UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM NEUROCIÊNCIAS

NAYARA FELICIDADE TOMAZ BRAZ

AVALIAÇÃO DO METABOLISMO ÓSSEO, COMPOSIÇÃO CORPORAL E QUALIDADE DE VIDA EM PACIENTES COM MIASTENIA GRAVIS.

BELO HORIZONTE – MG
2017

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Tese apresentada ao Programa de Pós-Graduação em Neurociências, da Universidade Federal de Minas Gerais, como requisito para obtenção do título de "Doutor".

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BELO HORIZONTE - MG
2017

B827a

Braz, Nayara Felicidade Tomaz.
Avaliação do metabolismo ósseo, composição corporal e qualidade de vida em pacientes com Miastenia Gravis [manuscrito]. / Nayara Felicidade Tomaz Braz. - - Belo Horizonte: 2017.
111f.: il.
Orientador (a): Antônio Lúcio Teixeira.
Coorientador (a): Adriana Maria Kakehasi.
Área de concentração: Neurociências.
Tese (doutorado): Universidade Federal de Minas Gerais, Faculdade de Medicina.

1. Miastenia Gravis. 2. Composição Corporal. 3. Qualidade de Vida. 4. Biomarcadores. 5. Dissertações Acadêmicas. I. Teixeira, Antônio Lúcio. II. Kakehasi, Adriana Maria. III. Universidade Federal de Minas Gerais, Faculdade de Medicina. IV. Título.

NLM: WE 555

Bibliotecário responsável: Fabian Rodrigo dos Santos CRB-6/2697

DEDICATÓRIA

A todos que contribuíram de alguma forma
para a realização da presente tese.
Especialmente meus mestres: minha mãe Simone,
e meu pai Francisco que investiram na educação dos seus filhos e sempre apoiaram os nossos sonhos.

AGRADECIMENTOS

Primeiramente a Deus pela dádiva da vida e por sempre guiar o meu caminho.

À minha mãe, por me ensinar ser determinada e otimista. Seu pensamento positivo e fé me ajudaram seguir em frente.

Ao meu pai, por ser meu exemplo de responsabilidade. Todo seu esforço em nossa criação serviu de motivação para eu buscar o sucesso.

Aos meus irmãos Guilherme e Nathália, que com a palavra amiga e de entusiasmo não me deixaram desistir nos momentos difíceis.

Ao Augusto por ser meu porto seguro e por todo apoio e companheirismo.

Ao meu orientador professor Dr. Antônio Lúcio pela confiança e pela dedicação ao estudo e à arte de ensinar.

À professora Dra. Adriana Kakehasi por todo conhecimento compartilhado e, sobretudo, pela amizade. Suas palavras de incentivo foram determinantes para eu lutar pelos meus objetivos.

Aos pesquisadores e professores do LIIM por todo aprendizado que obtive ao longo destes quatro anos e por todos os bons momentos que passamos juntos. Agradeço especialmente a Salvina, Belinha, Nat, Érica, prof. Léo, prof. Ana e prof. Jana.

Ao Dr. Rodrigo Santiago e aos residentes de neurologia do Hospital das Clínicas da UFMG pelo auxílio na seleção dos participantes do estudo.

Aos pacientes que participaram deste estudo, pela generosidade, disponibilidade e por contribuírem com o desenvolvimento científico.

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LISTA DE ABREVIATURAS E SIGLAS

25HOD – 25 dihidroxivitamina D

AC – anticorpo

ACh – acetilcolina

AChR – receptor de acetilcolina

ACTH – hormônio adrenocorticotrófico

Anti-AChR – anticorpo antirreceptor de acetilcolina

BIA – bioimpedância

DMO – densidade mineral óssea

DP – desvio padrão

DXA – absorciometria por dupla emissão de raios X

DKK – *Dickkopf 1*

EWGSOP – *European Working Group on Sarcopenia in Older People*

FGF-23 – Fator de crescimento do fibroblasto

GC – grupo controle

GCO – glicocorticoide oral

HAD – *Hospital Anxiety and Depression Scale*

HRQOL - *health-related quality of life*

IFN-β – interferon β

IL-1β – interleucina β

IL-6 – interleucina 6

IL-7 – interleucina 7

IMC – índice de massa corporal

IMMA– índice de massa muscular apendicular

JNM – junção neuromuscular

LEMS - Lambert-Eaton myasthenic syndrome

LRP-4 – proteína 4 relacionada ao receptor de lipoproteína de baixa densidade

M-CSF – fator de estimulação de colônia de macrófagos

MG – Miastenia Gravis

MGFA – *Myasthenia Gravis Foundation of America Clinical Classification*

MGQOL15 –*15-item Myasthenia Gravis Quality-of-Life Questionnaire*

MMA – massa muscular apendicular

MuSK – tirosina quinase músculo-específica

OC – osteocalcina

OPG – osteoprotegerina

OPN – osteopontina

OPIG – osteoporose induzida por glicocorticoide

PTH – hormônio paratireoide

RANK – receptor do fator nuclear kappa B

RANK-L – ligante do receptor ativador do fator nuclear kappa-B

RNM – ressonância nuclear magnética

SF-36 - *Medical Outcome Survey 36-Item Short-Form Health Survey*

SOST – esclerostina

TC – tomografia computadorizada

TNF-α – fator de necrose tumoral alfa

SUMÁRIO

1. INTRODUÇÃO

A miastenia gravis (MG) é uma doença neuromuscular caracterizada por fraqueza muscular com fatigabilidade. É uma doença autoimune causada pela presença de autoanticorpos que agem na membrana pós-sináptica da junção neuromuscular. Em cerca de 85% dos casos, esses anticorpos bloqueiam e/ou destroem os receptores do neurotransmissor acetilcolina (ACh) (Conti-Fine et al., 2006).

A origem da disfunção auto-imune em pacientes com MG é desconhecida, mas anormalidades tímicas, defeitos na regulação imunológica e hormônios sexuais desempenham papéis importantes em pacientes com anticorpos contra o receptor de Ach (anti-AChR) (Meriggioli, 2009; Akinin et al., 2013). O timo é essencial para a diferenciação das células T e para o estabelecimento da tolerância central. Como a sensibilização auto-imune contra o AChR provavelmente se desenvolve no timo, as citocinas inflamatórias podem desempenhar um papel crucial na patogênese da MG (Berrih-Aknin & Le Panse, 2014). Foi relatado que o timo de pacientes com MG apresenta maior produção de TNF-α e IL-6 do que o timo de indivíduos saudáveis (Cohen-Kaminsky et al., 1993).

O quadro clínico da MG varia de inclui sintomas puramente oculares como diplopia e ptose (MG ocular) a sintomas de fraqueza muscular generalizada tais como, disartria, disfagia, dispneia, e fraqueza muscular nos membros superiores e inferiores. Tais sintomas podem impactar negativamente suas atividades de vida diária e de função social, o que por sua vez, pode prejudicar a qualidade de vida dos pacientes (Utsugisawa et al., 2014). Outros fatores que podem influenciar a qualidade de vida desses pacientes são os sintomas ansiosos e depressivos (Ybarra et al., 2011).

O tratamento da MG inclui o uso de fármacos anticolinesterásicos para melhoria temporária da transmissão neuromuscular, uso de imunossupressores inespecíficos ou drogas imunomoduladoras e timectomia (Kumar et al., 2011). O uso de glicocorticóides (GC) é a terapia imunossupressora de primeira escolha para MG generalizada e a prednisona oral é o imunossupressor mais frequentemente usado (Sathasivam, 2011). Apesar dos benefícios clínicos dos glicocorticoides, seu uso pode causar efeitos adversos significativos. Dentre os efeitos de curto prazo tem-se insônia, obstipação, flatulência, alterações de humor e

hiperglicemia. Seu uso prolongado pode causar hipertensão arterial, hiperglicemia, catarata, glaucoma, aumento do peso, lipodistrofia, síndrome de Cushing, sarcopenia, diabetes e osteoporose (Neto et al., 2002; Henneicke et al., 2014; Gilhus et al., 2015). Diante da diversidade de sintomas apresentados pelos pacientes com MG, faz-se necessário avaliar o paciente de forma biopsicossocial e que o manejo seja multidisciplinar.

2. REVISÃO DA LITERATURA

2.1 Patogênese da miastenia gravis

A MG é a doença neuromuscular mais comum. A natureza da doença autoimune, inicialmente proposto em 1960, é agora firmemente estabelecida na MG onde a transmissão neuromuscular normal é interrompida pela ligação de autoanticorpos para proteínas envolvidas na sinalização na junção neuromuscular (JNM) (Lindstrom, 1976). Em 80% a 85% dos casos, estes anticorpos são dirigidos contra o receptor nicotínico de ACh do músculo esquelético e são detectáveis no soro do paciente (Conti-Fine et al., 2006).

Pelo menos três mecanismos estão envolvidos na perda de AChR funcionais em pacientes MG anti-AChR positivo: (1) raramente, bloqueio do AChR por anticorpos ligados aos sítios de ligação de acetilcolina (Burges 1990); (2) internalização acelerada e degradação de AChR causada por ligação cruzada de AChR pela imunoglobulina G (Engel 1979); (3) lise da placa terminal do músculo mediada por complemento resultando em distorção e simplificação da membrana pós-sináptica do músculo (Drachman et al, 1987). Os anticorpos anti-AChR são produzidos por mecanismos patogênicos dependentes de células B e mediada por células T, que ativam o sistema do complemento e levam a inflamação da membrana pós-sináptica do músculo (Tuzun et al., 2003).

Outra proteína da junção neuromuscular pós-sináptica, o receptor de tirosina quinase músculo-específica (MuSK) tem sido implicado em muitos dos 10% a 15% de pacientes que são seronegativos para anticorpos anti-AChR. Esses pacientes tem uma forma generalizada distinta da doença com circulação de anticorpos anti-MuSK (Hoch et al., 2001).

Recentemente foi descrito que em 2-45% dos pacientes seronegativos para anticorpos anti-AChR e anti-MuSK possuem autoanticorpos para LRP-4 (proteína 4 relacionada ao receptor de lipoproteína de baixa densidade), um receptor de agrina importante para a ativação de MuSK, para o agrupamento de AChR e a formação da JNM (Shen et al., 2013). Estes anticorpos inibem a ligação de LRP-4 ao seu ligante e predominantemente pertencem a subclasse de imunoglobulina G1, um ativador do complemento (Pevzner et al., 2012).

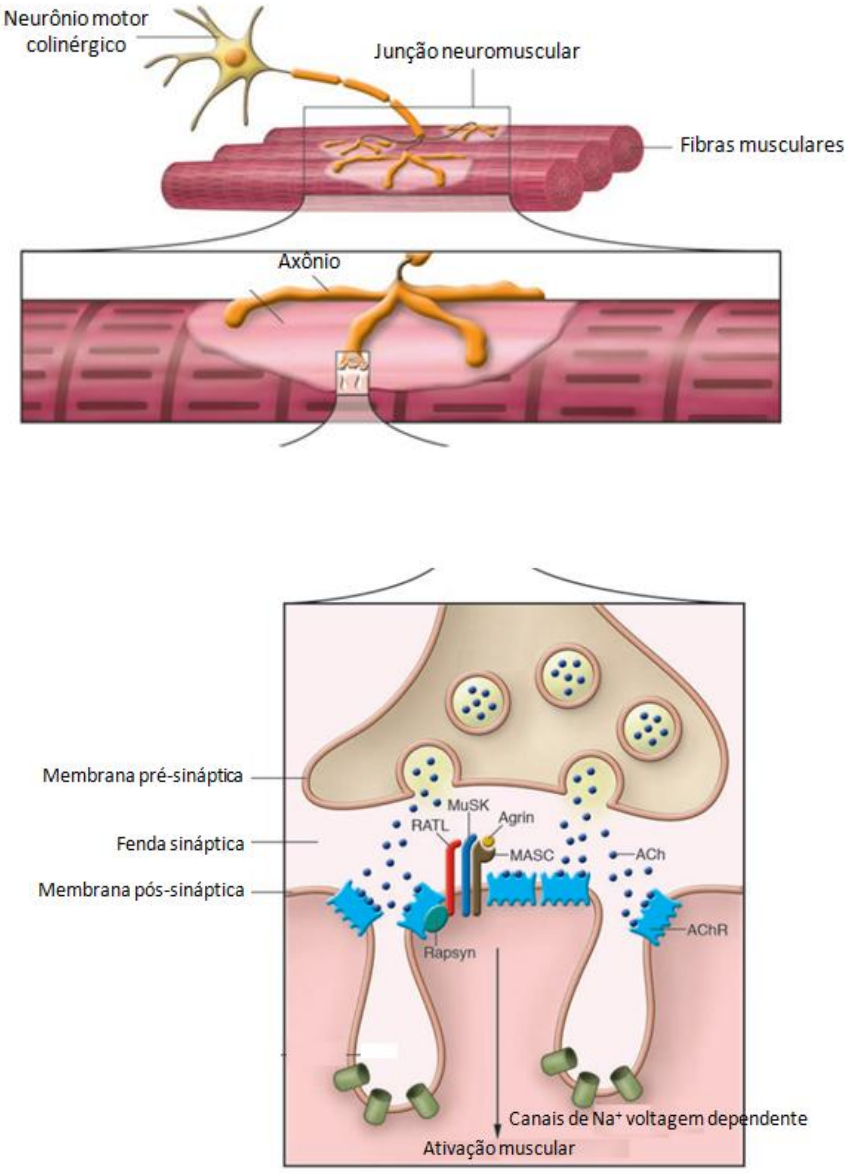

Figura 1- Estrutura da junção neuromuscular. Extraído de Conti-Fine et al., 2006.

O início da produção de anticorpos contra os AChR na MG autoimune ainda é desconhecida. Sabe-se que o timo pode influenciar a transmissão sináptica da JNM na MG que ocorre através da presença de um antígeno que transforma linfócitos virgens em linfócitos ativados (Pal et al., 2011). De acordo com estudos, 50% dos pacientes com MG positivos para anti-AChR apresentam hiperplasia tímica e 10% a 15% apresentam timoma (Pal et al., 2011). O timo de pacientes com MG contêm um número aumentado de linfócitos B que liberam anticorpos contra AChR. Em decorrência de estímulos desconhecidos, as células mioides do timo se danificam provocando uma falha no mecanismo inflamatório de linfócitos T propiciando um aumento descontrolado de anticorpos contra os AChR, produzidos pelos

linfócitos B (Munz et al., 2009). Outras hipóteses incluem a ocorrência de uma infecção viral que poderia alterar as propriedades da superfície da placa motora, tornando-a imunogênica ou na presença de antígenos virais ou bacterianos que compartilhem epítopos com o AChR, de modo que, quando uma pessoa é infectada, os anticorpos gerados contra o organismo estranho também podem reconhecer o AChR (Munz et al., 2009).

2.2 Sinais e sintomas da miastenia gravis

A MG é caracterizada clinicamente por fraqueza flutuante dos músculos extraoculares, orofaríngeos, axiais e/ou apendiculares, com sensibilidade e reflexos preservados. O grau de fraqueza muscular varia entre os dias e até mesmo de hora em hora e piora com a atividade e melhora no repouso. Os pacientes apresentam diferentes graus de ptose, diplopia, disartria, disfagia, dispneia, fraqueza facial ou fraqueza de músculos apendiculares ou axiais. A diplopia e a ptose são os sinais iniciais mais comuns e estão presentes em aproximadamente 85% dos pacientes (Grob et al., 2008).

A progressão da fraqueza generalizada geralmente ocorre dentro de dois anos do início da doença. A fraqueza dos músculos faciais é bastante comum e muitos pacientes com MG têm considerável fraqueza do fechamento palpebral com ou sem menor fraqueza facial. A fraqueza bulbar, cursando com disfagia, disartria ou dificuldade para mastigar, é o sintoma inicial em até 15% de pacientes (Grob et al., 2008). A fraqueza envolvendo músculos respiratórios é rara, mas pode ameaçar a vida, necessitando ação terapêutica imediata. Embora raro, uma distribuição de fraqueza cintura-membro ou mesmo fraqueza focal em grupos musculares individuais podem ocorrer (Rodolico et al., 2002).

A MG é dividida em subgrupos levando-se em consideração (Evoli et al., 2015):

- Idade de surgimento dos sintomas
    - MG precoce: surgimento antes dos 40 anos;
    - MG tardia: surgimento depois dos 40 anos.
- Tipo de autoanticorpo presente:
    - AChR;
    - MUSK;
    - LRP4;
    - Ausência: seronegativo.

- Grupos musculares acometidos:
    - Bulbares;
    - Generalizada.
- Alterações tímicas:
    - Hiperplasia tímica folicular;
    - Timoma;
    - Atrofia tímica.

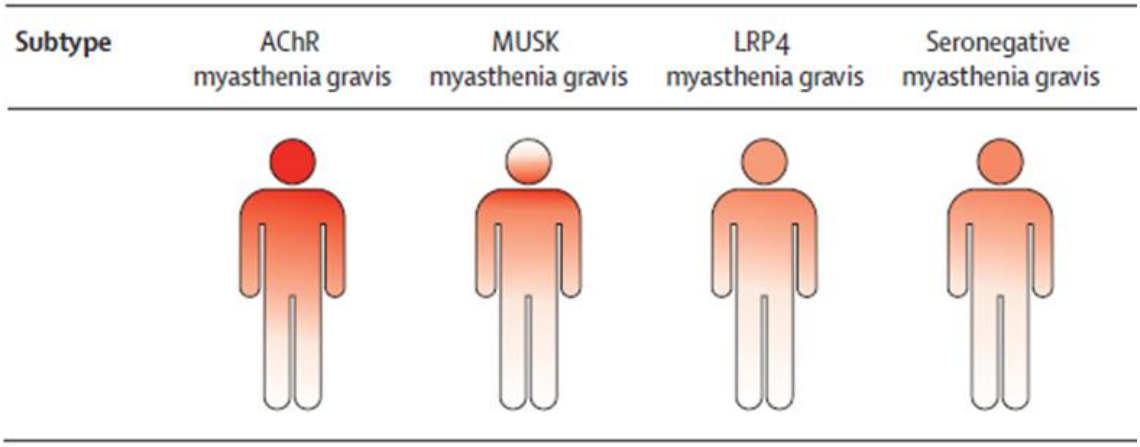

Figura 2- Distribuição da fraqueza muscular nos subtipos de Miastenia Gravis. Adaptado de: Guilhus et al., 2015.
AChR: receptor de acetilcolina; MUSK: tirosina quinase músculo-específica; LRP-4: proteína 4 relacionada ao receptor de lipoproteína de baixa densidade; LEMS: *Lambert-Eaton myasthenic syndrome.*

Muitos pacientes sofrem agravamento dos sintomas desencadeado por infecções, estresse emocional, cirurgias ou medicamentos, em especial durante os primeiros anos da doença. A progressão para gravidade máxima geralmente ocorre nos primeiros dois anos de início e remissões espontâneas duradouras são incomuns, mas foram relatadas em 10-20% de pacientes (Grob et al., 2008). O curso clínico da MG é variável, variando de remissão em um estágio inicial para uma exacerbação aguda e até mesmo a morte. Nas últimas décadas, o prognóstico melhorou consideravelmente, principalmente devido aos avanços no desenvolvimento farmacológico e da timectomia (Silvestri &Wolfe, 2012).

2.3 Epidemiologia

Os dados sobre a prevalência e incidência da MG variam amplamente com taxa de prevalência de 15 à 179 por milhão de habitantes (Carr et al., 2010) e taxa de incidência de 3-30 por milhão por ano (McGrogan et al., 2010). A maioria dos estudos é baseada em dados hospitalares. As altas taxas de prevalência e incidência relatadas derivaram dos poucos estudos de base populacional e prospectivos (McGrogan et al., 2010). A epidemiologia da MG para as duas últimas décadas mostra uma prevalência crescente (Carr et al., 2010) e os avanços na terapia com uma expectativa de vida normal, o envelhecimento da população, bem como a melhora na apuração dos casos são, provavelmente, as principais explicações para esse crescimento (Andersen et al., 2014).

A doença tem uma prevalência maior nas mulheres do que nos homens, com uma proporção aproximada do sexo feminino para masculino de 2:1 (Jacobson et al., 1997). MG na infância e adolescência é rara (Morita, 2001). As meninas são mais frequentemente afetadas do que os meninos em uma proporção de 1,3:1 em idades pré-púberes e 1,8:1 em idade peripuberal (Evoli et al., 1998). Sem tratamento, 20-30% dos pacientes morrerão em 10 anos, mas com a terapêutica adequada, a maioria dos pacientes são capazes de viver de forma produtiva (Aguiar et al., 2010).

No Brasil ainda não há estudos de base populacional com objetivo de analisar a distribuição da doença num determinado local. A maioria dos estudos brasileiros são pesquisas em clínicas especializadas com objetivo de explorar os aspectos clínicos e sócio-demográficos da doença e, ainda assim, existem estudos limitados no que se refere à análise desses aspectos de forma detalhada (Aguiar et al., 2010).

2.4 Diagnóstico

O diagnóstico da MG é baseado nos seguintes critérios: história pregressa, exame clínico e laboratorial, evidência clínica de fatigabilidade com recuperação em repouso, resposta clínica à administração de anticolinesterásicos, detecção de anticorpos de receptor de acetilcolina, diminuição da atividade elétrica na estimulação nervosa repetitiva; e exclusão de diagnósticos neurológicos alternativos (Meriggioli, 2009).

Há suspeita de MG em caso de história e presença de sinais de fraqueza flutuante dos músculos voluntários que piora no esforço e melhora em repouso. O diagnóstico é então confirmado através de: a) detecção de anticorpos séricos; b) estudos de eletromiografia que mostram diminuição do potencial de ação muscular em estimulações nervosas repetidas de baixa taxa ou jitter aumentado em EMG de fibra única; c) resposta clínica aos inibidores da acetilcolinesterase. Resultados positivos em b) e c) confirmam um defeito na transmissão neuromuscular pós-sináptica, enquanto a detecção de anticorpos estabelece o diagnóstico de MG (Meriggioli, 2009; Evoli et al., 2015).

Testes sorológicos de rotina incluem dosagem de anticorpos anti-AChR e anti-MuSK. Devido sua alta prevalência na MG, anti-AchR são os primeiros a serem testados quando se suspeita de MG por sinais clínicos. Pacientes com resultados negativos para anti-AchR devem ser testados para anticorpos anti-MuSK. A coexistência destes anticorpos é muito rara. Anticorpos anti-AChR e anti-MuSK são considerados específicos para MG e, na prática, sua detecção em pacientes com sintomas consistentes confirma o diagnóstico, sem necessidade real de teste adicional. Por outro lado, quando o resultado destes exames é negativo, a confirmação com eletromiografia é necessária (Meriggioli, 2009; Evoli et al., 2015).

2.5 Tratamento

A perspectiva para os pacientes com MG melhorou consideravelmente nos últimos anos, em grande parte devido aos avanços nos cuidados de medicina intensiva e a utilização de agentes imunomoduladores. A meta terapêutica é devolver ao paciente a função normal o mais rapidamente possível, minimizando os efeitos colaterais da terapia. O tratamento dos pacientes com MG deve ser individualizado de acordo com a extensão (ocular ou generalizada) e gravidade (leve a grave) da doença e a presença ou ausência de comorbidades (incluindo, mas não se limitando, a outras doenças autoimunes e timoma) (Meriggioli, 2009; Kumar & Kaminski, 2011).

O tratamento da MG divide-se em terapias de curta e longa duração. A primeira é usada durante a exacerbação da doença para obter uma melhora rápida (plasmaferese ou imunoglobulina) e a segunda busca imunossupressão crônica (Kumar & Kaminski, 2011). A Azatioprina tem sido utilizada no tratamento de MG por mais de 30 anos como tratamento

imunossupressor primário ou em combinação com prednisona. Seu modo de ação é através da inibição da síntese de purina levando a inibição da divisão ativa de linfócitos T e B. Azatioprina é geralmente fornecida a uma dose de 2 a 3 mg/kg dividido em dois ou três doses por dia (Kumar & Kaminski, 2011).

Dentre as drogas que atuam na transmissão muscular, a piridostigmina é a mais utilizada. Ela atua inibindo a acetilcolinesterase e, consequentemente, eleva a quantidade de acetilcolina endógena favorecendo a ligação entre a acetilcolina e seu receptor. Com isso, ocorre redução dos sintomas miastênicos. Doses iniciais de 30 a 60 mg de piridostigmina a cada 3 a 6 h, com ajuste da dose com base na eficácia clínica e efeitos adversos (Kumar & Kaminski, 2011).

O campo cirúrgico tem na timectomia sua participação na terapêutica da doença. O timo é o principal órgão produtor do anticorpo que bloqueia os receptores da acetilcolina na junção neuromuscular pós-sináptica levando o indivíduo a perda de força muscular (Sieb, 2013). Recente ensaio clínico randomizado avaliou a eficácia da timectomia em pacientes com MG sem timoma. Foram avaliados 136 pacientes que foram alocados no grupo de tratamento timectomia mais prednisona e no grupo que fez uso apenas de prednisona. Mostrou-se que a timectomia foi eficaz para melhora dos sintomas clínicos de pacientes com MG sem timoma e que esta melhora persistiu por três anos (Wolfe et al., 2016).

A prednisona oral é o imunossupressor mais frequentemente usado (Sathasivam, 2011). O mecanismo de ação dos glicocorticoides inclui a diminuição da produção de certas citocinas, a inibição de linfócitos T e o comprometimento da função monocítica e/ou macrofagocítica (Juel & Massey, 2005; Sathasivam, 2011). Apesar de não existirem estudos controlados sobre o efeito dos glicocorticoides, estudos observacionais registraram remissão ou melhora em 70-80% dos pacientes com MG tratados com glicocorticoides orais. Segundo esse estudo, o tempo médio para se obter pelo menos uma melhora foi de cerca de três meses e o benefício máximo foi atingido entre cinco e seis meses de tratamento (Sathasivam, 2011).

Apesar dos benefícios clínicos dos glicocorticoides, seu uso pode causar efeitos adversos significativos. Dentre os efeitos de curto prazo tem-se insônia, obstipação, flatulência, alterações de humor e hiperglicemia. Seu uso prolongado pode causar hipertensão arterial, diabetes, catarata, glaucoma, aumento do peso, lipodistrofia, síndrome de Cushing,

sarcopenia, osteoporose e diabetes (Neto et al., 2002; Henneicke et al., 2014; Gilhus et al., 2015). Além disso, a dose diária de GC foi associada a sintomas depressivos e ao prejuízo da qualidade de vida de pacientes com MG (Suzuki et al., 2011; Mourão et al., 2016).

3 JUSTIFICATIVA

Por ser a MG a principal doença neurológica com indicação de glicocorticoterapia prolongada, acredita-se que estes pacientes apresentem maior incidência de osteoporose e obesidade e maior comprometimento da qualidade de vida que indivíduos saudáveis. O conhecimento das complicações deste tratamento deve ser esclarecido. Estudo britânico que avaliou a adesão do consenso europeu de prevenção de osteoporose em pacientes em uso de glicocorticoterapia prolongada mostrou que pequena parte dos pacientes enquadrava-se no protocolo proposto, mostrando que pouca atenção tem sido dada aos riscos que a glicocorticoterapia prolongada oferece à saúde óssea desses pacientes (Gudbjornsson et al., 2002).

A MG é uma doença que ainda apresenta lacunas de conhecimento a serem exploradas e, no Brasil ainda não há estudos relacionados com o impacto da glicocorticoterapia na qualidade óssea e nos níveis de gordura corporal destes pacientes. Aspectos como idade, gênero, gravidade e tipo dos sinais e sintomas, terapia e medicação dos pacientes com MG são variáveis importantes na avaliação e/ou tratamento da doença. Assim, estes aspectos devem ser identificados a fim de traçar o melhor tipo de tratamento e avaliar necessidade ou não de tratamento desses pacientes.

Este trabalho justifica-se por se tratar de um tema relevante do ponto de vista clínico, já que a literatura a respeito do metabolismo ósseo, da composição corporal e de qualidade de vida em pacientes portadores de Miastenia Gravis, bem como a relação com biomarcadores plasmáticos, com glicocorticoterapia prolongada, é escassa.

4 OBJETIVOS

4.1 Objetivo Geral

Avaliar o metabolismo ósseo, a composição corporal e a qualidade de vida em pacientes com MG acompanhados no Ambulatório de Doenças Neuromusculares do Hospital das Clínicas da Universidade Federal de Minas Gerais.

4.2 Objetivos Específicos

Definir a prevalência de osteopenia e/ou osteoporose em pacientes com MG;

Avaliar o índice de sarcopenia e de adiposidade de pacientes com MG;

Verificar se há correlação entre a densidade mineral óssea e as variáveis clínicas e demográficas e a concentração de biomarcadores inflamatórios, do metabolismo ósseo e de adipocinas de pacientes com MG;

Verificar se há correlação entre a dose acumulada de prednisona e a densidade mineral óssea e a composição corporal de pacientes com MG.

Avaliar a qualidade de vida de pacientes com MG e verificar os fatores que a afeta.

Comparar o metabolismo ósseo, a composição corporal e a concentração plasmática de biomarcadores de pacientes com MG com indivíduos saudáveis.

5 PACIENTES E MÉTODOS

5.1 Delineamento do estudo

Trata-se de estudo observacional do tipo transversal com amostra de conveniência aprovado pelo Comitê de Ética em Pesquisa da UFMG sob parecer 501.655 (Anexo I). Foram incluídos pacientes de ambos os sexos, com MG em acompanhamento no Ambulatório de Doenças Neuromusculares do Hospital das Clínicas (HC) da UFMG. O grupo controle foi composto de indivíduos saudáveis, comparáveis pela idade e sexo, recrutados aleatoriamente na Faculdade de Medicina da UFMG e no ambulatório Jenny Faria do HC da UFMG. O grupo controle foi composto por alunos da pós-graduação e por funcionários administrativos da UFMG e do HC. As coletas de dados foram realizadas no período entre outubro de 2013 e junho de 2015.

5.2 Critérios de inclusão e exclusão do grupo MG

Foram incluídos pacientes portadores de MG em acompanhamento no ambulatório de Doenças Neuromusculares do Hospital das Clínicas da UFMG. Foram excluídos pacientes com infecção e uso de antibióticos ou anti-inflamatórios nas quatro semanas que antecederam a coleta de sangue, tratamento prévio para osteoporose e pacientes gestantes.

5.3 Critérios de inclusão e exclusão do grupo controle

Foram incluídos indivíduos sabidamente sem doença que não fizeram uso de antibiótico ou anti-inflamatório nas quatro semanas que antecederam a coleta de sangue. Foram excluídas mulheres, presença de doenças inflamatórias crônicas, relato ou diagnóstico conhecido de neoplasia ou infecção pelo HIV, uso atual de glicocorticoide sistêmico, insulina, estatina, hormônio de crescimento (GH), agente anabólico, terapia de reposição hormonal e gravidez ou lactação.

5.4 Coleta de dados

Para identificação do perfil clínico de todos os voluntários, foram coletados dados por meio de entrevista e preenchimento de protocolo específico (Anexo II). Todos os indivíduos do estudo assinaram o Termo de Consentimento Livre e Esclarecido (Anexo III). A dose acumulada de prednisona, isto é, a dose total de prednisona usada pelo paciente ao longo do curso de sua doença, foi calculada com base nos dados obtidos pela a revisão dos prontuários.

Outros tipos de corticosteroides por ventura empregados foram convertidos para a dose equivalente de prednisona e somados à dose acumulada. Registraram-se todos os medicamentos em uso pelo paciente no momento da avaliação. Foram registrados os resultados dos exames laboratoriais feitos pelos pacientes nos últimos seis meses.

5.5 Instrumentos de avaliação

5.5.1 *Myasthenia Gravis Foundation of America Clinical Classification (MGFA)* (anexo IV)

É conhecida como norma para pesquisar manifestações clínicas em MG por apresentar classificações separadas para pacientes com envolvimento puramente ocular (Classe I) daqueles com fraqueza da musculatura bulbar ou generalizada, e ainda especificando a fraqueza como leve (II), moderada (III) ou grave (IV).

As classificações II, III e IV podem ser subdivididas em A e B. O paciente é classificado com o "A" quando a fraqueza muscular predominante for os membros (musculatura generalizada) e "B" quando se destaca a musculatura bulbar dos demais sinais e sintomas. A sua aplicação destina avaliar o estágio histórico dos sinais e sintomas da miastenia gravis, ou seja, é uma a classificação definida pela gravidade da MG, bem como o predomínio dos grupos musculares envolvidos e maiores comprometidos ao longo da doença (Jaretzki et al., 2000).

5.5.2 MG Composite (anexo V)

É uma escala que consiste em 10 itens que avaliam sinais e sintomas da MG de acordo com sua manifestação, sendo três itens referentes às manifestações oculares, quatro para bulbares e o restante (três) para as generalizadas. Os itens referentes às formas oculares e generalizadas são examinados pelo avaliador a fim de analisar o valor ponderal para a fraqueza muscular. Já os itens referentes aos sintomas bulbares são coletas por meio do relato do paciente da sua melhor percepção em identificar o estágio das funções neurovegetativas como fala, mastigação, alimentação e respiração. A sua aplicação avalia o estágio atual dos sinais e sintomas da MG, pois o grau de flutuação e gravidade da MG, bem como o predomínio variável dos grupos musculares envolvidos, torna extremamente difícil a classificação definitiva desses pacientes como um todo (Burns et al., 2010).

5.5.3 *15-Item Myasthenia Gravis Quality-of-Life Questionnaire (MGQoL15)*" (anexo VI)

O MGQoL15 foi derivado a partir de uma escala de 60 itens de qualidade de vida relacionados à saúde específico de MG com objetivo principal de fornecer aspectos clínicos de forma eficaz, rápida e, fácil de usar para avaliar e interpretar a qualidade de vida dos pacientes com MG. Cada um dos seus itens pode ser pontuado de zero a quatro, compondo de uma pontuação máxima de 60 pontos. Assim, a distribuição do escore realizada foi 0 para "não", 1 "pouco", 2 " às vezes", 3 "frequentemente" e 4 "sempre". Em relação ao resultado final, quanto maior a pontuação pior considera-se a qualidade de vida do paciente (Burns et al., 2010). Esta escala foi traduzida e adaptada para o Brasil por Mourão e colaboradores em 2013 (Mourão et al., 2013).

5.5.4 *Hospital Anxiety and Depression Scale* (HAD)" (anexo VII)

A Escala HAD foi desenvolvida como objetivo avaliar, de forma breve, os níveis de ansiedade e depressão em doentes com patologia e sob tratamento ambulatorial. Possui 14 itens, dos quais sete são voltados para a avaliação da ansiedade (HAD-A) e sete para a depressão (HAD-D). Cada um dos seus itens pode ser pontuado de zero a três, compondo uma pontuação máxima de 21 pontos para cada escala. Os autores recomendam como ponto de corte para ambas as subescalas ≥ 9, sendo inferior a nove classificado sem alteração, entre 9 e 10 forma leve de ansiedade/depressão e, 11 e 14 forma moderada (Zigmond, 1983). A validação da versão em português da HAD é relatada em diversos grupos de pacientes, sendo realizada em pacientes da enfermaria de clínica médica por Botega (1995).

5.6 Coleta de sangue periférico e preparo do plasma para análise dos biomarcadores

Foram colhidos 9 ml de sangue periférico dos participantes, por um profissional qualificado, em coleta a vácuo em frascos heparina sódica, com ênfase nas normas de utilização de materiais pérfuro-cortantes para o descarte dos materiais. Após esse procedimento, os tubos foram centrifugados em 3000 g em uma centrífuga Fanem, por 10 minutos. O plasma foi retirado, utilizando pipetas, colocados em Eppendorfs e estocados em freezer a –80ºC.

5.7 Análise dos biomarcadores

Para as dosagens das proteínas e hormônios relacionadas ao metabolismo ósseo TNF-α, IL-1β, IL-6, ACTH, DKK_1 (Dickkopf), insulina, leptina, osteocalcina, osteopontina,

osteoprotegerina, PTH e SOST (esclerostina), fez-se a técnica Luminex® com o kit *Human Bone Metabolism* (Merck Millipore). As recomendações do fabricante foram seguidas para a análise. Brevemente, as proteínas padrões do kit foram delicadamente homogeneizadas e diluídas (diluições seriadas). Microesferas de capturas para cada proteína foram aliquotadas e misturadas para o ensaio. Para o ensaio, a mistura de microesferas de capturas foi agitada em vórtex e adicionado 25 µl da mistura em 25 µl de amostra e proteína padrão nas diluições indicadas. A placa foi incubada por 16-18 horas. Após incubação, as amostras foram lavadas e adicionou-se o anticorpo de detecção (25 µl) em todos os poços de análise, incubando por mais 2 horas. Transcorrido o tempo, adicionou-se a estreptavidina (50 µl), reincubando por mais 30 minutos. Após esse período, a placa foi lavada e ressuspendida em tampão de leitura (300 µl) para aquisição no equipamento MagPix (Merck Millipore), utilizando o software Exponent. Após leitura das amostras, os dados foram analisados pelo programa Analyst (Merck Millipore).

A adipocinas, adiponectina e resistina foram analisadas pela técnica ELISA (*enzyme-linked immunosorbent assay*) (DuoSet R&D Systems, Minneapolis, MN, USA). As citocinas IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ and IL-17 foram analisadas por citometria de fluxo com o Kit para citocinas Th1/Th2/Th17 (BD Biosciences, San Jose, CA, USA). A aquisição foi obtida com citômetro de fluxo FACSCanto II (BD Biosciences, San Jose, CA, USA). Os resultados foram obtidos pelo *software* FCAP Array v1.0.1 (Soft low Inc., Pecs, Hungary). Os resultados foram expressos em pg/ml. As análises foram realizadas no Laboratório Interdisciplinar de Investigação Médica da Faculdade de Medicina da Universidade Federal de Minas Gerais. As dosagens de 25-hidroxi vitamina D, cálcio iônico e fósforo foram feitas no Laboratório Geraldo Lustosa.

5.8 Composição corporal e densitometria óssea

A composição corporal e a densitometria óssea foram estimadas por absorciometria de raio-X de dupla energia (DXA), em aparelho Discovery W Hologic (Bedford, MA, USA), versão de software 3.3.01, do Hospital das Clínicas em data e horário definidos pela médica reumatologista Dra. Adriana Maria Kakehasi e os resultados foram enviados diretamente para o pesquisador deste estudo. As medidas foram realizadas após a remoção de todos os acessórios de metal na posição supina, com duração de quinze minutos.

Os resultados de densitometria óssea são apresentados através de valores absolutos de densidade mineral óssea (DMO) (g/cm^2) e de conteúdo mineral ósseo (BMC, g) que são os utilizados para monitorar as mudanças ao longo do tempo e através do T-score que é calculado em desvios-padrão (DP), tomando como referência a DMO média do pico da massa óssea em adultos jovens. Os critérios diagnósticos propostos pela OMS em 1994 baseiam-se neste dado, e classifica-se desta forma: até -1,0 DP em normal; -1,1 a -2,5 DP em osteopenia; abaixo de -2,5 DP em osteoporose; abaixo de -2,5 DP, na presença de fratura, em osteoporose estabelecida. Essa classificação está bem estabelecida para mulheres na pós-menopausa. O Z-score é utilizado para pacientes com menos de 50 anos de idade ou mulheres na pré-menopausa. Através do Z-score, calculado em DP, toma-se como referência a DMO média esperada para indivíduos da mesma idade, etnia e sexo. É importante ressaltar que resultados exibindo *Z*-score menor que -2,0 DP ou abaixo podem sugerir causas secundárias de osteoporose (Brandão et al., 2009).

Através do DXA, Baumgartner desenvolveu a definição operacional de sarcopenia como o Índice de Massa Muscular Apendicular (IMMA), obtido pela divisão da massa muscular apendicular (MMA) pelo quadrado da altura considerando o somatório da massa livre de gordura e osso dos membros superiores e inferiores (Baumgartner et al., 1998). A exemplo da definição de osteoporose são considerados sarcopênicos os indivíduos cujo IMMA é -2DP abaixo da média para uma população jovem de referência específico para o gênero. Os valores de corte menores que 5,45 kg/m^2 para mulheres e 7,26 kg/m^2 para homens foram aplicados (Baumgartner et al., 1998) e têm sido amplamente utilizados em estudos sobre sarcopenia.

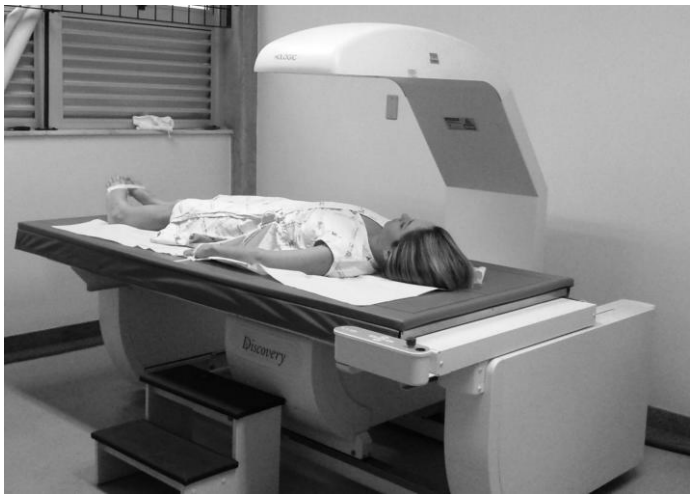

Figura 3- Realização do exame DXA para avaliação da composição corporal. Fonte: acervo pessoal.

ARTIGO I

Neurol Sci
DOI 10.1007/s10072-017-2964-z

ORIGINAL ARTICLE

Negative impact of high cumulative glucocorticoid dose on bone metabolism of patients with myasthenia gravis

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ABSTRACT

Purpose: to evaluate the frequency of low bone mass, osteopenia and osteoporosis in patients with myasthenia gravis (MG), and to investigate the possible association between bone mineral density (BMD) and plasma levels of bone metabolism markers and cytokines. **Methods**: 80 patients with MG and 62 controls BMD was measured in the right femoral neck and lumbar spine by dual-energy X-ray absorptiometry. Plasma concentrations of osteocalcin, osteopontin, osteoprotegerin, TNF-α, IL-1β, IL-6, DKK-1, sclerostin, insulin, leptin, adrenocorticotropic hormone, parathyroid hormone and FGF-23 were analyzed by Luminex®. **Results:** The mean age of patients was 41.9 years, with 13.5 years of length of illness, and mean cumulative dose of glucocorticoids 38,123 mg. Patients had significant reduction in BMD of the lumbar, the femoral neck and in the whole body when compared with controls. 14% MG patients had osteoporosis at the lumbar spine and 2.5% at the femoral neck. In comparison with controls, patients with MG presented lower levels of osteocalcin, adrenocorticotropic hormone, parathyroid hormone, sclerostin, TNF-α, and DKK-1, and higher levels of FGF-23, leptin and IL-6. There was a significant negative correlation between cumulative glucocorticoid dose and serum calcium, lumbar spine T-score, femoral neck BMD, T-score and Z-score. After multivariate analysis, higher TNF-α levels increased the likelihood of presenting low bone mass by 2.62. **Conclusion**: MG patients under corticotherapy presented low BMD and altered levels of bone markers.

Keywords: myasthenia gravis, bone mineral density, bone metabolism markers, osteoporosis, dual-energy X-ray absorptiometry.

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease characterized by fluctuating weakness of voluntary muscle and fatigability due to the impairment of synaptic transmission at the neuromuscular junction [1]. Treatment of MG can be schematically divided into short and long term strategies. The first is mainly based on plasmapheresis or intravenous immunoglobulins which are used during exacerbations of the disease to achieve a rapid improvement of muscle weakness. The second aims at chronic immunosuppression, and is based on glucocorticoids (GCs) and other corticosteroid-sparing agents [2]. Moderate- to high-doses of GC have multiple side-effects and one of the major concerns is their impact on bone metabolism leading to osteoporosis [3].

There are few reports concerning the frequency of osteoporosis in patients with MG on prolonged GC therapy. One study in Japan (n = 36) found a reduced bone mineral density (BMD) in 31% of women with MG and osteoporosis in 11.5% of patients with MG under GC therapy [4]. Conversely, a large-scale analysis from the UK General Practice Research Database evidenced that the overall risk of fracture in patients with MG is not statistically increased when compared with age- and gender-matched controls, irrespective of GCs use. Interestingly, fracture risk was increased only in patients with MG using antidepressant, anxiolytic or anticonvulsant drugs [5]. However, this was a retrospective study using electronic medical record data [5]. Recently, Konno et al (2015) have shown that 6% of MG patients in use of GCs (n = 283) presented osteoporotic fractures. Moreover, the presence of fracture was associated with the duration of GC treatment and significantly aggravated the quality of life in MG patients [6].

The pathogenesis of glucocorticoid-induced osteoporosis (GIOP) occurs through a number of mechanisms. Initially, GCs enhance osteoclastogenesis and bone resorption. GCs also increase osteoblast apoptosis, leading to a marked decrease in bone formation. In addition to their effects on osteoclasts and osteoblasts, GCs also increase osteocytes apoptosis that are thought to participate in the detection and healing of bone microdamage. Accelerated apoptosis of osteocytes could lead to diminished bone quality and bone strength [7]. Therefore, the impairment in bone formation seems to be the major effect of GCs. This is a complex mechanism which involves specific cytokines and bone metabolism markers [8].

This current study aimed to evaluate the frequency of low bone mass, osteopenia and osteoporosis in a sample of 80 patients with MG patients. Moreover, we assessed the putative

association between bone mass and plasma levels of bone metabolism markers in these patients.

MATERIAL AND METHODS

Subjects

This study included 80 patients with MG followed at the Neuromuscular Disease Outpatient Clinic, University Hospital, *Universidade Federal de Minas Gerais*, Belo Horizonte, Brazil.

Patients were diagnosed based on the Myasthenia Gravis Foundation of America (MGFA) criteria [9]. Briefly, MG diagnosis was based on clinical findings (fluctuating symptoms with easy fatigability and recovery after rest) along with amelioration of symptoms after use of acetylcholinesterase inhibitors, decremental muscle response to a train of low frequency repetitive nerve stimuli, or the presence of autoantibodies against skeletal muscle acetylcholine receptors. Exclusion criteria included pregnancy and presence of cancer. In addition, this study included a control group, comprising 62 subjects, age and gender matched, recruited from the community. Exclusion criteria for controls included pregnancy, presence of chronic inflammatory diseases, cancer, current use of systemic glucocorticoids, insulin, statins, growth hormone, anabolic agent or hormone replacement therapy. All subjects provided written informed consent before admission to the study. The Research Ethics Committee of the *Universidade Federal de Minas Gerais*, Brazil approved this study (Protocol number: CAAE-19045413.0.0000.5149).

Clinical assessment

Clinical status and severity of MG were determined by the recommendations of the MGFA [9]. Skeletal muscle force was evaluated by the Myasthenia Gravis Composite (MG Composite) scale that measures the stage of signs and symptoms of the disease, their degree of fluctuation and severity [10]. Participants were evaluated by a single previously trained researcher who collected socio demographic, clinical and anthropometric data.

The cumulative dose of prednisone, *i.e.* a total dose of prednisone used by the patient throughout the course of his disease, was based on data obtained by the careful review of medical records. Other corticosteroids (mainly methylprednisolone) were converted to the equivalent dose of prednisone, and added to the accumulated dose. All medications taken by the patient at the time of evaluation were registered.

Bone mineral density assessment

BMD in the lumbar spine (L1-L4), in the right femoral neck and in the whole body were measured by Dual-energy X-ray absorptiometry (DXA) provided by W Discovery equipment (Hologic, Bedford, MA, USA), software version 3.3.01. The scan images were analyzed using manufacturer specifications and normative data. Measurements were performed after removing all metal fittings. Patients were positioned in the supine position centered and straight on the densitometer table with knees and ankles bound together by a Velcro strap. The individual´s arms were positioned to maximize the space between the arms and torso. To help ensure that individuals do not spread their fingers and move them outside the scan field or under their buttocks (thereby confounding regional measurement), the fingers and thumb were bound together with an elastic or flexible plastic band. Additionally, the knees and ankles were strapped together to reduce the likelihood of patient movement. All measurements were performed by a single International Society of Clinical Densitometry-certified densitometrist blind to the clinical status of subjects.

The raw value of BMD was transformed into clinical values as T-score (the number of standard deviations, SD, by which a given BMD value differs from the mean reference value for young healthy adults) and Z-score (the number of SD by which a given BMD value differs from the mean reference value for an age- and gender-matched control). The diagnosis of osteoporosis was made according to the World Health Organization (WHO) criteria, represented by a T-score below –2.5 SD, and osteopenia as a T-score of < –1 to > –2.5 SD, for postmenopausal patients and men older than fifty years-old. To premenopausal patients and men younger than fifty, we used Z-score below –2.0 to diagnose low bone mass [11].

Analysis of bone metabolism markers and cytokines

Fasting blood samples (eight milliliters) were drawn by venipuncture in vacuum tubes containing heparin the morning of the clinical assessment. Blood was immediately centrifuged at 3,000 rpm for 10 min, 4°C. Plasma was collected and stored at -70°C until assayed.

Plasma levels of osteocalcin (OC), osteopontin (OPN), osteoprotegerin (OPG), tumor necrosis factor (TNF-α), interleukin 1 (IL)-1β, IL-6, adrenocorticotropic hormone (ACTH), Dickkopf (DKK-1), sclerostin (SOST), insulin, leptin, parathyroid hormone (PTH) and fibroblast

growth factor (FGF-23) were simultaneously measured by the Luminex® technique using the Human Bone Metabolism kit (Merck Millipore, Darmstadt, Germany). Briefly, the protein patterns of the kit were gently homogenized and diluted (serial dilutions). Capture beads for each protein were mixed into the assay. For the test, 25µl of capture beads'mix was added to the each well with 25µl of sample or standard protein. The plate was incubated for 16-18 hours. After incubation, the plate was washed and added 25µl of detection antibody (2 hours, room temperature). Next, streptavidin was added (50µl) and the plate was incubated for 30 minutes. After this period, the plate was washed and resuspended in fluid drive buffer (100µl) and acquired in Luminex'sxMAP® instruments (MAGPIX®, Merck Millipore, Darmstadt, Germany) using Exponent® software (LUMINEX, Austin, Texas, USA). Data were analyzed by MILLIPLEX® Analyst 5.1 (Merck Millipore, Darmstadt, Germany) and results were expressed as pg/mL.

Serum levels of 25OHD were measured by chemiluminescence immunoassay, ionic calcium (Ca) by an ion-selective electrode with automatic correction of pH and phosphorous (P) by standard colorimetric method ultraviolet.

Statistical analysis

All variables were tested for Gaussian distribution by the Kolmogorov-Smirnov normality test. Two groups (patients vs. controls) were compared by Mann–Whitney or Student's t tests when non-normally or normally distributed, respectively. Association between dichotomous variables was assessed with the chi-square test. Spearman's correlation analyses were performed to examine the relationship between clinical variables and plasma levels of bone metabolism markers.

Differences between normal DXA versus altered DXA (*i.e.*, low bone mass, osteopenia or osteoporosis) were examined with logistic regression analysis. Variables that reach a p value ≤ 0.20 in the univariate analyses were included in the logistic regression modeling. The goodness of fit of the final model was tested by the Hosmer–Lemeshow method, and odds ratios with 95% confidence intervals were obtained for each independent variable in the model. In this analysis, controls and patients with MG were assessed independently.

All statistical tests were two-tailed and were performed using a significance level of $\alpha=0.05$. Data were analyzed using the Statistical Package for the Social Sciences® version 20.0 (SPSS;

Chicago, IL, USA) and GraphPad Prism® version 5.0 (GraphPad Software, La Jolla, CA, USA).

RESULTS

Clinical and demographic features of MG patients

We evaluated 91 patients with MG, but six refused to participate in the study, three were pregnant and two were in treatment of breast cancer. Therefore, the study included 80 patients (60 women and 20 men). Of the 60 women evaluated, 33.3% were in postmenopause. Demographic, clinical and biochemical features of patients with MG are shown in Table 1. Patients and controls did not differ regarding age, gender and body mass index (BMI). Control group comprised 62 individuals (39 women and 23 men) with a mean ± SD age of 40.42 ± 13.62 years and mean BMI ± SD of 25.49 ± 5.42 kg/m^2.

Among MG patients, 31 underwent thymectomy (27 women and 4 men), four showed thymic hyperplasia and two had thymoma. Over the course of the disease, 52 patients had at least one myasthenic crisis. Some patients presented comorbidities, such as hypertension (33.8%), hypercholesterolemia (27.5%), diabetes mellitus (13.8%), hyperthyroidism (11.3%), cataract (11.3%) and glaucoma (7.5%).

Regarding current treatment, 87.5% of the patients were taking symptomatic medication (pyridostigmine) and 72.5% were in use of prednisone. The remaining MG patients (27.5%) have already used any GC during the course of the disease. There was no significant difference in cumulative dose of prednisone between the groups in current and past use of prednisone.

Twenty five patients (31.25%) had received primary prevention against GC-induced osteoporosis, such as the use of calcium carbonate and vitamin D. Ten patients (12,5%) developed at least one symptomatic bone fracture [humerus, radius, tibia (two patients each), fibula (four patients), thoracic vertebrae or lumbar vertebrae], which were due to falls from standing height or ankle sprain.

Ten percent of patients with MG presented 25HOD concentrations between 10-20 ng/ml, 50% between 20-30 ng/ml and 40% greater than 30 ng/ml. Mean serum levels of ionic calcium were 5.11 mg/dL (± 0.23) and phosphorous 3.43 mg/dL (± 0.69), featuring normal concentrations of these markers.

Bone mineral density

Bone densitometry results are shown in Table 2. According to the WHO criteria, the frequency of osteoporosis in MG patients were significantly higher than in CG ($p = 0.003$). Patients with MG presented lower lumbar BMD ($p = 0.041$), lumbar T-score ($p = 0.033$), femoral neck T-score ($p = 0.041$), total body bone mineral content ($p = 0.019$), total body BMD ($p = 0.001$), total body T-score ($p = 0.001$) and total body Z-score ($p = 0.008$) in comparison with controls. Severity of MG evaluated by MG composite was negatively associated with femoral neck BMD ($r = -0.248$; $p = 0.032$).

Sex-specific analyses showed that female patients with MG presented significant reduction in femoral neck T-score ($p = 0.03$), total body BMD ($p = 0.02$) and total body T-score ($p < 0.01$) in comparison with female control group. Male patients with MG presented only reduced total body Z-score ($p = 0.01$) in comparison with male controls. We also compared BMD between pre- and postmenopausal women with MG. As expected, postmenopausal women present lower lumbar BMD ($p = 0.01$), total body BMC ($p = 0.01$) and total body BMD ($p = 0.01$) in comparison with premenopausal women. We then stratified the MG group according to thymectomy status. Women who have undergone thymectomy present lower femoral neck Z-score ($p = 0.02$) than women who have not undergone thymectomy.

Subjects who were diagnosed with low bone mass, osteopenia and/or osteoporosis, received referral to an endocrinologist, rheumatologist or gynecologist, or received medical treatment prescribed by the attending neurologist. Figure 1 shows BMD by dual energy X-ray absorptiometry from a male 30 years-old MG patient.

Plasma levels of bone metabolism markers and cytokines

As shown in Table 3, MG patients presented lower levels of OC ($p = 0.000$), PTH ($p = 0.000$), ACTH ($p = 0.004$), TNF-α ($p = 0.034$), DKK-1 ($p = 0.000$) and SOST ($p = 0.000$), and higher levels of FGF-23 ($p = 0.001$), leptin ($p = 0.000$) and IL-6 ($p = 0.021$) when compared to control group.

There was a significant negative correlation between cumulative GC dose and ionic calcium levels ($r = -0.271$; $p = 0.020$), lumbar spine T-score ($r = -0.420$; $p = 0.029$), femoral neck BMD ($r = -0.277$; $p = 0.018$), femoral neck T-score ($r = -0.527$; $p = 0.006$) and femoral neck Z-score ($r = -0.241$; $p = 0.040$).

We found significant association between between MG composite and the plasma levels of ACTH (r = -0.300; p = 0.009), OC (r = -0.257; p = 0.026), SOST (r = -0.287; p = 0.013) and IL-1β (r = -0.307; p = 0.007).

Logistic regression models

Logistic regressions were performed to assess the likelihood of altered DXA result, *i.e.* low bone mass, osteopenia or osteoporosis. Among patients with MG, the model included ten independent variables (age, age at disease onset, total body BMD, cumulative prednisone dose, DKK-1, TNF-α, OPG, OPN, SOST, FGF-23). The full model with all predictors was statistically significant (Omnibus Tests of Model Coefficients = $p<0.001$), indicating that the model was able to distinguish normal versus altered DXA subjects. The full model explained between 52.4% (Cox & Snell R Square) and 71.8% (Nagelkerke R Square) of the variance of subjects, and correctly classified 91.7% of cases. As shown in Table 4A, age at onset, total body BMD and DKK-1 and TNF-α levels were statistically significant to the model. Interestingly, we observed that after controlling for all the mentioned variables, higher TNF-α levels increases the likelihood of presenting DXA alteration by 2.62.

Among controls, the model included five independent variables (age, total body BMD, BMI, DKK-1 and OC). The full model with all predictors was statistically significant (Omnibus Tests of Model Coefficients = $p<0.001$). The full model explained between 48.2% and 77.1% (Cox & Snell R Square and Nagelkerke R Square, respectively) of the variance of subjects, and correctly classified 91.9% of cases. As expected, only age and total body BMD were statistically significant to the model (Table 4B).

DISCUSSION

To the best of our knowledge, this is the first study to evaluate BMD by DXA and a comprehensive panel of bone metabolism markers in patients with MG. Our results showed that patients under high cumulative prednisone dose presented impairment of bone metabolism. BMD was significantly lower in relatively young patients with MG compared with healthy controls. In addition, cumulative GC dose negatively correlated with ionic calcium levels, lumbar spine T-score and femoral neck BMD, T-score and Z-score. Regarding that GCs are commonly used in the treatment with MG, these results highlight the relevance of systematically evaluating BMD in patients with MG.

MG is the main neurological disease treated with long term GCs. Nevertheless, only a few studies have evaluated the prevalence of osteoporosis in patients with MG. A retrospective cohort showed that patients with MG presented increased risk of developing osteoporosis regardless of GCs use, but the risk was higher among corticosteroid-treated individuals [12]. The risk of bone loss in GCs-treated patients with MG was found to be acceptable if prophylactic medication had been administered [4]. More recently, a cross-sectional study found that the duration of GCs therapy, but not the dose, was associated with osteoporotic fractures in patients with MG. In the current study, we confirmed that the use of GCs, more specifically their cumulative dose, was one of the major factors underlying bone metabolism impairment and, as consequence, osteopenia and osteoporosis in MG, especially in women.

GC-induced osteoporosis is the most common form of secondary osteoporosis [13]. Different rheumatologic panels recommend BMD measurement in individuals taking glucocorticoids in a daily dose ≥ 5 mg of prednisone or equivalent for ≥ three months [3, 14]. In the clinical practice, however, only part of patients on prolonged GCs therapy receives optimal primary prevention against GC-induced osteoporosis [15], and this was also noticed in the current cohort of patients with MG.

We evaluated which factors, including bone metabolism markers, were associated with osteopenia and/or osteoporosis in MG. As expected, age and BMD were significant predictors of osteopenia/osteoporosis in control subjects. Age at disease onset, BMD and DKK-1 and TNF-α levels were predictors of osteopenia/osteoporosis among patients with MG. After controlling for all confounding variables, higher TNF-α level increased the likelihood of presenting DXA alteration by 2.62 times.

Bone remodeling is a continuous and dynamic process with the participation of several cytokines that define the role of osteoblasts and osteoclasts in the chain of events leading to the formation and bone resorption. GCs adversely affect bone strength/quality in a number of ways. Specifically, the two main effects of GCs on bone metabolism are apoptosis induction in osteoblasts and osteocytes, thereby decreasing bone formation; and prolonging the lifespan of osteoclasts and increasing bone resorption, which results in increased net bone resorption [16, 17]. In the current study, patients with MG presented changes in circulating levels of bone formation markers: plasma levels of OC, PTH and ACTH were lower, while leptin and FGF-23 were higher in MG patients in comparison with controls. We also found changes in bone resorption markers. Patients presented lower plasma levels of SOST and TNF-α, but

higher levels of IL-6 when compared with controls. In addition, we observed 10% of vitamin D insufficiency, defined by as a 25OHD level of less than 20 ng/mL [18], in patients with MG. Vitamin D insufficiency induces bone resorption and bone loss, and subsequent decrease in BMD [19].

As far as we know, there is only one previous study that investigated serum concentration of bone markers in patients with MG [20]. Serum concentration of TNF-α, IL-7 and RANKL were assessed. Serum levels of IL-7 and RANKL, osteoclastogenic inflammatory cytokines, were increased in thymectomized MG women when compared with healthy controls [20].

We also observed that plasma levels of leptin were higher in patients with MG than controls. Leptin is a circulating hormone produced mainly by adipose tissue. Its biological and physiological roles in bone include increasing bone size, content and mineral density [21]. In addition, leptin appears to play an important role against GC-induced osteoporosis [21]. Accordingly, the increase in leptin levels in patients with MG might be due to the body fat redistribution that occurs secondary to GC use.

Patients with MG also presented higher plasma levels of FGF-23 compared with controls. FGF-23 is predominantly produced by osteocytes, and its principal actions involve sodium-dependent phosphate reabsorption inhibition and 1[alpha]-hydroxylase activity in the proximal tubule of the kidney, leading to phosphaturia and suppression of circulating $1.25(OH)_2D$ levels. Abnormal FGF-23 expression influences bone mineralization [22], and might contribute to reduced BMD observed in patients with MG.

Our patients with MG had lower plasma levels of ACTH, SOST and DKK-1 compared with controls. It is known that ACTH stimulates the secretion of GCs by the adrenal gland, while excessive doses of GCs inhibit the production of ACTH by the pituitary gland [23]. The genes DKK and SOST are mainly expressed in adult bone, and inhibit bone formation. Corroborating our results, Gifre and colleagues found reduced plasma concentration of DKK-1 in hematological patients (idiopathic thrombocytopenic purpura, hemolytic anemia) receiving GCs [8]. In our study, the suppression of ACTH by exogenous GCs may explain the decrease in DKK and in SOST levels.

In our study, TNF-α was associated with low bone mass. TNF-α is a potent bone resorption inducer [24]. TNF-α directly stimulates bone marrow osteoclastogenesis by increasing the

expression of c-Fms, the receptor for M-CSF. As a consequence of M-CSF stimulation, the differentiation and proliferation of progenitor osteoclasts cells occur. TNF-α also acts directly on the osteoclast precursor by enhancing receptor activator of nuclear factor kβ (RANK) signaling mechanisms, even in the absence of elevated levels of receptor activator of nuclear factor kβ ligand (RANKL) [24]. IL-6 is also known to induce osteoclast formation and bone resorption. IL-6 directly induces increased expression of RANKL and OPG in osteoblasts and regulates osteoclast progenitor cell differentiation into mature osteoclasts in states of increased bone turnover [25, 26].

Limitations of the current study include the cross-sectional design, the limited sample of patients and the fact that we did not evaluate GCs-naïve patients. Regarding the low incidence and prevalence rates of MG (around, respectively, 5.3 per million and 77.7 per million), it is very difficult to enroll naïve patients [27]. The risk of osteoporosis is multifactorial so, we cannot definitely state that osteoporosis in MG is solely due to GCs therapy. By contrast, the strict diagnosis criteria, the selection of controls with comparable age, gender, and BMI, and the analysis of clinical, bone density using DXA and bone metabolism parameters together can be regarded as strengths of the study.

In sum, patients with MG under long-term GC therapy presented changes in bone density and bone metabolism markers in comparison with controls. Our results point to the need of systematically evaluating bone density in patients with MG under GC therapy.

REFERENCES

[1] Farrugia ME, Vincent A (2010) Autoimmune mediated neuromuscular junction defects. Curr Opin Neurol 23:489-95.

[2] Kumar V, Kaminski HJ (2011) Treatment of Myasthenia Gravis. Curr Neurol Neurosci Rep 11:89-96.

[3] Grossman JM, Gordon R, Ranganath VK, Deal C, Caplan L, Chen W, Curtis JR, Furst DE, McMahon M, Patkar NM, Volkmann E, Saag KG (2010) American College of Rheumatology 2010 recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis. Arthritis Care Res 62:1515-26.

[4] Wakata N, Nemoto H, Sugimoto H (2004) Bone density in myasthenia gravis patients receiving long-term prednisolone therapy. Clin Neurol Neurosurg 106:139-41.

[5] Pouwels S, Boer A, Javaid MK, Jones DH, Verschuuren J, Cooper C, Leufkens HG, Vries F (2012) Fracture rate in patients with myasthenia gravis: the general practice research database. Osteoporos Int. 24(2):467-476.

[6] Konno S, Suzuki S, Masuda M, Nagane Y, Tsuda E, Murai H, Imai T, Fujioka T, Suzuki N, Utsugisawa K (2015) Association between Glucocorticoid-Induced Osteoporosis and Myasthenia Gravis: A Cross- Sectional Study. Plos One 10:1-7.

[7] Maricic M, Gluck O. Densitometry in Glucocorticoid-Induced Osteoporosis (2004) J Clin Densitom 7:359-63.

[8] Gifre L, Ruiz-Gaspà S, Monegal A, Nomdedeu B, Filella X, Guañabens N, Peris P (2013) Effect of glucocorticoid treatment on Wnt signalling antagonists (sclerostin and Dkk-1) and their relationship with bone turnover. Bone 57:272-76.

[9] Jaretzki A, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JG, Penn AS (2000) Myasthenia gravis. Recommendations for clinical research Standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. Neurology 55:1-14.

[10] Burns TM, Conaway M, Sanders DB (2010) The MG Composite. A valid and reliable outcome measure for myasthenia gravis. Neurology 74:1434-40.

[11] Kanis JA, Delmas P, Burckhardt P, Cooper C, Torgerson D (1997) Guidelines for diagnosis and management of osteoporosis. The European Foundation for Osteoporosis and Bone Disease. Osteoporos Int 7:390-406.

[12] Yeh JH, Chen HJ, Chen YK, Chiu HC, Kao CH (2014) Increased risk of osteoporosis in patients with myasthenia gravis. A population-based cohort study. Neurology 83:1-5.

[13] Buehring B, Viswanathan R, Binkley N, Busse W (2013) Glucocorticoid-induced osteoporosis: An update on effects and management. J Allergy Clin Immunol 132:1019-30.

[14] Pereira RMR, Carvalho JFC, Paula AP, Zerbini C, Domiciano DS, Gonçalves H, Danowski JS, Neto JFM, Mendonça LMC, Bezerra MC, Terreri MT, Imamura M, Weingrill P, Plapler PG, Radominski S, Tourinho T, Szejnfeld VL, Andrada NC (2012) Diretrizes para prevenção e tratamento da osteoporose induzida por glicocorticoide. Rev Bras Reumatol 52:569-93.

[15] Gudbjornsson B, Juliusson UI, Gudbjornsson FV (2002) Prevalence of long-term steroid treatment and the frequency of decision making to prevent steroid induced osteoporosis in daily clinical practice. Ann Rheum Dis 61:32-26.

[16] Mitra R (2011) Adverse Effects of Corticosteroids on Bone Metabolism: A Review. Am J Phy Med Rehabil 3:466-71.

[17] van Staa TP, Leufkens HGM, Cooper C (2002) The Epidemiology of Corticosteroid-Induced Osteoporosis: a Meta-analysis. Osteoporos Int 13:777-87.

[18] Holick FH (2007) Vitamin D Deficiency. N Engl J Med 357:266-81.

[19] Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B (2004) Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med 116:634-39.

[20] D'Amelio P, Grimaldi A, Bernabei P (2006) Immune system and bone metabolism: Does thymectomy influence postmenopausal bone loss in humans?. Bone 39:658-65.

[21] Ahmed HH, Morcos NYS, Eskander EF, Seoudi DMS, Shalby AB (2012) Potential role of leptin against glucocorticoid-induced secondary osteoporosis in adult female rats. Eur Rev Med Pharmacol Sci 16:1446-52.

[22] Shiguang L, Gupta, Quarles A, Darryl L (2007) Emerging role of fibroblast growth factor 23 in a bone–kidney axis regulating systemic phosphate homeostasis and extracellular matrix mineralization. Curr Opin Nephrol Hypertens 16:329-35.

[23] Henneicke H, Gasparini SJ, Brennan-Speranza TC, Zhou H, Seibel MJ (2014) Glucocorticoids and bone: local effects and systemic implications. Trends Endocrinol Metabol 25:197-211.

[24] Yao Z, Li P, Zhang Q, Schwarz EM, Keng P, Arbini A, et al (2006) Tumor necrosis factor-alpha increases circulating osteoclast precursor numbers by promoting their proliferation and differentiation in the bone marrow through up-regulation of c-Fms expression. J Biol Chem 281:11846-55.

[25] Franchimont N, Wertz S, Malaise M (2005) Interleukin-6: An osteotropic factor influencing bone formation? Bone 37:601-06.
[26] Ishimi Y, Miyaura C, Jin CH, Akatsu T, Abe E, Nakamura Y, et al (1990) IL-6 is produced by osteoblasts and induces bone resorption. J Immunol 145:3297-303.
[27] Carr AS, Cardwell CR, McCarron PO, McConville K (2010) A systematic review of population based epidemiological studies in Myasthenia Gravis. BMC Neurology 10:1-9.

TABLES

Table 1. Clinical, demographic and biochemical features of patients with myasthenia gravis (MG).

	MG patients
Gender (female/male)	60/20
Age in years (mean ± SD)	41.89 ± 14.17 (18 - 69)
Education level in years (mean ± SD)	9.28 ± 3.72 (4 - 23)
Body mass index in kg/m^2 (mean ± SD)	26.13 ± 8.04 (16.87 - 49.34)
Waist-hip ratio in cm (mean ± SD)	0.84 ± 0.26 (0 - 1.10)
Age at onset in years [mean ± SD (range)]	29.10 ± 13.48 (16 - 52)
Length of illness in years [mean ± SD (range)]	13.53 ± 10.05 (1 - 39)
MG Composite [mean ± SD (range)]	4.96 ± 5.02 (1 - 18)
Age at first myasthenic crisis in years [mean ± SD (range)]	35.20 ±15.82 (5 - 53)
Age at thymectomy in years, n = 31 [mean ± SD (range)]	32.50 ±11.83 (23 - 55)
Medications for MG (current use)	
Pyridostigmine [n (%) / mean daily dose]	70 (87.5%) / 236.71 mg
Prednisone [n (%) / mean daily dose]	58 (72.5%) / 14.5 mg
Azathioprine [n (%) / mean daily dose]	33 (41.3%) / 120 mg
Other immunosuppressant [n (%)]	7 (8.8%)
Cumulative glucocorticoid dose in mg [mean ± SD (range)]	38123.48 ± 41895.66 (60.0 - 232935.0)
MGFA	
I [n (%)]	10 (12.5%)
IIA [n (%)]	9 (11.3%)
IIB [n (%)]	7 (8.8%)
IIIA [n (%)]	15 (18.8%)
IIIB [n (%)]	4 (5.0%)
IVA [n (%)]	6 (7.5%)
IVB [n (%)]	5 (6.3%)
V [n (%)]	22 (27.5%)
25OHD in ng/mL [mean ± SD (range)]	31.20 ± 14.29 (10.90 – 117.00)
10-20 [n (%)]	8 (10)
20-30 [n (%)]	40 (50)
>30 [n (%)]	29 (36.3)
Ionic calcium mg/dL (mean ± SD)	5.11 ±0.23 (4.50 – 5.60)
Phosphorus mg/dL (mean ± SD)	3.43 ±0.68 (2.10 – 5.60)

Abbreviations: MG = Myasthenia Gravis; MGFA= Myasthenia Gravis Foundation of America Clinical Classification; 25OHD= 25-hydroxyvitamin D; SD= standard deviation.

Table 2. Bone mineral density analyses in patients with myasthenia gravis (MG) and control subjects.

	MG (*n* = 80)	**CG (*n* = 62)**	***p* value**
Lumbar spine			
BMD in g/cm^2 (mean ± SD)	0.93 ± 0.16	0.98 ± 0.11	**0.041**[a]
T-score (mean ± SD)	-1.29 ± 2.35	-0.43 ± 0.75	**0.033**[a]
Z-score (mean ± SD)	-0.90 ± 4.24	-0.45 ± 1.06	0.340[a]
Low bone mass [n (%)]	5 (6.3%)	4 (6.5%)	0.961
Osteopenia [n (%)]	8 (10.0%)	7 (11.3%)	0.494[b]
Osteoporosis [n (%)]	11 (13.8%)	1 (1.6%)	**0.008**[b]
Femoral neck			
BMD in g/cm^2 (mean ± SD)	0.87 ± 0.14	0.86 ± 0.12	0.621[a]
T-score (mean ± SD)	-0.80 ± 1.36	-0.29 ± 0.65	**0.041**[a]
Z-score (mean ± SD)	-0.49 ± 1.14	-0.21 ± 0.93	0.122[a]
Low bone mass [n (%)]	4 (5.0%)	0	0.134
Osteopenia [n (%)]	16 (20.0%)	3 (4.8%)	**0.003**[b]
Osteoporosis [n (%)]	2 (2.5%)	1 (1.6%)	0.631[b]
Total Body			
BMC in g/cm^2 (mean ± SD)	2033.84 ± 385.03	2204.87 ± 416.40	**0.019**[a]
BMD in g/cm^2 (mean ± SD)	1.03 ± 0.10	1.09 ± 0.96	**0.001**[a]
T-score (mean ± SD)	-1.29 ± 1.36	-0.23 ± 0.69	**0.001**[a]
Z-score (mean ± SD)	-0.63 ± 1.08	-0.21 ± 0.93	**0.008**[a]

Abbreviations: MG= myasthenia gravis group; CG= control group; SD= standard deviation; BMD= bone mineral density; BMC= bone mineral content.

[a]Mann-Whitney Test; [b]Qui-square test. **Bold** type indicates significant p values.

Table 3. Comparison between plasma concentrations of bone metabolism markers in patients with myasthenia gravis (MG) and the control group (CG).

Bone formation markers	**MG (*n* = 80)** Mean ± SD	**CG (*n* = 62)** Mean ± SD	***p* value**
OC (pg/mL)	5935.28 (±5165.05)	17006.85 (±25255.42)	**<0.001**[a]
OPN (pg/mL)	24991.29 (±28141.31)	17985.61 (±21651.96)	0.108[a]
PTH (pg/mL)	88.63 (±45.03)	131.37 (±90.33)	**<0.001**[a]
FGF-23 (pg/mL)	75.87 (±111.49)	63.24 (±173.13)	**0.001**[a]
ACTH (pg/mL)	0.29 (±0.62)	0.58 (±1.62)	**0.004** [a]
Leptin (pg/mL)	18545.08 (±14670.82)	9850.70 (±8682.96)	**<0.001**[a]
Insulin (pg/mL)	729.65 (±874.59)	1125.74 (±1780.50)	0.086[a]
IL-1β (pg/mL)	0.14 (±0.13)	0.14 (±0.12)	0.928[a]
IL-6 (pg/mL)	1.86 (±4.49)	0.90 (±1.85)	**0.021**[a]
TNF (pg/mL)	1.26 (±0.98)	2.92 (±4.44)	**0.034**[a]
DKK (pg/mL)	321.48 (±215.17)	597.12 (±443.40)	**<0.001**[a]
OPG (pg/mL)	463.06 (±226.42)	503.11 (±308.07)	0.375[a]
SOST (pg/mL)	2257.77 (±1100.97)	4173.90 (±3640.79)	**<0.001**[a]

Abbreviations: OC= osteocalcin; OPN= osteopontin; PTH= parathyroid hormone; FGF-23= fibroblast growth factor; ACTH= adrenocorticotropic hormone; IL-1β= interleukin 1; IL-6= interleukin 6; TNF= tumor necrosis factor; DKK1= Dickkopf; OPG= osteoprotegerin; SOST= sclerostin.

[a]: Mann-Whitney test. **Bold** type indicates significant *p* values.

Table 4. Logistic regression model for prediction of altered DXA.

A. Controls

	B	SE	Wald	Df	p Value	Odds Ratio	95% CI for Odds Ratio	
							Lower	Upper
Age	**0.257**	**0.093**	**7.710**	**1**	**0.005**	**1.293**	**1.079**	**1.551**
Total body BMI	**-63.410**	**26.234**	**5.842**	**1**	**0.016**	**0.000**	**0.000**	**0.000**
DKK-1	0.001	0.002	0.176	1	0.675	1.001	0.998	1.004
BMI	-0.344	0.180	3.661	1	0.056	0.709	0.499	1.008
OC	0.000	0.000	3.808	1	0.051	1.000	1.000	1.000
Constant	62.601	26.321	5.657	1	0.017	1.539E+27		

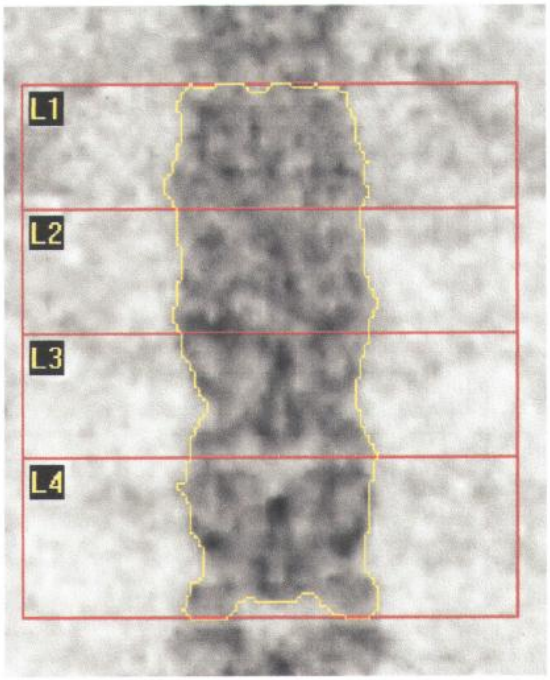
B. Patients with MG

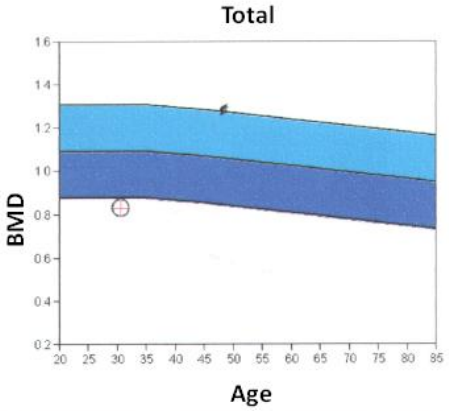
	B	SE	Wald	Df	p Value	Odds Ratio	95% CI for Odds Ratio	
							Lower	Upper
Age	0.006	0.054	0.013	1	0.908	1.006	0.906	1.118
Age at onset	**0.105**	**0.052**	**4.075**	**1**	**0.044**	**1.110**	**1.003**	**1.229**
Total body BMD	**-23.924**	**8.562**	**7.087**	**1**	**0.005**	**0.000**	**0.000**	**0.001**
Cumulative prednisone dose	0.000	0.000	2.438	1	0.118	1.000	1.000	1.000
DKK-1	**0.004**	**0.002**	**5.675**	**1**	**0.017**	**1.004**	**10..1**	**1.008**
TNF-α	**0.962**	**0.450**	**4.570**	**1**	**0.033**	**2.617**	**1.083**	**6.321**
OPG	-0.002	0.003	0.365	1	0.546	0.998	0.993	1.004
OPN	0.000	0.000	0.130	1	0.719	1.000	1.000	1.000
SOST	0.001	0.001	0.767	1	0.381	1.001	0.999	1.002
FGF-23	0.000	0.006	0.001	1	0.972	1.000	0.989	1.011
Constant	16.143	7.986	4.086	1	0.043	1.025E+7		

Altered DXA was considered when subjects presented low bone mass, osteopenia or osteoporosis.

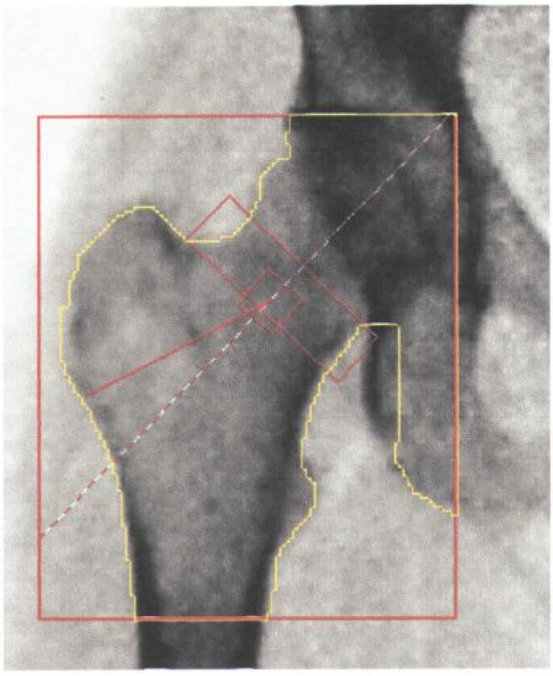
Abbreviations: BMI = body mass index; BMD = bone mineral density; CI = confidence interval; DKK-1 = dickkopf; df = degrees of freedom; DXA = dual-energy X-ray absorptiometry; FGF = fibroblast growth factor; MG = Myasthenia Gravis; OC = osteocalcin; OPG = osteoprotegerin; OPN = osteopontin; SE = standard error; SOST = sclerostin; TNF-α = tumor necrosis factor.

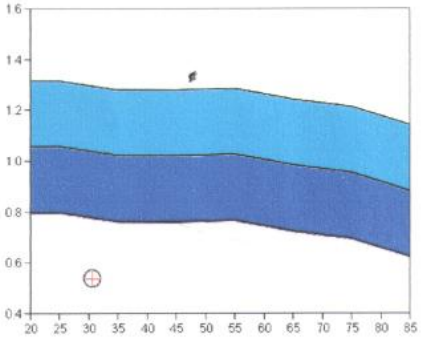
FIGURE



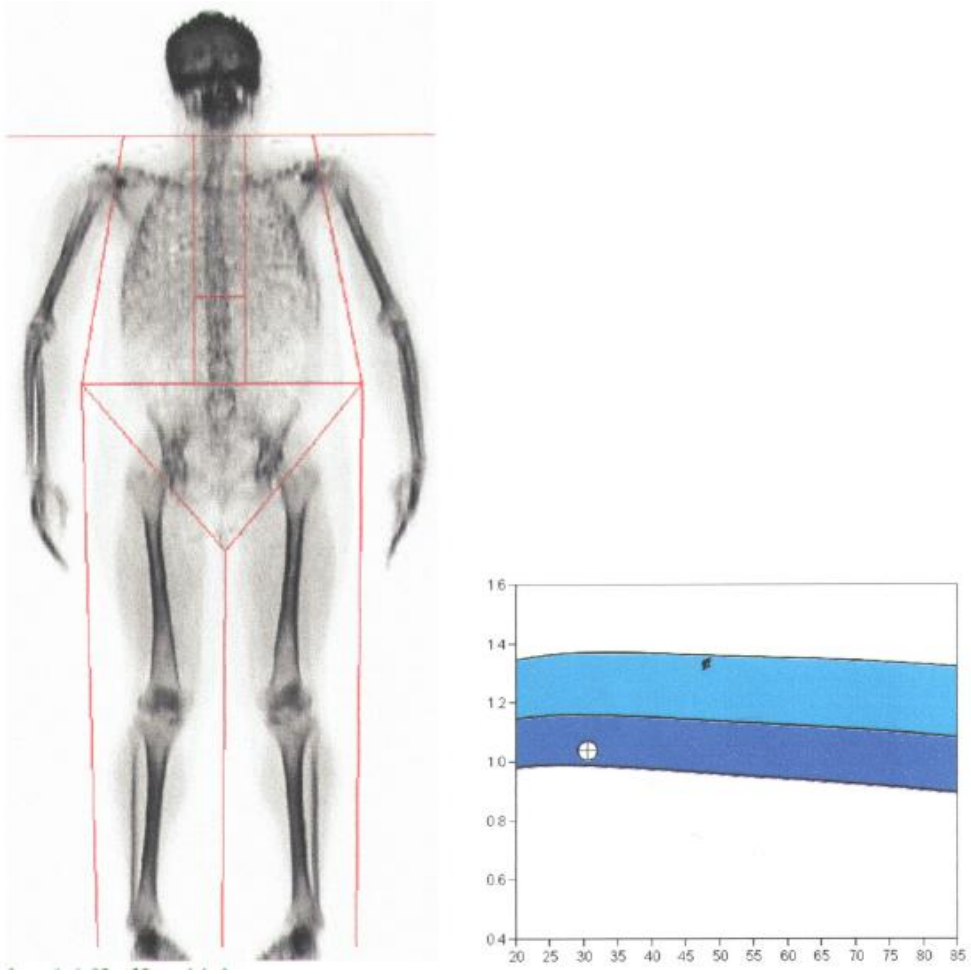


	Area (cm²)	BMC (g)	BMD (g/m²)	Z-score
Total	51.58	43.04	0.83	-2.3





	Area (cm²)	BMC (g)	BMD (g/m²)	Z-score
Total	45.06	24.32	0.54	-3.8



	Area (cm^2)	BMC (g)	BMD (g/m^2)	Z-score
Total	2147.02	2233.83	1.04	-1.3

Figure 1. Bone mineral density by dual energy X-ray absorptiometry from a myasthenia gravis patient, of lumbar spine (a), femoral neck (b) and total body (c). DXA results show osteoporosis in the right hip of a patient, 30 year-old with one year of MG duration, classified in class IVA, with score 5 in MG composite. He was currently taking 60 mg of prednisone per day, and had a cumulative GC dose of 41.440 mg

ARTIGO II

Journal of the Neurological Sciences 381 (2017) 169–175

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Body composition and adipokines plasma levels in patients with myasthenia gravis treated with high cumulative glucocorticoid dose

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Abstract

This study aimed to evaluate changes in body composition, *i.e.* overweight, obesity, fat accumulation and low lean body mass and plasma levels of adipokines in patients with MG. The study enrolled 80 patients with MG, and 62 controls. Body fat mass and body lean mass was analyzed by dual-energy X-ray absorptiometry technique (DXA). Plasma levels of leptin were analyzed by Luminex® and adiponectin and resistin were analyzed by ELISA. The mean age of patients with MG was 41.9 years, with 13.5 years of length of illness, and mean cumulative dose of glucocorticoids 38,123 mg. Our results showed that the frequency of obesity is higher in MG patients than in controls, and patients with MG presented higher body fat mass, android body adiposity and total body fat than controls. MG patients presented lower levels of resistin and higher levels of leptin in comparison with controls. There were no differences in the plasma levels of adiponectin. Higher total body fat and lower body lean mass were associated with increased severity of MG symptoms. This result points to the relevance of estimation of body composition in planning long-term care of MG patients.

Keywords: myasthenia gravis, obesity, adipokines, body composition, dual-energy X-ray absorptiometry.

INTRODUCTION

Myasthenia gravis (MG) is a neuromuscular disease characterized by muscle weakness that improves with rest. The most common form of MG is caused by antibodies against the postsynaptic membrane at the neuromuscular junction [1]. Patients with MG experience fluctuating and fatigable skeletal muscle weakness that often affects selected muscle groups. Usually, patients note weakness fluctuations from day to day or even from hour to hour. The most common manifestations include ptosis, diplopia, dysarthria, dysphagia, dyspnea, facial weakness, or fatigable limb or axial weakness [2].

The current management of MG includes the use of anticholinesterase drugs for temporary improvement of neuromuscular transmission, use of nonspecific immunosuppressant or immunomodulatory drugs and thymectomy [3]. The use of glucocorticoids (GCs) is the first-choice immunosuppressive therapy for generalized MG and has extensively been used in MG treatment mainly because of the rapid response onset [4]. Nevertheless, chronic use of GCs is limited by the numerous and frequent side effects. These side effects include but are not limited to osteoporosis, obesity, lipodystrophy, muscle atrophy, steroid myopathy, hypertension, impaired glucose tolerance, sodium/fluid retention, potassium loss, cataract, glaucoma, peptic ulcer and suppression of growth (children) [5-7].

Oral GCs use is particularly associated with obesogenic effects, such as body weight gain, altered energy expenditure and increased appetite [5, 8-11]. In addition, long-term GCs use results in changes in body fat composition known as GCs-induced lipodystrophy (GIL), featured by fat accumulation in facial (‘moon face’), dorsocervical (‘buffalo hump’, supraclavicular fat pads) and abdominal regions. GIL has been reported in subjects with inflammatory conditions that were subjected to long-term glucocorticotherapy, such as rheumatoid arthritis and systemic lupus erythematosus [12-15]. Patients under GCs consider weight gain one of the most distressing adverse events induced by these drugs, that impacts negatively in treatment adherence [5, 10]. It is worth mentioning that GIL is not only an aesthetic issue, but it has been associated with severe metabolic abnormalities including insulin resistance, dyslipidemia and glucose intolerance [16].

The pathophysiology of GIL is complex and involves changes in the expression of adipokines [17-19]. Adipokines are adipose tissue-derived peptides that contribute to the regulation of appetite and satiety, fat distribution, insulin secretion and sensitivity and energy expenditure [20]. Previous studies showed that GCs induce changes in plasma levels of adipokines [18,

19, 21]. For instance, one week of GCs use was associated with increase of leptin and adiponectin levels in physically fit women [21].

Because of long-term treatment with GCs, patients with MG may be predisposed towards unfavorable body composition characteristics and changes in circulating levels of adipokines. To the best of our knowledge, no previous studies addressed these issues in the context of MG. Therefore, this study was designed to investigate changes in body composition, *i.e.* overweight, obesity, fat accumulation and low lean body mass and plasma levels of adipokines in patients with MG. We hypothesize that patients with MG present changes in body composition and in the plasma levels of adipokines in comparison with controls.

METHODS

Subjects

This cross-sectional study was approved by the Research Ethics Committee of the *Universidade Federal de Minas Gerais*, Brazil (Permit number: 501.655) and all subjects provided written informed consent before admission to the study. This study included 80 patients with MG followed at the Neuromuscular Disease Outpatient Clinic, University Hospital, *Universidade Federal de Minas Gerais*, Belo Horizonte, Brazil. Patients were diagnosed based on the Myasthenia Gravis Foundation of America (MGFA) criteria [22]. Briefly, MG diagnosis was established considering clinical findings (fluctuating symptoms with easy fatigability and recovery after rest) along with amelioration of symptoms after use of acetylcholinesterase inhibitor, decremental muscle response to a train of low frequency repetitive nerve stimuli, or the presence of autoantibody against skeletal muscle acetylcholine receptors [22]. Exclusion criteria were pregnancy and previous osteoporosis treatment. In addition, this study included a control group comprising 62 age- and gender- matched subjects recruited from the community. Exclusion criteria for control group were the presence of risk factors for osteoporosis, pregnancy, presence of chronic inflammatory diseases, cancer, HIV infection, current use of GCs, insulin, statins, growth hormone, anabolic agents and hormone replacement therapy.

Clinical evaluation

Clinical status and severity of MG were determined according to the recommendations of the MGFA [22]. Muscle strength was evaluated by the MG Composite scale [23, 24]. Participants were evaluated by a single trained researcher (NB) who also collected sociodemographic, clinical and anthropometric data. Body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared (kg/m^2). Individuals were classified based on the criteria recommended by Bray (2003) [25] according to age, sex and fat body percentage (determined with DXA). Accordingly, individuals with BMI < 18.5 kg/m^2 are considered underweight; between 18.5 and 24.9 as normal; between 25 and 29.9 as overweight; and values > 30.0 indicate obesity [25]. The waist-to-hip ratio was calculated dividing waist circumference by hip circumference. Waist circumference was measured as the horizontal distance around the abdomen at the level of the umbilicus, and hip circumference was measured as the largest circumference between the waist and thighs.

The cumulative dose of prednisone - *i.e.* the total dose of prednisone used by the patient throughout the course of her/his illness - was based on data obtained from medical records. All medications taken by the patient at the time of evaluation were registered.

Body composition assessment

Whole body composition was measured by dual-energy X-ray absorptiometry (DXA) using the Discovery W equipment (Hologic, Bedford, MA, USA), software version 3.3. Measurements were performed in the supine position after removing all metal fittings. Scan images were analyzed using manufacturer specifications and normative data. All scans were performed by a single certified clinical densitometrist (AK). Daily calibration and quality control tests were performed according to the manufacturer's recommendations and different regions of interest were manually checked for maximal reliability. The DXA provides body characteristics accurately, including whole body fat and lean and mineral composition in various body compartments. DXA may be the preferred method to evaluate body composition as the whole body may be easily scanned, radiation exposure is low, and it is likely to be more accessible and more economical to obtain than computed tomography or magnetic resonance imaging [26, 27].

Using the DXA results, we evaluated body fat mass (Kg); body fat percentage (%); fat mass distribution (% fat trunk / % fat legs); android body adiposity (%); body lean mass (Kg); appendicular skeletal muscle mass (ASM, Kg); skeletal muscle mass index (SMI, calculated as lean mass / height^2) and sarcopenic index (appendicular lean + BMC / height^2).

Based on DXA data, sarcopenia is defined as appendicular skeletal muscle mass less than two standard deviations below the mean of a young reference group [28]. Accordingly, the cutoff values of 5.45 kg/m^2 for women and 7.26 kg/m^2 for men were applied in this study [28].

Biochemical measurements

Eight milliliters of peripheral blood samples were drawn by venipuncture in vacuum tubes containing heparin at the same day of the clinical assessment (between 8 – 11 AM). Blood was immediately centrifuged at 1,800 *g* for 10 min, 4°C. Plasma was collected and stored at -70°C until assayed.

Plasma levels of leptin were measured by the Luminex® technique using the Analyst 5.1 software for analysis (Merck Millipore, Darmstadt, Germany). Adiponectin and resistin levels were assessed by enzyme-linked immunosorbent assay (ELISA) following the

manufacturer’s instructions (DuoSet R&D Systems, Minneapolis, MN, USA). All results were expressed as ng/mL.

Statistical analysis

All variables were tested for Gaussian distribution by the Kolmogorov-Smirnov normality test. Two groups (patients vs. controls) were compared by Mann–Whitney or Student’s t tests when non-normally or normally distributed, respectively. Association between dichotomous variables was assessed with the chi-square test. Spearman's correlation analyses were performed to examine the relationship between clinical variables and plasma levels of adipokines. All statistical tests were two-tailed and were performed using a significance level of $p = 0.05$. Data were analyzed using the Statistical Package for the Social Sciences® version 22.0 (SPSS; Chicago, IL, USA) and GraphPad Prism® version 5.0 (GraphPad Software, La Jolla, CA, USA).

RESULTS

Clinical and demographic features of MG patients

Eighty patients with MG, including 60 women, were enrolled in this study. Demographic and clinical features of patients with MG and controls are shown in Table 1. Over the course of the disease, 53 (66.3%) patients with a mean age of 35 years (± 15.50) had at least one myasthenic crisis (45 patients had only one crisis, 3 had two crisis, 2 had three crisis and 3 had four crisis). Medical comorbidities in the MG group were: obesity (67.5%), hypertension (33.8%), hypercholesterolemia (27.5%), type 2 diabetes mellitus (13.8%), hyperthyroidism (11.3%), cataract (11.3%) and glaucoma (7.5%). Two patients reported the co-occurrence of other immune-related diseases (Grave's disease and autoimmune hepatitis). Patients and controls did not differ regarding age, gender and BMI.

DXA whole body composition

Anthropometric and DXA-derived body composition measures in MG patients and control subjects are shown in Table 2. Figure 1 shows a representative image of body composition obtained by DXA from a female patient with MG and a control woman. According to DXA results, the prevalence of obesity showed a trend toward being higher among patients with MG (n = 54, 67.5%) than in controls (n = 32, 51.6%). Patients with MG presented higher body fat mass ($p = 0.000$), android body adiposity ($p = 0.003$) and body fat percentage ($p = 0.001$) than controls. The sarcopenia index and sarcopenia frequency were similar among patients with MG and controls.

When MG patients were categorized into groups taking and not taking GCs, there were no significant differences in body composition between groups, except in the frequency of sarcopenia which was higher in patients under GC therapy ($p = 0.01$).

When stratified by sex, women with MG presented worse fat mass distribution ($p < 0.001$), higher android body adiposity ($p = 0.01$) and body fat percentage ($p < 0.001$), and lower body weight ($p < 0.001$), body lean mass ($p < 0.001$), appendicular skeletal muscle mass ($p < 0.001$), skeletal muscle mass index ($p < 0.001$) and sarcopenic index ($p < 0.001$) in comparison with men with MG.

Plasma levels of adipokines

As shown in figure 2, MG patients presented lower levels of resistin ($p = 0.019$) and higher levels of leptin ($p = 0.000$) in comparison with controls. We did not find any difference between patients with MG and controls regarding the levels of adiponectin ($p = 0.278$). There

were no significant differences in adipokines levels between MG patients taking and not taking GCs, and between premenopausal and postmenopausal women with MG. Plasma levels of leptin ($p < 0.001$) and adiponectin ($p = 0.01$) were significantly higher in women with MG than in men with MG.

Associations between disease-related factors and DXA-derived body composition in MG patients

As expected among MG patients, there was a negative correlation between body lean mass and total body fat (rho = -0.253, p = 0.023) and with duration of the disease (rho = -0.241, p = 0.032). Higher total body fat (rho = 0.321, p = 0.005, Figure 3d) and lower body lean mass (rho = -0.297, p = 0.009) were associated with higher severity of MG symptoms, as assessed by the MG Composite scale. Furthermore, plasma levels of resistin was associated with muscle weakness, also assessed by MG composite scale (rho = 0.394, p = 0.000).

We found a positive correlation between android body adiposity and plasma levels of leptin (rho = 0.682, p = 0.000, Figure 3a). In addition, higher cumulative doses of GCs (rho = 0.325, p = 0.005) and lower body lean mass (rho = -0.281, 0.012) were associated with higher levels of adiponectin (Figures 3b and 3c, respectively). There was no significant association between cumulative doses of GCs and body lean mass or total body fat mass.

In the control group, we found a positive correlation between plasma levels of leptin and body fat percentage (rho = 0.567, $p < 0.001$), BMI (rho = 0.352, p = 0.005) and android body adiposity (rho = 0.516, $p < 0.001$). Plasma levels of adiponectin and resistin were negatively associated with body fat mass (rho = -0.458, $p < 0.001$; rho = -0.465, $p < 0.001$, respectively) and with body lean mass (rho = -0.521, $p < 0.001$; rho = -0.588, $p < 0.001$, respectively).

DISCUSSION

To the best of our knowledge, this is the first study of whole body composition with DXA in patients with MG. Herein, we evaluated body composition and plasma levels of adipokines in patients with MG and controls. Our results showed that the frequency of obesity is higher in MG patients than in controls, and patients with MG presented higher body fat mass, android body adiposity and total body fat than controls. Interestingly, higher total body fat and lower body lean mass were associated with increased severity of MG symptoms.

The observed changes in body composition may be due to long-term use of GCs by patients with MG. Although there are other serious side effects associated with the use of GCs, body weight gain, increased appetite and lipodystrophy also harm patients and can reduce treatment adherence [11, 29]. Furthermore, an increase in fat mass can lead to negative metabolic consequences such as insulin resistance, diabetes and hypertension [14].

Patients with MG presented higher levels of leptin in comparison with controls. Leptin was originally conceptualized as a satiety hormone primarily secreted by adipose tissue. However, leptin it is also produced by other cell types, and has actions, for instance, in the lungs. Oral GCs increase leptin levels, the same effect observed with increasing body weight [11]. Studies have reported an increase in leptin levels with both long- and short-term GCs use [21, 30, 31]. Furthermore, circulating levels of leptin are directly proportional to body fat mass [20]. As expected, higher plasma levels of leptin were negatively associated with greater android adiposity. In our study, increased leptin levels in patients with MG are possibly due to GC therapy combined with the observed body composition change.

We found that patients with MG presented lower plasma levels of resistin when compared with controls. Resistin is mainly expressed in macrophages and monocytes both within and outside adipose tissue. It acts as an inflammatory cytokine and is associated with insulin resistance and glucose tolerance impairment [32]. Previous studies reported increased levels of resistin in inflammatory conditions, such as ankylosing spondylitis, rheumatoid arthritis, inflammatory bowel disease [33-36]. Only one previous study investigated circulating levels of resistin in patients with MG [37]. Contrary to our results, higher resistin levels in patients with MG in comparison with controls were reported. The patients from this study had not been treated with cholinesterase inhibitors or immunosuppressive drugs in the six months

antedating resistin measurement [37]. It is possible that the use of GC by our patients resulted in decreased levels of resistin.

Adiponectin has been proposed to play important roles in the regulation of energy homeostasis, insulin sensitivity and food intake [38]. Although studies have reported an increase in leptin levels with both long- and short-term GCs treatments, the effects on adiponectin levels have been more controversial [21, 30, 31]. In the current study we did not find a significant difference in the plasma levels of adiponectin between controls and patients with MG. However, cumulative GCs dose was positively associated with adiponectin levels.

The current study has several limitations. We did not evaluate GCs-naïve patients. Since the study design was cross-sectional, it was not possible to make any cause-effect inference on the relationship between MG characteristics and body composition alterations. Prospective studies are warranted to determine causal relationships. Furthermore, we do not have any information regarding physical activity, nutritional aspects and/or dietary behaviors of the subjects enrolled in the study. By contrast, the strict diagnostic criteria, the selection of controls with comparable age, and gender, and the analysis of clinical, body composition using DXA and metabolic biomarkers (adipokines) together can be regarded as strengths of the study.

In sum, patients with MG present changes in body composition that are accompanied by changes in adipokines levels. The observed changes are possibly due, at least in part, to the long-term use of GCs. Since MG patients usually present high cumulative dose of GCs, and this treatment can increase body fat/adiposity, the estimation of body composition is important in planning long-term care of MG patients.

Conflicts of interest: Nayara Felicidade Tomaz Braz, Natalia Pessoa Rocha, Érica Leandro Marciano Vieira, Rodrigo Santiago Gomez, Adriana Maria Kakehasi and Antonio Lucio Teixeira declare that they have no conflict of interest.

Acknowledgements: This study was funded by Brazilian government funding agencies *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG), Brazil.

REFERENCES

[1] Farrugia ME, Vincent A. Autoimmune mediated neuromuscular junction defects. Curr Opin Neurol 2010;23:489-95.

[2] Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. Muscle Nerve 2008;37:141-49.

[3] Kumar V, Kaminski HJ. Treatment of Myasthenia Gravis. Curr Neurol Neurosci Rep 2011;11:89-96.

[4] Bedlack RS, Sanders DB. Steroid treatment for myasthenia gravis: steroids have an important role. Muscle Nerve 2002;25:117-21.

[5] Fardet L, Kassar A, Cabane J, Flahault A. Corticosteroid-induced adverse events in adults: frequency, screening and prevention. Drug Saf 2007;30:861-81.

[6] Schakman O, Gilson H, Thissen JP. Mechanisms of glucocorticoid-induced myopathy. J Endocrinol 2008;197:1-10.

[7] Braz NFT, Rocha NP, Vieira ELM, Gomez RS, Barbosa IG, Malheiro OB, Kakehasi AM, Teixeira AL. Negative impact of high cumulative glucocorticoids dose in bone metabolism of patients with myasthenia gravis. Neurol Sci 2017.

[8] Tataranni PA, Larson DE, Snitker S, Young JB, Flatt JP, Ravussin E. Effects of glucocorticoids on energy metabolism and food intake in humans. Am J Physiol 1996;271:317-25.

[9] Cheskin LJ, Bartlett SJ, Zayas R, Twilley CH, Allison DB, Contoreggi C. Prescription medications: a modifiable contributor to obesity. South Med J 1999;92:898-904.

[10] Arena C, Morin AS, Blanchon T, Hanslik T, Cabane J, Dupuy A, et al. Impact of glucocorticoid-induced adverse events on adherence in patients receiving long-term systemic glucocorticoids therapy. Br J Dermatol 2010;163:832-7.

[11] Berthon BS, MacDonald-Wicks LK, Wood LG. A systematic review of the effect of oral glucocorticoids on energy intake, appetite, and body weight in humans. Nutr Res 2014;34:179-90.

[12] Fardet L, Cabane J, Kettaneh A, Lebbé C, Flahault A. Corticosteroid-induced lipodystrophy is associated with features of the metabolic syndrome. Rheumatol 2007;46:1102-6.

[13] Lilleby V, Haugen M, Morkrid L, Frey Froslie K, Holven KB, Forre O. Body composition, lipid and lipoprotein levels in childhood-onset systemic lupus erythematosus. Scand J Rheumatol 2007;36:40-7.

[14] Dao HH, Do QT, Sakamoto J. Abnormal body composition phenotypes in Vietnamese women with early rheumatoid arthritis. Rheumatol 2011;50:1250-58.
[15] Popescu C, Bojinca V, Opriş D, Ionescul R. Dual X-ray absorptiometry whole body composition of adipose tissue in rheumatoid arthritis. Rom J Intern Med 2015;53:237-247.
[16] Vigouroux C, Caron-Debarle M, Le Doura C, Magréa J, Capeau J. Molecular mechanisms of human lipodystrophies: From adipocyte lipid droplet to oxidative stress and lipotoxicity. Int J Biochem Cell Biol 2011;43:862-76.

[17] Vigouroux C, Maachi M, Nguyen TH, Coussieu C, Gharakhanian S, Funahashi T, et al. *Serum adipocytokines* are related to lipodystrophy and metabolic disorders in HIV-infected men under antiretroviral therapy. AIDS *2003*;17:1503-11.

[18] Rieth N, Jollin L, Le Panse, B, Lecoq AM, Arlettaz A, De Ceaurriz J, et al. Effects of short term corticoid injection on food intake and adipokines in healthy recreationally trained men. Eur J App Physiol 2009;105:309-13.
[19] Fardet L, Antuna-Puente B, Vatier C, Cervera P, Touati A, Simon T, Capeau J, Fe`ve B, Bastard JP. Adipokine profile in glucocorticoid-treated patients: baseline plasma leptin level predicts occurrence of lipodystrophy. Clin Endocrinol 2013;78:43-51.

[20] Fasshauer M, Blüher M. Adipokines in Health and Disease. Trends Pharmacol Sci 2015;36:461-70.

[21] Jollin L, Rieth N, Thomasson R, Amiot V, Lasne F, Collomp K. Changes in adipokines but not in body composition after one week of prednisone intake in physically fit women. Endocr 2013;43:444-46.

[22] Jaretzki A, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JG, Penn AS. Myasthenia gravis. Recommendations for clinical research Standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. Neurology 2000;55:1-14.

[23] Burns TM, Conaway M, Sanders DB. The MG Composite. A valid and reliable outcome measure for myasthenia gravis. Neurology 2010;74:1434-40.
[24] Braz NFT, Rocha NP, Vieira ELM, Gomez RS, Barbosa IG, Malheiro OB, Kakehasi AM, Teixeira AL. Negative impact of high cumulative glucocorticoid dose on bone metabolism of patients with myasthenia gravis. Neurol Sci 2017.

[25] Bray GA. Contemporary diagnosis and management of obesity and the metabolic syndrome, 3rd edn. Handbooks in health care: Newton, PA, 2003.

[26] Albanese CV, E Diessel, HK Genant. Clinical applications of body composition measurements using DXA. J Clin Densitom 2003;6:75-85.

[27] Kendler DL, Borges JLC, Fielding RA, Itabashi A, Krueger D, Mulligan K, Camargos BM, Sabowitz B, Wu CH, et al. The Official Positions of the International Society for Clinical Densitometry: Indications of Use and Reporting of DXA for Body Composition. J Clin Densitom 2013;4:496-507.

[28] Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ, Lindeman RD. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol 1998;147:755-63.

[29] Mok CC, To CH, Ma KM. Changes in body composition after glucocorticoid therapy in patients with systemic lupus erythematosus. Lupus 2008;17:1018-22.

[30] Uddén J, Bjorntorp P, Arner P, Barkeling B, Meurling L, Rossner S. Effects of glucocorticoids on leptin levels and eating behaviour in women. J Intern Med 2003;253:225-31.

[31] Cimmino MA, Andraghetti G, Briatore L, Salani B, Parodi M, Cutolo M, et al. Changes in adiponectin and leptin concentrations during glucocorticoid treatment: a pilot study in patients with polymyalgia rheumatica. Ann N Y Acad Sci 2010;1193:160-3.

[32] Filková M, Haluzík M, Gay S, Senolt L. The role of resistin as a regulator of inflammation: Implications for various human pathologies. Clin Immunol 2009;133:157-70.

[33] Konrad A, Lehrke M, Schachinger V, Seibold F, Stark R, Ochsenkühn T, et al. Resistin is an inflammatory marker of inflammatory bowel disease in humans. Eur J Gastroenterol Hepatol 2007;19:1070-74.

[34] Senolt L, Housa D, Vernerová Z, Jirásek T, Svobodová R, Veigl D, et al. Resistin in rheumatoid arthritis synovial tissue, synovial fluid and serum. Ann Rheum Dis 2007;66:458-63.

[35] Kocabas H, Kocabas V, Buyukbas S, Melıkoglu MA, Sezer I, Butun B. The serum levels of resistin in ankylosing spondylitis patients: a pilot study. Rheumatol Int 2012;32:699-02.

[36] Demirci S, Aynal A, Demirci K, Demirci S, Arıdoğan BC. The Serum Levels of Resistin and Its Relationship with Other Proinflammatory Cytokines in Patients with Alzheimer's Disease. Clin Psychopharmacol Neurosci 2017;15:59-63.

[37] Zhang DQ, Wang R, Li T, Li X, Qi Y, Wang J, Yang L. Remarkably increased resistin levels in anti-AChR antibody-positive myasthenia gravis. J Neuroimmunol 2015;283:7-10.

[38] Mohiti-Ardekani J, Soleymani-Salehabadi H, Owlia MB, Mohiti A. Relationships between serum adipocyte hormones (adiponectin, leptin, resistin), bone mineral density and bone metabolic markers in osteoporosis patients. J Bone Miner Metab 2014;32:400-4.

TABLES

Table 1. Demographic and clinical features of myasthenia gravis patients.

	MG patients	**Control group**
Gender (female/male)	60/20	39/23
Age in years (mean ± SD)	41.89 ± 14.171 (18 - 69)	40.42 ± 13.62
Length of illness in years [mean ± SD (range)]	13.53 ± 10.05 (1 - 39)	-
MG Composite [mean ± SD (range)]	4.96 ± 5.02 (1 - 18)	-
Medications for MG (current use)		
Pyridostigmine [n (%) / mean daily dose]	70 (87.5%) / 236.71 mg	-
Prednisone [n (%) / mean daily dose]	58 (72.5%) / 14.5 mg	-
Azathioprine [n (%) / mean daily dose]	33 (41.3%) / 120 mg	-
Other immunosuppressants [n (%)]	7 (8.8%)	-
Cumulative glucocorticoid dose in mg [mean ± SD (range)]	38123.48 ± 41895.66 (60.0 - 232935.0)	-
MGFA		
I [n (%)]	10 (12.5%)	-
IIA [n (%)]	9 (11.3%)	-
IIB [n (%)]	7 (8.8%)	-
IIIA [n (%)]	15 (18.8%)	-
IIIB [n (%)]	4 (5.0%)	-
IVA [n (%)]	6 (7.5%)	-
IVB [n (%)]	5 (6.3%)	-
V [n (%)]	22 (27.5%)	-
Sedentary lifestyle [n (%)]	62 (77.5%)	-
Waist-to-hip ratio	0.84 ± 0.26	-

Abbreviations: MG= myasthenia gravis; MGFA= Myasthenia Gravis Foundation of America Clinical Classification; SD= standard deviation.

Table 2. Anthropometric and DXA-derived body composition measures in myasthenia gravis patients and control subjects.

	MG (*n* = 80)	**CG (*n* = 62)**	***p* value**
Height (m)	1.62 ± 0.83 (1.47 – 1.93)	1.64 ± 0.92 (1.49 – 1.90)	0.060 [a]
Weight (Kg)	72.93 ± 16.65 (40 – 135)	68.13 ± 11.31 (46 – 90)	0.127 [a]
BMI in kg/m^2 (mean ± SD)	27.17 ± 6.18 (16.87 – 49.84)	25,48 ± 5.42 (17.64 - 44.20)	0.092 [a]
Body Fat mass (Kg)	28.91 ± 9.96 (8.49 – 65.24)	16.93 ± 7.35 (5.14 – 40.33)	**0.000** [a]
Fat mass distribution (%)	1.32 ± 3.04 (0.61 – 28.20)	0.99 ± 0.22 (0.61 – 1.55)	0.389 [a]
Android body adiposity (%)	42.61 ± 9.37 (15.7 – 58.5)	38.34 ± 6.93 (20.1 – 56.1)	**0.003** [a]
Body fat percentage (%)	39.62 ± 8.02 (17.1 – 58.6)	35.68 ± 6.73 (18.5 – 50.1)	**0.001** [a]
Normal weight (%)	10 (12.5)	8 (12.9)	0.943 [b]
Overweight *n* (%)	16 (20)	22 (35.5)	**0.039** [b]
Obesity *n* (%)	54 (67.5)	32 (51.6)	0.055 [b]
Body lean mass (kg)	41.08 ± 10.03 (20.04 – 83.60)	26.86 ± 8.00 (0.48 – 42.65)	**0.000** [a]
ASM (Kg)	17.84 ± 4.88 (7.30 – 36.98)	18.84 ± 5.23 (11.10 – 36.99)	0.306[c]
Skeletal muscle mass index (kg/m^2)	6.58	6.84	0.488 [c]
Sarcopenic index (kg/m^2)	7.08 ± 1.36 (3.53 – 10.40)	7.28 ± 1.43 (4.81 – 11.53)	0.400 [a]
Sarcopenia *n* (%)	15 (18.8)	17 (27.4)	0.220 [b]

Abbreviations: DXA= dual-energy X-ray absorptiometry; MG= myasthenia gravis group; CG= control group; SD= standard deviation; BMI= body mass index; ASM= appendicular skeletal muscle mass.

[a] *t*-student test; [b] Chi-Square Test; [c] Mann-Whitney Test. **Bold** type indicates significant p values.

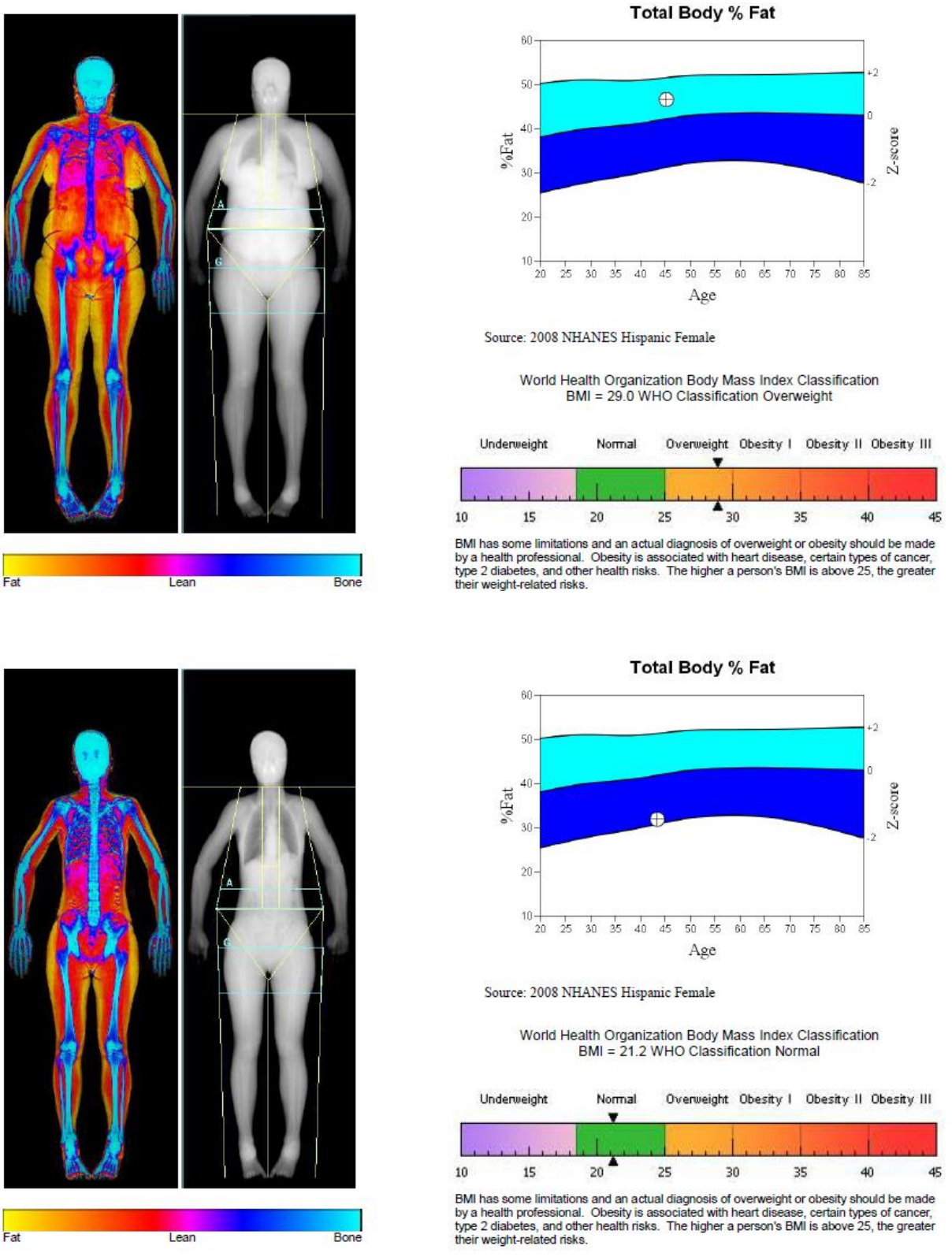

Figure 1. Whole body dual-energy X-ray scan images acquired from a woman with myasthenia gravis (1) and a control (2). The MG patient was 45 years-old, overweight (BMI = 29.0), with 46.6% body fat percentage. She has 11 years of MG duration, was classified in class V, with score 6 in MG composite and had cumulative GCs dose of 47.700 mg. The

control woman was 43 years-old and presented normal weight (BMI = = 21.2), and 31.9% body fat percentage. Red color indicates area that is predominantly fat-free mass (lean soft tissue and bone) and yellow color indicates area that is predominantly adipose tissue.

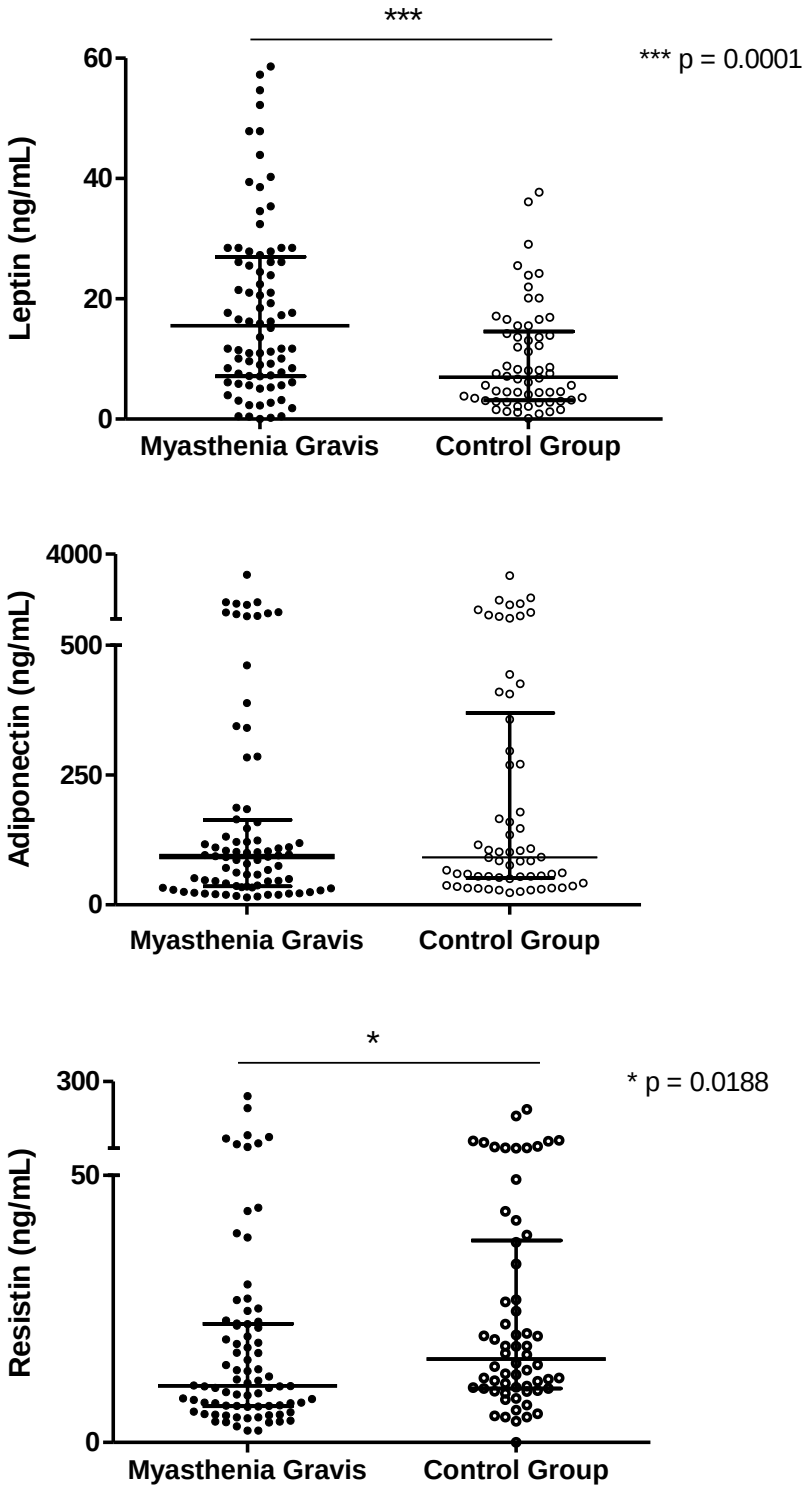

Figure 2- Comparison between plasma concentrations of adipokines in patients with myasthenia gravis and the control group.

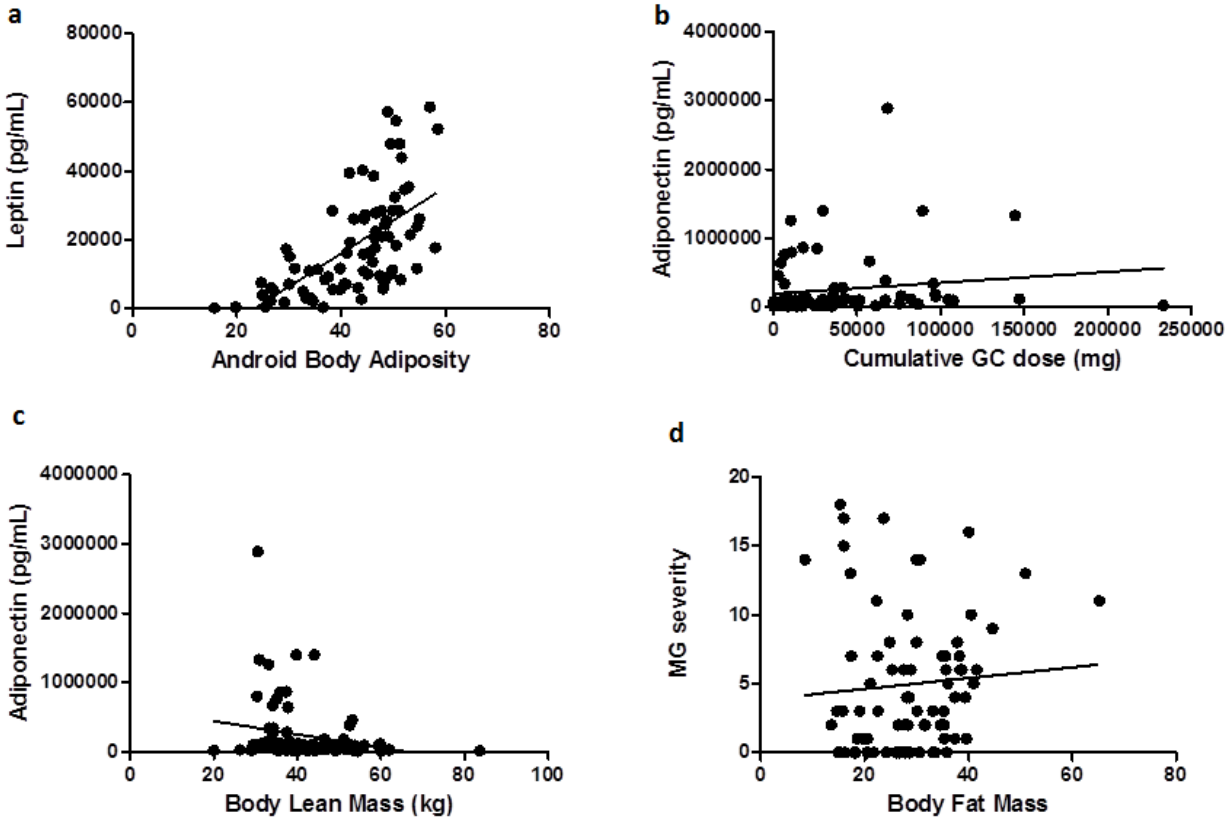

Figure 3. Correlations between plasma levels of leptin and android body adiposity (a); adiponectin levels and GC cumulative doses (b); adiponectin levels and body lean mass (c); and MG severity, as assessed by MG composite scale, and body fat mass (d).

ARTIGO III

Serum levels of interleukin-6 are related to muscle strength in Myasthenia Gravis patients

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Financial disclosure: This study was funded by Brazilian government funding agencies *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG), Brazil.

Conflicts of interest: Nayara Felicidade Tomaz Braz, Salvina Maria de Campos, Érica Leandro Marciano Vieira, Izabela Guimarães Barboza, Natália Pessoa Rocha, Rodrigo Santiago Gomez, Adriana Maria Kakehasi and Antonio Lucio Teixeira declare that they have no conflict of interest.

ABSTRACT

Objectives: We aimed to measure the plasma levels of cytokines in patients with MG and controls, and to investigate potential associations between them and clinical parameters. **Methods:** We evaluated 80 medicated patients and 50 healthy controls. IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ and IL-17 were measured by cytometric bead array. **Results:** The plasma levels of all measured cytokines were reduced in patients with MG. Patients under current glucocorticoids (GC) presented higher levels of IL-10 than who was past GC. There was an association between IL-6 and muscle strength of MG patients. **Conclusion:** Immunomodulatory treatment significantly affected the levels of circulating cytokines in patients with MG. IL-6 levels are associated with muscle strength in patients with MG.

Keywords: myasthenia gravis, immune system, cytokines, IL-6, muscle strength.

INTRODUCTION

Myasthenia gravis (MG) is a chronic autoimmune disease caused by antibodies directed to the postsynaptic membrane at the neuromuscular junction. Three main antigenic targets for disease-inducing antibodies have been described in MG: acetylcholine receptor (AChR), muscle specific tyrosine kinase (MuSK) and lipoprotein receptor-related protein 4 (LRP4). In approximately 80% of all cases, MG is caused by pathogenic auto-antibodies against the AChR (Hoch et al., 2001; Bates & Stassen, 2002; Higuchi et al., 2011).

The origin of the autoimmune dysfunction in MG patients is unknown, but thymic abnormalities, defects in immune regulation and sex hormones play major roles in patients with anti-AChR antibodies (Meriggioli, 2009; Akinin et al., 2013). The thymus is essential for T-cell differentiation and for the establishment of central tolerance. As autoimmune sensitization against the AChR likely develops in the thymus, inflammatory cytokines may play a crucial role in MG pathogenesis (Berrih-Aknin & Le Panse, 2014). It had been reported that MG thymus has higher production of TNF-α and IL-6 than normal tissue (Cohen-Kaminsky et al., 1993).

Despite these facts, only a few studies have evaluated the circulating levels of cytokines in MG patients (Uzawa et al., 2014; Xie et al., 2016; Molin et al., 2017). In the current study, we aimed at measuring the plasma levels of cytokines in patients with MG and controls. In addition, we investigated whether the levels of cytokines were associated with clinical parameters.

METHODS

Subjects

This study included 80 patients with MG followed at the Neurology Outpatient Clinic, University Hospital, *Universidade Federal de Minas Gerais* (UFMG), Brazil. Patients were diagnosed based on the Myasthenia Gravis Foundation of America (MGFA) criteria (Jaretzki 2000). MG diagnosis was based on typical clinical symptoms along with improvement of symptoms with acetylcholinesterase inhibitors, decremental muscle response to a train of low frequency repetitive nerve stimuli, or the presence of autoantibody against AChR. These patients were clinically stable and without modification of the treatment regimen for at least one month. Exclusion criteria included pregnancy, and presence of cancer.

This study also recruited a control group comprising age- and gender-matched subjects recruited from the community. Exclusion criteria for controls included pregnancy, presence of any chronic inflammatory diseases, cancer, and current use of systemic glucocorticoids, insulin, statins, growth hormone, anabolic agent or hormone replacement therapy.

All subjects provided written informed consent before admission to the study. The Research Ethics Committee of UFMG approved this study (Protocol number: CAAE-19045413.0.0000.5149).

Clinical assessment

Myasthenia Gravis Composite (MG Composite) scale was used to evaluate the degree of muscle weakness fluctuation and the severity of typical symptoms of MG (Burns 2010; Mourão 2015). This scale comprises 10 items evaluating ocular (3 items), bulbar (3 items), respiratory (1 item), neck (1 item), and limb (2 items) signs and symptoms (Burns 2010; Mourão 2015). Participants were evaluated by a single previously trained researcher.

The cumulative dose of prednisone, *i.e.* a total dose of prednisone used by the patient throughout the course of his disease, was calculated based on medical record data (Braz et al., 2017; Braz et al., 2017).

Cytokine Assessment

Fasting blood samples were drawn by venipuncture in vacuum tubes containing heparin in the morning of the clinical assessment. Blood was immediately centrifuged at 3,000 rpm for 10 min, 4°C. Plasma was collected and stored at -70°C until assayed.

Multiple cytokines [interleukin (IL)-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF), interferon (IFN)-γ, and IL-17] were simultaneously measured by flow cytometry using the Cytometric Bead Array (CBA) Human Th1/Th2/Th17 Cytokine Kit (BD Biosciences, San Jose, CA, USA). Acquisition was performed using a FACSCanto II flow cytometer (BD Biosciences, San Jose, CA, USA). The instrument was checked for sensitivity and overall performance with Cytometer Setup and Tracking beads (BD Biosciences) prior to data acquisition. Quantitative results were generated using FCAP Array v1.0.1 software (Soft low Inc., Pecs, Hungary).

Statistical analysis

All variables were tested for Gaussian distribution by the Kolmogorov-Smirnov normality test. Two groups (patients vs. controls) were compared by Mann–Whitney or Student's t tests when non-normally or normally distributed, respectively. Spearman's correlation analyses were performed to examine the relationship between clinical variables and plasma level of cytokines. All statistical tests were two-tailed and were performed using a significance level of α=0.05. Data were analyzed using the Statistical Package for the Social Sciences® version 20.0 (SPSS; Chicago, IL, USA) and GraphPad Prism® version 5.0 (GraphPad Software, La Jolla, CA, USA).

RESULTS

Demographic, clinical and biochemical features of patients with MG and controls are shown in table 1. The patients had a mean age of 29.10 years at disease onset. Sixty-four (80%) patients were classified as early onset MG subtype (< 40 years of age). Sixty percent of the sample was positive for anti-AChR antibody detection. Thirty-one patients underwent thymectomy, and four showed thymic hyperplasia and two had thymoma. The co-occurrence of other immune-related diseases (Grave's disease, chronic autoimmune hepatitis) was reported in four patients.

Regarding current treatment, 87.5% of the patients were taking symptomatic medication (pyridostigmine) and 72.5% were in use of prednisone. The remaining MG patients (27.5%) have already used GC during the course of the disease. There was no significant difference in cumulative dose of prednisone between the groups in current and past use of prednisone.

As shown in table 2, MG patients presented lower levels of IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL-17 in comparison with controls.

In subgroup analyses, patients under current GC therapy (n = 58; 1.96 pg/ml ± 1.82) had higher levels of IL-10 in comparison with patients not taking GC (n = 22; 1.40 pg/ml ± 0.23) (p = 0.04). Thymectomy did not influence the levels of cytokines. There were no differences in the plasma levels of the cytokines between the seropositive and seronegative patients.

We also investigated the potential association between clinical parameters (i.e. MG composite, disease duration, cumulative GC dose, and current GC dose) and cytokine levels. There was a negative correlation between IL-6 and MG composite score (rho = -0.233, p = 0.04).

DISCUSSION

In the current study, the plasma levels of IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL-17 were reduced in treated patients with MG. Uzawa et al., (2014) investigated the serum levels of 27 cytokines/chemokines in 47 seropositive and untreated MG patients and observed that IL-4 was lower in MG patients than in the control group and that IL-15 and vascular endothelial growth factor (VEGF) was higher in the MG group. There were no significant differences in the plasma concentration of other molecules: IL-1ra; IL-2; IL-5; IL-6; IL-7; IL-8; IL-9; IL-10; IL-12; IL-13; IL-17; CCL11; FGFB; G-CSF; GM-CSF; IFN-γ; CXCL10; CCL3; CCL4; CCL5; TNFα; IL-1β; and PDGF (Uzawa, 2014). Xie et al., (2015) compared the plasma level of IL-17 in untreated MG patients and healthy controls and observed that early-onset women without thymoma and who had not received immunotherapy presented higher levels of IL-17 than healthy controls (Xie 2015).

Patients under current GC presented higher levels of IL-10. Previous studies have shown that IL-10 can be up-regulated by GC (Visser 1998; Visser 2003), synergistically work at T-cell activation (Almawi 2002). Interestingly, Yilmaz et al (2014), evaluated B cells from patients with MG under immunosuppressive treatment and found that B cell derived IL-6, IL-10 and TNF-α are down-regulated in MG (Yilmaz et al (2014).

We then, investigated the association between the plasma levels of cytokines and we found a negative correlation between IL-6 and MG composite, that is, the higher the IL-6 levels, the lower the severity of MG symptoms, indicating lower muscle weakness. IL-6 is a myosin associated with hypertrophic muscle growth and myogenesis through regulation of the proliferative capacity of muscle stem cells (Serrano et al., 2008; Munõz-Cánoves et al., 2013). Altogether, IL-6 may play a protective role on the muscle symptoms in MG patients and could be considered a staging biomarker.

The current study has several limitations. Since the study design was cross-sectional, it was not possible to make any cause-effect inference on the relationship between characteristics of the illness and the plasma levels of cytokines. Furthermore, we did not evaluated GC-naïve patients. By contrast, the strict diagnosis criteria, the subtypes of the disease and the evaluation of biomarkers can be regarded as strengths of the study.

Immunomodulatory treatment significantly affect the levels of circulating cytokines in patients with MG. IL-6 levels are associated with muscle strength in patients with MG.

REFERENCES

Hoch, W., Mc Conville, J., Helms, S. et al. 2001. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. Nat. Med. 7:365-368.

Bates, M.D., Stassen, M.H.W. 2002. The role of antibodies in myasthenia gravis. J. Neurol. Sci. 202:5-11.

Higuchi, O., Hamuro, J., Motomura, M. et al. 2011. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. Ann. Neurol. 69:418-422.

Meriggioli, M.N. 2009. Myasthenia Gravis: Immunopathogenesis, Diagnosis, and Management. Continuum. Lifelong. Learning. Neurol. 15(1):35–62.

Berrih-Aknin, S., Rozen, Le Panse. 2014. Myasthenia gravis: A comprehensive review of immune dysregulation and etiological mechanisms. J. Autoimm. 52:90-100.

Berrih-Aknin, S. 2014. Myasthenia Gravis: Paradox versus paradigm in autoimmunity. J. Autoimm. 52:1-28.

Cohen-Kaminsky, S., Devergne, O., Delattre, R.M., Klingel-Schmitt, I., Emilie, D., Galanaud, P., et al. 1993. Interleukin-6 overproduction by cultured thymic epithelial cells from patients with myasthenia gravis is potentially involved in thymic hyperplasia. Eur. Cytokine. Netw. 4:121-32.

Uzawa, A., Kawaguchi, N., Himuro, K., Kanai, T., Kuwabara, S. 2014. Serum cytokine and chemokine profiles in patients with myasthenia gravis. Clin. Experim. Immun. 176:232-237.

Xie, Y., Li, H., Jiang, B., Li, T., Kaminski, H.J., Kusner, L.L. 2016. Elevated plasma interleukin-17A in a subgroup of Myasthenia Gravis Patients. Cytokine. 78:44–46.

Molin, C.J., Westerberg, E., Punga, A.R. 2017. Profile of upregulated inflammatory proteins in sera of Myasthenia Gravis patients. Scientific Reports. 7:397:1-9.

Jaretzki, A., Barohn, R.J., Ernstoff, R.M., Kaminski, H.J., Keesey, J.G., Penn, A.S. 2000. Myasthenia gravis. Recommendations for clinical research Standards. Task Force of the

Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. Neurology. 55:1-14.

Burns, T.M., Conaway, M., Sanders, D.B. 2010.The MG Composite. A valid and reliable outcome measure for myasthenia gravis. Neurology. 74:1434-40.

Mourão, A.M., Araújo, C.M., Barbosa, L.S.M., Gomez, R.S., Burns, T.M., Lemos, S.M.A., Teixeira, A.L. 2013. Brazilian cross-cultural translation and adaptation of the "Questionnaire of Life Quality Specific for Myasthenia Gravis - 15 items". Arq. Neuropsiquiatr. 71(12):955-958.

Braz, N.F.T., Rocha, N.P., Vieira, E.L.M., Gomez, R.S., Barbosa, I.G., Malheiro, O.B., Kakehasi, A.M., Teixeira, A.L. 2017. Negative impact of high cumulative Glucocorticoids dose in bone metabolism of patients with myasthenia gravis. Neurological. Sciences. 38(8):1405-1413.

Braz, N.F.T., Rocha, N.P., Vieira, E.L.M., Gomez, R.S., Kakehasi, A.M., Teixeira, A.L. 2017. Body composition and adipokines plasma levels in patients with myasthenia gravis treated with high cumulative glucocorticoid dose. J. Neurol. Sci.15:169-175.

Visser, J.T. 2003. Sparing interleukin-10 receptor expression by glucocorticoids is a prerequisite for the synergistic immunosuppressive effects of interleukin-10 and glucocorticoids. Transplantation. 27;76:1534-5.

Visser, J., van Boxel-Dezaire, A., Methorst, D., et al. 1998. Differential regulation of interleukin-10 (IL-10) and IL-12 by glucocorticoids in vitro. Blood; 91:4255-4264.

Almawi, W.Y., Melemedjian, O.K. 2002. Negative regulation of nuclear factor-kB activation and function by glucocorticoids. J. Mol. Endocrinol. 28:69-78.

Yilmaz, V., Oflazer, P., Aysal, F., Parman, Y.G., Direskeneli, H., Deymeer, F., Saruhan-Direskeneli, G. 2015. B cells produce less IL-10, IL-6 and TNF-a in myasthenia gravis. Autoimmunity. 48(4):201-7.

Serrano AL, Baeza-Raja B, Perdiguero E, Jardí M, Muñoz-Cánoves P. 2008. Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. Cell Metab. 7(1):33-44.

Muñoz-Cánoves P, Scheele C, Pedersen BK, Serrano AL. 2013. Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword? FEBS J. 280(17):4131-48.

Table 1. Clinical and demographic features of patients with myasthenia gravis (MG) and controls.

Characteristics	**MG (*n* = 80)**	**CG (*n* = 50)**
Gender		
Female	60	30
Male	20	20
Age (years ± SD)	41.89[#] (±14.171)	42.94 ± 13.76
Education (years ± SD)	9.288[#] (±3.728)	-
Age at onset (years ± SD)	29.10[#] (±13.483)	-
Length of disease (years ± SD)	13.53[#] (±10.053)	-
Cumulative glucocorticoid dose (mg)	38123.477[#] (±41895.659)	-
Age at thymectomy (years ± SD)	32.50 (±11.829)	-
Age at crisis (years ± SD)	35.20 (±15.828)	-
Mean MG composite	4.96[#] (±5.021)	-
MGFA		
I	10[*]	-
IIA	9[*]	-
IIB	7[*]	-
IIIA	15[*]	-
IIIB	4[*]	-
IVA	6[*]	-
IVB	5[*]	-
V	22[*]	-

CG: control group; MGFA: Myasthenia Gravis Foundation of America Clinical Classification; [#]value average. [*]absolute value; SD: standard deviation.

Table 2. Comparison between plasma concentrations of biomarkers in patients with myasthenia gravis (MG) and the control group (CG).

Biomarkers (pg/mL)	**MG (*n* = 80)** Mean ± SD	**CG (*n* = 50)** Mean ± SD	***p* value**
IL-2	2.44 ± 0.38	24.41 ± 23.32	**<0.001**[a]
IL-4	2.12 ± 0.25	29.95 ± 110.05	**<0.001**[a]
IL-6	3.70 ± 7.97	9.78 ± 10.03	**<0.001**[a]
IL-10	1.81 ± 1.59	5.07 ± 3.28	**<0.001**[a]
IL-17	12.25 ± 9.43	558.79 ± 262.27	**<0.001**[a]
IFN-γ	1.20 ± 0.26	19.52 ± 24.65	**<0.001**[a]
TNF-α	1.39 ± 0.33	16.02 ± 52.05	**<0.001**[a]

Abbreviations: MG= myasthenia gravis group; CG= control group; SD= standard deviation. [a]Mann-Whitney Test; [b]Qui-square test. **Bold** type indicates significant p values.

ARTIGO IV

Journal of Clinical Neuroscience 50 (2018) 41–44

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Clinical commentary

Muscle strength and psychiatric symptoms influence health-related quality of life in patients with myasthenia gravis

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Abstract

Myasthenia gravis (MG) is a neuromuscular autoimmune disease characterized by skeletal muscle weakness which can impact motor function and, furthermore, produce negative impact on the health-related quality of life (HRQOL). **Objective:** To evaluate the predictors for HRQOL in patients with MG. **Methods:** Eighty patients were evaluated with the MG Foundation of America classification and the MG Composite scale. HRQOL was estimated by the MGQOL15, while anxious and depressive symptoms were evaluated with the Hospital Anxiety and Depression Scale (HAD). **Results:** The mean age of patients was 41.9 years with mean illness duration of 13.5 years. Almost half of the patients (43.75%) had significant anxiety and more than a quarter (27.50%) had depressive symptoms. Factors that influenced the HRQOL in MG were skeletal muscle weakness and anxiety and depressive symptoms ($p < 0.001$ in logistic regression model). **Conclusion:** Anxiety and depressive symptoms, besides motor symptoms, influence HRQOL in MG. Mental health must be a clinical focus in addition to the treatment of somatic symptoms during the course of MG.

Keywords: myasthenia gravis; health-related quality of life; anxiety; depression; muscle weakness.

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease caused by antibodies directed to the postsynaptic membrane at the neuromuscular junction. In approximately 80% of all cases, MG is associated with antibodies against AChR [1].

Symptoms of MG range from only ocular symptoms such as diplopia and ptosis (ocular MG) to more generalized symptoms, including dysarthria, dysphagia, chewing and breathing difficulty, and limb weakness (generalized MG). Such symptoms influence social functioning of the patients, impacting negatively on their health-related quality of life (HRQOL) [2-5]. Other factors may influence HRQOL in MG such as the number and severity of myasthenic crisis and drug treatment [6]. Furthermore, psychiatric symptoms such as anxiety and depression have been associated with poor self-reported HRQOL [6-8].

There are only few studies that investigated the determinants of HRQOL in Brazilian patients with MG [6, 7]. Hence, the objective of this study was to determine the factors associated with HRQOL in a Brazilian sample of subjects with MG.

METHODS

Subjects

This study comprised 80 patients with MG followed at the Neuromuscular Disease Outpatient Clinic, University Hospital, Federal University of Minas Gerais, Belo Horizonte, Brazil. Patients were diagnosed based on the Myasthenia Gravis Foundation of America (MGFA) criteria [9]. Exclusion criteria included pregnancy, and presence of cancer. This study was approved by the Research Ethics Committee of the Federal University of Minas Gerais, Brazil (Protocol number: CAAE-19045413.0.0000.5149). All subjects provided written informed consent.

Clinical assessment

Clinical status and severity of MG were determined following the recommendations of the MGFA [9]. Skeletal muscle strength was evaluated with the Myasthenia Gravis Composite (MG Composite) scale that measures the degree of muscle weakness and comprises 10 items evaluating ocular (3 items), bulbar (3 items), respiratory (1 item), neck (1 item), and limb (2 items) signs and symptoms [10]. Participants were evaluated by a single previously trained researcher who collected socio demographic, clinical and anthropometric dates.

A total dose of glucocorticoids used by the patient throughout the course of his disease, so called, cumulative dose, was calculated. All medications taken by the patient were recorded. The HRQOL was assessed with the Brazilian version of the Questionnaire of Life Quality Specific for Myasthenia Gravis - 15 Items (MGQOL15) [2,6]. Each item is scored from zero to four according to its frequency, scoring a maximum of 60, with the higher the score, the worse the perceived HRQOL.

Symptoms of anxiety and depression were evaluated with the Hospital Anxiety and Depression Scale (HAD). The HAD comprises 14 items, seven to assess anxiety and seven depression [11]. The authors recommend as cutoff for both subscales ≥ 9, being less than nine classified as without clinically significant symptoms, between 9 and 10 mild form of anxiety / depression, 11 and 14 moderate form and, more than 14 severe form [12].

Statistical analysis

The Kolmogorov-Smirnov test was used to analyze the normality of the data. The patients with anxiety and depressive symptoms were compared by Mann–Whitney or Student's t tests when non-normally or normally distributed, respectively. Association between dichotomous variables was assessed with the chi-square test. Spearman's correlation analyses were performed to examine the relationship between clinical variables and quality of life.

We performed linear regression analyses testing possible associations between HRQOL in patients with MG and selected parameters: age, gender, years of education, age at onset, MG composite, number of myasthenic crises, cumulative glucocorticoids dose and total HAD scores. Variables with a univariate correlation with a p-value < 0.20 were tested in the final model.

All statistical tests were two-tailed and were performed using a significance level of α=0.05. Data were analyzed using the Statistical Package for the Social Sciences® version 20.0 (SPSS; Chicago, IL, USA) and GraphPad Prism® version 5.0 (GraphPad Software, La Jolla, CA, USA).

RESULTS

Clinical and demographic features of MG patients

Eighty patients with MG were evaluated (60 women and 20 men). Sociodemographic and clinical features are shown in table 1. The patients had a mean age of 29.10 years at disease onset. Sixty-four (80%) patients were classified as early onset MG subtype (< 40 years of age) and were more frequently female (52 females and 12 males), whereas patients with late onset MG (> 40 years of age) were 8 females and 8 males. The most common initial symptoms were hip flexor muscle weakness, diplopia and ptosis. The frequency of all items composing the MG Composite is described in table 2.

Over the course of the disease, 53 (66.3%) patients had at least one myasthenic crisis (45 patients had only one crisis, 3 had two crisis, 2 had three crisis and 3 had four crisis) with a mean age of 35 years (± 15.50). Comorbidities were present: obesity (67.5%), hypertension (33.8%), hypercholesterolemia (27.5%), osteoporosis (14%), type 2 diabetes mellitus (13.8%), hyperthyroidism (11.3%), cataract (11.3%), glaucoma (7.5%). Four patients reported the co-occurrence of other immune-related diseases (gout, Grave's disease, fibromyalgia, chronic autoimmune hepatitis).

Regarding current treatment, 87.5% of the patients were taking symptomatic medication (pyridostigmine) and 72.5% were in use of prednisone. The remaining MG patients (27.5%) have already used corticosteroids during the course of the disease. There was no significant difference in cumulative dose of prednisone between the groups in current and past use of prednisone.

According to the HAD scale, 35 (43.75%) patients had clinically meaningful anxiety symptoms (13 with mild, 15 moderate and seven with severe anxiety) and 22 (27.50%) had clinically meaningful depression (eight with mild, seven moderate and seven with severe depressive symptoms).

Patients with MG had a mean total score of 16 (±12.86) in MGQOL15. Patients with anxiety and depressive symptoms presented higher scores in MGQOL15 than patients without these symptoms, which means that they have worse healthy-related quality of life ($p = 0.04$ and $p = 0.02$, respectively).

There was a positive correlation between MGQOL15 and MG composite (rho = 0.45; p = 0.00), total HAD score (rho = 0.48; p = 0.00), HAD anxiety subscale score (rho = 0.36; p = 0.00) and HAD depression subscale score (rho = 0.51; p = 0.00).

We analyzed the effects of a priori selected confounders (age, gender, years of education, age at onset, MG composite, number of myasthenic crises, cumulative glucocorticoids dose and total HAD scores) on HRQOL scores. Only total HAD and MG composite scores independently contributed to quality of life at patients with MG (see table 3).

DISCUSSION

Health-related quality of life is defined as “individuals’ perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns” being used to quantify the degree to which a disease influences on self-reported perception of health [13,14]. HRQOL can complete the clinical evaluation and may provide valuable data on physical, mental and social functioning for an accurate decision about patient management [5].

Several studies have evaluated HRQOL in patients with MG using the Medical Outcome Survey 36-Item Short-Form Health Survey (SF-36), however, the results were discordant [15-18]. For instance, Paul et al. observed that quality of life and well-being of patients with MG did not differ markedly from the general population [16]. Conversely, Boldingh et al. reported that patients with MG had lower scores in the SF-36 than healthy controls [4].

In the current study, we investigated HRQOL using a disease-specific questionnaire previously validated for Brazilian patients [6]. MGQoL15 has items relevant to the illness, such as items dedicated to vision, speaking, chewing and swallowing. We observed that HRQOL is positively correlated with muscle weakness as assessed by the MG Composite. In addition to muscle strength, anxiety and depressive symptoms were associated with HRQOL similarly to previous studies. In the study of Twork et al. (2010), for instance, 1.518 patients with MG answered to a mail questionnaire that included the SF-36 questions. HRQOL was mainly affected by impaired mobility and depression [19]. Mourão et al. (2016) evaluated HRQOL in 69 patients with MGQoL15 and reported that determinants of HRQOL were muscle strength, current prednisone dose and the levels of anxiety and depression [6].

There are restricted data on the frequency of psychiatric disorders in MG patients [6-8, 20]. The frequency of anxiety symptoms was remarkably high (43.75%) in our sample, and more than a quarter of patients (27.50%) had depressive symptoms. These results are also in line with previous studies on psychiatric symptoms in MG [7, 21, 22]. Psychiatric disorders in MG are partly due to disease characteristics: incapacitating, life-threatening, chronic and unpredictable [23]. Nevertheless, other factors, including treatment, may play a role in the occurrence of psychiatric symptoms in MG.

Depressive symptoms have been associated with current dose of oral glucocorticoids, duration of disease, and muscle weakness [24]. Aysal et al. (2013) evaluated anxiety and depressive symptoms with Hamilton Rating Scale 17-item version in patients with MG and reported that disease gravity and stressful life events were associated with depressive symptoms, while disease gravity, treatment modalities, and gender were associated with anxiety symptoms [25].

The current study has limitations. Since the study design was cross-sectional, it was not possible to make any cause-effect inference on the relationship between characteristics of the illness and HRQOL. Prospective studies are warranted to determine causal relationships. By contrast, the strict diagnosis criteria, the evaluation of HRQOL, motor and psychiatric symptoms using disease-specific tools can be regarded as strengths of the study.

In sum, besides motor symptoms, anxiety and depressive symptoms influence HRQOL in MG. Mental health management must be a clinical focus in addition to the treatment of somatic symptoms during the course of MG.

Financial disclosure: This study was funded by Brazilian government funding agencies *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG), Brazil.

REFERENCES

[1] Bates MD, Stassen MHW. The role of antibodies in myasthenia gravis. J Neurol Sci 2002;202:5-11.

[2] Mourão AM, Araújo CM, Barbosa LSM, Gomez RS, Burns TM, Lemos SMA, et al. Brazilian cross-cultural translation and adaptation of the "Questionnaire of Life Quality Specific for Myasthenia Gravis - 15 items". Arq Neuropsiquiatr 2013;7:955-58.

[3] Utsugisawa K, Suzuki S, Nagane Y, Masuda M, Murai H, Imai T, et al. Health-related quality-of-life and treatment targets in myasthenia gravis. Muscle Nerve 2014;50:493-500.

[4] Boldingh MI, Dekker L, Maniaol AH, Brunborg C, Lipka AF, Niks EH, et al. An up-date on health-related quality of life in myasthenia gravis -results from population based cohorts. Health Qual Life Outcomes 2015;13:1-14.

[5] Cioncoloni D, Casali S, Ginanneschi F, Carone M, Veronica B, Rossi A, et al. Major motor-functional determinants associated with poor self-reported health-related quality of life in myasthenia gravis patients. Neurol Sci 2016;37:717-23.

[6] Mourão AM, Gomez RS, Barbosa LS, Freitas DS, Comini-Frota ER, Kummer A, et al. Determinants of quality of life in Brazilian patients with myasthenia gravis. Clinics 2016;71:370-4.

[7] Ybarra MI, Kummer A, Frota ERC, Oliveira JTD, Gomez RS, Texeira AL. Psychiatric disorders in myasthenia gravis. Arq Neuropsiquiatr 2011;69:176-79.

[8] Basta IZ, Pekmezovic TD, Peric SZ, Kisić-Tepavčević DB, Rakočević-Stojanović VM, Stević ZD, et al. Assessment of health-related quality of life in patients with myasthenia gravis in Belgrade (Serbia). Neurol Sci 2012;33:1375-81.

[9] Jaretzki A, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JG, Penn AS. Myasthenia gravis. Recommendations for clinical research Standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. Neurology 2000;55:1-14.

[10] Burns TM, Conaway M, Sanders DB. The MG Composite. A valid and reliable outcome measure for myasthenia gravis. Neurology 2010;74:1434-40.

[11] Botega NJ, Bio MR, Zomignani MA, Garcia C, Pereira WA. Transtornos de humor em enfermarias de clínica médica e validação de escala de medida (HAD) de ansiedade e depressão. Rev Saúde Pública 1995;29(5):355-63.

[12] Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983;67:361-70.

[13] Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473-83.

[14] Guyatt GH, Feeny DH, Patrick DL. Measuring health related quality of life. Ann Intern Med 1993;118:622-29.

[15] Padua L, Evoli A, Aprile I, Caliandro P, Mazza S, Padua R, et al. Health-related quality of life in patients with myasthenia gravis and the relationship between patient-oriented assessment and conventional measurements. Neurol Sci 2001;22:363-9.

[16] Paul RH, Nash JM, Cohen RA, Gilchrist JM, Goldstein JM. Quality of life and well-being of patients with myasthenia gravis. Muscle Nerve 2001;24:512-6.

[17] Kulkantrakorn K, Sawanyawisuth K, Tiamkao S. Factors correlating quality of life in patients with myasthenia gravis. Neurol Sci 2010;31:571-3.

[18] Yang Y, Zhang M, Guo J, Ma S, Fan L, Wang X, et al. Quality of life in 188 patients with myasthenia gravis in China. Int J Neurosci 2015;1-8.

[19] Twork S, Wiesmeth S, Klewer J, Pöhlau D, Kugler J. Quality of life and life circumstances in German myasthenia gravis patients. Health Qual Life Outcomes 2010,8:129.

[20] Kulaksizoglu IB. Mood and anxiety disorders in patients with myasthenia gravis: aetiology, diagnosis and treatment. CNS Drugs 2007,21:473-81.

[21] Lundeen J, Fisher J, Kothari MJ. Frequency of anxiety in myasthenia gravis. J Clin Neuromuscul Dis 2004;6:9-12.

[22] Sitek EJ, Bilinska MM, Wieczorek D, Nyka WM. Neuropsychological assessment in myasthenia gravis. Neurol Sci 2009;30:9-14.

[23] Magni G, Micaglio GF, Lalli R, Bejato L, Candeago MR, Merskey H, et al. Psychiatric disturbances associated with myasthenia gravis. Acta Psychiatr Scand 1988;77:443-45.

[24] Suzuki Y, Utsugisawa K, Suzuki S, Nagane Y, Masuda M, Kabasawa C, et al. Factors associated with depressive state in patients with myasthenia gravis: a multicentre cross-sectional study. BMJ 2011;19:1-7.

[25] Aysal F, Karamustafalioğlu O, Ozçelik P, Yilmaz M, Karamustafalioğlu N, Yumrukçal H, et al. The Relationship of Symptoms of Anxiety and Depression with Disease Severity and Treatment Modality in Myasthenia Gravis: A Cross-sectional Study. Arch Neuropsychiatry 2013;50:295-30.

Table 1. Clinical and demographic features of myasthenia gravis patients.

Characteristics	**MG (*n* = 80) ± SD**	**(%)**
Gender		
Female	60	75
Male	20	25
Age (years)	41.89[#] (±14.171)	-
Education (years)	9.288[#] (±3.728)	-
Age at onset (years)	29.10[#] (±13.483)	-
Length of disease (years)	13.53[#] (±10.053)	-
Cumulative glucocorticoid dose (mg)	38123.477[#] (±41895.659)	-
Age at thymectomy (years)	32.50 (±11.829)	-
Mean MG composite	4.96[#] (±5.021)	-
MGFA		
I	10[*]	12.5
IIA	9[*]	11.3
IIB	7[*]	8.8
IIIA	15[*]	18.8
IIIB	4[*]	5.0
IVA	6[*]	7.5
IVB	5[*]	6.3
V	22[*]	27.5
HAD anxiety subscale score	7.68[#] (±4.453)	-
Mild	13*	16.3
Moderate	15*	18.8
Severe	7*	8.8
HAD depression subscale score	6.52[#] (±4.686)	-
Mild	8*	10
Moderate	7*	8.8
Severe	7*	8.8
MGQoL15	16.23[#] (±12.858)	-

MGFA: Myasthenia Gravis Foundation of America Clinical Classification; HAD: Hospital Anxiety and Depression Scale; MGQoL15: Myasthenia Gravis Quality-of-Life Questionnaire; [#]value average. [*]absolute value; SD: standard deviation.

Table 2- Frequency of symptoms and severity of muscle weakness according to MG Composite Scale.

Grade	**0**	**1**	**2**	**3**	**4**
Ptosis, upward gaze (physician examination)	49 (61.3%)	14 (17.5%)	2 (2.5%)	11 (13.8%)	-
Double vision on lateral gaze, left or right (physician examination)	46 (57.5%)	15 (18.8%)	1 (1.3%)	7 (8.8%)	7 (8.8%)
Eye closure (physician examination)	52 (65%)	22 (27.5%)	2 (2.5%)	-	-
Talking (patient history)	64 (80%)	12 (15%)	-	-	-
Chewing (patient history)	67 (83.8%)	9 (11.3%)	-	-	-
Swallowing (patient history)	59 (73.8%)	1 (1.3%)	16 (20%)	-	-
Breathing (thought to be caused by MG)	61 (76.3%)	15 (18.8%)	-	-	-
Neck flexion or extension (weakest) (physician examination)	57 (71.3%)	15 (18.8%)	1 (1.3%)	3 (3.8%)	-
Shoulder abduction (physician examination)	63 (78.8%)	1 (1.3%)	10 (12.5%)	1 (1.3%)	1 (1.3%)
Hip flexion (physician examination)	44 (55%)	2 (2.5%)	22 (27.5%)	6 (7.5%)	2 (2.5%)

Table 3- Logistic regression model for variables association with poor HRQOL.

				95% CI	
	B	SE	p Value	Lower	Upper
MG composite	1.24	0.24	< **0.001**	0.76	1.72
Total HAD score	0.66	0.15	< **0.001**	0.32	0.96
Constant	0.71	2.75	0.79	-4.79	6.20

DISCUSSÃO

Do nosso conhecimento, este é o primeiro estudo sobre a composição corporal com DXA em pacientes com MG. Avaliamos a composição corporal, a densidade mineral óssea (densidade mineral óssea) e os níveis plasmáticos de adipocinas e marcadores de metabolismo ósseo em pacientes com MG e indivíduos saudáveis. Nossos resultados mostraram que a freqüência de obesidade é maior nos pacientes com MG do que nos controles. Os pacientes com MG também apresentaram maior massa gorda corporal, maior adiposidade da região androide e maior percentual de gordura corporal total do que os controles. Além disso, maior gordura corporal total e menor massa magra foram associados com aumento da gravidade dos sintomas da MG.

De interesse foi o fato de que pacientes com alta dose acumulada de prednisona apresentaram comprometimento do metabolismo ósseo. A DMO foi significativamente menor em pacientes relativamente jovens com MG em comparação com controles saudáveis. Além disso, a dose acumulada de GC correlacionou-se negativamente com os níveis de cálcio iônico, T-score da coluna lombar e DMO, T-score e Z-score do colo do femural. Como os GC são comumente usados no tratamento com MG, esses resultados destacam a relevância da avaliação sistemática da DMO em pacientes com MG.

Avaliamos a qualidade de vida (QV) com uso de questionário específico para MG e validado no Brasil e observamos que a QV é negativamente influenciada pela fraqueza muscular e por sintomas de ansiedade e depressivos.

No presente estudo, os níveis plasmáticos de IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ e IL-17 estavam reduzidos em pacientes tratados com MG. Observou-se também associação negativa entre a IL-6 e a força muscular, ou seja, quanto maior o nível de IL-6, maior a gravidade dos sintomas MG, o que indica maior fraqueza muscular.

O presente estudo apresenta limitações. Uma vez que o desenho do estudo foi transversal, não foi possível fazer inferência causa-efeito sobre a relação entre as características da doença e as variáveis níveis plasmáticos de citocinas. Além disso, não foi possível avaliar pacientes virgens de tratamento. Em contrapartida, os critérios de diagnóstico rigorosos, a inclusão de diversos subtipos da doença, o uso de ferramentas validadas para esta população e a avaliação de biomarcadores plasmáticos podem ser considerados como pontos fortes do estudo.

CONCLUSÃO

Este estudo foi o primeiro registro formal das características clínico-laboratoriais, incluindo avaliação do metabolismo ósseo e da composição corporal, dos pacientes com MG tratados no serviço de neurologia do Hospital das Clínicas da UFMG. Foi desenvolvido dentro de um conjunto de ações pensadas com o intuito de proporcionar um melhor atendimento e tratamento aos pacientes no que diz respeito não só à abordagem da sua doença em si, mas também das várias comorbidades que os acometem.

No presente estudo observou-se que:

1. Pacientes com MG em uso prolongado de GC apresentam maior prevalência de baixa massa óssea e obesidade que indivíduos saudáveis.

2. A alta dose acumulada de GC e a concentração plasmática de TNF-α estão associadas com a redução da densidade mineral óssea dos pacientes com MG.

3. A gordura corporal total e a concentração plasmática de IL-6 estão associadas com maior gravidade da doença.

4. Os fatores que influenciam negativamente a qualidade de vida destes pacientes são a fraqueza muscular e os sintomas ansiosos e depressivos.

Assim, este estudo formado pela união de ciência básica e com a neurociência clínica, trata-se de trabalho científico com repercussão clínica e social. Com ele esperamos contribuir para o manejo biopsicossocial de pacientes com MG e, assim, melhorar a qualidade de vida destes pacientes.

REFERÊNCIAS

Aguiar AAX, Carvalho AF, Costa CMC, Fernandes JMA, D'Almeida JAC, Furtado LETA, Cunha FMB. Myasthenia gravis in Ceará, Brazil. Clinical and epidemiological aspects. **Arq Neuropsiquiatr.** 2010;68(6):843-48.

Andersen JB, Heldal AT, Engeland A, Gilhus NE. Myasthenia gravis epidemiology in a national cohort; combining multiple disease registries. **Acta Neurol Scand**. 2014;129: 26-31.

Baumgartner R, Koehler K, Gallagher D, Romero L, Heymstleld SB, Ross RR, Garry PJ, Lindeman RD. Epidemiology of sarcopenia among the elderly in New Mexico. **Am J Epidemiol**. 1998;147:755-63.

Berrih-Aknin S, Le Panse R. Myasthenia gravis: a comprehensive review of immune dysregulation and etiological mechanisms. **J Autoimmun**. 2014;52:90-100.

Botega NJ, Bio MR, Zomignani MA, Garcia C JR, Pereira WA. Transtornos de humor em enfermarias de clínica médica e validação de escala de medida (HAD) de ansiedade e depressão. **Rev Saúde Pública.** 1995;29(5):355-63.

Brandão CMA, Camargos BM, Zerbini CA. Posições oficiais 2008 da Sociedade Brasileira de Densitometria Clínica. **Arq Bras Endocrinol Metab**. 2009;53(1):107-12.

Burges J, Wray DW, Pizzighella S, Hall Z, Vincent A. A myasthenia gravis plasma immunoglobulin reduces miniature endplate potentials at human endplates in vitro. **Muscle Nerve**. 1990;13:407-13.

Burns TM, Conaway M, Sanders DB. The MG Composite. A valid and reliable outcome measure for myasthenia gravis. **Neurology**. 2010;74:1434-40.

Burns TM, Conaway M, Sanders DB. Construct and concurrent validation of the MG-QOL15 in the practice setting. **Muscle Nerve**. 2010;41(2):219-26.

Carr AS, Cardwell CR, MCCarron PO, MCConville J. A systematic review of population based epidemiological studies in Myasthenia Gravis. **BMC Neurol.** 2010;18:10-46

Cohen-Kaminsky S, Delattre RM, Devergne O, Klingel-Schmitt I, Emilie D, Galanaud P, Berrih-Aknin S. High IL-6 gene expression and production by cultured human thymic epithelial cells from patients with myasthenia gravis. **Ann N Y Acad Sci**. 1993;21;681:97-9.

Conti-Fine BM, Milani M, Kaminski HJ. Myasthenia gravis: past, present and future. **J Clin Invest.** 2006;116(11):2843-54.

Drachman DB, de Silva S, Ramsay D, Pestronk A. Humoral pathogenesis of myasthenia gravis. **Ann N Y Acad Sci.** 1987;505:90-105.

Engel AG. The immunopathological basis of acetylcholine receptor deficiency in myasthenia gravis. **Prog Brain Res. 1979;**49:423-34.

Evoli A, Batocchi AP, Bartoccioni E, Lino MM, Minisci C, Tonali P. Juvenile myasthenia gravis with prepubertal onset. **Neuromuscul Disord.** 1998;8:561-67.

Evoli A, Iorio R, Bartoccioni E. Overcoming challenges in the diagnosis and treatment of myasthenia gravis. **Expert Review of Clinical Immunology**. 2015;12(2):157-68.

Gilhus NE, Nacua A, Andersena JB, Owea JF. Myasthenia gravis and risks for comorbidity. European Journal of Neurology. **European Journal of Neurology**. 2015;22:17-23.

Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. **Muscle Nerve**. 2008;37:141-49.

Gudbjornsson B, Juliusson UI, Gudbjornsson FV. Prevalence of long-term steroid treatment and the frequency of decision making to prevent steroid induced osteoporosis in daily clinical practice. **Ann Rheum Dis**. 2002;61:26-32.

Henneicke H, Gasparini SJ, Brennan-Speranza TC, Zhou H, Seibel MJ. Glucocorticoids and bone: local effects and systemic implications. **Trends in Endocrinology and Metabolism**. 2014;25(4):197-211.

Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent . Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. **Nat Med.** 2001;7(3):365-68.

Jacobson DL, Gange SJ, Rose NR, Graham NM. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. **Clin Immunol Immunopathol**. 1997;84:223-43.

Jaretzki A, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JG, Penn AS. Myasthenia gravis. Recommendations for clinical research Standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. **Neurology.** 2000;55:1-14.

Juel VC, Massey JM. Autoimmune Myasthenia Gravis: Recommendations for Treatment and Immunologic Modulation. **Curr Treat Options Neurol**. 2005;7(1):3-14.

Kumar V, Kaminski HJ. Treatment of Myasthenia Gravis. **Curr Neurol Neurosci Rep**.2011;11:89-96.

Lindstrom JM, Einarson BL, Lennon VA, Seybold ME. Pathological mechanisms in experimental autoimmune myasthenia gravis. I. Immunogenicity of syngeneic muscle acetylcholine receptor and quantitative extraction of receptor and antibodyreceptor complexes from muscles of rats with experimental automimmune myasthenia gravis. **J Exp Med**. 1976;144:726-38.

MCGrogan A, Sneddon S, De Vries CS. The incidence of myasthenia gravis: a systematic literature review. **Neuroepidemiology.** 2010;34:171-83.

Meriggioli MN. Myasthenia Gravis: Immunopathogenesis, Diagnosis, and Management. Continuum Lifelong Learning Neurol 2009;15(1):35–62.

Morita MP, Gabbai AA, Oliveira AS, Penn AS. Myasthenia gravis in children: analysis of 18 patients. **Arq Neuropsiquiatr.** 2001;59:681-5.

Mourão AM, Araújo CM, Barbosa LSM, Gomez RS, Burns TM, Lemos SMA, Teixeira AL. Brazilian cross-cultural translation and adaptation of the "Questionnaire of Life Quality Specific for Myasthenia Gravis - 15 items". **Arq Neuropsiquiatr**. 2013;71(12):955-58.

Munz C, Lunemann JD, Getts MT, Miller SD. Antiviral immune responses: triggers of or triggered by autoimmunity? **Nat Rev Immunol**. 2009;9(4):246-58.

Neto. Consenso Brasileiro de Osteoporose. **Bras Reumatol**. 2002;46(6):343-54.

Pal J, Rozsa C, Komoly S, Illes Z. Clinical and biological heterogeneity of autoimmune myasthenia gravis. **J Neuroimmunol**. 2011;231(1-2):43-54.

Pevzner A, Schoser B, Peters K, Cosma NC, Karakatsani A, Schalke B, Melms A, Kro¨ger S. Anti-LRP4 autoantibodies in AChR- and MuSK-antibodynegative myasthenia gravis. **J Neurol.** 2012;259:427-35.

Rodolico C, Toscano M, Autunno S, Messina S, Nicolosi C, Aguennouz M, Laurà M, Girlanda P, Messina C, Vita G. Limb-girdle myasthenia: clinical, electrophysiological and morphological features in familial and autoimmune cases. **Neuromuscul Disord.** 2002;12:964-69.

Sathasivam S. Current and emerging treatments for the management of myasthenia gravis. **Ther Clin Risk Manag**. 2011;7:313-23.

Shen C, Lu Y, Zhang B, Figueiredo D, Bean J, Jung J, Wu H, Barik A, Yin DM, Xiong WC, Mei L. Antibodies against low-density lipoprotein receptor–related protein 4 induce myasthenia gravis. **J Clin Invest**. 2013;123(12):5190-5202.

Sieb JP. Myasthenia gravis: an update for the clinician. Clin Exp Immunol. 2014;175:408-18.

Silvestri NJ, Wolfe GI. Myasthenia gravis. **Semin Neurol.** 2012;32:215-26.

Suzuki Y, Utsugisawa K, Suzuki S, Nagane Y, Masuda M, Kabasawa C, et al. Factors associated with depressive state in patients with myasthenia gravis: a multicentre cross-sectional study. BMJ 2011;19:1-7.

Tüzün E, Scott BG, Goluszko E, Higgs S, Christadoss P. Genetic evidence for involvement of classical complement pathway in induction of experimental autoimmune myasthenia gravis. **J Immunol.** 2003;171:3847-54.

Utsugisawa K. Association between Glucocorticoid-Induced Osteoporosis and Myasthenia Gravis: A Cross- Sectional Study. **Plos One** 2015;10:1-7.

Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo HC, Marx A, et al. Randomized Trial of Thymectomy in Myasthenia Gravis. **N Engl J Med.** 2016;11;375:511-22.

Ybarra MI, Kummer A, Frota ERC, Oliveira JTD, Gomez RS, Texeira AL. Psychiatric disorders in myasthenia gravis. **Arq Neuropsiquiatr** 2011;69:176–179.

Zigmond AS, Snaith RP. The hospital anxiety and depression scale. **Acta Psychiatr Scand** 1983;67:361-70.

ANEXO I – Aprovação do COEP

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Projeto: CAAE –19045413.0.0000.5149

Interessado(a): Prof. Antônio Lúcio Teixeira Junior
Departamento de Clinica Médica
Faculdade de Medicina- UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 18 de dezembro de 2013, o projeto de pesquisa intitulado **"Estudo do metabolismo ósseo em pacientes portadores de Miastenia Gravis em uma amostra de centro terciário"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 - Unidade Administrativa II - 2º andar - Sala 2005 - Cep: 31270-901 - BH-MG
Telefax: (031) 3409-4592 - e-mail: coep@prpq.ufmg.br

ANEXO II - Protocolo Miastenia Gravis

Nome: _____ **Data:** **___/___/___**

Data de nascimento:___/___/___ Idade: ____anos **Registro:**_____

Telefone:_____

Endereço:

_____

Data do retorno: ___/___/___ Data da densitometria: ___/___/___

Peso: _____ Altura: _____ IMC: _____ Cintura: _____ Quadril: _____ RCQ: _____

Escolaridade: _____ Ocupação:

_____

Idade início dos sintomas: _____anos **Tempo de doença: _____**

Anticorpos positivos: ( ) Sim ( ) Não ( ) Anti-receptor ACh ( ) Anti-MUSK ( ) Anti-TPO

ENMG (__/___/__) Positiva: ( ) Negativa: ( ) Taxa de decremento TER:___ Jitter (fibra única):

TC de tórax (___/___/___):

_____

Timectomia: ( ) Sim ( ) Não Se sim, data: **___/___/___** Idade: **_____**

Anatomopatologia do timo:

_____

Crise miastênica: ( ) Sim ( ) Não Se sim, data: **___/___/___** Idade: **_____**

Classificação MGFA:

I – Somente envolvimento ocular.

II – Fraqueza leve afetando outra musculatura além da ocular:

IIA- predomínio de fraqueza em musculatura apendicular e/ou axial;

IIB- predomínio de fraqueza em musculatura orofaríngea e/ou respiratória.

III- Fraqueza moderada afetando outra musculatura além da ocular:

IIIA- - predomínio de fraqueza em musculatura apendicular e/ou axial;

IIIB – predomínio de fraqueza em musculatura orofaríngea e/ou respiratória.

IV- fraqueza intensa afetando outra musculatura além da ocular:

IV A – predomínio de fraqueza em musculatura apendicular e/ou axial;

IV B – predomínio de fraqueza em musculatura orofaríngea e/ou respiratória.

V – intubação com ou sem necessidade de ventilação mecânica, exceto quando empregada durante rotina pós-operatória.

Comorbidades Clínicas:

- Hipertensão arterial: ( ) Sim ( ) Não - Glaucoma: ( ) Sim ( ) Não
- Diabetes Mellitus : ( ) Sim ( ) Não - Catarata: ( ) Sim ( ) Não
- Disfunção Tireoidiana ( ) Sim ( ) Não. Se sim, qual? _____
- Dislipidemia: ( ) Sim ( ) Não. Se sim, qual? _____
- Tabagismo: ( ) Sim ( ) Não ( ) Ex Anos-maço: _____
- Etilismo: ( ) Sim ( ) Não ( ) Ex

Sedentarismo: ( ) Sim ( ) Não. Se não for sedentário, especificar qual atividade física e frequência semanal de prática de esportes

_____

Medicações de uso atual (data de início e dose)**:**

Piridostigmina: _____

Prednisona (dose atual): _____ Tempo sem prednisona: _____

CaCO3 + Vit. D3: _____ Tempo de uso: _____

Alendronato: _____ Tempo de uso: _____

Imunossupressor (data de início e dose): _____

Outras drogas de importância ao metabolismo ósseo (data de início e dose)**:**

- Diuréticos: _____
- Omeprazol: _____
- Carbonato de cálcio + Vitamina D3: _____

Já fez uso de imunossupressor antes? (Anotar dose e tempo de uso)

( ) azatioprina _____

( ) ciclosporina _____

( ) ciclofosfamida _____

Já fez uso das seguintes medicações anteriormente? (Anotar qual e tempo de uso)

( ) Diuréticos _____

( ) Omeprazol _____

( ) Carbonato de cálcio + vitamina D3 _____

MG composite: _____

Ptose: ____

Diplopia: ____

Fechamento ocular: ____

Fala: ____

Mastigação____

Deglutição: ____
Respiração: ____
Flexão/extensão cervical: ____
Deltoide: ____
Iliopsoas: ____

Fratura óssea pós MG? ( ) Sim ( ) Não ( ) Sintomática ( ) Assintomática Data: **___/___/___**

Idade: _____ Local acometido: _____

Densitometria óssea (___/___/___): _____

Diagnóstico de: ( ) osteopenia ( ) osteoporose ( ) massa óssea preservada

Dosagens: Data: **___/___/___**

Colesterol total_____ LDL: _____ HDL_____ VLDL: _____ TG: _____

Glicemia de jejum: _____ TSH: _____ T3: _____ T4: _____

Calciúria 24 horas: _____ Fosfatase alcalina: _____

25-OH-Vitamina D: _____ cálcio iônico: _____ fósforo:_____ Paratormônio: _____

Ciclo menstrual: ( ) regular ( ) irregular ( ) climatério Tempo de menopausa_____

Em uso de contraceptivo: ( ) sim ( ) não Nome: _____

ANEXO III - Termo de Consentimento Livre e Esclarecido

Título da Pesquisa: "Estudo do metabolismo ósseo em pacientes portadores de Miastenia Gravis em uma amostra de centro terciário".

Natureza da pesquisa: o Sr. (Sra.) está sendo convidado (a) a participar desta pesquisa que tem como finalidade estudar alterações do metabolismo do osso em paciente portadores de miastenia gravis em uso de corticosteroides (dexametasona, prednisona, prednisolona). É sabido que a exposição prolongada a corticosteroides pode levar a alterações do osso, como osteoporose, predispondo o paciente a fraturas. No entanto, o metabolismo ósseo ainda não foi bem estudado em pacientes com miastenia gravis. Este estudo visa elucidar as implicações do uso de corticosteroides na saúde óssea de pacientes com miastenia gravis.

1. **Participantes da pesquisa**: serão avaliados pacientes com miastenia gravis em uso de corticosteroides, atendidos no Ambulatório de Neuromuscular do Hospital das Clínicas da UFMG (HC-UFMG).

2. **Envolvimento na pesquisa**: ao participar deste estudo o Sr. (Sra.) permitirá que os pesquisadores realizem avaliações clínicas e laboratoriais específicas. O Sr. (Sra.) tem liberdade de se recusar a participar em qualquer fase da pesquisa, sem qualquer prejuízo. Sempre que quiser poderá pedir mais informações sobre a pesquisa à pesquisadora e, se necessário ao Comitê de Ética em Pesquisa.

3. **Sobre a entrevista e testes**: Serão coletados dados clínicos referentes à sua idade, sexo e informações relacionadas à miastenia gravis, uso de medicações e sobre sua saúde óssea por meio de questionário padronizado. Esta coleta ocorrerá durante suas consultas de rotina no Ambulatório de Neuromuscular do HC-UFMG. Em data definida pelo pesquisador, serão coletadas amostras de sangue e será realizado o exame de densitometria óssea. Nas amostras de sangue coletadas, serão realizados exames laboratoriais específicos para estudo do metabolismo ósseo. A densitometria óssea consiste em exame indolor, em que o Sr. (Sra.) será exposto a uma baixa incidência a raios X, praticamente inócuos à sua saúde. Com este exame, será possível obter informações quanto à sua densidade óssea e a presença ou não de osteopenia ou osteoporose.
4. **Riscos e desconforto**: a participação nesta pesquisa não traz riscos significativos à sua saúde. Os riscos são os mesmos de uma coleta comum de sangue e à exposição de uma radiografia de tórax.
5. **Confidencialidade**: todas as informações coletadas neste estudo são estritamente confidenciais. Somente os pesquisadores terão conhecimento dos dados pessoais. Os resultados do estudo não o (a) citará nominalmente.

6. **Benefícios**: ao participar desta pesquisa o Sr. (Sra.) poderá ter o benefício do diagnóstico de uma alteração no seu metabolismo ósseo decorrente da exposição a corticosteroides. Neste caso, medidas com relação ao tratamento da miastenia gravis poderão ser tomadas. Esperamos ainda que este estudo traga informações relevantes sobre a relação entre uso prolongado de corticosteroides em pacientes com miastenia gravis e metabolismo ósseo nestes pacientes.
7. **Pagamento**: o Sr. (Sra.) não terá nenhum tipo de despesa para participar desta pesquisa, bem como nada será pago por sua participação.

Após estes esclarecimentos, solicitamos o seu consentimento de forma livre para participar desta pesquisa. Portanto preencha, por favor, os itens que se seguem:

Consentimento Livre e Esclarecido

Tendo em vista os itens acima apresentados, eu, de forma livre e esclarecida, manifesto meu consentimento em participar da pesquisa.

_____

Nome do Participante da Pesquisa

_____

Assinatura do Participante da Pesquisa

_____

Assinatura do Pesquisador

_____

Assinatura do Orientador

CONTATOS

Pesquisadores: Nayara Felicidade Tomaz Braz (31) 9755-5050

Orientador: Antônio Lúcio Teixeira Júnior (31) 35409-8073.

Comitê de Ética em Pesquisa da UFMG: (31) 3409-4592.

ANEXO IV – *Myasthenia Gravis Foundation of America Clinical Classification* (*MGFA*)

Classe I: Puramente ocular	Qualquer fraqueza dos músculos oculares; Pode ter fraqueza no fechamento dos olhos; Todos os outros grupos musculares apresentam força normal.
Classe II: Leve II A	Fraqueza predominantemente em membros; Pode ter menor participação de músculos da orofaringe.
II B	Fraqueza predominantemente em músculos da região orofaringe e/ou das vias respiratórias; Pode ter participação igual ou menor de membros.
Classe III: Moderado III A	Fraqueza predominantemente em membros; Pode ter menor participação de músculos da orofaringe.
III B	Fraqueza predominantemente em músculos da região da orofaringe e/ou das vias respiratórias; Pode ter participação igual ou menor de membros.
Classe IV: Grave IV A	Fraqueza predominantemente em membros; Pode ter menor participação de músculos da orofaringe.
IV B	Fraqueza predominantemente em músculos da região da orofaringe e/ou das vias respiratórias; Pode ter participação igual ou menor de membros.
Classe V: Definido por Intubação	Definido por intubação, com ou sem mecânica ventilação, exceto quando utilizado durante a rotina do manejo cirúrgico. O uso de via alternativa de alimentação, sem intubação, classifica-se o paciente em classe IVB.

ANEXO V – MG Composite

Ptose, olhar fixo para cima (exame médico)	>45 segundos = 0	11-45 segundos = 1	1-10 segundos = 2	Imediato = 3
Diplopia, olhar fixo para direita e/ou esquerda (exame medico)	>45 segundos = 0	11-45 segundos = 1	1-10 segundos = 3	Imediato = 4
Fechamento Ocular (exame médico)	Normal = 0	Fraqueza leve (aberto com esforço) = 0	Fraqueza moderada (aberto facilmente) = 1	Fraqueza severa (não é possível manter os olhos fechados) = 2
Fala (anamnese)	Normal = 0	Discurso pouco articulado ou hipernasalidade de forma intermitente = 2	Discurso pouco articulado ou hipernasalidade de forma constante, porém inteligível = 4	Dificuldade em compreender o discurso falado = 6
Mastigação (anamnese)	Normal = 0	Fraqueza para comer alimentos sólidos = 2	Fraqueza para comer alimentos pastosos/líquidos = 4	Via alternativa de alimentação = 6
Deglutição (anamnese)	Normal = 0	Raros episódios de asfixia ou dificuldade para engolir = 2	Problema freqüente em engolir, exigindo mudanças na dieta = 5	Via alternativa de alimentação = 6
Respiração (decorrente da MG)	Normal = 0	Falta de ar com esforço = 2	Falta de ar no repouso = 4	Ventilação mecânica = 9
Flexão ou Extensão (mais fraca) cervical (exame médico)	Normal = 0	Fraqueza leve = 1	Fraqueza moderada* = 3	Fraqueza grave = 4
Abdução do ombro (exame médico)	Normal = 0	Fraqueza leve = 2	Fraqueza moderada* = 4	Fraqueza grave = 5
Flexão do quadril (exame médico)	Normal = 0	Fraqueza leve = 2	Fraqueza moderada* = 4	Fraqueza grave = 5
Fraqueza moderada* deve ser interpretada como fraqueza que equivale cerca de 50%.				
Escore total:				

FONTE: PUBMED, ed. 2012

ANEXO VI - *15-Item Myasthenia Gravis Quality-of-Life Questionnaire* (*MGQOL15*)

Nome: _____ Data:_____ Registro: _____

Questionário de Qualidade de Vida Especifico para Miastenia Gravis (MG-QOL15)					
Por favor, Marque a afirmativa verdadeira (referente às semanas passadas)					
Perguntas:	Não	Pouco	As vezes	Frequentemente	Sempre
	0	1	2	3	4
1. Eu estou frustrado por causa da miastenia gravis.					
2. Eu tenho dificuldade para usar meus olhos por causa da miastenia gravis.					
3. Eu tenho dificuldade para comer por causa da miastenia gravis.					
4. Eu limitei a minha atividade social por causa da miastenia gravis.					
5. A miastenia gravis limita minha capacidade de ter divertimento e atividades de lazer					
6. Eu tenho dificuldade para atender as necessidades da minha família por causa da miastenia gravis.					
7. Eu tenho que fazer os meus planos torno da miastenia gravis.					
8. Minhas habilidades profissionais e minha posição no trabalho (cargo) foram afetadas negativamente por causa da miastenia gravis.					
9. Eu tenho dificuldade para falar por causa da miastenia gravis.					
10. Eu tenho problemas para dirigir por causa da miastenia gravis.					
11. Eu estou deprimido por causa da miastenia gravis.					
12. Eu tenho dificuldade para andar por causa da miastenia gravis.					
13. Eu tenho dificuldade de passear em lugares públicos por causa da miastenia gravis.					
14. Sinto-me sobrecarregado por causa da miastenia gravis.					
15. Eu tenho dificuldade para realizar meus cuidados pessoais (higiene) por causa da miastenia gravis.					
Total:					

ANEXO VII - *Hospital Anxiety and Depression Scale* (HAD)

Quadro 1 – Escala Hospitalar de Ansiedade e Depressão

Este questionário ajudará o seu médico a saber como você está se sentindo. Leia todas as frases. Marque com um "X" a resposta que melhor corresponder a como você tem se sentido na ÚLTIMA SEMANA. Não é preciso ficar pensando muito em cada questão. Neste questionário as respostas espontâneas têm mais valor do que aquelas em que se pensa muito. Marque apenas uma resposta para cada pergunta.

A 1) Eu me sinto tenso ou contraído:
- 3 ( ) A maior parte do tempo
- 2 ( ) Boa parte do tempo
- 1 ( ) De vez em quando
- 0 ( ) Nunca

D 2) Eu ainda sinto gosto pelas mesmas coisas de antes:
- 0 ( ) Sim, do mesmo jeito que antes
- 1 ( ) Não tanto quanto antes
- 2 ( ) Só um pouco
- 3 ( ) Já não sinto mais prazer em nada

A 3) Eu sinto uma espécie de medo, como se alguma coisa ruim fosse acontecer:
- 3 ( ) Sim, e de um jeito muito forte
- 2 ( ) Sim, mas não tão forte
- 1 ( ) Um pouco, mas isso não me preocupa
- 0 ( ) Não sinto nada disso

D 4) Dou risada e me divirto quando vejo coisas engraçadas:
- 0 ( ) Do mesmo jeito que antes
- 1 ( ) Atualmente um pouco menos
- 2 ( ) Atualmente bem menos
- 3 ( ) Não consigo mais

A 5) Estou com a cabeça cheia de preocupações:
- 3 ( ) A maior parte do tempo
- 2 ( ) Boa parte do tempo
- 1 ( ) De vez em quando
- 0 ( ) Raramente

D 6) Eu me sinto alegre:
- 3 ( ) Nunca
- 2 ( ) Poucas vezes
- 1 ( ) Muitas vezes
- 0 ( ) A maior parte do tempo

A 7) Consigo ficar sentado à vontade e me sentir relaxado:
- 0 ( ) Sim, quase sempre
- 1 ( ) Muitas vezes
- 2 ( ) Poucas vezes
- 3 ( ) Nunca

D 8) Eu estou lento para pensar e fazer as coisas:
- 3 ( ) Quase sempre
- 2 ( ) Muitas vezes
- 1 ( ) De vez em quando
- 0 ( ) Nunca

A 9) Eu tenho uma sensação ruim de medo, como um frio na barriga ou um aperto no estômago:
- 0 ( ) Nunca
- 1 ( ) De vez em quando
- 2 ( ) Muitas vezes
- 3 ( ) Quase sempre

D 10) Eu perdi o interesse em cuidar da minha aparência:
- 3 ( ) Completamente
- 2 ( ) Não estou mais me cuidando como deveria
- 1 ( ) Talvez não tanto quanto antes
- 0 ( ) Me cuido do mesmo jeito que antes

A 11) Eu me sinto inquieto, como se eu não pudesse ficar parado em lugar nenhum:
- 3 ( ) Sim, demais
- 2 ( ) Bastante
- 1 ( ) Um pouco
- 0 ( ) Não me sinto assim

D 12) Fico esperando animado as coisas boas que estão por vir:
- 0 ( ) Do mesmo jeito que antes
- 1 ( ) Um pouco menos do que antes
- 2 ( ) Bem menos do que antes
- 3 ( ) Quase nunca

A 13) De repente, tenho a sensação de entrar em pânico:
- 3 ( ) A quase todo momento
- 2 ( ) Várias vezes
- 1 ( ) De vez em quando
- 0 ( ) Não sinto isso

D 14) Consigo sentir prazer quando assisto a um bom programa de televisão, de rádio ou quando leio alguma coisa:
- 0 ( ) Quase sempre
- 1 ( ) Várias vezes
- 2 ( ) Poucas vezes
- 3 ( ) Quase nunca