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PROGRAMA DE PÓS-GRADUAÇÃO EM
CIÊNCIAS DA SAÚDE

Famiely Colman Machado de Machado

INTERVENÇÃO FONOTERAPÊUTICA EM PACIENTES COM SEQUELA DE LEISHMANIOSE MUCOSA E CUTÂNEA

Tese de Doutorado

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Famiely Colman Machado de Machado

Professor-orientador: Marcus Miranda Lessa
Co-orientador (a): Carla Aparecida Cielo

Tese apresentada ao Colegiado do PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE, da Faculdade de Medicina da Universidade Federal da Bahia, como pré-requisito obrigatório para a obtenção do grau de Doutora em Ciências da Saúde.

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FRONTISPÍCIO

Conheça todas as teorias,
Domine todas as técnicas,
Mas ao tocar uma alma humana
Seja apenas outra alma humana.
(Carl G. Jung)

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LISTA DE ABREVIATURAS

†A-Asperity (Aspereza)

A- Asthenia (Astenia)

APQ- Amplitude Pertubation Quotient (Quociente de Perturbação da Amplitude)

CEP- Comitê de Ética em Pesquisa

CL-Cutaneous Leishmaniasis

COM-HUPES- Complexo Hospitalar Universitário Professor Edgard Santos

CONEP- Comissão Nacional de Ética em Pesquisa

DSH- Degree of Sub-harmonics (Grau dos Componentes Subharmônicos)

DUV- Degree of Voiceless (Grau de Segmentos não Sonorizados)

DVB- Degree of Voice Breaks (Grau de Quebra da Voz)

EBE-Espectrografia de Banda Estreita

EBL-Espectrografia de Banda Larga

EVTSO-Exercício Vocal de Trato Semiocluído

f0- Frequência Fundamental

F1-Primeiro Formante

F2-Segundo Formante

F3-Terceiro Formante

F4-Quarto Formante

fhi- Maximum f0 (f0 Máxima)

flo- Minimum f0 (f0 Mínima)

I- Instability (Instabilidade)

***Jita*-** Absolute Jitter (*Jitter* Absoluto)

***Jitt*-** Jitter percentage (*Jitter* Percentual)

LabVoz-Laboratório de Voz

LC- Leishmaniose Cutânea

LCD-Leishmaniose Cutânea Difusa

LD- Leishmaniose Disseminada

LM-Leishmaniose Mucosa

MDVPA- *Multi Dimension Voice Program Advanced*

ML-Mucosal Leishmaniasis

NBS- spectrographic analysis of narrowband

NHR- Harmonic Noise Ratio (Proporção Ruído-Harmônico)

NSH- Number of Sub-harmonic Segments (Número de Segmentos Subharmônicos)

NUV- Number of Unvoice Segments (Número de Segmentos não Sonorizados)

NVB- Number of Voice Breaks (Número de Quebras Vocais)

R- Hoarseness (Rouquidão)

RAP- Relative Average Perturbation of the Pitch (Média Relativa da Perturbação da Frequência)

RASATI-Rouquidão, Aspereza, Soprosidade, Astenia,Tensão, Intensidade

RTS-*Real Time Spectrogram*

S- Thunder (Soprosidade)

sAPQ- Smoothed Amplitude Perturbation Quotient (Quociente de Perturbação da Amplitude Suavizado)

ShdB- Shimmer in dB (*Shimmer* em dB)

Shim- Shimmer Percent (*Shimmer* Percentual)

SOVT-Semi-Occluded Vocal Tract Voice

SPI- Soft Phonation Index (Índice de Fonação Suave)

sPPQ- Smoothed Pitch Perturbation Quotient (Quociente de Perturbação do *Pitch* Suavizado)

STD- Standard Deviation of f0 (Desvio-Padrão da f0)

TCLE- Termo de Consentimento Livre e Esclarecido

TMF- Tempos Máximos de Fonação

T-Tension (Tensão)

UFBA- Universidade Federal da Bahia

UFSM-Universidade Federal de Santa Maria

vAm- Amplitude Variation (Variação da Amplitude)

vf0- Variation of f0 (Variação da f0)

VTI- Índice de Turbulência da Voz

WBS- spectrographic analysis of wide band

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I. RESUMO

INTERVENÇÃO FONOTERAPÊUTICA EM PACIENTES COM SEQUELA DE LEISHMANIOSE MUCOSA E CUTÂNEA

INTRODUÇÃO: A Leishmaniose Tegumentar é uma doença estigmatizante, considerada um grave problema de saúde pública. Apresenta três formas clínicas clássicas, dentre elas as formas Cutânea e a Mucosa. A primeira afeta principalmente os membros superiores e inferiores, com lesões ulceradas, que podem ser múltiplas ou únicas. Já a segunda, atinge o trato respiratório superior, com lesões destrutivas, que podem afetar a voz, deglutição e a respiração dos pacientes. **OBJETIVO:** Caracterizar a voz e verificar a resposta vocal à intervenção fonoterapêutica dos pacientes com sequela de Leishmaniose Mucosa e sequela de Leishmaniose Cutânea. **MÉTODOS:** Foi coletada a emissão vocal /a:/ de 22 participantes de cada grupo (total de 44 casos) para a análise computadorizada da voz através do programa *Real Time Spectrogram* da *Kay PENTAX*® e do *Multi Dimensional Voice Program Advanced* e para análise perceptivoauditiva, através da escala RASATI. **RESULTADOS:** Antes da fonoterapia, os participantes com Leishmaniose Mucosa tiveram resultado estatisticamente significativo, onde 5 (27,7%) participantes apresentaram qualidade vocal astênica, e alteração dos parâmetros de medidas de frequência, perturbação de frequência, ruído e medidas de sub-harmônicos. Já dos participantes com Leishmaniose Cutânea, 8 (36,4%) apresentaram instabilidade vocal de grau 1. Após a fonoterapia, viu-se que os pacientes com Leishmaniose Cutânea apresentaram redução no grau de aspereza e melhora nos parâmetros acústicos de perturbação de frequência. O grupo com sequela de Leishmaniose Mucosa apresentou redução das medidas de segmentos sub-harmônicos. Apenas o grupo com sequela de Leishmaniose Cutânea teve resultados estatisticamente significativos quanto à espectrografia, com melhora dos seguintes parâmetros: intensidade da cor do traçado, presença de ruído, substituição de harmônicos por ruído, definição e regularidade de harmônicos, regularidade das baixas frequências e de todo o espectrograma e para anti-ressonância. Não houve diferença estatisticamente significativa quanto ao Perfil do Comportamento vocal. **CONCLUSÃO:** Os dois grupos apresentaram alterações vocais em diferentes graus antes da terapia vocal, sendo que os pacientes com Leishmaniose Mucosa apresentam graus mais severos. Após a intervenção fonoaudiológica, os participantes com sequela de Leishmaniose Cutânea tiveram mais benefícios vocais após a execução da técnica, possivelmente por não apresentarem lesões no trato vocal.

Palavras-Chave: 1.Voz; 2.Leishmaniose Mucosa; 3.Leishmaniose Cutânea; 4.Fonoterapia; 5.Distúrbios da voz. 6.Espectrografia.

II. OBJETIVOS

II.1. Objetivo geral

Avaliar a voz e os efeitos da fonoterapia em pacientes com sequelas da Leishmaniose Mucosa e Cutânea.

II.2. Objetivos específicos

- Caracterizar a voz dos pacientes com LM e LC após o tratamento medicamentoso;
- Analisar as mudanças vocais que ocorrem após a intervenção fonoterapêutica nos pacientes com alteração vocal.

III. INTRODUÇÃO

As Leishmanioses são causadas por diferentes espécies de parasitas do gênero *Leishmania* e transmitidas através da picada das fêmeas dos insetos de gênero *Phlebotomus* (Velho mundo) e *Lutzomia* (Novo Mundo) (Diptera: Psychodidae, Phebotominae) chamados de flebotomíneos [(1)]. Essa doença apresenta duas formas principais: a Leishmaniose Tegumentar, classificada em tegumentar americana e tegumentar do velho mundo, e a Leishmaniose Visceral [(2,3)].

Essa afecção figura como um grave problema de saúde pública no mundo, sendo considerada como uma das seis enfermidades infectoparasitárias de maior importância [(1,4)]. A Leishmaniose Tegumentar Americana (LTA) está presente desde o sul dos Estados Unidos até o norte da Argentina [(5)]. No Brasil, encontra-se em larga escala por todo o território, desde a Amazônia até os estados do Sul, sendo que as regiões Norte e Nordeste são as mais afetadas com a maior incidência entre os habitantes das áreas rurais [(5)], tendo como principal causadora a *Leishmania (Viannia) braziliensis* [(6)].

Na LTA evidenciam-se quatro tipos clássicos de apresentação clínica: cutânea (LC), disseminada (LD), difusa (LCD) e mucosa [(2,3)]. A primeira forma clínica é responsável pela maioria dos casos de LTA, sendo tipificada pela presença de lesões ulceradas, de formato arredondado, com bordas elevadas e bem delimitadas com granulações grosseiras [(2,7)]. Normalmente, limita-se à derme e acomete áreas expostas como membros e, de forma menos frequente, pavilhões auriculares [(8,9)]. Já a LD é uma forma rara [(10)], caracterizada pelo aparecimento de múltiplas lesões ulceradas, eritematosas, pápulas, que poderão acometer vários segmentos corporais, envolvendo com frequência a face e o tronco [(2)]. No caso da LCD, as

lesões são nodulares ou tubérculos com infiltrações cutâneas pronunciadas. Geralmente essas lesões não cicatrizam espontaneamente e são normalmente são resistentes ou tratamento medicamentoso [11,12]. A LM, terceira forma clínica, é representada por lesões destrutivas localizadas nas mucosas do trato aerodigestivo superior (boca, nariz, faringe, laringe) [2,3,13], sendo que nas lesões crônicas e avançadas pode haver mutilações com perda parcial ou total do nariz, lábios, pálpebras, causando deformidades e consequente estigma social [2].

A LM não aparece em todos os pacientes, apenas em cerca de 5% a 10% [14,15] daqueles que apresentam uma lesão cutânea primária [16]. Geralmente, em função das suas lesões ocorrem queixas de obstrução nasal, epistaxe, eliminação de crostas, disfagia, odinofagia, disfonia, dispneia e tosse. Além disso, as sequelas das lesões como retração da pirâmide nasal, perfuração do septo nasal ou do palato e destruição da úvula podem interferir nas funções de deglutição, respiração, fala e vocalização [13].

Durante o quadro de Leishmaniose, as pregas vocais podem estar se movendo bem, entretanto a fonação pode ser fraca e o controle muscular de tensão pode estar prejudicado por formação granulomatosa e subsequente fibrose. Mesmo o tratamento da leishmaniose ocorrendo de forma satisfatória, muitas vezes a voz não retornará ao normal e a luz da laringe poderá estar reduzida [17], sendo de suma importância a atuação/ reabilitação fonoaudiológica [13,17].

Considerando o exposto, vê-se a necessidade de entender que tipo de voz o indivíduo com sequela de Leishmaniose Tegumentar apresenta. Além disso, com a escassez de estudos sobre a terapia fonoaudiológica nesses casos, pretende-se

compreender as mudanças vocais que ocorrem frente à estimulação com técnica vocal.

IV. REVISÃO DE LITERATURA

IV.1. Leishmaniose Tegumentar

A LT é uma doença endêmica [18,19], infecciosa e não contagiosa [19,20]. É geralmente negligenciada e ignorada quando se trata de doenças tropicais brasileiras [18,19], cuja distribuição já foi assinalada em aproximadamente 98 países, acometendo em torno de 1,5 milhões de pessoas ao ano [4,15]. A mesma tem grande incidência no Brasil, em que se estimam pelo menos 20 mil casos ao ano, sendo a região nordeste a mais afetada. [15,18].

Os aspectos envolvidos nas diferentes formas e no agravamento da LTA ainda não estão bem esclarecidos [15]. Supõe-se que o polimorfismo da doença dependa de diferentes fatores como a resposta imune e características genéticas do hospedeiro humano, características de algumas cepas do parasita e elementos ligados aos vetores envolvidos na transmissão [15,20-23] e, mais recentemente, a presença do vírus de RNA de Leishmania 1 [15,24] pode estar relacionada com os desfechos dessa doença [15]. Desta forma, essa diversidade de fatores torna difícil a compreensão da patogênese dessa patologia [15,25].

Diferentes espécies de protozoários do gênero *Leishmania* podem causar a LTA [20]. No Brasil, reconhece-se como causadores da forma tegumentar principalmente a *L. (V.) braziliensis*, L (V.) *guyanensis* e *L. (L.) amazonenses* [20,26,27] e, mais raramente, a L. (V.) laisoni, L. (V.) naiffi e L. (V.) shawi, L. (V.) lindenbergi [7,18,19,28]. A principal ocasionadora da LM é a L. brasiliensis, contudo já foi observada uma significativa incidência de LM originária da L. guyanensis, especialmente ao norte do Rio Amazonas [18,29].

Os parasitos do tipo *Leishmania* só completam o seu ciclo evolutivo passando por pelo menos dois hospedeiros (digenéticos) e se apresentam nas formas fundamentais de promastigota e amastigota. Na primeira, são encontradas no tubo digestivo dos flebotomíneos. Já na segunda forma, são intracelulares obrigatórias e encontradas nos hospedeiros vertebrados [2,19,20,30]. Esses protozoários são passados por pequenos dípteros bastante pilosos e reconhecidos em função da sua característica de asas entreabertas e levantadas durante o pouso [19,20].

Esses insetos são chamados de flebotomíneos devido à sua família Psychodidai e subfamília Phebotominae, não sendo definidos como mosquitos e exibindo nomes não científicos como: mosquito-palha, cangalha e tatuíra [19,20]. Destes, apenas as fêmeas se alimentam de sangue (hematófogas), contaminando-se, ao sugá-lo, com a *Leishmania* que evoluirá no seu tubo digestivo [20,23,31].

O seu habitat preferencial é o ambiente silvestre, entretanto, os homens e animais que residem próximos a regiões de mata podem ser picados por esses insetos [20,32]. A contaminação do homem e de animais não silvestres tem ocorrido, dentre outras coisas, por fatores como o desmatamento das áreas florestais, a expansão de fronteiras agrícolas [20,33,34], a construção de novas estradas, intensa urbanização [33-36], projetos de irrigação [35], a implantação de áreas de garimpo [33,34], que fazem com que o homem entre, cada vez mais, em contato com o transmissor dessa doença.

Em alguns estudos é possível observar que indivíduos do sexo masculino são mais acometidos que os do sexo feminino [7,37]. Essa prevalência seria fundamentada com base nas atividades laborais ou de lazer

(caça, pesca ou acampamento) exercidas pelos homens [38,39], visto que a menor ocorrência dos casos de LTA entre as mulheres seria devido a pouca atividade da mulher na área de agricultura, sendo que essa enfermidade ainda mantém natureza especialmente de ciclo silvestre resultante da ação antrópica e ocupacional [39].

Quanto à disseminação da doença entre as faixas etárias, ressalta-se a importância da possibilidade de contágio nas áreas domiciliar, peridomiciliar e ocupacional, dado que se verificam crianças abaixo de seis anos e idosos, que geralmente se encontram dentro de casa, enquanto as pessoas em idade produtiva, entre 25 e 50 anos, apresentam maior risco de ter a doença em virtude das suas ocupações laborais na floresta, normalmente agricultura ou extrativismo [33,37,39]. Outra forma de infecção das crianças ocorreria através da contaminação de animais domésticos que serviriam de atrativo aos vetores [34,37].

Longe de ser uma doença que leve à morte, a LTA tem grande impacto na vida social das pessoas, trazendo transtornos de ordem psicológica, social e econômica [20,40]. Assim, a infecção tegumentar pode ser assintomática ou produzir tipos clínicos diferentes como a forma cutânea, mucosa, disseminada e difusa, com características diferentes, afetando pele e mucosas, [19] rosto e membros [20].

IV.2. Manifestações Clínicas: Leishmaniose Cutânea e Leishmaniose Mucosa

A LC e a LM são duas das principais formas clínicas da LTA, sendo que a primeira é considerada mais simples do que a segunda. Alguns autores mostram que 5% a 10% dos pacientes que fazem tratamento ou se curam da LC podem desenvolver LM [14,15].

IV.2.1 Diagnóstico da LTA

O diagnóstico da LTA é feito através de diversos testes, caracterizado como clínico e epidemiológico. O método padrão ouro na detecção da Leishmaniose é a Reação em Cadeia da Polimerase (PCR), pois apresenta alta sensibilidade e especificidade, principalmente na determinação da espécie do parasita [11,18,28,41,42].

Além disso, há a Intradermo Reação de Montenegro [28,43], com 90% de sensibilidade e especificidade, sendo confiável e largamente utilizada para a triagem de indivíduos com suspeita de LTA [43], apresentando, geralmente, resposta fortemente positiva [11,28,42]. No entanto, segundo alguns autores, este teste não é muito adequado para as lesões mucosas em função das mesmas frequentemente serem secundárias às cutâneas [7,18,44].

Ainda, o diagnóstico deve ser composto pelos testes sorológicos, que tem por objetivo identificar anticorpos anti-*Leishmania* no sangue periférico [28], porém são limitados devido ao baixo nível de anticorpos circulantes [18,43].

Podem ser empregados ainda testes histopatológicos (demonstração direta do parasito na amostra da biópsia da lesão), parasitológicos (isolamento em cultivo *in vitro* - meios de cultivo) [11,28,42], exames por vídeo (videolaringoscopia e videoendoscopia nasal) que possibilitam averiguar detalhes das lesões mucosas suspeitas de LTA [42,45].

Quanto ao diagnóstico diferencial da LTA, esta inclui as seguintes patologias: paracoccidiodomicose, carcinoma epidermóide, carcinoma basocelular, linfomas, rinofima, rinosporodiose, entomoftoromicose, hanseníase virchowiana, sífilis terciária, perfuração septal traumática ou por uso de drogas, rinite alérgica, sinusite, sarcoidose, granulomatose de Wegener, actinomicose, histoplasmose, dentre outras doenças raras [42,43,46].

Além da complexidade envolvida no diagnóstico da LC e da LM, ainda há a possibilidade do diagnóstico tardio, em razão da existência de múltiplos fatores que podem estar ligados a aspectos educacionais, sociais, econômicos, geográficos [43] como a falta de conhecimento dos profissionais que fazem o atendimento primário sobre essa doença [7,18,43,44]; a dificuldade de acesso ao sistema de saúde por parte dos indivíduos acometidos por essa afecção, dado que muitos moram na zona rural [18,29]; a falta de disponibilidade de métodos diagnósticos apropriados para a detecção dessa patologia no sistema de saúde [7,18,43,44]; e a falta de conhecimento e percepção dos próprios indivíduos acerca dessa doença, demorando, assim, para procurar o atendimento de saúde [43].

IV.2.2 Características Clínicas da Leishmaniose Cutânea

A LC é uma doença crônica e benigna [28,47], sendo a forma mais comum de LTA, que pode não se manifestar ao longo de anos ou evoluir para lesões cutâneas. Acredita-se que algumas pessoas possam estar infectadas, contudo não desenvolvem a doença, a julgar por encontrar, algumas vezes, indivíduos em áreas endêmicas com resultado positivo para o teste de Montenegro sem história clínica prévia e sem cicatrizes compatíveis com a LTA [28].

As lesões características de LC podem se apresentar de forma única ou múltipla, ulceradas [20,42], geralmente indolor, de aparência arredondada ou ovalada, com base eritematosa, infiltrada e aspecto firme, com bordas circunscritas e elevadas, de fundo avermelhado e com granulações grosseiras [42,48]. Frequentemente acometem áreas expostas do corpo, como os membros [20,47].

Inicialmente, nas lesões primárias podem ocorrer nodulações, posicionadas de modo profundo na hipoderme, ou serem pequenas pápulas, semelhantes à picada de inseto, que se desenvolve em tamanho e profundidade (lesões pápulo-tuberosas) e ulcerando no vértice. Por outro lado, as úlceras que demoram a evoluir podem caracterizar-se como ulcero-vegetantes em virtude da proliferação do fundo granuloso [28,42].

Havendo associação de infecção bacteriana, poderá ocorrer dor no local da ferida e produção de exsudato seropurulento que ao dessecar-se em crostas recobre total ou parcialmente o fundo da úlcera [42,48]. No entanto, eczema na pele envolvendo a úlcera pode aparecer quando ocorre infecção secundária e uso de produtos tópicos [3,28, 42].

Na ocorrência de cura, as lesões cutâneas podem deixar como sequelas cicatrizes atróficas achatadas de plano liso, com áreas de pouca ou muita pigmentação e com fibroses. Além disso, em alguns casos, a cicatriz pode ficar hipertrófica ou não ser reconhecida por sua cor, tamanho, forma e local [28,42].

Existe a possibilidade da LC ser curada mesmo sem tratamento, ocorrendo a cura espontânea dentro de alguns meses ou poucos anos. Porém, há a possibilidade dessas lesões estarem ativas por muitos anos e coexistindo com lesões mucosas de surgimento posterior. [3,28].

IV.2.3 **Características clínicas da Leishmaniose Mucosa**

A LM está presente por todas as regiões do Brasil [2,19], no entanto sua maior incidência está situada na região Nordeste e depois na região Norte [15,49]. Essa alta incidência é esclarecida por alguns autores como ocorrência da demora com a qual os pacientes iniciam o tratamento, atrelada à evolução dos casos [20,50], além de relacionar esse aparecimento à busca ativa pelos casos suspeitos e notificação mais eficiente [51].

Normalmente o aparecimento da LM se dá após a cicatrização da lesão cutânea primária (2 a 5% dos casos), mas também pode ocorrer simultaneamente ao quadro de LC ou, eventualmente, como forma inicial de apresentação da doença [20,52,53]. Alguns autores creditam esse aparecimento a metástases por via hemática [7,18,50] ou linfática [7,18], relatando ser atípico o contágio através do contato direto da lesão mucosa com a cutânea como no caso de um recém nascido de mãe com lesão cutânea em mamilo e que desenvolveu a lesão mucosa na cavidade oral [7].

Além disto, algumas pessoas com LM afirmam não disporem de tratamento adequado ou até mesmo não terem recebido tratamento para a lesão cutânea inicial, demonstrando, com isso, que as curas espontâneas ou tratamentos irregulares e/ou curtos estejam contribuindo para o aparecimento dessa forma clínica, considerando-se esses elementos como de risco para o aparecimento da LM [10,20,48,50,53,54]. Também, para alguns autores pode haver envolvimento da mucosa nasal sem doença cutânea anterior, sugerindo que a penetração da *Leishmania* tenha ocorrido ao nível do límen, ou seja, na transição cutâneo-mucosa da estrutura do nariz [7].

Tudo isso mostra o quão difícil é a compreensão do desenvolvimento das lesões mucosas, que podem estar ligadas ao hospedeiro, parasita, à magnitude da resposta imunológica [7,18,28,44], aos fatores socioeconômicos, ambientais e a contiguidade [18].

As lesões mucosas podem configurar-se como destrutivas, potencialmente mutilantes, atingindo as vias aerodigestivas superiores [18,20,55-60]. O comprometimento mucoso na LTA é considerado uma manifestação grave da doença, capaz de impossibilitar a pessoa para o trabalho e retirá-la do convívio social [20]. Entre os homens a forma mucosa é mais comum, além desses exibirem mais perfuração septal e haver propagação das lesões para estruturas externas de cavidade nasal [37].

Essas lesões mucosas são definidas como de evolução lenta, situadas nas mucosas do nariz, boca, faringe e laringe [52,53,56-60]. Em consequência a esses acometimentos pode haver obstrução nasal (epistaxe) [2,7,18,29,43] relacionado à rinorreia e dor leve, edema e hiperemia de mucosa septal

anterior, presença de nodulações [7,18,29,42], espessamento da mucosa dos seios paranasais, dor facial, cefaleia, hiposmia, coceira [57], formação de lesão granulomatosa [7,18,29,43] que poderá evoluir com perfuração septal, edema de nariz com espessamento de pele, queda de suporte nasal (nariz de tapir ou anta) [7,18,29,61], pirâmide nasal volumosa [7,18,29], bronco-aspiração levando à morte, sequelas cicatrizantes extensas, com tendência a sinéquias e estenoses de orifícios naturais [43,59,60].

O estadiamento das lesões na mucosa nasal subdivide-se entre os seguintes estágios: I. Nodulação sem ulceração; II. Ulceração superficial; III. Ulcerações profundas; IV perfuração septal; V. destruição do arcabouço nasal e alteração da estrutura facial. No primeiro estágio os pacientes podem ser assintomáticos ou ter sintomas leves como, por exemplo, rinorreia sem sangramento ou obstrução nasal. O segundo estágio pode ser caracterizado por lesões com sangramento, além de que podem estar presentes os sintomas do estágio inicial. Já no terceito estágio, observa-se crostas sangrentas no septo nasal, na concha inferior e no assoalho da fossa nasal. Os pacientes também podem ter sensação de dor na pirâmide nasal, secreção de coágulo sanguíneo, rinorreia com sangue e importante obstrução nasal. Já a quarta fase se caracteriza pela necrose da cartilagem no septo nasal anterior e em alguns casos as colunas nasais podem estar comprometidas. Nessa fase, a cartilagem septal é perfurada com acentuada infiltração do septo posterior. Na última fase, pode-se ter a total destruição da coluna nasal e a ponto do nariz pode desabar. A região dorsal da pirâmide nasal em alguns casos pode estar perfurada. Extensas crostas com aparência sangrenta podem ser observadas

devido ao alargamento da cavidade nasal, representada pela destruição das cartilagens do septo e da concha inferior [62].

Ademais, outros sintomas que podem ser evidenciados nesses casos são a disfonia, odinofagia, rinorreia, entre outros [13], que podem modificar as funções do sistema estomatognático, como a respiração, deglutição e mastigação, e prejudicar aspectos vocais [13,17]. Além disto, muitos pacientes podem demonstrar dor ao deglutir e ou sialorreia devido à presença de lesões no palato duro e mole, úvula, gengiva e faringe [7,17,18,42,48,63], podendo manifestar disfagia [17]. De mais a mais, a mastigação pode ser prejudicada em vista do envolvimento das gengivas e interstícios dentários, onde granulações grosseiras podem estar presentes e proeminentes, chegando ao lábio superior, muitas vezes poupando a língua [17,64].

Todas essas implicações da LM podem impactar nos âmbitos social, psicológico e econômico dos indivíduos afetados por essa enfermidade [2,19], estigmatizando o paciente e prejudicando os relacionamentos dentro e fora do ambiente familiar [20,40], causando exclusão social e diversos transtornos emocionais [19,20].

Além disso, há uma convicção equivocada de que a Leishmaniose pode ser transmitida por partilhar refeições ou por contato corporal, retirando, assim, as pessoas acometidas do convívio social, reforçando o padrão de exclusão [65] e fragilizando o indivíduo que remodela a forma como se vê, motivando emoções de ansiedade, desesperança, agressividade, luto, raiva, medo [66]. Em função disso, essa doença também pode ser considerada ocupacional, refletindo-se em perdas no campo econômico [19,67].

Contribuindo para que o convívio com a doença se torne mais doloroso, durante o período de tratamento o paciente passa por uma fase vulnerável resultante de uma moléstia com recuperação incerta, ademais encara exaustão física decorrente dos efeitos de muitos medicamentos. Outra circunstância que coopera para a manifestação do modo como as pessoas reagem diante desta realidade é a ocorrência de linguagem inapropriada por parte de profissionais que atuam na área da saúde, que não conseguem explicar de forma clara o desafio que o paciente terá pela frente, fazendo com que os pacientes banalizem a doença, se automedicando e tentando explicá-la de maneira fantasiosa [66].

Ademais, o estigma psicossocial acarretado por essa doença ainda não foi mensurado pelos serviços de vigilância em saúde [31,43]. Existem sugestões na literatura para a melhora da capacitação de equipes quanto aos conhecimentos técnicos, aos aspectos psicológicos e às praticas relacionadas à doença e aos doentes, para que possa ocorrer a adoção de medidas de prevenção que levem em conta o conhecimento a respeito da doença, as atitudes e as práticas da população referentes às condições de vida e ao trabalho das pessoas; o estabelecimento de relação dinâmica entre o conhecimento do profissional que vai atuar com as medidas educativas e a vivência da população em seus fatores sociais, econômicos, políticos e culturais [2, 20,68].

IV.2.4 Tratamento das Leishmanioses Cutânea e Mucosa

No tratamento das Leishmanioses é importante frisar que o tratamento precoce da LC pode diminuir a prevalência das formas mais graves de LTA,

bem como sua morbidade e custos com tratamento [37,39], considerando que a maioria dos pacientes que desenvolvem a LM tem histórico de LC [38].

Isto posto, o tratamento preconizado para a LC é com o uso de antimonial pentavalente (Sb^{+5}), em que a dose é calculada a partir mgSb^{+5}/Kg/dia, podendo utilizar-se dois tipos de antimoniais pentavalentes: antimoniato de N-metilglucamina e o estibogluconato de sódio (este último não é comercializado no Brasil) [3,28].

Recomenda-se que a dose empregada esteja entre 10 e 20mg Sb^{+5}/Kg/dia, priorizando-se 15mg Sb^{+5}/Kg/dia tanto para adultos quanto para crianças durante 20 dias seguidos nos casos de LC. Caso não haja cicatrização completa dentro de três meses, depois do tratamento, deverá avaliar-se novamente o paciente. Se houver necessidade, o esquema terapêutico deverá ser repetido, porém a duração da série precisará ser de 30 dias [28].

Em contrapartida, no acometimento mucoso a dose sugerida é de 20mg Sb^{+5}/Kg/dia ao longo de 30 dias, via parenteral. Caso não haja cicatrização completa em até três meses ao final do tratamento, o esquema deverá ser repetido. Se não houver resposta frente ao tratamento, deverá ser avaliada a associação terapêutica de outra droga [20,28,55;58].

Tem-se observado o sucesso do emprego da pentoxifilina como coadjuvante (imunomodulador) no tratamento da Leishmaniose Tegumentar, com desfecho de cura em menor tempo quando comparado ao tratamento convencional. O seu papel é atuar como adjuvante, não tem indicação como medicamento isolado, mas sim em associação ao antimoniato de meglumina. Além disso, o uso de pentoxifilina diminuiu a toxicidade do tratamento com o

antimoniato, pois contribuiu para a melhora da taxa de cura e proporciona cura mais rápida, o que evitaria a exposição a um segundo ciclo de tratamento com antimoniato de meglumina, especialmente lesivo em pacientes mais vulneráveis como idosos e aqueles que apresentam progressão da doença com lesões agressivas e desfigurantes [3].

Nos casos onde não há resposta satisfatória ao tratamento, poderão ser utilizadas drogas de segunda escolha como, por exemplo, a anfotericina B e as pentamidinas (sulfato de pentamidina e mesilato de pentamidina). A primeira droga apresenta excelente atividade *in vitro* na destruição de Leishmania intra e extracelular. É a primeira a ser escolhida para os casos de gestantes e a segunda para os casos onde não se obtém resposta ao tratamento com antimonial pentavalente ou na impossibilidade de seu uso. Já para as pentamidinas, existem poucos estudos realizados na América sobre as mesmas como terapêutica da LTA. Essas drogas são comumente utilizadas como segunda escolha para o tratamento da LTA em áreas endêmicas dos continentes Americano, Asiático e Africano [28].

Quanto aos efeitos colaterais do tratamento, o antimonial pentavalente pode desencadear efeitos colaterais como artralgia, mialgia, anorexia, náuseas, vômitos, plenitude gástrica, epigastralgia, pirose, dor abdominal, prurido, febre, fraqueza, cefaleia, tontura, palpitação, insônia, nervosismo, choque pirogênico, edema e insuficiência renal aguda [2,3,31,58]. Atualmente não existem estudos sobre medicamentos usados no tratamento da Leishmaniose e suas consequências para os aspectos vocais dessa população.

Com isso, percebe-se a necessidade de um plano de vigilância e controle epidemiológico, entomológico e de reservatórios, para combater as populações de insetos vetores de doenças e controlar o aumento de casos, assim como devem ocorrer medidas educativas (atividades de conscientização sobre a doença, distribuição de repelentes, melhorias nas condições de trabalhos, moradia e infraestrutura) voltadas à comunidade [33,39,37,69], contribuindo para a melhoria da qualidade de vida, em especial dos moradores de zonas rurais [33].

V. Atuação Fonoaudiológica na Leishmaniose: Interação vocal e as Sequelas da Leishmaniose Mucosa

A produção vocal ocorre no trato vocal, fruto da harmonia da respiração, fonação e ressonância, capaz de manifestar as emoções e representar a personalidade dos indivíduos [70,71]. Quando há um desequilíbrio nesse conjunto, pode-se obter como resultado um sintoma denominado disfonia [70,72] que se caracteriza pela dificuldade ou alteração na emissão vocal que afete a voz natural [73,74], associada a variadas etiologias [70].

Observa-se que a presença de LM pode causar lesões que, às vezes, levam à alteração da face (desfiguração) dos indivíduos [60] e a diversos sintomas como, disfonia, odinofagia, rinorreia, entre outros [13]. Com isso, é possível que essas pessoas apresentem modificações nas funções do sistema estomatognático (respiração, deglutição, mastigação) e na produção da voz [13,17].

Em função dos sintomas de rinorreia, crostas nasais, epistaxe, hiposmia, perfuração septal, queda do suporte nasal [7,17,75], os sujeitos podem apresentar problemas como respiração forçada [7] ou oral. Além disso, muitos pacientes apresentam dor para deglutir e ou sialorreia devido à presença de lesões no palato duro e mole, úvula, gengiva e faringe [7,17,18], podendo manifestar disfagia [17]. Para mais, a mastigação pode ser prejudicada em vista do envolvimento das gengivas e interstícios dentários, onde podem se desenvolver granulações grosseiras e proeminentes, chegando ao lábio superior, muitas vezes poupando a língua [17,64]. Com esses agravos, pode haver comprometimento da fala, ocorrendo distorções de fonemas ou dificuldades articulatórias.

A voz nesses pacientes pode estar alterada devido à localização das lesões, que se mostram presentes no nariz, na cavidade oral, na laringe e na faringe, acarretando transformações na qualidade vocal como um todo [13]. Há inflamação laríngea generalizada particularmente na região dos seios piriformes. As pregas vocais podem estar se movendo bem, mas a fonação pode ser fraca e a contração muscular pode estar prejudicada por formação granulomatosa e subsequente fibrose [17,64].

Considerando-se a localização das lesões presentes na LM, que podem trazer alterações vocais, modificações nas funções do sistema estomatognático, que podem aumentar os prejuízos psicológicos e sociais, observa-se a necessidade da intervenção Fonoaudiológica nesses indivíduos. Contudo, há apenas um estudo sobre a intervenção fonoterapêutica nesses casos [76]. Já para a LC, ainda não existem estudos que abordem as características vocais desses indivíduos.

Tal trabalho [76], mostra que 88,5% dos participantes apresentaram alteração da qualidade vocal. Tanto os pacientes com lesão na cavidade oral e faringe quanto os com lesão na laringe tinham queixa de disfonia e apresentavam alteração vocal. Também foi realizada fonoterapia após a resposta favorável ao tratamento de leishmaniose, em que se verificou melhora estatisticamente significativa nos parâmetros de grau geral da rouquidão, rugosidade, soprosidade, tensão, *Shimmer* e Tempo Máximo de Fonação (TMF). Contudo, cinco pacientes continuaram com alteração vocal após a terapia, sendo que os autores creditam isso à associação de mais de um sítio de lesão. Além disso, entre os pacientes do estudo havia tabagistas e etilistas, participantes com hipertensão, diabetes, tuberculose, hanseníase e com

síndrome de imunodeficiência humana, o que pode ter sido fator de contribuição para a manutenção da disfonia.

V.1 **Intervenção Fonoterapêutica**

A voz é parte integrante da nossa comunicação social, personalidade individual e expressão de emoções [71,77]. Estabelece-se como o meio mais rápido e fácil de nos comunicarmos [71,78], enriquecendo a transmissão da mensagem articulada, agregando à palavra o conteúdo emocional, a entonação, a expressividade, de modo que os seres humanos possam ser reconhecidos e identificados através das características vocais [71,79,80].

Ela se constitui em um processo complexo, que envolve a realização de um sinal vocal pela laringe mais a filtragem desta pelo trato vocal. Algumas particularidades da voz decorrem da laringe e outros advêm em forma de contribuição fornecida pelo filtro do trato vocal [81,82].

Quando há alteração vocal decorrente de um distúrbio funcional e/ou orgânico do trato vocal, ocorre um sintoma denominado disfonia, que pode ser expressa por sinais como cansaço ou esforço ao falar, rouquidão, pigarro ou tosse persistente, sensação de aperto ou peso na garganta, falhas na voz, falta de ar para falar, afonia, ardência ou queimação na garganta, entre outros [83,84].

Para que a voz ocorra de forma satisfatória, é necessário que o aparato fonador tenha um condicionamento muscular favorável [85,86]. A fim de evitar a disfonia, podem ser utilizados exercícios vocais que, normalmente, causam aumento da temperatura do tecido muscular e do fluxo sanguíneo, diminuindo o número de prejuízos para o trabalho muscular [85,86].

A terapia vocal inclui informações sobre ergonomia da voz, que compreende todas as ações que facilitem a comunicação verbal e as questões relacionadas à saúde vocal [87,88]. Seu intuito é proporcionar ao paciente a melhor voz possível, sempre considerando as limitações intrínsecas a cada indivíduo [89,90,91]. A prática clínica moderna tem buscado terapias de curtos períodos e que apresentem resultados mais eficazes de forma mais objetiva [88].

A terapia deve envolver determinados objetivos e etapas a serem realizados para uma repercussão positiva, englobando orientação, conscientização, treinamento vocal com exercícios mais específicos [92-95]. Esta parte inicial pode ser considerada uma fonoterapia de base, pois com explicação e prevenção o paciente poderá, sistematicamente, tentar reverter os comportamentos vocais inadequados [95,96].

Estudos mostram que os indivíduos que passam por programas de terapia vocal apresentam benefícios significativos na qualidade de vida e na comunicação [88,97]. Exercícios vocais têm sido propostos para favorecer fechamento glótico, aumentar pressão subglótica e intensidade da voz, estabilizar qualidade vocal e frequência fundamental, além de proporcionar melhora global do sistema funcional da fala [88,93;98].

Dentre as técnicas fonoterapêuticas, encontram-se os Exercícios Vocais de Trato Semiocluído (EVTSO), que tem indicação tanto para os casos de alteração vocal, como para os casos de vozes normais, onde se busca apenas o aperfeiçoamento vocal, por meio do favorecimento da economia e eficiência vocal [91,93, 99,100,101].

Este tipo de técnica vocal é realizado através da oclusão parcial da região anterior do trato vocal, que se torna constrito ou alongado, promovendo a ressonância retrorreflexa em direção às pregas vocais. Inúmeras variações destes estão descritas no contexto das recentes pesquisas como, por exemplo, os vibrantes, fricativos, sons nasais, firmeza glótica, /b/ prolongado, constrição labial, fonação em tubos, Finger Kazoo, Vogais arredondadas, entre outras [91,100,102].

Estudos sobre EVTSO mostram que a oclusão do trato vocal modifica a impedância acústica e gera ressonância retroreflexa, afastando as pregas vocais na vibração, reduzindo os riscos de trauma e equilibrando as pressões sub e supraglótica, com economia vocal, além de verificar que os mesmos podem ser utilizados em distúrbios vocais, incluindo a hipernasalidade, aquecimento e aperfeiçoamento vocal [86,91,102].

Esses exercícios do EVTSO expandem a área do trato vocal, enquanto o fechamento glotal e o fluxo de ar são mantidos, assim reduzindo a dose de vibração e o trauma de adução nas pregas vocais [102]. Exercícios como esse promovem o aumento do conforto fonatório, reduzem a tensão, e a resistência vocal que é essencial para os pacientes [102].

A terapia da voz com o EVTSO tem efeito positivo sobre a voz porque torna a emissão vocal mais eficiente e econômica [103]. Sobre os efeitos imediatos dos exercícios de EVTSO (Finger Kazoo e Fonação em tubos), alguns autores [104] apresentaram resultados positivos e similares quanto à avaliação acústica e autoavaliação perceptivoauditiva. Na avaliação acústica, viu-se diminuição da frequência fundamental e a avaliação perceptivoauditiva

indicou melhoras substancialmente visíveis após a fonação em tubos de ressonância.

Quando observado os efeitos da técnica fonoterapêutica de fricativo sonoro /ž/, analisados após duas séries de 15 repetições, verificou-se redução do ruído glótico, melhora da Proporção Harmônico-Ruído, diminuição do cociente de contato entre as pregas vocais, melhora do tipo de voz e da ressonância e da sensação autorreferida de voz mais clara e limpa [105].

Um estudo que analisou o tempo de execução da técnica de vibração sonorizada de língua constatou que para os homens a avaliação perceptivoauditiva evidenciou melhoras vocais após o quinto minuto de execução. Contudo, as sensações desagradáveis e sinais laringoscópicos, como hiperemia e muco, aumentaram proporcionalmente ao aumento de tempo da execução da técnica [106].

Em outra pesquisa realizada sobre os efeitos imediatos do exercício de sopro sonorizado na voz de idosos, de ambos os sexos, onde foi feita coleta de emissão regular, de emissão da fala espontânea e após a aplicação de técnica vocal, observou-se diferença significativa entre a emissão regular e fala espontânea. Porém, viu-se que as emissões após o exercício vocal foram consideradas melhores na maioria dos casos [98].

Quando avaliadas as modificações vocais ocorridas antes, imediatamente após a execução da técnica fonoterapêutica de Finger Kazoo e após cinco minutos de silêncio absoluto, através da espectrografia, em indivíduos adultos do sexo feminino, observou-se que, após o exercício vocal, a Espectrografia de Banda Larga apresentou melhora nos parâmetros de

intensidade do traçado, da definição dos Formantes, e regularidade do traçado. Porém não houve mudanças na largura de banda dos Formantes, na definição de F1 e na presença de ruído nas altas, médias e baixas frequências. Em contrapartida, a Espectrografia de Banda Estreita, mostrou aumento da intensidade do escurecimento do traçado das altas frequências e de todo o espectrograma, melhora da regularidade do traçado, melhora da definição dos harmônicos. Porém, houve piora da presença de ruído entre os harmônicos [107].

Na avaliação de 14 meninas de um coro infantil amador, antes e após um programa de aquecimento vocal que incluía alguns EVTSO (sons fricativos, nasais e vibrantes de lábios e língua), não foram observadas diferenças em relação aos momentos pré e pós-exercícios. Além disso, não houve preocupação em se analisar a qualidade perceptivoauditiva das vozes, apenas observá-las visualmente e identificar possíveis mudanças nos parâmetros [108].

Com a realização de exercícios de sons de apoio nasal, fricativos sonoros e vibrantes de lábios e língua, 13 professoras apresentaram redução significativa no grau de tensão vocal [109].

Os benefícios que os EVTSO proporcionam estão sendo cada vez mais evidenciados em pesquisas que procuram expor resultados de duas ou mais avaliações. Ainda há poucos estudos, mas se percebe preocupação cada vez maior em pesquisar os efeitos específicos desses exercícios em cada nível fonatório [91].

V.1.1 Sons Nasais

A técnica de sons nasais é um exercício de trato vocal semiocluído, considerada facilitadora do equilíbrio fonatório [95,102,110]. É uma das técnicas mais utilizada desde tempos remotos no canto e no teatro [93].

Também pode receber outras denominações, como *humming*, técnica de ressonância ou de colocação da voz na máscara. Sua aplicação vai desde o método de voz ressonante a casos de tratamento das disfonias, principalmente as hiperfuncionais, caracterizadas pelo excesso de tensão muscular [92,95,110,111; 112,113], bem como casos de fenda triangular médio-posterior e lesão nodular que necessita de redução na concentração e no esforço vocal [114].

Alguns autores classificam os sons nasais como sons de apoio, ou seja, facilitadores da emissão, que podem promover um equilíbrio funcional no intuito de uma eficiência fonatória [93,114,115].

Devido à interação fonte e filtro que a técnica traz, há energia retroflexa, que pode, além de minimizar o impacto sofrido pelas pregas vocais durante a fonação e reduzir os riscos de trauma, melhorar a ressonância e a projeção vocal [95,102,110].

Os sons nasais têm por objetivo diminuir o foco de ressonância baixo, aumentando a oralidade por meio da ressonância nasal, sem deixar a voz com nasalidade adicional. Ainda, quando se percebe dificuldade por parte do paciente em executar a técnica de forma adequada, esse exercício pode ser emitido em “três andares” (como técnica terapêutica), colaborando na passagem de uma máxima nasalidade para uma máxima oralidade [93,114,115].

Esta técnica deve ser realizada de forma confortável e natural, em que, durante a emissão de um “hum” sustentado, haja uma fonação adequada, de preferência com a mandíbula abaixada e lábios fechados [116]. Para mais, também haveria benefícios da realização da técnica com a língua solta no soalho da boca, com percepção da vibração na área da máscara (nariz e boca), sendo praticada todos os dias, pelo menos por cinco minutos. Alguns autores relatam que som revelaria a naturalidade da voz de cada pessoa e, com este treinamento vocal, haveria uma voz mais potente e com longevidade [117].

O *humming* é um som musical, leve, produzido com a consoante /m/, de boca fechada que ocasiona vibrações na laringe e na faringe, na boca e no nariz, inclusive nos seios nasais, dentes, lábios, pele e mucosas [118]. Com a emissão prolongada de “hum” em diferentes frequências, para que exista um espaço dentro da boca, o paciente deve imaginar que emite a vogal “u”, de lábios fechados e, depois, procurar, variar este som, subindo e descendo três tons, entre agudos e graves [119].

Os principais benefícios da técnica de som nasal são: o equilíbrio ressonantal; enriquecimento da energia harmônica do sinal glótico; redução e equilíbrio da hipertensão laríngea, mandibular e de todo o aparato fonador; suavização da emissão; melhora da projeção vocal; equilíbrio do ataque vocal; aumento dos tempos máximos de fonação (TMF); melhora do automonitoramento vocal; [92,95,110,111,112,115]; favorecimento da agilidade dos músculos respiratórios; alívio da sobrecarga das pregas vocais e adequada vibração de suas bordas [120].

O som nasal pode ainda interferir no modo de vibração das pregas vocais, gerando um maior número de harmônicos e, desse modo, um som mais rico e com qualidade vocal com menos ruído [121]. Ademais, esse exercício é primordial para o desenvolvimento da percepção auditiva, relaxamento das tensões e distribuição da energia entre as três principais cavidades ressonantais [111].

Verificou-se a percepção do impacto na qualidade da voz de 20 indivíduos sem alterações vocais, utilizando-se três técnicas: vibração de língua, técnica de ressonância com o /m/ sustentado e sopro continuado. Os participantes executaram os três exercícios em ordem casual, durante três minutos. Após os exercícios de som nasal 80% dos participantes apresentaram melhora na qualidade vocal e sentiram as vozes mais resistentes, fortes, fáceis e claras [122].

A cavidade nasal e suas estruturas adjacentes tem grande importância para a fonação, sobretudo para a ressonância. Ao utilizar-se o som nasal, em que a energia sonora é disseminada para as cavidades supralaríngeas, pode-se perceber a vibração dos tecidos da face, bem como uma fonação mais fácil pelo fator de relaxamento das estruturas laríngeas, faríngeas e orais [111,114,115].

Foram observados os efeitos de um programa fonoterapêutico utilizando a técnica de sons nasais em casos de disfonias hiperfuncionais, em um grupo de mulheres com idade entre 19 e 60 anos. Constatou-se melhora das medidas acústicas sugestivas de ruído à emissão vocal e efeitos positivos sobre o tecido e o fechamento das pregas vocais [95].

Em outro estudo com o objetivo de comparar a efetividade de duas intervenções fonoaudiológicas, aquecimento vocal (que incluía sons fricativos, vibrantes e nasais) e treino respiratório, em professores com idade entre 20 e 60 anos, observou-se que houve redução estatisticamente significativa da frequência fundamental, sugerindo provável diminuição do esforço vocal e do hiperfuncionamento da musculatura laríngea nos participantes desse grupo [123].

VI. ARTIGO

ARTIGO 1

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Phonotherapeutic Intervention in Patients with Mucosal Leishmaniasis Sequelae

Famiely Colman Machado de Machado [*], Marcus Miranda Lessa[†], Carla Aparecida Cielo[‡], Tássia Milenna de Oliveira Souza[*‡], Fernanda Ventin Prates de Souza[×], Camila Sampaio Ribeiro[Ω], Juliana Almeida Silva[Ψ], Edgar Marcelino de Carvalho Filho[∞], Paulo Roberto Lima Machado[↕], Thaynara Montagner[Ω*], Mailane Mota Muniz[♯], Thaynara Montagner[↕∞], Daniela Gonçalves[×∞], Carolina Cincurá[+]

Institution where it was held: Health Certer Dr. Jackson Lemos Costa, Corte de Pedra, Municipality of Tancredo Neves (BA), Brazil.

(*) Graduate Program in Health Science (Doctorede Degree), Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(†)Associate Researcher of the Immunology Department of Professor Edgard Santos University Hospital of the Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(‡) Department of Speech-Language Pathology and Post-Graduation Program in Human Communication Disorders, Federal University of Santa Maria-UFSM-Santa Maria (RS), Brazil. Productivity Exchange 2 CNPq.

(*‡) Otorhinolaryngologist and Master in Health Sciences, Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(×) Dermatologist and Doctorate in Health Sciences, Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(Ω) Dermatologist and Master in Health Sciences, Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(Ψ) Biologist and Post-Doctorate in Health Sciences, Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(∞)Researcher at the Gonçalo Muniz Institute (Fiocruz-Bahia) and Associate Researcher at the Immunology Service at the Edgard Santos University Hospital, Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(↕)Coordinator and Associate Researcher of the Immunology Service of Professor Edgard Santos University Hospital of the Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(+)Associate Researcher of the Immunology Department of Professor Edgard Santos University Hospital of the Federal University of Bahia-UFBA-Salvador (BA), Brazil.

Address for correspondence: Famiely Colman Machado de Machado, Avenida Dom João VI, número 378, complemento 03, Salvador (BA), 40285-001, Brazil. Tel: +55 07130130528. E-mail address: famycolman@yahoo.com.br

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ABSTRACT

Purpose: To characterize the voice before and after speech-language intervention, with Humming nasal sound in patients with sequelae Mucosal Leishmaniasis (ML) and Cutaneous Leishmaniasis (CL). **Methods**: Collection of phonation /a:/ from 44 pacients with ML and CL for perceptual voice analysis and computed acoustic. The Wilcoxon Nonparametric Test and Fisher's exact test were used, with significance level of 5%. **Results**: It was observed, pre-speech therapy, that 27.7% of participants with ML presented asthenic vocal quality, and for the acoustics characteristics there was a statistically significant result for measures of frequency, frequency disturbance, noise and sub-harmonic measurements, indicating phonatory instability, weakness and noise emission giving the emission a feeling of vocal weakness. After therapy, the sub-harmonic segment measurements for the group with ML, showing reduction noise emission. Patients with CL had more grade 1 instability (36.4%), indicating tremor in vocal tract structures. After speech therapy, this group presented a reduction in the degree of roughness and reduction of the frequency disturbance measures, indicating a decrease in tension in the larynx and pharynx. **Conclusion**: Even after completing treatment for LM, patients may experience vocal changes due to the sequelae of the disease, like vocal alterations due to nasal lesions or in other locations that interfere in the correct vocal emission. As for participants with CL, no vocal changes would be expected, since these patients present thorax, leg and arm lesions that would not cause problems for the voice. Nevertheless, the two groups of participants presented vocal changes to different degrees before vocal therapy. However, it was observed that patients with ML present vocal alterations with more severe degrees. After the speech-language intervention, the participants of both groups showed vocal improvement, but the group with CL presented more vocal benefits, possibly due to the previous vocal alterations not being so severe.

Keywords: Voice, Mucosal Leishmaniasis, Cutaneous Leishmaniasis, Voice disorders

INTRODUCTION

Leishmaniasis is an infectious parasitic disease of great concern to public health. Brazilian Northeast suffers a significant incidence of this disease, mainly American Tegumentary Leishmaniasis, although it is possible to find it from the south of the United States to the north of Argentina [1-4].

This disease is characterized for having several clinical presentations and can cause disfiguring and disabling lesions with severe psychosocial consequences. There are three classical types of clinical presentation: Mucosal Leishmaniasis (ML), cutaneous Leishmaniasis (CL) and Disseminated Leishmaniasis (DL) [2,5].

ML causes destructive lesions of the upper respiratory tract which can cause from a small perforation of the nasal septum to the destruction and collapse of the entire nasal pyramid. Approximately 5% patients healed from CL, develop ML after months or years [5-9]. Some of the symptoms of this pathology are: nasal obstruction, cough, rhinorrhea and epistaxis. However, these symptoms and the complaints reported by the patients such as dysphagia, odynophagia, dysphonia, dyspnea, depend on the area affected by the LM (mouth, nose, pharynx, and larynx) [10,11].

Dysphonia, one of the complaints reported by those patients, can significantly impact their quality of life due to communication impairment, which, consequently, affects social and professional relationships [12,13]. In addition, there is the psychological factor [14], that can further worsen the quality of life and voice of those patients [15].

Voice rehabilitation of patients with ML can rely on voice therapy programs that significantly benefit communication [16,17] such as semi-occluded vocal tract (SOVT) exercises indicated either in cases of voice disorders or in cases of individuals with normal voice but, who pursue voice economy and efficiency [18-20].

These exercises are performed by occluding the anterior region of the vocal tract, promoting retroflex resonance towards the vocal folds [17], which generates increased phonatory comfort and vocal resistance, in addition to reducing laryngeal tension [21, 22].

An example of these exercises is the nasal sound “humming”, which provokes phonation harmony. This vocal technique ensures adequate interaction between source and filter, minimizing the impact suffered by the vocal folds during emission. With this, the risk of lesions is reduced and resonance and vocal projection are improved [23]. These effects are only possible because of the energy dissipated towards the supra-laryngeal cavities. In this way, the vibration generated in the facial tissues will allow lighter phonation due to the relaxation of laryngeal, pharyngeal and oral structures [24,25].

Thus, we intend to verify and compare the auditory perceptual and acoustic vocal characteristics before and after the speech-language intervention, with Humming nasal sound, in patients with sequelae of ML and CL.

Method and Patients

1. Study design

A cross-sectional analytical study, of experimental nature was conducted at the Dr. Jackson Lemos Costa Health Clinic located in the village of Corte de Pedra, in the municipality of Tancredo Neves in southeastern Bahia, from February 2016 to May 2017. Patients were divided into two groups (Participants with sequelae of CL) and (Participants with sequelae of ML), with the purpose of characterizing their voices and verifying the vocal changes occurred after the application of vocal technique. To this end, the technique was applied in only one session, where three series of nasal sound were performed, collecting the voice of the participants before and after the technique.

All participants signed an informed free consent term - TCLE (acronym in Portuguese) in accordance to the ethical principles established in Norm 466/12, of the National Committee of Ethics in Research and approved by the Committee of Ethics in Research of COMHUPES-UFBA, under number 43/2015.

2. Patient Selection

All participants selected for the study, according to inclusion and exclusion criteria, had already completed the treatment for Leishmaniasis. After the speech-language evaluations, the participants who presented vocal alterations were referred for vocal treatment, and the patients had the choice to continue the treatment.

Participants were selected according to the following inclusion criteria: aged from 18 years, aiming at avoiding the alterations of the voice change period [26,27], to 70 years to include older male patients who are more frequently affected by ML [28,29]; participants who had ML and CL, and were diagnosed by parasitology, histology, culture, immunology (provided by the Immunology Service - SIM- of HUPES), and otorhinolaryngology; with concluded medical treatment for leishmaniasis; with sequelae of nasal, buccal, pharyngeal and laryngeal lesions diagnosed by a physician, with or without voice complaints; who signed the TCLE.

As for exclusion criteria, patients with the following characteristics were excluded from the study: smokers, alcohol consumers [27,30]; those who did not finish the drug treatment for ML or CL, those with history of voice therapy, history of Diffuse, Disseminated or Mucocutaneous (concomitant) Leishmaniasis, those with history of

respiratory diseases (bronchitis, asthma, allergic rhinitis, sinusitis); who had the flu on the day of the evaluation; who presented a history of neurological problems [27,30]; who reported other granulomatous diseases (paracoccidioidomycosis, squamous cell carcinoma, basal cell carcinoma, lymphomas, rhinophyma, rhinosporidiosis, entomophthoromycosis, wegener granulomatosis, Wirchowian leprosy, tertiary syphilis, tuberculosis, sarcoidosis, and cocaine-induced midline granuloma); who had other laryngeal pathologies (minimal structural changes in the coverage and organic lesions) [30]; who had traumatic septal perforation or by drug use diagnosed by a physician [31].

For the performance of exclusion and inclusion criteria it was done anamnesis, including questions on complaints of voice disorders, aspects that could interfere in voice performance or in the execution of the evaluations, use of larynx aggressive agents that could favor the emergence of laryngeal disorders, respiratory diseases, date of ATL diagnosis and voice conditioning through speech-therapy techniques and otorhinolaryngological evaluation, including oroscopy, rhinoscopy and videolaryngoscopy were carried out targeting the identification of laryngeal or other disorders in accordance to the exclusion criteria; and evaluation of the Stomatognathic System of CL patients, who can present facial deformities, observing the posture, mobility and tonicity of these structures

3. Sample

All participants in this study were enrolled in a post-treatment follow-up list of Leishmaniasis. Patients were called for review via telephone call. At the time of the medical appointment, participants were informed about the research and questioned about their interest in participating in the study.

The sample was composed of 63 participants interested in the research. Participants were numbered and randomized, using the simple random sampling process until reaching a minimum of 44 cases. After being subjected to the inclusion and exclusion criteria, two participants with sequelae of CL were excluded because they did not complete all the examinations and one of them because he presented a nodular lesion on the right vocal fold (Figure 1). On the other hand, only one participant with sequelae of ML was excluded because he presented a granulomatous

disease (paracoccidioidomycosis) (Figure 1), and was referred to monitoring and specific treatment.

Thus, four participants were excluded, being necessary to continue the lottery until reaching the ¨n¨ determined by sample size calculation. Therefore, according to sample size calculation assuming a level of significance of 5% (α), a sample power of 80% (1-β), and a simple random sampling process for the statistical analyses, sample size was 22 participants per group, totaling 44 cases.

4. Data Collection

To collect vowel /a:/ emission, patients were asked to be in orthostatic position and to emit the sound in a sustained manner in usual pitch and loudness, at maximum phonation time and without using the respiratory reserve [27,30,32]. Emission was recorded in a digital *Zoom* Model Q3 *Handy Recorder* professional voice and video recorder, with PCM audio format, quantization of 16*bits*, capture frequency of 96kHz, keeping a mouth-recorder distance of four centimeters and 90º, in a quiet environment [27,30,32].

Collection was carried out immediately before and after the complete execution of the Nasal Sound vocal technique, in just one session, performed by the participants in maximum phonation time, in three series of 15 repetitions [27,33,34], with a 30-second rest between series, with the patients sitting and in complete silence [27,35]. The technique was demonstrated and monitored by a speech therapist to avoid incorrect performance [27,36,37].

To perform the technique, the participants closed their lips and clenched their teeth. Gradually they opened their teeth only slightly, keeping their jaws relaxed and their tongue on the floor of the mouth. Then they emitted the prolonged "hum" sound, imagining emitting the sound of the vowel / u /, with perception of the vibration in the mask area (nose / mouth) [23]. During the technique, participants were sitting with their feet flat on the floor, an upright spine with no cervical dislocation, with a 90 ° angle between the chin and the neck, without increasing the muscular contraction of the shoulder girdle, maintaining the constant rhythm between one repetition and another, without making use of the expiratory reserve and, avoiding the fluctuation or variability of pitch and / or loudness [27,33,36,38].

Ingestion of 250 ml water was allowed [39], considering a possible vocal tract dryness with increased air flow. However, this did not interfere at the glottic level until the final vowel emission was collected, since water reached the larynx in a systemic way, not interfering with the results of the research.

Concerning vowel /a:/ emission, auditory perceptive evaluation was performed through the RASATI scale that evaluates hoarseness (R), level of roughness (A), breathiness (S), asthenia (A), strain (T) and instability (I), using the following rating scale: 0 = normal: when no voice alteration is perceived by the listener; 1=slight, when the alteration is evident and 3= for extreme voice alterations [40]. This evaluation was conducted by four speech-therapists judges, with expertise in the voice area, who did not participate as authors of this study and were blinded about the purposes of the research. The information that the evaluators received was on the age and gender of the patients, and the recordings were delivered through a file storage and sharing service based on the “cloud computing” concept with individual accounts [30].

To this analysis it was carried out an inter-rater agreement through the Kappa coefficient. The interpretation of the test is as follows: values less than 0 correspond to no agreement; values between 0 and 0.19 represent poor agreement; values between 0.20 and 0.39 correspond to good agreement; values between 0.40 and 0.59 correspond to moderate agreement; values from 0.60 to 0.79 correspond to strong agreement; and values between 0.80 and 1 correspond to an almost perfect agreement [41]. It was observed a strong agreement between the judges, with an index of 0.78. Only the answers of these judges were selected and grouped to the data analysis, due to a greater reliability.

Also, a computed acoustic analysis was performed, using vowel /a:/ emission and the *Multi Dimensional Voice Program Advanced* (MDVPA) from Kay Pentax, in which vocal attack was eliminated and the end of emission discarded so that these stretches did not alter signal analysis, once the ends of prolonged emissions usually present decreases of amplitude and frequency, creating therefore the interval of five seconds for the analysis window, which was the smallest sustain time obtained in the group [27,30,42].

Threshold values for males proposed by the MDVPA software [30,33,43] were used as reference of the extracted measures. In addition, the fundamental frequency

parameters were analyzed considering the following values as reference of normality: 80 Hz to 150 Hz for males [44]. The measures automatically extracted by MDVPA, with sampling rate of 44 kHz and 16 *bits*, were the frequency measures: f0; maximum f0 (fhi); minimum f0 (flo); Standard deviation of f0 (STD); frequency perturbation measures: absolute *Jitter* (Jita); *Jitter* percentage (Jitt); pitch relative average perturbation (RAP); pitch perturbation quotient (PPQ); smoothed pitch perturbation quotient (sPPQ); variation of f0 (vf0); amplitude perturbation: *Shimmer* in dB (ShdB); *Shimmer* percentage (Shim); amplitude perturbation quotient (APQ); smoothed amplitude perturbation quotient (sAPQ); amplitude variation (vAm); noise measures: noise-to-harmonics ratio (NHR); voice turbulence index (VTI); soft phonation index (SPI); voice break measures: degree of vocal breaks (DVB); number of vocal breaks (NVB); measures of voiceless or unvoiced segments: number of unvoiced segments (NUV); degree of unvoiced segments (DUV); measures of subharmonic segments: degree of subharmonic components (DSH); number of subharmonic components (NSH) [27,30,43].

5. Statistical Analysis

SPSS 17.0 was used for the statistical analysis, including social-demographic data and clinic characteristics. In addition, to compare de auditory measures before and after applying the vocal technique to the group with sequelae of ML and the comparison between the group with sequelae of CL and ML, Wilcoxon non-parametric test was applied having in mind that 80% of the distributions were not normal, with a level of significance of 5% ($p<0.05$). On the other hand, Fisher exact test was used to compare the data of the RASATI scale, before and after vocal treatment between groups (ML and CL).

RESULTS

The sample was composed of 44 participants, 22 in each group, all males, with average age of 57.59 ± 6.49 for the participants with sequelae of ML and 35.13 ± 11.28 for the participants with sequelae of CL. All participants were from the rural zone, occupationally active, most of them exerting activities in the field (Table 1).

Of the 22 patients with ML, 72.72 % presented lesions only in the nose (Table 2), and the symptoms more reported during the illness period were nasal crusts (86.36 %), epistaxis (36.36 %), nasal obstruction (31.81 %), rhinorrhea (27.27 %), anosmia (13.3 %), hyposmia (4.54 %) and dysphonia (4.54 %). Concerning the disease staging degree: 9.52 % presented grade I, 28.57 % grade II, 23.80 % grade III, 28.57 % grade IV and 9.52 % grade V.

The comparison of the results of the RASATI scale between groups with ML and CL before the speech therapy showed statistically significant results, with 27.7% participants with ML showing the voice quality asthenia, indicating that this characteristic is more frequently associated with ML. In contrast, more patients with CL had instability grade 1 (36,4 %) than patients with ML (Table 3).

After applying the nasal sound technique, participants of both groups presented reduction of the grade of hoarseness, although those of the CG presented greater reduction than those of the SG, with statistically significant results (Table 3).

Table 4 shows, that in the comparison of ML with CL before the application of the vocal technique, statistically significant results were obtained for frequency measures (fhi, STD), frequency perturbation measures (vF0), noise measures (SPI) measures of voiceless or unvoiced segments (NUV), measures of subharmonic segments (NSH), indicating association of these parameters with the group with ML, which presented higher values than the CL group. However, the CL group presented higher SPI (noise measure) to emission than the ML group.

On the other hand, after the speech therapy intervention there was statistically significant reduction of the frequency perturbation measures (PPQ, sPPQ, vF0) for the CL group and of the measures of subharmonic segments (NSH, DSH) and of STD) for the ML group.

Table 5 shows the acoustic measures of the MDVPA, before and after applying the vocal technique, only for the group ML, which presented statistically significant reduction of the noise parameters (VTI, NHR), although with increase of the SPI. Moreover, there was increase of the degree of unvoiced segments (DUV).

Figure 1 shows the laryngoscopic characteristics of patients with LM and LC sequelae.

DISCUSSION

Throughout Brazilian territory, the predominance of Leishmania (Viannia) brasiliensis [45] was observed, with a large growth since 2000, with the state of Bahia being the second, after Maranhão, to report [46] and, on the other hand, the southern region of the country presented the lowest number of cases [47].

The LM and CL are two of the main clinical forms of LTA, the former being considered simpler than the latter. Some authors have shown that 5% to 10% of patients who receive treatment or cure for CL may develop ML [5,8].

The CL is a chronic and benign disease [48, 49], being the most common form of ATL, which may not manifest itself over the years or evolve to cutaneous lesions. The characteristic CL lesions may present as single or multiple, ulcerated [14, 50], usually painless, round or oval in appearance, with erythematous, infiltrated and firm appearance, with circumscribed and elevated borders, reddish and with coarse granulations [51, 52]. They often affect exposed areas of the body, such as limbs [14, 50].

Usually, the appearance of ML occurs after primary skin lesion healing (2 to 5% of cases), but it can also occur simultaneously to the CL frame [14,53, 54]. However, the true incidence of LM in Brazil is not known, often due to the lack of notification of these cases [55].

The mucosal lesions can be configured as destructive, potentially mutilating, reaching the upper aerodigestive pathways [56, 57, 58,59]. Mucosal involvement in ATL is considered a serious manifestation of the disease, which makes it impossible for the person to work and withdraw from social interaction [14]. Among men, the mucosal form is more common, besides they exhibit more septal perforation and there is propagation of the lesions to external nasal cavity structures [28].

In this study, 59.09 % (n=26) participants were aged between 25 and 50 years, all were rural dwellers and most of them worked in agriculture. Studies show that there may be variation concerning the spread of cases among the different age groups, indicating the possibility of household, peridomiciliar and occupational transmission, because children under six and the elderly are generally indoors, while people of productive age have higher risk of having the disease because of their occupations in the forest, usually agriculture or extractivism (Table 1) [28,60,61].

The sample of this study was composed of males and most studies show that males are more affected by ATL [10,28], that the mucosal form is more frequent and that they can develop more severe forms of the disease, with high incidence of perforations and involvement of the external structures of the nasal cavity [28]. This prevalence of ATL among males, may occur due to their occupational or leisure activities such as hunting, fishing or camping which are still more common among them (Table 1) [60,62].

ML has an insidious onset and although it presents scarce initial symptomatology [63], has lesions with a great destructive potential [64], affecting the mucosa and cartilage of the upper airways and digestive tract [7,58,59,63,65,66], disfiguring and disabling the individuals affected by this disease [7,58,59,63,66,]. Among the symptoms frequently reported in the literature [10,31,64,67], this study found higher occurrence of nasal crusts, epistaxis, nasal obstruction and rhinorrhea during the course of the disease (Table 2).

As a result of the symptoms of rhinorrhea, nasal crusts, epistaxis, hyposmia, septal perforation, collapse of the nasal support (tapir nose) [9,10,68], individuals may present breathing problems, such as breathing in a forced manner [10]. Also, many patients have pain when swallowing, and sialorrhea, caused by lesions on the hard and soft palate, uvula, gingiva and pharynx [10,64,68], and may manifest dysphagia [68]. Moreover, mastication may be impaired because of the involvement of the gums and dental interstices, where coarse and prominent granulations may develop, reaching the upper lip, often sparing the tongue [51,68]. All this can cause speech impairment, with phoneme distortions or articulatory difficulties.

Clearly, the most prevalent symptoms indicate the nasal region as the most affected site (72.72 %) (Table 2). The literature shows that most lesions are located in the nose [10,31,64,67], and that the involvement of the nasal mucosa can occur without previous cutaneous disease, revealing that Leishmania penetration occurred at the cutaneous-mucosal transition of the nose structure [10]. Moreover, it is believed that parasite metastasis can be brought to the upper airways and digestive tract through lymphatic or hematogenous route [10,64] and unusually by direct contact of the mucosa with a cutaneous lesion as in the case of a newborn whose mother has a cutaneous lesion on the nipple and who developed a mucosal lesion in the mouth [10].

In addition, some studies [7,10,29,31,64,69] have shown that, following the nasal site, lesions may appear on the palate, pharynx, larynx and gum. However, in the present study, it was observed that most of the lesions occurred just in the nasal site (Table 2). In addition, even without the presence of lesions in the larynx, we observed lower incidence of dysphonia, which, according to some authors, may be associated with lesions on the pharynx and oral cavity [11]. Moreover, this symptom may also be due to presbyphonia, since the CL group is composed of participants with higher age [30,70,71]. Even considering these results, the development of mucosal lesions is still not well understood. Several factors are related to these lesions such as, the parasite, the host and the magnitude of the immunological response [10,49,64,72], as well as socioeconomic and environmental factors or even contiguity [64].

The voice of these patients may be altered due to the location of the lesions sequelae, which were present on the nose, oral cavity, larynx and pharynx, causing changes in resonance and vocal quality [73]. There is generalized laryngeal inflammation particularly in the region of the pyriform sinuses. The vocal folds may be moving well, but the phonation may be weak (asthenic) and the muscle contraction tension may be impaired by granulomatous formation and subsequent fibrosis [51, 68].

Although no laryngeal lesions were found in this research (Figure 1), it is known that voice production depends on the harmony between breathing, phonation and resonance which express the emotions and represent people's personality [74,75]. Thus, when there is no harmony in that set, one of the consequences may be dysphonia [74,76], which will impair or alter voice emission, affecting natural voice [77,78].

The auditory perceptive analysis of the vocal characteristics of the groups studied evidenced a statistically significant association of asthenia grade 2 with the ML group (27.7 %) and of voice instability grade 1 with the CL group (36.4 %) (Table 3). Asthenia gives a feeling of weakness to emission and instability is the abnormal change of the voice [79]. In the case of asthenia, it was also found that, acoustically, the values Jitter and Shimmer were higher when compared to the CL group (Table 4), however, without statistical significance. Studies have shown that this voice quality may be related to high indices of theses acoustic parameters [80].

Moreover, asthenia may be related to glottic insufficiency, age (usually present in the elderly) and to the etiology of vocal fold paralysis [80,81]. Therefore, in this study,

asthenia associated with the ML group may be explained because the participants with ML were older (57.59 ± 6.49), which may favor laryngeal alterations, such as the presence of vocal crevices or reduction of vocal fold mucosa, which can lead to glottic insufficiency [30]. However, some authors report that clinical manifestation of muscular hypofunction, would not yet be installed in the laryngeal mechanism of voice production [82], and also, that uncontrolled pulmonary air may favor the decrease of pneumophonic efficiency [83] which may cause voice fatigue [84,85], generating asthenia which would involve everybody from the age of 60 years.

On the other hand, vocal instability brings a shivering characteristic to vocal tract structures [70,86], which may be related to the decline in muscle strength of the larynx and aerodynamics of the pulmonary current [83], usually linked to the voice of older people [87], not to mention the occurrence due to greater adduction of the vocal folds. It is possible that the vocal deviation presented by this group is related to their vocal habits or even due to greater laryngeal constriction. This constriction would reduce transglottic flow, which may lead to pneumo-phono-articulatory incoordination [88]. Furthermore, it was verified that 13.63% of these participants presented incomplete vocal coaptation, which may have been compensated by laryngeal hypercon- straction (Figure 1).

Concerning the vocal acoustic characteristics, the measure of the variation of the fundamental frequency (vF0), was higher for the participants with ML (Table 4), indicating association of this group, with statistically significant results. These high values may be due to the anatomical characteristics of male larynx, with greater extent and volume of the vocal folds, which favors phonatory instability, once these measures are related to instability of the cycle-to-cycle signal or, in short term and to the control over the phonatory system [44,90]. In addition, it was observed that this parameter may be related to hoarseness and voice roughness. [91], which were also characteristics presented by the group with ML, without statistical significance, although with a high percentage and rough voice yet with moderate degree. However, it should be pointed out that these vocal deviations can also be attributed to the age of the participants [30].

Furthermore, the frequency measures (STD, Fhi) and the number of subharmonic segments (NSH) were also associated with the ML group, with values higher than the CL group and statistically significant. Similar to vF0, the high result of these measures

may also be related to the discrete instability in frequency maintenance [30]. This can occur due to resonance alteration, caused by the imbalance in the air direction due to nasal changes (for example, nasal obstruction). Such changes may trigger oral breathing, which will lead to changes in muscle function, leading to compensatory effort in the musculature of the larynx, which would trigger emission instability [73].

Three quarters of patients with ML presented higher values of the number of unvoiced segments (NUV) than the CL group, with statistically significant values (Table 4). This measure is characterized by disrupted periodicity of the sound wave, reflected as noise or irregular emission. There are studies that show that higher values of these measures are associated with males [92]. However, this was not confirmed in this research, once only three quarters of ML participants presented high values and that the CL group was also composed by males. Moreover, people who go through the aging process may exhibit instability of the voice quality and increase of voice breaks and or frequency changes, which would justify the increase in the ML group [30].

The patients with CL presented statistically significant association with the noise measure SPI, indicating higher presence of noise to phonation than the ML group. These high values suggest inadequate closure of the vocal folds [30,42], or can occur as consequence of bass voices (as in males) and aerial turbulences due to non-linear phonation [93]. To avoid voice disorders, voice exercises can be used, which usually cause increase of the muscle tissue temperature and of the blood flow, reducing the impairment of the muscular work [94,95]. In this context SOVT exercises can be used. They are performed by partial occlusion of the anterior portion of the vocal tract, which becomes constrict or lengthened, promoting retroflex resonance towards the vocal folds. Some variations of these exercises are described in recent researches, such as nasal sounds [17,23] used in this work.

After speech therapy intervention, there was improvement of the acoustic parameters in both groups. However, there was statistically significant reduction of the measures of frequency perturbation (vF0, PPQ, sPPQ) for the CL group (Table 4). As the technique of nasal sounds is an exercise that helps to shift the resonant focus from hypo to hyper, it reduces the tension of the larynx and pharynx, acting as a springboard of voice projection to the space, which will reduce low resonance and

increase the oral component of nasal resonance, producing richer harmonics series and bringing more stability to emission [23,96,97].

In addition, there was a statistically significant reduction of the measures of sub-harmonic segments (DSH, NSH) for the participants with ML (Table 4). The measures of the sub-harmonic components allow measuring the presence of these low intensity components located between the harmonics. Their decrease after the vocal technique shows reduction of the noise to emission. In the same way, the decrease of the parameters of unvoiced segments, which represent the interruption of the sound wave, indicates reduction of the irregularities during vocalization [44]. Moreover, there was statistically significant improvement of the frequency measure STD, suggesting greater emission stability (Table 4).

In addition, when only the group with ML was analyzed, before and after the speech therapy intervention (Table 5), improvement of the noise parameters (VTI, NHR, SPI) was observed, indicating regularization in the vibration of the laryngeal structures [42,89], which may have occurred due to the benefit brought to vocal folds coaptation, as well as by the better channeling of the aerial flow to the resonance cavities, suggesting improved glottic closure during phonation [42]. In the same way, the decrease in the parameters of unvoiced segments, which represent the interruption of the sound wave, indicates reduction of the irregularities during vocalization [44].

Concerning the vocal characteristic of roughness (Table 3), it can be observed that after applying the speech therapy technique, participants of both groups presented reduction of this vocal type, although the reduction of the CL group was greater than that of the ML group, with statistically significant results. Roughness usually occurs in a noisy and unpleasant way, poor in harmonics and rich in noise [98], emerging as a consequence of the rigidity of the covering of the vocal folds and or caused by muscle rigidity due to tension increase [99], bringing vibration irregularity to emission [99]. Thus, the reduction of this vocal characteristic may have occurred due to the smoothing of the emission, the reduction of the laryngeal hypertonicity and the improvement of the vibration of the vocal folds, favored by the nasal sounds technique [23,96,97].

Finally, we have to point out that this study has some limitations such as the possible interference of presbylarynx on ML group vocal disorders and that the study

was conducted only with males. Further studies should use a younger public, and, if possible include the female population, even though ML is more common among males.

CONCLUSION

The purpose of this research was to improve the understanding of the relationship between Leishmaniasis and voice, especially the Mucosal Leishmaniasis. We verified that even without the presence of sequelae of laryngeal lesions, people with ML may present voice disorders caused by sequelae of nasal lesions or in other sites, which interfere in the correct vocal emission. We highlight asthenia and the most alterations of the acoustic measures (fhi, STD, vF0, SPI, NSH, DSH) associated with the ML group, which presented the greatest vocal deviations when compared to the CL group. This may have occurred due to the impact of the disease on laryngeal structures, as well as due to voice disorders caused by age and gender.

The CL group also presented vocal changes, marked by vocal instability along with noise measurements (SPI). However, the changes did not present great severity when compared to the ML group. These alterations can be attributed to the incorrect vocal habits of the laryngeal hypercontraction performed in an attempt to compensate for incomplete vocal coaptation.

Despite the disfiguring lesions of ML, which lead to greater vocal impairment of individuals with this disease, we proved that vocal intervention, even over a short period of time, considerably benefits the voice of these patients, improving their quality of life and voice. The results of this study can stimulate the creation of measures of vocal promotion and prevention and better speech therapy strategies. We also verified voice disorders in the CL group, which was not expected, because it was a group characterized by lesions in regions that are not related to the vocal apparatus. However, similar to the ML group, the speech therapy brought significant improvement of the acoustic and auditory perceptual voice parameters.

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Figure 1. Laryngoscopic characteristics of patients with leishmaniasis sequelae

Laryngoscopy Characteristics		
	Mucosal Leishmaniasis	**Cutaneous Leishmaniasis**
	N(%)	
Mobility	22(100%)	22 (100%)
Symmetry	22 (100%)	22 (100%)
Complete Glotic Coaptation	21(95,45%)	19(86,36%)
Incomplete Glotic Coaptation		
Irregular Slit	1 (4,54%)	2 (9,09%)
Triangular posterior Slit		1 (4,54%)
Presence of Mucosal wave	22 (100%)	22 (100%)
Supraglottic Activity	0 (0%)	0 (0%)
Participantes excluídos		
Vocal nodules		X*
Paracoccidioidomycosis	X*	

Table 1. Demographic distribution of patients with Cutaneous Leishmaniasis and Mucosa

	Mucosal Leishmaniasis		Cutaneous Leishmaniasis	
Variables	N	%	N	%
Occupation				
Farmer	20	90,90	16	95,45
Bricklayer	2	9,09	0	0
Unemployed	0	0	1	4,54
Previous history of Leishmaniasis				
Yes	11	50	4	18,18
No	11	50	18	81,81
Age				
Age	Mean	Standart Deviation	Mean	Standart Deviation
	57,59	±6,49	35,13	±11,28

Legend: N:Number of subjects. %:Percentage.

Table 2. Location of lesions of patients with Leishmaniasis befor drug treatment

	Mucosal leishmaniasis	
Location of Injuries	**N**	**%**
Nose	16	72,72
Nose+Mouth	1	4,54
Nose+Pharynx	3	13,63
Mouth+Pharynx	1	4,54
Nose+Pharynx+Mouth	1	4,54

Table 3. Comparison of auditory perceptual parameters between the Mucous and Cutaneous Leishmaniasis groups, before and after the vocal technique

		Mucosal Leishmaniasis								Cutaneous Leishmaniasis								
		0		1		2		3		0		1		2		3		p-value
	R	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	0,087
		6	27,3	7	31,8	4	18,2	5	22,7	11	50	6	27,3	5	22,7	0	0	
	A†	6	27,3	5	22,7	7	31,8	4	18,2	13	59,1	4	18,2	5	22,7	0	0	0,077
BEFOR	S	19	86,4	2	9,1	1	4,5	0	0	22	100	0	0	0	0	0	0	0,223
	A	16	72,7	1	4,5	5	22,7	0	0	21	95,5	1	4,5	0	0	0	0	0,048*
	T	16	72,7	4	18,2	1	4,5	0	0	17	77,3	2	9,1	3	13,6	0	0	0,511
	I	18	81,8	4	18,2	0	0	0	0	10	45,5	8	36,4	4	18,2	0	0	0,023*
A	R	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	p-value
F		10	45,5	8	36,4	4	18,2	0	0	11	50	10	45,5	1	4,5	0	0	0,479
T	A†	6	27,3	10	45,5	6	27,3	0	0	18	81,8	4	18,2	0	0	0	0	0,000*
E	S	21	95,5	1	4,5	0	0	0	0	22	100	0	0	0	0	0	0	1,000
R	A	17	77,3	4	18,2	1	4,5	0	0	21	95,5	1	4,5	0	0	0	0	0,185
	T	18	81,8	4	18,2	0	0	0	0	14	63,6	1	4,5	1	4,5	0	0	0,345
	I	20	90,9	2	9,1	0	0	0	0	14	63,6	6	27,3	2	9,1	0	0	0,083

Legend Fisher's exact test. * Statistically significant values (p <0.05). N: number of subjects. %: Percentage.

R: Hoarseness; †A: Asperity; S: Thunder; A: Asthenia; T: Tension; I: Instability.

Table 5. Comparison of vocal technique application between Cutaneous and Mucous Leishmaniasis Groups through acoustic means of MDVPA

		ML			CL			
		Medium	**Q1**	**Q3**	**Medium**	**Q1**	**Q3**	**p-value**
BEFOR	**F0**	161,35	124,65	200,02	142,57	131,99	165,91	0,330
	Fhi	205,89	152,49	225,67	147,19	135,29	185,18	0,017*
	Fl0	133,67	99,26	173,60	137,84	128,59	158,62	0,734
	STD	3,63	1,93	2,17	1,75	1,27	2,24	0,003*
	Jita	59,59	25,36	131,40	36,86	24,52	62,88	0,142
	Jitt	0,93	0,38	1,74	0,52	0,36	0,91	0,119
	RAP	0,56	0,21	1,06	0,28	0,20	0,56	0,124
	PPQ	0,52	0,21	0,98	0,28	0,21	0,50	0,121
	sPPQ	0,86	0,59	1,91	0,66	0,48	0,86	0,051
	vF0	1,91	0,99	5,66	1,21	0,88	1,47	0,019*
	ShdB	0,33	0,15	0,76	0,29	0,25	0,43	0,751
	Shim	3,68	1,81	8,27	3,29	2,79	4,86	0,769
	APQ	3,14	1,91	6,37	3,19	2,63	4,76	0,805
	sAPQ	6,27	3,45	8,46	6,25	4,33	8,47	0,664
	vAm	15,79	8,63	22,52	13,70	10	18,83	0,769
	NHR	0,15	0,12	0,22	0,13	0,11	0,15	0,074
	VTI	0,068	0,04	0,086	0,055	0,044	0,068	0,152
	SPI	4,62	3,09	6,26	7,41	5,75	9,46	0,002*
	DVB	0,000	0,000	0,000	0,000	0,000	0,000	0,153
	NVB	0,000	0,000	0,000	0,000	0,000	0,000	0,153
	NUV	0,000	0,000	12,25	0,000	0,000	0,000	0,031*
	DUV	0,000	0,000	6,94	0,000	0,000	0,12	0,109

	DSH	0,000	0,000	1,22	0,000	0,000	0,000	0,206
	NSH	0,000	0,000	1,25	0,000	0,000	0,000	0,034*
		Medium	**Q1**	**Q3**	**Medium**	**Q1**	**Q3**	**p-value**
	F0	162,94	135,425	193,33	154,75	134,92	177,37	0,330
	Fhi	168,03	139,39	211,84	156,98	139,02	185,57	0,285
	Fl0	138,76	107,19	180,34	151,22	131,25	169,19	0,565
	STD	2,17	1,63	3,60	1,62	1,15	2,41	0,017*
	Jita	54,04	28,66	111,35	34,22	20,82	65,56	0,149
	Jitt	0,91	0,47	1,57	0,47	0,33	0,95	0,119
	RAP	0,55	0,28	0,95	0,27	0,19	0,57	0,093
A	**PPQ**	0,65	0,21	1,06	0,28	0,19	0,53	0,022*
F	**sPPQ**	0,69	0,56	1,64	0,55	0,49	0,81	0,050*
T	**vF0**	1,31	1,05	2,50	1,15	0,85	1,46	0,022*
E	**ShdB**	0,35	0,17	0,62	0,27	0,19	0,32	0,231
R	**Shim**	3,81	1,61	6,04	3,12	2,09	3,50	0,366
	APQ	3,15	1,93	5,38	2,83	1,96	3,46	0,519
	sAPQ	5,55	3,67	7,94	5,29	4,12	6,88	0,796
	vAm	13,35	8,78	19,66	11,42	6,97	13,64	0,130
	NHR	0,13	0,12	0,15	0,13	0,12	0,14	0,372
	VTI	0,052	0,044	0,07	0,047	0,037	0,061	0,213
	SPI	6,76	4,20	10,05	6,65	5,18	10,14	0,769
	DVB	0,000	0,000	0,000	0,000	0,000	0,000	0,317
	NVB	0,000	0,000	0,000	0,000	0,000	0,000	0,317
	NUV	0,000	0,000	4,25	0,000	0,000	0,000	0,081
	DUV	0,000	0,000	2,31	0,000	0,000	0,000	0,081
	DSH	0,000	0,000	1,16	0,000	0,000	0,000	0,043*
	NSH	0,000	0,000	1,20	0,000	0,000	0,000	0,018*

Legend Wilcoxon Ratings Test. Q1: first quartile. Q2: Second quartile. Q3: Third quartile. The data marked with (*) are statistically significant.

Abbreviations: f0, fundamental frequency; fhi, highest fundamental frequency; flo lowest fundamental frequency; STD, Standard Deviation of f0; Jitta, Absolute Jitter; RAP: Média relativa da perturbação do *pitch;* PPQ: quocientede perturbação do *pitch*.; sPPQ, Smoothed Pitch Perturbation Quotient; vf0: Variação da f0;ShdB, Shimmer in dB; APQ: quociente de perturbação da amplitude; sAPQ, Smoothed Amplitude Perturbation Quotient; vAm: variação da amplitude; NHR, Noise to Harmonic Ratio; VTI, Voice Turbulence Index; SPI, Soft Phonation Index; DVB, Degree of Voice Breaks; NVB, Number of Voice Breaks; NUV, Number of Unvoiced Segments; DUV, Degree of Voiceless; DSH, Degree of Sub-harmonics; NSH, Number of Sub-harmonic Segments.

Table 6. Acoustic measurements of the MDVPA before and after vocal technique (Nasal Sounds) in the Leishmaniasis Mucosa group

	Before vocal technique			After vocal technique			
	Medium	**Q1**	**Q3**	**Medium**	**Q1**	**Q3**	**p-value**
F0	161,35	124,65	200,02	162,94	135,425	193,33	0,838
Fhi	205,89	152,49	225,67	168,03	139,39	211,84	0,262
Fl0	133,67	99,26	173,60	138,76	107,19	180,34	0,610
STD	3,63	1,93	2,17	2,17	1,63	3,60	0,176
Jita	59,59	25,36	131,40	54,04	28,66	111,35	0,424
Jitt	0,93	0,38	1,74	0,91	0,47	1,57	0,980
RAP	0,56	0,21	1,06	0,55	0,28	0,95	0,949
PPQ	0,52	0,21	0,98	0,65	0,21	1,06	0,775
sPPQ	0,86	0,59	1,91	0,69	0,56	1,64	0,321
vF0	1,91	0,99	5,66	1,31	1,05	2,50	0,187
ShdB	0,33	0,15	0,76	0,35	0,17	0,62	0,759
Shim	3,68	1,81	8,27	3,81	1,61	6,04	0,775
APQ	3,14	1,91	6,37	3,15	1,93	5,38	0,849
sAPQ	6,27	3,45	8,46	5,55	3,67	7,94	0,638
vAm	15,79	8,63	22,52	13,35	8,78	19,66	0,243
NHR	0,15	0,12	0,22	0,13	0,12	0,15	0,039*
VTI	0,068	0,04	0,086	0,052	0,044	0,07	0,024*
SPI	4,62	3,09	6,26	6,76	4,20	10,05	0,050*
DVB	0,000	0,000	0,000	0,000	0,000	0,000	0,500
NVB	0,000	0,000	0,000	0,000	0,000	0,000	0,750
NUV	0,000	0,000	12,25	0,000	0,000	4,25	0,310
DUV	0,000	0,000	6,94	0,000	0,000	2,31	0,014*
DSH	0,000	0,000	1,22	0,000	0,000	1,16	0,820
NSH	0,000	0,000	1,25	0,000	0,000	2,00	0,625

-**Legend** Wilcoxon Ratings Test. Q1: first quartile. Q2: Second quartile. Q3: Third quartile. The data marked with (*) are statistically significant.

Abbreviations: f0, fundamental frequency; fhi, highest fundamental frequency; flo lowest fundamental frequency; STD, Standard Deviation of f0; Jitta, Absolute Jitter; RAP: Média relativa da perturbação do *pitch;* PPQ: quocientede perturbação do *pitch*.; sPPQ, Smoothed Pitch Perturbation Quotient; vf0: Variação da f0;ShdB, Shimmer in dB; APQ: quociente de perturbação da amplitude; sAPQ, Smoothed Amplitude Perturbation Quotient; vAm: variação da amplitude; NHR, Noise to Harmonic Ratio; VTI, Voice Turbulence Index; SPI, Soft Phonation Index; DVB, Degree of Voice Breaks; NVB, Number of Voice Breaks; NUV, Number of Unvoiced Segments; DUV, Degree of Voiceless; DSH, Degree of Sub-harmonics; NSH, Number of Sub-harmonic Segments

Table 7. Correlation between the parameters from RASATI scale and the acoustic vocal measures

	Hoarseness (R)		Roughness (A)		Breathiness (S)		Asthenia (A)		Strain (T)		Instability (I)	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**P**	**R**	**P**	**r**	**p**
f0(Hz)	0.094	0.484	0.356	0.006*	-0.122	0.363	-0.051	0.705	0.008	0.952	0.128	0.338
fhi(Hz)	0.214	0.107	0.291	0.027*	0.171	0.199	0.142	0.288	-0.118	0.379	0.106	0.428
flo(Hz)	-0.84	0.531	0.309	0.018*	-0.395	0.002*	-0.210	0.114	0.128	0.337	0.165	0.215
STD (Hz)	0.378	0.003*	0.208	0.117	0.413	0.001*	0.301	0.022*	-0.167	0.209	0.199	0.134
Jita(µs)	0.447	<0.001*	-0.111	0.408	0.420	0.001*	0.409	0.001*	-0.259	0.050*	-0.152	0.254
sPPQ(%)	0.275	0.037*	0.028	0.833	0.278	0.034*	0.311	0.017*	-0.211	0.111	0.096	0.474
ShdB (dB)	0.633	<0.001*	0.120	0.369	0.447	<0.001*	0.318	0.015*	-0.244	0.065	-0.163	0.222
sAPQ(%)	0.378	0.003*	0.165	0.216	0.522	<0.001*	0.223	0.092	-0.198	0.136	0.146	0.274
NHR	0.511	<0.001*	0.120	0.368	0.394	0.002*	0.249	0.060	-0.070	0.600	0.022	0.872
VTI	0.124	0.353	0.332	0.011*	0.176	0.187	0.127	0.343	-0.100	0.454	0.031	0.817
SPI	0.100	0.455	-0.338	0.009*	0.224	0.091	0.189	0.155	-0.156	0.243	-0.379	0.003*
DVB (%)	0.261	0.048*	-0.100	0.456	0.348	0.007*	0.176	0.186	-0.131	0.328	0.117	0.381
NVB	0.259	0.050	-0.100	0.456	0.340	0.009*	0.176	0.186	-0.131	0.327	0.124	0.352
NUV	0.304	0.020*	0.031	0.816	0.198	0.136	0.268	0.042*	-0.185	0.164	0.135	0.311
DUV (%)	0.304	0.020*	0.031	0.816	0.198	0.136	0.268	0.042*	-0.185	0.164	0.136	0.308
DSH (%)	0.560	<0.001*	-0.015	0.914	0.197	0.138	0.204	0.125	0.042	0.756	0.019	0.887
NSH	0.562	<0.001*	0.008	0.951	0.207	0.119	0.197	0.138	0.049	0.714	0.031	0.819

Fftr(Hz)	-0.161	0.229	-0.099	0.459	-0.050	0.708	0.098	0.464	0.096	0.475	-0.190	0.153
Fatr(Hz)	-0.021	0.874	-0.145	0.278	-0.119	0.374	0.200	0.133	-0.075	0.577	0.167	0.210
FTRI (%)	0.185	0.165	0.032	0.814	0.277	0.035*	0.313	0.017*	0.008	0.950	0.160	0.230
ATRI (%)	0.217	0.102	0.050	0.711	0.067	0.616	0.325	0.013*	-0.018	0.894	0.293	0.026*

*Notes: **Spearman's*** correlation test. p: statistical significance. r: value of the correlation coefficient. Data marked with an asterisk (*) are statistically significant.

Notes: R, hoarseness; A, roughness; B, breathiness; A, asthenia; T, tension; I, instability.

Abbreviations: f0, fundamental frequency; fhi, highest fundamental frequency; flo lowest fundamental frequency; STD, Standard Deviation of f0; Jitta, Absolute Jitter; sPPQ, Smoothed Pitch Perturbation Quotient; ShdB, Shimmer in dB; sAPQ, Smoothed Amplitude Perturbation Quotient; NHR, Noise to Harmonic Ratio; VTI, Voice Turbulence Index; SPI, Soft Phonation Index; DVB, Degree of Voice Breaks; NVB, Number of Voice Breaks; NUV, Number of Unvoiced Segments; DUV, Degree of Voiceless; DSH, Degree of Sub-harmonics; NSH, Number of Sub-harmonic Segments; Fftr, f0-Tremor Frequency; Fatr, Amplitude Tremor Frequency; FTRI, f0-Tremor Intensity Index; ATRI, Amplitude Tremor Intensity Index.

ARTIGO 2

“Spectrographic Analysis Before and After Phonotherapeutic Intervention in Patients With Tegumentary Leishmaniasis” **Plos One** [submetido, vide Normas de Publicação no ANEXO 3 e comprovante de envio no ANEXO 6].

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Spectrographic Analysis Before and After Phonotherapeutic Intervention in Patients With Tegumentary Leishmaniasis

Famiely Colman Machado de Machado [*], Carla Aparecida Cielo[†], Tássia Milenna de Oliveira Souza[*‡], Fernanda Ventin Prates de Souza[×], Camila Sampaio Ribeiro[Ω], Juliana Almeida Silva[Ψ], Edgar Marcelino de Carvalho Filho[∞], Paulo Roberto Lima Machado[↕], Thalita Barbosa Nunes[♯], Mailane Mota Muniz[♯], Thaynara Montagner [×∞], Daniela Gonçalves[×∞], Carolina Cincurá[+], Marcus Miranda Lessa [‡]

Institution where it was held: Health Certer Dr. Jackson Lemos Costa, Corte de Pedra, Municipality of Tancredo Neves (BA), Brazil.

(*) Graduate Program in Health Science (Doctorate Degree), Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(†) Department of Speech-Language Pathology and Post-Graduation Program in Human Communication Disorders, Federal University of Santa Maria-UFSM-Santa Maria (RS), Brazil. Productivity Exchange 2 CNPq.

(*‡) Otorhinolaryngologist and Master in Health Sciences, Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(×) Dermatologist and Doctorate in Health Sciences, Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(Ω) Dermatologist and Master in Health Sciences, Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(Ψ) Biologist and Post-Doctorate in Health Sciences, Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(∞)Researcher at the Gonçalo Muniz Institute (Fiocruz-Bahia) and Associate Researcher at the Immunology Service at the Edgard Santos University Hospital, Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(↕)Coordinator and Associate Researcher of the Immunology Service of Professor Edgard Santos University Hospital of the Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(♯)Speech Therapist, Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(×∞)Speech Therapist, Federal University of Santa Maria-UFSM- Santa Maria (RS), Brazil

(+)Associate Researcher of the Immunology Department of Professor Edgard Santos University Hospital of the Federal University of Bahia-UFBA-Salvador (BA), Brazil.

(‡)Associate Researcher of the Immunology Department of Professor Edgard Santos University Hospital of the Federal University of Bahia-UFBA-Salvador (BA), Brazil.

Address for correspondence: Famiely Colman Machado de Machado, Avenida Dom João VI, número 378, complemento 03, Salvador (BA), 40285-001, Brazil. Tel: +55 07130130528. E-mail address: famycolman@yahoo.com.br

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SUMMARY

Dysphonia may have an organic or functional cause and, in this context, lesions of the upper aerodigestive tract, as in Leishmaniasis Mucosa, can contribute to this vocal state. As vocal treatment we have, for example, the vocal exercise "humming", that acts in the vocal quality, resonance and vocal projection. To verify if there was voice improvement of the participants with sequelae of Mucous and Cutaneous Leishmaniasis, through spectrographic analysis, before and after the application of vocal exercise "humming". The vocal emission / a: of 22 participants from each group (total of 44 cases) was collected for computerized voice analysis through Kay PENTAX®'s Real Time Spectrogram program. Also, the protocol of the Profile of Vocal Behavior was applied, in order to characterize the vocal habits of these individuals. Only the sequelae group of cutaneous leishmaniasis had statistically significant results, with improvement of the following parameters: intensity of the tracing color, presence of noise, substitution of harmonics by noise, definition and regularity of harmonics, regularity of low frequencies and of the entire spectrogram and for anti-resonance. There was no statistically significant difference in the Voice Behavior Profile. Thus, possibly the group with Cutaneous Leishmaniasis sequela presented better vocal performance due to the absence of sequels in the upper aerodigestive tract. In addition, both groups were classified as a serious risk when presenting vocal changes, which may be justified by laryngeal motor adjustments performed to compensate for the sequelae of mucosal lesions and incorrect vocal habits practiced by the groups.

Key Words: Voice, Mucosal Leishmaniasis, Cutaneous Leishmaniasis, Voice disorders, Espectrography.

INTRODUCTION

For vocal quality without deviations, an adequate interaction between laryngeal and aerodynamic muscle forces is required [1-3]. In addition, healthy vocal habits are important for vocal emission to occur in a balanced manner [4,5].

However, emission without deviations is not always possible, given dysphonia, characterized by the presence of vocal symptoms such as tiredness when talking, hoarseness, throat clearing, burning throat, voice failure, breathlessness, which may be of organic or functional origin [6,7], that bring a mark of unpleasantness to the voice.

In this context, Mucosal Leishmaniasis (ML), evidenced by destructive lesions in the upper respiratory tract, affects areas ranging from the mouth to the larynx, which may lead to septal perforation [8-12], with consequences for vocal emission, including dysphonia [13,14].

The elective treatment for dysphonia is speech therapy, excluding cases of organic etiology that will require other priority interventions. The purpose of speech therapy is to deactivate the functional adjustment and to obtain muscular balance that provides a stable emission, with passages of the notes, without breaks or irregularities, besides establishing the fundamental frequency within the expected normal range for the gender and age of the individual [15-17].

Aiming at benefits for vocal emission, some studies have shown the positive results of Semi-Occluded Vocal Tract Voice (SOVT) exercises, which will act through retroreflex resonance [18-21]. These exercises are performed with semi-occlusion at some point in the vocal tract, providing economy and vocal efficiency, as well as increased loudness [20,21].

One of the vocal techniques used in this group is the humming nasal sound. Such exercise can soften vocal quality, improve resonance and voice projection [16,22]. Using this vocal technique, it is intended to verify if there was vocal improvement through spectrographic analysis, before and after the application of vocal exercise “humming” in patients with sequelae of ML and cutaneous leishmaniasis (CL). In addition, the vocal behavior of these individuals will be characterized.

PATIENTS AND METHODS

1. Study Design

This is an experimental analytical cross-sectional research of the quantitative type, performed at the Dr. Jackson Lemos Costa Health Post, located in the village of Corte de Pedra, Tancredo Neves municipality in southeastern Bahia, in February 2016 to May 2017.

Patients were divided into two groups (Participants with sequelae of CL) and (Participants with sequelae of ML), with the purpose of characterizing their voices and verifying the vocal changes occurred after the application of vocal technique. To this end, the technique was applied in only one session, where three series of nasal sound were performed, collecting the voice of the participants before and after the technique

All participants signed an informed free consent term - TCLE (acronym in Portuguese) in accordance to the ethical principles established in Norm 466/12, of the National Committee of Ethics in Research and approved by the Committee of Ethics in Research of COMHUPES-UFBA, under number 43/2015.

2. Patient Selection

2.1 Inclusion criteria

All participants selected for the study, according to inclusion and exclusion criteria, had already completed the treatment for Leishmaniasis. After the speech-language evaluations, the participants who presented vocal alterations were referred for vocal treatment, and the patients had the choice to continue the treatment.

Participants were selected according to age, from 18 years of age, in order to avoid changes in the period of voice changes [23,24], to 70 years to include older male patients who are more frequently affected by ML [12,25]; participants who had ML and CL; and were diagnosed by parasitology, histology, culture, immunology (provided by the Immunology Service - SIM- of HUPES), and otorhinolaryngology; with concluded medical treatment for leishmaniasis; with sequelae of nasal, buccal, pharyngeal and laryngeal lesions diagnosed by a physician, with or without voice complaints; who signed the TCLE.

2.2 Exclusion Criteria

The study excluded patients with the following characteristics: tobacco and / or alcohol users [24,26]; those who did not finish the drug treatment for ML or CL, those with history of voice therapy, history of Diffuse, Disseminated or Mucocutaneous (concomitant) Leishmaniasis, those with history of respiratory diseases (bronchitis, asthma, allergic rhinitis, sinusitis); who had the flu on the day of the evaluation; who presented a history of neurological problems [24,26]; who reported other granulomatous diseases (paracoccidioidomycosis, squamous cell carcinoma, basal cell carcinoma, lymphomas, rhinophyma, rhinosporidiosis, entomophthoromycosis, wegener granulomatosis, Wirchowian leprosy, tertiary syphilis, tuberculosis, sarcoidosis, and cocaine-induced midline granuloma); who had other laryngeal pathologies (minimal structural changes in the coverage and organic lesions) [26]; who had traumatic septal perforation or drug use diagnosed by a doctor [27].

In order to apply the criteria mentioned above, we proceeded with the anamnesis, formed by questions about complaints of vocal alteration, aspects that could interfere with vocal performance or the performance of evaluations, use of aggressive agents to the larynx that could favor the appearance of laryngeal disorders, respiratory diseases, time of diagnosis of ATL, and vocal conditioning through speech therapy techniques. Besides that, otorhinolaryngological evaluation including oroscopy, rhinoscopy and videolaryngoscopy were carried out targeting the identification of laryngeal or other disorders in accordance to the exclusion criteria; and evaluation of the Stomatognathic System of CL patients, who can present facial deformities, observing the posture, mobility and tonicity of these structures

3. Sample

All participants in this study were enrolled in a post-treatment follow-up list of Leishmaniasis. Patients were called for review via telephone call. At the time of the medical appointment, participants were informed about the research and questioned about their interest in participating in the study.

The sample was composed of 63 participants interested in the research. Participants were numbered and randomized, using the simple random sampling process until reaching a minimum of 44 cases. After being subjected to the inclusion

and exclusion criteria, two participants with sequelae of CL were excluded because they did not complete all the examinations and one of them because he presented a nodular lesion on the right vocal fold (Figure 1). On the other hand, only one participant with sequelae of ML was excluded because he presented a granulomatous disease (paracoccidioidomycosis) (Figure 1), and was referred to monitoring and specific treatment.

Thus, four participants were excluded, being necessary to continue the lottery until reaching the ¨n¨ determined by sample size calculation. Therefore, according to sample size calculation assuming a level of significance of 5% (α), a sample power of 80% (1-β), and a simple random sampling process for the statistical analyses, sample size was 22 participants per group, totaling 44 cases.

4. Data Collection

Initially, the questionnaire “Vocal Behavior Profile” was applied (Villela, Behlau, 1998[28]). Participants answered the test in the presence of the speech therapist, in which doubts were promptly answered. This test raises questions about how to speak (vocal intensity), use of breathing during speech, speech rate, water intake, use of self-medication, among other questions. It aims to evaluate the perception capacity of the subjects regarding the impact of voice on their quality of life [(29)].

Assessment questions are measured on a scale from zero to four for occurrence and frequency of situations, where “0” = never, “1” = rare occurrence, “2” = low frequency, “3” = high occurrence, “4 ”= Constant. After the evaluation, the Speech Therapist performed the sum of the points and classified the participants in relation to the vocal type as follows: 1) The behaved (up to 15 points), 2) The candidate for vocal problems (from 16 to 30 points), 3) The serious risk (from 31 to 50 points), 4) The champion of abuse (over 51 points). At the end, all participants received guidance on vocal hygiene.

To collect vowel /a:/ emission, patients were asked to be in orthostatic position and to emit the sound in a sustained manner in usual pitch and loudness, at maximum phonation time and without using the respiratory reserve [(24,26,30,31)]. Emission was recorded in a digital *Zoom* Model Q3 *Handy Recorder* professional voice and video recorder, with PCM audio format, quantization of 16*bits*, capture

frequency of 96kHz, keeping a mouth-recorder distance of four centimeters and 90º, in a quiet environment [24,26,30,31].

Collection was carried out immediately before and after the complete execution of the Nasal Sound vocal technique, in just one session, performed by the participants in maximum phonation time, in three series of 15 repetitions[24,31,32,33], with a 30-second rest between series, with the patients sitting and in complete silence [24,34]. The technique was demonstrated and monitored by a speech therapist to avoid incorrect performance [24,35,36].

To perform the technique, the participants closed their lips and clenched their teeth. Gradually they opened their teeth only slightly, keeping their jaws relaxed and their tongue on the floor of the mouth. Then they emitted the prolonged "hum" sound, imagining emitting the sound of the vowel / u /, with perception of the vibration in the mask area (nose / mouth) [22]. During the technique, participants were sitting with their feet flat on the floor, an upright spine with no cervical dislocation, with a 90 ° angle between the chin and the neck, without increasing the muscular contraction of the shoulder girdle, maintaining the constant rhythm between one repetition and another, without making use of the expiratory reserve and, avoiding the fluctuation or variability of pitch and / or loudness [24,32,35,37].

Ingestion of 250 ml water was allowed[31,38], considering a possible vocal tract dryness with increased air flow. However, this did not interfere at the glottic level until the final vowel emission was collected, since water reached the larynx in a systemic way, not interfering with the results of the research [24,32].

The spectrograph on the emission of the vowel /a:/ was carried out through the Real Time Spectrogram program (RTS) of Kay Pentax®, being eliminated the vocal attack and the end of emission in order not compromise the signal analysis, as long emissions can submit finals with decreases of amplitude and frequency. For the analysis, a five-second interval was created based on the shortest time of sustentation obtained in the group [39].

This analysis provided the spectrographic wideband (WBS) filtered in 100points (646Hz) and narrowband (NBS) with filter of 1024points (63.09Hz), with a sampling rate of 11 kHz and 16bit and resolution of 5 kHz. In WBS, the F were classified as

aspects: the intensity of the color of the tracing (F1, F2, F3 and F4); intensity of the color of the tracing of the high, low and medium frequencies; intensity of the tracing of the entire vocal spectrogram; 2) definition and regularity of the tracing (F1, F2, F3 e F4); 3) trace regularity for high, low and mid frequencies and throughout the vocal spectrogram [39,40,41,42]; 4) Formants Bandwidth (F1, F2, F3 e F4); 5) Damping immediately above F1, high, low and mid frequencies and throughout the vocal spectrogram.

For NBS, the following aspects were considered: 1) the intensity of the color of the tracing at high, low and medium frequencies tracing and tracing throughout the vocal spectrogram; 2) presence of noise at high, low and medium frequencies and in the entire vocal spectrogram; 3) replacement of harmonics by noise at high, low and medium frequencies and the vocal spectrogram; 4) definition and regularity of the harmonics; 5) number of harmonics; 6) presence of subharmonics [39,40,41,42].

Three speech-language pathologists with experience in the voice area - not authors of the study and not aware to the research objectives, carried out this evaluation in an individual approach. Speech therapists who acted as judges of the study were aware only on age and gender of the participants. All judges received the spectrographs and the protocol to mark their answers [26,39,42] in individual accounts through a storage service and sharing files, supported on a concept of cloud computing.

Inter-rater agreement was made using the Lin coefficient, interpreted as follows: values below 0.90 correspond to poor agreement; values between 0.90 and 0.95 correspond to moderate agreement; values between 0.95 and 0.99 correspond to the strong agreement; values greater than 0.99 equate to near perfect agreement [44]. Among the judges, the agreement was moderate, with an index of 0.95. The data of these judges were chosen and grouped for data analysis in view of greater reliability.

The evaluation of the spectrographs was performed according to a linear analog scale that presents scores from “zero” to “ten”. The answers of the judges were converted into numbers from zero to 100, corresponding to 100mm of the scale, through direct reading with millimeter ruler to perform the tabulation and analysis of results [42,45,46].

In assessing the intensity of the trace (F, high, medium and low frequencies and the entire spectrogram), the degree of darkening of the trace was considered, which may vary from black (strong intensity) to light gray (low intensity) [43,47], where ten corresponded to the highest and zero the lowest intensity of the stroke color.

The definition of formants and harmonics was measured according to their visibility, demarcation and symmetry, where zero was related to the lowest and ten to the highest definition. The regularity of the trace is linked to its continuity and stability, being considered zero for total irregularity and ten for total regularity of the trace. Noise visualization on the spectrogram was based on a dotted or shaded image and evaluated according to the degree of presence and darkness of the dotted or shaded, with zero total noise absence and ten maximum noise presente [43,47].

For noise harmonic replacement, zero was considered for no noise harmonic replacement and ten for total noise harmonic replacement. In the item number of harmonics, zero represented the absence of harmonics and ten the complete filling of the harmonic spectrographic image. Regarding the presence of subharmonics, the absence of subharmonics and ten the presence of subharmonics throughout the spectrogram were considered zero [42,43,47,48]. And for the anti-resonance (or damping effect) topic, pointed out as acoustic muffling due to sound damping, when it appeared visibly and apparently it was judged as ten and zero for its non-visualization [49].

5. Statistical Analysis

Comparison of spectrographic parameters before and after vocal technique application within the ML and CL groups using the non-parametric Wilcoxon test, considering that 80% of the distributions were not normal, with a significance level of 5% ($p < 0.05$). In addition, the Vocal Behavior Profile of each group was analyzed by comparing the level of vocal abuse between the groups using Fisher's Exact test. For all statistical analyzes, SPSS 17.0 was used.

RESULTS

The sample of this study consisted only of males, with a mean age of 57.59 ± 6.49 for the group with ML and a mean of 35.13 ± 11.28 for the participants with CL sequel. Most of the participants carry out activities in the field, all of them living in rural areas.

Table 1 shows the comparison of WBS before and after vocal technique for participants with ML and CL sequelae. In this study, it was possible to observe a statistically significant result for the group with CL sequelae, indicating improvement for the intensity parameters of F1, low frequency regularity and the whole spectrogram, and damping of medium frequencies.

Table 2, when comparing the NBS before and after the use of vocal technique between the leishmaniasis groups, showed that the group with CL sequelae had statistically significant results, where there was an increase in the color intensity parameter of the tracing to low frequency and across the spectrum, decreased presence of noise for high frequencies and replacement of harmonics with noise at low frequencies, and increased definition and regularity of harmonics for low frequencies.

Regarding the Vocal Behavior Profile of the participants in this study, 54.5% of participants with ML sequelae and 50% of participants with CL sequelae were considered serious risk for the development of vocal disorders. However, the results were not statistically significant. Among the abuse and misuse of vocal use and adverse conditions to vocal health, the most frequent among participants with ATL were as follows: 1) Speak in strong intensity (strong voice); 2) Talking for a long time; 3) Speak too fast; 4) Talk for a long time without hydrating; 5) Clear throat constantly; 6) Coughing too much; 7) Keep sound, radio or TV on while talking; 8) Drink little water; 9) Have too much coffee or tea; 10) Eat fatty or excessively spicy foods.

DISCUSSION

In agreement with previous studies [50-,53] on the prevalence of American Cutaneous Leishmaniasis (ATL) in men due to their labor activities (forest raids and working with domestic animals)[50-54], this study also evidenced a total sample of rural dwellers linked to activities in the field.

ATL is not an infectious or contagious disease, and presents as its classic clinical forms CL and ML. It is observed in the CL papules which develop into ulcers of the skin and / or mucous membranes that may be single, multiple, widespread or diffuse, have elevated edges and a generally painless granular bottom [55-57]. In ML, frequent lesions are seen in the nose, mouth and throat, manifesting as warty, papular, nodular, localized or diffuse plaques [11,57,58].

In the spectrography, the formant arrangement in WBS is directly related to the vocal tract conformation during the production of the analyzed sound, especially with the conformation of lips, tongue, soft palate and mandible, besides the conformation of the oral and pharyngeal cavities resulting from the various mobilizations of these articulators [59,60].

Participants with CL sequelae, after performing the vocal technique, showed a statistically significant increase in the intensity of the F1 trace color (WBS) and low frequencies (Table 1), as well as for the whole vocal spectrum (NBS) (table 2). These results may be linked to the fact that these participants did not have, and in this study did not present, lesions in the upper aerodigestive tract, as happens in the ML. This could have contributed to the participants' performance and, consequently, to the positive results after the vocal technique application.

In addition, the improvement in stroke color intensity may also be justified by the increase in vocal tract impedance generated by voice exercise. This increase would protect the glottis due to the high air pressure in the supraglottic region, which would trigger an increase in glottic pressure. As a result, the vocal folds would move away, minimizing the impact on the return of their contact medially, balancing the pressures at the level of the glottis and vocal tract [19,22,61,62]. Thus, these adjustments would favor a less vocal effort and higher vocal efficiency [19,63-65], softening the emission and decreasing laryngeal hypertonicity [16,22,66].

Linked to these results, the group with CL sequelae showed a statistically significant improvement in tracing regularity for low frequencies and for the entire spectrogram, for mean frequency anti-resonance (WBS) (Table 1), and increased definition and regularity. Low Frequency Harmonics (NBS) (Table 2). These results may have been obtained due to the intense mobilization of the mucosa, caused by the increased airflow during the vocal technique. Thus, the vocal fold vibrates more synchronously, improving the laryngeal signal through mucus layer renewal and mucosal homogenization, with a positive impact on vocal resonance, projection and emission [19,22,67,68].

The group with CL sequelae also showed a statistically significant result for the noise presence parameter and the replacement of harmonics by noise at low frequencies (Table 2), in which these parameters decreased. This improvement may have happened due to the vocal technique favoring the emission stability and agility of the respiratory muscles [22]. These benefits will lead to enrichment of harmonic energy, demonstrating improved pneumophonoarticulatory coordination and projection and vocal resonance, reduction and balance of laryngeal, mandibular hypertension and the entire phonatory apparatus [19,22,48,59,69].

Importantly, even without statistically significant results for the ML group, there was an improvement in the means presented by the group before and after the vocal technique. This shows that this group has benefits with exercise for the voice. However, due to the sequelae of lesions presented in the upper aerodigestive tract, it would be necessary to use the combination of multiple vocal exercises to promote significant vocal improvement in this population.

Another aspect that may predispose or influence the onset of dysphonia is the incorrect vocal habits performed in daily life. As an example within this studied group, we have the act of clearing our throat, talking too much, shouting, talking loudly, coughing, drinking too little water. In addition, general health conditions, anthropometric factors, and individual susceptibility may also favor the onset of dysphonia to some degree [7,69,70].

Tracing the profile of vocal behavior through the protocol used in this research, it was found that there was no statistically significant difference between the groups, showing that both practiced almost the same amount of vocal abuse, and most

participants were classified as serious risk of voice problems. This may also shed some light on why individuals with CL sequelae presented vocal alterations without sequelae of upper aerodigestive tract lesions, since this type of sequelae would likely imply alterations in vocal emission.

However, the group with sequelae of ML presented more participants classified as champion of vocal abuse when compared to the group with sequel of CL. Probably this may have been due to the inadequate muscle adjustments of these participants in an attempt to compensate for the sequelae that most compromise the vocal tract with negative impact on vocal emission [70].

However, vocal self-perception by participants through protocols may have the disadvantage that these individuals do not know how to self-evaluate, since many participants rely on the lack of information and knowledge about voice [71,72,73].

CONCLUSION

Thus, it is demonstrated that both groups studied showed improvement in vocal quality. However, only the group with CL sequelae presented statistically significant results. The vocal improvement of this group was evidenced by the increase in the color intensity of the stroke, the regularity and definition of harmonics and the decrease in anti-resonance after the vocal technique was performed.

The group with CL sequelae may have responded better to vocal therapy when compared to the group with ML sequelae, due to the absence of sequelae in the upper aerodigestive tract, which would allow better performance in performing vocal exercise.

Regarding the Vocal Behavior Profile, both groups were classified as a serious risk for the development of vocal disorders. Although these results are not statistically significant, this may be influenced by laryngeal motor adjustments performed to compensate for the sequelae of mucosal lesions and incorrect vocal habits practiced by the groups.

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Table 1. Spectrographic (WBS) comparison before and after vocal thechnique among patients with LT

TABLE 1: Spectrographic (WBS) comparison before and after vocal thechnique among patients with LT

		Mucosal Leishmashmaniasis					Cutaneous Leishmaniais				
		Before		After			Before		After		
		Mean±SD	Median	Mean ± SD	Median	p-value	Mean ± SD	Median	Mean ± SD	Median	p- value
Intensity of the F	F1	80,91±80	15,09	92,73±10,77	100	0,540	78,18±15,31	80	81,82±19,91	90	0,027*
	F2	70 ±16,61	70	79,55±13,96	80	0,606	67,27±14,20	65	71,82±14,35	70	0,085
	F3	66,82±16,44	70	73,64±20,59	80	0,820	65,91±17,63	60	70±16,03	70	0,218
	F4	62,73±20,04	65	71,36±22,52	80	0,991	62,73±17,23	60	67,73±17,71	70	0,233
	Low Frequencies	66,82±18,61	65	76,82±13,58	80	0,828	63,18±18,09	60	72,27±16,01	70	0,096
	Average Frequencies	67,73±14,45	65	76,36±15,59	80	0,566	66,82±14,27	60	72,27±16,01	70	0,337
	High Frequencies	80,45±15,57	80	90,45±12,52	100	0,532	77,73±15,40	75	82,73±15,48	85	0,073
	All Spectrum	74,09±10,07	70	80,45±9,50	80	0,360	71,36±12,83	70	76,36±12,92	80	0,252
Definition and regularity of the F	F1	80±16,33	80	90,91±14,11	100	0,346	70,45±26,81	80	77,73±22,66	80	0,016*
	F2	65,91±17,90	65	77,27±15,17	80	0,721	61,36±24,16	65	66,82±19,61	70	0,066
	F3	64,09±20,15	65	75,91±15,93	80	0,803	60,91±23,88	60	65±19,94	65	0,054
	F4	61,82±20,84	60	73,64±17,60	80	0,896	58,64±23,36	60	65,45±20,17	70	0,093
Regularity	Low Frequencies	64,09±20,62	65	74,55±15,95	80	0,553	59,09±24,08	60	62,73±21,19	60	0,037*
	Average Frequencies	65,00±18,19	65	74,55±15,34	75	0,552	60±23,09	60	65±20,64	70	0,098
	High Frequencies	78,64±17,26	75	84,09±22,60	95	0,261	69,09±25,80	70	75,91±22,81	80	0,113
	All Spectrum	67,73±15,71	70	78,64±10,37	80	0,327	59,09±24,86	65	62,73±22,92	70	0,004*

Bandwidth	F1	42,27±30,85	40	43,64±40,53	40	0,196	30±24,10	30	24,55±23,44	20	0,155
	F2	45,45±18,70	50	47,73±27,41	50	0,179	37,27±20,51	35	34,55±17,92	30	0,081
	F3	45±19,21	50	45,00±28,41	45	0,497	39,55±22,77	45	38,18±19,42	45	0,407
	F4	46,82±20,79	50	46,82±26,43	45	0,264	40,00±21,15	40	39,09±20,68	40	0,303
Anti-resonance	F1	31,82±30,65	30	21,36±28,66	0	0,830	32,27±27,06	30	27,27±26,93	25	0,291
	Low Frequencies	38,64±21,44	40	31,82±21,3	30	0,318	45±20,41	50	44,09±24,81	50	0,083
	Average Frequencies	39,09±20,68	40	30±22,03	30	0,236	45±21,10	50	44,09±23,23	50	0,032*
	High Frequencies	39,09±19,73	40	29,09±22,86	30	0,695	35,45±24,82	35	32,27±27,59	35	0,767
	All Spectrum	43,18±21,018	50	37,27±23,54	40	0,511	47,73±20,22	50	46,82±23,17	50	0,150

Legend: Wilcoxon Nonparametric Test. WBS: spectrographic analysis of wide band. SD:standard deviation. F: Formante . *Data marked with an asterisk (*) are statistically significant.

Table 2. Spectrografic comparison (NBS) showing before and after the vocal thechnique between the groups ML and CL

		Mucosal Leishmashmaniasis					Cutaneous Leishmaniais				
		Before		After			Before		After		
		Mean±SD	Median	Mean±SD	Median	p-value	Mean±SD	Median	Mean±SD	Median	p-value
Intensity of the F	Low Frequencies	62,73±18,56	65	70,91±16	80	0,551	57,73±22,45	50	66,82±17,28	70	0,027*
	Average Frequencies	63,64±15,59	70	70,91±16,87	75	0,504	62,27±19,98	55	67,73±17,16	65	0,096
	High Frequencies	71,36±15,21	75	80,45±15,26	80	0,442	71,82±18,16	70	75±15,66	80	0,066
	All Spectrum	66,82±12,86	70	77,27±11,20	80	0,753	63,64±17,33	55	68,18±15,31	70	0,005*
Presence of Noise	Low Frequencies	51,82±26,66	50	51,82±22,39	50	0,687	47,27±23,33	50	48,18±23,42	50	0,601
	Average Frequencies	46,36±26,28	40	39,55±21,26	40	0,397	49,09±21,80	50	45±22,83	50	0,361
	High Frequencies	45,45±24,04	50	34,55±20,17	35	0,142	55±23,24	60	51,82±21,52	45	0,011*
	All Spectrum	47,27±20,04	50	51,36±18,33	50	0,867	48,18±20,38	50	48,64±19,09	50	
Harmonics Raplacement	Low Frequencies	41,82±20,38	40	48,73±26,96	50	0,107	32,27±20,91	30	31,36±19,83	30	0,016*
	Average Frequencies	38,18±20,61	40	30,09±21,47	30	0,054	27,73±17,97	20	29,55±16,17	30	0,971
	High Frequencies	30,91±26,17	25	25,91±23,43	25	0,695	32,27±22,23	30	33,18±17,01	30	0,111
	All Spectrum	44,55±20,86	45	41,91±22,21	45	0,181	36,36±23,41	30	36,36±19,16	30	0,306
Definition and regularity of harmonics	Low Frequencies	66,82±16,15	70	75,91±15,63	80	0,952	63,18±19,36	70	65,91±18,93	70	0,018*
	Average Frequencies	55,45±15,34	55	61,82±18,93	60	0,764	55,45±20,40	60	57,27±18,81	55	0,323
	High Frequencies	57,27±16,38	50	62,73±16,38	60	0,830	51,36±23,36	55	55±21,10	55	0,144
	All Spectrum	62,73±14,20	65	67,73±13,06	70	0,971	58,64±19,09	60	62,73±18,04	60	0,087

Number of harmonics	Low Frequencies	65±15,35	65	70,00±16,03	70	0,858	65,45±20,17	65	59,09±22,86	50	0,101
	Average Frequencies	67,27±15,17	70	72,73±16,09	70	0,381	70,91±17,70	70	67,73±16,88	70	0,350
	High Frequencies	76,36±19,65	80	78,64±24,35	85	0,567	80,45±14,30	80	78,64±13,20	80	0,332
	All Spectrum	70,91±12,30	70	76,82±10,86	80	0,553	68,18±17,08	70	64,09±17,63	60	0,007
Presence of subharmonics	Low Frequencies	40,45±24,97	40	43,18±28,18	40	0,308	34,09±26,84	20	29,09±22,86	20	0,073
	Average Frequencies	40±24,68	40	44,55±28,40	40	0,102	29,09±25,61	20	30,91±22,65	25	0,091
	High Frequencies	28,18±30,02	10	35±31,13	25	0,923	25,91±28,39	10	27,73±27,06	15	0,513
	All Spectrum	42,27±24,08	50	46,82±26,25	50	0,361	37,27±29,94	25	34,55±22,40	30	0,124

Legend: Wilcoxon Nonparametric Test. NBS: spectrographic analysis of narrowband. . SD:standard deviation. F: Formante . *Data marked with an asterisk (*) are statistically significant.

Table 3. Vocal Bahavior Profile of ATL patients (p-valor=0,683)

	Mucosal Leishmaniasis		Cutaneous Leishmaniasis	
	n	%	n	%
The Behaved (up to 15 points)	0	0,0	2	4,5
Candidate for vocal problems (16 to 30 points)	7	31,8	8	34,1
The srious risk (31 to 50 points)	12	54,5	10	50,0
The Champion of abuses (above 51 points)	3	13,6	2	11,4
Total	22		22	

Legend: n: sample. %: percentage . p: statistical significance. *Data marked with an asterisk (*) are statistically significant.

VII. DISCUSSÃO

Neste estudo, pode-se observar que 59,09 % (n=26) dos participantes tem idade entre 25 e 50 anos, sendo que todos são moradores da zona rural e a maioria trabalha com agricultura. Estudos mostram que pode haver variação em relação à disseminação de casos entre as diversas faixas etárias indicando a possibilidade de transmissão domiciliar, peridomiciliar e ocupacional, pois se observa que crianças abaixo de seis anos e idosos geralmente se encontram dentro de casa, enquanto as pessoas em idade produtiva apresentam maior risco de ter a doença em virtude das suas ocupações laborais na floresta, normalmente agricultura ou extrativismo (Tabela 1, artigo 1) [18-20,29,33,37,39].

A amostra deste estudo foi composta por homens, em que se observa que a maioria dos estudos mostra os homens como os mais acometidos pela LTA [7,37], sendo que entre eles a forma mucosa é mais comum, além de poder desenvolver formas mais graves da doença com alta incidência de perfuração e envolvimento de estruturas externas de cavidade nasal [37]. Essa prevalência de LTA entre os homens pode ocorrer devido as suas atividades ocupacionais ou de lazer como caça, pesca ou acampamento, que ainda são mais praticadas pelo sexo masculino (Tabela 1, Artigo 1) [18,19,20,29,38; 39,124].

A LM tem início insidioso e, apesar de apresentar pouca sintomatologia inicialmente [58], tem lesões com um grande potencial destrutivo [18], acometendo a mucosa e cartilagem do trato aerodigestivo superior [55-60], desfigurando e incapacitando os indivíduos acometidos por essa doença [56-60]. Dentre os sintomas frequentemente evidenciados pela literatura [7,18,29,43], na fase do tratamento

medicamentoso desses pacientes, houve maior ocorrência da presença de crostas nasais, perfuração septal, epistaxe, obstrução nasal e rinorreia (Tabela 2, Artigo 1).

Em função dos sinais e sintomas de rinorreia, crostas nasais, epistaxe, hiposmia, perfuração septal, queda do suporte nasal (nariz de tapir) [7,17,75], os sujeitos podem apresentar problemas na respiração, efetuando-a de modo forçado [7]. Além disso, muitos pacientes apresentam dor para deglutir, sialorreia devido à presença de lesões no palato duro e mole, úvula, gengiva e faringe [7, 17,18], podendo manifestar disfagia [17]. Para mais, a mastigação pode ser prejudicada em vista do envolvimento das gengivas e interstícios dentários, onde pode se desenvolver granulações grosseiras e proeminentes, chegando ao lábio superior, muitas vezes, poupando a língua [17,64]. Com esses agravos, ainda pode haver comprometimento da fala, ocorrendo distorções de fonemas ou dificuldades articulatórias.

Claramente, percebe-se por meio dos sintomas de maior prevalência que o sítio mais acometido foi o nasal (95,45%) (Tabela 2, Artigo 1). A literatura mostra que a grande parte das lesões estão situadas no nariz [7,18,29,43], que podem ocorrer devido ao envolvimento da mucosa nasal sem doença cutânea anterior, revelando que a penetração da Leishmania tenha ocorrido ao nível do límen, ou seja, na transição cutâneo-mucosa da estrutura do nariz [7]. Além do mais, acredita-se que as metástases do parasita possam ser levadas até as vias aerodigestivas superiores através do meio linfático ou hematogênico [7,18] e sendo incomum pelo contato direto da mucosa com a lesão cutânea como no caso de um recém nascido de mãe com lesão cutânea em mamilo e que veio a desenvolver lesão mucosa na boca [7].

Ademais, alguns estudos [7,18,43,57,62,125] mostram que, seguindo o sítio nasal, podem aparecer lesões no palato, na faringe, laringe e gengiva. Contudo, no

presente estudo, observou-se que a maioria das lesões foi exclusivamente nasal (72,72%) (Tabela 2, Artigo 1). Todavia, mesmo sem a presença de lesões na laringe, observou-se o sintoma de disfonia em menor incidência que, segundo alguns autores, pode estar associada às lesões em faringe e cavidade oral [13]. Além do mais, esse sintoma também pode ser decorrente da presbifonia, visto que os pacientes com LM apresentam os participantes com idades mais elevadas [126,127,128]. Embora com esses resultados, o desenvolvimento das lesões mucosas ainda não é bem compreendido, sendo relacionado a essas lesões elementos como, por exemplo, o parasita, o hospedeiro, a magnitude da resposta imunológica [7,18,28,44], bem como, fatores socioeconômicos e ambientais, e até mesmo a contiguidade [18].

A voz nesses pacientes pode estar alterada devido à localização das lesões, que se mostram presentes no nariz, na cavidade oral, na laringe e na faringe, acarretando transformações na ressonância, qualidade vocal [76]. Há inflamação laríngea generalizada particularmente na região dos seios piriformes. As pregas vocais podem estar se movendo bem, mas a fonação pode ser fraca (astênica) e a tensão da contração muscular pode estar prejudicada por formação granulomatosa e subsequente fibrose [17,64].

Apesar dos pacientes, na fase anterior ao tratamento, não terem apresentado lesões laríngeas (Figura 1, Artigo 1), sabe-se que a produção vocal depende da harmonia entre a respiração, fonação e ressonância, que manifestarão as emoções e representarão a personalidade das pessoas [70,71]. Então, quando existe uma desarrmonia nesse conjunto, uma das consequências pode ser a disfonia [70,72], que trará dificuldade ou alteração na emissão vocal, afetando a voz natural [73,74].

Quando analisadas perceptivoauditivamente as características vocais dos grupos estudados, evidenciou-se a associação estatisticamente significativa da astenia de grau 2 aos participantes com LM (27,7 %) e da instabilidade vocal de grau 1 aos participantes com LC (36,4 %) (Tabela 3, Artigo 1). A astenia confere à emissão uma sensação de fraqueza e a instabilidade é a variação anormal da voz [129]. No caso da astenia, viu-se também que, acusticamente, os valores de *Jitter* e *Shimmer* foram mais elevados quando comparadas ao grupo com LC (Tabela 4, Artigo 1), porém sem significância estatística. Estudos mostram que essa qualidade vocal pode estar relacionada a altos índices desses parâmetros acústicos [130].

Além disso, a astenia pode estar ligada a insuficiência glótica, faixa etária (geralmente presente em idoso) e à etiologia de paralisia de prega vocal [130,131]. Assim, neste estudo, a astenia associada ao grupo com LM pode ser justificada tendo em vista que esses participantes apresentaram média de idade maior (57,59 ± 6,49), o que pode favorecer alterações laríngeas como, por exemplo, a presença de fendas vocais ou redução da mucosa de prega vocal, que podem levar à insuficiência glótica [127]. Contudo, alguns autores referem que quadros de hipofunção muscular ainda não estariam instalados no mecanismo laríngeo da produção vocal [132], além do que o descontrole de ar pulmonar pode favorecer diminuição da eficiência pneumofônica [133] que pode ocasionar cansaço vocal [134, 135], gerando a astenia que envolveria todas as faixas etárias, a partir dos 60 anos.

Em contrapartida, a instabilidade vocal traz uma característica de tremor às estruturas do trato vocal [126,136], podendo se relacionar com o declínio nas forças musculares da laringe e aerodinâmicas da corrente pulmonar [133], normalmente ligada à voz de pessoas com idade elevada [137], sem falar na ocorrência devido à maior adução das pregas vocais. Esta redução do fluxo transglótico, poderá gerar

incoordenação pneumofonarticulatória [113]. Além do mais, foi verificado que 13,63% desses participantes apresentaram coaptação vocal incompleta, que pode ter sido compensada pela hiperconstrição laríngea (Figura 1, Artigo 1).

Mesmo sem significância estatística, pode-se constatar que os participantes com sequela de LM também apresentaram qualidade vocal marcada por rouquidão de grau 1 (31,8 %), aspereza grau 2 (31,8 %), soprosidade grau 1 (9,1 %); tensão grau 1 (18,2 %) e instabilidade grau 1 (18,2 %), assim como o grupo com sequela de LC apresentou características vocais de rouquidão grau 1 (27,3 %), aspereza grau 2 (22,7 %), astenia grau 1(4,5 %), tensão grau 2 (13,2 %), sendo que o grupo com sequela de LM apresentou características vocais com maior grau de desvio (Tabela 3, Artigo 1). Assume-se esse resultado considerando, além do impacto da LM sobre a estrutura laríngea, as alterações vocais em decorrência da idade dos participantes desse grupo. Estudos mostram que há uma deterioração das características vocais em virtude do aumento da idade [126, 127,138]

Em relação às características vocais acústicas, viu-se que a medida de variação da frequência fundamental (vF0), foi maior para o grupo com LM (Tabela 4, Artigo 1), mostrando associação a esse grupo, com resultados estatisticamente significativos. Essa medida pode ter seus valores elevados devido às características anatômicas da laringe masculina, com maior extensão e volume das pregas vocais, o que favorece a instabilidade fonatória, uma vez que tais medidas estão relacionadas com a instabilidade do sinal ciclo-a-ciclo ou em curto termo, e com o controle sobre o sistema fonatório [82,139]. Além disso, observou-se que esse parâmetro pode estar relacionado à rouquidão e à aspereza vocal [140], que também foram características vocais apresentadas pelo grupo com sequela de LM, sem significância estatística, mas com uma porcentagem elevada, e a voz áspera ainda

com grau moderado. Contudo, salienta-se que esses desvios vocais também podem contar com a atuação da idade dos participantes sobre eles [127].

Para mais, as medidas de frequência (STD, Fhi) e a medida de número de segmentos sub-harmônicos (NSH) também foram associadas ao grupo com sequela de LM, com valores superiores ao grupo com sequela de LC e estatisticamente significativos. Assim como a vF0, o resultado elevado dessas medidas também pode estar relacionado à discreta instabilidade na sustentação da frequência [127]. Isso pode ocorrer devido à alteração ressonantal, causada pelo desequilíbrio no direcionamento do ar em função das alterações nasais (por exemplo, a obstrução nasal). Tais alterações podem desencadear a respiração oral, que gerará alterações nas funções musculares, acarretando esforço compensatório na musculatura da laringe, que desencadearia a instabilidade na emissão [76].

Três quartos dos participantes do grupo com LM apresentaram valores maiores para as medidas de número de segmentos não sonorizados (NUV) em relação ao grupo com LC, com valores estatisticamente significativos (Tabela 4, Artigo 1). Esta medida é caracterizada pela interrupção da constância da onda sonora, aparecendo como irregularidade ou ruído à emissão. Existem estudos que mostram que valores mais elevados dessas medidas podem estar ligados ao sexo masculino [141]. Todavia, nessa pesquisa, isso não se aplica visto que apenas três quartos dos participantes do grupo com LM tiveram esses valores elevados e que o grupo com LC também é composto pelo sexo masculino. Ademais, as pessoas que passam pelo processo de envelhecimento, podem exibir uma qualidade vocal instável e aumento de quebras de sonoridade e ou variações de frequência, o que justificaria esse aumento para os participantes com LM [127].

Quanto aos pacientes com LC, este apresentou associação estatisticamente significativa com a medida de ruído, SPI, indicando maior presença de ruído à fonação quando comparado aos indivíduos com LM. Seus valores elevados podem sugerir fechamento inadequado das pregas vocais [127,142], ou podem ocorrer em decorrência de vozes graves (como no sexo masculino) e turbulências aéreas em virtude do fato da fonação não ser linear [143]. A fim de evitar as alterações vocais, podem ser utilizados exercícios de voz que, normalmente, causam aumento da temperatura do tecido muscular e do fluxo sanguíneo, diminuindo o número de prejuízos para o trabalho muscular [85,86]. Nesse contexto, podem ser utilizados os EVTSO, executados através da oclusão parcial da região anterior do trato vocal, que se torna constrito ou alongado, promovendo a ressonância retroreflexa em direção às pregas vocais e, diversas variações destes estão descritas no contexto das recentes pesquisas como, por exemplo, os sons nasais [88,95] utilizados nesse trabalho.

Após a intervenção fonoterapêutica, houve melhora dos parâmetros acústicos para ambos os grupos. Todavia, deu-se uma redução estatisticamente significativa das medidas de perturbação de frequência (vF0, PPQ, sPPQ) para os participantes com LC (Tabela 4, Artigo 1). Como a técnica dos sons nasais é um exercício que ajuda a deslocar o foco de ressonância de inferior para superior, isso reduz a tensão da laringe e da faringe, funcionando como um trampolim de projeção da voz no espaço, que diminuirá a ressonância baixa e aumentará o componente oral da ressonância nasal, produzindo uma série mais rica de harmônicos e trazendo maior estabilidade à emissão [95,110,112].

Além disso, ocorreu redução estatisticamente significativa das medidas de segmentos sub-harmônicos (DSH, NSH) para o grupo com LM (Tabela 4, Artigo 1).

As medidas de componentes sub-armônicos permitem medir a presença desses componentes de baixa intensidade situados entre os harmônicos, com sua diminuição após a técnica vocal, mostram uma diminuição do ruído à emissão. Do mesmo modo, para os parâmetros de segmentos surdos que representam a interrupção da onda sonora, sua diminuição indica diminuição das irregularidades durante a vocalização [82]. Ademais, houve melhora estatisticamente significativa para a medida de frequência STD, sugerindo maior estabilidade da emissão (Tabela 4, Artigo 1).

Além disso, quando analisado apenas o grupo dos pacientes com sequela de LM antes e após a intervenção fonoaudiológica (Tabela 5, Artigo 1), constatou-se melhora nos parâmetros de ruído (VTI, NHR, SPI), indicando regularização na vibração das estruturas laríngeas [138,142], que pode ter ocorrido pelo beneficio trazido à coaptação das pregas vocais, assim como pelo melhor direcionamento do fluxo aéreo para as cavidades de ressonância, sugerindo melhora no fechamento glótico durante a fonação [142]. Do mesmo modo, para os parâmetros de segmentos surdos que representam a interrupção da onda sonora, sua diminuição indica menos irregularidades durante a vocalização [82].

Em relação à característica vocal de aspereza (Tabela 3, Artigo 1), pode-se verificar que após a aplicação da técnica fonoaudiológica, os participantes de ambos os grupos apresentaram redução desse tipo vocal, sendo que o grupo com LC apresentou maior redução quando comparado com o grupo com LM, com resultados estatisticamente significativos. A aspereza normalmente se dá de forma ruidosa e desagradável, pobre em harmônicos e rica em ruídos [144], aparecendo em consequência de rigidez da cobertura das pregas vocais e ou ocasionada pela rigidez da musculatura em razão do aumento de tensão [145], imprimindo à emissão

irregularidade vibratória [145]. Assim, a redução dessa característica vocal pode ter acontecido em função da suavização da emissão, da redução da hipertonicidade laríngea e da melhora da vibração das PPVV, favorecidas pela técnica dos sons nasais [95,110,112].

Ademais, foi possível observar que, mesmo sem significância estatística, os participantes com LM, após a fonoterapia, não apresentaram mais soprosidade e tensão de grau 2. Dos 22,7 % dos participantes com astenia de grau 2, apenas 4,5 % continuou apresentando essa característica e, quanto à instabilidade, dos 18,2 % dos sujeitos que apresentavam grau 1, apenas 9,1 % continuaram a apresentar esse tipo vocal de grau 1. Em relação aos participantes com LC, também houve diminuição da rouquidão de grau 2, eliminação da aspereza de grau 2, redução da tensão e instabilidade de grau 1 e 2 (Tabela 3, Artigo 1), evidenciando os benefícios do som nasal [95,110,112].

Na espectrografia, a disposição dos formantes na EBL tem relação direta com a conformação do trato vocal durante a produção do som analisado, principalmente com a conformação de lábios, língua, palato mole e mandíbula, além da conformação das cavidades oral e faríngea decorrentes das diversas mobilizações desses articuladores [146,147].

Verificou-se que os participantes com sequela de LC, após a execução da técnica vocal, apresentaram aumento estatisticamente significativo da intensidade da cor do traçado de F1 (EBL) e das baixas frequências (tabela 1, Artigo 2), assim como para todo o espectro vocal (EBE) (tabela 2, Artigo 2). Esses resultados podem estar ligados ao fato desses participantes não terem e, neste trabalho não apresentaram, lesões no trato aerodigestivo superior, como acontece na LM. Isso poderia ter

colaborado no desempenho dos participantes e por consequência nos resultados positivos após a aplicação da técnica vocal.

Além disso, a melhora da intensidade da cor do traçado pode também ser justificada pelo aumento da impedância do trato vocal, gerada pelo exercício de voz. Esse aumento protegeria a glote em virtude da elevada pressão aérea na região supraglótica, que desencadearia um aumento de pressão na glote. Em consequência, ocorreria o afastamento das pregas vocais, minimizando o impacto no retorno do seu contato medialmente, equilibrando as pressões no nível da glote e do trato vocal [80,95,148,149]. Assim, esses ajustes favoreceriam uma produção vocal com menor esforço e maior eficiência vocal [80,98,150,151], suavizando a emissão e diminuindo a hipertonicidade laríngea [95,110,144].

Ligado a esses resultados, o grupo com sequela de LC apresentou melhora estatisticamente significativa da regularidade do traçado para as baixas frequências e para todo o espectrograma, para antirressonância das médias frequências (EBL) (tabela 1, Artigo 2), e aumento da definição e regularidade de harmônicos para as baixas frequências (EBE) (tabela 2, Artigo 2). Esses resultados podem ter sido obtidos devido a intensa mobilização da mucosa, acarretada pelo aumento do fluxo aéreo durante a execução da técnica vocal. Assim, a prega vocal vibra de forma mais sincronizada, melhorando o sinal laríngeo através da renovação da camada de muco e homogeneização da mucosa, com impacto positivo sobre a ressonância, projeção e emissão vocal [80,95,99,152].

O grupo com sequela de LC também apresentou resultado estatisticamente significativo para o parâmetro de presença de ruído e substituição de harmônicos por ruído nas baixas frequências (tabela 2, Artigo 2), em que houve diminuição desses

parâmetros. Essa melhora pode ter acontecido em função da técnica vocal favorecer a estabilidade da emissão e agilidade dos músculos respiratórios [95]. Esses benefícios acarretarão enriquecimento da energia harmônica, demonstrando melhora na coordenação pneumofonoarticulatória e na projeção e ressonância vocal, redução e equilíbrio da hipertensão laríngea, mandibular e de todo o aparato fonador [80,86,95, 101;120,146].

É importante salientar que, mesmo sem resultados estatisticamente significativos para o grupo de LM, observou-se melhora das médias apresentadas pelo grupo no antes e após a técnica vocal. Isso mostra que esse grupo apresenta benefícios com o exercício para a voz. Contudo, devido às sequelas das lesões apresentadas no trato aerodigestivo superior, seria necessário utilizar a associação de múltiplos exercícios vocais para promover uma melhora vocal significativa dessa população.

Outro aspecto que pode predispor ou influenciar o aparecimento de disfonia são os hábitos vocais incorretos realizados no cotidiano. Como exemplo dentro deste grupo estudado, temos o ato de pigarrear, falar demasiado, gritar, falar em forte intensidade, tossir, ingestão de pouca água. Além disso, as condições de saúde geral, os fatores antropométricos e a suscetibilidade individual também podem favorecer o aparecimento da disfonia em algum grau [120,153,154].

Traçando-se o perfil do comportamento vocal, através do protocolo utilizado nesta pesquisa, viu-se que não houve diferença estatisticamente significativa entre os grupos, mostrando que ambos praticaram quase a mesma quantidade de abusos vocais, sendo que a maioria dos participantes foi classificada como risco sério para apresentar problemas de voz. Isso também pode esclarecer um pouco mais o

porquê dos indivíduos com sequelas de LC terem apresentado alterações vocal, sem terem sequelas de lesões no trato aerodigestivo superior, uma vez que esse tipo de sequela provavelmente implicaria em alterações na emissão vocal.

Entretanto, o grupo com sequela de LM apresentou mais participantes classificados como campeão de abusos vocais quando comparado com o grupo com sequela de LC. Provavelmente isso pode ter ocorrido devido aos ajustes musculares inadequados desses participantes na tentativa de compensar as sequelas que comprometem mais o aparelho fonador com impacto negativo na emissão vocal [120].

Todavia, a autopercepção vocal realizada pelos participantes através de protocolos pode contar com a desvantagem desses indivíduos não saberem se autoavaliar, visto que muitos participantes contam com a falta de informação e conhecimento sobre a voz [132,155,156].

Por fim, observa-se que este estudo conta com algumas limitações como, por exemplo, a possível interferência da presbilaringe nas alterações vocais dos participantes com LM e a intervenção realizada apenas em homens. Para futuros estudos, recomenda-se que se trabalhe com um público mais jovem e, se possível, incluir a população feminina, mesmo sabendo que a LM é mais comum entre os homens.

VIII. PERSPECTIVAS DE ESTUDO

Verificar e comparar as características vocais entre os pacientes com Leishmaniose Mucosa e Cutânea, após a intervenção Fonoterapêutica, com um Programa de terapia com Técnicas variadas, em virtude dos resultados positivos das análises das medidas acústicas e da avaliação perceptivoauditiva, apresentados nesta pesquisa. Desta forma, verificar qual o grupo apresenta melhores resultados nos parâmetros perceptivoauditivos e nas medidas acústicas, a fim de beneficiar os resultados terapêuticos desses pacientes, minimizando os impactos das sequelas à voz desses indivíduos.

Correlacionar as características vocais dos participantes com Leishmaniose Mucosa e seu estadiamento da lesão mucosa nasal, bem como analisar as consequências dos medicamentos utilizados, pentoxifilina e antimonial pentavalente, para o tratamento da Leishmaniose no aparato fonador e na voz dos pacientes com Leishmaniose Mucosa e Cutânea.

IX. CONCLUSÃO:

- A astenia e a maioria das alterações das medidas acústicas (fhi, STD, vF0, SPI, NSH, DSH) foi associadas ao grupo com sequela de LM, que apresentou os maiores desvios vocais quando comparado ao grupo LC.
- O grupo com sequela de LC também apresentou alterações vocais, sem considerável gravidade, marcadas por instabilidade vocal junto com medidas de ruído (SPI), o que não era esperado, pois era um grupo caracterizado por lesões em regiões não relacionadas ao aparelho vocal.
- A intervenção fonoterapêutica, mesmo em curto espaço de tempo, houve beneficio vocal, para ambos os grupos, verificado através da melhora dos parâmetros perceptivoauditivos e das medidas acústicas.
- Apenas o grupo com sequela de LC apresentou resultados espectrográficos estatisticamente significativos, evidenciados através do aumento na intensidade da cor do traçado, na regularidade e definição de harmônicos e na diminuição da antirressonância, após a execução da técnica vocal.
- Em relação ao Perfil do Comportamento Vocal, os participantes dos dois grupos foram classificados como risco sério para o desenvolvimento de alterações vocais, apesar desses resultados não serem estatisticamente significativos.

X. SUMMARY

Phonotherapeutic Intervention in Patients with Leishmaniosis Mucosal and Cutaneous sequelae

INTRODUCTION: Leishmaniasis is a stigmatizing disease, considered a serious public health problem. It presents three classic clinical forms, among them Cutaneous and Mucosa forms. The former affects upper and lower limbs, with ulcerated lesions, which may be multiple or unique. Second, it reaches the upper respiratory tract, with destructive lesions, which can affect the voice, swallowing and breathing of the patients. **OBJECTIVES**: To characterize the voice and verify the vocal response to speech therapy intervention. **METHODS**: The vocal emission / a: of 22 participants from each group (total of 44 cases) was collected for the computerized analysis of the voice through the Kay PENTAX® Real Time Spectrogram and the Multi Dimensional Voice Program Advanced and for perceptualaudit analysis through of the RASATI scale. **RESULTS**: Before the speech therapy, participants with Mucosa Leishmaniasis had a statistically significant result, where 5 (27.7%) participants presented asthenic vocal quality, and altered parameters of frequency measurements, frequency disturbance, noise and sub-harmonic measurements. Of the participants with Cutaneous Leishmaniasis, 8 (36.4%) presented grade 1 vocal instability. After the speech therapy, patients with Cutaneous Leishmaniasis showed a reduction in the degree of roughness and an improvement in acoustic parameters of frequency disturbance. The group with sequelae of Leishmaniasis Mucosa presented reduction of the measurements of sub-harmonic segments. Only the sequelae group of Cutaneous Leishmaniasis had statistically significant results regarding spectrography, with improvement of the following parameters: color intensity of the trace, presence of noise, substitution of harmonics for noise, definition and regularity of harmonics, regularity of low frequencies and the whole spectrogram and for anti-resonance. There was no statistically significant difference in the Voice Behavior Profile. **CONCLUSION**: Both groups presented vocal alterations in different degrees before vocal therapy, and patients with Mucosal Leishmaniasis presented more severe degrees. After speech therapy intervention, participants with Cutaneous Leishmaniasis sequela had more vocal benefits after performing the technique, possibly because they did not present lesions in the vocal tract.

Keywords: 1. Voice; 2. Mucosal Leishmaniasis; 3. Cutaneous Leishmaniasis; 4. Speech Therapy; 5. Voice disorders. 6. Espectrography.

XI. REFERÊNCIAS BIBLIOGRÁFICAS

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XII APÊNDICES

XII.1. APÊNDICE 1

Termo de Consentimento Livre e Esclarecido

Título do Estudo: **Intervenção Fonoterapêutica em pacientes com Leishmaniose Mucosa**

Pesquisador Responsável: **Famiely Colman Machado de Machado**

O (A) Senhor (a) está sendo convidado (a) a participar de uma pesquisa. Por favor, leia este documento com bastante atenção antes de assiná-lo. Caso haja alguma palavra ou frase que o (a) senhor (a) não consiga entender, converse com o pesquisador responsável pelo estudo ou com um membro da equipe desta pesquisa para esclarecê-los. A proposta deste termo de consentimento livre e esclarecido (TCLE) é explicar tudo sobre o estudo e solicitar a sua permissão para participar do mesmo.

OBSERVAÇÃO: Esse termo será elaborado em duas vias, rubricadas em todas as suas páginas e assinadas, ao seu término, pelo convidado a participar da pesquisa. Caso o participante de pesquisa não tenha condições de ler e/ou compreender este TCLE, o mesmo poderá ser assinado e datado por um membro da família ou responsável legal pelo mesmo, assim como pelo pesquisador responsável.

Objetivo do Estudo: Os objetivos do estudo são: Caracterizar a voz e verificar a eficácia da terapia vocal em participantes de pesquisa com sequelas de lesões de Leishmaniose Mucosa no trato aerodigestivo superior (boca, nariz, laringe, faringe). Além disso, pretende-se Investigar a voz dos participantes de pesquisa com LM antes e após o tratamento medicamentoso e analisar a eficácia da fonoterapia nos participantes de pesquisa com LM.

Duração do Estudo: A duração total do estudo é de quatro anos. A sua participação no estudo será de aproximadamente 30 minutos nas duas fases da pesquisa.

Descrição do Estudo: Participarão do estudo aproximadamente 30 indivíduos, sendo 15 participantes de pesquisa com Leishmaniose Mucosa e 15 com Leishmaniose cutânea. Este estudo será realizado durante as consultas habituais dos participantes de pesquisa, no posto de saúde Dr. Jackson Lemos Costa, situado na vila de Corte de Pedra (BA). O (a) Senhor (a) foi escolhido (a) a participar do estudo porque apresenta idade entre 18 e 60 anos; por ser homem ou mulher; por apresentar diagnóstico prévio parasitológico, histológico, de cultivo, imunológico e otorrinolaringológico para leishmaniose cutânea ou mucosa; por iniciar o tratamento medicamentoso para leishmaniose; por exibir lesões nasais, bucais, faríngeas e laríngeas; por apresentar ou não queixas vocais; por ter respondido o questionário de anmnese; e por ter lido, aceitado e assinado este termo.

O (a) Senhor (a) não poderá participar do estudo caso seja usuário (a) de tabaco e/ou álcool; não queira realizar o tratamento medicamentoso; por ter realizado anteriormente terapia vocal; por apresentar outras formas de Leishmaniose (difusa, disseminada ou mucocutânea); por ter doenças respiratórias (asma, bronquite, sinusite); por estar gripado no dia da avaliação vocal; por ter histórico de problemas neurológicos; caso seja mulher, estar no período menstrual ou gestante; e caso não concorde e não queira assinar o TCLE.

Procedimento do Estudo: Inicialmente o senhor (a) responderá a uma anmnese com perguntas sobre queixa de alteração vocal, aspectos que possam interferir na *performance* vocal ou na execução das avaliações, uso de agentes agressivos à laringe que possam favorecer o aparecimento de afecções laríngeas, doenças respiratórias, época de diagnóstico da Leismaniose, e condicionamento vocal através de terapia vocal. Posteriormente, o (a) senhor será convidado a realizar a avaliação otorrinolaringológica, que também acontecerá no posto de saúde Dr. Jackson Lemos Costa. Nessa avaliação, o médico otorrinolaringologista examinará sua garganta envolvendo a língua com uma gaze, segurando-a para fora, logo após um tubo fininho será colocado pela boca ou pelo nariz, até o fundo da garganta, para gravar as imagens das pregas vocais numa fita de vídeo ou DVD. Durante o exame, você terá que pronunciar alguns sons. Dependendo da sensibilidade de cada pessoa, o tubo poderá provocar o reflexo de vômito, mas o uso de anestésico em *spray* pode evitar isso.

Logo em seguida, será coletada a emissão vocal da vogal /a:/, solicitando-se ao senhor (a) estar em pé e com os braços ao longo do corpo, emitindo o som da letra “a” de forma sustentada. Essa emissão será gravada com gravador de voz profissional. Ao final do tratamento medicamentoso, a sua voz será coletada novamente, conforme dito anteriormente.

Caso o senhor (a) apresente alteração vocal ao final do tratamento para Leishmaniose, será convidado a participar da segunda etapa da pesquisa, que se refere à terapia vocal. Nesta fase, o senhor (a) será convidado (a) a participar da Terapia Breve Intensiva, realizada em 20 sessões terapêuticas, não invasivas, durante um mês e por no mínimo 30 minutos, em que será solicitado ao (a) senhor (a) soprar em um canudinho, realizando três séries de seis vezes, com intervalos de cinco minutos para descanso. Os resultados dos exames serão fornecidos pessoalmente ou através de um telefonema da examinadora para o (a) senhor (a), ficando ao seu critério a forma de recebê-los.

Riscos Potenciais, Efeitos Colaterais e Desconforto: Os procedimentos que serão utilizados no decorrer da pesquisa não oferecem riscos à sua saúde, apenas poderá sentir desconforto durante uma das avaliações (otorrinolaringológica), na qual o médico examinará sua garganta e poderá usar um anestésico, a fim de evitar náuseas, e você poderá ter uma sensação desagradável na garganta, e um gosto ruim na boca, que poderá permanecer durante alguns minutos. Durante a avaliação de voz e a terapia vocal, o (a) senhor (a) poderá sentir um pouco de sede e leve cansaço após falar as letras solicitadas.

Benefícios para o participante: Como benefícios diretos, o (a) senhor (a) terá o parecer dos examinadores sobre o seu exame otorrinolaringológico e o comportamento vocal e, caso seja necessário, o (a) senhor (a) receberá encaminhamento para avaliações mais completas e ou para profissionais específicos. Da mesma forma, será beneficiado pelo aperfeiçoamento vocal que a terapia de voz propicia. Além disso, com sua participação até o final do estudo, você estará contribuindo com o aumento e a melhoria do conhecimento sobre o tema abordado.

Compensação: Você não receberá nenhuma compensação para participar desta pesquisa e também não terá nenhuma despesa adicional.

Participação Voluntária/Desistência do Estudo: Sua participação neste estudo é totalmente voluntária, ou seja, você somente participa se quiser. A não participação no estudo não implicará em nenhuma alteração no seu acompanhamento médico tão pouco alterará a relação da equipe médica com o mesmo. Após assinar o consentimento, você terá total liberdade de retirá-lo a qualquer momento e deixar de participar do estudo se assim o desejar, sem quaisquer prejuízos à continuidade do tratamento e acompanhamento na instituição.

Novas Informações: Quaisquer novas informações que possam afetar a sua segurança ou influencias na sua decisão de continuar a participação no estudo serão fornecidas a você por escrito. Se você decidir continuar neste estudo, terá que assinar um novo (revisado) Termo de Consentimento informado para documentar seu conhecimento sobre novas informações.

Em Caso de Danos Relacionados à Pesquisa: Em caso de dano pessoal, diretamente causado pelos procedimentos ou tratamentos propostos neste estudo (nexo causal comprovado), o participante tem direito a tratamento médico na Instituição, bem como às indenizações legalmente estabelecidas.

Utilização de Registros Médicos e Confidencialidade: Todas as informações colhidas e os resultados dos testes serão analisados em caráter estritamente científico, mantendo-se a confidencialidade (segredo) do participante de pesquisa a todo o momento, ou seja, em nenhum momento os dados que o identifique serão divulgados, a menos que seja exigido por lei. Os registros médicos que trazem a sua identificação e esse termo de consentimento assinado poderão ser inspecionados por agências reguladoras e pelo CEP. Os resultados das avaliações realizadas (gravações, questionários e exame de garganta) serão armazenados num banco de dados, em que ficarão sob responsabilidade da pesquisadora responsável Fonoaudióloga Famiely Colman Machado de Machado e do orientador Dr. Marcus Miranda Lessa, num banco de dados para utilização em publicações científicas atuais e futuras, sem jamais revelar a identidade dos participantes.

Quem Devo Entrar em Contato em Caso de Dúvida: Em qualquer etapa do estudo você terá acesso aos profissionais responsáveis pela pesquisa para esclarecimento de eventuais dúvidas. Os responsáveis pelo estudo nesta instituição são a pesquisadora responsável Fonoaudióloga Famiely Colman Machado de Machado e o orientador Dr. Marcus Miranda Lessa, que poderão ser encontrados no Ambulatório de Otorrinolaringologia e Fonoaudiologia do Complexo Hospitalar Universitário Prof. Edgar Santos (HUPES), no posto de saúde Dr. Jackson Lemos Costa, ou nos respectivos telefones (71) 9369-4256 e (71) 3235-1635.

Em caso de dúvidas com respeito aos aspectos éticos deste estudo, você poderá consultar:

Pesquisador(a) Responsável: Famiely Colman Machado de Machado

Endereço: Ambulatório Magalhães Neto, rua Augusto Vianna, s/n- Canela

Sala: Ambulatório de Otorrinolaringologia Andar: 2 andar Horários de Atendimento:Segunda a sexta, das 8horas até o 12horas.

Salvador (BA) - CEP: 4011060 Fone: (71) 3235-1635/ E-mail: famycolman@yahoo.com.br

CEP/HUPES- Comitê de Ética em Pesquisa

Hospital Universitário Prof. Edgard Santos- UFBA

Salvador (BA) - CEP: 4011060

Fone: (71) 3283-8043 / E-mail: cep.hupes@gmail.com

Declaração de Consentimento

Concordo em participar do estudo intitulado "Intervenção Fonoterapêutica em pacientes com Leishmaniose Mucosa".

Li e entendi o documento de consentimento e o objetivo do estudo, bem como seus possíveis benefícios e riscos. Tive oportunidade de perguntar sobre o estudo e todas as minhas dúvidas foram esclarecidas. Entendo que estou livre para decidir não participar dessa pesquisa. Entendo que ao assinar este documento, não estou abdicando de nenhum dos meus direitos legais.

Eu autorizo a utilização dos meus registros médicos (prontuário médico) e os resultados das avaliações realizadas (gravações, questionários, exame de garganta e de audição) pelo pesquisador, autoridades regulatórias e pelo Comitê de Ética e Pesquisa (CEP) da instituição.

_____

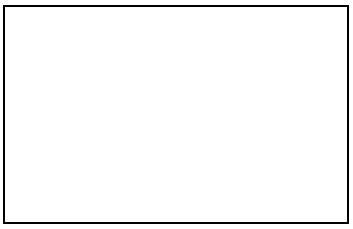
_____

Nome do Sujeito de Pesquisa Letra de Forma ou à Máquina Data

_____

Assinatura do Sujeito de Pesquisa

Impressão Datiloscópica



_____ RG:

Nome do Sujeito de Pesquisa

_____

_____

Nome do Representante Legal do Sujeito de Pesquisa Data

Letra de Forma ou à Máquina (quando aplicável)

_____

Assinatura do Representante Legal do Sujeito de Pesquisa

Letra de Forma ou à Máquina (quando aplicável)

_____ _____

Nome da pessoa obtendo o Consentimento Data

_____

Assinatura da Pessoa Obtendo o Consentimento

_____ _____

Nome do Pesquisador Principal Data

_____

Assinatura e Carimbo do Pesquisador Principal

_____ _____

XII.2. APÊNDICE 2

INSTRUMENTOS/QUESTIONÁRIOS DE COLETA DE DADOS

Questionário de Anamnese

1. Dados de identificação

Número do participante: DN:_____

Endereço:_____ Profissão:_____

Data:_____ Telefone:_____

2. Questionário

a) Apresenta queixa de voz: ( ) sim ( ) não

b) Época em que foi diagnosticado com Leishmaniose: ___________

c) Está em tratamento para Leishmaniose? Data de início: ___________

c) Apresenta histórico de doenças neurológicas: ( ) sim ( ) não Qual?_____

d) Apresenta infecções respiratórias agudas: ( ) sim ( ) não Qual?_____

e) Apresenta alterações endocrinológicas: ( ) sim ( ) não Qual?_____

f) Você fuma: ( ) sim ( ) não Quantos maços de cigarro por dia?_____

g) Você bebe: ( ) as vezes ( ) socialmente ( )frequentemente

h) Têm histórico de cirurgia laríngea ou já realizou algum procedimento de cabeça e pescoço: ( ) sim ( ) não Qual?_____

i) Já realizou fonoterapia: ( ) sim ( ) não

j) Já realizou aula de canto: ( ) sim ( ) não

l) Participa de corais: ( ) sim ( ) não Quantas vezes na semana?_____

m) Faz uso de algum medicamento: ( ) sim ( ) não Qual?_____

XII.3. APÊNDICE 3

Protocolo de Avaliação Vocal Acústica Espectrográfica

Juiz: _____

Espectrografia nº:_____

ESPECTROGRAFIA VOCAL DE BANDA LARGA

1. Intensidade da cor do traçado

Neste item, zero corresponde à total ausência de cor do traçado e 10 corresponde à extrema intensidade de cor do traçado espectrográfico.

1.1 Do primeiro formante Escore ____/100

1.2 Do segundo formante Escore ____/100

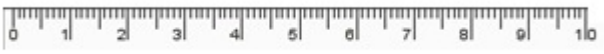
1.3 Do terceiro formante Escore ____/100

1.4 Do quarto formante Escore ____/100

1.5 Das baixas frequências Escore ____/100

1.6 Das médias frequências Escore ____/100

1.7 Das altas frequências Escore ____/100



1.8 No espectrograma vocal como um todo Escore ____/100

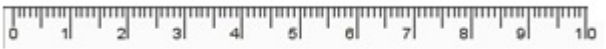
2. Definição e regularidade dos formantes

Neste item, zero corresponde à total irregularidade e indefinição e 10 à máxima regularidade e definição dos formantes.

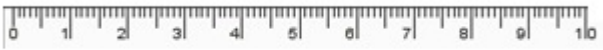
3.1 Do primeiro formante Escore ____/100

3.2 Do segundo formante Escore ____/100

3.3 Do terceiro formante Escore ____/100



3.4 Do quarto formante Escore ____/100



3. Regularidade do traçado

Neste item, zero corresponde à total irregularidade e 10 à máxima regularidade do traçado.

3.1 Das estrias verticais nas baixas frequências Escore ____/100

3.2 Das estrias verticais nas médias frequências Escore ____/100

3.3 Das estrias verticais nas altas frequências Escore ____/100

3.4 No espectrograma vocal como um todo Escore ____/100

4. Largura de banda

Neste item, zero corresponde à totalmente reduzida/ausente e 10 à totalmente aumentada.

4.1 Do primeiro formante Escore ____/100

4.2 Do segundo formante Escore ____/100

4.3 Do terceiro formante Escore ____/100

4.4 Do quarto formante Escore ____/100

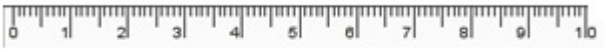
5. Anti-ressonância/*damping* /amortecimento/queda de intensidade/ressonância negativa

5.1 Imediatamente acima do primeiro formante Escore ____/100

5.2 Nas baixas frequências Escore ____/100

5.3 Nas médias frequências Escore ____/100

5.4 Nas altas frequências Escore ____/100



5.5 No espectrograma vocal como um todo Escore ____/100

ESPECTROGRAFIA VOCAL DE BANDA ESTREITA

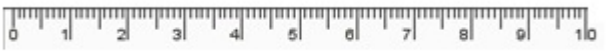
1. Intensidade da cor do traçado

Neste item, zero corresponde à total ausência de cor do traçado e 10 corresponde à extrema intensidade de cor do traçado espectrográfico.

1.1 Das baixas frequências Escore ____/100

1.2 Das médias frequências Escore ____/100

1.3 Das altas frequências Escore ____/100



1.4 No espectrograma vocal como um todo Escore ____/100

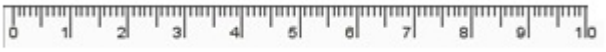
2. Presença de ruído

Neste item, zero corresponde à ausência total de ruído e 10 ao máximo de ruído (imagem sombreada) presente.

2.1 Nas baixas frequências Escore ____/100

2.2 Nas médias frequências Escore ____/100

2.3 Nas altas frequências Escore ____/100



2.4 No espectrograma vocal como um todo Escore ____/100

3. Substituição de harmônicos por ruído

Neste item, zero corresponde à ausência de substituição de harmônicos por ruído e 10 à total substituição de harmônicos por ruído.

3.1 Nas baixas frequências Escore ____/100

3.2 Nas médias frequências Escore ____/100

3.3 Nas altas frequências Escore ____/100

3.4 No espectrograma vocal como um todo Escore ____/100

4. Definição e regularidade de harmônicos

Neste item, zero corresponde à total irregularidade e indefinição e 10 à total regularidade e definição dos harmônicos.

4.1 Nas baixas frequências Escore ____/100

4.2 Nas médias frequências Escore ____/100

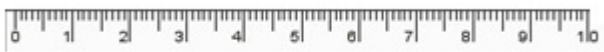
4.3 Nas altas frequências Escore ____/100

4.4 No espectrograma vocal como um todo Escore ____/100

5. Número de harmônicos

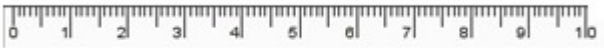
Neste item, zero corresponde à ausência de harmônicos e 10 ao preenchimento completo da imagem espectrográfica por harmônicos.

5.1 Nas baixas frequências Escore ____/100



5.2 Nas médias frequências Escore ____/100

5.3 Nas altas frequências Escore ____/100



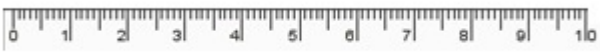
5.4 No espectrograma vocal como um todo Escore ____/100

6. Presença de sub-harmônicos

Neste item, zero corresponde à ausência de sub-harmônicos e 10 à presença de sub-harmônicos em todo o espectrograma.

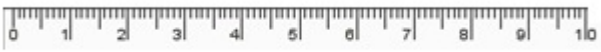
6.1 Nas baixas frequências Escore ____/100

6.2 Nas médias frequências Escore ____/100



6.3 Nas altas frequências Escore ____/100

6.4 No espectrograma vocal como um todo Escore ____/100



XIII.ANEXOS

XIII.1. ANEXO 1

Protocolo de Avaliação Vocal Perceptivoauditiva - Escala RASATI

Juíza:_____

Data de entrega __/__/__ Data de retorno:___/___/___

Total de vozes:_____

Voz nº_____

QUALIDADE VOCAL:

Rouca (0) ausente (1) discreto (2)moderado (3)intenso

Áspera (0) ausente (1) discreto (2)moderado (3)intenso

Soprosa (0) ausente (1) discreto (2)moderado (3)intenso

Astênica (0) ausente (1) discreto (2)moderado (3)intenso

Tensa (0) ausente (1) discreto (2)moderado (3)intenso

Instabilidade (0) ausente (1) discreto (2)moderado (3)intenso

Normal (0) ausente

XIII.2. ANEXO 2

Ofício do Comitê de Ética

PARECER CONSUBSTANCIADO DO CEP

PlataForma Brasil — MINISTÉRIO DA SAÚDE - Conselho Nacional de Saúde - Comissão Nacional de Ética em Pesquisa – CONEP

FOLHA DE ROSTO PARA PESQUISA ENVOLVENDO SERES HUMANOS

1. Projeto de Pesquisa:
Intervenção Fonoterapêutica em pacientes com Leishmaniose Mucosa.

2. Número de Participantes da Pesquisa:
30

3. Área Temática:

4. Área do Conhecimento:
Grande Área 4. Ciências da Saúde

PESQUISADOR RESPONSÁVEL

5. Nome:
Famiely Colman Machado de Machado

6. CPF:	7. Endereço (Rua, n.º):		
018.483.740-54	COMENDADOR PEREIRA DA SILVA 1/99999 BROTAS SALVADOR BAHIA 40285040		
8. Nacionalidade:	9. Telefone:	10. Outro Telefone:	11. Email:
BRASILEIRO	(71) 9369-4256		famycolman@yahoo.com.br

12. Cargo:

Termo de Compromisso: Declaro que conheço e cumprirei os requisitos da Resolução CNS 466/12 e suas complementares. Comprometo-me a utilizar os materiais e dados coletados exclusivamente para os fins previstos no protocolo e a publicar os resultados sejam eles favoráveis ou não. Aceito as responsabilidades pela condução científica do projeto acima. Tenho ciência que essa folha será anexada ao projeto devidamente assinada por todos os responsáveis e fará parte integrante da documentação do mesmo.

Data: 30 / 06 / 2015

Famiely C. M. de Machado
Assinatura

INSTITUIÇÃO PROPONENTE

13. Nome:	14. CNPJ:	15. Unidade/Órgão:
Hospital Universitário Prof. Edgard Santos-UFBA	15.180.714/0002-87	

16. Telefone:	17. Outro Telefone:
(71) 3283-8141	

Termo de Compromisso (do responsável pela instituição): Declaro que conheço e cumprirei os requisitos da Resolução CNS 466/12 e suas Complementares e como esta instituição tem condições para o desenvolvimento deste projeto, autorizo sua execução.

Responsável: Antônio Carlos Nascimento Lemos CPF: 217276270-91

Cargo/Função: Superintendente do HUPES

Data: 14 / 07 / 2015

Assinatura

PATROCINADOR PRINCIPAL

Não se aplica.

XIII.3. ANEXO 3

Norma de publicação de revista

Journal of Voice

INSTRUCTIONS TO AUTHORS

Scope

The *Journal of Voice* includes clinical and research articles that are of interest to all professionals of all backgrounds. Papers are solicited on all aspects of voice, including basic voice science, acoustics, anatomy, synthesis, medical and surgical treatment of voice problems, voice therapy, voice pedagogy, and studies in other areas that increase the knowledge of normal (including performance) and abnormal vocal function in adults and children. Review articles will also be considered.

Manuscript Submission

All manuscripts must be submitted via the Elsevier Editorial System (EES) at http://ees.elsevier.com/jvoice . You will be instructed to enter the manuscript title, type, authors, abstract, and keywords and to upload your cover letter, manuscript text (including references, figure legends, etc.), and figures (see below for further information on figures). It is advisable to save the complete manuscript as a word-processing document (MS Word is preferred) and then upload it into EES.

All materials submitted for publication, including solicited articles and supplements, are subject to editorial review and revision. Only previously unpublished material will be considered for publication. Material submitted to the *Journal* must not be under consideration for publication elsewhere. All accepted manuscripts become the property of the *Journal* and may not be reproduced without the written permission of the Editor and the Publisher.

Copyright

In compliance with current U.S. Copyright law, transfer of copyright from author to publisher or its designee must be explicitly stated in writing to enable the publisher to assure maximum dissemination of the author's work. A copy of the agreement, executed and signed by the author(s), is required with each manuscript submission. The form to be used is available from the Editor and Publisher. No manuscript can be published without a signed copyright transfer.

Form of Manuscript

Manuscripts should be submitted in English. The paper should be divided into sections with appropriate section headings. Pages must be numbered sequentially with the first page of the manuscript being page 1 (title page and abstract page are not numbered). Authors are cautioned to type, where possible, all mathematical and chemical symbols, equations, and formulas and to identify all unusual symbols the first time they are used. Author(s) will use the *American Medical Association Manual of Style*, 9th ed., as a reference guide for writing purposes.

Cover Letter

Please include a cover letter indicating the name, mailing address, email address, telephone number, and fax number of the person to whom correspondence, proofs, and reprint requests are to be sent.

Title Page

The title page should contain the title, list of authors with affiliations, and complete mailing address, email address, telephone number, and fax number of the author to whom correspondence, proofs, and reprint requests are to be sent. If the research was presented at a meeting, the name of the meeting, location, and date should be given.

Abstract

The abstract must be included twice--once alone, where indicated by EES, and once as a part of the whole manuscript. It should be factual, comprehensive, and presented in a structured abstract format. Limit the abstract to 250 words. Do not cite references in the abstract. Limit the use of

abbreviations and acronyms. Use the following subheads: Objectives/Hypothesis, Study Design (randomized, prospective, etc.), Methods, Results, and Conclusions. Abbreviations and general statements (e.g., "the significance of the results is discussed") should be avoided.

Body of Paper

The beginning of the manuscript should be an introduction to the topic discussed including references to related literature, followed by a statement of the purpose and, where applicable, specific questions to be answered by the research. Typically, this section is followed by labeled sections with a sequence similar to Methods, Results, Discussion, and Conclusions.

References

References should follow the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (http://www.icmje.org/). References are to be supplied in order of citation in the text, numbered consecutively, and typed double-spaced. Sample references are given below of a journal article and a book.

1. Sataloff RT. Professional singers: the science and art of clinical care. *Am J Otolaryngology*. 1981;2: 251-266.
2. Sataloff RT, Myers DL. Cancer of the Ear and Temporal Bone. In: Gates, Ed. ***Current therapy on Otolaryngology- Head & neck surgery.*** 3rd ed. Toronto and Philadelphia: B.C. Decker; 1987:157-160.

Volume and issue numbers, specific beginning and ending pages, and name of translator should be included where appropriate.

Journal title abbreviations should follow the practices of *Index Medicus.* Provide all author names when there are seven or fewer co-authors. If there are more than seven co-authors, list only the first three and use et al. Authors are responsible for the bibliographic accuracy of all references. "Personal communications" and "unpublished observations" should be indicated within the text but excluded from the reference list (such communications and observations should be used only with the permission of those cited).

Symbols and Abbreviations

Use of symbols and abbreviations should conform to those provided by professional standards publications such as the American National Standard Letter Symbols and Abbreviations for Quantities Used in Acoustics Y10.11-1984, and the American National Standard Acoustical Terminology S1.1-1994. These two publications are available from the American National Standards Institute, 11 West 42nd Street, New York, NY 10018, 212-642-4900.

Accuracy of Data

For all studies dealing with instrumental quantities, a statement of the "error of measurement" should be included. For studies dealing with judgments, a statement concerning the procedure for determining the "reliability" of the judgments is expected.

Glossary

Authors are encouraged to define or explain jargon, and technical or novel language (or expressions) for terms not commonly known across the audiologic professions. These terms and explanations can be placed in a glossary table. If few, the terms can be explained in the text.

Tables

All tables must be cited sequentially in the text, numbered, and supplied with suitable explanatory legends and headings. Tables should not be supplied typed within the body of the manuscript. They must be separately uploaded into EES. Tables should be self-explanatory and should supplement, rather than duplicate, the material in the text.

Figures and Illustrations

All figures and illustrations must be cited sequentially in the text, numbered, and supplied with legends. Figures, illustrations, and legends should not be supplied within the body of the manuscript. Each individual figure must be separately uploaded into EES. Legends to figures should be brief, specific,

and explanatory. They should not unduly repeat information already given in the text. Magnification and stain should be provided where appropriate. All photographs and illustrations documenting any postoperative change must be labeled with the postoperative interval.

Figures should be submitted in electronic format, preferably in EPS or TIF format. Figures should be created using graphics software such as Photoshop or Illustrator. DO NOT USE PowerPoint, Corel Draw, or Harvard Graphics. COLOR figures submitted with the manuscript will appear in black and white in print unless the author agrees to pay fees associated with color reproduction. They will appear on the website in color at no extra charge. When color images appear in print in black and white, the black and white contrast will diminish, so choose distinct color contrasts and/or patterns for best conversion to black and white images.

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If a figure has been taken from previously copyrighted material, the legend must give full credit to the original source, and letters of permission must be submitted with the manuscript. Articles appear in both the print and online versions of the *Journal*, and wording of the letter should specify permission in both forms of media. Failure to get electronic permission rights may result in the images not appearing in the online version.

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Manuscripts received by the *Journal* are read by two or three reviewers who are knowledgeable in the topic in question. The role of the reviewer(s) is to read the manuscript critically, comment on possible or needed changes, and assist the Editor in making a decision concerning the acceptance or rejection of the manuscript for publication. Final page proofs sent to the author(s) can be changed only minimally.

Research Subjects

Research studies reported in manuscripts submitted to the *Journal of Voice* must abide by the ethical principles for the protection of human and animal subjects. The *Journal* endorses those principles found in the Belmount Report: Ethical Principles and Guidelines for the Protection of Human Subjects (1979, Office of the Protection from Research Risks Report, Bethesda, MD: U.S. Dept. of Health and Human Services); the Guide for the Care and Use of Laboratory Animals (DHEW Publication No. (NIH) 80-23, Revised 1978, Reprinted 1980, Office of Science and Health Reports, DDR/NIH, Bethesda, MD 20205); and the World Medical Association Declaration of Helsinki

guidelines (JAMA. 1997;277:925-926). To be considered for publication, studies involving human research subjects ordinarily require a statement indicating Institutional Review Board approval and/or compliance with the Guidelines specified.

XIII.4. ANEXO 4

Recibo de aceite do artigo 1

Your manuscript JVOICE_2018_359_R1 has been accepted4 Yahoo/Entrada

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Ref: JVOICE_2018_359_R1
Title: Phonotherapeutic Intervention in Patients with Mucosal Leishmaniasis Sequelae
Journal: Journal of Voice
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I am pleased to inform you that your paper has been accepted for publication. My own comments as well as any reviewer comments are appended to the end of this letter.
Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check. You can read more about this here.
Meanwhile, you will be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact.
Thank you for submitting your work to Journal of Voice. We hope you consider us again for future submissions.

Kind regards,
Robert T. Sataloff, MD, DMA, FACS
Editor-in-Chief
Journal of Voice

XIII.5. ANEXO 5

Norma de publicação de revista

Submission Guidelines
Style and Format

Copyediting manuscripts

Prior to submission, authors who believe their manuscripts would benefit from professional editing are encouraged to use language-editing and copyediting services. Obtaining this service is the responsibility of the author, and should be done before initial submission. These services can be found on the web using search terms like “scientific editing service” or “manuscript editing service.”

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Manuscript Organization

Manuscripts should be organized as follows. Instructions for each element appear below the list.

Beginning section	*The following elements are required, in order:* • Title page: List title, authors, and affiliations as first page of manuscript • Abstract • Introduction
Middle section	*The following elements can be renamed as needed and presented in any order:* • Materials and Methods • Results • Discussion • Conclusions (optional)
Ending section	*The following elements are required, in order:* • Acknowledgments • References • Supporting information captions (if applicable)
Other elements	• Figure captions are inserted immediately after the first paragraph in which the figure is cited. Figure files are uploaded separately. • Tables are inserted immediately after the first paragraph in which they are cited. • Supporting information files are uploaded separately.

Please refer to our downloadable sample files to ensure that your submission meets our formatting requirements:

Title	Length	Guidelines	Examples
Full title	250 characters	Specific, descriptive, concise, and comprehensible to readers outside the field	Impact of cigarette smoke exposure on innate immunity: A *Caenorhabditis elegans* model Solar drinking water disinfection (SODIS) to reduce childhood diarrhoea in rural Bolivia: A cluster-randomized, controlled trial
Short title	100 characters	State the topic of the study	Cigarette smoke exposure and innate immunity SODIS and childhood diarrhoea

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Viewing Figures and Supporting Information in the compiled submission PDF
The compiled submission PDF includes low-resolution preview images of the figures after the reference list. The function of these previews is to allow you to download the entire submission as quickly as possible. Click the link at the top of each preview page to download a high-resolution version of each figure. Links to download Supporting Information files are also available after the reference list.
Parts of a Submission

Title

Include a full title and a short title for the manuscript.

Titles should be written in sentence case (only the first word of the text, proper nouns, and genus names are capitalized). Avoid specialist abbreviations if possible. For clinical trials, systematic reviews, or meta-analyses, the subtitle should include the study design.

Author list

Authorship requirements

All authors must meet the criteria for authorship as outlined in the authorship policy. Those who contributed to the work but do not meet the criteria for authorship can be mentioned in the Acknowledgments. Read more about Acknowledgments.

The corresponding author must provide an ORCID iD at the time of submission by entering it in the user profile in the submission system. Read more about ORCID.

Author names and affiliations

Enter author names on the title page of the manuscript and in the online submission system.

On the title page, write author names in the following order:

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- Last name (surname, family name)

Each author on the list must have an affiliation. The affiliation includes department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country. Authors have the option to include a current address in addition to the address of their affiliation at the time of the study. The current address should be listed in the byline and clearly labeled “current address.” At a minimum, the address must include the author’s current institution, city, and country.

If an author has multiple affiliations, enter all affiliations on the title page only. In the submission system, enter only the preferred or primary affiliation. Author affiliations will be listed in the typeset PDF article in the same order that authors are listed in the submission.

Author names will be published exactly as they appear in the manuscript file. Please double-check the information carefully to make sure it is correct.

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Contributions will be published with the final article, and they should accurately reflect contributions to the work. The submitting author is responsible for completing this information at submission, and we expect that all authors will have reviewed, discussed, and agreed to their individual contributions ahead of this time.

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Read about publication fee assistance.

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The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

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The Abstract should:

- Describe the main objective(s) of the study
- Explain how the study was done, including any model organisms used, without methodological detail
- Summarize the most important results and their significance
- Not exceed 300 words

Abstracts should not include:

- Citations
- Abbreviations, if possible

Introduction

The introduction should:

- Provide background that puts the manuscript into context and allows readers outside the field to understand the purpose and significance of the study
- Define the problem addressed and why it is important
- Include a brief review of the key literature
- Note any relevant controversies or disagreements in the field
- Conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved

Materials and Methods

The Materials and Methods section should provide enough detail to allow suitably skilled investigators to fully replicate your study. Specific information and/or protocols for new methods should be included in

detail. If materials, methods, and protocols are well established, authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

Protocol documents for clinical trials, observational studies, and other **non-laboratory** investigations may be uploaded as supporting information. We recommend depositing **laboratory protocols** at protocols.io. Read detailed instructions for depositing and sharing your laboratory protocols.

Human or animal subjects and/or tissue or field sampling

Methods sections describing research using human or animal subjects and/or tissue or field sampling must include required ethics statements. For details, consult the reporting guidelines for specific study types.

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PLOS journals require authors to make all data underlying the findings described in their manuscript fully available without restriction, with rare exception.

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For smaller data sets and certain data types, authors may provide their data within supporting information files accompanying the manuscript. Authors should take care to maximize the accessibility and reusability of the data by selecting a file format from which data can be efficiently extracted (for example, spreadsheets or flat files should be provided rather than PDFs when providing tabulated data).

For more information on how best to provide data, read our policy on data availability. PLOS does not accept references to "data not shown."

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1. Describe your step-by-step protocol on protocols.io
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Methods sections of manuscripts adding new zoological, botanical, or fungal taxon names to the literature must follow the guidelines for new taxon names.

Results, Discussion, Conclusions

These sections may all be separate, or may be combined to create a mixed Results/Discussion section (commonly labeled “Results and Discussion”) or a mixed Discussion/Conclusions section (commonly labeled “Discussion”). These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn.

Authors should explain how the results relate to the hypothesis presented as the basis of the study and provide a succinct explanation of the implications of the findings, particularly in relation to previous related studies and potential future directions for research.

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Acknowledgments

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.

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- Manuscripts on preprint servers, providing the manuscript has a citable DOI or arXiv URL.

Do not cite the following sources in the reference list:

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References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., “We used the techniques developed by our colleagues [19] to analyze the data”). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts.

Make sure the parts of the manuscript are in the correct order *before* ordering the citations.

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Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of the references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the “Vancouver” style. Example formats are listed below. Additional examples are in the ICMJE sample references.

A reference management tool, EndNote, offers a current style file that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

Journal name abbreviations should be those found in the National Center for Biotechnology Information (NCBI) databases.

Source	Format
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (*Ailuropoda melanoleuca*). Genet Mol Res. 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005. *Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.*
Accepted, unpublished articles	Same as published articles, but substitute “Forthcoming” for page numbers or DOI.
Online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14
Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.

Source	Format
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity; 1991. Preprint. Available from: arXiv:1403.3301v1. Cited 17 March 2014.
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 29 Jan 2014. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html Cited 17 March 2014.
New media (blogs, web sites, or other written works)	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09
Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

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Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 20 MB in size.

Authors may use almost any description as the item name for a supporting information file as long as it contains an “S” and number. For example, “S1 Appendix” and “S2 Appendix,” “S1 Table” and “S2 Table,” and so forth.

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Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and

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- Enter the given data DOI into the full Data Availability Statement, which is requested in the Additional Information section of the PLOS submission form. Then provide the URL passcode in the Attach Files section.

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- Ensembl
- Entrez Gene
- FlyBase
- InterPro
- Mouse Genome Database (MGD)
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Upon submission, authors must confirm that the manuscript, or any related manuscript, is not currently under consideration or accepted elsewhere. If related work has been submitted to *PLOS ONE* or elsewhere, authors must include a copy with the submitted article. Reviewers will be asked to comment on the overlap between related submissions.

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Read our policies on related manuscripts.

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All research involving human participants must have been approved by the authors' Institutional Review Board (IRB) or by equivalent ethics committee(s), and must have been conducted according to the principles expressed in the Declaration of Helsinki. Authors should be able to submit, upon request, a statement from the IRB or ethics committee indicating approval of the research. We reserve the right to reject work that we believe has not been conducted to a high ethical standard, even when formal approval has been obtained.

Subjects must have been properly instructed and have indicated that they consent to participate by signing the appropriate informed consent paperwork. Authors may be asked to submit a blank, sample copy of a subject consent form. If consent was verbal instead of written, or if consent could not be obtained, the authors must explain the reason in the manuscript, and the use of verbal consent or the lack of consent must have been approved by the IRB or ethics committee.

All efforts should be made to protect patient privacy and anonymity. Identifying information, including photos, should not be included in the manuscript unless the information is crucial and the individual has provided written consent by completing the Consent Form for Publication in a PLOS Journal (PDF). Download additional translations of the form from the Downloads and Translations page. More information about patient privacy, anonymity, and informed consent can be found in theInternational Committee of Medical Journal Editors (ICMJE) Privacy and Confidentiality guidelines.

Manuscripts should conform to the following reporting guidelines:

- Studies of diagnostic accuracy: STARD
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- Microarray experiments: MIAME
- Other types of health-related research: Consult the EQUATOR web site for appropriate reporting guidelines

Methods sections of papers on research using human subjects or samples must include ethics statements that specify:

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- **Whether informed consent was written or oral**. If informed consent was oral, it must be stated in the manuscript:
    - Why written consent could not be obtained
    - That the Institutional Review Board (IRB) approved use of oral consent
    - How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

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- Define categories in as much detail as the study protocol allows
- Justify their choices of definitions and categories, including for example whether any rules of human categorization were required by their funding agency
- Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: “Caucasian” should be changed to “white” or “of [Western] European descent” (as appropriate); “cancer victims” should be changed to “patients with cancer.”

For papers that include identifying, or potentially identifying, information, authors must download the Consent Form for Publication in a PLOS Journal, which the individual, parent, or guardian must sign once they have read the paper and been informed about the terms of PLOS open-access license. The signed consent form should not be submitted with the manuscript, but authors should securely file it in the individual's case notes and the methods section of the manuscript should explicitly state that consent authorization for publication is on file, using wording like:

The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

For more information about *PLOS ONE* policies regarding human subjects research, see the Publication Criteria and Editorial Policies.

Clinical trials

Clinical trials are subject to all policies regarding human research. *PLOS ONE* follows the World Health Organization's (WHO) definition of a clinical trial:

A clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes [...] Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioural treatments, process-of-care changes, preventive care, etc.

All clinical trials must be registered in one of the publicly-accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors). Authors must provide the trial registration number. Prior disclosure of results on a clinical trial registry site will not affect consideration for publication. We reserve the right to inform authors' institutions or ethics committees, and to reject the manuscript, if we become aware of unregistered trials.

PLOS ONE supports prospective trial registration (i.e. before participant recruitment has begun) as recommended by the ICMJE's clinical trial registration policy. **Where trials were not publicly registered before participant recruitment began**, authors must:

- Register all related clinical trials and confirm they have done so in the Methods section
- Explain in the Methods the reason for failing to register before participant recruitment

Clinical trials must be reported according to the relevant reporting guidelines, i.e. CONSORT for randomized controlled trials, TREND for non-randomized trials, and other specialized guidelines as

appropriate. The intervention should be described according to the requirements of the TIDieR checklist and guide. Submissions must also include the study protocol as supporting information, which will be published with the manuscript if accepted.

Authors of manuscripts describing the results of clinical trials must adhere to the CONSORT reporting guidelines appropriate to their trial design, available on the CONSORT Statement web site. Before the paper can enter peer review, authors must:

- Provide the registry name and number in the methods section of the manuscript
- Provide a copy of the trial protocol as approved by the ethics committee and a completed CONSORT checklist as supporting information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.
- Include the CONSORT flow diagram as the manuscript's “Fig 1”

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The methods section must include the name of the registry, the registry number, and the URL of your trial in the registry database for each location in which the trial is registered.

Animal research

All research involving vertebrates or cephalopods must have approval from the authors' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s), and must have been conducted according to applicable national and international guidelines. Approval must be received prior to beginning research.

Manuscripts reporting animal research must state in the Methods section:

- The full name of the relevant ethics committee that approved the work, and the associated permit number(s).
- Where ethical approval is not required, the manuscript should include a clear statement of this and the reason why. Provide any relevant regulations under which the study is exempt from the requirement for approval.
- Relevant details of steps taken to ameliorate animal suffering.

Example ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Minnesota (Protocol Number: 27-2956). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Authors should always state the organism(s) studied in the Abstract. Where the study may be confused as pertaining to clinical research, authors should also state the animal model in the title.

To maximize reproducibility and potential for re-use of data, we encourage authors to follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for all submissions describing laboratory-based animal research and to upload a completed ARRIVE Guidelines Checklist to be published as supporting information.

Non-human primates

Manuscripts describing research involving non-human primates must report details of husbandry and animal welfare in accordance with the recommendations of the Weatherall report, *The use of non-human primates in research*, including:

- Information about housing, feeding, and environmental enrichment.
- Steps taken to minimize suffering, including use of anesthesia and method of sacrifice, if appropriate.

Random source animals

Manuscripts describing studies that use random source (e.g. Class B dealer-sourced in the USA), shelter, or stray animals will be subject to additional scrutiny and may be rejected if sufficient ethical and scientific justification for the study design is lacking.

Unacceptable euthanasia methods and anesthetic agents

Manuscripts reporting use of a euthanasia method(s) classified as unacceptable by the American Veterinary Medical Association or use of an anesthesia method(s) that is widely prohibited (e.g., chloral hydrate, ether, chloroform) must include at the time of initial submission, scientific justification for use in the specific study design, as well as confirmation of approval for specific use from their animal research ethics committee. These manuscripts may be subject to additional ethics considerations prior to publication.

Humane endpoints

Manuscripts reporting studies in which death of a regulated animal (vertebrate, cephalopod) is a likely outcome or a planned experimental endpoint, must comprehensively report details of study design, rationale for the approach, and methodology, including consideration of humane endpoints. This applies to research that involves, for instance, assessment of survival, toxicity, longevity, terminal disease, or high rates of incidental mortality.

Definition of a humane endpoint

A humane endpoint is a predefined experimental endpoint at which animals are euthanized when they display early markers associated with death or poor prognosis of quality of life, or specific signs of severe suffering or distress. Humane endpoints are used as an alternative to allowing such conditions to continue or progress to death following the experimental intervention ("death as an endpoint"), or only euthanizing animals at the end of an experiment. Before a study begins, researchers define the practical observations or measurements that will be used during the study to recognize a humane endpoint, based on anticipated clinical, physiological, and behavioral signs. Please see the NC3Rs guidelines for more information. Additional discussion of humane endpoints can be found in this article: Nuno H. Franco, Margarida Correia-Neves, I. Anna S. Olsson (2012) How "Humane" Is Your Endpoint? — Refining the Science-Driven Approach for Termination of Animal Studies of Chronic Infection. PLoS Pathog 8(1): e1002399 doi.org/10.1371/journal.ppat.1002399.

Full details of humane endpoints use must be reported for a study to be reproducible and for the results to be accurately interpreted.

For studies in which death of an animal is an outcome or a planned experimental endpoint, authors should include the following information in the Methods section of the manuscript:

- The specific criteria (i.e. humane endpoints) used to determine when animals should be euthanized.
- The duration of the experiment.
- The numbers of animals used, euthanized, and found dead (if any); the cause of death for all animals.
- How frequently animal health and behavior were monitored.
- All animal welfare considerations taken, including efforts to minimize suffering and distress, use of analgesics or anaesthetics, or special housing conditions.

If humane endpoints were not used, the manuscript should report:

- A scientific justification for the study design, including the reasons why humane endpoints could not be used, and discussion of alternatives that were considered.
- Whether the institutional animal ethics committee specifically reviewed and approved the anticipated mortality in the study design.

Observational and field studies

Methods sections for submissions reporting on any type of field study must include ethics statements that specify:

- Permits and approvals obtained for the work, including the full name of the authority that approved the study; if none were required, authors should explain why
- Whether the land accessed is privately owned or protected
- Whether any protected species were sampled
- Full details of animal husbandry, experimentation, and care/welfare, where relevant

Paleontology and archaeology research

Manuscripts reporting paleontology and archaeology research must include descriptions of methods and specimens in sufficient detail to allow the work to be reproduced. Data sets supporting statistical and phylogenetic analyses should be provided, preferably in a format that allows easy re-use. Read the policy.

Specimen numbers and complete repository information, including museum name and geographic location, are required for publication. Locality information should be provided in the manuscript as legally allowable, or a statement should be included giving details of the availability of such information to qualified researchers.

If permits were required for any aspect of the work, details should be given of all permits that were obtained, including the full name of the issuing authority. This should be accompanied by the following statement:

All necessary permits were obtained for the described study, which complied with all relevant regulations.

If no permits were required, please include the following statement:

No permits were required for the described study, which complied with all relevant regulations.

Manuscripts describing paleontology and archaeology research are subject to the following policies:

- **Sharing of data and materials.** Any specimen that is erected as a new species, described, or figured must be deposited in an accessible, permanent repository (i.e., public museum or similar

institution). If study conclusions depend on specimens that do not fit these criteria, the article will be rejected under *PLOS ONE*'s data availability criterion.

- **Ethics.** *PLOS ONE* will not publish research on specimens that were obtained without necessary permission or were illegally exported.

Systematic reviews and meta-analyses

A systematic review paper, as defined by The Cochrane Collaboration, is a review of a clearly formulated question that uses explicit, systematic methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review. These reviews differ substantially from narrative-based reviews or synthesis articles. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies.

Reports of systematic reviews and meta-analyses must include a completed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist and flow diagram to accompany the main text. Blank templates are available here:

- Checklist: PDF or Word document
- Flow diagram: PDF or Word document

Authors must also state in their "Methods" section whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as supporting information and provide the registry number in the abstract.

If your article is a systematic review or a meta-analysis you should:

- State this in your cover letter
- Select "Research Article" as your article type when submitting
- Include the PRISMA flow diagram as Fig 1 (required where applicable)
- Include the PRISMA checklist as supporting information

Meta-analysis of genetic association studies

Manuscripts reporting a meta-analysis of genetic association studies must report results of value to the field and should be reported according to the guidelines presented in *Systematic Reviews of Genetic Association Studies* by Sagoo *et al.*

On submission, authors will be asked to justify the rationale for the meta-analysis and how it contributes to the base of scientific knowledge in the light of previously published results. Authors will also be asked to complete a checklist (DOCX) outlining information about the justification for the study and the methodology employed. Meta-analyses that replicate published studies will be rejected if the authors do not provide adequate justification.

Personal data from third-party sources

For all studies using personal data from internet-based and other third-party sources (e.g., social media, blogs, other internet sources, mobile phone companies), data must be collected and used according to company/website Terms and Conditions, with appropriate permissions. All data sources must be acknowledged clearly in the Materials and Methods section.

Read our policy on data availability.

In the Ethics Statement, authors should declare any potential risks to individuals or individual privacy, or affirm that in their assessment, the study posed no such risks. In addition, the following Ethics and Data Protection requirements must be met.

For interventional studies, which impact participants' experiences or data, the study design must have been prospectively approved by an Ethics Committee, and informed consent is required. The Ethics Committee may waive the requirement for approval and/or consent.

For observational studies in which personal experiences and accounts are not manipulated, consultation with an Ethics or Data Protection Committee is recommended. Additional requirements apply in the following circumstances:

- If information used could threaten personal privacy or damage the reputation of individuals whose data are used, an Ethics Committee should be consulted and informed consent obtained or specifically addressed.
- If authors accessed any personal identifying information, an Ethics or Data Protection Committee should oversee data anonymization. If data were anonymized and/or aggregated before access and analysis, informed consent is generally not required.

Note that Terms of Use contracts do not qualify as informed consent, even if they address the use of personal data for research.
See our reporting guidelines for human subjects research.

Cell lines

Authors reporting research using cell lines should state when and where they obtained the cells, giving the date and the name of the researcher, cell line repository, or commercial source (company) who provided the cells, as appropriate.

Authors must also include the following information for each cell line:

For *de novo* (new) cell lines, including those given to the researchers as a gift, authors must follow our policies for human subjects research or animal research, as appropriate. The ethics statement must include:

- Details of institutional review board or ethics committee approval; AND
- For human cells, confirmation of written informed consent from the donor, guardian, or next of kin

For established cell lines, the Methods section should include:

- A reference to the published article that first described the cell line; AND/OR
- The cell line repository or company the cell line was obtained from, the catalogue number, and whether the cell line was obtained directly from the repository/company or from another laboratory

Authors should check established cell lines using the ICLAC Database of Cross-contaminated or Misidentified Cell Lines to confirm they are not misidentified or contaminated. Cell line authentication is recommended – e.g., by karyotyping, isozyme analysis, or short tandem repeats (STR) analysis – and may be required during peer review or after publication.

Blots and gels

Please review *PLOS ONE's* requirements for reporting blot and gel results and providing the underlying raw images.

Antibodies

Manuscripts reporting experiments using antibodies should include the following information:

- The name of each antibody, a description of whether it is monoclonal or polyclonal, and the host species.
- The commercial supplier or source laboratory.
- The catalogue or clone number and, if known, the batch number.
- The antigen(s) used to raise the antibody.
- For established antibodies, a stable public identifier from the Antibody Registry.

The manuscript should also report the following experimental details:

- The final antibody concentration or dilution.
- A reference to the validation study if the antibody was previously validated. If not, provide details of how the authors validated the antibody for the applications and species used.

We encourage authors to consider adding information on new validations to a publicly available database such asAntibodypedia or CiteAb.

Small and macromolecule crystal data

Manuscripts reporting new and unpublished three-dimensional structures must include sufficient supporting data and detailed descriptions of the methodologies used to allow the reproduction and validation of the structures. All novel structures must have been deposited in a community endorsed database prior to submission (please see our list of recommended repositories).

Small molecule single crystal data

Authors reporting X-Ray crystallographic structures of small organic, metal-organic, and inorganic molecules must deposit their data with the Cambridge Crystallographic Data Centre (CCDC), the Inorganic Crystal Structure Database (ICSD), or similar community databases providing a recognized validation functionality. Authors are also required to include the relevant structure reference numbers within the main text (e.g. the CCDC ID number), as well as the crystallographic information files (.cif format) as Supplementary Information, along with the checkCIF validation reports that can be obtained via the International Union of Crystallography (IUCr).

Macromolecular structures

Authors reporting novel macromolecular structures must have deposited their data prior to submission with the Worldwide Protein Data Bank (wwPDB), the Biological Magnetic Resonance Data Bank (BMRB), the Electron Microscopy Data Bank (EMDB), or other community databases providing a recognized validation functionality. Authors must include the structure reference numbers within the main text and submit as Supplementary Information the official validation reports from these databases.

Methods, software, databases, and tools

PLOS ONE will consider submissions that present new methods, software, databases, or tools as the primary focus of the manuscript if they meet the following criteria:

Utility
The tool must be of use to the community and must present a proven advantage over existing alternatives,

where applicable. Recapitulation of existing methods, software, or databases is not useful and will not be considered for publication. Combining data and/or functionalities from other sources may be acceptable, but simpler instances (i.e. presenting a subset of an already existing database) may not be considered. For software, databases, and online tools, the long-term utility should also be discussed, as relevant. This discussion may include maintenance, the potential for future growth, and the stability of the hosting, as applicable.

Validation
Submissions presenting methods, software, databases, or tools must demonstrate that the new tool achieves its intended purpose. If similar options already exist, the submitted manuscript must demonstrate that the new tool is an improvement over existing options in some way. This requirement may be met by including a proof-of-principle experiment or analysis; if this is not possible, a discussion of the possible applications and some preliminary analysis may be sufficient.

Availability
If the manuscript's primary purpose is the description of new software or a new software package, this software must be open source, deposited in an appropriate archive, and conform to the Open Source Definition. If the manuscript mainly describes a database, this database must be open-access and hosted somewhere publicly accessible, and any software used to generate a database should also be open source. If relevant, databases should be open for appropriate deposition of additional data. Dependency on commercial software such as Mathematica and MATLAB does not preclude a paper from consideration, although complete open source solutions are preferred. In these cases, authors should provide a direct link to the deposited software or the database hosting site from within the paper. If the primary focus of a manuscript is the presentation of a new tool, such as a newly developed or modified questionnaire or scale, it should be openly available under a license no more restrictive than CC BY.

Software submissions

Manuscripts whose primary purpose is the description of new software must provide full details of the algorithms designed. Describe any dependencies on commercial products or operating system. Include details of the supplied test data and explain how to install and run the software. A brief description of enhancements made in the major releases of the software may also be given. Authors should provide a direct link to the deposited software from within the paper.

Database submissions

For descriptions of databases, provide details about how the data were curated, as well as plans for long-term database maintenance, growth, and stability. Authors should provide a direct link to the database hosting site from within the paper.

Read the PLOS policy on sharing materials and software.

New taxon names

Zoological names

When publishing papers that describe a new zoological taxon name, PLOS aims to comply with the requirements of the International Commission on Zoological Nomenclature (ICZN). Effective 1 January 2012, the ICZN considers an online-only publication to be legitimate if it meets the criteria of archiving and is registered in ZooBank, the ICZN's official registry.

For proper registration of a new zoological taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Anochetus boltoni *Fisher* ***sp. nov.*** *urn:lsid:zoobank.org:act:B6C072CF-1CA6-40C7-8396-534E91EF7FBB*

You will need to contact Zoobank to obtain a GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper.

Please also insert the following text into the **Methods** section, in a sub-section to be called “Nomenclatural Acts”:

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix “http://zoobank.org/”. The LSID for this publication is: urn:lsid:zoobank.org:pub: XXXXXXX. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS [author to insert any additional repositories].

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Botanical names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature, and apply only to seed plants, ferns, and lycophytes.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Solanum aspersum S.Knapp, sp. nov. [urn:lsid:ipni.org:names:77103633-1] Type: Colombia. Putumayo: vertiente oriental de la Cordillera, entre Sachamates y San Francisco de Sibundoy, 1600-1750 m, 30 Dec 1940, J. Cuatrecasas 11471 (holotype, COL; isotypes, F [F-1335119], US [US-1799731]).

Journal staff will contact IPNI to obtain the GUID (LSID) after your manuscript is accepted for publication, and this information will then be added to the manuscript during the production phase

In the **Methods** section, include a sub-section called “Nomenclature” using the following wording:

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to IPNI, from where they will be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix http://ipni.org/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)].

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Fungal names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Hymenogaster huthii. Stielow et al. 2010, sp. nov. [urn:lsid:indexfungorum.org:names:518624]

You will need to contact either Mycobank or Index Fungorum to obtain the GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper. Effective January 2013, all papers describing new fungal species must reference the identifier issued by a recognized repository in the protologue in order to be considered effectively published.

In the **Methods** section, include a sub-section called “Nomenclature” using the following wording (this example is for taxon names submitted to MycoBank; please substitute appropriately if you have submitted to Index Fungorum):

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to MycoBank from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number contained in this publication to the prefix http://www.mycobank.org/MB/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)].

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

XIII.5. ANEXO 6

Recibo de submissão do artigo 2

PLOS ONE
PONE-D-19-22588
Spectrographic Analysis Before and After Phonotherapeutic Intervention in Patients With Tegumentary Leishmaniasis
PLOS ONE

Dear Mrs DE MACHADO,
Thank you for submitting your manuscript entitled 'Spectrographic Analysis Before and After Phonotherapeutic Intervention in Patients With Tegumentary Leishmaniasis' to PLOS ONE. Your assigned manuscript number is PONE-D-19-22588.

We will now begin processing your manuscript and may contact you if we require any further information. You will receive an update once your manuscript passes our in-house technical check; you can also check the status of your manuscript by logging into your account at https://www.editorialmanager.com/pone/.

If you have any inquiries or other comments regarding this manuscript please contact plosone@plos.org. Thank you for your support of PLOS ONE.

Kind regards,
PLOS ONE