EFEITO DA HIDROXICLOROQUINA NA FUNÇÃO ENDOTELIAL DE IDOSOS COM APNEIA DO SONO – ESTUDO HOLD

TESE

Letícia Maria Tedesco Silva

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

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Autor: Letícia Maria Tedesco Silva

Orientador: Prof. Dr. Denis Martinez

Coorientador: Prof. Dr. Sandro Cadaval Gonçalves

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Aos voluntários do estudo pela participação e altruísmo

“O destino ajuda os corajosos”

Provérbio latino

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS EM PORTUGUÊS

AOS – apneia obstrutiva do sono

FMD – dilatação fluxo-mediada da artéria braquial

HCQ – hidroxicloroquina

IAH – índice de apneia-hipopneia

IC – intervalo de confiança

IDR - índice de distúrbio respiratório

NO – óxido nítrico

PAT – tonometria arterial periférica

RERA - despertares relacionados ao esforço respiratório

TNF-α – fator de necrose tumoral alpha

RESUMO

Objetivos/Hipótese: Hidroxicloroquina (HCQ) previne mortalidade cardiovascular em pacientes com doenças reumatológicas possivelmente por inibir inflamação. Apneia do sono (AOS) causa inflamação, disfunção endotelial e aumenta mortalidade cardiovascular. HCQ melhoraria a função endotelial de idosos com AOS. *Métodos:* Incluímos 29 participantes com idade maior que 65 anos e AOS moderada-grave. Avaliou-se função endotelial por meio de dilatação fluxo-mediada da artéria braquial (FMD) e tonometria arterial periférica (PAT) no *baseline* e oito semanas após tratamento com HCQ (grupo HCQ) ou placebo (grupo placebo). Avaliamos também o índice de apneia-hipopneia (IAH) com poligrafia portátil antes e após o tratamento. *Resultados:* Não houve diferença estatisticamente significativa na função endotelial entre o grupo HCQ e placebo. O IAH do grupo HCQ reduziu de maneira estatisticamente significativa. *Conclusão:* HCQ não melhorou a função endotelial de idosos com AOS após 8 semanas de tratamento em comparação ao placebo. Tratamento com HCQ pode melhorar a AOS.

PALAVRAS-CHAVE: Apneia do sono; hidroxicloroquina; dilatação fluxo-mediada da artéria braquial; tonometria arterial periférica; função endotelial

ABSTRACT

Objectives/Hypothesis: Hydroxychloroquine (HCQ) prevents cardiovascular mortality in patients with rheumatic diseases, possibly by inhibiting inflammation. Obstructive sleep apnea (OSA) causes inflammation, endothelial dysfunction and increases cardiovascular mortality. HCQ could improve the endothelial function of older adults with OSA. *Methods:* We included twenty-nine participants older than 65 years and with moderate-severe OSA in the study. Endothelial function was assessed by flow-mediated dilation of the brachial artery (FMD) and peripheral arterial tonometry (PAT) at baseline and eight weeks after treatment with HCQ (HCQ group) or placebo (placebo group). We also assessed the apnea-hypopnea index (AHI) with home portable monitoring at baseline and follow-up. Results: There was no statistically significant difference in endothelial function between the HCQ group and the placebo group. The HCQ group presented a statistically significant reduction in AHI. Conclusion: HCQ did not improve endothelial function in older adults with sleep apnea after 8 weeks of treatment compared to placebo. HCQ treatment can improve OSA.

KEYWORDS: Sleep apnea; hydroxychloroquine; flow-mediated dilation of the brachial artery; peripheral artery tonometry; endothelial function

INTRODUÇÃO

A apneia obstrutiva do sono (AOS) é caracterizada por colapso repetido da via aérea durante o sono, que leva à interrupção do fluxo de ar e à hipóxia intermitente. Nosso grupo publicou estudos demonstrando que a hipóxia intermitente causa estresse oxidativo[1], inflamação e danos a lipídios e proteínas[2]. Portanto, a AOS pode ser considerada uma doença inflamatória crônica de baixo grau.[3] A inflamação em pacientes com AOS ocorre tanto em nível local, na via aérea,[4,5] quanto sistêmico[6,7]. A prevalência de AOS aumenta com a idade, atingindo valores em torno de 80%.[8] Idade avançada é considerada um estado inflamatório por si só e tem sido chamada de "*inflammaging*".[9] A inflamação crônica inicia e propaga a disfunção endotelial, que está associada à aterosclerose acelerada e maior morbidade e mortalidade cardiovascular.[10,11] AOS prejudica a função endotelial[12,13] e aumenta o risco cardiovascular[14]. O tratamento da AOS parece reduzir a inflamação e melhorar a função endotelial.[15,16] Os dois métodos principais usados para avaliação não invasiva da função endotelial são dilatação mediada por fluxo da artéria braquial (FMD) e tonometria da artéria periférica (PAT).

A hidroxicloroquina (HCQ), um medicamento antimalárico de baixo custo e efeitos colaterais raros, é usada há muitos anos no tratamento de doenças reumáticas, como lúpus eritematoso sistêmico e artrite reumatoide. Sharma et al. relataram em um estudo retrospectivo envolvendo 1.266 pacientes com artrite reumatoide uma associação entre o uso de HCQ e uma redução de 72% no risco de um desfecho composto por síndrome coronariana aguda, revascularização cardíaca, acidente vascular cerebral, acidente isquêmico transitório, doença

arterial periférica e morte súbita.[17] Os mecanismos que envolvem essa redução do risco cardiovascular não são claros, mas parece razoável supor que uma melhora na função endotelial possa estar envolvida.

O tratamento com HCQ em células endoteliais da veia umbilical humana reduziu marcadores de estresse oxidativo.[18] Em modelos animais, HCQ melhorou a vasodilatação dependente do endotélio.[19-21] Estudos de coorte demonstraram que HCQ também reduz os níveis de colesterol em pacientes com lúpus e artrite reumatóide.[22-25] Além disso, em pacientes com doenças reumáticas, o uso do HCQ tem sido associado a uma menor incidência de diabetes tipo 2. [26-28]

Especulamos que a diminuição da mortalidade cardiovascular observada em pacientes com doenças reumáticas que usaram HCQ pode ter ocorrido devido à melhora da função endotelial e consequente prevenção da formação de placa aterosclerótica. Um dos mecanismos de disfunção endotelial visto na AOS é mediado pela hipóxia intermitente e a consequente formação de radicais livres de oxigênio que causam estresse oxidativo. O HCQ, por suas propriedades anti-inflamatórias e efeitos favoráveis na redução de diversos fatores de risco cardiovascular, pode melhorar a função endotelial em pacientes com AOS. Além disso, o efeito da HCQ em doenças não reumáticas, mas com um componente inflamatório, como a AOS, nunca foi estudado.

REVISÃO DA LITERATURA

Apneia Obstrutiva do Sono

Apneia obstrutiva do sono (AOS) é caracterizada por recorrentes episódios de colapso parcial ou total da parte superior das vias aéreas durante o sono, resultando em redução (hipopneia) ou ausência (apneia) de fluxo de ar por pelo menos 10 segundos associado com despertar ou queda na saturação de oxigênio no sangue superior a 3% a partir da linha de base, conforme demonstrado na Erro! Autoreferência de indicador não válida.. Os “despertares relacionados ao esforço respiratório” ou, em inglês, “*respiratory effort-related arousal”* (RERA) foram incluídos ao diagnóstico da AOS em 2014, na 3ª edição da classificação internacional dos distúrbios do sono. Os *RERAs* são caracterizados por uma sequência de respirações, com esforço respiratório crescente, resultando em despertar, sem queda na saturação de oxigênio. Visto que cada evento respiratório, seja apneia, hipopneia ou RERA, leva a despertar transitório, a doença apneia do sono causa em primeiro lugar fragmentação do sono e alteração de sua arquitetura normal. Como consequência, se exacerba a atividade do sistema nervoso autônomo. A AOS é importante causa de sonolência diurna excessiva, contribuindo para a redução da qualidade de vida, prejuízo ao desempenho no trabalho e aumento de risco de acidentes com veículos motorizados.[29] AOS está associada a um aumento da incidência de hipertensão, diabetes mellitus tipo 2, fibrilação atrial, insuficiência cardíaca, doença arterial coronariana, acidente vascular cerebral e morte.[30-32] AOS é

diagnosticada através do exame de polissonografia, que pode ser realizado em casa (poligrafia portátil) ou em laboratório.

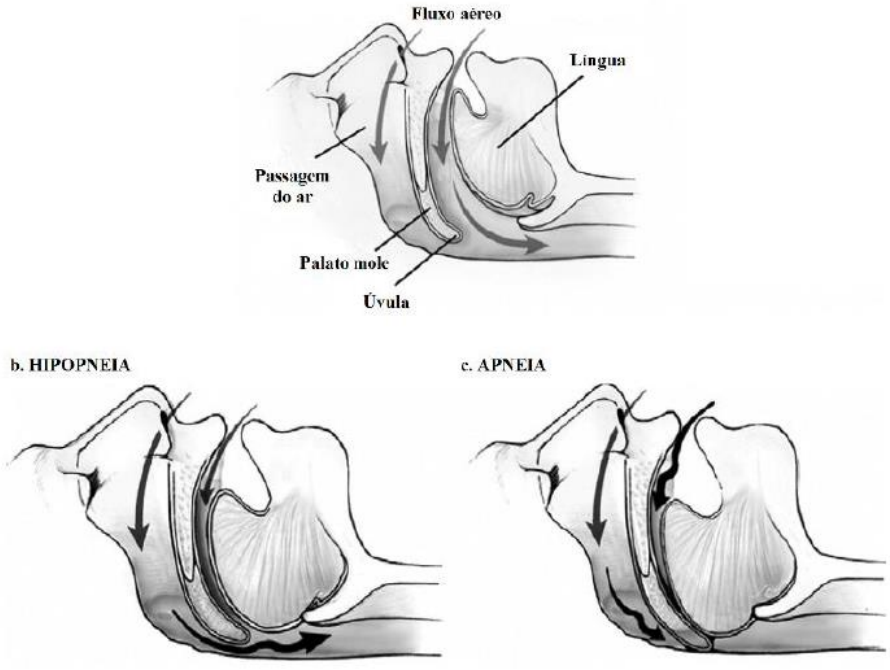

Figura 1 - Obstrução parcial e completa das vias aéreas, resultando em hipopneia e apneia, respectivamente. Adaptada de Somers et al[30]

Classificação

A presença e gravidade da AOS são normalmente quantificadas pelo índice de apneia-hipopneia (IAH), definido como o número de apneias mais hipopneias por hora de sono (ou hora de gravação no exame de poligrafia). Os "despertares relacionados ao esforço respiratório" ou, em inglês, "*respiratory effort-related arousal"* (RERA) foram incluídos ao diagnóstico da AOS em 2014, na 3ª edição da classificação internacional dos distúrbios do sono. Somando eventos de apneia, hipopneia e RERA, e os dividindo pelo total de horas de sono obtêm-se o índice de distúrbio respiratório - IDR (**Tabela 1**).

Tabela 1 - Classificação da gravidade da apneia obstrutiva do sono

IAH ou IDR	Classificação
< 5	Normal
5 – 14	Leve
15 – 29	Moderada
≥ 30	Grave

Classificação conforme a Academia Americana de Medicina do Sono[33]

Epidemiologia

O Wisconsin Sleep Cohort Study[34], um estudo de coorte com 602 participantes publicado em 1993, mostrou pela primeira vez que 9% das mulheres e 24% dos homens com idade entre 30 e 60 anos nos EUA tinham IAH>5. A prevalência de AOS é aproximadamente duas vezes mais comum em homens do que em mulheres e aumenta com a idade. Em 2013, foram publicados novos dados dessa mesma coorte, agora com 1.520 participantes, que demonstraram aumento da prevalência de AOS. A prevalência de AOS moderada a grave foi de 10% em homens entre 30-49 anos, 17% em homens entre 50-70 anos, 3% em mulheres entre 30-49 anos e 9% em mulheres entre 50-70 anos. Comparando os achados de 1993 e 2013, se observam taxas de aumento da prevalência de AOS em adultos em aproximadamente 30%.

No Multi-Ethnic Study of Atherosclerosis, a prevalência de AOS em adultos de 54 a 93 anos excedeu 60%, com AOS moderada a grave presente em 30,3% dos brancos, 32,4% dos afro-americanos, 38,2% dos hispânicos indivíduos e 39,4% dos participantes de ascendência chinesa.[35] A AOS está

associada ao sobrepeso e à obesidade. Entre indivíduos de 30 a 49 anos com índice de massa corporal (IMC) inferior a 25, a prevalência de AOS entre os homens é de 7,0% e entre as mulheres é de 1,4%, em comparação com 44,6% entre os homens e 13,5% entre as mulheres com IMC de 30 a 39. A associação de AOS com obesidade e sexo masculino diminui com a idade.[36]

Um estudo epidemiológico de base populacional conduzido em 2010 no Brasil analisou a prevalência de AOS. Dos 1.042 voluntários com idades entre 20 e 80 anos que realizaram polissonografia, 55% eram do sexo masculino e 60% apresentavam IMC maior que 25 kg/m². A prevalência total de apneia obstrutiva do sono foi de 32,8%. Os fatores independentes associados à apneia obstrutiva do sono foram sexo masculino (OR 4,1; IC 95% 2,9 – 5,8), obesidade (OR 10,5; IC 95% 7,1 – 15,7) e idade superior a 60 anos (OR 34,5; IC 95% 18,5 – 64,2).[8] Nesse estudo, a prevalência de AOS em idosos foi de 80 a 95%, conforme demonstrado na **Figura 2**.

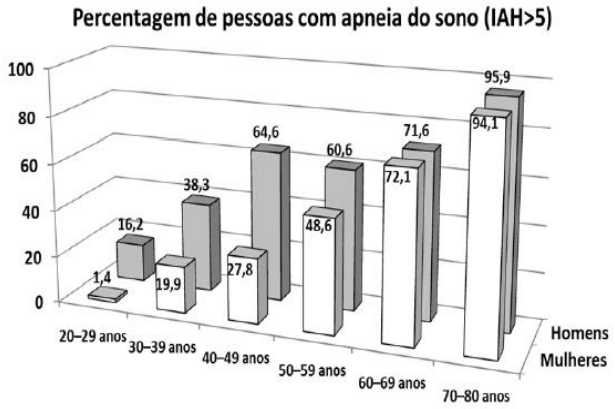

Figura 2 - Prevalência de apneia obstrutiva do sono (IAH>5) por sexo e idade no estado de São Paulo, Brasil. Adaptada de Tufik et al.[9]

Fisiopatologia

Os mecanismos fisiopatológicos subjacentes à apneia obstrutiva do sono estão bem estabelecidos. A contração dos músculos dilatadores das vias aéreas superiores é necessária para manter a patência das vias aéreas durante a inspiração. O músculo dilatador mais importante da via aérea superior é o músculo genioglosso, que se contrai com cada inspiração para evitar o colapso posterior da língua, auxiliado pelos músculos levantadores e tensores palatinos (avançando e elevando o palato mole) e os músculos gênio-hióideo e estilofaríngeo (colapso medial oposto das paredes laterais da faringe).[37] A maioria pessoas com AOS têm uma via aérea superior estreita, geralmente causada por deposição de gordura nas regiões parafaríngeas ou, menos frequentemente, por anormalidades da estrutura craniofacial[38]. Essas anormalidades incluem defeitos anatômicos clinicamente evidentes, como micrognatia e retrognatia, ou sutis alterações vistas apenas em radiografia, como posicionamento inferior do osso hióide e menor comprimento mandibular e maxilar, o que resulta em pequeno volume maxilo-mandibular. A contribuição relativa do tecido mole e anormalidades ósseas para AOS diferem entre os indivíduos e entre populações; por exemplo, para a mesma gravidade de AOS, indivíduos caucasianos tendem a ter mais excesso de peso, enquanto os asiáticos têm mais anormalidades craniofaciais.[39]

As principais causas e fatores de risco da apneia obstrutiva do sono são idade avançada, obesidade e sexo masculino. A idade, por exemplo, leva à sarcopenia e flacidez da musculatura abdutora da faringe, mesmo na ausência de obesidade[40]. A obesidade, por acúmulo de gordura perifaríngea, diminui a

área transversal da faringe, aumentando a sua colapsabilidade. Sexo feminino é protetor contra apneia obstrutiva do sono apenas na idade reprodutiva. Alguns fatores de causalidade modificáveis da apneia obstrutiva do sono são o etilismo e o tabagismo.

As apneias obstrutivas e as hipopneias resultam em alterações nas pressões intratorácicas, hipoxemia intermitente e despertares. Embora esses despertares geralmente não acordem o paciente, essa fragmentação do sono é a principal causa do excesso de sonolência em indivíduos com AOS. Hipoxemia intermitente, particularmente com hipercapnia concomitante, ativa o sistema nervoso simpático, o que causa elevação aguda e crônica da pressão arterial sistêmica. O nível sanguíneo elevado de catecolaminas reduz a sensibilidade insulínica e, em modelo animal, promoveu apoptose das células beta pancreáticas, o que poderia explicar a associação de AOS com diabetes mellitus independente do índice de massa corporal.[41,42]

Os episódios repetidos de hipóxia intermitente levam à formação de espécies reativas de oxigênio, o que contribui para dano vascular, alterações metabólicas e inflamação sistêmica.[3]

Manifestações Clínicas

Os sintomas mais comuns da AOS são fadiga, sensação de sono não reparador, cansaço ou falta de energia. Um sintoma comum e com potencial de graves consequências é a sonolência. Uma revisão sistemática concluiu que na história e exame físico, queixa de respiração interrompida ou sensação de sufocamento durante o sono são os indicadores mais específicos de AOS, enquanto o ronco é sensível, porém não é específico.[43] Sonolência diurna excessiva é relatada por grande proporção dos pacientes com AOS encaminhados para clínicas do sono.[44,45] Um estudo populacional relatou noctúria (pelo menos 2 vezes por noite) em 37,4% dos indivíduos com IAH de pelo menos 20 /h em comparação com 25,6% daqueles com um IAH inferior a 20 eventos/h.[46] Dor de cabeça matinal (ocorrendo pelo menos metade dos dias) é duas vezes mais comum em indivíduos com AOS do que na população em geral. As dores de cabeça, caracterizadas por sensação de pressão bilateral, resolvem horas após o despertar e são de etiologia desconhecida.[47] O refluxo gastroesofágico noturno é aproximadamente duas vezes mais comum em pacientes com AOS.[48] Os fatores de risco e manifestações clínicas da AOS podem ser vistos na **Tabela 2**.

Tabela 2– Fatores de risco e Manifestações Clínicas de AOS

Fator de risco	Odds ratio
Sobrepeso vs. eutrófico	2.3 – 3.4
Obeso vs. eutrófico	4.0 – 10.5
Sexo masculino	1.7 – 3.0
Idade (a cada 10 anos de aumento)	1.4 – 3.2
Status de pós-menopausa	2.8 – 4.3
Manifestação Clínica	**Prevalência, %**
Sonolência excessiva, fadiga, sono não reparador	73 - 90
Ronco	50 – 60
Pausas respiratórias, sufocamento ou engasgo	10 – 15
Noctúria (2 ou mais vezes por noite)	30
Refluxo gastroesofágico noturno	50 – 75
Cefaleia matinal	12 – 18

Razão de chance (odds ratio) e prevalência baseadas nos estudos referenciados no texto.[16,17]

Diagnóstico

O diagnóstico de síndrome da apneia obstrutiva do sono é feito relacionando os achados da polissonografia com as queixas clínicas do paciente. Os questionários disponíveis para avaliar o risco de AOS incluem o Questionário de Berlim[49], desenvolvido para uso no ambiente de atenção primária, e o questionário STOP-Bang[50,51], desenvolvido para triagem pré-operatória. A Escala de Sonolência de Epworth[52] é amplamente utilizada na clínica e em pesquisas para avaliar sonolência, mas apresenta baixa sensibilidade para AOS. Metanálise demonstrou que o questionário com maior acurácia para detecção de AOS é o STOP-Bang. [53] Os achados de exame físico são pouco específicos para AOS, embora seja aproximadamente duas vezes mais comum em indivíduos com sobrepeso e 4 vezes mais comum em indivíduos com obesidade em

comparação com indivíduos sem sobrepeso ou obesidade. Nosso grupo mostrou pela primeira vez que o formato das bochechas pode ter acurácia diagnóstica próxima da do STOP-Bang.[54] O exame das vias aéreas superiores pode identificar anormalidades anatômicas, como hipertrofia tonsilar, macroglossia ou retrognatia, mas os achados normais do exame físico das vias aéreas superiores não excluem AOS. A confirmação diagnóstica requer testes de polissonografia ou poligrafia portátil quando a avaliação clínica sugerir AOS.

O teste de diagnóstico padrão ouro para AOS é a polissonografia em laboratório, durante a qual os parâmetros respiratórios e do sono são monitorados **(Figura 3)**.[55] Uma polissonografia típica baseada em laboratório inclui medidas de (1) fluxo de ar oronasal usando cânula nasal conectada a transdutor de pressão ou através de sensor térmico; (2) esforço respiratório com faixas de indutância torácica e abdominal; (3) saturação de oxigênio por oximetria de pulso digital; (4) ronco usando microfone afixado sobre a traqueia ou filtrando os sinais de baixa frequência do sistema transdutor de pressão da cânula nasal; (5) estágios do sono e despertares usando eletroencefalograma, eletrooculograma e eletromiograma do queixo; (6) achados do eletrocardiograma; (7) posição corporal; e (8) movimento das pernas. Os testes laboratoriais são trabalhosos e causam inconvenientes a alguns pacientes. O custo da polissonografia em laboratório é aproximadamente três vezes maior que o custo do teste de AOS em casa.[56]

O teste de AOS em casa (poligrafia portátil) cresce em importância para diagnosticar AOS. Consiste em medidas de fluxo de ar, esforço respiratório e saturação de oxigênio, mas não contempla estagiamento do sono ou registro de movimentos das pernas. [57] Os sensores são colocados pelo próprio paciente em

casa, seguindo as instruções de profissional da saúde ou por meio de vídeo instrutivo. O teste de AOS em casa em nossa experiência obteve alta sensibilidade (96%) e especificidade 64%, sendo comparável à polissonografia de laboratório.[58]

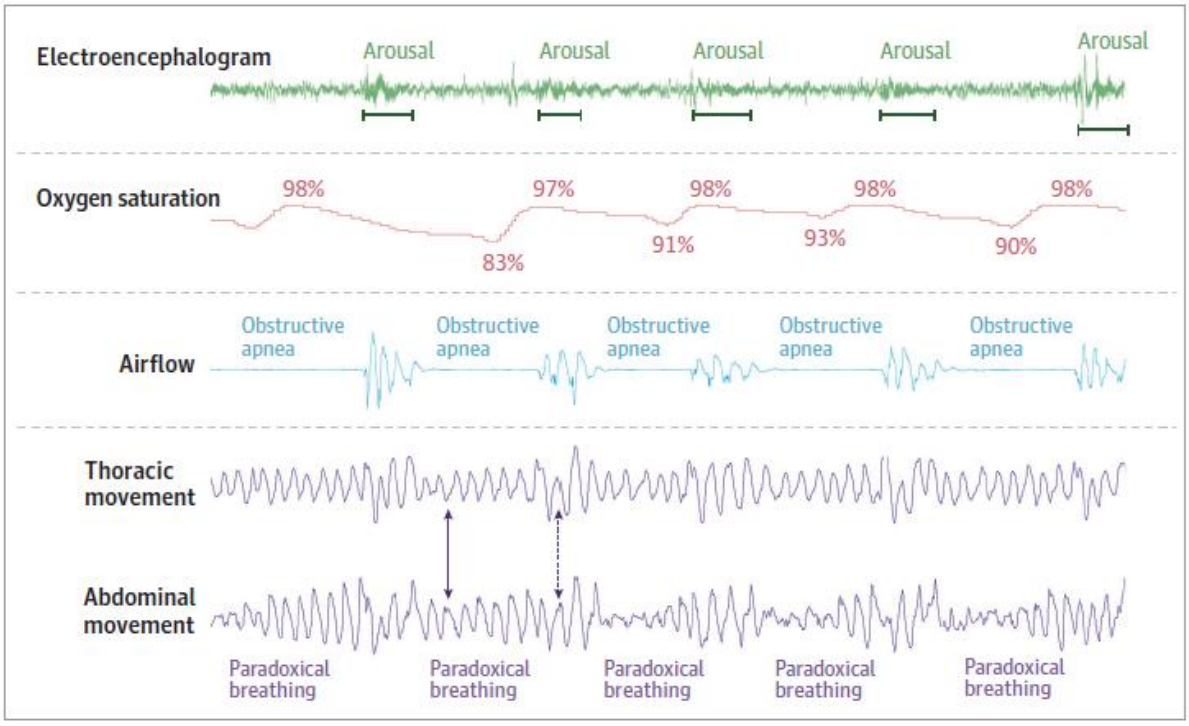

Figura 3 – Polissonografia demonstrando eventos de AOS

Tratamento

O padrão áureo no tratamento da AOS é o uso dos chamados equipamentos de pressão aérea positiva (PAP), em inglês. Os três tipos principais formam um A, B, C; AutoPAP, BiPAP ou CPAP. A PAP impede o colapso da via aérea durante o sono e é indicado para todos os pacientes que tenham AOS e sintomas, independente do IAH na tentativa de aliviar o sintoma. O CPAP elimina os eventos respiratórios durante seu uso.[59] Os benefícios do CPAP são proporcionais à aderência, havendo relação direta dessa com a melhora dos sintomas[60] e a redução da pressão arterial[61].

Algumas modificações comportamentais, tais como evitar dormir na posição supina e ingerir álcool próximo à hora de dormir, podem ser eficazes no tratamento da AOS. A perda de peso reduz o IAH[62] e deve ser recomendada para todos os pacientes com AOS e sobrepeso ou obesidade. No estudo randomizado Sleep AHEAD, 264 pacientes com sobrepeso/obesidade com diabetes tipo 2 mellitus e AOS passaram por intervenção de perda de peso por dieta e exercícios ou um controle de aulas sobre o manejo adequado do diabetes. Em 1 ano, o grupo de modificação de estilo de vida apresentou redução de peso 10,2 kg maior que o grupo controle e uma redução do IAH de 9,7 eventos por hora. Os participantes que tiveram uma perda de peso superior a 10kg apresentaram maior queda no IAH.[63]

Há evidência que atividade física pode melhorar a AOS independentemente da perda de peso.[64] Exercícios físicos também atuam como fatores protetores para AOS. Indivíduos que praticavam atividade física de 1 a 2 horas por semana apresentaram 38% menor risco de AOS em comparação com indivíduos que não praticavam exercícios físicos, e esse valor sobe para 61%

nos que praticavam de 3 a 6 horas por semana, após ajuste para idade, sexo, hábitos corporais e sonolência diurna.[65]

Os aparelhos orais (dispositivos de reposicionamento mandibular) são opções de tratamento eficazes, principalmente para indivíduos com AOS leve a moderada. 69 Uma metanálise de 2015 de 34 ensaios clínicos randomizados descobriu que esses dispositivos estavam associados a uma redução média no IAH de 13,6 (IC 95%, 12,0-15,3) eventos por hora. 75

A modificação cirúrgica das vias aéreas superiores é adequada para pacientes selecionados e frequentemente recomendada para pacientes sintomáticos incapazes de tolerar a terapia com CPAP. O procedimento mais extensamente estudado é a uvulopalatofaringoplastia. Outros procedimentos incluem faringoplastia de parede lateral e redução cirúrgica da língua. As estruturas ósseas da face também podem ser modificadas para controlar a AOS. O procedimento mais bem estudado é o avanço maxilomandibular, no qual a via aérea superior é alargada por meio de osteotomias maxilares e mandibulares bilaterais com bons resultados. [66]

As terapias farmacológicas testadas em indivíduos com AOS incluem drogas propostas para aumentar o tônus muscular das vias aéreas ou o *drive* ventilatório. A maioria dessas terapias foi estudada em pequenos ensaios, muitas vezes com administração por uma única noite, e nenhuma demonstrou eficácia claramente.[67] Um estudo de 2019 avaliou a combinação de um inibidor da recaptação da norepinefrina (atomoxetina) e a oxibutinina, que possui ação antimuscarínica. Neste estudo cruzado, randomizado e de dose única, 20 pacientes com IAH mediano de 28 eventos/hora, a terapia combinada reduziu o IAH em uma mediana (intervalo interquartil) de 15,9 (7,3-35,3) eventos por hora

em comparação com o placebo.[68] Em um ensaio randomizado de 73 pacientes com gravidade semelhante de AOS, o agonista do receptor de canabinoide dronabinol reduziu o IAH médio (desvio padrão) em 12,9 (4,3) após 6 semanas de terapia.[69] Embora promissores, esses tratamentos permanecem sob investigação. O tratamento com montelucaste, um antagonista do receptor de leucotrieno com efeitos anti-inflamatórios, reduziu o IAH em crianças (diferença média de -2,7 eventos/h; IC 95% -5,6 a 0,3) com melhora na saturação de oxigênio mais baixa de 89,5 para 92 quando comparado ao placebo.[70] Em um estudo randomizado com 26 participantes, o tratamento com montelucaste não afetou o IAH de adultos com AOS, no entanto, o tempo total de sono e a porcentagem do sono no estágio R aumentaram significativamente.[71]

Apneia Obstrutiva do Sono e Doença Cardiovascular

As doenças cardiovasculares são responsáveis por 20% dos óbitos de brasileiros acima de 30 anos e são a morbimortalidade principal em idosos.[72] Em 2014 foram publicadas quatro metanálises sobre mortalidade da AOS. Em 2016, revisão sistemática e metanálise avaliou a mortalidade geral e cardiovascular associada à AOS com e sem a terapia CPAP. Foram incluídos 27 estudos de coorte, totalizando 3.162.083 participantes, cuja idade variava de 20 a 81 anos. Ao avaliar mortalidade cardiovascular, AOS se aumentou significativamente o risco desse desfecho (HR 2.73; 95%CI, 1.94–3.85). Significativa redução na mortalidade cardiovascular foi constatada nos 7 estudos que analisaram o efeito do CPAP na AOS comparando com pacientes com AOS que não realizaram tratamento (HR 0.37; 95 % CI 0.16–0.54), sem heterogeneidade significativa (I^2= 5.7 %, P= 0.35).[14]

Apneia obstrutiva do sono é fator de risco independente para acidente vascular cerebral (AVC).[73] Além disso, o tratamento da AOS com CPAP parece se associar a menor risco de AVC, conforme demonstrado em revisão sistemática e metanálise publicada em 2019 que incluiu 13 estudos (9 ensaios clínicos randomizados e 4 coortes). O efeito, entretanto, é observado apenas na análise de subgrupo que incluiu pacientes com AOS moderada-grave e com boa aderência ao CPAP (mais de 4 horas de uso em 70% ou mais do período analisado).[74]

Pacientes com apneia, comparados com a população geral, possuem mais chance de apresentar hipertensão[75,76], cardiopatia isquêmica[77-79] e doença cerebrovascular[42,80]. Em 2004, comitê do National Heart, Lung, and Blood

Institute propôs linhas de pesquisa para corrigir os déficits de conhecimento sobre as consequências dos distúrbios do sono nas cardiopatias.[81] A American Heart Association e o American College of Cardiology publicaram consenso indicando a necessidade de pesquisas como a nossa que abordem as principais consequências cardiovasculares da AOS.[82] As razões principais para AOS estar associada a doenças cardiovasculares são os períodos de hipóxia intermitente e os repetidos despertares noturnos principiados pelas apneias. Os despertares noturnos causam hiperatividade crônica do simpático e a hipóxia intermitente causa estresse oxidativo[1], o que ocasiona inflamação[83], surgimento de disfunção endotelial e, mais tarde, aterosclerose. A associação entre doença arterial coronariana e AOS parece ser consistente.[84] Na análise transversal da coorte do Sleep Heart Health Study, observa-se aumento de 27% no risco para coronariopatia nos indivíduos do quartil mais alto de IAH.[85] A AOS aumenta a chance de apresentar cardiopatia isquêmica em 65%[86] e de sofrer infarto em 71%[87]. Nosso grupo demonstrou que AOS é um fator de risco mais robusto para doença arterial coronariana do que os fatores clássicos como colesterol, em uma amostra excluindo obesos mórbidos, diabéticos e fumantes[88].

Função Endotelial

O endotélio, por área isolada, é um dos maiores órgãos do corpo, composto por trilhões de células, pesando mais de 1 kg e cobrindo quase 3m², em um homem de 70 kg.[89] Interage com múltiplos sistemas e tem sido implicado na patogênese de doenças neurológicas, renais, hepáticas, vasculares, dermatológicas, imunológicas e cardiovasculares. Esse tecido altamente especializado é responsável pela homeostase vascular através da regulação do tônus arteriolar, da agregação plaquetária, adesão de leucócitos e da oferta de oxigênio aos tecidos pelos eritrócitos. A regulação do tônus é feita pela secreção do vasodilatador óxido nítrico (NO). Danos ao endotélio e disfunção desse tecido estão associados a surgimento de hipertensão arterial e aterosclerose. A disfunção endotelial se caracteriza pela redução de expressão da enzima NO sintase, que diminui a síntese de NO e aumenta expressão de moléculas de adesão leucocitária pelas células endoteliais, tais como VCAM-1. Ocorre então secreção de fatores pró-trombóticos e de citocinas pró-inflamatórias, que propiciam o surgimento da lesão aterosclerótica.

Essa alteração é reconhecida por ser a conexão mais crítica entre fatores de risco e doença clínica estabelecida, manifestando-se, portanto, como o primeiro sinal de doença cardiovascular.[90] Muitos mecanismos moleculares e celulares estão envolvidos com o dano endotelial e envelhecimento vascular. A inflamação e o estresse oxidativo fazem parte desses mecanismos e podem ser considerados alvos terapêuticos futuros para prevenção de doença cardiovascular.[11] Artigo de revisão sobre o tema defende que a maior compreensão da função endotelial propicia não apenas o entendimento da fisiopatologia da doença cardiovascular, mas também a oportunidade de

tratamento clínico, detecção precoce de doenças, estratificação de risco cardiovascular e avaliação de resposta terapêutica. [91]

Apneia Obstrutiva do Sono, Estresse Oxidativo e Inflamação

Os ciclos de hipóxia e reoxigenação observados nos pacientes com AOS (Figura 3) levam ao desequilíbrio entre a produção de espécies reativas de oxigênio/nitrogênio e o sistema de defesa antioxidante, resultando em um sério distúrbio da homeostase redox.[92] As espécies reativas de oxigênio (ROS), além de causarem danos às biomoléculas e alterarem as funções celulares, também funcionam como moléculas sinalizadoras em condições fisiológicas e fisiopatológicas, acionando a cascata inflamatória. As ROS causam danos ao endotélio e estimulam a expressão das moléculas de adesão de leucócitos (L-selectina, integrinas) e moléculas de adesão endotelial relacionadas (E-selectina, P-selectina, ICAM-1, VECAM-1), o que leva à alteração da função micro e macro vascular observada nos pacientes com OAS.

O estresse oxidativo pode ser avaliado pela presença de biomarcadores em sangue venoso periférico, como catalase, superóxido dismutase, e glutationa peroxidase. Sinais indiretos de estresse oxidativo são a vasorreatividade prejudicada, alteração da espessura da camada íntima dos vasos ou até por biópsia vascular. Estudos demonstraram que pacientes com AOS possuem aumento da liberação de superóxido por leucócitos[93,94], redução da biodisponibilidade de NO[95,97], maior concentração de lipídios, proteínas e DNA oxidado[98-102], redução da capacidade antioxidante[103] e redução da enzima sintetizadora de NO em biópsias[104]. Nosso grupo publicou estudos demonstrando que hipóxia intermitente causa estresse oxidativo, inflamação e

dano a lipídios e a proteínas.[2] Esses são apenas alguns dos diversos dados que apontam para uma associação entre AOS e estresse oxidativo.

O estresse oxidativo altera as vias de sinalização e ativa as respostas inflamatórias/imunológicas por meio de interações aumentadas das células sanguíneas com as células endoteliais, ocasionando lesão endotelial. A hipóxia intermitente ativa peptídeos derivados do endotélio sensíveis à hipóxia, como endotelina e fator de crescimento endotelial vascular (VEGF), levando à angiogênese, ao aumento da coagulação e à diminuição da fibrinólise, bem como à perturbação dos mecanismos de reparo vascular.[105-107] Encontrou-se uma relação entre a função endotelial avaliado por FMD e os níveis de ADMA (inibidor da produção de NO) , peptídeo solúvel derivado de NOX2 e isoprostanos (marcadores de estresse oxidativo)[108-110], confirmando que a disfunção endotelial vista na AOS está associada ao estresse oxidativo e à inflamação **(Figura 4)**[111].

Assim, é aceito que apneia do sono leva ao estresse oxidativo, que leva à disfunção endotelia e à inflamação sistêmica.[3,112] Estudos sugerem que, além da inflamação sistêmica, ocorre inflamação localizada nas vias aéreas dos pacientes com AOS, provavelmente devido ao estresse mecânico dos repetidos colapsos das paredes da faringe.[4,5] AOS é considerada uma doença inflamatória crônica, assim como aterosclerose, e inflamação crônica está intimamente relacionada ao surgimento de doenças cardiovasculares. A inflamação sistêmica e local em pacientes com AOS é resultado da hipoxemia intermitente crônica[113,114], ronco[115], estresse oxidativo e fragmentação e privação do sono[116]. Nas últimas duas décadas, muitos estudos investigaram as interações entre AOS e inflamação em adultos e crianças, e alguns estudos tentaram elucidar os

biomarcadores envolvidos na AOS e suas comorbidades associadas. Em pacientes com AOS, se observa níveis elevados de biomarcadores inflamatórios, tais como proteína C-reativa (PCR), fator de necrose tumoral alpha (TNF-α), interleucina-6 (IL-6) e interleucina-8 (IL-8).[117] Metanálise com foco em AOS e inflamação observou que, em comparação com o grupo controle, os pacientes com AOS apresentavam níveis significativamente mais elevados de PCR, TNF-α, lL-6, IL-8, ICAM, VCAM e selectinas. O estudo indica que os fatores inflamatórios mais proeminentes apresentados na AOS incluem IL-1, IL-6 e PCR. Além disso, concluiu que as alterações nos níveis de citocinas estavam relacionadas com a idade, índice de massa corporal e IAH dos pacientes.[118] A terapia com CPAP reduz esses marcadores inflamatórios[119,120] Um estudo transversal conduzido por Bouloukaki e colegas incluiu 1.053 pacientes com AOS sem outras comorbidades agrupados de acordo com o IAH em grupos de controle, AOS leve, moderada e grave. Eles coletaram sangue venoso de todos os indivíduos para medir os níveis de PCR, fibrinogênio e taxa de sedimentação de eritrócitos. Os níveis de PCR e fibrinogênio foram significativamente mais elevados no grupo com AOS grave em comparação com o grupo com AOS leve. Curiosamente, todos esses biomarcadores, exceto o fibrinogênio, foram correlacionados com o tempo de sono com saturação de oxigênio inferior a 90%.[121] Estudos semelhantes conduzidos por outros pesquisadores demonstraram resultados convergentes; PCR foi positivamente correlacionado com IAH, mesmo ajustando para IMC.[122]

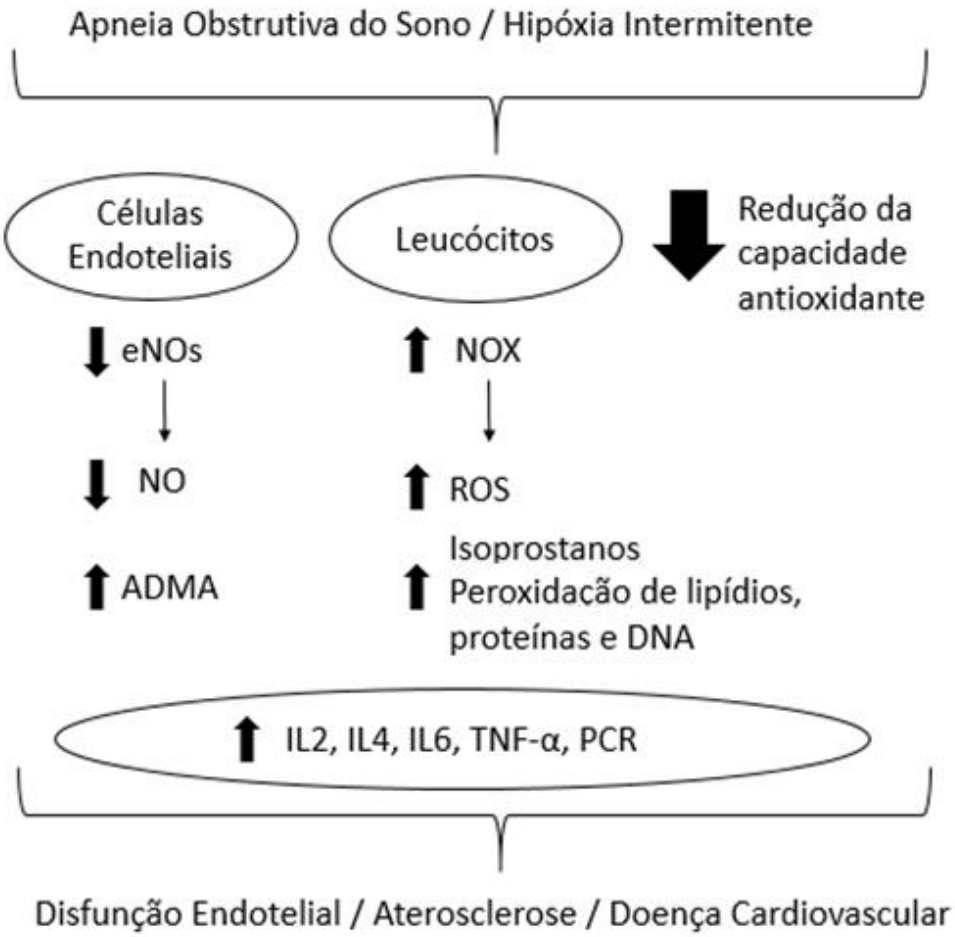

Figura 4 - Estresse oxidativo como via intermediária da doença cardiovascular associada à AOS. A hipóxia intermitente característica da AOS leva ao aumento da capacidade oxidativa de leucócitos por meio da ativação de NOX. Espécies reativas de oxigênios (ROS) excessivamente produzidos aumentam a peroxidação lipídica e a formação de isoprostano. A biodisponibilidade do NO é reduzida pela diminuição da expressão de eNOS e sua inibição pelo ADMA. Finalmente, a capacidade antioxidante é prejudicada nos pacientes afetados, e a cascata inflamatória é ativada. ADMA: dimetilarginina assimétrica; eNOS: óxido nítrico sintase endotelial; NO: óxido nítrico; NOX: NADPH oxidase; ROS: espécies reativas de oxigênio; IL2: interleucina-2; IL4: interleucina-4; IL6: interleucina-6; TNFα: fator de necrose tumoral alpha; PCR: proteína C-reativa

Apneia Obstrutiva do Sono e Disfunção Endotelial

Dados de metanálise de 28 estudos, publicada em 2017, totalizando 1.496 pacientes com AOS e 1.135 controles, concluíram que a função endotelial avaliada por FMD no grupo AOS era reduzida (diferença %FMD −3.07; IC 95% −3.71 a −2.43; P<0.01), **Figura 5**.[12] A hipótese mais aceita para explicar esse achado é que a hipóxia intermitente eleva a produção de espécies reativas de oxigênio (ROS), causando o estresse oxidativo e menor atividade da eNOS. Isso causa atenuação da produção de NO, o principal vasodilatador, pelo endotélio e comprometimento da função endotelial. Acredita-se que a interrupção desta delicada homeostase vascular leva a uma sequência de eventos patológicos, incluindo vasoconstrição excessiva, aumento das moléculas de adesão, ativação da cascata inflamatória com liberação de citocinas, oxidação aumentada de lipoproteínas, ativação de estados pró-trombóticos, e a formação da placa aterosclerótica. Essa deficiência de NO pode ser um dos mecanismos fisiopatológicos envolvidos no desenvolvimento da hipertensão vista em pacientes com AOS, entretanto mais estudos são necessários para confirmar essa suposição.[123]

A associação entre disfunção endotelial e AOS foi inicialmente considerada ambígua por existirem numerosos traços comuns entre pacientes com disfunção endotelial e com AOS. Idade, obesidade, tabagismo e consumo de álcool eram fatores citados como causas potenciais das duas condições, confundindo eventual relação de causa-efeito. Metanálise de Wang et al concluiu que a AOS moderada/grave se associa à disfunção endotelial, ao aumento da rigidez arterial e ao aumento dos níveis séricos de marcadores inflamatórios. Os dados da metarregressão sugerem que o efeito adverso da

AOS moderada-grave na função endotelial não é modificado por confundidores potenciais, tais como o IMC.[124] Análises transversais de estudos populacionais e estudos de caso-controle demonstraram consistentemente associação entre a gravidade da apneia obstrutiva do sono e o prejuízo da vasodilatação dependente de endotélio. Embora a maioria dos estudos utilize técnicas que avaliam a função endotelial da macrovasculatura, a associação ocorre tanto a níveis macro quanto microvasculares.[125] Experimentos *in vitro* demonstram que o soro de pacientes com AOS prejudica a migração de células endoteliais coronarianas.[126]

Metanálise publicada em 2015 avaliando o efeito do CPAP na função endotelial de pacientes com AOS concluiu melhora após tratamento (diferença média %FMD 2.92; IC 95% 2.21-3.63; P < .001). As análises de sensibilidade indicaram efeito protetor do CPAP na função endotelial.[16] Outra metanálise, publicada em 2019, incluiu estudos prospectivos de pacientes afetados por AOS leve a grave tratados CPAP, cirurgia, aparelho intraoral ou terapia farmacológica. A função endotelial avaliada por FMD foi medida antes e depois do tratamento. Nessa metanálise, o tratamento da AOS mostrou um impacto positivo na função endotelial (diferença média = 2.58; IC 95% 1.95-3.20; P <0,00001), **Figura 6.** [15] Os dados apontam, portanto, para uma associação entre disfunção endotelial e AOS, que aparentemente é reversível mediante tratamento que acarrete na interrupção/redução das apneias.

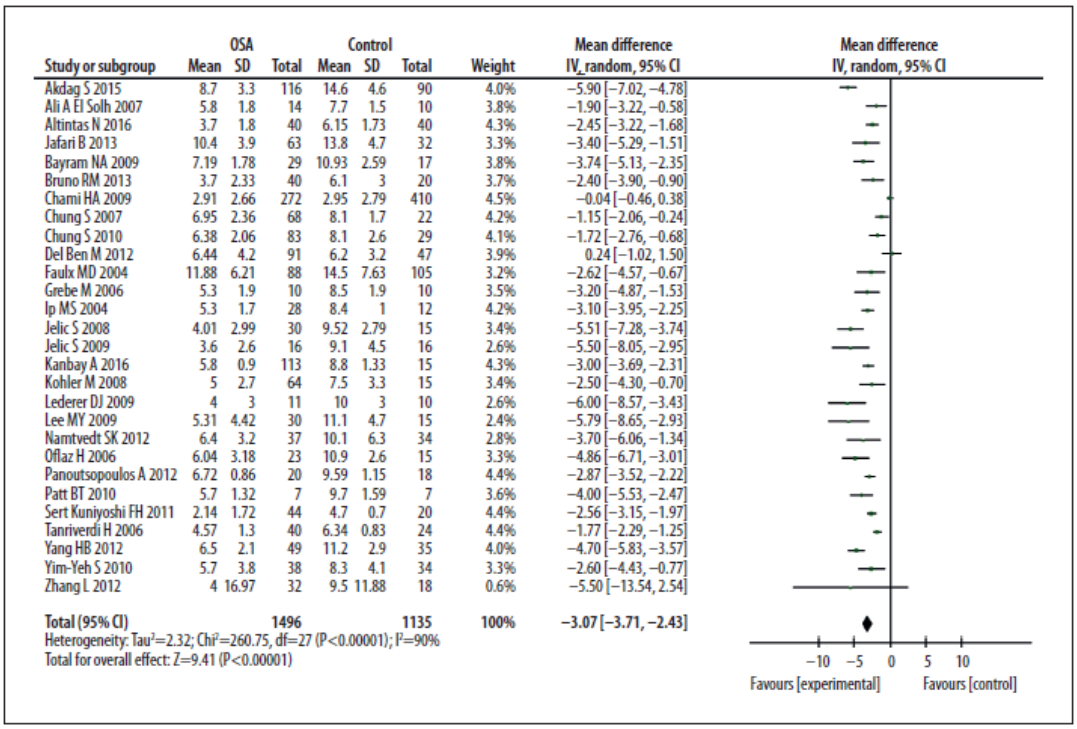

Figura 5 – Metanálise comparando a função endotelial avaliada por FMD em pacientes com apneia do sono e controles

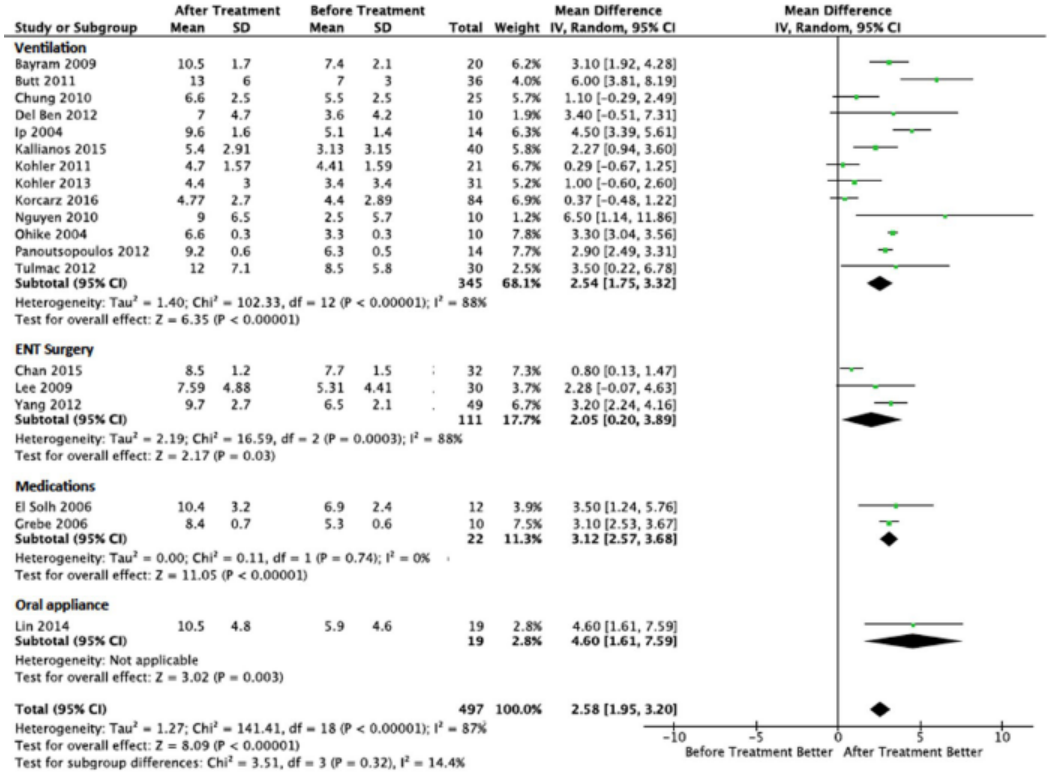

Figura 6 – Metanálise demonstrando a melhora da função endotelial avaliada por FMD com diversos tipos de tratamento para apneia do sono

Dilatação Fluxo-Mediada da Artéria Braquial

A avaliação da dilatação fluxo-mediada (FMD, em inglês) foi introduzida como uma abordagem não invasiva para examinar a função vasodilatadora in vivo. Consiste em provocar isquemia do membro superior durante 5 minutos e avaliar a vasodilatação endotélio-dependente em percentagem de aumento do diâmetro da artéria braquial (**Figura 7**). Os parâmetros calculados na FMD são: fluxo, diâmetro da artéria braquial, estresse de cisalhamento medido pela área sob a curva de fluxo.

Os resultados da FMD quantificam a função arterial endotélio-dependente mediada por NO, sendo usado como marcador indireto da higidez vascular.[127] Metanálise que incluiu 32 estudos e 15 mil indivíduos concluiu que o resultado da FMD é preditor independente de evento cardiovascular e morte. Cada 1% a mais de dilatação na FMD associa-se a risco 10% menor de evento cardiovascular ou morte, **Figura 8**. Nesse estudo, o efeito preditivo da FMD braquial foi mais substancial para mortalidade cardiovascular do que para mortalidade geral, sugerindo que a função endotelial comprometida seja fator de risco predominantemente cardiovascular. Além disso, o risco cardiovascular associado à pior função endotelial é maior em pacientes com doença cardiovascular já existente em comparação com pacientes sem doença estabelecida.[10]

O método apresenta algumas limitações que devem ser levadas em consideração. Pequenas alterações na abordagem metodológica podem impactar criticamente a variabilidade e diminuir a reprodutibilidade do resultado do exame. Destaca-se, portanto, a importância de treinamento para a aplicação rigorosa do método atualizado e padronizado para reduzir o erro de aferição da

dilatação fluxo-mediada e, consequentemente, melhorar sua confiabilidade em estudos clínicos. A aderência ao uso de diretrizes ao se fazer uso dessa técnica comprovadamente aumenta a reprodutibilidade dos resultados.[128] Estudo publicado em 2019 no *Hypertension* que correlacionou a função coronariana durante cateterismo cardíaco com a função vascular avaliada por FMD concluiu que a dilatação fluxo mediada da artéria braquial foi fortemente correlacionada com a mudança induzida pela acetilcolina no diâmetro da artéria coronária ($r= 0,77$; $P< 0,0001$) e foi forte indicador de disfunção da artéria coronária (acurácia de 78%), o gráfico da correlação pode ser visualizado na **Figura 9**.[129]

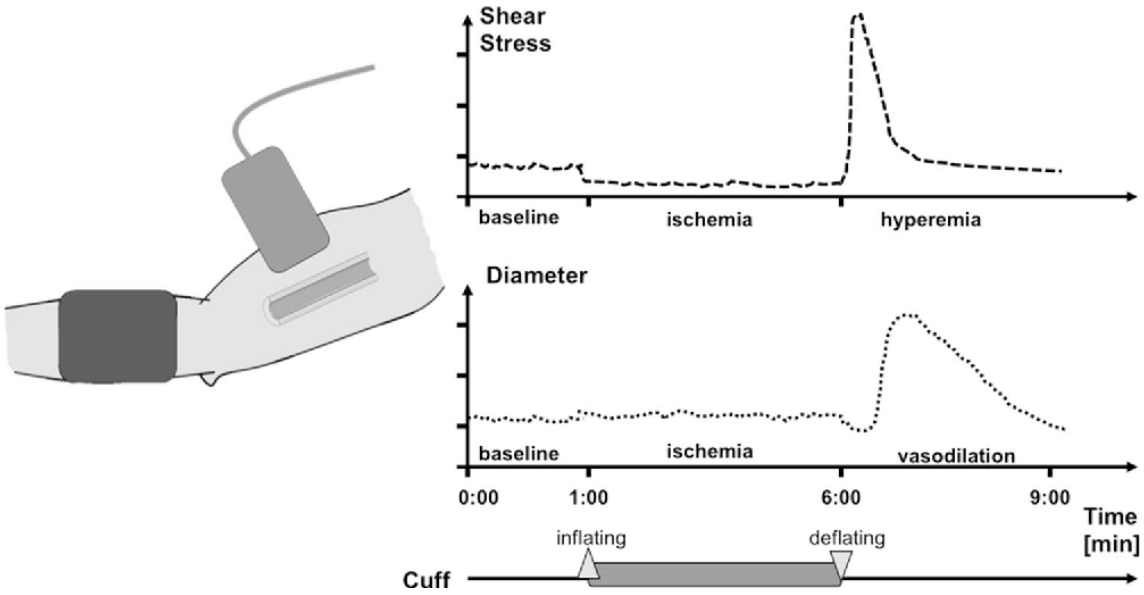

Figura 7 - Representação esquemática da avaliação da dilatação mediada por fluxo da artéria braquial: o desenho mostra a posição do manguito no antebraço e da sonda de ultrassom no braço. Os gráficos mostram o comportamento das mudanças na taxa de cisalhamento e diâmetro durante a linha de base, isquemia de 5 minutos e após a liberação do manguito. Adaptada de Ghiadoni et al[130]

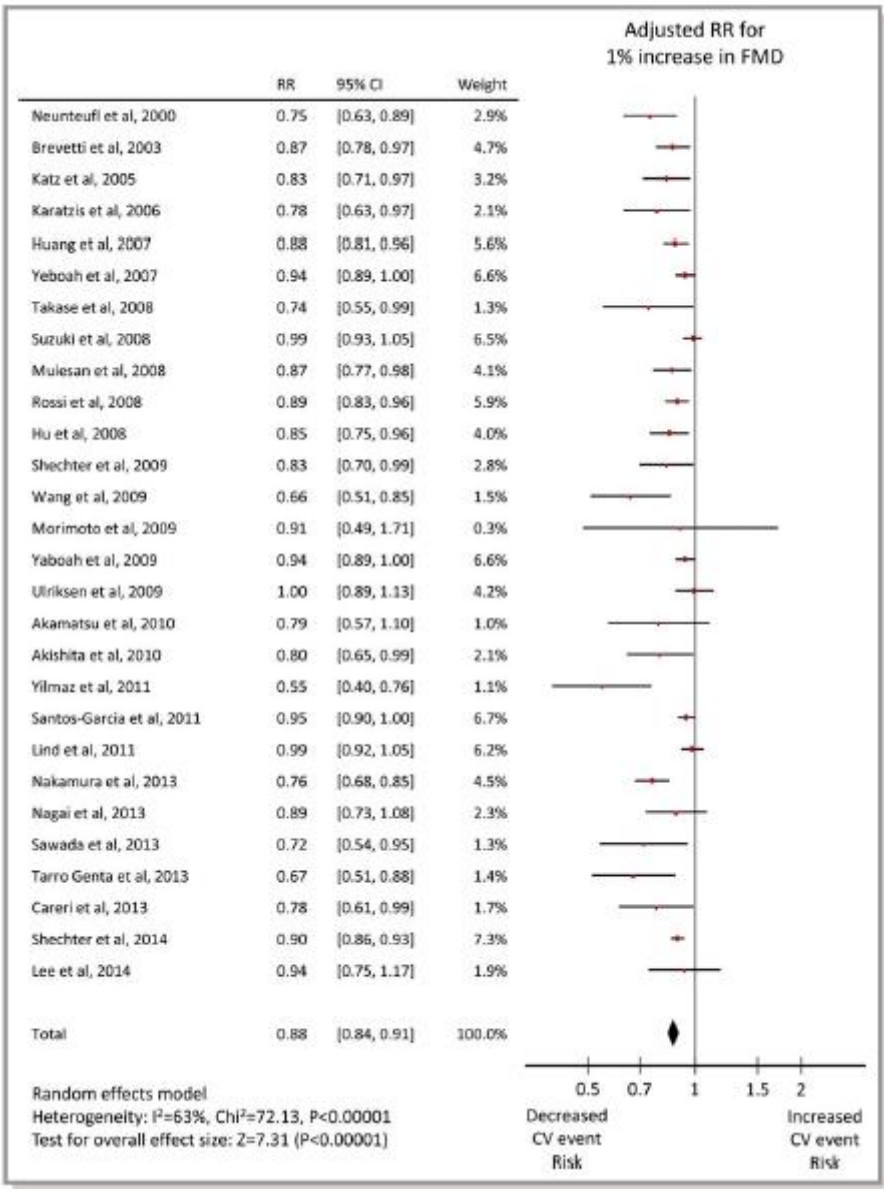

Figura 8 – Metanálise avaliando o valor prognóstico da FMD para eventos cardiovasculares

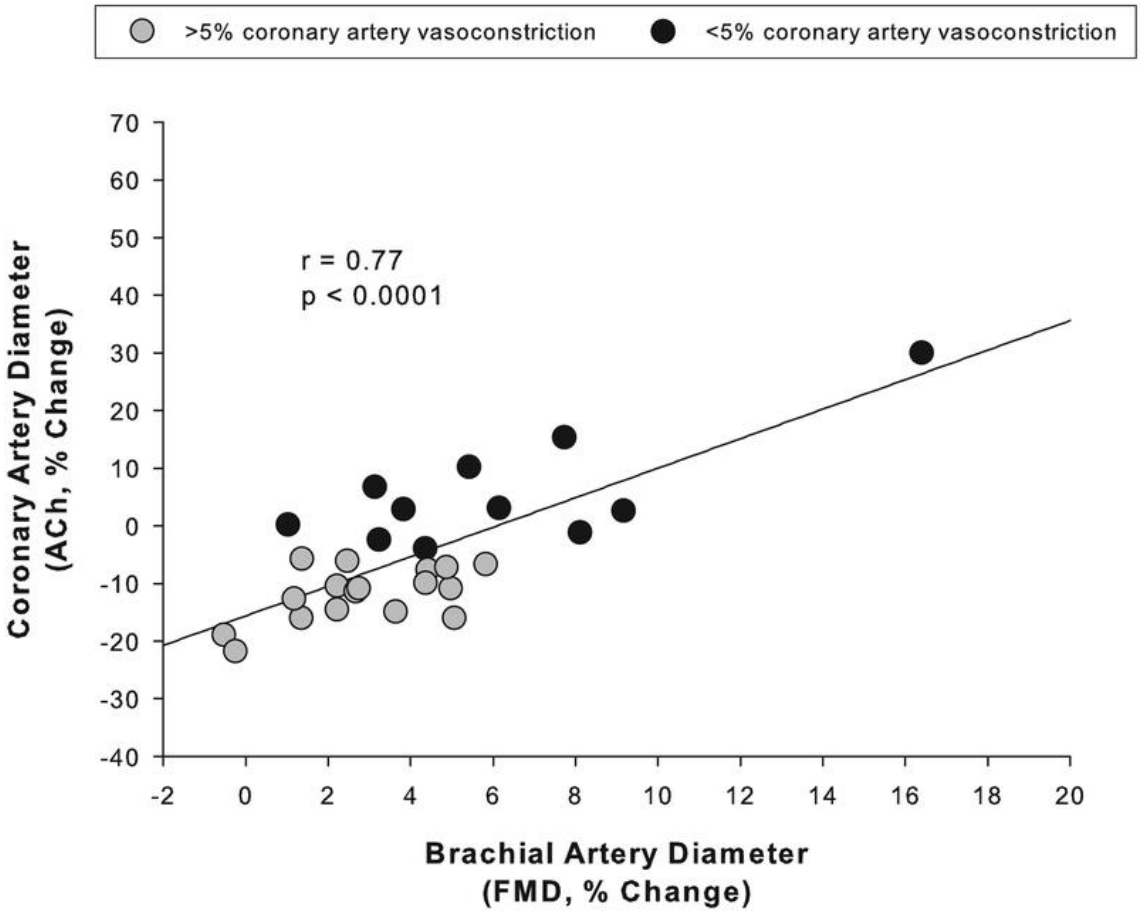

Figura 9 - Relação entre a função da artéria coronária e braquial Correlação de Pearson entre a alteração percentual no diâmetro da artéria descendente anterior esquerda com acetilcolina (ACh) e a alteração percentual no diâmetro da artéria braquial com dilatação mediada por fluxo (FMD).

Tonometria Arterial Periférica

A tonometria arterial periférica (PAT, em inglês) destaca-se como um método não invasivo, acurado e seguro de avaliar a função endotelial na prática clínica. O método foi incorporado a diversos estudos populacionais e multicêntricos, como o Framingham Heart Study. Baseia-se nos mesmos mecanismos fisiológicos que a técnica de FMD, induzindo isquemia transitória no dedo por 5 minutos como estímulo para vasodilatação reativa. Inicialmente, um sensor de pressão é colocado ao redor do dedo e inflado acima da pressão sistólica após uma gravação de linha de base; o manguito é desinflado após 5 minutos para medir a hiperemia reativa, conforme mostrado na **Figura 10**. Na FMD se mede o diâmetro da artéria braquial, na PAT, o resultado avaliado é a amplitude do volume do pulso arterial do dedo. Dentre suas vantagens em relação à FMD podem-se citar sua independência de operador e interpretador que medem o diâmetro da artéria braquial e o fornecimento de resultados imediatos, automaticamente calculados. O índice da função endotelial obtido por PAT se correlaciona significativamente com a função endotelial coronariana.[131]

O índice de hiperemia reativa (RHI) avaliado por PAT é preditor de risco cardiovascular em pacientes de alto risco, segundo diversos autores.[132-135] O método também apresenta boa reprodutibilidade, segundo os estudos de Brant et al e Reisner et al.[136,137]

Em dois grandes estudos populacionais, somando mais de 10 mil participantes, observa-se correlação apenas modesta entre FMD e PAT.[138,139] Por essa razão, se decidiu incluir os dois métodos no estudo, visando a quantificar aspectos da macrocirculação e microcirculação, e a possibilitar comparação entre os dois métodos.

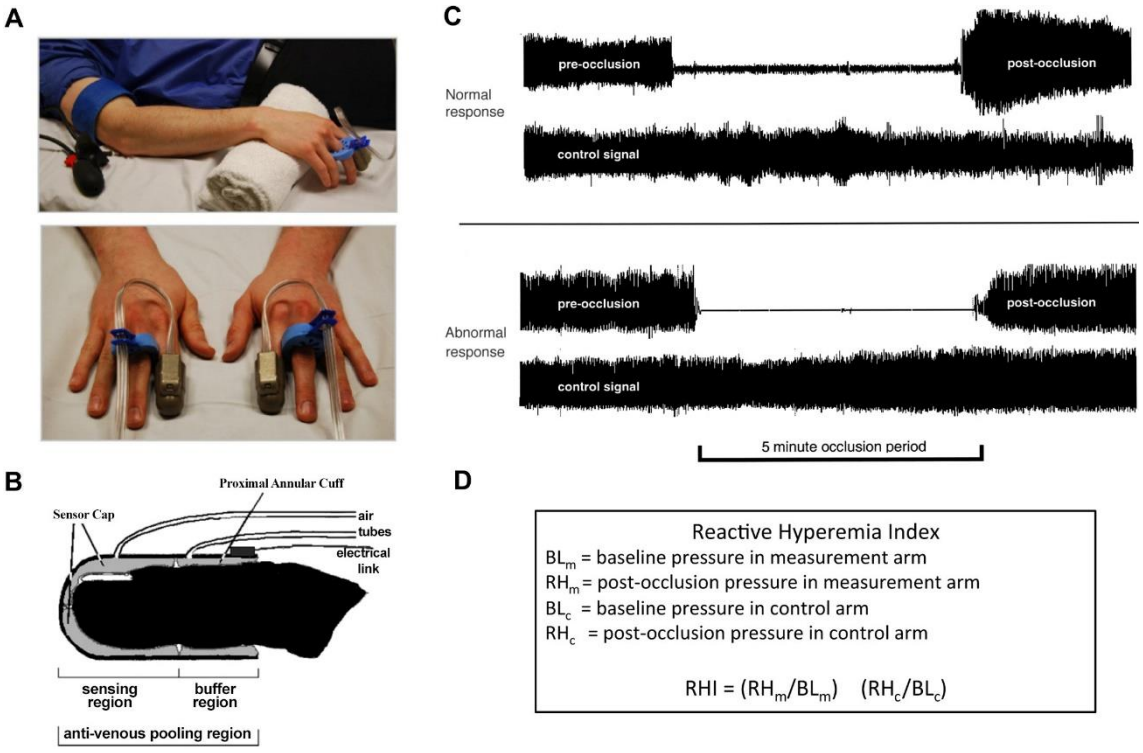

Figura 10 - Hiperemia reativa avaliada tonometria arterial periférica. **A**: um manguito oclusivo é colocado em torno de um braço. Os braços do sujeito são repousados em uma posição ligeiramente elevada para facilitar a drenagem venosa do dedo. [Adaptado de Hamburgo e Benjamin[140]] **B**: biossensores sensíveis à pressão semelhantes a dedais são colocados no dedo médio de cada mão. [Adaptado de Celermajer et al.[141].] **C**: após o estabelecimento de uma linha de base estável, o manguito é insuflado no braço de medição por um período de tempo predeterminado, eliminando as pulsações de pressão no dedo. Depois de decorrido o período de oclusão, o manguito do braço é desinflado. O aumento sanguíneo pós-isquemia faz com que a amplitude da pressão arterial do dedo aumente. [Adaptado de Bonetti et al.[142].] **D**: o índice de hiperemia reativa (RH) (RHI) é determinado pelo cálculo da razão entre a pressão pós-isquemia (RHm) e a pressão basal (BLm) nos braços de medição e controle (RHc e BLc respectivamente). Essa proporção é então calculada, produzindo o RHI. Rosenberry R, Nelson MD. Reactive hyperemia: a review of methods, mechanisms, and considerations. Am J Physiol Regul Integr Comp Physiol. 2020;318(3):R605-R618.

Hidroxicloroquina

O fármaco antimalárico HCQ, por suas propriedades anti-inflamatórias, é comumente utilizado para o tratamento de doenças reumáticas, em particular artrite reumatóide (AR) e doenças do tecido conjuntivo tais como lúpus eritematoso sistêmico (LES). HCQ reduz a ativação do sistema de imunidade inato por inibição da estimulação dos receptores tipo Toll[143], que podem desempenhar um papel importante na ativação de células inflamatórias em pacientes ateroscleróticos[144]. Além de seus efeitos anti-inflamatórios, HCQ possui outras propriedades que podem ser úteis no tratamento de doença arterial coronariana[145]. Estudos de coorte mostraram que a HCQ diminui o colesterol de pacientes com lúpus e artrite reumatoide.[22-25] Além disso, na artrite reumatoide, o uso de HCQ tem sido associada a menor incidência de diabetes tipo 2[26-28], níveis mais baixos de hemoglobina glicosilada (HbA1c)[146] e melhor sensibilidade à insulina e função de células beta[147]. Em dois estudos randomizados de diabéticos com inadequado controle glicêmico, a HCQ reduziu significativamente os níveis de glicose[148,149].

Sharma et al.[17] relataram em estudo retrospectivo, envolvendo 1266 pacientes com artrite reumatoide, associação de HCQ com redução de 72% do risco de desfecho composto de síndrome coronariana aguda, revascularização cardíaca, acidente vascular cerebral, acidente isquêmico transitório, doença arterial periférica e morte súbita. Tomadas em conjunto, as evidências derivadas de estudos em pacientes com doenças reumáticas, convergem para um papel de HCQ na redução de risco cardiovascular. Outros estudos avaliando o efeito de anti-inflamatórios em desfechos cardiovasculares apontam para possível benefício com o uso dessa classe de drogas. No ensaio clínico COLCOT, com

4.745 pacientes, o uso diário de 0,5mg de colchicina, um potente anti-inflamatório, reduziu em 33% a ocorrência de novo desfecho cardiovascular em pacientes que já tinham apresentado infarto do miocárdio.[150] O uso do anti-inflamatório canakinumab, testado no ensaio clínico randomizado CANTOS, quando usado na dose de 150 mg trimestralmente aplicada, reduziu em 17% a incidência de desfechos cardiovasculares, sem efeito observado na mortalidade geral.[151]

Alguns estudos sugerem que a HCQ também reduz a produção de citocinas importantes na patogênese da aterosclerose, como interleucina-1e 6 e fator alfa de necrose tumoral (TNF-α)[152,153]. A terapia de bloqueio do TNF-α foi associada com risco reduzido de doença arterial coronariana entre os pacientes com artrite reumatoide[154]. Nos pacientes com lúpus, a cloroquina inibe a síntese de vários membros da família das metaloproteinases de matriz, especialmente de MMP-9[155], enzimas capazes de degradar o colágeno intersticial na capa fibrótica da placa aterosclerótica[156]. Além disso, HCQ pode ter propriedades antitrombóticas[157,158]. Em camundongos, HCQ reduziu o tamanho e a duração do trombo[159], e provocou diminuição da espessura da parede vascular e progressão da aterosclerose[160]. Em um estudo caso-controle, entre pacientes com lúpus, o uso de HCQ foi associado a um risco reduzido de complicações tromboembólicas[161].

Especulamos que a redução de mortalidade cardiovascular observada em pacientes reumáticos que usaram HCQ pode ter se dado por melhora na função endotelial e consequente prevenção da formação de placa aterosclerótica. Nesse trabalho publicado em 2018, que avaliou o efeito da HCQ em células endoteliais de veias umbilicais humanas, constatou-se o efeito benéfico da HCQ

na resposta inflamatória endotelial através da supressão de TNF-α e consequente inibição da adesão endotelial-leucocitária, vista pela redução da das moléculas ICAM-1 e VCAM-1. O estudo sugere que HCQ pode ser uma abordagem promissora para o tratamento da doença vascular inflamatória.[162] Outro estudo publicado em 2019 avaliou efeito da HCQ em células endoteliais aórticas humanas após terem sido colocadas em contato com anticorpos antifosfolipídicos, que induzem disfunção endotelial. Nesse cenário, HCQ aumentou a produção endotelial de NO, melhorando a vasodilatação endotélio-dependente e consequentemente a função endotelial.[19] Em um modelo animal de lúpus, a aorta dos camundongos mostrou redução das respostas vasodilatadoras endotélio-dependentes e maior contração à fenilefrina, que foram normalizadas após tratamento com HCQ na dose de 10mg/kg/dia por 5 semanas. Observou-se também aumento de espécies reativas de oxigênio NOX-1 e p47 (phox) nos camundongos com lúpus, que apresentaram redução após tratamento com HCQ.[20] Outro estudo usando modelo animal de lúpus também demonstrou aumento da biodisponibilidade de NO e redução das espécies reativas de oxigênio no grupo de ratos tratado com HCQ.[21] Em novas análises do efeito da HCQ em células endoteliais humanas de veias umbilicais, HCQ inibiu a produção de 8-isoprostano e fosfato de dinucleotídeo de nicotinanamida adenina (NADPH), marcadores de estresse oxidativo.[18] Considerando que um dos mecanismos da disfunção endotelial vista na AOS é mediada por hipóxia intermitente e consequente formação de radicais livres de oxigênio que ocasionam estresse oxidativo, testar o efeito da HCQ na função endotelial nesses pacientes é justificável. No entanto, não encontramos estudo abordando o efeito da HCQ sobre a função endotelial em pacientes com AOS.

Efeitos adversos comuns da HCQ ocorrem em 1-10% dos usuários, incluem anorexia, labilidade emocional, cefaleia, visão borrada, dor abdominal, náusea, erupção cutânea e prurido. Raramente, em 0,1-1% dos usuários, pode ocorrer síndrome de Stevens-Johnson, cardiomiopatia e retinopatia. Ocorrem geralmente com uso prolongado e em altas doses.

JUSTIFICATIVA

Considerando-se ser a AOS uma doença crônica com componente inflamatório, assim como as condições reumáticas, supõe-se que a HCQ, por seus efeitos favoráveis na redução de vários fatores de risco cardiovascular e aparente melhora da função endotelial em modelos animais e *in vitro*, possa melhorar a disfunção endotelial associada à AOS.

Considerando o baixo custo da HCQ, a alta prevalência de AOS em idosos e a elevada morbimortalidade das doenças cardiovasculares, especialmente as decorrentes de doença arterial coronariana, justifica-se a busca por abordagens exequíveis em atenção primária que possam reduzir esses desfechos. Assim, o uso de HCQ em idosos com AOS e em alto risco de doença arterial coronariana representa abordagem inteiramente nova para prevenção desta doença.

HIPÓTESE CONCEITUAL

A ação anti-inflamatória da HCQ melhora função endotelial, avaliada por FMD e PAT, em pacientes com AOS moderada/grave.

OBJETIVOS

Objetivo geral

Testar em ensaio clínico randomizado o efeito da HCQ sobre a função endotelial e seus correlatos em idosos com índice de apneia/hipopneia maior que 15 eventos por hora.

Objetivos específicos

Testar em ensaio clínico randomizado o efeito da HCQ sobre:

- A função endotelial mensurada por tonometria arterial periférica e pela dilatação fluxo mediada da artéria braquial.
- O índice de apneia-hipopneia fornecido pelo exame de poligrafia portátil
- Os níveis séricos de proteína C-reativa
- O perfil glicêmico, avaliado por níveis séricos de glicose e HbA1c.
- O perfil lipídico, avaliado por níveis séricos de triglicerídios, colesterol total e colesterol HDL
- Comparar a função endotelial mensurada por tonometria arterial periférica com a função endotelial mensurada pela dilatação fluxo mediada da artéria braquial nos mesmos indivíduos para embasar avaliações sobre a qualidade dos métodos

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ARTIGO 1

Effects of Hydroxychloroquine on endOthelial function in eLDerly with sleep apnea (HOLD): study protocol for a randomized clinical trial

Title

Effects of Hydroxychloroquine on endOthelial function in eLDerly with sleep apnea (HOLD): study protocol for a randomized clinical trial

Names protocol contributors

Leticia Maria Tedesco Silva, Antonio Cortes, Beatriz Rossi, Liliana Boll, Gustavo Waclawosky, Bruna Eibel, Sandro Gonçalves, Maria Claudia Irigoyen, Denis Martinez

Abstract

BACKGROUND: Sleep apnea and coronary artery disease are prevalent and relevant diseases. The mechanism by which sleep apnea leads to coronary artery disease remains unclear. Intermittent hypoxia, caused by sleep apnea, leads to inflammation and consequent endothelial dysfunction. Endothelial dysfunction precedes the development of atherosclerotic disease and the occurrence of cardiovascular events. Agents that potentially act to improve endothelial function can help prevent cardiovascular events. Patients using immunomodulators due to rheumatic diseases have a lower prevalence of cardiovascular diseases. However, the potential cardio protective effect of these drugs in patients without autoimmune diseases is not clear. Hydroxychloroquine (HCQ) is an immunomodulator used to treat rheumatoid arthritis and systemic lupus erythematosus. In addition to its anti-inflammatory properties, HCQ reduces cholesterol and blood glucose levels and has antithrombotic effects. The drug is inexpensive and widely available. Adverse effects of HCQ are rare and occur more frequently with high doses.
OBJECTIVE: In this randomized clinical trial, the effect of HCQ treatment on endothelial function will be tested in seniors with sleep apnea.
METHODS: We will recruit participants over the age of 65 and with moderate-severe sleep apnea from an ongoing cohort. We chose to use this sample already evaluated for sleep apnea for reasons of convenience, but also because the elderly with sleep apnea are vulnerable to heart disease. Endothelial function will be assessed by examining flow-mediated dilation of the brachial artery, the gold standard method, considered an independent predictor of cardiovascular events in the general population and by peripheral arterial tonometry, the most recent and most easily obtained method. Hydroxychloroquine will be used at a dose of 400 mg/daily for eight weeks.
DISCUSSION: Our study aim to obtain evidence, albeit preliminary, of the efficacy of hydroxychloroquine in improving endothelial function and reducing cardiovascular risk markers. If the improvement occurs, we plan to design a randomized multicenter clinical trial to confirm the findings.

TRIAL REGISTRATION: ClinicalTrials.gov Identifier: NCT04161339
KEYWORDS: hydroxychloroquine; endothelial function; sleep apnea
Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

Administrative information

Title {1}	Effects of Hydroxychloroquine on endOthelial function in eLDerly with sleep apnea (HOLD): study protocol for a randomized clinical trial
Trial registration {2a and 2b}.	ClinicalTrials.gov, ID: NCT04161339. Registered on November, 2019. Anti-Inflammatory Drug and Endothelial Function (HOLD)
Protocol version {3}	March 2021, version 1.0
Funding {4}	FAPERGS / CAPES / HCPA / IC-FUC/RS
Author details {5a}	Leticia Maria Tedesco Silva, Antonio Cortes, Beatriz Rossi, Liliana Boll, Gustavo Waclawosky, Bruna Eibel, Sandro Gonçalves, Maria Claudia Irigoyen, Denis Martinez
Name and contact information for the trial sponsor {5b}	FAPERGS - Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul Address: Av. Borges de Medeiros, 261 - 2º andar - Centro Histórico, Porto Alegre - RS, 90020-021, Brazil Phone: +55 51 3221-4922
Role of sponsor {5c}	The study sponsor had no role in the design, collection, management, analysis or interpretation of data, nor in the writing of the report.

Introduction

Background and rationale {6a}

Obstructive Sleep Apnea (OSA) and Cardiovascular Disease

In meta-analysis, the prevalence of sleep apnea in the general adult population ranges from 6% to 17%, reaching 49% at advanced ages.[163] In a Brazilian epidemiological study, OSA affected 80 to 95% of the elderly.[164] The most damaging consequence of OSA is an increase in cardiovascular morbidity and mortality.[165] Cardiovascular diseases are responsible for 20% of deaths of Brazilians over 30 years old and lead to increased morbidity and mortality in over 30% of the elderly.[166] Prospective population studies have shown that individuals with severe untreated sleep apnea have a higher risk of general and cardiovascular mortality, independent of traditional cardiovascular risk factors.[167] Apnea patients, compared to the general population, are twice as likely to have hypertension[168], ischemic heart disease[169] and cerebrovascular disease[170]. In 2004, the National Heart, Lung, and Blood Institute committee proposed lines of research to enlighten the consequences of sleep disorders in heart disease.[171] In 2008, the American Heart Association and the American College of Cardiology published a consensus indicating the need for researches like ours that addresses the major cardiovascular consequences of sleep apnea.[172] The main reasons why sleep apnea is associated with cardiovascular diseases are the periods of intermittent hypoxia and the repeated nocturnal awakenings precipitated by apneas. Apnea occurs when anatomical and / or functional changes in the airway, associated with loss of tone in the pharyngeal dilator muscles during sleep, lead to airway collapse and interruption of airflow. Nighttime awakenings cause chronic sympathetic hyperactivity and intermittent hypoxia causes oxidative stress, which causes inflammation, the onset of endothelial dysfunction and, later, atherosclerosis. The association between coronary artery disease and sleep apnea appears to be consistent. In a cross-sectional analysis of the Sleep Heart Health Study cohort, individuals in the highest quartile of apnea-hypopnea index present a 27% risk increase for coronary artery disease. Sleep apnea increases the chance of having ischemic heart disease by 65%[173] and having a heart attack by 71%[174]. Our group demonstrated that sleep apnea is a more robust risk factor for coronary artery disease than classic factors such as cholesterol, in a sample excluding morbidly obese, diabetic and smokers.[175]

Endothelial Function

The endothelium, by isolated area, is one of the largest organs in the body, composed of trillions of cells, weighing more than 1 kg and covering almost 3m² in a 70 kg adult male.[176] It interacts with multiple systems and has been implicated in the pathogenesis of neurological, renal, liver, vascular, dermatological, immunological and cardiovascular diseases. It is a highly specialized tissue, responsible for vascular homeostasis through the regulation of arteriolar tone, platelet aggregation, smooth muscle cell growth and leukocyte adhesion. The endothelium regulates the vascular tone through the secretion

vasodilator, such as nitric oxide (NO) and vasoconstrictors such as endothelin. Damage to the endothelium and dysfunction of this tissue are associated with the development of arterial hypertension and atherosclerosis. There are many molecular and cellular mechanisms involved with endothelial damage and vascular aging. Since inflammation and oxidative stress are part of these mechanisms, tackling with these two processes can be considered as future therapeutic targets for the prevention of cardiovascular disease.[177] A review article on the topic states that a greater understanding of endothelial function provides not only a grasp of the pathophysiology of cardiovascular disease, but also an opportunity for clinical treatment, early detection of diseases, stratification of cardiovascular risk and evaluation of therapeutic response.[178]

Flow-Mediated Dilatation of the Brachial Artery

The assessment of flow-mediated dilation (FMD) was first introduced in the 1990s as a non-invasive approach to examining vasodilator function in vivo. The FMD result quantifies the endothelium-dependent arterial function mediated by nitric oxide, being used as an indirect marker of vascular health.[179] A meta-analysis that included 32 studies and 15,000 individuals concluded that the FMD result is an independent predictor of cardiovascular event and death. Each 1% dilation increase in the FMD result was associated with a 10% lower risk of cardiovascular event or death. In that study, the predictive effect of brachial FMD was more substantial for cardiovascular mortality than for general mortality, suggesting that impaired endothelial function is predominantly a cardiovascular risk factor.[180]
The method has limitations that must be considered. Small changes in the methodological approach can critically influence results and decrease the exam reproducibility. Therefore, the compliance of guidelines with updated and standardized methodology is indicated to reduce measurement errors and improve FMD reliability in clinical studies.[181]

Peripheral Arterial Tonometry

Peripheral arterial tonometry (PAT) is a non-invasive method of assessing endothelial function in clinical practice. The method was incorporated into several population and multicenter studies, such as the Framingham Heart Study. The results are based on digital pulse amplitude variation during reactive hyperemia induced by a 5-minute forearm cuff occlusion. It is operator/interpreter independent and provide immediate results, which are advantages compared to FMD. The reactive hyperemia index (RHI) obtained by PAT is considered to correlate significantly with coronary endothelial function.[182] PAT is a predictor of cardiovascular risk in high-risk patients, according to several authors.[183-186] The method also has good reproducibility, according to studies by Brant et al and Reisner et al.[187] In two large population studies, totaling more than 10,000 participants, there is only a modest correlation between FMD and PAT.[188,189] Therefore, both methods will be used in this study, in order to quantify aspects of both macro and microvascular circulation and compare the two methods.

Endothelial Function and Obstructive Sleep Apnea

Sleep apnea is related to systemic inflammation, oxidative stress and endothelial dysfunction.[190,191] These factors are protagonists in the process that leads patients with sleep apnea to develop cardiovascular disease. The association between endothelial dysfunction and sleep apnea was initially considered ambiguous because there are numerous common features among patients with endothelial dysfunction and sleep apnea. Age, obesity, smoking and alcohol consumption were factors cited as potential causes of the two conditions, confusing a possible cause-effect relationship. A meta-analysis by Wang et al concluded that moderate / severe sleep apnea was significantly associated with endothelial dysfunction, increased arterial stiffness and increased serum levels of inflammatory markers. The meta-regression data suggest that the adverse effect of moderate-severe sleep apnea on endothelial function is not modified by potential confounders, such as body mass index.[192] Cross-sectional analyses of population studies and case-control studies consistently demonstrated an association between obstructive sleep apnea and impaired endothelium-dependent vasodilation.[193] In-vitro experiments demonstrate that the serum of patients with sleep apnea impairs the migration of coronary endothelial cells.[194] Our group published studies demonstrating that intermittent hypoxia causes oxidative stress[195], inflammation and damage to lipids and proteins.[196]

Hydroxychloroquine

The hydroxychloroquine was initially an antimalarial drug. Due to its anti-inflammatory properties, it is commonly used to treat rheumatic diseases, in particular rheumatoid arthritis and connective tissue diseases such as systemic lupus erythematous. HCQ reduces the activation of the innate immunity system by inhibiting the stimulation of Toll-like receptors[197], which can play an important role in the activation of inflammatory cells in atherosclerotic patients.[198] Some studies suggest that hydroxychloroquine also reduces the production of cytokines important in the pathogenesis of atherosclerosis, such as interleukin-1 and 6 and tumor necrosis factor alpha (TNF-α).[199,200] TNF-α block therapy was associated to a risk reduction of coronary artery disease among patients with rheumatoid arthritis.[201]

In addition to its anti-inflammatory effects, HCQ has other properties that may be beneficial in the treatment of coronary artery disease.[202] In patients with lupus, chloroquine inhibits the synthesis of several members of the matrix metalloproteinase family, especially MMP-9[203], enzymes capable of degrading interstitial collagen in the fibrotic layer of the atherosclerotic plaque.[204]

Cohort studies have shown that HCQ lowers cholesterol in patients with lupus and rheumatoid arthritis.[205-208] In addition, in rheumatoid arthritis, the use of HCQ has been associated with a lower incidence of type 2 diabetes[209-211], lower levels of glycosylated hemoglobin (HbA1c)[212] and better sensitivity to insulin and beta cell function[213]. In two randomized studies of diabetics with inadequate glycemic control, HCQ significantly reduced glucose levels.[214,215]

In addition, HCQ may have antithrombotic properties.[216,217] In mice, HCQ reduced the size and duration of the thrombus, and caused a decrease in the thickness of the vascular wall and progression of atherosclerosis.[218,219] In a case-control study, among patients with lupus, the use of HCQ was associated with a reduced risk of thromboembolic complications.[220]

Sharma et al. reported in a retrospective study involving 1266 patients with rheumatoid arthritis an association between HCQ use and a 72% reduction risk of an outcome composed of acute coronary syndrome, cardiac revascularization, stroke, transient ischemic accident, peripheral arterial disease and sudden death.[221]

Evidences from studies in patients with rheumatic diseases converges to a role for HCQ in reducing cardiovascular risk. However, its cardiovascular effects in patients at higher cardiovascular risk but without rheumatic diseases is unknown.

Some common side effects of hydroxychloroquine, occurring in 1-10% of users, include anorexia, emotional labiality, headache, blurred vision, abdominal pain, nausea, rash and itching. Rarely, in 0.1-1% of high-dose users, Stevens-Johnson syndrome, cardiomyopathy and retinopathy may occur.

Overall

Considering that sleep apnea is a chronic disease with an inflammatory component, as well as rheumatic conditions, HCQ, due to its favorable effects in the reduction of several cardiovascular risk factors, may improve endothelial dysfunction associated with sleep apnea.

Considering the low cost of HCQ, the high prevalence of sleep apnea in the elderly and the high morbidity and mortality of cardiovascular diseases, the search for feasible approaches in primary care that can reduce these outcomes is justified. Thus, the use of HCQ in patients with sleep apnea and at high risk for coronary artery disease represents an entirely new approach to this disease.

Objectives {7}

Main Objective

To test in a randomized clinical trial the effect of hydroxychloroquine on endothelial function and its correlates, in elderly people with sleep apnea.

Specific Objectives

- To test in a randomized clinical trial the effect of hydroxychloroquine on endothelial function measured by peripheral arterial tonometry.
- To test in a randomized clinical trial the effect of hydroxychloroquine on endothelial function measured by flow-mediated dilation of the brachial artery.
- Compare the endothelial function measured by peripheral arterial tonometry with the endothelial function measured by the mediated flow dilation of the brachial artery in the same individuals to support assessments of the quality of the methods
- Test in a randomized clinical trial the effect of hydroxychloroquine on inflammatory markers such as reactive C-protein.

- Test in a randomized clinical trial the effect of hydroxychloroquine on glycemic homeostasis, assessed by fasting glucose and HbA1c levels.
- Test in a randomized clinical trial the effect of hydroxychloroquine on the lipid profile
- To test in a randomized clinical trial the effect of hydroxychloroquine on the apnea-hypopnea index and the mean and minimum O2 saturations assessed by portable sleep monitoring device

Trial design {8}

This exploratory study was designed as a randomized researcher and patient blinded controlled trial with a primary endpoint of change in endothelial function after eight weeks of treatment with hydroxychloroquine. Randomization will be performed as block randomization with a 1:1 allocation ratio.

Methods: Participants, interventions and outcomes

Study setting {9}

The study is conducted in the research laboratory of two academic hospitals in Porto Alegre, Brazil: Hospital de Clínicas de Porto Alegre and Instituto de Cardiologia do Rio Grande do Sul.

Eligibility criteria {10}

Inclusion Criteria

- People over 65 years old
- Apnea-hypopnea index greater than 15 events per hour

Exclusion Criteria

- Contraindication to the use of hydroxychloroquine (porphyria, retinopathy, severe hepatic or renal dysfunction, neuropathy and / or muscle disease).
- Rheumatic diseases
- Chronic infections
- Serious, terminal or disabling disease
- Previous ECG with evidence of long QT interval
- Previous or current treatment for obstructive sleep apnea

Who will take informed consent? {26a}

Members of our research group, formed by nurses, physical educators, nutritionists, medical doctors and physiotherapists, will obtain informed consent from potential trial participants. After a brief explanation of the study made by phone, we will schedule an

appointment for those who are willing to participate. In this appointment, all aspects and details of the study will be explained to the potential participant and the informed written consent will be signed and given a copy to the participant.

Interventions

Explanation for the choice of comparators {6b}

Since there is no standard treatment available for endothelial dysfunction, we decided to compare hydroxychloroquine with placebo. The placebo group will receive capsules identical to those of hydroxychloroquine, containing the same excipient as the industrialized product.

Intervention description {11a}

The intervention group will receive a 400 mg hydroxychloroquine capsule daily for eight weeks, to be taken with food or milk during lunchtime. Hydroxychloroquine is generally prescribed at a daily dose of 6.5 milligrams (or less) per kilogram of body weight (using this formula, the dosage for a 70-kilogram person would be 455 mg/day). Considering patients newly diagnosed with lupus take 400 mg once daily, we decided to use the same dose due to its known safety and efficacy.

Criteria for discontinuing or modifying allocated interventions {11b}

The intervention will be discontinued in case of any severe adverse effect or withdrawal of participant consent

Strategies to improve adherence to interventions {11c}

Every 2 weeks after starting the intervention, participants will receive a call to monitor adherence and adverse effects. At the eight-week follow-up visit, participants will have their unused capsules counted and recorded on the case report form.

Relevant concomitant care permitted or prohibited during the trial {11d}

The participants are advised to not initiate any new care during the trial. The participant will be excluded from the trial if any new concomitant care needs to be initiated.

Outcomes {12}

Outcomes

Main Outcome

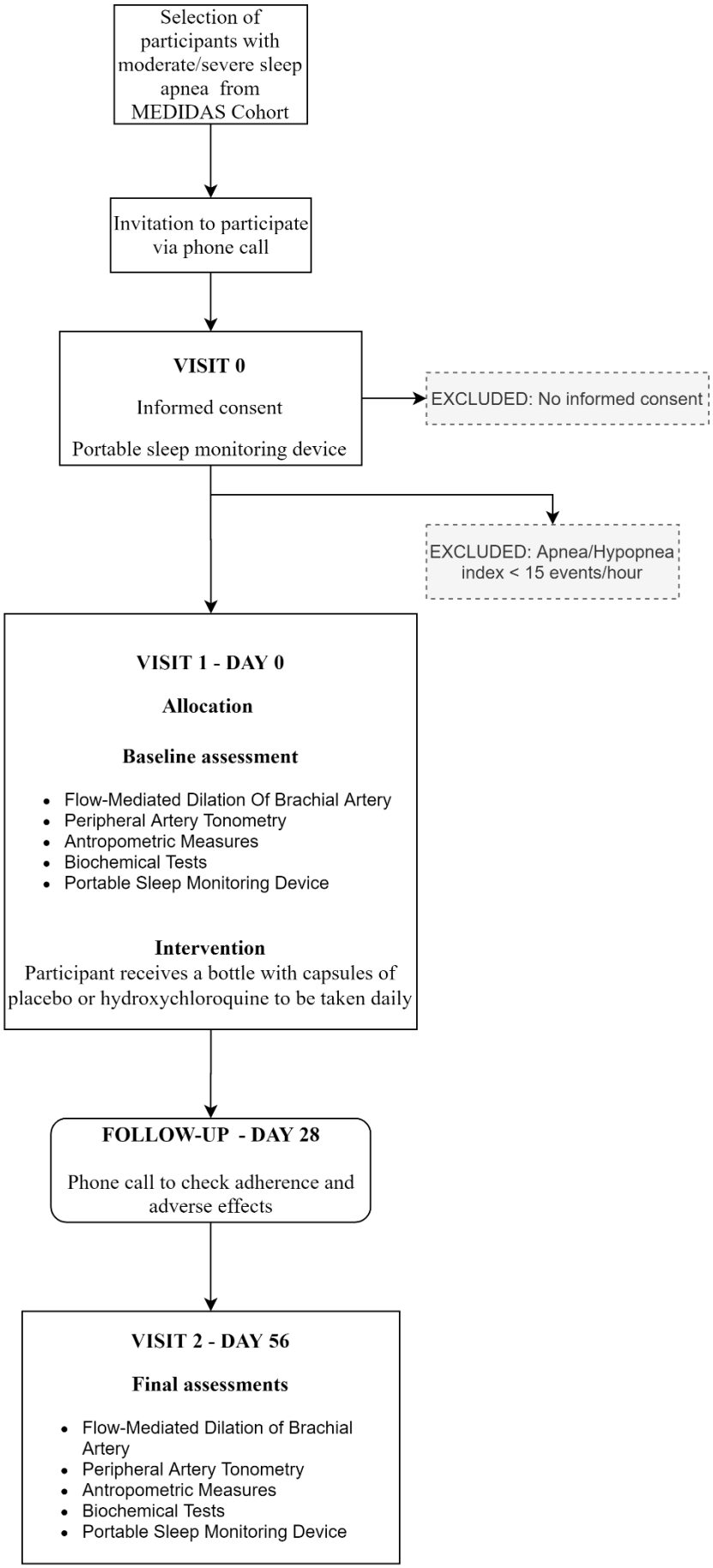
- Difference between the two groups on endothelial function measured by FMD (change of % of dilatation of brachial artery diameter from baseline) and PAT (change of reactive hyperemia index of finger from baseline) after eight week of intervention.

Secondary Outcomes

- Difference between the two groups on changes from baseline in concentration of PCR blood levels
- Difference between the two groups on changes from baseline in the blood levels of HbA1c fraction
- Difference between the two groups on changes from baseline in blood levels of fasting glucose
- Difference between the two groups on changes from baseline in lipid profile (blood levels of fasting total cholesterol, high-density lipoprotein cholesterol and triglycerides)
- Difference between the two groups on changes from baseline in the apnea-hypopnea index and in the mean and minimum oxygen saturation measured with the portable sleep monitoring device
- Difference between the two groups on changes from baseline in weight
- Difference between the two groups on changes from baseline in systolic blood pressure and diastolic blood pressure
- Comparison between rate of adverse effects on intervention x control group.

Participant timeline {13}

Figure 1. shows the time schedule of the study.


Selection of participants with moderate/severe sleep apnea from MEDIDAS Cohort
Invitation to participate via phone call
VISIT 0
Informed consent
Portable sleep monitoring device
EXCLUDED: No informed consent
EXCLUDED: Apnea/Hypopnea index < 15 events/hour
VISIT 1 - DAY 0
Allocation
Baseline assessment
Flow-Mediated Dilation Of Brachial Artery
Peripheral Artery Tonometry
Antropometric Measures
Biochemical Tests
Portable Sleep Monitoring Device
Intervention
Participant receives a bottle with capsules of placebo or hydroxychloroquine to be taken daily
FOLLOW-UP - DAY 28
Phone call to check adherence and adverse effects
VISIT 2 - DAY 56
Final assessments
Flow-Mediated Dilation of Brachial Artery
Peripheral Artery Tonometry
Antropometric Measures
Biochemical Tests
Portable Sleep Monitoring Device

Sample size {14}
Based on the effect size estimate of 0.3 standard deviations in the response of endothelial function after HCQ, for a 95% power and 0.05 level of alpha error probability, the sample size was estimated at 20 participants per group. Allowing for extra recruitment to compensate for losses, the sample size will be 25 participants per group, with a sum of 50 individuals.

Recruitment {15}
Patients will be recruited by telephone. We will contact participants in the MEDIDAS cohort study who meet the inclusion criteria for this study. The MEDIDAS cohort study is composed by elderly with different degrees of obstructive sleep apnea, recruited from a local basic health unit, after positive screening for sleep apnea symptoms. .

Assignment of interventions: allocation

Sequence generation {16a}

Participants will be randomly assigned to either control or experimental group with a 1:1 allocation as per a computer generated allocation list (https://www.randomizer.org/) using permuted blocks of four.

Concealment mechanism {16b}
The bottles with hydroxychloroquine or placebo will be numbered in the handling pharmacy that will produce the capsules. Only the pharmacist will have access to the allocation list. Each participant will match the bottle with the number that participant will receive in the plan. Both patients and researchers involved with either primary or secondary outcome measures will be blinded to the patient's allocation.

Implementation {16c}
Someone from outside the research team will generate the allocation sequence.

Assignment of interventions: Blinding

Who will be blinded {17a}
Trial participants, researchers involved in the study, outcome assessors will be blinded.

Procedure for unblinding if needed {17b}
Unblinding will be permissible in case of severe adverse effects. The pharmacist with the allocation list will be contacted to inform the assigned group of the participant in question.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Biochemical tests

Biochemical assessment will be performed on all participants at the beginning of the study and after 8 weeks of treatment. The exams will be held early in the morning, with participants fasting. Blood samples will be collected in five different sampling tubes with a capacity of 9 ml. The samples will be taken to the laboratory to measure plasma levels of: ultra-sensitive C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides, glucose and glycated hemoglobin.

Peripheral arterial tonometry

The microvascular endothelial function will be determined by an automatic device (EndoPAT2000, Itamar Medical, Cesarea, Israel), with the technique already described. The cuff will be placed on the non-dominant arm, 2 cm above the cubital fossa, and RH-PAT tests on the pulp of each index finger will be performed. The basal pulse amplitude will be measured for 5 minutes. Arterial flow will be interrupted on one side for 5 minutes by insufflation of the cuff, with an occlusion pressure of 200 mmHg, or 60 mmHg above systolic blood pressure. After 5 minutes of occlusion, the cuff is deflated and reactive hyperemia, the RH-PAT sign, is registered in both hands for an additional 5 minutes. The contralateral finger is a control for changes in systemic vasodilation. The reactive hyperemia index (RHI) is automatically calculated by the RH-PAT equipment according to the manufacturer's formula. RHI is defined as the ratio between post-deflation pulse amplitude, 90 to 150 seconds after the cuff is released, and the mean baseline pulse amplitude. This result is divided by the corresponding proportion of the control finger, and multiplied by a correction factor in relation to the baseline. Low RHI values are related to an inadequate endothelial vasodilator response.

Flow-mediated dilation of the brachial artery

High-resolution ultrasound equipment (Esaote) will be used to assess arterial endothelial function. A high frequency transducer will be used to obtain longitudinal images of the brachial artery walls. The images of diameter and arterial flow, at each moment of the protocol, will be recorded simultaneously on a computer with the aid of a capture card (Easycap). To minimize operational errors, both the transducer and the subject's arm will be positioned and maintained in the same position during the examination. Baseline images will be recorded for 1 minute and then the pressure cuff on the forearm will be inflated to 200 mmHg and maintained for 5 minutes, characterizing reactive hyperemia. After deflating the cuff, 3 minutes of endothelium-dependent brachial artery dilation will be recorded. After the exam, the images recorded in MPEG will be converted to MP4 and then analyzed using specific software (Cardiovascular Suite) for the diameter and arterial flow before and after reactive hyperemia.

Portable sleep monitoring device

The portable sleep monitoring device Somnocheck Micro (Weinmann Medical Technology, Hamburg, Germany) will be attached to the patient’s wrist. The patient will

also use a nasal cannula to register airflow. The device will be programed to turn-on at 22pm and turn-off at 10 am. A combination of photoplethysmography-derived pulse wave analysis and respiratory flow signals may enable differentiation between obstructive and central apnea and provide information regarding the extent of sleep fragmentation. In detail, respiratory effort derives by analyzing fluctuations of the pulse wave analysis signal caused by intrathoracic pressure changes during spontaneous breathing cycles. Central apneas, RERAs, mean and minimum oxygen saturation and the apnea-hypopnea index will be reported according to the rules of the American Academy of Sleep Medicine. Portable sleep monitoring device will be performed before and after 8 weeks of treatment.

Plans to promote participant retention and complete follow-up {18b}

In order to promote participant retention and complete follow-up, all participants will be asked to provide, at the beginning of the study, their updated contact informations.

Data management {19}

The web-based application REDCap will be used for data entry and storage. Original study forms will be entered and kept on file at the participating site. Participant files will be maintained in storage for a period of 2 years after completion of the study.

Confidentiality {27}

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with limited access. All laboratory specimens, reports, data collection, process, and administrative forms will be identified by a coded ID [identification] number only to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

Normally distributed data will be presented as mean and standard deviation. Data not normally distributed as median and interquartile range. The natural logarithmic transformation of the variable will be used to correct its non-normal distribution in analyses that assume the normal data distribution. The intervention arm (hydroxychloroquine) will be compared against the control for all primary analysis. We will use chi-squared test for binary outcomes, and T-test for continuous outcomes. We will examine the residual to assess model assumptions and goodness-of-fit. The group x time interaction will be tested using generalized estimating equations. We will use the Bonferroni method to appropriately adjust the overall level of significance for multiple primary outcomes, and secondary outcomes. Results with a probability <0.05 of alpha error will be considered statistically significant. Statistical analyses will be performed using SPSS software (SPSS Inc., Chicago, IL, USA

Interim analyses {21b}
No interim analysis will be performed.

Methods for additional analyses (e.g. subgroup analyses) {20b}
There is no plan to conduct any subgroup or adjusted analyses.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}
Participants who withdraw consent for continued follow-up will be included in the analysis by modern imputation methods for missing data.The effect that any missing data might have on results will be assessed via sensitivity analysis of augmented data sets.

Oversight and monitoring

Composition of the data monitoring committee, its role and reporting structure {21a}
Due to the short duration of the trial and known minimal risks, a data monitoring committee is not needed.

Adverse event reporting and harms {22}
Hydroxychloroquine use may have potential risks for the participants. It is possible that the ingestion of the drug causes side effects (loss of appetite, emotional instability, headache, blurred vision, pain in the belly, nausea, itching and spots on the skin), described in the informed consent. To monitor these side effects, follow-up phone-calls will be made every four weeks and adverse events will be investigated for open-ended questions, including general symptoms, such as headache, nausea and blurred vision. An adverse event that meets the criteria for a serious adverse will be reported to the institutional ethics committee. A serious adverse event for this study is any untoward medical occurrence that is believed by the investigators to be causally related to study-drug and results in any of the following: Life-threatening condition, severe/permanent disability or prolonged hospitalization

Frequency and plans for auditing trial conduct {23}
There is no plan for auditing trial conduct.

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

Important protocol changes as well as changes in eligibility criteria, outcomes or analyses will be communicated to the investigators, institutional ethics committee, trial participants, and trial registries.

Dissemination plans {31a}

The investigators will communicate relevant trial results to participants by telephone call and printed material. The publication in a peer reviewed journal is mandatory for the thesis author have her PhD title acknowledged. The results database will be made available at the university repository.

Discussion

The COVID pandemics forced changes in protocol. The research center was closed and volunteers were instructed to cancel the visits. This incurred in the loss of 4 subjects. An interim analysis was performed to check for the need to continue recruiting. A new sample size calculation was performed. The large number necessary to obtain 80% power made the project unviable. The investigators decided to abandon the project as initially designed. The finding of a small but significant reduction in the OSA severity may be hypothesis-generating. We may continue searching for possible inflammatory mechanisms and therapeutic targets of OSA.

Trial status

The recruitment began on 03/19. Due to the changes in protocol during COVID pandemic, recruitment will not be resumed.

Abbreviations

HCQ - Hydroxychloroquine

OSA - Obstructive Sleep Apnea

NO - Nitric Oxide

FMD - Flow-mediated dilation

PAT - Peripheral artery tonometry

RHI - Reactive hyperemia index

TNF-α - Tumor Necrosis Factor alpha

Declarations

Acknowledgements

Not applicable.

Authors' contributions {31b}

LMTS and DM are the chief investigators, they conceived the study, led the proposal and protocol development. SG, MCI, LB were the lead trial methodologist. BE, GW, BC,AC contributed to the development of the proposal. All authors read, approved, and agreed to the publication of the final manuscript. All named authors adhere to the authorship guidelines of Trials.

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Availability of data and materials {29}

Not applicable.

Ethics approval and consent to participate {24}

All participants will be asked to sign the approved informed consent form prior to participation in the study. The study was approved by the Ethics Committee of the Hospital de Clinicas de Porto Alegre under the number 2017–0472. This committee is accredited by the Office of Human Research Protection as an Institutional Review Board (IRB0000921). The study has been registered under the number NCT04161339 at https://clinicaltrials.gov/ct2/ show/ NCT04161339. The research will be conducted according to the guidelines and regulatory standards for research involving human beings, Resolution No. 466/2012 of the National Health Council.

Consent for publication {32}

Not applicable.

Competing interests {28}

The authors declare that they have no competing interests.

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Authors' information (optional)

Graduate Program in Cardiology and Cardiovascular Sciences, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. 2 Cardiology Unit, Hospital de Clinicas de Porto Alegre (HCPA), UFRGS, Porto Alegre, RS, Brazil. 3 Medical School, UFRGS, Porto Alegre, RS, Brazil.

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ARTIGO 2 –

Effect of Hydroxychloroquine on Endothelial Function in Older Adults with Moderate-Severe Sleep Apnea: a Randomized Clinical Trial

EFFECT OF HYDROXYCHLOROQUINE ON ENDOTHELIAL FUNCTION IN OLDER ADULTS WITH MODERATE-SEVERE SLEEP APNEA: A RANDOMIZED CLINICAL TRIAL

Leticia Maria Tedesco Silva[1], Beatriz Rossi[3], Liliana Boll[3], Gustavo Waclawosky[3], Bruna Eibel[3], Sandro Cadaval[1,2], Maria Claudia Irigoyen[4], Denis Martinez[1,2]

[1]Graduate Studies Program in Cardiology, School of Medicine, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil;

[2]Division of Cardiology, HCPA, Porto Alegre, RS, Brazil

[3]Division of Cardiology, IC-FUC RS, Porto Alegre, RS, Brazil

Reprints requests and correspondence: Denis Martinez, MD, PhD, Division of Cardiology, Interdisciplinary Sleep Research Laboratory - Hospital de Clínicas de Porto Alegre; Universidade Federal do Rio Grande do Sul, Ramiro Barcelos, 2350, Porto Alegre, RS, Brazil Zip Code: 90035-903 Telephone: +5551-33598289 Fax: +5551-30222282 E-mail: denis@sono.com.br

ABSTRACT

BACKGROUND: Obstructive sleep apnea (OSA) is considered a low-grade chronic inflammatory disease. OSA impairs endothelial function and increases cardiovascular mortality. Hydroxychloroquine (HCQ), an anti-inflammatory drug, seems to reduce cardiovascular mortality. In animal and *in vitro* models, HCQ improved endothelial function. Its effects on endothelial function of patients with OSA is not established.

OBJECTIVES: To investigate the effect of hydroxychloroquine on endothelial function in adults with untreated moderate-severe OSA.

METHODS: In this placebo-controlled randomized trial, adults older than 65 years with an apnea-hypopnea index (AHI) greater than 15 events/hour were allocated to receive either 400mg of HCQ or placebo daily for eight-weeks. The randomization was computer-generated and pharmacy-controlled. Participants and outcome evaluators were blinded to the group allocation. Home sleep apnea test and measurements of flow-mediated dilation of brachial artery (FMD) and peripheral artery tonometry (PAT) were performed at baseline and follow-up in a research facility. The primary outcomes were the change in FMD (Δ%FMD) and change in PAT reactive-hyperemia index (ΔRHI) and the secondary outcomes was the effect of HCQ on AHI, glucose and lipid profile, blood pressure, and C-reactive protein. Generalized estimating equations were used to verify time*group interaction.

RESULTS: Fourteen patients were randomly assigned to the HCQ group and fifteen patients to the placebo group between April 2019 and May 2020 with no losses to follow-up. The recruitment was interrupted due to COVID-19 pandemic. Mean Δ%FMD was 0.35 (95% CI −4.26 to 4.97) in the placebo group and 0.48 (95% CI −4.08 to 5.04) in the HCQ group. Mean ΔRHI was 0.02 (95% CI -0.07 to 0.11) in the placebo group and 0.05 (95% CI -0.24 to 0.13) in the HCQ group. Mean ΔAHI was 7 (95% CI -1 to 15) in the placebo group and -4 (95% CI -11 to 2) in HCQ group. P value for time*group interaction were 0.97, 0.74 and 0.04, respectively. No important adverse events have occurred.

CONCLUSIONS: HCQ does not seem to alter endothelial function measured by FMD and PAT in older adults with OSA. The finding of a slight but significant improvement in OSA severity suggests that some specific inflammatory mechanisms may participate in OSA causation that deserves further investigation.

CLINICAL TRIAL: Anti-Inflammatory Drug and Endothelial Function (HOLD), ClinicalTrials.gov number: NCT01945801, https://clinicaltrials.gov/ct2/show/ NCT01945801

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KEYWORDS: Sleep apnea; hydroxychloroquine; flow-mediated dilation of the brachial artery; peripheral artery tonometry; endothelial function

INTRODUCTION

Obstructive sleep apnea (OSA) is characterized by repetitive collapse of upper airway that leads to intermittent hypoxia. Our group published studies demonstrating that intermittent hypoxia causes oxidative stress[222], inflammation and damage to lipids and proteins.[223] OSA has been considered a chronic low-grade inflammatory disease.[224] Inflammation in OSA patients

occurs at both the airway[225,226] and systemic levels[227,228]. OSA prevalence increases with age reaching values around 80%.[229] Old age is considered an inflammatory state by itself and has been called "inflammaging".[230] Chronic inflammation initiates and propagates endothelial dysfunction, which is associated with atherosclerosis, and higher cardiovascular morbidity and mortality.[231,232] The endothelial dysfunction seen in OSA is triggered by intermittent hypoxia and the consequent formation of oxygen free radicals that cause oxidative stress thus impairing endothelial function[233] and increasing cardiovascular risk[234]. Treating OSA seems to reduce inflammation and improve endothelial function.[235-238] The two main methods used for non-invasive assessment of endothelial function are flow-mediated dilation of brachial artery (FMD) and peripheral artery tonometry (PAT).

Hydroxychloroquine (HCQ), a low-cost antimalarial drug with rare side effects has been used to treat rheumatic diseases for decades. In a retrospective study, HCQ use in patients with rheumatoid arthritis was associated with lower cardiovascular risk.[239] The mechanisms involving this cardiovascular risk reduction are not clear, but it seems reasonable to assume that an improvement in endothelial function may be part of it. HCQ treatment in human umbilical vein endothelial cells reduced markers of oxidative stress.[240] In animal models, HCQ improved endothelium-dependent vasodilation.[241-243]

We hypothesized that the decrease in cardiovascular risk observed in patients with rheumatic diseases who used HCQ may have been due to improvement in endothelial function and consequent prevention of the formation of atherosclerotic plaque. HCQ, due to its anti-inflammatory properties and favorable effects in the reduction of several cardiovascular risk factors, may improve endothelial function in patients with OSA.

Therefore, our objective was to test the effect of HCQ on endothelial function of older patients with OSA

METHODS

Trial design

This exploratory study was designed as a randomized researcher and patient blinded controlled with placebo trial with a primary endpoint of change in endothelial function after eight weeks of treatment with hydroxychloroquine. Randomization was performed as block randomization with a 1:1 allocation ratio.

Participants

Participants who meet the inclusion criteria were recruited from an on-going cohort by telephone. Adults older than 65 years old with different degrees of obstructive sleep apnea, recruited from a local basic health unit, after positive screening for sleep apnea symptoms, compose the cohort. The inclusion criteria was having an AHI greater than 15 events per hour. The exclusion criteria were contraindication to the use of hydroxychloroquine (porphyria, retinopathy, severe hepatic or renal dysfunction, neuropathy and / or muscle disease), rheumatic diseases, chronic infections, terminal or disabling disease, previous ECG with evidence of long QT interval and current or previous treatment for OSA. The study was conducted in the research laboratory of two academic hospitals.

Participants attended three clinic visits: 1) to sign informed consent forms and to pick up home sleep apnea test (HSAT) device; 2) at the time of randomization to receive the flasks with medication or placebo and to perform baseline evaluation; 3) after 8 weeks of intervention to perform follow-up evaluation. After 4 weeks of intervention, participants received a phone call to verify adherence and adverse effects.

Interventions

The intervention group received a 400 mg hydroxychloroquine capsule daily for eight weeks, to be taken with food or milk during lunchtime. The placebo group received capsules identical to those of hydroxychloroquine, containing the same excipient as the industrialized product.

Outcomes

The primary outcome was the difference between the two groups on endothelial function measured by FMD (change of % of dilatation of brachial artery diameter from baseline – Δ%FMD) and PAT (change of reactive hyperemia index of finger from baseline - ΔRHI) after eight weeks of intervention. The secondary outcomes were the difference between the two groups regarding changes in PCR, HbA1c fraction, fasting glucose, total cholesterol, high-density lipoprotein cholesterol and triglycerides blood levels. Other pre-specified outcomes were the changes in AHI, systolic and diastolic blood pressure. We also assessed the reported number of adverse effects in each group.

Biochemical tests

Biochemical assessment was performed on all participants at the beginning of the study and after 8 weeks of treatment. The exams were held early in the morning, with participants fasting. The samples were taken to the laboratory to measure plasma levels of: ultra-sensitive C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides, glucose and glycated hemoglobin.

Peripheral arterial tonometry (PAT)

The microvascular endothelial function was determined by an automatic device (EndoPAT2000, Itamar Medical, Cesarea, Israel). We placed one biosensor on the pulp of each index finger. The cuff was placed on the non-dominant arm, 2 cm above the cubital fossa. The basal pulse amplitude was measured for 5 minutes. Arterial flow was interrupted on one side for 5 minutes by insufflation of the cuff, with an occlusion pressure of 200 mmHg, or 60 mmHg above systolic blood pressure. After 5 minutes of occlusion, the cuff is deflated and reactive hyperemia, the RH-PAT sign, is registered in both hands for an additional 5 minutes. The contralateral finger is a control for changes in systemic vasodilation. The reactive hyperemia index (RHI) is automatically calculated by the EndoPAT equipment according to the manufacturer's formula. RHI is defined as the ratio between post-deflation pulse amplitude, 90 to 150 seconds after the cuff is released, and the mean baseline pulse amplitude. This result is divided by the corresponding proportion of the control finger, and multiplied by a correction factor in relation to the baseline. Low RHI values correlate to an inadequate endothelial function.

Flow-mediated dilation of the brachial artery (FMD)

Endothelial function was assessed by FMD of the brachial artery following a technical procedure described in the literature.[244] Participants came to the laboratory in a fasting state; they were instructed to take their medication and refrain from physical exercise. The measurements were taken in a quiet, dark room at controlled temperatures (23–24°C). Participants lay down in the supine position with their arm extended at a ~40° angle of the trunk. Following a 20-minute rest, blood flow velocity and diameter of the brachial artery were simultaneously measured by a blinded investigator. A rapid deflation cuff (Incoterm ™ EC500; Porto Alegre, Brazil) was positioned on the forearm 5 cm distal to the antecubital fossa. Brachial artery B-mode images were taken at the distal third of the arm using a linear multifrequency transducer (12 MHz) attached to a high-resolution Doppler ultrasound machine (Esaote MyLab™70 XVision, Genoa, Italy). Baseline diameter scans were recorded over 1 minute. The cuff was then inflated to 200 mmHg for 5 minutes. Image recordings were resumed 20 seconds before cuff deflation and continued for 3 minutes thereafter. Real-time Doppler ultrasound video signal was recorded using a USB video card (EasyCAPture; China) and data was stored for offline analysis. Blood flow velocity and diameter of the brachial artery analyses were performed using edge-detection and wall-tracking software (Cardiovascular Suite™; Pisa, Italy). Blood flow was calculated at 30 Hz from synchronized blood flow velocity and diameter of the brachial artery data. Peripheral vascular resistance of the forearm was calculated as the mean blood pressure divided by blood flow. FMD was calculated as the percentage change in peak diameter following cuff deflation from the preceding baseline diameter. The time to peak diameter was calculated from the point of cuff deflation to the maximum post-deflation diameter. Shear rate (SR) an estimate of shear stress without viscosity (four times mean blood velocity divided by diameter) was calculated and described as the area under the curve (SR-AUC) from cuff deflation to the peak dilation (Dawson, Cable et al. 2018).

Home sleep apnea monitoring (HSAT)

The portable sleep monitoring device Somnocheck Micro (Weinmann Medical Technology, Hamburg, Germany) was attached to the patient's wrist and a nasal cannula was used to register airflow. The device was programed to turn-on at 22pm and turn-off at 10 am. A combination of photoplethysmography-derived pulse wave analysis and respiratory flow signals may enable differentiation between obstructive and central apnea and provide information regarding the extent of sleep fragmentation. In detail, respiratory effort derives by analyzing fluctuations of the pulse wave analysis signal caused by intrathoracic pressure changes during spontaneous breathing cycles. Central apneas, RERAs, mean and minimum oxygen saturation and the apnea-hypopnea index were reported according to the rules of the American Academy of Sleep Medicine. Home sleep apnea monitoring was performed before and after 8 weeks of treatment.

Sample size

Based on a Cohen's F effect size estimate of 0.3 in the response of endothelial function after HCQ, for a 95% power and 0.05 level of alpha error probability, the sample size was estimated at 20 participants per group.

Randomization and blinding

A member of our research lab not involved in this study generated the allocation sequence with permuted blocks of four using a website (https://www.randomizer.org/). Participants were randomly assigned to control or intervention group with a 1:1 allocation ratio.

The bottles with hydroxychloroquine or placebo were numbered in the handling pharmacy that produced the capsules. Only the pharmacist and the previous cited member of our research lab had access to the allocation sequence. Each participant matched the bottle with the number in the sequence. Both patients and researchers involved with either primary or secondary outcome measures were blinded to the patient's allocation.

Statistical methods

Normally distributed data was presented as mean and standard deviation. Data not normally distributed as median and interquartile range. The natural logarithmic transformation of the variable was used to correct its non-normal distribution in analyses that assume the normal data distribution. The intervention arm (hydroxychloroquine) was compared with the control for all primary and secondary outcomes. We used chi-squared test for binary outcomes, and T-test for continuous outcomes. Pearson's correlation was used to verify association between %FMD, RHI and AHI. We examined the residual to assess model assumptions and goodness-of-fit. The group x time interaction was tested using generalized estimating equations. We used the Bonferroni method to appropriately adjust the overall level of significance for multiple primary outcomes, and secondary outcomes. Results with a probability <0.05 of alpha error were considered statistically significant. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). Considering that a significant P value may be misleading without a substantial effect size that vouches the clinical significance[245], we calculated the effect size for repeated measures designs and considered significant if larger than 0.3.[246]

RESULTS

Eligible participants were recruited between April 2019 and March 2020 among 272 patients with moderate-severe OSA identified in an on-going cohort study. The last follow-up visit happened in May 2020. Fourteen patients were randomly assigned to the HCQ group and fifteen patients to the placebo group (***Figure 1***). Twenty-nine participants successfully completed the baseline and follow-up endothelial function testing and visits. The analysis was performed only by intention-to-treat. One participant was excluded from analysis of FMD data due to poor vascular imaging. All participants had the diagnosis of moderate-severe OSA

confirmed by HSAT. Clinical characteristics were similarly distributed between the two groups (***Table 1***).

Figure 2 displays the individual values and *Erro! Fonte de referência não encontrada.* displays the means of RHI, %FMD and AHI in both groups at baseline and follow-up. Time x group interaction was non-significant for endothelial function estimated by either RHI or % FMD. Hydroxychloroquine group presented a reduction in AHI, with a moderate effect size (P= 0.04, d= 0.56). The effect of HCQ on other secondary outcomes was non-significant, although the effect size of HCQ treatment on reducing fasting glucose, total cholesterol and triglycerides blood levels was moderate, as shown in ***Table 3***. The values of other FMD-derived variables, such as vascular resistance and shear stress can be found in ***Table 4***.

In the HCQ group, the correlations between ΔRHI and Δ%FMD, between ΔRHI and ΔAHI, and between Δ%FMD and ΔAHI were non-significant. No important reactions to treatment were observed in neither group. Symptoms reported by the participants in the adverse-effect questionnaire are shown in ***Table 5***. More symptoms were reported in the placebo group than in the HCQ group.

DISCUSSION

In this study, we investigated the effect of HCQ treatment in older adults with OSA. As far as we know, this was the first randomized clinical trial evaluating treatment with HCQ on patients with OSA. Eight weeks of treatment with HCQ did not improve endothelial function evaluated with PAT and FMD in older adults with moderate-severe OSA. In addition, HCQ significantly reduced AHI in those patients.

Most previous studies evaluated the endothelial effect of HCQ on animal models of rheumatic diseases and on *in vitro* models of endothelial cells. HCQ treatment in human umbilical vein endothelial cells reduced the TNF-α-induced endothelial-leukocyte adhesion and expression of ICAM-1 and VCAM-1[247] and inhibited the production of 8-isoprostane and nicotinanamide adenine dinucleotide phosphate (NADPH), markers of oxidative stress[18].The study suggested that HCQ may be a promising approach for the treatment of inflammatory vascular disease. Another study published in 2019 evaluated the effect of HCQ in mice and in human aortic endothelial cells after contact with antiphospholipid antibodies, which induce endothelial dysfunction. In this scenario, HCQ increased the endothelial production of NO, improving endothelium-dependent vasodilation and consequently endothelial function.[19] Similar findings were seen in this previous study, in which aortae from lupus mice showed reduced endothelium-dependent vasodilator responses to acetylcholine and enhanced contraction to phenylephrine, which were normalized by after 5 weeks of treatment with hydroxychloroquine. There was also an increase in reactive oxygen species NOX-1 and p47 (phox) in lupus mice, that decreased after treatment with hydroxychloroquine.[20] Another study using an animal model of lupus demonstrated an increase in the bioavailability of NO and a reduction in reactive oxygen species in the HCQ group.[21] Considering that intermittent hypoxia leads to oxidative stress and endothelial dysfunction in OSA and that HCQ seemed to mitigates oxidative stress and improve endothelial function, we hypothesized that HCQ treatment would improve endothelial function in patients with OSA. In our trial, however, HCQ did not affect endothelial function. A possible explanation for our finding is that, against our hypotheses, few participants had an impaired endothelial function, and inflammatory risk, as indicated by C - reactive protein levels above 0.3. In designing future trials, this aspect should be considered; impaired endothelial function and high inflammatory risk should be among the inclusion criteria when testing the effect of anti-inflammatory drugs on endothelial dysfunction. Furthermore,

some participants used medications that could alter endothelial function, such as statins and calcium channels antagonists, which could have affected our results. However, excluding these participants would reduce the external validity of our study, since hypertension and dyslipidemia are prevalent diseases among older adults. Considering the shortage of previous studies, it was difficult to determine the adequate time of treatment for this trial. A longer period of treatment with HCQ could have changed our results. Although FMD is an operator-dependent method, the reproducibility of the results of the examiner in our study had been previously tested and variability was within appropriate limits as by the new guidelines. Thus, technical issues with FMD are unlikely to be a significant limitation.

The slight reduction in the AHI presented by the group treated with HCQ in our trial may be due to reduction of the airway inflammation seen in patients with OSA. In a meta-analysys, treatment with montelukast, a cysteinyl leukotriene receptor antagonist with anti-inflammatory effects, reduced AHI in children (medium difference of -2.7 events/h; 95% confidence interval -5.6 to 0.3) when compared to placebo.[248] In a randomized trial with 26 participants, montelukast treatment did not affect the AHI of adults with OSA, however, total sleep time and percent of stage R sleep significantly increased.[249] This finding may explain why patients with Sjögren's syndrome treated with HCQ presented a better sleep quality. In this retrospective study, 383 patients with Sjögren's Syndrome were divided into the HCQ-treated group (n=230) and non-treated group (n=153), the period of treatment ranging from four to 41 months. One hundred eighteen patients (51.3%) in the HCQ-treated group reported a good sleep, according to the Pittsburgh Sleep Quality Index, against 58 (37.9%) in the non-treated group.[250] However, the researchers did not evaluate the mechanisms involved in this improvement of sleep quality seen in HCQ-treated group. Considering the much higher prevalence of OSA in patients with Sjögren's Syndrome[251], it is reasonable to hypothesize that the improvement may have been due to a HCQ effect in the apnea-hypopnea index. The improvement we found with HCQ treatment is comparable with other non-PAP treatments for sleep apnea. For instance, a decrease in BMI of 3 kg/m² was associated with a decrease of 11 events/hour in the AHI in a systematic review and comparison of meta-analysis.[252]

Cohort studies have shown that HCQ was associated to lower cholesterol levels in patients with lupus and rheumatoid arthritis.[253-256] Furthermore, in patients with rheumatic diseases, the use of HCQ has been associated with a lower incidence of type 2 diabetes.[257-259] Our results showed a moderate, though non-significant, effect size of HCQ treatment on reducing fasting glucose and total cholesterol blood levels, confirming previous findings and warranting future research in OSA patients.

Our study present some limitations that must be taken into account. The need to stop the trial due to COVID pandemic led to a smaller than calculated sample size, which reduced the power of the study. In addition, an overestimation of the estimated effect size when calculating the sample size may have occurred, leading us to assume a too small sample size.

CONCLUSION

HCQ does not seem to alter endothelial function in older adults with OSA. Besides the previously cited limitations, the originality and relevance of our research question taken together with our findings make this trial hypothesis-generating. Considering the reduction of AHI seen in HCQ group, investigating the effect of HCQ on OSA is warranted.

REGISTRATION

Anti-Inflammatory Drug and Endothelial Function (HOLD), ClinicalTrials.gov number: NCT01945801, https://clinicaltrials.gov/ct2/show/ NCT01945801

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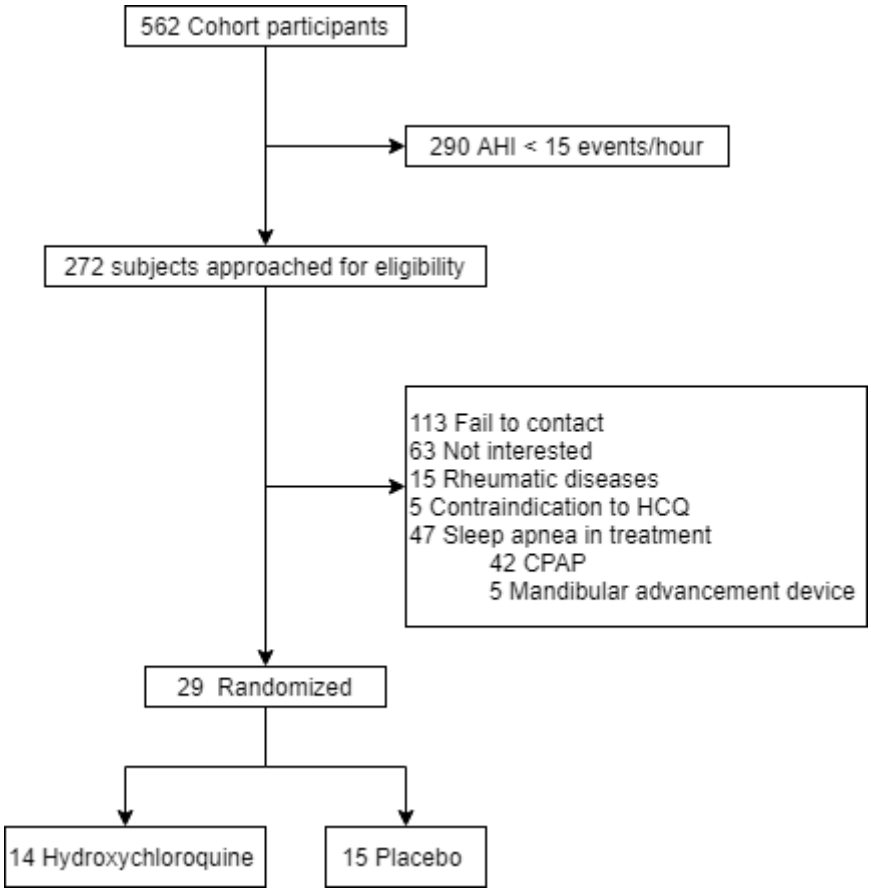

Figure 1 – Flow diagram of study's participants. There were no losses to follow-up after randomization.

Table 1- Characteristics of the patients at baseline by study group (n = 29)

Characteristics	Placebo (n=15)	Hydroxychloroquine (n=14)	P value
Sex (male)	7 (47)	9 (64)	0.34
Age (years)	73 ± 3	72 ± 3	0.26
Body mass index (kg/m²)	28.3 ± 3.7	28.9 ± 5.4	0.74
Apnea/Hypopnea index (events/hour)	37 ± 16	35 ± 13	0.66
Smoking status:			
Current	0	2 (14)	0.16
Past	7 (47)	3 (21)	0.16
Systolic Blood Pressure (mm Hg)	138 ± 19	135 ± 14	0.62
Diastolic Blood Pressure (mm Hg)	77 ± 12	78 ± 10	0.85
Arterial Hypertension [n, (%)]	13 (87)	7 (50)	0.05*
Antihypertensive drugs:			
Diuretics (n)	4	5	-
Betablockers (n)	6	1	-
Calcium channel blockers (n)	3	1	-
Angiotensin II receptor antagonists (n)	3	3	-
Angiotensin converting enzyme inhibitors (n)	6	1	-
Glycosylated hemoglobin (%)	6.55 ± 1.11	6.56 ± 1.86	0.98
Fasting Blood Glucose (mg/dL)	117 ± 41	112 ± 43	0.79
Diabetes	4 (27)	3 (21)	0.74
Total Cholesterol (mg/dL)	199 ± 57	185 ± 23	0.39
HDL-Cholesterol (mg/dL)	50 ± 9	51 ±16	0.92
Triglycerides (mg/dL)	155 ± 87	150 ± 69	0.84
Dyslipidemia	14 (93)	7 (50)	0.009*
Creatinine (mg/dL)	0.99 ± 0.23	0.91 ± 0.21	0.30
C-Reactive Protein (mg/dL)	0.27 ± 0.23	0.28 ± 0.26	0.86
FMD variables:			
% FMD	2.46 ± 7.02	3.63 ± 4.45	0.61
Diameter of the brachial artery (mm)	4.55 ± 0.59	4.61 ± 0.96	0.83
Peak diameter (mm)	4.67 ± 0.71	4.77 ± 0.93	0.75
Time to peak (s)	75 ± 46	65 ± 50	0.59
Mean SR-AUC (s, 10^3)	27.05 ± 19.91	25.69 ± 31.29	0.89
Vascular resistance	1.05 ± 1.87	0.79 ± 1.02	0.66
PAT variables:			
Reactive hyperemia index (RHI)	0.77 ± 0.04	0.71 ± 0.59	0.43
Abnormal RHI [n (%)]	7 (47)	6 (40)	

Data are presented as mean ± standard deviation or as number (%). *Hydroxycloroquine vs. placebo (Student's t-test, Pearson Chi-square); significant at $p \leq 0.05$. We considered abnormal % FMD values < 0.

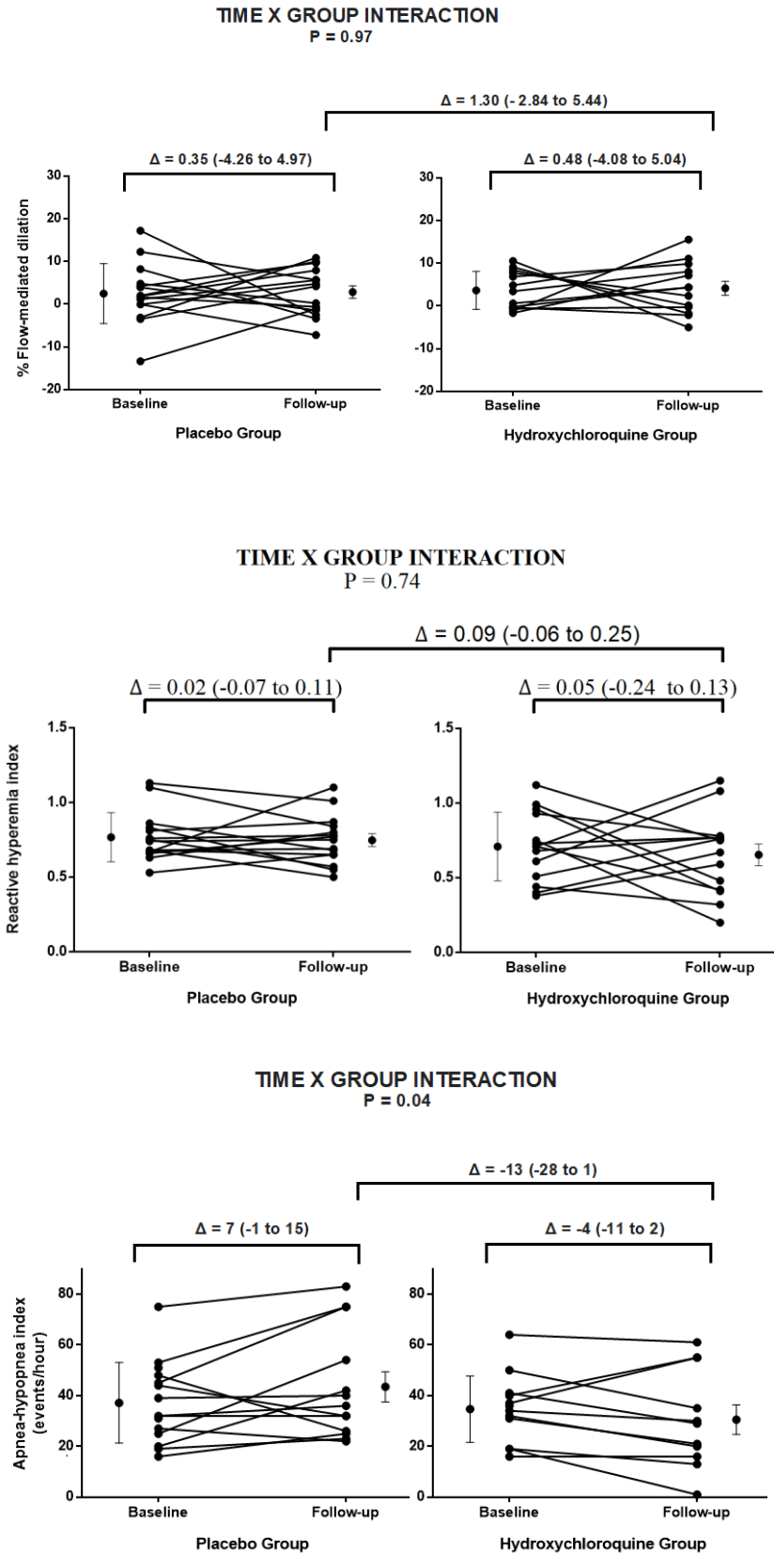

Figure 2 - Changes in reactive hyperemia-index, apnea-hipopnea-index and % flow-mediated dilation of brachial artery in placebo and HCQ group at baseline and after eight weeks of treatment

Table 2- Primary outcome measures in the study participants

	Placebo		Hydroxychloroquine		P for time x group interaction	Effect size treatment vs. placebo
Outcome	Baseline	Mean Δ	Baseline	Mean Δ		
% FMD	2.46 ± 1.75	0.35 ± 2.35	3.63 ± 1.18	0.48 ± 2.33	0.97	0.14
RHI	0.77 ± 0.04	0.36 ± 0.18	0.71 ± 0.59	0.04 ± 0.14	0.20	0.26

Data are shown as estimated marginal means ± standard error and as estimated marginal mean difference (follow-up minus baseline) ± standard error**.**

Table 3- Secondary outcome measures in the study participants

	Placebo		Hydroxychloroquine		P for Time × group interaction	Effect size
	Baseline	Follow-up	Baseline	Follow-up		
AHI – events/hour	37±16	43±21	35±13	31±19	**0.04**	**0.56**
Fasting glucose – mg/dL	116±41	115±39	112±42	106±35	0.45	**0.33**
Glycated hemoglobin – %	6.5±1.1	6.5±1.1	6.5±1.9	6.5±1.6	0.61	0
Total Cholesterol - mg/dL	199±57	187±45	185±23	163±26	0.27	**0.63**
HDL Cholesterol – mg/dL	50±9	52±10	51±16	49±13	0.20	0.17
Triglycerides – mg/dL	155±87	140±83	150±69	121±44	0.23	**0.34**
C-reactive protein – mg/dL	0.26±0.23	0.29±0.29	0.28±0.26	0.18±0.11	0.10	0.24
Systolic Blood Pressure – mmHg	138±19	137±18	135±14	134±14	0.98	0.17
Diastolic Blood Pressure - mmHg	77±12	76±9	78±10	77±6	0.92	0.10

Data are shown as estimated marginal means ± standard error.

Table 4- Diameter of the brachial artery, time to peak, shear rate and vascular resistance in study participants.

	Placebo		Hydroxychloroquine	
	Baseline	Follow-up	Baseline	Follow-up
Diameter of the brachial artery (mm)	4.55 ± 0.59	4.31 ± 0.66	4.61 ± 0.96	4.61 ± 0.82
Peak diameter (mm) - FMD	4.67 ± 0.71	4.41 ± 0.65	4.77 ± 0.93	4.79 ± 0.85
Time to peak (s) - FMD	75 ± 46	99 ± 47	65 ± 50	73 ± 43
Mean SR-AUC (s, 10^3) - FMD	27.05 ± 19.91	38.80 ± 18.82	25.69 ± 31.29	29.63 ± 22.48
Vascular resistance	1.05 ± 1.87	1.08 ± 1.51	0.79 ± 1.02	0.64 ± 0.46

Data are shown as estimated marginal means ± standard error.

Table 5 - Adverse effects in each group

Symptoms	Placebo N=15	Hydroxychloroquine N=14
Skin eruption	1	2
Inappetence	1	0
Headache	2	1
Nausea	2	0
Agitation	1	0
Tinnitus	0	0
Fussiness	1	1
Blurred vision	1	1
Diarrhea	1	0
Hypotension	0	0
Flutter	2	1
Dyspnea	2	0
Ataxia	0	0
Dysuria	1	1
Tremor	0	1
Muscular pain	2	0
Total of symptoms	17	8
Total of participants reporting symptoms	5	6

Number of participants in each group that checked “Yes” for the symptom in the adverse-effect questionnaire

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CONCLUSÕES E CONSIDERAÇÕES FINAIS

Neste estudo, investigamos o efeito do tratamento com HCQ por oito semanas em idosos com AOS. Até onde sabemos, este foi o primeiro ensaio clínico randomizado avaliando o efeito da HCQ em pacientes com AOS. A maioria dos estudos anteriores constatou um efeito benéfico da HCQ na função endotelial em modelos animais de doenças reumáticas e em modelos *in vitro* de células endoteliais.

Em nosso estudo, entretanto, a HCQ não afetou a função endotelial, avaliada por PAT e FMD, de idosos com AOS. Embora não significativo, o tamanho de efeito do tratamento com HCQ nos níveis sanguíneos de glicose em jejum, triglicerídeos e colesterol total é moderado, confirmando achados anteriores e garantindo pesquisas futuras em pacientes com AOS.

O achado de leve, mas significativa melhora na gravidade da AOS após tratamento com HCQ sugere que alguns mecanismos inflamatórios específicos podem participar da causação da AOS, o que merece investigação adicional em ensaios clínicos com maior amostragem.

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