percentages of normal and impaired, according to the cutoff points validated for the Brazilian population. Categorical variables were described by percentage and N.

Association analysis was performed between outcome variables (cognitive results categorized as normal or impaired) and contextual variables (e.g. medications, other associated diseases, a clinical diagnosis of depression, sex, marital status, BDI score and Epworth score) using the Fisher's exact test, except for the MG clinical classification, for which the Pearson chi-square test was used. Subsequently, with associations established at $p \leq 0.2$, the Poisson regression with robust variance was used. The linearity of the quantitative variables was analyzed and it was found that the assumption of linearity was maintained. In addition, the presence of multicollinearity was assessed using the variance inflation factor (VIF) estimates, noting that the cutoff points are good (close to 1) indicating that the variables are not multicollinear. The statistical significance of the odds ratio indices was assessed using the Wald test. The model's adjustment was assessed using the Hosmer and Lemeshow test. Also, a Pearson correlation was performed between cognitive test scores, contextual variables and questionnaires based on their gross values, except for the correlation between the MMSE and MoCA tests. For these two, the Spearman's correlation test was applied. To verify the possible influence of thymomas on cognitive performance, we compared the cognitive scores of patients with and without thymomas by means of the t-test. The adopted statistical significance level was $p < 0.05$.

3. Results

Eighty-eight patients with MG were initially included; of these, 49 patients were excluded for the following reasons: 39 did not come to the scheduled assessment date, eight did not want to participate in the study, one was hospitalized and one was under 18 years old. The final sample of this study consisted of 39 subjects diagnosed with generalized MG. The socio-demographic data are presented in Table 1.

Regarding the cognitive battery, there was a predominance of impairments, (according to the cutoff points specific to the Brazilian population) on the MOCA screening test (66.7%) and on the subtasks of immediate memory (59.0%) and recent memory (56.4%). from the RAVLT test. Regarding the self-perception questionnaires, 23.1% of patients presented scores which suggested excessive daytime sleepiness (EDS), based on data from the Epworth questionnaire, and 41.02% presented scores suggestive of depression, according to the BDI (Table 1).

Regarding drug treatment, there was a predominance of anticholinesterase inhibitor (92.3%) use, followed by glucocorticosteroids (CG) (59.0%). Most patients used more than one type of medication (Table 1). The MGFA clinical classification scores distributed a similar proportion of patients among class 1 (only ocular weakness), class 2 (mild weakness) and class 3 (moderate weakness), whereas only a smaller number of patients were assessed as class 4 (severe weakness). (Table 2).

Correlation analyses were performed between the gross scores of the cognitive tests, clinical variables and questionnaire scores, as presented in Tables 3 and 4. Positive correlations were found between all cognitive tests and level of education, showing that the higher the education, the higher the test scores. Age correlated only with the RAVLT test, demonstrating that the younger the patient, the better the individuals' performance on memory tasks. Regarding quality of life, no correlation was found between MG-QOL and cognitive tests. On the other hand, a positive correlation was established between MG-QOL and MGCS,

showing that patients with a poorer perception of quality of life also had more severe motor impairments.

The motor scale (MGCS) only correlated with the RAVLT subtask that evaluates short-term memory (A6) ($p = <0.001$ and R-0.663); thus, patients with less motor impairment due to the disease presented higher performances on the memory test. In addition, the same patient sample was divided into subjects with and without thymoma. Subsequently, an analysis was performed and no statistical difference was observed between the two groups (Table 5).

After the Poisson regression analysis with robust variance, it was found that patients diagnosed with depression had a prevalence ratio (PR) = 1,887 (CI: 1,166 - 3,054) for lower MoCA scores, as well as PR = 9,533 (CI: 1,600 - 56,788) for impairment on PVF tasks and PR= 12,426 (IC: 2,177 - 70,931) for SVF tasks. Participants who used GCs and presented BDI scores indicating depression showed PR = 11,227 (CI: 1.736 - 72.604) and PR = 0.351 (CI: 0.13-0.904), respectively, representing lower scores on the RAVLT subtask that assesses short-term memory (A6). Therefore, there is a significant PR for the presence of cognitive deficits in patients with depression and who used GCs. It was not observed na association between the MGFA clinical classification scores and cognitive tasks (Tables 6 and 7).

4. Discussion

To the best of our knowledge, so far, this study has been the first to investigate cognitive performance in patients with MG from the Brazilian population. The results of this study showed worst performance in tasks related to memory in patients with MG. Moreover, this change was associated with depression and the use of GC. These data corroborate the findings of the systematic reviews by Mao et al. (2015) [16] and Paul et al. (2001) [1], in which the authors point out that, although there are several studies also pointing to a cognitive decline in MG, they did not exclude the possibility that cognitive function may have been affected by

other aspects such as sleep apnea, depression and Type 1 drug use. In their review, Paul et al. (2001) [1] already mentioned the adverse effect of high doses of drugs, such as prednisone, as well as depression on the cognitive functioning of patients with MG. Besides this, our results show cognitive decline in the same functions highlighted by other studies, such as memory [5, 6, 7, 8, 9, 10] and executive functions[8, 10]

There are few studies in specialized literature that analyzed the interference of MG medication, depression and EDS on cognitive performance. Three studies [9, 10, 11] found a higher incidence of cognitive impairment in patients with depression or scores suggestive of depression in self-perception questionnaires. Three other studies describe an analysis of cognitive performance and the use of MG medication. Bartel and Lotz (1995) [30] published work about a possible association between medications and cognitive impairment. In a linear regression analysis, Marra et al. (2009) [14] found that longer treatment time with CG seemed to be correlated with better performance on attentional tasks and long-term verbal memory, contrary to the evidence in our sample. Interestingly, Jordan et al. (2017) [15] found no association at all between the use of acetylcholinesterase inhibitors and performance on cognitive tests.

Additionally, with our sample, we investigated whether the presence of thymomas could influence patients' cognitive performance. Our data corroborated other studies [14, 15] which found that they did not influence cognitive performance.

4. 1 Memory decline and glucocorticosteroid use

Regarding the association between impairments in short-term memory retention and the use of GC, several studies [31, 32, 33, 34, 35] have been found about other clinical populations (e.g. patients with asthma, rheumatoid arthritis, kidney transplants and non-CNS systemic diseases), in which participants showed significantly worse performance on memory and attention tests when compared to the control group. Also, research [36, 37] on healthy

volunteers, with no history of systematically prescribed corticosteroid therapy, noted a significant reduction in performance on memory-related tasks after first-time prednisone use.

Apparently, this associated decline is not only restricted to human subjects. Similar findings were published on rats treated with GC. A significant 20% delay during memory testing was found when compared to controls. What is more, in the study group, the rate of neuronal degeneration was twice as high in the prefrontal cortex and approximately 10 times higher in the hippocampus than in control animals [38]. Nevertheless, it bears stressing that, while there is evidence of cognitive impairment after the use of GC by MG patients, only two studies have investigated this association. Even so, data were not conclusive. Additionally, they investigated only prednisone [14] and AI [15].

GCs are endogenous steroid hormones consisting of 21 carbons secreted by the adrenal cortex, defined as corticosteroids. In humans, cortisol is known to influence behavior, mood and cognitive performance. The pathophysiology of the adverse effects of using synthetic GC is still unclear. Some studies indicate that this may be due to functional changes caused by alterations in gamma-aminobutyric acid signaling, increased glutamate concentration in synapses, concomitant elevation of cytosolic calcium, synthetic GC-induced reduction in hippocampal glucose uptake, altered adrenal steroid receptor density or neurotransmitter content and dendritic atrophy, any of which may result in smaller hippocampal volumes [31, 36, 39].

Another issue under debate is whether cognitive changes are experienced only during the first weeks of therapy, as in some cases side effects proved reversible with dose reduction or suspended treatment [31, 36, 39]. However, no longitudinal studies on MG patients have been found so far.

Therefore, the risk of memory impairment should be considered before starting treatment with GCs. Thus, along with the other forms of monitoring already routinely

performed on patients treated with GCs, cognitive aspects should also be taken into consideration - such as memory - in addition to psychiatric symptoms such as depression [32].

4. 2 Losses in cognitive function associated with depression

In our sample, there was a significant prevalence ratio of depression in participants who showed cognitive impairment on screening tests, where verbal fluency and memory are concerned. These results corroborate the data in the literature that patients with depression present cognitive decline, regardless of severity (i.e. whether mildly or severely depressed). Studies have shown poorer performance on: memory tests (on tasks such as coding, retrieval, recall and recognition) [40, 41, 42, 43, 44], sustained and/or selective attention, psychomotor deceleration (such as reaction time, information, writing and drawing tasks) [41, 42, 45], verbal fluency [40, 42, 43, 46] and executive function [41, 42, 44, 45].

It is known that, after treatment for depression, some cognitive impairment is compensated; however, performance levels usually do not reach pre-morbid status. Thus, depression and residual cognitive deficits are believed to be caused by underlying and related brain dysfunction [40].

The causes of these deficits can be explained by three different theories: 1) the stress hypothesis, which proposes that performance on stress tasks is disproportionately impaired in depressed patients when compared with their performance on automatic tasks; 2) the cognitive velocity hypothesis, which states that depression is characterized by cognitive slowness and that this deceleration may be at the root of other cognitive impairments and 3) the hypothesis of impairment of executive control functions which, in turn, is underlying to the hypothesis of effort [47]. Our study fortify the theories 1 and 2 of this authors [47].

4.3 Limitations of the study

The cross-sectional and exploratory design of the study presented limitations, since it did not allow for analysis of the causal factors of the cognitive decline found in the

sample. Thus, we identified a need for longitudinal studies that could explain if cognitive impairment is due to the pathophysiology of the disease or whether it is associated with other clinical aspects.

Another limitation of the study is that it was carried out at a public hospital which was reference in the treatment of MG patients. This may have led more severe patients to our recruitment universe, as they require more specialized care. As such, the representative power of the sample may have been reduced. Moreover, we cannot analysed de correlation between antibodies and cognitive performance because the exam was not available in public health system

In addition, considering the known differences linked to the education and socioeconomic levels of other populations, the scarcity of studies on the Brazilian population with MG did not allow for a comparison between the scores found in this sample.

5. Conclusion

Given the above, in this sample participants with MG presented a worst performance in tasks of executive function and immediate and recent memory were observed in. These are not associated with the time and severity of the disease. However, a significant prevalence ratio was found for poorer memory performance in patients diagnosed with depression and in patients using GC. As regards QOL, only the motor scale showed a positive correlation, suggesting that patients with a poorer perception of quality of life also suffered from more severe motor restrictions.

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Tables

Table 1: Descriptive analysis of contextual variables and cognitive scores.

	Minimum	Maximum	Average	Standard deviation	Normal	Impaired
Age	18	84	51.08	17.133	-	-
Education	0	18	9.31	4,372	-	-
Length of illness	1	38	13.92	9,696	-	-
MMSE	16	30	26.05	3.203	56.4% (22)	43.6% (17)
MoCA	10	29	22.38	4,982	33.3% (13)	66.7% (26)
PVF	0	63	31.00	14.220	79.5% (31)	20.5% (8)
SVF	4	25	16.49	5.201	87.2% (34)	12.8% (5)
A1-A5	15	68	37.38	11,762	41.0% (16)	59.0% (23)
A6	0	15	6.77	4.055	56.4% (22)	43.6% (17)
A7	0	15	6.32	4.101	43.6% (17)	56.4% (22)
MG-QOL	0	46	16.36	15.276	-	-
MGCS	0	34	11.22	8.619	-	-
BDI	0	41	12.49	11.546	-	-
Epworth	0	22	8.21	5,447	61.5% (24)	23.1% (9)

Medication	Use % (N)	No use % (N)
Immunomodulators	51.3 (20)	48.7 (19)
Acetylcholinesterase inhibitors	92.3 (36)	7.7 (3)
Glucocorticosteroids	59.0 (23)	41.0 (16)
Antidepressants	38.5 (15)	61.5 (24)
-	Yes % (N)	No % (N)
Associated Diseases	74.4 (29)	25.6 (10)
Depression[a]	17.9 (7)	82.05 (32)
Thymoma	51.2 (20)	43.5 (17)

MMSE = Mini Mental State Examination; MoCA = Montreal Cognitive Assessment; PVF = Phonological verbal fluency; SVF = semantic verbal fluency; A1-A5 = immediate memory; A6 = short term memory retention; A7 = recent memory; MG-QOL = MG quality of life scale; MGCS = Quantitative Myasthenia Gravis Score; [a]according to medical record

Table 2: Descriptive Analysis of Disease Classification and BDI

BDI classification % (N)		MG classification % (N)	
No depression	48.7 (19)	Class 1	28.2 (11)
Mild to moderate depression	12.8 (5)	Class 2	33.3 (13)
Moderate to severe depression	20.5 (8)	Class 3	30.8 (12)
Severe depression	7.7 (3)	Class 4	7.7 (3)
Missing data	10.3 (4)		

MG = Myasthenia Gravis; BDI = Beck Depression Inventory.

Table 3: Correlations between clinical variables and cognition

Cognitive Tests	Age		Education		Length of illness	
	p	r	p	r	p	r
MMSE	0.738	-	<0.001	0.602 [2]	0.728 [2]	-
MoCA	0.380 [2]	-	<0.001	0.695 [2]	0.806 [2]	-
PVF	0.455 [2]	-	<0.001	0.623 [2]	0.773 [2]	-
SVF	0.336 [2]	-	<0.001	0.628 [2]	0.367 [2]	-
A1-A5	<0.00 1	- 0.591	0.001	0.50 8 [2]	0.778 [2]	-
A6	<0.001	-0,663 [2]	0.097 [2]	-	0.502 [2]	-
A7	<0.001	-0,643 [2]	0.014	0.397 [2]	0.324 [2]	-

MMSE = Mini Mental State Examination; MoCA = Montreal Cognitive Assessment; Phonological verbal fluency = PVF; Semantic verbal fluency = SVF; A1-A5 = immediate memory; A6 = short term retention memory; A7 = recent memory; [1]Correlation of Spearman's; [2] Pearson Correlation

Table 4: Correlations between questionnaires and cognitive tests

	MG-QOL		BDI		Epworth	
	p	r	p	r	p	r
Age	0.306 [2]	-	0.157 [2]	-	0.328 [2]	-
Education	0.352 [2]	-	0.609 [2]	-	0.313 [2]	-
Length of illness	0.114 [2]	-	0.776 [2]	-	0.967 [2]	-
MMSE	0.101 [2]	-	0.001	-0.551 [2]	0.582 [2]	-
MoCA	0.628 [2]	-	0.155 [2]	-	0.678 [2]	-
PVF	0.995 [2]	-	0.253 [2]	-	0.822 [2]	-
SVF	0.609 [2]	-	0.157 [2]	-	0.571 [2]	-
A1-A5	0.796 [2]	-	0.218 [2]	-	0.675 [2]	-
A6	0.048	0.777 [2]	0.617 [2]	-	0.162 [2]	-
A7	0.333 [2]		0.713 [2]	-	0.111 [2]	-
MG-QOL	-	-	0.007	0.449 [2]	0.491 [2]	-
MGCS	<0.001	0.775 [2]	0.054 [2]	-	0.085 [2]	-
BDI	0.007	0.449 [2]	-	-	0.089 [2]	-
Epworth	0.491 [2]	-	0.089 [2]	-	-	-

MMSE = Mini Mental State Examination; MOCA = Montreal Cognitive Assessment ; Phonological verbal fluency = PVF; Semantic verbal fluency = SVF; A1-A5 = immediate memory; A6 = short term memory retention; A7 = recent memory; BDI = Beck Depression Inventory; MG quality of life scale = MG-QOL; Quantitative Myasthenia Gravis Score = MGCS; [1] Correlation of Spearman's ; [2] Pearson Correlation

Table 5: Correlation between thymomas and cognitive tests

	Thymoma mean (± SD)	No thymoma mean (± SD)	p value
MMSE	25.90 (3.68)	26.0 (2.81)	0.200
MoCA	22.25 (5.51)	22.59 (4.71)	0.605
PVF	31.20 (10.57)	29.35 (16.77)	0.049
SVF	15.90 (5.65)	16.94 (12.57)	0.341
A1-A5	37.90 (12.57)	36.35 (11.73)	0.707
A6	7.50 (3.42)	5.88 (4.74)	0.193
A7	7.37 (3.53)	5.24 (4.63)	0.178

MMSE = Mini Mental State Examination; MOCA = Montreal Cognitive Assessment; Phonological verbal fluency = PVF; Semantic verbal fluency = SVF; A1-A5 = immediate memory; A6 = short term memory retention; A7 = recent memory; T test

Table 6: Association between cognitive tests and contextual variables

	MMSE	MoCA	PVF	SVF	A1 - A5	A6	A7
Other diseases[1]	0.721	0.056 *	0.086 *	1.00	0.264	0.464	0.062
Depression[1]	0.300 *	0.073 *	0.002 *	0.032 *	0.678	0.438	1,000
Antidepressants	-	-	0.037	-	-	-	-
Immunomodulators[1]	1,000	0.741	0.695	0.661	0.748	0.341	0.743
Inhibitors[1]	0.243	0.253	1,000	1,000	0.557	1,000	0.562
Glucocorticosteroids[1]	0.325	1,000	0.109 *	0.631	0.509	0.001 *	0.047 *
Sex[1]	0.168 *	0.714	1,000	1.00	0.726	0.299	0.504
Marital Status[1]	0.106 *	1,000	1,000	0.349	1,000	0.05 *	0.342
BDI[1] classification	0.182 *	1,000	0.207	0.312	0.727	0.041 *	0.484
Epworth[1] classification	0.698	0.681	1,000	0.545	1,000	0.021 *	1,000
MG[2] classification	0.897	0.898	0.414 *	0.630	0.340	0.406	0.355

[1]Fisher 's Exact Test; [2]Pearson Chi-Square; * Proven association; MMSE = Mini Mental State Examination; MoCA = Montreal Cognitive Assessment; PVF = Phonological verbal fluency; SVF = semantic verbal fluency; A1-A5 = immediate memory; A6 = short term memory retention; A7 = recent memory.

Table 7: Regression Analysis between cognitive tests and contextual variables

	MMSE			MoCA			PVF			SVF			A6			A7º
	p	PR	CI	p	PR	CI	p	PR	CI	p	PR	CI	p	RP	IC	p
Glucocorticosteroids	-	-	-	-	-	-	0.154	-	-	-	-	-	0.011	11,22	1.73-72.60	0.071
Other diseases	-	-	-	0.163	-	-	-	-	-	-	-	-	-	-	-	0.123
Depression (1)	0.223	-	-	0.010	1,88	1,16-3,05	0.013	9,53	1,600-56,78	0.005	12,42	2,17-70,93	-	-	-	-
Depression (2)	-	-	-	0.009	0.274	0.18-1.25	0.084	-	-	-	-	-				
Class 2	0.010	0.20	0.06-0.68	0.793	-	-	0.321	-	-	0.343	-	-	0.225	-	-	-
Class 3	0.025	0.25	0.07-0.84	0.236	-	-	0.437	-	-	0.338	-	-	0.058	3,72	0.95-14,46	-
Class 4	0.055	0.23	0.05-1.02	0.611	-	-	0.275	-	-	0.278	-	-	0.103	-	-	-
Marital status	0.412	-	-	-	-	-	-	-	-	-		-	0.160	-	-	-
BDI	0.174	-	-	-	-	-	-	-	-	-	-	-	0.030	0.35	0.13-0.90	-
Epworth	-	-	-	-	-	-	-	-	-	-	-	-	0.786	-	-	-
Sex	0.053	3,04	0.98-9.42	-	-	-	-	-	-	-	-	-	-	-	-	-

Poisson regression with robust variance; ºgross analysis - p=0.057; MMSE=Mini Mental State Examination; MoCA= Montreal Cognitive Assessment; PVF = phonological verbal fluency; SVF = semantic verbal fluency; A1-A5 = immediate memory; A6 = short term memory retention; A7 = recent memory; BDI = Beck Depression Inventory; PR = prevalence ratio ; CI = confidence interval; (1) no inclusion of the antidepressant use variable; (2) inclusion of antidepressant use variable

6 CONCLUSÃO

A partir dos dados encontrados nessa pesquisa, verificou-se que os pacientes com MG apresentavam um padrão perceptivo-auditivo e acústico disartria, com alteração nas bases motoras fonação, respiração e articulação, sendo a fonação a base motora com maior percentual de alteração e a respiração com maior diferenciação perceptiva-auditiva entre indivíduos com MG e controles.

Verificou-se também que os pacientes com MG apresentaram disartria independente da gravidade e do tempo de doença. Além disso, verificou-se que o questionário de autopercepção de fala não foi sensível para diferenciar os pacientes com maior e menor comprometimento de fala. Com relação ao uso de medicamentos observou-se pior produção da fonação em pacientes que usam glucocorticoides.

No que diz respeito ao perfil cognitivo, verificou-se um prejuízo nas funções executivas e memoria imediata e recente nos pacientes com Miastenia Gravis, quando analisados os escores obtidos a partir de dados normativos dos testes para população brasileira. Além disso, encontrou-se uma associação entre o déficit na função de memória com presença de depressão e uso de glucocorticoides. Quanto a qualidade de vida, apenas a escala motora apresentou correlação positiva, sugerindo que pacientes com pior percepção de qualidade de vida também apresentavam restrições motoras mais severas.

ANEXOS

Anexo 1 – Carta de Aprovação no Comitê de Ética

UFRGS - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE DA UNIVERSIDADE FEDERAL

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: INCIDÊNCIA DE DISFAGIA E DISARTRIA EM MIASTENIA GRAVIS: UM ESTUDO DE COORTE

Pesquisador: MAIRA ROZENFELD OLCHIK

Área Temática:

Versão: 3

CAAE: 60718016.4.0000.5327

Instituição Proponente: Hospital de Clínicas de Porto Alegre

Patrocinador Principal: Fundo de Incentivo à Pesquisa e Eventos

DADOS DO PARECER

Número do Parecer: 1.887.088

Apresentação do Projeto:

A Miastenia Gravis (MG) é uma doença auto-imune causada, na maior parte dos casos, pela presença de anticorpos circulantes contra os receptores nicotínicos da acetilcolina, localizados na membrana póssináptica da junção neuromuscular. Seus sintomas clássicos são por fraqueza e fadiga progressivas dos músculos esqueléticos, que se agrava com o esforço e melhora com o repouso. Além disso, pode-se observar sintomas oculares (diplopia e ptose) e sintomas bulbares (disartria, disfagia, mastigação fraca, disartria e fraqueza na musculatura facial e dificuldades respiratórias). Desta forma, o objetivo deste estudo é descrever o perfil disfagia e disartria de pacientes com MG. Serão convidados a participar deste estudo todos os indivíduos com MG (confirmada por meio de verificação do prontuário HCPA) que são atendidos no Ambulatório de Neuromuscular do HCPA. Será realizada uma avaliação da fala e da deglutição de todos os participantes, por meio de protocolos clínicos e de questionários de auto-percepção. Será realizado o exame de videofluoroscopia nos pacientes que apresentarem disfagia conforme a avaliação clínica. Com intervalos anuais entre as avaliações todos os pacientes serão chamados para re-avaliação, da mesma forma como ocorreu a primeira.

Objetivo da Pesquisa:

Objetivo Primário:

Endereço: Rua Ramiro Barcelos 2.350 sala 2227 F
Bairro: Bom Fim **CEP:** 90.035-903
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3359-7640 **Fax:** (51)3359-7640 **E-mail:** cephcpa@hcpa.edu.br

Descrever o perfil disfagia e disartria de pacientes com MG.

Objetivos Secundários:
• Avaliar o perfil preditivo para MG e disfagia e disartria de distintas variáveis: idade, sexo, escolaridade, tempo de doença, idade de inicio e classificação clinica de MG;
• Investigar associações entre os achados da videofluoroscopia da deglutição com o tempo de doença e classificação clinica de MG;
• Correlacionar o grau da disfagia com idade, sexo, escolaridade, tempo de doença, idade de início e classificação clinica de MG.
• Correlacionar o grau da disartria com idade, sexo, escolaridade, tempo de doença, idade de início e classificação clinica de MG.
• Investigar as associações entre achados da avaliação clínica com a videofluoroscopia da deglutição.
• Investigar o perfil evolutivo da disfagia e disartria em pacientes com MG através do acompanhamento longitudinal destes pacientes.

Avaliação dos Riscos e Benefícios:
Riscos:
Os possíveis riscos e possíveis desconfortos decorrentes da participação na pesquisa são: exposição à radiação (equivalente à 70 Raio-X de tórax) e ao contraste durante o exame de videofluoroscopia.
Desconforto ao paciente caso ocorra alguma dificuldade de ingerir os alimentos, durante a avaliação clínica e o exame, como tosse e engasgo. Tempo necessário para a participação no estudo.
Benefícios:
Os possíveis benefícios decorrentes da participação na pesquisa são contribuir para o aumento do conhecimento sobre o assunto estudado, e poderá beneficiar futuros pacientes.

Comentários e Considerações sobre a Pesquisa:
Estudo interessante que pode contribuir com informações sobre disfagia e disatria na MG. Serão selecionados pacientes acometidos por MG, todos oriundos do ambulatório de Doenças Neuromusculares do Departamento de Neurologia do Hospital de Clínicas de Porto Alegre (HCPA). Será utilizado o processo de amostragem por conveniência. Todos os pacientes com MG atendidos

no ambulatório de Doenças Neuromusculares do HCPA serão convidados a participar do estudo.
Atualmente, são atendidos em média 100 pacientes com MG no presente ambulatório.
Critérios de inclusão
• Possuir diagnóstico de MG.
• Aceitar participar do estudo.
Critérios de exclusão
• Outras doenças neurológicas associadas.
• Diagnóstico de doença sistêmica que cause disfagia/ disartria ou alteração cognitiva (por exemplo: tumor de cabeça e pescoço).
Etapas do estudo
1. Seleção dos participantes: todos os indivíduos com MG (confirmada por meio de verificação do prontuário HCPA) que são atendidos no Ambulatório de Neuromuscular do HCPA serão convidados a participar do estudo.
2. Marcação da avaliação: Será agendado um horário para aplicação dos protocolos de avaliação no Centro de Pesquisas Clínicas do HCPA
3. Avaliação: os protocolos serão aplicado no CPC com data e hora marcadas previamente.
4. Videofluoroscopia: será realizada nos pacientes que apresentarem disfagia conforme a avaliação clínica. Após a avaliação clínica será então agendado um horário com os pacientes selecionados para realização da videofluoroscopia no Serviço de Radiologia do HCPA. O intervalo entre as duas avaliações será de no máximo 7 dias.
5. Follow-up: com intervalos anuais entre as avaliações todos os pacientes serão chamados para reavaliação, da mesma forma como ocorreu a primeira.

Considerações sobre os Termos de apresentação obrigatória:
Apresenta TCLE.

Recomendações:
Nada a recomendar.

Conclusões ou Pendências e Lista de Inadequações:
As pendências emitidas para o projeto no parecer 1.825.553 e 1.863.176
foram adequadamente respondidas pelos pesquisadores, conforme cartas de resposta adicionadas

em 25/11/2016 e 22/12/2016. Não apresenta novas pendências.

Considerações Finais a critério do CEP:

Lembramos que a presente aprovação (versão projeto de 25/11/2016, TCLE de 22/12/2016 e demais documentos que atendem às solicitações do CEP) refere-se apenas aos aspectos éticos e metodológicos do projeto. Para que possa ser realizado o mesmo deve estar cadastrado no sistema WebGPPG em razão das questões logísticas e financeiras.

O projeto somente poderá ser iniciado após aprovação final da Comissão Científica, através do Sistema WebGPPG.

Qualquer alteração nestes documentos deverá ser encaminhada para avaliação do CEP. Informamos que obrigatoriamente a versão do TCLE a ser utilizada deverá corresponder na íntegra à versão vigente aprovada.

A comunicação de eventos adversos classificados como sérios e inesperados, ocorridos com pacientes incluídos no centro HCPA, assim como os desvios de protocolo quando envolver diretamente estes pacientes, deverá ser realizada através do Sistema GEO (Gestão Estratégica Operacional) disponível na intranet do HCPA.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_794526.pdf	22/12/2016 16:16:10		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	tcle_revisado.doc	22/12/2016 16:16:00	Annelise Ayres	Aceito
Outros	carta_plataforma_2.docx	22/12/2016 16:15:48	Annelise Ayres	Aceito
Outros	carta_plataforma.docx	25/11/2016 13:31:30	Annelise Ayres	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	tcle_mg_revisado.doc	25/11/2016 13:30:27	Annelise Ayres	Aceito
Projeto Detalhado / Brochura Investigador	proj_doc_mg_final_revisado.doc	25/11/2016 13:30:14	Annelise Ayres	Aceito

Endereço: Rua Ramiro Barcelos 2.350 sala 2227 F
Bairro: Bom Fim **CEP:** 90.035-903
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3359-7640 **Fax:** (51)3359-7640 **E-mail:** cephcpa@hcpa.edu.br

Declaração de Pesquisadores	declaracao_participantes.pdf	30/09/2016 19:30:41	MAIRA ROZENFELD OLCHIK	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	tcle_mg_submetido.doc	21/09/2016 11:05:36	MAIRA ROZENFELD OLCHIK	Aceito
Projeto Detalhado / Brochura Investigador	proj_doc_mg_final_submetido.doc	21/09/2016 11:04:41	MAIRA ROZENFELD OLCHIK	Aceito
Folha de Rosto	folhaderostoprojetoMG.pdf	21/09/2016 11:03:02	MAIRA ROZENFELD OLCHIK	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PORTO ALEGRE, 04 de Janeiro de 2017

Assinado por:
José Roberto Goldim
(Coordenador)

Endereço: Rua Ramiro Barcelos 2.350 sala 2227 F
Bairro: Bom Fim **CEP:** 90.035-903
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3359-7640 **Fax:** (51)3359-7640 **E-mail:** cephcpa@hcpa.edu.br

Anexo 2 - Normas para publicação na revista Neuromuscular Disorders

Types of Paper

Research Articles

Regular original research articles should be sent to the main Editorial Office. There is no restriction on length though most articles are between 2500 and 6000 words long. Please contact the Editorial Office if you wish to discuss. The Editor-in-Chief or an appropriate Executive Associate Editor will handle the submission. (For more information on Executive Associate Editors please see Editor's Commentary. Neuromuscular Disorders, Volume 26, Issue 1, January 2016, Pages 1–4.)

Animal Models for Neuromuscular Diseases

Gillian Butler-Browne will be allocated research articles submitted under this section. There is no restriction on length though most articles are between 2500 and 6000 words long. Please contact the Editorial Office if you would like to discuss.

Veterinary Myology

Diane Shelton will be pleased to receive research articles covering clinical or investigative aspects of spontaneously occurring myopathies, neuropathies or disorders of neuromuscular transmission in domestic animals. There is no restriction on length though most articles are between 2500 and 6000 words long. Please contact the Editorial Office if you would like to discuss.

In addition to submitting regular original research articles, letters and meeting reports, we invite readers to submit interesting articles to the special sections listed below. All items should be submitted online in the usual way to the main Editorial Office in London, with the relevant article type selected from the drop-down menu. If you wish to discuss anything with section editors prior to submission please refer to the journal homepage online or the inside front cover of the printed journal for up-to-date contact information of each section editor.

Reviews

Review papers should cover recent, important developments related to diagnosis, pathogenesis or therapy of a neuromuscular disorder. They can be either in-depth and comprehensive, or short, mini-reviews. Please include an abstract and key words. Reviews will be directed to Anders Oldfors who will co-ordinate peer review. There is no upper limit on the length though most articles do not exceed 6000 words. Please contact the Editorial Office if you would like to discuss.

Case Reports

Case reports should be of interest to the multidisciplinary readership of Neuromuscular Disorders and have a neuromuscular component. Topics such as sensory neuropathies and ataxias are of limited interest to our readership. Case reports should not exceed 2000 words and may include up to three tables or figures and a maximum of 25 references. They should take the form of Title, Abstract (up to 150 words), Introduction, Case Report, Discussion, Acknowledgements and References. Please note that key clinical information must be included in the abstract. Case reports will be directed to Beril Talim who will co-ordinate the editorial process.

Picture of the Month

Please send an interesting picture, clinical, pathological or imaging, of clinical challenge or interest. This should be accompanied by a brief case presentation and discussion, highlighting the special features of the picture, in no more than 300 words and up to three references (no abstract is required). The picture should be the main part of the presentation and be of adequate size and good quality.

Clinical Casebook

Contributions will be welcome for this section for cases that show a conflict of interpretation between the clinical and the investigative aspects of a case, with a view to raising questions, promoting thinking and discussion and potentially opening new channels of research to advance our knowledge.

Historical Reports

We welcome articles of historical interest. These can be sent to the Editorial Office in the first instance and will be redirected to the Historical Section Editor.

ENMC Workshop Reports

These submissions will be treated as a report on a workshop, with the convenor(s) listed as corresponding author(s). They will not be subjected to peer review and, after approval by the Editor, will be published in the next available issue of the journal. The workshop report should be concise and follow the agenda of the workshop - it has the nature of a workshop report, not of a review article (setting the stage for future developments).

The length of a report will vary depending on the number of topics discussed. Workshop reports need to be succinct, focusing on the new information. The references should be confined to those directly relevant to the workshop. Up to three tables, figures or photos may be included. No abstract is required.

1. The basic format of the ENMC-based workshop reports will be the same as in the past with a TITLE reflecting the number of the ENMC workshop, the number if appropriate of the topic workshop and the location and date.
2. A full list of all PARTICIPANTS will be included at the end of the report, with their city and country. This list will also include any ENMC representative as appropriate with [ENMC] after their name.
3. Full ACKNOWLEDGEMENT will be given to ENMC and all its sponsoring organisations at the end of the report using the exact wording as requested by ENMC as one of the conditions in their original letter of acceptance of the workshop.
4. In principle, only the workshop organizers will be the author(s) of the workshop report. The organizers are to make sure that the tasks of all workshop participants regarding the preparation of the meeting report will have been discussed prior to closing the workshop. All workshop participants will be included in the "ENMC XXXX Workshop Study Group*", so that they can be found in PubMed as co-authors of the workshop report. The workshop participants/report authors will be mentioned in an Appendix under the asterisk. The maximum number of authors for a workshop report (including the "ENMC study group") will be five – so a maximum of four (organizer) names can be used for the workshop report. The list of authors will be included on the first page of the report, under the title, with a similar format to original papers in the journal. A full but preferably brief address can be included for each author, and the corresponding author for proofs and reprints should also be indicated.
5. As in the past, these reports will not be subjected to any peer review and it will be assumed that the content has the approval of all participants of the workshop. Once approved by the editor, the report will be given priority publication in the next available issue of the journal.
6. Keywords can be provided for reference.

Contact details for submission

Authors may send queries concerning the submission process, manuscript status or journal procedures to the Editorial Office (jane.miller@ucl.ac.uk).

Before You Begin

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Description of variants (mutations)

Authors are required to follow the recommendations of the HGVS to describe sequence variants (see http://www.HGVS.org/mutnomen/ for a summary of the current recommendations).

Submission of data to a genetic database

In keeping with the recommendations of the Human Variome Project (Cotton RG et al 207. Nat Genet 39:433 http://www.nature.com/ng/journal/v39/n4/full/ng2024.html) authors submitting a manuscript to *Neuromuscular Disorders* are required to submit all variants and phenotype descriptions to a public database prior to acceptance. Authors must declare the status of database submission in their covering letter upon submission to the journal. In addition, authors should indicate in their manuscript the database(s) to which they have submitted the variants, and provide the URL. For further information and links to gene variant databases either use GeneSymbol.lovd.nl (e.g. TP53.lovd.nl) or visit the following website: http://www.hgvs.org/dblist/dblist.html.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places: 1. A summary declaration of interest statement in the title page file (if double-blind) or the manuscript file (if single-blind). If there are no interests to declare then please state this: 'Declarations of interest: none'. This summary statement will be ultimately published if the article is accepted. 2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches. More information.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

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Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, culture or any other characteristic, and should use inclusive language throughout.

Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (e.g. 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess').

Contributors

Each author is required to declare his or her individual contribution to the article: all authors must have materially participated in the research and/or article preparation, so roles for all authors should be described. The statement that all authors have approved the final article should be true and included in the disclosure.

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

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Acknowledgements

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[4] Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, editors. Introduction to the electronic age, New York: E-Publishing Inc; 2009, p. 281–304.

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[5] Cancer Research UK. Cancer statistics reports for the UK, http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/; 2003 [accessed 13 March 2003].

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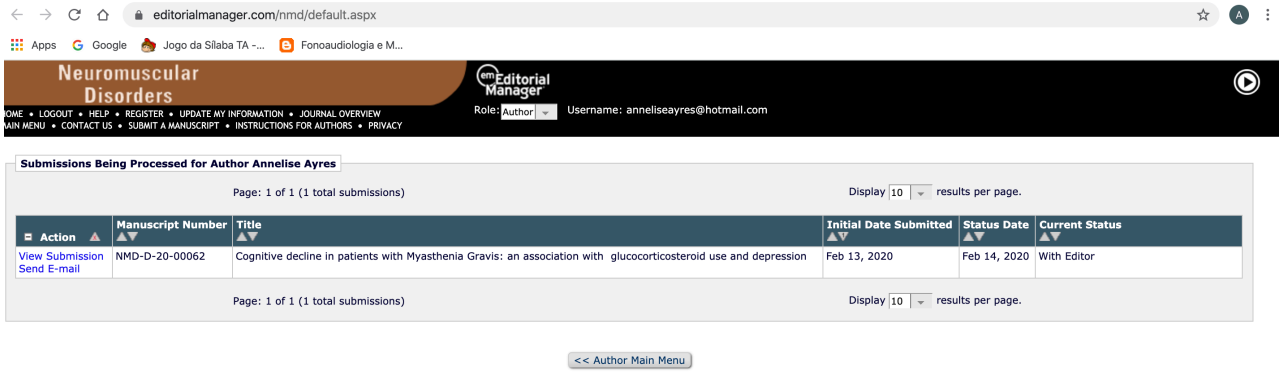

Anexo 4 - Normas para publicação na revista Folia Phoniatrica & Logopaedica

About the Journal

Aims and Scope

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Journal Sections

Consensus Committee Reviews

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