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Émille Dalbem Paim

Uso de recursos físicos na reabilitação fonoaudiológica em oncologia.

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É justo que muito me custe

aquilo que muito vale!

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RESUMO

Esta tese teve como objetivo contribuir para o avanço da reabilitação fonoaudiológica no câncer de cabeça e pescoço, fortalecendo a base de evidências científicas para o uso seguro de dispositivos terapêuticos como eletroestimulação e fotobiomodulação (FBM) na prática clínica. **Métodos artigo 1:** Esta revisão sistemática, conduzida de acordo com as diretrizes PRISMA e registrada no PROSPERO, avaliou a eficácia da estimulação elétrica neuromuscular (EEM) no tratamento da disfagia radioinduzida. Utilizando as bases de dados MEDLINE/PubMed, Embase, Cochrane Central Register of Controlled Trials (CENTRAL), Scielo e PEDro, foram identificados quatro estudos. **Resultados artigo 1:** A análise dos dados demonstrou que a EEM, associada a exercícios, promoveu benefício na deglutição, especialmente na redução do resíduo faríngeo e na qualidade de vida. No entanto, a heterogeneidade dos estudos e o risco de viés, avaliados pela ferramenta ROB 2.0, limitam a generalização dos resultados. **Métodos artigo 2:** série de casos com seis pacientes com trismo severo, submetidos a sessões de terapia e, no caso do grupo experimental, à aplicação de FBM, que investigou a eficácia de diferentes terapias no tratamento do trismo radioinduzido, uma condição que limita a abertura da boca e causa dor, geralmente após radioterapia na região da cabeça e pescoço. Foram comparadas duas abordagens: terapia miofuncional oral (TMO) isolada e TMO combinada com FBM. **Resultado artigo 2:** Os resultados demonstraram que a combinação de TMO e FBM proporcionou melhora significativa na abertura bucal, redução da dor e melhor qualidade de vida dos pacientes, em comparação ao grupo que recebeu apenas a TMO. **Métodos artigo 3:** Este estudo *in vitro* investigou o efeito da FBM com comprimentos de onda de 658 nm e 830 nm e doses de 1 J, 3 J e 6 J/cm² sobre a viabilidade e proliferação de células de carcinoma de células escamosas (CEC) das linhagens CAL27 e SCC09. **Resultados artigo 3:** Os resultados demonstraram que a aplicação da FBM não promoveu alterações significativas na viabilidade e proliferação celular, quando comparada ao grupo controle, em nenhuma das condições testadas. **Conclusão:** baseado nos achados descritos nesta tese, destaca-se a relevância da eletroestimulação e da FBM como ferramentas promissoras na reabilitação fonoaudiológica de pacientes com câncer de cabeça e pescoço. No entanto, são necessárias mais pesquisas para elucidar os mecanismos de ação dessas terapias e otimizar seus protocolos de aplicação. Os estudos que compõe este trabalho podem servir como base para o desenvolvimento de novas pesquisas e para a implementação de protocolos de tratamento mais eficazes e individualizados para esses pacientes.

Palavras-chave: Fotobiomodulação; Terapia por Estimulação Elétrica; Neoplasias; Reabilitação; Fonoaudiologia; Neoplasias de Cabeça e Pescoço; Técnicas In Vitro.

ABSTRACT

This thesis aimed to contribute to the advancement of speech-language rehabilitation in head and neck cancer, strengthening the scientific evidence base for the safe use of therapeutic devices such as electrostimulation and photobiomodulation (PBM) in clinical practice. **Methods article 1:** This systematic review, conducted in accordance with the PRISMA guidelines and registered in PROSPERO, evaluated the efficacy of neuromuscular electrical stimulation (EEM) in the treatment of radioinduced dysphagia. Using the MEDLINE/PubMed, Embase, Cochrane Central Register of Controlled Trials (CENTRAL), Scielo and PEDro databases, four studies were identified. **Results article 1:** Data analysis demonstrated that EEM, associated with exercises, promoted benefits in swallowing, especially in reducing pharyngeal residue and in quality of life. However, the heterogeneity of the studies and the risk of bias, assessed by the ROB 2.0 tool, limit the generalization of the results. **Methods article 2:** case series with six patients with severe trismus, submitted to therapy sessions and, in the case of the experimental group, to the application of PBM, which investigated the efficacy of different therapies in the treatment of radiation-induced trismus, a condition that limits mouth opening and causes pain, usually after radiotherapy in the head and neck region. Two approaches were compared: oral myofunctional therapy (OMT) alone and OMT combined with PBM. **Result article 2:** The results demonstrated that the combination of OMT and PBM provided significant improvement in mouth opening, pain reduction and better quality of life of patients, compared to the group that received only OMT. **Methods article 3:** This in vitro study investigated the effect of PBM with wavelengths of 658 nm and 830 nm and doses of 1 J, 3 J and 6 J/cm² on the viability and proliferation of squamous cell carcinoma (SCC) cells of the CAL27 and SCC09 cell lines. **Results of article 3:** The results demonstrated that the application of PBM did not promote significant changes in cell viability and proliferation, when compared to the control group, in any of the conditions tested. **Conclusion:** based on the findings described in this thesis, the relevance of electrostimulation and PBM is highlighted as promising tools in the speech-language rehabilitation of patients with head and neck cancer. However, more research is needed to elucidate the mechanisms of action of these therapies and optimize their application protocols. The studies that make up this work can serve as a basis for the development of new research and for the implementation of more effective and individualized treatment protocols for these patients.

Keywords: Photobiomodulation; Electric Stimulation Therapy; Neoplasms; Rehabilitation; Speech Therapy; Head and Neck Neoplasms; In Vitro Techniques.

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LISTA DE ABREVIATURAS E SIGLAS

ATP	Adenosina trifosfato
CCP	Câncer de cabeça e pescoço
CCEo	Carcinoma de células escamosas oral
CCO	Câncer de cavidade oral
CEC	Carcinoma de células escamosas
CFFa	Conselho Federal de Fonoaudiologia
CG	Control Group (Grupo Controle)
CNAs	Cópias cromossômicas
CTC	Células tronco cancerígenas
CT	Chemotherapy
DNA	Ácido Desoxirribonucleico
EENM	Estimulação elétrica neuromuscular
EROs	Espécies reativas de oxigênio
FDA	Food and drug administration
FBM	Fotobiomodulação
GEE	Modelos de equações de estimações generalizadas
GTQ	Gothenburg Trismus Questionary
GRADE	Grading of recommendation assessment development and evaluation
GY	Gray
HA	Hyoid anteriorization
HE	Hyoid elevation
HNC	Head neck cancer
HPV	Papilomavírus Humano
Hz	Hertz
IMRT	Intensity Modulated Radiotherapy
J	Joule
LASER	Light Amplification by Stimulated Emission of radiation
LBI	Laser de baixa intensidade
mA	Milliamperes
MDADI	MD Anderson Dysphagia Inventory
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
nm	nanômetros
NMES	Neuromuscular Electrical Stimulation
NO	Oxido Nítrico
OMT	Oral myofunctional therapy
OTT	Oral transit time
PAS	Penetration and aspiration scale
PBM	Photomiomodulation
PRISMA	Preferred Reporting Items for Systematic Reviews
PROSPERO	Prospective Register of Systematic Reviews

PTT	Pharyngeal transit time
QT	Quimioterapia
QOL	Quality of life
RT	Radioterapia
SCC	Squamous cell carcinoma
SRB	Sulforodamine B
TCLE	Termo de Consentimento Livre e Esclarecido
TENS	*Transcutaneous electric nerve stimulation*
TMO	Terapia miofuncional orofacial
VAS	Visual analogue scale
uS	Largura de Pulso

SUMÁRIO

1 CÂNCER DE CABEÇA E PESCOÇO

Câncer é o nome dado à doença originada pela proliferação celular irrestrita causada por uma série de alterações somáticas do ácido desoxirribonucleico (DNA), sendo que grande parte destas modificações decorrem da sequência real, podendo advir por erros aleatórios de replicação, exposição a agentes carcinogênicos ou defeitos no processo de reparo do DNA.[1]

O câncer de cabeça e pescoço constitui um relevante desafio para a saúde mundial, com uma incidência estimada em 900.000 novos casos por ano, representando a terceira causa mais comum de óbito por câncer no mundo.[2] No contexto brasileiro, a carga dessa doença é particularmente expressiva, com projeções de 15.100 e 7.790 novos casos por 100.000 habitantes para os tumores de cavidade oral e laringe, respectivamente, em 2024. Esses dados posicionam essas neoplasias entre os dez mais incidentes no país, evidenciando a necessidade de estratégias de prevenção, diagnóstico precoce e tratamento eficaz.[2]

A incidência deste tipo de câncer é maior no sexo masculino que em média surge aos 59,6 anos de idade.[2] Os sítios anatômicos acometidos distribuem-se de forma divergente conforme as características sociodemográficas, mas os mais prevalentes são cavidade oral seguido de laringe, orofaringe e hipofaringe.[2, 3]

O carcinoma de células escamosas (CEC) de cabeça e pescoço com acometimento de sítios anatômicos do trato aerodigestivo superior representa a terceira causa mais comum de óbito por câncer no mundo e no Brasil, configurando-se como um dos cânceres de maior incidência conforme estimativa para 2024-2025[2]. O desenvolvimento de CEC de cabeça e pescoço resulta da interação de fatores ambientais, ocupacionais e herança genética, tratando-se, portanto, de uma doença multifatorial.[4]

Cerca de 75% dos casos são diagnosticados tardiamente, o que influencia diretamente no prognóstico e sobrevida do paciente. Como consequência disto, em torno de 40% a 60% apresentam recidiva locorregional e 20% a 30% evoluem com metástases à distância.[3]

Os sintomas diferem em função da região acometida, mas de um modo geral, os mais prevalentes são: dor, otalgia reflexa, disfonia e disfagia que leva ao prejuízo do estado nutricional e dispneia.[5] O tratamento para tumores de cabeça e pescoço, comumente pode impactar nas funções de fonação, deglutição, audição e mastigação, além das comorbidades estéticas que se relacionam diretamente com a interação social e a qualidade de vida.[5]

O papilomavírus humano (HPV) surgiu como um fator etiológico significativo, particularmente para cânceres orofaríngeos. Os CECs orofaríngeos HPV-positivos têm aumentado em incidência, especialmente em países desenvolvidos, devido a mudanças nos comportamentos sexuais e à prevalência de tipos de HPV de alto risco, como o HPV-16[6]. Os cânceres HPV-positivos tendem a ocorrer em pacientes mais jovens e têm um prognóstico melhor em comparação aos cânceres HPV-negativos.[6]

A incidência de cânceres orofaríngeos HPV-positivos varia globalmente. Nos Estados Unidos e em partes da Europa, o HPV é responsável por 60-70% dos cânceres orofaríngeos, enquanto as taxas são menores em outras regiões como Ásia e Índia. Em contraste, o carcinoma nasofaríngeo, que é mais comum em certas regiões endêmicas, está intimamente associado à infecção pelo vírus Epstein-Barr.[6]

O aumento dos cânceres orofaríngeos HPV-positivos mudou o cenário epidemiológico, com esses cânceres agora ultrapassando o câncer cervical como a malignidade mais comum relacionada ao HPV nos Estados Unidos. Tumores HPV-positivos frequentemente apresentam características clínicas e patológicas distintas, como morfologia não queratinizante e melhor resposta ao tratamento.[6]

No geral, a prevalência de CEC em cânceres de cabeça e pescoço é moldada por uma interação complexa de fatores de estilo de vida, infecções virais e tendências epidemiológicas regionais. A crescente incidência de cânceres orofaríngeos relacionados ao HPV destaca a natureza evolutiva dessas malignidades e ressalta a importância de medidas preventivas, como a vacinação contra o HPV, que podem impactar as taxas de incidência futuras.[7]

1.1 Particularidades do carcinoma de células escamosas

Os CECs são um grupo heterogêneo de tumores que se originam em células epiteliais escamosas, que revestem as superfícies mucosas da cavidade oral, faringe, laringe e seios nasais. Exibem várias características celulares distintas que são consistentes em vários locais anatômicos.[4]

Histologicamente, as células cancerosas apresentam: atipia celular, com alterações em seu núcleo, citoplasma e forma, perdendo a organização característica das células normais; pleomorfismo, culminando em tamanhos e formas variadas; hipercromasia, onde o núcleo é geralmente maior e com pigmentação elevada devido ao aumento de cromatina; mitoses atípicas, com número elevado e anormal de divisões celulares; perda da polaridade e invasão local.[4]

São caracterizados pela presença de queratinização e pontes intercelulares. A queratinização se refere à formação de queratina dentro das células tumorais, que pode ser observada como pérolas de queratina ou queratinização de células individuais. Pontes intercelulares são conexões desmossômicas entre células tumorais, indicativas de sua origem epitelial.[8, 9]

Molecularmente, mostram altos níveis de transcritos que codificam queratinas e proteínas relevantes para junções intercelulares e polaridade celular. Os CECs apresentam diversas alterações genéticas, como mutações em genes supressores de tumor (p53, p16) e oncogenes (EGFR, MYC) e instabilidade genômica devido à alta taxa de mutações. As células cancerosas expressam marcadores tumorais específicos, como p16, Ki-67 e EGFR, que podem ser utilizados para o diagnóstico e prognóstico da doença.[10]

As alterações genéticas são complexas e envolvem ativação do oncogene e inativação do gene supressor de tumor. Alterações comuns incluem mutações em TP53 e CDKN2A, bem como amplificações de EGFR e CCND1. [10] Os CECs HPV-positivos frequentemente exibem mutações no gene PIK3CA e perda de TRAF3, enquanto os relacionados ao tabagismo mostram mutações de perda de função quase universais em TP53 e CDKN2A.[10]

Também é possível identificar alterações recorrentes no número de cópias cromossômicas (CNAs), como ganhos em 3q e 5p, e perdas em 3p e 9p. Essas alterações genéticas estão associadas a programas multigênicos ligados à diferenciação epitelial-mesenquimal, crescimento, integridade genômica, dano oxidativo, morte e inflamação.[4]

O perfil genético e molecular dos tumores é determinante para a caracterização fenotípica, prognóstico e resposta terapêutica. Alterações genéticas recorrentes, incluindo mutações e alterações epigenéticas, influenciam a ativação de vias de sinalização que regulam a proliferação celular, a invasividade, a metástase e a resistência a terapias.[4, 10]

Em termos de composição celular, podem exibir heterogeneidade significativa e a caracterização celular é fundamental para estabelecer o diagnóstico.[4] Para além disso, tal identificação pode ser um preditor importante de agressividade do tumor e resposta ao tratamento, assim como permite o desenvolvimento de terapias direcionadas.[11]

As opções terapêuticas variam conforme a localização anatômica do tumor, tipo histológico, extensão da lesão primária, comprometimento dos linfonodos cervicais, morbidade esperada e associada a cada modalidade de tratamento, condição clínica e opção do paciente.[12] Dentre as principais alternativas estão a ressecção cirúrgica, seguida ou não de radioterapia (RT), associada ou não a quimioterapia (QT).[4, 12] Tais tratamentos implicam em perdas estruturais e/ou declínios nas funções estomatognáticas por efeitos adversos dos tratamentos adjuvantes.[4]

O CCP é uma doença complexa que pode impactar significativamente na qualidade de vida dos pacientes. A perda da capacidade de se comunicar, de se alimentar e de interagir socialmente pode gerar grande sofrimento emocional e social.[5] Nesse contexto, a fonoaudiologia emerge como uma área crucial, oferecendo ferramentas e técnicas para reabilitar as funções comprometidas e promover a recuperação desses indivíduos restaurando assim a autonomia.[13]

2. FONOAUDIOLOGIA NO CCP

A avaliação fonoaudiológica realizada antes, durante e após o tratamento oncológico permite identificar as alterações nas funções estomatognáticas e as especificidades de cada caso.[13] Com base nos resultados da avaliação, é elaborado um plano de tratamento individualizado e personalizado, com o objetivo de restaurar ou otimizar as funções de deglutição, voz e mastigação, frequentemente afetadas pelos tratamentos oncológicos para CCP.[14]

A reabilitação fonoaudiológica busca, sobretudo, promover a segurança na alimentação, a inteligibilidade na comunicação e a reintegração social do paciente.[14]

Deste modo, surge uma preocupação com o tempo. O processo de reabilitação associado deve considerar a heterogeneidade nas taxas de sobrevida. A sobrevida global (SG) em 10 anos do câncer orofaríngeo p16-positivo apresenta o prognóstico mais favorável para 87% dos pacientes, seguido pelos cânceres na cavidade oral (69%), laringe (67%), orofaringe p16-negativa (56%) e hipofaringe (51%). Desta forma, quanto antes houver ganho funcional nas funções estomatognáticas, antes este indivíduo retorna para sua vida com independência, dignidade e prazer. De um modo geral, a reabilitação tende a ser tardia e com planejamento a longo prazo, principalmente se o tratamento oncológico envolver RT.[15]

A RT, apesar dos avanços tecnológicos, provoca alterações na configuração muscular, resultando em fibrose da musculatura esquelética, redução da sensibilidade, linfedema laríngeo, redução da elevação laríngea e prejuízo na proteção das vias aéreas.[16-18]

Estas alterações compõem um cenário por vezes crônico, desafiador e torna complexa a reabilitação. Também é comum observar hipossalivação e/ou aumento da viscosidade da saliva, que leva ao acúmulo de resíduos faríngeos (devido à dificuldade de clareamento) e/ou aspiração silente (durante ou após a deglutição).[18, 19]

Devido à complexidade das alterações, múltiplas abordagens são necessárias, como adaptações das consistências dietéticas, instrumentos utilizados, ritmo e volume de oferta, manobras de proteção e mudança da postura do paciente. Além disso, o treinamento muscular orientado pelo fonoaudiólogo é indicado para reduzir a fibrose muscular, melhorando a força laringofaríngea e a mobilidade durante a deglutição.[19]

A prática de exercícios profiláticos de deglutição em pacientes com CCP submetidos à RT demonstra potencial para prevenir ou atenuar a disfagia induzida pelo tratamento. No entanto, a heterogeneidade dos protocolos de exercícios, a falta de consenso sobre a melhor abordagem e a complexidade da adesão dos pacientes representam desafios para a implementação dessa estratégia na prática clínica.[20] A personalização dos programas de exercícios, considerando as características individuais de cada paciente, a utilização de exames objetivos como a videofluoroscopia e a colaboração entre diferentes profissionais da saúde podem contribuir para a otimização dos resultados da reabilitação.[21]

Em Fononcologia, recursos como a estimulação elétrica neuromuscular (EENM) e a fotobiomodulação (FBM), muitas vezes, carecem de maiores investigações para que se possa compreender a aplicabilidade, o tamanho do efeito e para garantir o uso seguro, dada a peculiaridade da população oncológica.[22]

2.1 Eletroestimulação na Fononcologia

A eletroestimulação é uma técnica utilizada desde 1980 para reabilitação muscular, porém a aplicabilidade para a disfagia induzida por RT ainda é controversa.[23] [24] Trata-se de um método não invasivo de aplicação da corrente elétrica que, por meio de agentes físicos, estimula respostas fisiológicas locais e/ou sistêmicas, a depender da especificidade da corrente elétrica e do objetivo terapêutico.[24]

Apesar do instrumento ter sido inserido na prática clínica fonoaudiológica nos últimos anos, sua utilização vem ganhando abrangência em virtude dos benefícios demonstrados por meio de evidência científica.[23]

O uso da eletroestimulação (e sua gama de parâmetros e possibilidades) visa, principalmente, acelerar o processo de reabilitação, o qual tende a ser prolongado em âmbito oncológico.[23] Entre suas funções encontram-se a estimulação sensorial e neuromuscular, que podem promover modificações na configuração e composição das fibras musculares.[25] Além disso, evidências demonstram aumento do tamanho e número de fibras musculares, maior recrutamento de unidades motoras, redução do edema e melhora da perfusão sanguínea dos tecidos.[25]

É importante lembrar que o benefício depende também da ação conjunta de outras técnicas já presentes no cotidiano do fonoaudiólogo e que o uso deste recurso em Fononcologia está condicionado à formação específica em Eletroterapia e uma base sólida de conhecimento acerca de anatomofisiologia,

fisiologia do exercício e oncologia. Esta tríade sustenta o uso seguro desta estratégia, tornando-a um complemento potencializador e não substituto.[23]

Assim como em outros aspectos que permeiam a atuação do Fonoaudiólogo, o uso da eletroterapia exige a individualização e a customização dos parâmetros. Para que o estímulo gere o efeito desejado deve-se levar em consideração alguns fatores como: tecido (músculos, glândulas, receptores sensoriais, dentre outros), limiar desejado (sensorial, sensório-motor e motor), tamanho da estrutura alvo, eletrodos, acessórios e tipo de corrente.[24] Além disso, existe a necessidade de configurar a frequência, largura de pulso, duração do estímulo, intensidade, dentre outros parâmetros a depender da corrente escolhida.[24]

Na região da cabeça e pescoço, a eletroestimulação possui algumas desvantagens, como a pobre reprodutibilidade da contração devido à variabilidade de posicionamento dos eletrodos e tolerância dos pacientes, proximidade das estruturas, inconveniência de utilizar múltiplos eletrodos em estruturas pequenas, maior tendência a alergias e irritações na pele quando comparado a outras regiões do corpo, fadiga precoce devido à alta demanda energética que deve ser acionada nos contextos funcionais.[26]

Desde a aprovação do *Food and Drug Administration* (FDA) em 2001[27] acerca do uso da eletroestimulação para reabilitação da disfagia, surgiram diversas evidências quanto ao uso do recurso, porém em oncologia o número é expressivamente menor quando comparado a outras patologias de base como o Acidente Vascular Cerebral (AVC), por exemplo.[28]

Na oncologia, a incorporação deste recurso no tratamento dos pacientes vem crescendo exponencialmente, porém ainda existe uma lacuna de conhecimento que vem sendo preenchida gradualmente pelas evidências.[23]

O número restrito de estudos pode estar associado à dificuldade em realizar ensaios clínicos adequadamente randomizados devido à diversidade dos tumores e tratamentos, o que, por vezes, impossibilita a homogeneidade da amostra. Atualmente, a heterogeneidade dos participantes e dos protocolos de intervenção reduz a qualidade da evidência e aumenta o risco de viés. Isso influencia diretamente na tomada de decisão do fonoaudiólogo quanto ao uso do recurso nesta população.[22, 23]

Quanto à disfagia, a eletroestimulação emerge como uma promissora estratégia para acelerar a recuperação funcional, especialmente quando induzida por RT. Estudos sugerem que a estimulação elétrica pode modular a expressão de fatores de crescimento, como o TGF-β1, reduzindo a fibrose muscular e promovendo a regeneração tecidual. Ao diminuir a deposição de colágeno e aumentar a expressão de queratina, a eletroestimulação contribui para a restauração da homeostase muscular, otimizando a deglutição.[29]

Um estudo avaliou o efeito da eletroestimulação em 60 pacientes com disfagia induzida pela radioterapia para tumor de nasofaringe e identificou melhora significativa na segurança e eficácia da deglutição quando comparado ao grupo que não utilizou a técnica[30] reforçando a ideia de que a eletroestimulação agrega benefício quando comparada à terapia isolada.[31]

Por meio de estímulo sensorial e motor, em pacientes com câncer de laringe, se identificou redução do grau da disfagia e dos episódios de penetração e/ou aspiração laringotraqueal principalmente para a consistência mel em um estudo brasileiro.[32] Tais efeitos foram obtidos com a estimulação sensorial e motora diretamente na musculatura supra e infrahióidea e 80Hz de frequência e 700uS de largura de pulso com variação de intensidade para atingir os limiares desejados.[32]

Tais evidências vão de encontro a um estudo de Langmore et al (2016), no qual não foi identificado efeito da eletroestimulação na deglutição de pacientes com efeitos tardios da RT.[33] No entanto, os participantes progrediram a consistência alimentar e tiveram influência positiva na qualidade de vida. O que permite a reflexão quanto à percepção do paciente frente ao estímulo de um equipamento e, diante do desejo de melhora, ter apresentado um efeito placebo.[33]

Salienta-se que a maior parte das evidências atuais é baseada na análise do efeito da eletroestimulação com frequências altas (70-80Hz).[23] Há uma tendência de frequências abaixo de 40-50Hz recrutarem um maior número de fibras de contração lenta (tipo I), que são mais resistentes à fadiga, enquanto frequências mais altas recrutam fibras de contração mais rápida (tipo II) e menos resistentes à fadiga. [34]

A possibilidade de variar as configurações de acordo com o objetivo desejado é importante e favorece a reabilitação, pois os alvos terapêuticos mudam ao longo do tratamento de acordo com o desempenho do paciente, e devem ser adaptadas às particularidades de cada indivíduo.[34]

Já em relação à hipossalivação, a aplicação da corrente *Transcutaneous Electric Nerve Stimulation* (TENS) com frequência de 50 Hz e largura de pulso de 250 μs, diretamente sobre as glândulas salivares, demonstra um potencial significativo para aumento do fluxo salivar em pacientes com câncer de cabeça e pescoço submetidos à RT.[22] O fluxo salivar, uma vez aumentado, não regrediu até o final dos 180 dias de acompanhamento dos participantes, esse fato reforça e ideia de recuperação, mesmo que parcial, da função glandular.[22]

Embora o mecanismo exato ainda não esteja completamente elucidado, acredita-se que a estimulação do nervo auriculotemporal e a modulação da atividade parassimpática contribuam para o aumento do fluxo salivar.[35] Estudos clínicos têm demonstrado que a TENS pode induzir um aumento significativo e duradouro da produção salivar, melhorando significativamente a qualidade de vida desses pacientes.[22 36]

Uma pesquisa identificou redução de cerca de 50,0% da quantidade de saliva já em 10Gy, na segunda semana de RT, sendo que já havia queixa de xerostomia e diminuição do paladar além do aumento da viscosidade da saliva que a tornava mais difícil de deglutir e gerenciar.[36]

Em relação aos parâmetros da TENS, observa-se que a estimulação realizada diretamente sobre as glândulas salivares com frequência de 50Hz e 250 μS de largura de pulso[22] tem demonstrado aumento significativo do fluxo salivar em protocolos que envolvem 8 sessões por mês (2x na semana).[22]

Mesmo com escassez de literatura, comparado a outras patologias, a eletroestimulação parece ter um efeito positivo sobre as funções estomatognáticas, ainda que heterogêneo e, portanto, sua aplicação requer maior investigação. Dada a peculiaridade dos pacientes oncológicos, um outro fator que pode contribuir para o uso, ainda limitado, deste recurso em Fononcologia é o desconhecimento quanto ao mecanismo de ação da corrente elétrica na célula tumoral, o que traz à tona reflexões acerca da segurança da aplicação na região de tratamento.

2.1.2. Segurança do uso da eletroestimulação no CCP

Uma possibilidade levantada é que a estimulação elétrica poderia ter um efeito direto sobre a formação de novos vasos sanguíneos e esse processo, conhecido como angiogênese, é um evento chave no desenvolvimento, cicatrização de feridas e formação de tumor. A célula tumoral se prolifera rapidamente e de forma descontrolada, o que causa alterações significativas na carga da superfície celular e torna o tumor mais polarizado em relação às regiões circundantes.[37]

Pensando neste mecanismo, sabe-se que a eletroestimulação não tem capacidade de modificar o DNA das células e, além disso, pesquisas indicam que a corrente elétrica equilibra a polarização ao redor das células tumorais o que favorece, inclusive, o tratamento oncológico realizado na região, embora esse não seja do âmbito fonoaudiológico.[12]

O receio de promover crescimento desordenado das células tumorais, recorrência de doença e/ou metástases diante do estímulo elétrico fez com que por muitos anos se evitasse o uso desse recurso em regiões de tratamento oncológico.[38]

Linkov (2012)[39] investigou o impacto da estimulação elétrica em modelo animal com carcinoma de células escamosas e percebeu que, mesmo com a aplicação sobre a lesão, não houve mudança no tamanho do tumor e/ou outros efeitos adversos. Este achado possibilita justificar que a eletroestimulação no cenário da malignidade não aumenta a atividade tumoral.

Importante ressaltar que se deve avaliar cautelosamente a indicação/finalidade para a qual será utilizada a eletroestimulação, além do momento que aquele paciente se encontra, pois o próprio tumor e os tratamentos podem provocar dor e desconforto e, inclusive, dependendo da região, exacerbar essa sensação.

Antes de optar pela técnica deve-se aguardar a completa reconstrução da pele, que sofre alteração durante a RT, pois a aderência de eletrodos e fitas adesivas pode prejudicar o processo de recuperação. Em nossa experiência é comum, durante a aplicação da eletroestimulação, identificar boa tolerância dos pacientes a altas intensidades. No entanto, pode ocorrer: predomínio sensorial unilateral - em função do método e plano radioterápico, dose e região de tratamento -, sensação e visualização de tremor facial, aquecimento da região, formigamento e sensação dolorosa - causada pela contração muscular -, havendo remissão deles após o encerramento da sessão.[26]

De um modo geral, a técnica é de boa tolerância, não havendo referências a efeitos adversos causados pela utilização da eletroestimulação[26]. A aplicação da eletroestimulação na região da cabeça e pescoço, quando o câncer é localizado neste sítio, exige conhecimentos que vão além da formação em eletroestimulação e que são particulares à atuação em Fononcologia.

A eletroestimulação neuromuscular tem se mostrado uma promissora ferramenta na reabilitação fonoaudiológica em pacientes oncológicos. Embora os mecanismos de ação ainda não estejam completamente elucidados, a modulação da excitabilidade neuronal e a ativação de vias metabólicas relacionadas à regeneração muscular e glandular são hipóteses plausíveis. No entanto, a heterogeneidade dos protocolos de tratamento, incluindo parâmetros de estimulação, dificulta a definição de diretrizes clínicas claras.[23] A personalização do tratamento, considerando as características individuais de cada paciente e o estágio da doença, é fundamental para o uso seguro do recurso.

2.2 Fotobiomodulação na Fononcologia

O termo LASER, acrômio da expressão inglesa *Light Amplification by Stimulated Emission of Radiation* (Amplificação da luz por emissão estimulada de radiação, em português) consiste em uma radiação eletromagnética, unidirecional, monocromática, com feixe estreito, propagação paralela (colimação) e com as ondas dos fótons em fase (coerência).[40, 41]

A terapia com LASER de baixa intensidade (LBI) é uma das modalidades dentro da FBM cujo comprimento de onda varia de luz vermelha (600-700 nanômetros [nm]) ao infravermelho (700-1000 nm), e a densidade de energia é medida em Joules (J/cm2)[40]. Para ser classificado como LBI, o equipamento deve apresentar potência inferior a 500 mW, podendo operar em modo contínuo ou pulsado, com dose máxima de 35 J/cm^3. Essa classificação é baseada na capacidade do LBI de modular processos biológicos por meio da bioestimulação ou bioinibição, dependendo do protocolo de aplicação.[40, 41].

Os resultados da FBM estão associados à sua absorção nas células através de fotorreceptores, facilitando o transporte de cálcio e a respiração mitocondrial, o que resulta em reparo e regeneração tecidual mais acelerado.[40, 42] Além disso, provoca redução do processo inflamatório e alívio do quadro álgico.[41] Pode também aumentar a expressão de citocinas anti-inflamatórias e diminuir as pró-inflamatórias, principalmente por estimular as células imunológicas no local da lesão.[42]

A eficácia da FBM no tecido alvo depende dos parâmetros utilizados, como fonte de luz, comprimento de onda, densidade de energia, estrutura do pulso de luz e duração da aplicação do laser [41]. Essas respostas terapêuticas da FBM foram amplamente atribuídas a três principais vias fotorresponsivas: homeostase de energia mitocondrial, receptores ou transportadores de membrana celular e ativação do fator de crescimento extracelular (TGF-β1) [40].

Da Silva et al. (2023)[43] analisou os efeitos FBM em diferentes níveis biológicos, incluindo molecular, celular e sistêmico. Essa interação promove a produção de moléculas sinalizadoras, as quais, por sua vez, desencadeiam uma série de eventos moleculares, incluindo a ativação de fatores de transcrição e a produção de moléculas efetoras. Em nível celular, esses eventos moleculares induzem uma variedade de respostas, tais como proliferação celular, migração celular, diferenciação celular e apoptose.

As mitocôndrias contêm cromóforos que absorvem fótons da FBM, sendo a enzima Citocromo C oxidase o principal componente celular que absorve a luz vermelha. Localizada na unidade IV da cadeia respiratória mitocondrial, essa absorção resulta na atividade de diversas moléculas como óxido nítrico (NO), ATP, íons cálcio (Ca^{2+}), EROs e outras moléculas sinalizadoras. A FBM estimula os elétrons nos cromóforos, promovendo a glicólise e a produção de ATP. Além disso, ativa vários fatores de transcrição e incentiva a produção de espécies reativas de oxigênio[41].

Em um nível mais amplo, os efeitos da FBM manifestam-se em alterações fisiológicas sistêmicas, como a modulação do processo inflamatório, a promoção da reparação tecidual e da cicatrização de feridas, a redução do edema e da dor, bem como a melhora do desempenho muscular.[43]

No âmbito da fonoaudiologia, tal recurso vem ganhando espaço em diversas áreas de atuação, ainda que careça de evidências científicas robustas para nortear o clínico quanto às indicações e o uso seguro[44]. Em 2021, o Conselho Federal de Fonoaudiologia (CFFa), por meio de resolução específica, normatizou a utilização da FBM como recurso terapêutico complementar aos procedimentos clínicos fonoaudiológicos tradicionais. A resolução autoriza a aplicação da FBM nas modalidades direta, indireta, adaptada ou transdérmica, permitindo sua utilização em intervenções sistêmicas.[45]

Os fonoaudiólogos participantes de uma pesquisa indicaram que a técnica de irradiação mais utilizada na área da voz consiste na aplicação direta na região laríngea, precedendo a realização dos exercícios vocais. O comprimento de onda predominantemente utilizado situa-se entre 808 e 830 nanômetros (nm). A dosagem da FBM variou conforme o objetivo terapêutico, sendo empregada uma dose de 3 a 5 Joules (J) em casos de processos inflamatórios nas pregas vocais e uma dose de 6 a 9 Joules para o aperfeiçoamento ou condicionamento vocal.[46]

Considerando-se a escassez de estudos sobre a efetividade da FBM no tratamento fonoaudiológico, a regulamentação é necessária para legitimar e nortear o uso em caráter exploratório, com a mínima garantia de não maleficência ao paciente. O uso da FBM pode otimizar a terapia na clínica vocal, motricidade orofacial, disfagia, paladar e xerostomia.[45, 46]

A despeito de um número crescente de estudos investigando a aplicabilidade da FBM no tratamento de disfonia e na otimização da performance vocal em indivíduos saudáveis, a literatura científica ainda não dispõe de um corpo de evidências suficientemente robusto para sustentar recomendações clínicas definitivas. Embora haja um esforço crescente na condução de pesquisas nessa área, os estudos disponíveis até o momento são incipientes e apresentam limitações metodológicas que impedem a generalização dos resultados.

Uma série de casos revela o efeito da FBM com 635 nm e uma dose de 3 J/cm2 sobre complicações orais advindas da RT. Para disfagia e xerostomia, foram realizadas 5 aplicações com intervalo de 24h e para paladar e, para odinofagia, 10 sessões. Ainda que se trate de uma série de casos heterogênea, todos participantes apresentaram melhora nas suas disfunções.[47] Ressalta-se o fato de que em se tratando de desempenho muscular para disfagia, a conduta sempre será a complementação com a tecnologia e não o uso isolado como sugerido nesta pesquisa.

Os resultados de Castro et al. (2020) em modelos animais sugerem que a FBM pode modular o metabolismo energético muscular através da ativação de vias de sinalização específicas.[48] Embora estudos clínicos em humanos sejam necessários para confirmar esses achados, os resultados pré-clínicos são promissores e indicam um potencial terapêutico para a FBM no tratamento da sarcopenia e outras condições musculoesqueléticas.[48, 49] A associação da técnica com o exercício físico, como demonstrado por Kumar et al. (2024), pode potencializar os efeitos benéficos dessa terapia, oferecendo uma abordagem multifatorial para a reabilitação muscular.[49]

Para trismo induzido por RT, também é possível encontrar série de casos em que os participantes apresentaram maior ganho na abertura de boca quando associaram a terapia manual orofacial a FBM quando comparado àqueles que realizaram somente a terapia isoladamente (Artigo 2). Tal diferença se deu principalmente devido a tolerância às técnicas que foi maior nos indivíduos submetidos a FBM.[50]

Apesar dos avanços na pesquisa sobre a FBM, ainda não há evidências suficientes para garantir a segurança de sua aplicação em pacientes com CCP submetidos a tratamento conservador ou com margens cirúrgicas comprometidas. A ausência de diretrizes específicas dificulta a identificação de pacientes elegíveis para esse recurso terapêutico, principalmente considerando a necessidade de direcionar o tratamento para regiões com histórico de mutação.[51]

2.2.1 Segurança do uso da FBM no CCP

O artigo publicado por S. Sonis [51] , intitulado “O impacto da FBM na resposta tumoral à radiação pode ser afetado pela heterogeneidade tumoral?”, constitui uma relevante contribuição para o campo em crescimento da pesquisa sobre FBM, com foco em medidas *in vitro* e moleculares. Este comentário levanta uma questão pertinente sobre a possível variação na resposta tumoral à FBM, a qual pode estar associada à heterogeneidade tumoral. Embora essa questão seja de grande relevância, ela se relaciona sobretudo à segurança do uso da FBM no contexto do cuidado ao paciente oncológico.

Dada a heterogeneidade genômica dos tumores, parece provável que o efeito da FBM sobre o comportamento tumoral, assim como ocorre com medicamentos ou agentes biológicos, não seja uniforme. Isso pode fornecer uma explicação para algumas das contradições observadas em estudos in vitro com culturas celulares relatados na literatura. Mesmo tumores com caracterização histológica semelhante (como CEC) apresentam variações, sendo que 35% deles expressam desregulações na via PI3K, um alvo comum da FBM.[52] É fundamental reconhecer as limitações dos estudos in vitro em oncologia, em comparação com a abordagem sistêmica e os resultados clínicos observados nos ensaios.

Comparativamente, pesquisas anteriores mostraram efeitos mistos da FBM no CEC, conforme disposto na tabela 1. Alguns estudos relataram inibição da proliferação de células tumorais, enquanto outros observaram aumento da mesma.[53, 54] Estudos *in vivo* também mostraram resultados conflitantes em relação à progressão do tumor.[55,56] Vamos refletir um pouco sobre esses estudos a seguir.

O efeito da FBM na radiosensibilização foi abordado em um estudo *in vitro* com CEC (SCC9, Cal-27, A431 e HaCaT) antes da RT. As células foram submetidas à FBM (660 nm, 300 J/cm²), sendo avaliada a viabilidade celular, capacidade de formação de colônias, migração e níveis de espécies reativas de oxigênio (ROS). Os resultados demonstraram que a pré-exposição à FBM potencializou os efeitos da radioterapia, resultando em uma redução significativa na proliferação e sobrevivência celular, bem como em um aumento da apoptose e da inibiação da migração celular. O achado de um aumento nos níveis de ROS sugere que o mecanismo de radiosensibilização pode estar relacionado ao estresse oxidativo induzido pela FBM.[57]

O estudo de Sperandio et al. (2013)[58] investigou os efeitos da terapia a laser de baixa intensidade na agressividade de linhagens celulares displásicas e de cavidade oral, com foco na via de sinalização Akt/mTOR. A pesquisa demonstrou que FBM pode aumentar a agressividade das células displásicas orais (DOK) e das células cancerígenas orais (SCC9 e SCC25). Foi observado um aumento na expressão de proteínas associadas à progressão e invasão celular, como pAkt, pS6 e Ciclina D1 e essa modulação levou a um fenótipo celular mais agressivo. Essas descobertas sugerem que, embora a FBM tenha aplicações terapêuticas, seu uso em pacientes com lesões orais displásicas ou malignas deve ser abordado com cautela devido ao potencial de aumento da agressividade do tumor.

Rhee et al. (2023) demonstraram a FBM com comprimento de onda de 650 nm e dose de 30 J/cm² acelerou o crescimento tumoral em um modelo ortotópico de câncer de tireoide anaplásico em camundongos. A análise imuno-histoquímica revelou um aumento significativo na expressão de HIF-1α e p-Akt nas células tumorais dos animais tratados com FBM, sugerindo a ativação da via Akt/HIF-1α. Concomitantemente, observou-se uma diminuição na expressão de TGF-β1, um potente supressor tumoral. Esses resultados indicam que a FBM por meio da laserterapia de baixa intensidade ao modular negativamente a via TGF-β1 e ativar a via Akt/HIF-1α, promove a proliferação celular e a resistência à apoptose em células de câncer de tireoide anaplásico.[59]

Em contraponto Ibarra et al. (2020)[60] examinaram os efeitos da FBM na viabilidade celular e no fenótipo das células-tronco cancerígenas (CTC) no CEC oral. Os resultados indicaram que uma única aplicação com 3 J/cm aumentou a viabilidade celular, enquanto as aplicações diárias com 3 J/cm e 6 J/cm diminuíram. É importante ressaltar que as aplicações diárias não promoveram a autorrenovação do CTC nem alteraram o fenótipo CD44/ESA. Além disso, houve diminuição no número de esferas e na expressão do gene BMI1 relacionado ao CSC após FBM diária com 6 J/cm que pode estar associado à resistência terapêutica e à agressividade do tumor.

Antunes et al (2017)[61] apresentaram evidências clínicas preliminares sugerindo que a FBM pode modular a resposta tumoral em pacientes com câncer de cabeça e pescoço. Ao aplicar FBM com parâmetros específicos (660 nm, 100 mW, 4 J/cm²) em 94 pacientes, os autores observaram uma melhora significativa na sobrevida livre de progressão e na resposta completa ao tratamento.

A aplicação da FBM exige uma compreensão detalhada dos parâmetros de luz (como comprimento de onda, fluência, energia, tempo e cronograma: dose por fração, número de sessões por semana e número total de sessões), bem como o reconhecimento da importância dos estudos in vivo em animais e seres humanos e in vitro com culturas celulares.[55]

Tal importância é justificada no estudo de Pinheiro et al (2002)[62] onde houve influência do comprimento de onda sobre a proliferação celular de H.Ep.2

de tumores laríngeos e revelou que o comprimento de onda 635 nm, em uma faixa de dosagem entre 0,04 e 0,48 J/cm², não induziu um aumento significativo na taxa de proliferação celular. Contudo, a utilização de 670 nm, com doses variando entre 0,05 e 0,6 J/cm², demonstrou uma tendência de aumento na proliferação celular, sendo este efeito mais evidente a partir de doses de 0,08 J/cm². A comparação entre os grupos irradiados com os diferentes comprimentos de onda sugere que a irrradiação de 670 nm pode estimular, ainda mais, a proliferação celular.

A irradiação de células cancerígenas *in vitro* também resultou em uma atividade proliferativa aumentada com uma densidade de energia relativamente baixa, em 1 J/cm² com o comprimento de onda 830 nm. Tal efeito, conforme os autores, foi associado a níveis elevados de expressão de proteína pAKT, pERK e Ki67 e progressão do ciclo celular. A exposição a uma densidade de energia de 2 J/cm² resultou em um ligeiro aumento na proliferação de células cancerígenas, ainda que sem significância estatística.[63]

Tabela 1.Revisão de estudos que utilizaram a FBM em carcinoma de células escamosas

Estudo	Objetivo	Comprimento de onda (nm)	Dose (J/cm^2)	Número de Aplicações	Linhagem Celular ou Região tumoral	Resultados Principais
Antunes et al. (2017)[61]	Avaliar a sobrevida global, livre de doença e livre de progressão dos pacientes com CCP submetidos a FBM	660	1 e 4	Aplicações diárias, 5x na semana com duração média de 45,7 dias.	Câncer de cabeça e pescoço (orofaringe, nasofaringe, hipofaringe)	Melhora significativa na resposta completa ao tratamento, sobrevivência livre de progressão e tendência para melhor sobrevivência geral
Bamps et al. (2016)[63]	Avaliar o efeito biostimulante da FBM em células tumorais de CCEo	830	0, 1 ou 2	-	SCC15, SQD9, SCC61, células epiteliais de tonsilas	Aumento da proliferação em células de HNSCC com 1 J/cm², sem efeito nas células epiteliais normais. Aumento de pAKT, pERK, Ki67 e progressão do ciclo celular.
Barasch et al. (2020)[55]	Investigar o efeito da PBM em um modelo ortotópico de CEC oral	660 ou 660 + 850	18.4 ou 3.4	5 ou 1	Cal-33	Não houve proteção do tumor contra os efeitos letais da RT.
Da Silva et al. (2020)[43]	Avaliar os efeitos da FBM na proliferação e apoptose de CEC oral	660	5	3	SCC-9	Aumento da proliferação celular em doses menores; inibição da apoptose.
Ibarra et al. (2020) [60]	Avaliar o efeito da FBM na viabilidade celular e no fenótipo de células-tronco cancerígenas em CCEo	660	3 e 6	Uma única aplicação e aplicação diária por três dias consecutivos	SCC9 (ATCC), CA1, LUC4	Aumento da viabilidade celular com FBM única de 3 J/cm². Redução da viabilidade celular com FBM diária de 3 ou 6 J/cm². Redução do número de esferas e expressão de BMI1 com FBM diária de 6 J/cm².
Martins et al. (2020)[64]	Explorar os efeitos da FBM no CEC com base na proliferação celular, migração e sobrevivência de células	660	4	Três sessões de irradiações foram aplicadas com intervalos de 6 h	HN6 e HN13	Diminuição na proliferação celular de uma linhagem celular (HN6) quando cultivada sob condições de estresse nutricional. Não houve diferenças significativas entre os grupos controle e

	tumorais e sua população de células-tronco cancerígenas					FBM em relação à migração celular, sobrevivência e porcentagem de CSC
Pinheiro et al. (2002)[62]	Avaliar o efeito da FBM em CEC de laringe	635 e 670	04 - 0,48	7 dias consecutivos	H.Ep.2	Aumento da proliferação com 670 nm quando comparada às células controle e irradiadas com 635 nm.
Ravera et al. (2023) [65]	Avaliar a segurança dos parâmetros de tratamento de FBM na proliferação ou sobrevivência celular. Investigar quaisquer efeitos anticâncer dos tratamentos de fBM. Viabilidade celular, metabolismo, estresse oxidativo e marcadores de expressão pró e antiapoptótica foram investigados.	810	0, 0.25, 0.50, 0.75, 1.00, 1.25	Única	OHSU-974 FAcorr	Afetou a fosforilação oxidativa, causou um switch metabólico para a glicólise anaeróbica, reduziu a atividade da catalase, desequilibrou a produção de estresse oxidativo e as defesas antioxidantes, podendo estimular as vias celulares pró-apoptóticas
Schalch et al. (2019)[54]	Investigar o efeito da FBM na proliferação celular	660 e 780	1.4 , 2.7 2.7 , 5.4 8.1 , 1.4	1	SCC9	Irradiação com 780 nm (70 mW, 4 J/cm 2) foi mais segura e levou à redução da viabilidade celular, à indução de apoptose e à redução da capacidade de migração das células tumorais.
Sperandio et al. (2017) [58]	Investigar o efeito da FBM na agressividade de células displásicas e cancerígenas orais	660 ou 780	2.05, 3.07 ou 6.15	Uma aplicação em cada dose	DOK, SCC9, SCC25	Aumento da expressão de pAkt, pS6 e Cyclin D1, produção de uma isoforma agressiva de Hsp90, aumento da agressividade celular.
Tabosa ATL et al. (2022)[57]	Investigar o efeito da FBM antes da radioterapia em células de OSCC	660	300	1	SCC9, Cal-27, A431, HaCaT	Radiosensibilização das células de OSCC, redução da proliferação, sobrevivência clonogênica, migração e aumento da morte celular e ROS

FBM: fotobiomodulação; CCEo: carcinoma de células escamosas orais; CEC: carcinoma de células escamosas; MMPs: metaloproteinases de matriz; ROS: espécies reativas de oxigênio; J: joule; - não consta a informação; CSC: células tronco cancerígenas

Essas observações *in vitro* destacam a importância da dosimetria e da definição rigorosa dos parâmetros da FBM para uso clínico. Contudo, os dados *in vitro* parecem confirmar a existência de uma variabilidade na resposta à FBM entre diferentes linhagens celulares, e até mesmo dentro da mesma linhagem, dependendo das condições locais ou do cronograma de tratamento[51].

Esses resultados indicam que a resposta ao FBM não é uniforme entre os diferentes tipos tumorais, destacando a necessidade de estudos *in vivo* adicionais e a personalização da terapia com base na caracterização molecular e genômica das malignidades antes do tratamento. Em se tratando do uso seguro do recurso, a abordagem padronizada para aplicação de FBM em oncologia não é a mais adequada e em se tratando de segurança a aplicação direta sobre células tumorais é questionável.[51]

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2 OBJETIVOS

Este estudo tem como objetivo contribuir para o avanço da reabilitação fonoaudiológica no câncer de cabeça e pescoço, fortalecendo a base de evidências científicas para o uso seguro de dispositivos terapêuticos como eletroestimulação e fotobiomodulação na prática clínica.

Os objetivos específicos incluíram:

1) Avaliar os efeitos da eletroestimulação e fotobiomodulação na reabilitação de efeitos adversos da radioterapia para câncer de cabeça e pescoço.
    a. Artigo 1: Electrical Stimulation for Treatment of Dysphagia Post Head Neck Cancer: A Systematic Review and Meta-Analysis.
    b. Artigo 2: Orofacial Myofunctional Therapy with and without Photobiomodulation in the Rehabilitation of Radiation-Induced Trismus: Case Series.
2) Avaliar os efeitos da aplicação da fotobiomodulação em carcinoma de células escamosas.
    a. Artigo 3: Efeito da FBM na viabilidade e proliferação celular de carcinoma de células escamosas.

3 ARTIGO 1

Electrical Stimulation for treatment of dysphagia post head neck cancer: A systematic Review and Meta-Analysis

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THIEME OPEN ACCESS

Systematic Review 339

Electrical Stimulation for Treatment of Dysphagia Post Head Neck Cancer: A Systematic Review and Meta-Analysis

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Abstract

Introduction Dysphagia induced by radiotherapy in the head and neck region comprises a challenging scenario and sometimes difficult rehabilitation due to the severity of the adverse effects. Some resources such as electrical stimulation have emerged as an alternative to complement the therapeutic process, but there is still no consensus on its use.

Objective The purpose of the present study was to evaluate, through a meta-analysis, the effect of electrical stimulation on the rehabilitation of dysphagia generated after head and neck cancer treatment.

Data Synthesis Four randomized controlled trials with a total of 146 participants were included. The age of the participants was 58.37 ± 1.8 years old and there was a predominance of males. The time to start the intervention ranged from 50.96 ± 40.12 months after cancer treatment. The intervention showed great heterogeneity regarding the positioning of the electrodes, parameters, duration of the stimulus, number of sessions, and intensity. No difference was identified in the following aspects: oral transit time, hyoid elevation, penetration and/or aspiration after electrostimulation. The quality of the evidence ranged from very low to moderate and high risk of bias.

Conclusion In this meta-analysis, we found weak evidence for small and moderate swallowing benefits in patients after radiotherapy for head and neck cancer in short-term clinical trials.

Keywords
- electrical stimulation
- dysphagia
- head and neck neoplasms
- rehabilitation

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Introduction

Dysphagia is a highly prevalent symptom in head neck cancer (HNC).[1] It is estimated that between 31 and 79% of patients with HNC experience swallowing disorders after surgery and radiotherapy (RT), two common modalities for HNC treatment.[2] Regardless of the etiology, dysphagia causes negative impacts on respiratory and nutritional functions, leading to an increase in the rate of aspiration pneumonia, malnutrition, and dehydration problems with impairment to health and quality of life (QOL).[3]

Surgery compromises swallowing due to the extension of the procedures and their reconstructions, which often leads to major structural damage and tissue loss.[4] Radiotherapy (RT), despite technology advances, causes changes in muscle configuration and muscle fibrosis, reduces sensitivity, contributes to laryngeal edema, reduces laryngeal elevation, and impairs airway protection.[5–7] These associated factors often cause chronic dysphagia, which makes it difficult to rehabilitate and due to the predominantly irreversible nature of these changes, many patients still have swallowing difficulties for years after the end of treatment.[8]

It is also commonly observed, among other clinical signs and symptoms, hyposalivation and/or increased salivary viscosity[9] that lead to the accumulation of pharyngeal residues (due to the difficulty of cleaning) and silent aspiration (during or after swallowing).[5–8] Hyposalivation also requires attention as it impairs the preparation of the food bolus and a tendency to food residue in the pharynx, which, in addition to discomfort, impairs the QOL of patients.

Hyposalivation and radioinduced fibrosis are two major challenges in daily practice. This requires a multiple approach, with adaptation of food consistency and utensils used, rhythm and volume of each portion, airway protection maneuvers and posture change. In addition, muscle training guided by the speech therapist is indicated to reduce fibrosis, improving laryngopharyngeal strength and mobility during swallowing.[10,11]

There is a large number of publications available in the literature that address interventions to prevent or rehabilitate muscle dysfunctions related to dysphagia.[12–14] Some studies evaluate therapeutic interventions that start before cancer treatment, others during, and some in the late phase after the end of antineoplastic treatment.[15,16] Apparently, there is no consensus on which intervention should be used as a care routine, not even on the ideal start time.

More recently, some instruments, such as neuromuscular electrical stimulation (NMES), have emerged to streamline the swallowing rehabilitation process.[16–19] One of the hypotheses that tries to explain the beneficial effects of electrical stimulation on muscle preservation involves reducing the production of TGF-β1, considered an important marker of the degree of severity of muscle fibrosis.[20,21]

There is evidence that NMES may promote improvement in muscle homeostasis, including changes in the type of muscle fiber.[21] After RT, the skeletal musculature of the head and neck region tends to change its muscular configuration, with a predominance of type 1 muscle fibers.[22] Type 1 muscle fibers are responsible for slow contractions and are more resistant to fatigue; however, when it comes to swallowing, it is necessary to have a muscular balance so that, in addition to resistance, there is adequate strength and hyolaryngeal mobility.[23] In this scenario, possibly, electrostimulation would enhance muscle regeneration if associated with exercises.

Some studies show potential in the technique for recovery of salivary flow,[24,25] and for dysphagia several methodologies and protocols are suggesting a lack of consensus.[21] In addition, there is still a hesitation to apply electrical stimulation on areas where cancer treatment has been carried out. This hesitancy may be related to the hypothesis that this technique could stimulate the growth or proliferation of cancer cells after treatment. Despite that, there is no published research that validates this apprehension as a scientific fact. On the other hand, it has already been demonstrated in some studies that electrical stimulation shows no positive correlation to tumorigenesis.[26] This uncertain paradigm is delaying the rehabilitation process by not having NMES being used earlier on patients' treatment, which could possibly prevent chronic effects on swallowing.[26]

This fact makes it difficult for the therapist to define whether to use (or not) this resource. Therefore, the purpose of the present study was to evaluate, through a meta-analysis, the effect of electrical stimulation on the rehabilitation of dysphagia generated after HNC treatment.

The present review follows the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) guidelines[27] and, subsequently, it was registered in the International Prospective Register of Systematic Reviews (PROSPERO), with the following identification: CRD42020200248.

Methods

Clinical trials using NMES for rehabilitation of dysphagia in all languages were included when according to the characteristics of the participants (a): adults ($\geq$ 18 years old) who underwent RT for the treatment of head and neck tumors, with or without surgery, with any stage or severity of dysphagia objectively diagnosed by tests such as videofluoroscopy of swallowing or endoscopic evaluation of swallowing with fiber optics, the result of these tests being concluded with reliable validated measures, such as the Penetration-Aspiration Scale (PAS).[28]

Proposed interventions (b): electrical stimulation alone or electrical stimulation with concomitant exercises. There was no restriction for the electrical stimulation protocol. Intervention effectiveness assessment and outcome measures (c): intervention effectiveness was classified according to swallowing function (degree of dysphagia based on objective examinations and validated scales). All validated quantitative scores measuring swallowing function in patients with dysphagia were accepted. Study types (d): Clinical trials and quasi-clinical trials examining the effect of electrical stimulation alone or electrical stimulation performed during exercise treatment for dysphagia were included. Case reports and systematic reviews were excluded.

Search Strategy

A comprehensive electronic search strategy of MEDLINE/PubMed, Embase, the Cochrane Central Register of Controlled Trials (CENTRAL), Scielo, and PEDro was performed from their earliest record to September, 2021. The search strategies can be seen in the ►**Supplementary Material Appendix 1**.

Study Selection

Two authors independently scanned the titles and abstracts, excluding obviously irrelevant studies. The full text of relevant articles was evaluated according to prespecified eligibility criteria. Disagreements were resolved through discussion with the corresponding author.

Data Extraction

Two reviewers independently extracted data using a predefined data recording form and discussed disagreements by consensus. The following data were extracted: details of the study design, patient characteristics (etiology, number of patients, age, sex), intervention protocol (frequency, intensity, duration, and stimulation setting) and control group (swallowing management, exercises, sham), as well as swallowing function outcomes and assessment timing. The means and standard deviations (SDs) of change scores (change from baseline) were extracted. When data were not reported, the posttreatment mean and SD were extracted. When articles only provided the median and quartiles, the mean and SD were estimated. If important data were not available, attempts were made to contact the author by e-mail. When the authors did not respond to requests, the study was excluded.

Statistical Analysis

The results of four studies were statistically compiled in meta-analyzes for four outcomes of interest: laryngeal elevation, laryngeal anteriorization, QOL and Penetration-Aspiration Scale (PAS).[27] Since the studies measured the same outcomes using different methods, a data compilation performed on standardized mean difference (SMD) calculates in Hedges g (M1-M2/s), where is the grouped SD; M1 is the mean of the intervention group and M2 is the mean of the control group),[29] a measure of the size of the effect in which each addition of the unit to the final result indicates that the groups differ by 1 SD. The interpretation of the findings in SMD in the present study concluded the traditional cutoff points: 0.2 represents a small effect size; 0.5 represents a medium effect size; and 0.8 represents a large effect size.[30]

All analyzes were conducted using the method of inverse of variances and the DerSimonian and Laird estimator for $\tau 2$ in a random effects model, which allows to statistically incorporate the variability between studies in the final effect estimate. For continuous outcomes, data from change in relation to the baseline were preferred to deal with a more efficient analysis that confers greater statistical power when compared to the analysis of post-treatment values only. The data for each group were computed to calculate the effect size in Hedges g, presented in SMD and 95% confidence interval (CI). To include results from studies that did not report data on mean and SD in meta-analyzes, conversion of data from median to mean and interquartile range to SD was performed. In some circumstances, primary studies reported results without a measure of dispersion for values of change from baseline, such as SD or standard error. In these cases, the standard deviation was imputed from the calculation of a correlation coefficient using the SDs known from the other primary studies included in the systematic review, strictly according to Cochrane's guidance (Chapter 16.1.3.2). All analyzes were conducted using RStudio software version 1.3.1093 (R Foundation, Vienna, Austria) with the 'meta' package in R language (version 4.0.3).

Risk of Bias

Two authors (Sugueno L. A. and Macagnan F. E.) assessed the risk of bias in individual studies, independently, using the recent modified Cochrane Collaboration's Risk of Bias assessment tool (RoB 2.0) for randomized clinical trials (RCTs).[31] ROBINS-I: a tool for assessing risk of bias in non-randomized studies of interventions (NRSIs) was used for only included NRSI [32]

Each study was evaluated in relation to the following five domains: 1) bias due to the randomization process; 2) bias due to deviations from the intended interventions; 3) bias due to the lack of outcome data; 4) bias in measuring the result; 5) bias in the selection of the reported result. The risk of bias judgments were: a) low risk of bias; b) some concerns; and c) high risk of bias. If an individual domain was considered at a certain level of risk of bias, the overall risk of bias for that study was considered to be at least as severe.

Any disagreements were resolved by discussion with other three authors (Sugueno L. A., Macagnan F. E. and Zanella V. G.). An online web app robin was used to visualizing the risk of bias assessments as "traffic light" plots of the domain-level judgment for each individual result; and "weighted bar" plots of the distribution of risk-of-bias judgment within each bias domain.

Evaluation of Heterogeneity

Statistical heterogeneity was assessed quantitatively using the I^2 statistic, in addition to the $\chi 2$ significance test. The interpretation of statistical heterogeneity followed Cochrane's guidelines. An I^2 up to 40% represents negligible heterogeneity; 30 to 60% represents moderate heterogeneity; 50 to 90% represents substantial heterogeneity; and 75 to 100% represents built-in heterogeneity.

Evaluation of the Quality of Evidence by the Grade System

The overall quality of the evidence was assessed according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.[33] For each outcome, the quality of the evidence was formed as 'high' and subsequently graded down to the 'moderate', 'low', or 'very low' quality levels, depending on the assessment of five criteria: risk of bias from individual studies, indirect indemnity, heterogeneity, imprecision, and risk of publication bias.

Results

A total of 65 studies were yielded. Using the EndNote7 exact duplicate finder, 56 studies remained after the duplication. Forty-five studies were excluded after analyzing the title and summary. After further revision of the full text of the 11 articles, 3 involved other rehabilitation techniques (not electrical stimulation) and 5 were case series. No additional articles were included by manually searching for reference lists and citation tracking. Finally, 4 studies involving 146 patients were considered eligible for qualitative analysis.[16–19] For the meta-analysis, two studies were included for oral transit time (OTT),[16,17] two for penetration and aspiration scale (PAS),[17,18] two for hyoid anteriorization (HA),[17,18] two for hyoid elevation (HE),[17,18] and three for quality of life (QL)[17,19] as shown in ►**Figure 1**.

Study Data

The information extracted from the included studies is summarized in ►**Table 1**. The articles were published between 2009 and 2016. The study included 146 participants aged 58.37 ± 1.8 years old and there was a male predominance (70%). The time to start the intervention ranged from

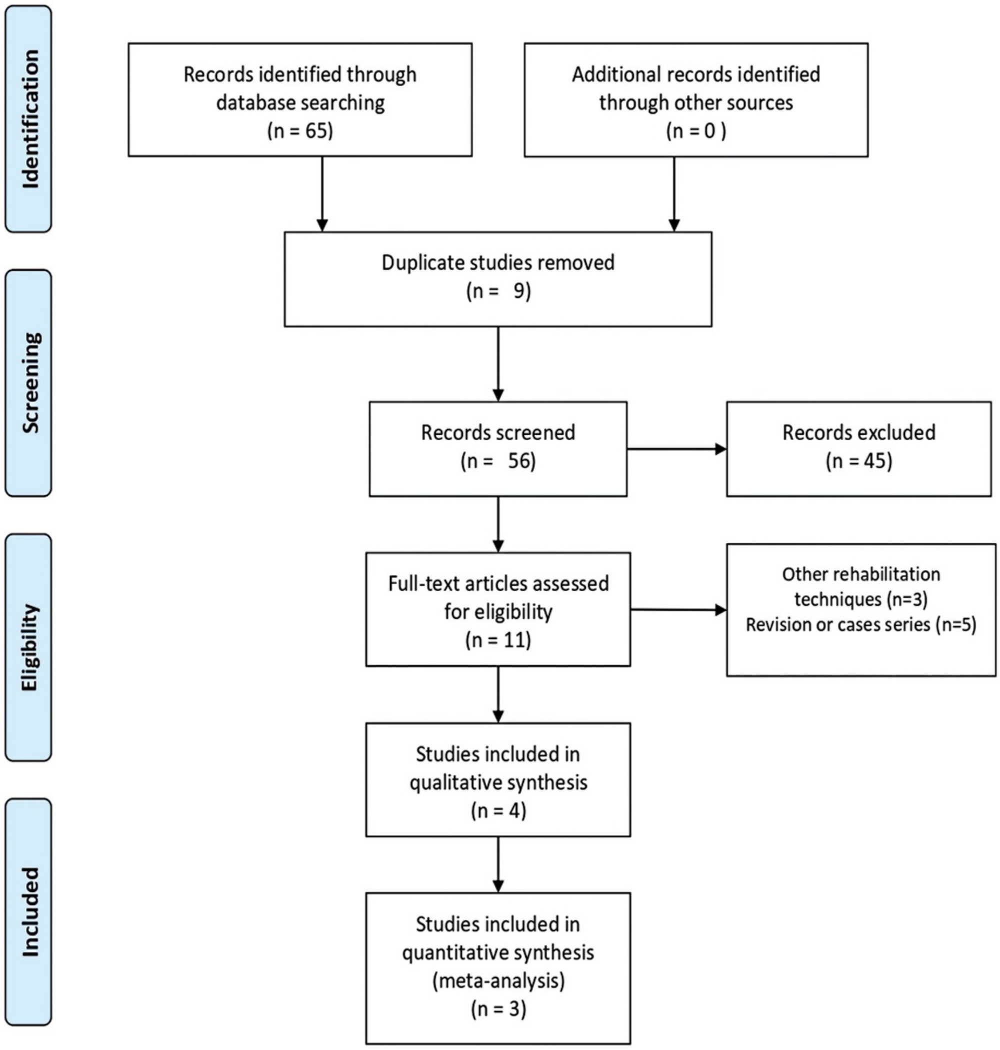

Fig. 1 Flow chart of search and study selection.

Table 1 Characteristics of included studies

Author, Year	n (total)	n (GI / GC)	Age (mean ± SD, years)	Male (%)	RT (%)	IMRT (%)	TPT (mean ± SD, %)	GI	GC	Analyzed Parameters
Langmore et al., 2016[18]	40	20/20	61.9 ± 6.9	85,7	100	51.5	53.7 ± 60.9	FES + exercises	exercises+ SHAM*	PAS, OPSE, LE, AL, RP, QOL
Lin et al., 2011[17]	20	10/10	54.2 ± 2.6	60	100	NR	9.1 ± 4.0	FES+ exercises	exercises	PAS, OTT, PTT, PDT; LE, AL, PR, QOL
Long et al., 2013[16]	60	31/29	56.1 ± 0.5	48.3	100	NR	63.1 ± 6.5	FES+ exercises	exercises	WST , OTT, SRT , PTT, LCD
Ryu et al., 2009[19]	26	14/12	61.4 ± 10.6	86.1	50	NR	NR	FES+ exercises	exercises+ SHAM**	FDS;CDS ASHA NOMS, MDADI

Abbreviations: AL, anteriorization laryngeal; ASHA NOMS, American speech-language-hearing association national outcome measurement system; FDS, functional dysphagia scale; GC, Control group; GI, intervention group; LCD, laryngeal closure duration; LE, laryngeal elevation; MDADI, M.D. Anderson dysphagia inventory; NR, not reported; OPSE, oropharyngeal swallow efficiency; OTT, oral transit time; PAS, penetration and aspiration scale; PDT, pharyngeal delay time ; PTT, pharyngeal transit time; QOL, quality of life; RP, residue pharyngeal; RT, radiotherapy; SD, standard deviation; SHAM, stimulation electrical* off or TENS**; SRT, swallow reaction; TPT, time post treatment; WST, water swallow test.

50.96 ± 40.12 months after cancer treatment. With the exception of one study,[21] the entire sample involved exclusive or associated radiotherapy as a treatment modality. Only one study described the RT method by intensity modulated radiotherapy (IMRT).[18]

Regarding the region of cancer treatment, two studies involved only the nasopharynx,[16,17] and the others included in addition to the nasopharynx the oral cavity, the oropharynx, the larynx, and the hypopharynx.[18,19]

Neuromuscular Electrical Stimulation Data

The application of NMES showed high heterogeneity as to the position of the electrodes, parameters and protocols, duration of the stimulus, number of sessions, and intensity. All studies used a functional electrical stimulation (FES) current and the frequencies varied from 70 to 80 Hz, the pulse width from 300 to 700 uS, and the other parameters such as up and down ramp, time on and time off were described by only one article,[18] the others used equipment with closed programming, which did not allow changes.[16–18] The duration of the electrical stimulus varied from 30[19] to 60[16–18] minutes and the maximum intensity was reported in only one study,[16] the others reported as the maximum level of tolerance.[16–19] Adverse effects were not mentioned by any study, the other information on the application of electrical stimulation can be found in ►**Table 2**.

Swallowing Data

Different scales were found to assess the biomechanics of swallowing and oral intake, showing high heterogeneity. None of the selected outcomes were homogeneously assessed in all included studies.

All included studies associated exercises with the main intervention through NMES.[16–19] In the control groups, in addition to general guidelines for food, exercises traditionally used for swallowing rehabilitation were also invented. One study,[19] in addition to the exercises and NMES, used a dilation balloon.

Among the analyzed outcomes, studies included oral transit time (OTT) (DMP = - 1.19; 95%CI: - 3.47–1.10),[18,19] pharyngeal transit time (PTT),[18,19] pharyngeal residue (PF),[19] hyoid anteriorization (HA) (DMP = 0.15; 95%CI: - 0.30–0.60),[19,20] hyoid elevation (HE) (DMP = - 0.26; 95%CI: - 0.64–0.11),[19,20] penetration and/or aspiration (PAS) (DMP = - 0.21; 95%CI: - 1.66–1.24).[19,20] The meta-analysis information for OTT, HE, HA, PAS and QOL is shown in ►**Figure 2**.

Despite the great interest in evaluating the effect of electrical stimulation on the pharyngeal residue, it was not possible due to the variability in measurement, and it is not possible to unify it. To assess the degree of penetration and/or aspiration, the most used scale was the PAS.[27]

Three studies evaluated QOL, of which one[20] used the Head and Neck Cancer Inventory (HNCI), which includes aspects related to swallowing, and two[19,20] used the MD Anderson Dysphagia Inventory (MDADI), which is specifically validated to assess the impact of dysphagia on QOL.

Table 2 Characteristics of electrical stimulation

Author Year	Parameters	Position of electrodes	Duration (minutes)	FR (per week)	Equipment	Intensity (mA)
Langmore et al., 2016[18]	FR 70Hz; 300Us (130-300); TON (2-4); TOFF (12-16); RO (2-4); RD 0	1 channel in the submandibular region	5 warm up + 60 with exercises	6x	BMR Neuro Tech (NT)2000	NR
Lin et al., 2011[17]	80Hz , 700Us	3A/3B	60	1-3x	Vital Stim	MTI
Long et al., 2013[16]	80 Hz, 700 Us	3A/3B	60	5x	Vital Stim	0-25 increasing 5 until MTI
Ryu et al., 2009[19]	80 Hz, 700 Us	3A/3B	30	5x	Vital Stim	MTI

Abbreviations: FR, Frequency; MTI, maximum tolerated intensity; NR, not reported; RD, ramp down; RO, ramp On; TOFF, OFF time; TON, On Time; Us, pulse width.

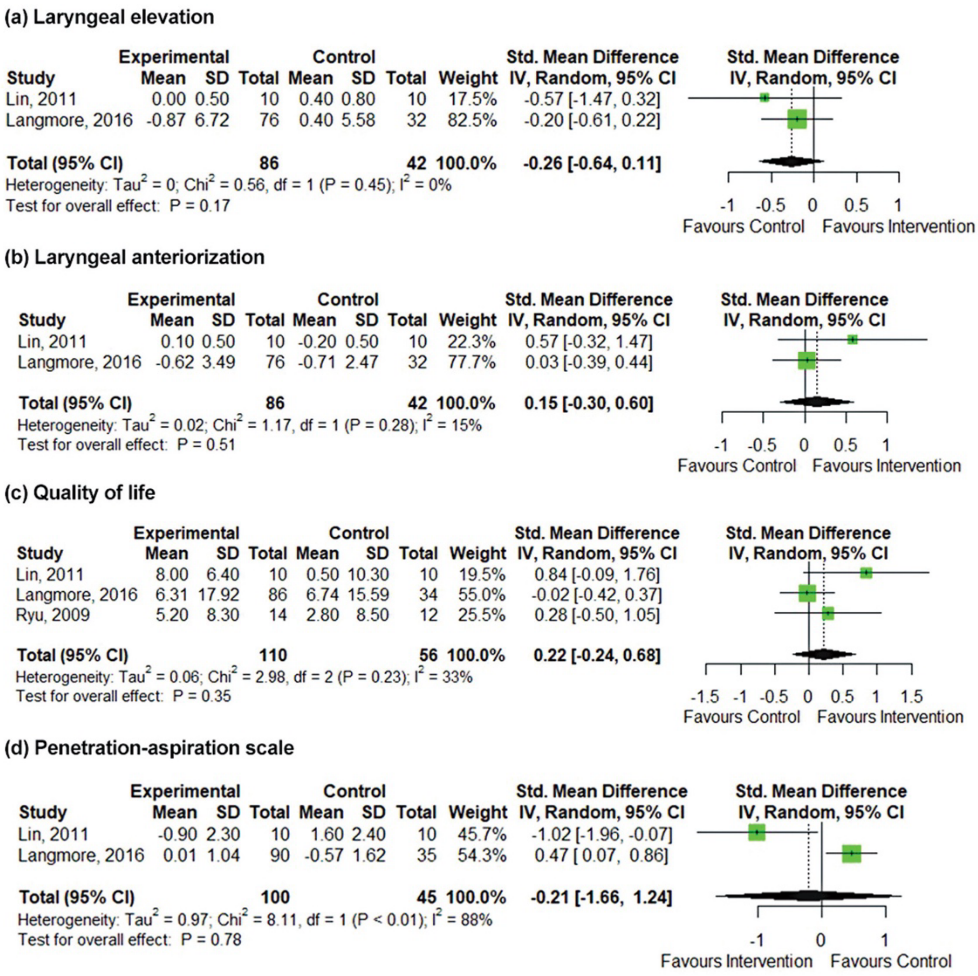

Fig. 2 Forest plot: effects of e on Hyoid Elevation (HE) (A), Hyoid Anteriorization (HA) (B), Quality of Life (C), and Penetration Aspiration Scale (D).

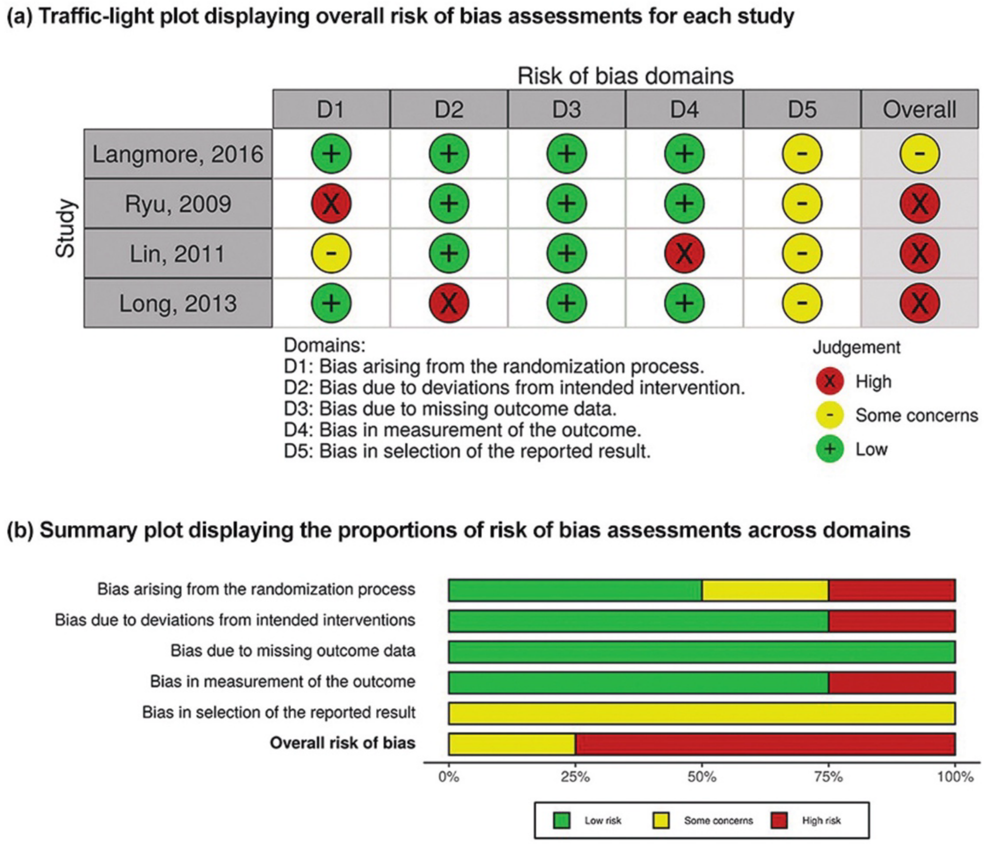

Fig. 3 Assessment of the risk of bias (A) and risk of bias summary (B).

Risk of Bias

The risk of bias for the included studies was assessed with ROB 2.0, and the results are presented in ►**Figure 3**. Three studies[16,17,19] were assessed as high risk of bias due to measurement of the outcome and randomization process, deviation from intended intervention, measurement of the outcome, and one had some concerns in the selection of reported results.[18]

Assessment of the Quality of Evidence

The Summary of Findings (►**Table 3**) presents an assessment of the quality of the evidence by the GRADE system, with judgments for each outcome. The quality of the evidence ranged from very low to moderate. The justifications for each judgment are assessed in greater detail in ►**Table 3**.

Discussion

After analyzing the data produced in the present systematic review, we found no beneficial effects of NMES treatment on swallowing rehabilitation of patients with NHC treated with RT. The results showed that, after treatment with NMES, the performance in the different swallowing tests was similar to that of the volunteers in the control group. The studies showed important discrepancies in relation to the time of completion of the cancer treatment and the beginning of the intervention and involved patients with acute and chronic effects on swallowing, making the findings variable, heterogeneous, and, sometimes, inaccurate. Among all the analyzes performed, only the laryngeal excursion demonstrated a certain benefit with electrical stimulation. However, the risk of bias was high, and the quality of evidence measured using the GRADE scale ranged from very low to moderate.

Although NMES has shown a positive effect on swallowing[16,17,19] and on QOL,[16–19] these results cannot be generalized due to the losses related to the randomization and blinding process that sometimes was not clearly described, showing heterogeneity in the design and instruments used, data analysis, reduced sample size, high number of losses and analysis without intention to treat, nonassessment of adherence and divergence in intervention protocols despite using the same NMES technique. These aspects are reflected on the

Table 3 Summary of results: effects of electrical stimulation on different aspects of swallowing

Population: head and neck cancer patients Context: outpatient and/or hospitalized Intervention: electrical stimulation Comparison: usual care or placebo					
Outcomes[a]	Participants, *n* (Studies, *n*)	Compiled Studies	Statistical heterogeneity (I^2, %)	Effect Size (DMP, 95% CI)	Quality of evidence (GRADE)
Hyoid Elevation	128 (2)	17,18	0.0%	DMP, -0.26 (-0.64 a 0.11)	⊕⊕⊕◯ MODERATE [b]
Hyoid Anteriorization	128 (2)	17,18	15.0%	DMP, 0.15 (-0.30 a 0.60)	⊕◯◯◯ VERY LOW [b,c,d]
Quality of life	166 (3)	17–19	33.0%	DMP, 0.22 (-0.24 a 0.68)	⊕⊕⊕◯ MODERATE [b]
Oral transit time	80 (2)	16,17	94.0%	DMP, -1.19 (-3.47 a 1.10)	⊕⊕⊕◯ MODERATE [b]
Penetration-aspiration scale	145 (2)	17,18	88.0%	DMP, -0.21 (-1.66 a 1.24)	⊕◯◯◯ VERY LOW [b,c,d]

Abbreviations: DMP, standardized mean difference; 95% CI, 95% confidence interval.
[a]Means represent the post-treatment value of each group and DMP represents the standardized difference in Hedges g between groups in the post-treatment means GRADE approach to assess the quality of evidence.
[b]Graduated down to a level due to risk of bias in primary studies, assessed by the RoB 2 instrument.
[c]Graduated down to one level due to important statistical heterogeneity in the final result, not explained by subgroup analyzes or meta-regression.
[d]Graduated down to a level due to high inaccuracy, with a low number of participants included (< 200) and wide confidence intervals that simultaneously encompass clinically relevant benefits and harms.

grade going downwards in the analysis of the quality of the evidence through GRADE, in which there was important statistical heterogeneity in the final result and high imprecision, with a low number of participants included (< 200) and wide confidence intervals that encompass simultaneously clinically relevant benefits and harms. The previous reported difficulties were found in the study that did not identify a benefit with NMES on swallowing.[18]

The parameters of the electrical stimulus used in each therapeutic program vary significantly between studies. The frequency of the electric pulse ranged from 70[18] to 80 Hz,[16,17,19] the pulse width range from 300[18] to 700 ms,[16,17,19] the total time per electrical treatment session varied from 30 to 60 minutes, and the intensity of the electric current was reported in only 1 study (maximum of 25 mA); in the other clinical trials, the intensity was progressively increased up to the maximum tolerance level. The muscular contraction produced by NMES can be controlled by the manipulation of the parameters of frequency, intensity, and duration of the impulse. There is a tendency for frequencies < 40 to 50 Hz to recruit a greater number of slow contraction fibers (type I), which are more resistant to fatigue, while higher frequencies recruit faster contraction fibers (type II) that are less resistant to fatigue.[34–37] The possibility to vary the configurations according to the desired objective is important and favors rehabilitation since the therapeutic targets change along the way according to the performance of the patient; however, only one study used open programming equipment that allows the configuration of the therapy adapting to the particularities of each individual.[18]

If we analyze the composition of the suprahyoid musculature, which is the main musculature responsible for the hyolaryngeal excursion, it is possible to notice that 45.7% is constituted by type 2 fibers, 34.7% by type 1 fibers, and 19.5% by type 2X or hybrid fibers. In this concept, using programming during electrostimulation that mainly recruits type II fibers (70 Hz) as observed in the included studies[16–19] may, in fact, not promote gains in hyolaryngeal excursion or produce a limited effect size. Individualizing the protocols, reflecting on muscle composition, and varying the parameters considering the recovery of muscle homeostasis prior to radiotherapy (low, medium, and high frequencies) may be the way to recruit the musculature as a whole and promote a functional change in swallowing.

The anatomical location where the electrodes were placed was another factor that differed between studies. The supra and infrahyoid segments were stimulated in three studies,[16,17,19] while only one stimulated the suprahyoid region,[18] specifically in submandibular and mylohyoid sections. A meta-analysis analyzed the effect of NMES in different populations, in which studies involving supra- and infrahyoid stimulation demonstrated greater potential for rehabilitation of the swallowing biomechanics.[38] This is a particularly important aspect to be considered in radioinduced fibrosis, where the swallowing movement as a whole is compromised. Selective stimulation of the suprahyoid region may limit the extent of the benefit of the rehabilitation of hyolaryngeal mobility. If there is no contraindication for the placement of the electrodes in the supra- and infrahyoid muscles, electrical stimulation of both regions must be encouraged. Perhaps this is also a justification for the divergence in results between studies.

Another aspect that can influence the depth of the electrical impulse and the ability to overcome radiation-induced fibrosis is the pulse width. Most studies involving electrical stimulation for dysphagia rehabilitation are directed to patients with neurological disorders, especially after stroke. However, it is not possible to generalize the findings in these studies to the cancer population, especially after head and neck cancer and RT sequelae. This is because there are anatomical changes caused by the surgery and in the muscle configuration, which makes it difficult to create and apply a single protocol (parameters and electrode positions) for all patients.

In our clinical experience, the best results in relation to hyolaryngeal excursion and sensory changes promoted by RT occur with the development of individualized programs and with the modification of parameters and electrode positioning in the course of rehabilitation in association with exercises. The difficulty of carrying out research with patients with head and neck cancer is understood, and the need to standardize information to reduce the risk of bias but to evaluate different parameters stratifying the sample by groups after time after treatment, tumor region, age and staging seems to favor the understanding of which parameters and in which patients the relationship of the use of NMES may be more favorable. This reflection allows us to infer the reason why there is so much divergence in the literature and the difficulty in deciding as to whether or not to use NMES as an ally in the rehabilitation of dysphagia.

In all studies included in the present systematic review, electrical stimulation was associated with exercises traditionally used for swallowing rehabilitation. Therefore, one must consider the variations in the therapeutic electrical stimulation procedures, as well as the different exercise protocols employed. In addition to electrostimulation, a study used balloon dilation after all sessions of NMES.[16] There is evidence that balloon dilation promotes food transit benefits since it expands the digestive tract, whether pharyngeal or esophageal, especially in cases of stenosis.[39] The dilation balloon, in this case, is characterized as a concomitant intervention, with confusion bias, as it is not possible to analyze separately what was the effect of NMES and what was the effect of the dilation balloon on swallowing since there is evidence that the latter directly interferes with function.[38,39]

In addition to the different aspects related to the electrical stimulation protocols and exercise program, the adherence to swallowing exercises plays a central role in the prognosis of dysphagia rehabilitation, but only one study clearly reported the measures used to control the involvement of the patients in the program. In the study by Lin et al.,[17] adherence control was carried out every 2 weeks by means of a telephone call. In addition to checking adherence, telephone contact was also used to encourage participation and reinforce the importance of home exercises for the rehabilitation program, this monitoring possibly reflected in the large size of the effect obtained (0.91).

The time that elapses after the cancer treatment ends determines the type of side effects observed. Treating the effects of the acute phase increases the chances of obtaining better results in the rehabilitation of dysphagia. However, one of the studies included in the review did not report this information.[19] In the other studies,[16–19] there was great variability, but a larger size of the intervention effect was observed in a study[17] in which the time between the end of cancer treatment and the beginning of the intervention was shorter. It is possible to assume that the prognosis of dysphagia rehabilitation is more favorable when instituted early, because the longer the time, the greater the difficulty of management due to chronicity, especially in the case of radioinduced fibrosis. However, the limited number of articles included and the great variability found in the post-RT time do not allow this inference ($50,96 \pm 40,12$ months).

There is evidence to show that radiotherapy affects the muscle repair mechanism, significantly reducing the number of satellite cells that are responsible in part for this regeneration.[40,41] The dose-response effect is well-established, because the greater the severity of fibrosis, the greater the limitation of the muscle response to training,[42] although the skeletal muscle has the ability to modify its structure and function in response to factors such as denervation, exercise, and electrical stimulation.[42]

Exercise is one of the most effective strategies for maintaining and recovering muscle function; coupled with this, there is evidence that NMES is a method that improves performance and muscle structure as a whole.[43] The mechanism of NMES on swallowing is still unclear, but there are some theories, one of which is the possibility of the electric current promoting greater resistance to swallowing when the infrahyoid muscle is stimulated, with which the individual should make a greater effort to overcome the NMES barrier and thus improve its range of motion during the hyolaryngeal excursion. In addition, there is evidence that NMES can promote neural adaptations through afferent feedback to the spinal cord during contractions triggered by stimulation, increased isometric strength and modification of the type of muscle fiber.[43]

Another aspect that has not been tested in this population is the associated use of NMES with resistance exercises. The literature has shown gains in hyolaryngeal excursion and movement speed during swallowing[43], aspects that are altered in radio-induced dysphagia. The surveys included do not specify the exercises used and generally approach the exercises or involved Supersupraglottic, Mendelsohn, Swallows Effortful[18], but with null results or with a reduced effect size both in the NMES group and in the group that performed only the exercises. It is believed that to overcome the scenario of radioinduced fibrosis and the impact it causes on swallowing, higher intensity training is necessary, with exercises that involve resistance and use resources as allies of therapy; in this case, NMES.

Despite the limitations identified after careful analysis, the positive findings found in the studies cannot be ruled out,[16,17,19] such as: increased speed of hyoid movement, reduced stasis in pyriform recesses, less impairment in swallowing over 3 months. It was noticed that in studies in which there was a gain in the hyolaryngeal excursion[16,17,19] (HE and HA) both in range of motion and speed, a reduction in the PAS scale was also identified. This may be associated with the importance of this mechanism in swallowing and airway protection, which impacted on the reduction of episodes of penetration and/or laryngotracheal aspiration. These functional changes, even if slight, may be related to NMES and changes in muscle structure and function that promote the reactivation of genes that were inactivated and modify the skeletal muscle phenotype[44] and the configuration of muscle fibers. Predominance of type 2A fibers and increased expression of the *MyHC2A* gene, which represents an increase in muscle strength and rapid contraction, are interdependent factors for swallowing that demand a quick and precise response and are potentiated through NMES.[38,40,41]

All included studies that evaluated QOL identified a positive change after NMES, mainly in aspects related to swallowing function.[16,17,19] Only one of the studies did not identify an effect on swallowing; however, interestingly, in the assessment of QOL, patients reported improvement mainly in aspects related to the speed of eating and were already able to eat food in public without discomfort.[18] Perhaps the sensitivity of the instruments used to measure such outcomes was not sufficient to detect changes in the swallowing function, since it is unlikely that, if the individuals followed the exercise protocol (twice a day, 6 days a week), there have not been changes over the course of 3 months.

However, new studies should be encouraged, because, with larger samples and with greater methodological rigor, it is possible that the results lead to a favorable indication of the use of NMES for the rehabilitation of dysphagia in this population. Finally, it is essential that the assessment of dysphagia be more uniform, that the protocol of the swallowing rehabilitation exercise program must be better described and also more standardized. The anatomical location of the electrode attachment points should consider the wider involvement of the swallowing muscles and the parameters of the electric stimulation therapy need to be better described, especially in relation to the intensity of the electric current.

Final Comments

In the present meta-analysis, we found weak evidence for small and moderate swallowing benefits in patients after RT for HNC in short-term clinical trials. Due to the limited quality of the evidence, our findings require further confirmation with robust randomized controlled trials.

Ethics Approval

The present review follows the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) guidelines and, subsequently, it was registered in the International Prospective Register of Systematic Reviews (PROSPERO), with the following identification: CRD42020200248

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Conflict of Interests

The authors have no conflict of interests to declare.

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Case Series and Review of the Literature

Orofacial Myofunctional Therapy with and without Photobiomodulation in the Rehabilitation of Radiation-Induced Trismus: Case Series

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ABSTRACT

Photobiomodulation (PBM) as a therapeutic technology is justified by the biochemical changes caused in the intracellular environment, such as increased production of adenosine triphosphate and activation of antioxidant enzymes, allowing early recovery and maintenance of the homeostasis and proper functioning. This case report aimed to describe the effect of orofacial myofunctional therapy associated or not with photobiomodulation in the rehabilitation of radio-induced trismus in 6 patients. Two intervention modalities were performed, with three patients undergoing OMT isolated and the other two subjects undergoing oral myofunctional therapy associated with photobiomodulation therapy (OMT+PBM). All participants completed the radiotherapy between 3 and 15 months before starting the trismus rehabilitation. The mouth opening was 21.00mm for the patients who underwent exclusive OMT and reached 30.25mm at the end of the rehabilitation (difference of 9.25mm), but for the other three patients submitted to OMT+PBM, it went from 8.4mm to 31.5mm (difference of 23,1mm). It was observed that patients who performed PBM+OMT had greater tolerance to the protocol exercises and less pain report. OMT+PBM was a good combination for trismus rehabilitation and could be considered in further randomized clinical trials.

Introduction

Head and neck neoplasms can have adverse effects on swallowing, chewing, breathing and speech, and can deteriorate quality of life of patients [1]. Radiotherapy can be started with the curative objective, adjuvant (pre or postoperatively) or as palliative treatment, but the side effects that occur when normal cells located within the treatment area suffer temporary or permanent damage. The sequelae resulting from this treatment depend on the number of doses and intensity of exposure and may manifest during or after treatment conclusion [2]. Among the most prevalent complications is trismus, defined as a restriction in mouth opening that can be caused by tumor infiltration into the masticatory muscles and/or the temporomandibular joint; by radiotherapy itself when it involves these muscles in the radiation field; or even a combination of both [3].

Radio-induced trismus originates from fibrosis in the masticatory muscles, which, when affected by irradiation, initially reacts through an abnormal proliferation of fibroblasts, accentuating the synthesis of collagen that leads to the formation of thick fibrous tissue [4]. The diagnostic criterion for trismus is usually defined by mouth openings smaller than 35 millimeters (mm) [5-7]. The subjective diagnosis, based on the patient's clinical complaint such as locking, difficulty opening the mouth, and muscle stiffening should be considered; however, it is less reliable from a scientific point of view, so measurement using a caliper is indicated.

Some therapy options have been used in the rehabilitation of radio-induced trismus, including photobiomodulation therapy (PBM), also known as LASER therapy or low-level LASER therapy. It is a therapeutic approach that modulates biological activity through the use of light in red and infrared wavelengths, which causes positive

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therapeutic results, including a significant reduction of inflammatory processes, pain relief, prevention of fibrosis and improvement in wound healing and tissue regeneration [8-10].

This technique can be used alone or associated with orofacial myofunctional therapy seeking a significant gain in strength, considerably reducing levels of muscle fatigue, thus optimizing the performance of musculoskeletal functions related to speech; dysphagia, through improvements in saliva flow; improvements in chewing and swallowing functions; muscle toning or relaxation; modulation of inflammation in the treatment of facial paralysis; stimulation and regeneration of the injured nerve and post-surgical recovery, providing faster reduction of edema [11]. This case report was conducted to describe the effect of conventional speech therapy with and without PBM to aid in the rehabilitation of radio-induced trismus.

Case Presentation

This study describes the interventions performed in six patients diagnosed with severe radio-induced trismus. Study was approved by the Research Ethics Committee under opinion number 5.106.387. All participants signed an informed consent form. Prior to the interventions, all participants underwent clinical anamnesis, mouth opening measurement, visual analogue scale (VAS) and Gothenburg Trismus Questionnaire (GTQ). The classification regarding the mouth opening measure was according to the severity of restriction being considered trismus values equal to or less than 35 mm [5-7].

All participants were instructed to remain with their necks in a neutral position and to open their mouths as wide as possible, avoiding excessive pain. A 6 inches (150mm) stainless steel digital caliper was used, from the Stainless Hardened brand. Three measurements were taken and the largest was considered. The GTQ is used as a screening to measure the impact of trismus on aspects related to dietary limitations, fatigue and muscle tension, as well as problems related to the jaw. In each domain, the research subject answered the questions by marking the most convenient answer qualitatively (e.g., responding about the existence of jaw fatigue as “not at all”, “mild”, “moderate”, “severe”, “very severe”) and scores were assigned to the items in such a way that a higher score meant worse performance in quality of life with regard to trismus [12]. The VAS aimed to subjectively measure the perception of pain experienced by patients before and after therapeutic procedures. Patients should answer about their degree of pain, with “0” meaning total absence of pain and “10” the maximum level of pain bearable by the subject.

Interventions

Two intervention modalities were performed: 1) Oral Myofunctional Therapy (OMT) for three patients and OMT plus photobiomodulation therapy (OMT+PBM) for the other three patients. The OMT consists of three mandibular mobility exercises and two traction exercises that should be performed three times a day, daily for five weeks [13]. In addition to performing the OMT programme for mouth opening at home, the participants underwent two weekly sessions in the clinic, supervised by the speech therapist, lasting 30 minutes each, for adjustments, monitoring of the evolution and clinical conditions.

The PBM was conducted with 100 Watts (W) of power and an output spot with an area of 3.3 mm^2 (MMO® Brand, LASER duo model). The dosimetric parameters were: red wavelength (660nm) and infrared (808nm) separately irradiated, energy of 6 joules (J) per point, fluence of 199.98 J/cm^2, type of continuous emission, which were applied for 60 seconds in each point [10]. The application was punctual, with contact, extra-oral, bilaterally on the temporal muscle (9 points), temporomandibular joint (TMJ) (4 points) and masseter muscle (6 points) (Figure 1). Participants were also instructed to perform OMT for mouth opening three times a day throughout all treatment period (five weeks). After the end of the protocol, all patients were reassessed and followed up in outpatient speech therapy.

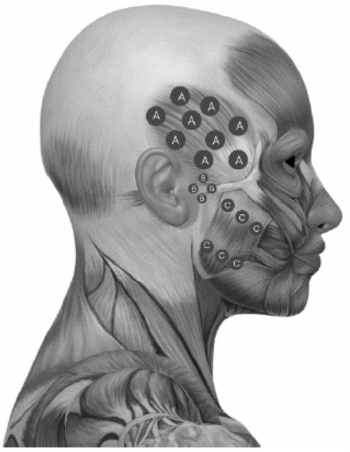

Figure 1: PBM application points. **A)** Temporal muscle. **B)** Temporomandibular joint (TMJ). **C)** Masseter muscle.
Source: Adapted from a picture available on google images.

The final sample consisted of six participants, all male, aged between 53 and 76 years, who completed the radiotherapy treatment between 3 and 15 months before the start of the speech therapy intervention for trismus. Below are the sociodemographic data of the participants (Table 1).

Table 1: Sociodemographic data of the participants.

	OMT			**PBM + OMT**		
Patients	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Sex	M	M	M	M	F	M
Age	76	64	52	56	33	57
Diagnosis	HPV-related oropharyngeal SCC	Oral cavity SCC	Oropharyngeal SCC	Oropharyngeal SCC	Oral cavity SCC	Oropharyngeal SCC
Cancer Staging	T4N2M0	pT4pN0M0	T4acN1M0	T4acN2Mx	T1cN0M0-I	pT2pN2bM0

Time after RT (months)	3	32	5	12	6	6
Method of RT	2D	2D	2D	2D	2D	2D
Dose of RT	70 Gy	70 Gy	60 Gy	70 Gy	60 Gy	70 Gy
Intervenção realizada	OMT	OMT	OMT	PBM+OMT	PBM+OMT	PBM+OMT

M: Male; RT: Radiation Therapy; HPV: Human Papiloma Virus; SCC: Squamous Cell Carcinoma; Gy: Gray; OMT: Orofacial Myofunctional Therapy isolated; PBM+OMT: Photobiomodulation associated with Orofacial Myofunctional Therapy; P: Patient (1, 2, 3, 4, 5, 6). *Difference between the pre-intervention and after 3 months of Follow-Up.

Table 2 shows the results of the pre- and post-intervention mouth opening measurement, as well as the reassessment performed three months after the end of the protocol. Regarding the mouth opening measure, all participants had pre-intervention values below normal parameters. After ten sessions of orofacial myofunctional therapy alone (OMT) and photobiomodulation therapy associated with the conventional modality (PBM+OMT), all participants reached values above the normal level (35mm) [5-7].

Table 2: Mouth opening measurement (mm).

Patients	Before intervention	After intervention	Follow-up (3 months)	Diference*
P1 - OMT	26.4	44	48.2	17.6
P2 - OMT	15.6	16.5	16.8	1.2
P3 - OMT	25.5	26.2	27	1.5
P4 - PBM+ OMT	11.5	36.6	42.2	30.7
P5 - PBM+ OMT	5.3	25.5	24.5	19.2
P6 - PBM+ OMT	15.2	43.2	46.5	31.3

Mm: Millimeters; OMT: Orofacial Myofunctional Therapy isolated; PBM+OMT: Photobiomodulation associated with Orofacial Myofunctional Therapy; P: Patient (1, 2, 3, 4, 5, 6). *Difference between the pre-intervention and after 3 months of Follow-Up.

There was total pain reduction in patients who received OMT+PBM. Patients who received only OMT reported mild pain after the end of the intervention, which remained until the third month of follow-up (Table 3).

Table 3: Pain evaluation (VAS).

Patient	Before intervention	After intervention	Follow-up (3 months)
P1 - OMT	9	3	2
P2 - OMT	9	9	9
P3 - OMT	8	2	2
P4 - PBM+ OMT	8	0	0
P5 - PBM+ OMT	10	0	0
P6 - PBM+ OMT	10	2	0

Mm: Millimeters; OMT: Orofacial Myofunctional Therapy isolated; PBM+OMT: Photobiomodulation associated with Orofacial Myofunctional Therapy; P: Patient (1, 2, 3, 4, 5, 6). *Difference between the pre-intervention and after 3 months of Follow-Up.

Regarding the level of referred pain, a total reduction was observed in three patients three months after the end of the PBM protocol associated with orofacial myofunctional therapy (PBM+OMT). The other three patients who received only orofacial myofunctional therapy (OMT) reported mild pain after the end of the intervention, which remained after three months. Table 3 shows the data referring to the level of pain reported by the patients, before and after the end of the interventions and after the 3 months reassessment.

Table 4: Association between trismus and quality of life.

Patient	Before intervention	After intervention	Follow-up (3 months)
P1 - OMT	51	6	1
P2 - OMT	50	20	29
P3 - OMT	52	40	39
P4 - PBM+ OMT	57	10	9
P5 - PBM+ OMT	53	20	0
P6 - PBM+ OMT	60	10	9

OMT: Orofacial Myofunctional Therapy isolated; PBM+OMT: Photobiomodulation associated with Orofacial Myofunctional Therapy; P: Patient (1, 2, 3, 4, 5, 6).

Table 4 shows the results of the quality-of-life questionnaire for patients with trismus (Gothenburg Trismus Questionnaire - GTQ). Before the interventions, all patients had significant impairment, with scores between 51 and 60 in the domains related to mouth opening, jaw-related problems, eating limitations, fatigue and orofacial muscle tension. Three months after the end of the treatment protocols, the subjects showed a reduction in the domains evaluated by the GTQ.

Discussion

These cases report the possibility of using PBM in the rehabilitation of trismus when associated with OMT. It was possible to assume that the PBM promotes a synergic effect to the exercises therapy. Probably, mouth opening recovery can benefit from this association, and the long-term effects of combination therapy indicate that PBM may also be acting in the prevention of radio-induced fibrosis.

In view of the damage to the masticatory muscles caused in these patients, mainly by radio-induced fibrosis, it is essential to carefully evaluate the moment of initiation of the intervention as well as to use assertive strategies aimed at maintaining homeostasis and muscle functionality. In recent years, research using electrostimulation and PBM have emerged in order to add to therapeutic programmes aimed at the rehabilitation of trismus.

The intervention protocol proposed in this case series was tolerated by the participants with no expression of unwanted side effects or discomfort. On the contrary, a reduction in pain was observed, especially in patients undergoing PBM associated with OMT. Another factor that allows us to reflect is the fact that fatigue was reduced in participants who underwent PBM, this factor can influence the patient's adherence to therapy, possibly the use of this resource allows greater acceptability and tolerance of OMT. As for the time after the end of RT, the cases had a variability of 3 to 32 months and, despite including patients with chronic adverse effects, an increase in mouth opening was observed both in those who underwent OMT only and those who had the association of PBM. However, considering the potential for maintaining muscle homeostasis promoted by myofunctional exercise and PBM, it is understood that the sooner the intervention is started, the more promising the results can be.

Radio-induced trismus originates from fibrosis in the masticatory muscles, when located in the radiation area, causing reduced mandibular mobility. Radio-induced fibrosis is a major challenge in rehabilitation as it can last for years after the end of treatment. This is due to the persistence of the alteration, chronic local inflammation that can be characterized by the presence of activated T cells and macrophages that produce various chemical mediators of inflammation, such as prostaglandins (PGs), interleukin-6 (IL-6), Tumor Necrosis Factor (TNF), interferon alpha (IF-ά), transforming growth factor beta (TGF-b) and connective tissue growth factor (CTGF/CCN2) [14, 15].

During fibrogenesis, TGF-b is the main cytokine responsible for the increased production and decreased collagen degradation that occurs after radiotherapy. CCN2 is a multifunctional heparin-binding glycoprotein that is expressed at low levels in normal tissues but overexpressed in fibrotic tissues. This overexpression of CCN2 has been associated with fibrosis in various tissues such as skin and muscle [16].

One study compared therapy through exercises associated with ultrasound and low-intensity PBM and isolated OMT, a configuration similar to the proposal of this series of cases where three patients performed the exercise program exclusively and three the exercises associated with the application of PBM. In synergy with the results of Elgohary *et al*., it was possible to observe a significant benefit of LASER associated with mouth opening exercises compared to exercise therapy alone [17]. These results deserve an in-depth look as it can be an interesting strategy for the rehabilitation process of these patients and in a shorter period of time.

One of the factors that influence the analysis of training efficiency in this population is patient adherence to training, and this control is understood as arduous and complex in most Randomized Clinical Trials (RCTs). Our study analysed adherence through a form that the patient filled in every time he performed the scheduled exercise. This remote control allows us to evaluate with greater precision the efficiency of the strategies and the viability of the disposition of the number of exercises and daily series [17].

A systematic review analysed proposed strategies for trismus in patients with head and neck cancer in RCTs and identified similarity in benefit of proposed interventions despite high variability in exercise planning. In addition, the assessment of the quality of evidence and the risk of bias provided important information about the heterogeneity of the studies and reinforced the need to evaluate the association of therapeutic resources such as PBM with exercises and to follow a methodological rigor that makes it possible to propose protocols. Likewise, analysing and understanding the biochemical effect of PBM and the best way to use this resource is a determining factor of its effectiveness [18].

The effect of light is photochemical and not thermal and, for this reason, light triggers biochemical changes within cells, which can be compared to the process of photosynthesis in plants, where photons are absorbed by cellular chromophores, triggering chemical changes. Low energy density is offered, but high enough for the target cell to use it in a way that stimulates its membrane and/or organelles. Thus, the cell is induced to biomodulation, that is, it will seek to reestablish the state of normality in the affected region [19].

Although many studies show that PBM acts in the effective modification of biological functions, the complex mechanism with which this resource exerts its therapeutic effects has not yet been fully understood, since its effectiveness may vary according to the different states of the affected tissue, cell type, inferred irradiation parameters, among other factors [20]. The most acceptable theory is that the enzyme cytochrome c oxidase (CcO), released by red and infrared light, acts mainly to increase the production of adenosine triphosphate (ATP), the energy required for cellular functioning, causing a short burst of reactive oxygen species (ROS), which also acts as an antioxidant, helping the body's homeostasis [21].

When PBM is applied to target tissues, it involves four different types of interactions which are reflection, transmission, scattering and absorption. The wavelength is the main factor that determines the penetration depth and energy absorption of the LASER in the tissue. LASERs have precise specificity for tissue components known as chromophores that absorb light of specific wavelengths. The primary chromophores in intraoral soft tissue are melanin, haemoglobin and water, and in hard tissues they are water (H_2O) and hydroxyapatite (HA). In general, longer wavelengths will have greater affinity for H2O and HA, while shorter wavelengths are absorbed by pigmented tissue and blood elements [22].

There is evidence on the effect of infrared PBM for the treatment of trismus, but our proposal in this case series analysed the effect of red and infrared PBM. It is possible to infer from the results identified that associating the two modalities can promote superior results in mouth opening. Low power PBM plays a preventive role during radiotherapy and may promote a decrease in the risk of trismus, and a protective effect for muscles, ligaments and other tissues associated with stomatognathic functions. The anti-inflammatory effect and analgesic properties of photobiomodulation therapy together with the stimulation of tissue repair and cell proliferation are the main mechanisms associated with its therapeutic effects.

I Effect of LASER on Skeletal Muscle

The best results observed in patients undergoing low-power LASER, in relation to mouth opening, can be attributed to the regeneration of skeletal muscles, through the activation of quiescent satellite cells, leading to their proliferation and, consequently, reduction of the inflammatory process. Another important point is the ability to reduce oxidative stress, a factor related to degenerative changes such as the development of fibrosis. Regarding cellular mechanisms, the effect of the technique directly impacted the capacity of the mitochondrial respiratory chain, leading to greater production of adenosine triphosphate (ATP) and culminating in higher energy levels [23, 24].

The level of muscle fatigue tends to be influenced by LASER due to the increase in the microcirculation of the irradiated anatomical region, which allows the re-establishment of muscle resistance, which may explain the greater tolerance of P4, P5 and P6 (PBM+OMT group) to exercise protocol. As for fatigue, P2, which only after orofacial myofunctional therapy obtained a score of 29 in the GTQ, indicating that higher hand muscle levels end fatigue three months of treatment [12]. Considering the value equal to or greater than 35 mm as a criterion of normality, patients with PBM therapy reached levels above the established standard after the intervention. The mean mouth opening in millimeters in patients P1, P2 and P3 increased from 26.4, 15.6 and 25.5 mm to 48.2 mm, 16.8 and 27mm (difference of 17.6, 1.2, 1.5 mm), while P4, P5 and P6, submitted to the association of PBM, went from 11.5, 5.3 and 15.2mm to 42.2, 24.5 and 46.5mm (difference of 30.7, 19.2 and 31.3mm). The results obtained and described in this case series demonstrate that photobiomodulation therapy brings benefits to the treatment of radioinduced trismus when associated with OMT, which contrasts with the data reported by Serique *et al*. where smaller gains were observed with the use of exclusive photobiomodulation [25].

LASER has already demonstrated its ability to optimize muscle function under hypoxic conditions such as mechanical stress, fatigue and neurogenic inflammation, which are responsible for electrolyte and metabolic changes present in patients with radio-induced trismus. These effects are thought to be primarily due to mitochondrial activation, resulting in an increase in electron transport, cellular respiration, oxygen consumption, and ATP production. In addition, it initiates signaling pathways that lead to the activation of various transcription factors and modulate the levels of cytokines, growth factors, and inflammatory mediators. Direct effects on somatosensory and/or motor nerves also participate in muscle relaxation and analgesia induced by laser therapy through neural blockade of nociceptors and inhibition of motor nerves [26-28].

II Effect of LASER on Pain Relief

The effectiveness of low-level LASER in the treatment of pain originating from soft tissue trauma, including those related to radiotherapy, can be attributed to the indirect reduction of edema, bleeding, neutrophilic activity, provocative cytokines and enzymatic action. This treatment modality reduces the characteristic pain observed more markedly in the three cases undergoing photobiomodulation therapy in this series, resulting in better tissue repair, as lymphatic vessel regeneration is accelerated [29, 30].

Literary data validate the effectiveness of photobiomodulation in the management of pain, acute or chronic, related to the impairment of the muscles involved in mouth opening, leading to a reduction in the need for analgesic drugs and maintenance of low pain symptoms in the long term. The analgesic action is related, especially, to the endorphin release that occurs during the systemic process of the LASER in the tissues. Our study observed a reduction in pain levels in all cases, but more markedly in those submitted to the association of exercises with low-frequency LASER. Other studies carried out in similar populations obtained very similar numerical results, with an average reduction of 8 points on the Visual Analogue Scale after the LASER protocol [17, 31].

It is concluded that orofacial myofunctional therapy associated with photobiomodulation promoted greater gains in mouth opening when compared to cases in which OMT was performed isolated. More studies are needed, including a larger sample in an RCT to strengthen this hypothesis.

Conflicts of Interest

None.

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5 ARTIGO 3 (Será submetido para publicação na revista Dysphagia)

EFFECT OF PHOTOBIOMODULATION ON VIABILITY AND PROLIFERATION OF SQUAMOUS CELL CARCINOMA *IN VITRO*

Effect of photobiomodulation on viability and proliferation of squamous cell carcinoma

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Data availability statements

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data are in controlled access data storage.

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ABSTRACT

Head and neck cancer has a multifactorial etiology and significant cellular heterogeneity, which makes treatment difficult and contributes to high mortality rates. The need to combine different therapeutic modalities, such as surgery, chemotherapy, and radiotherapy, increases patient toxicity and requires a multidisciplinary approach to optimize results and improve quality of life. In recent years, photobiomodulation (PBM) has gained ground in tissue rehabilitation and regeneration. However, its effect on squamous cell carcinoma tumor cells lacks investigation. This study evaluated the effects of PBM on head and neck squamous cell carcinoma (SCC) based on cell proliferation and viability. We explored the behavior of two cell lines, SCC09 and CAL27, under 5 sequential applications of PBM using the Fluence HTM® device and laser pens of different wavelengths (830 nm and 658 nm) with irradiation doses of 1, 3, and 6 J/cm². To ensure standardization and avoid unwanted reflections, a specific adapter was developed, maintaining a fixed distance of 20 mm between the light source and the cells in culture, on a black surface. Cell viability was measured through the MTT ([3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide]) experiment and proliferation was evaluated through the Sulforhodamine B (SR-B) assay. No significant effects on cell proliferation or viability of the evaluated cell lines were identified. PBM did not demonstrate a significant effect on cell proliferation and viability under the experimental conditions tested. However, the variability found in other studies highlights the need for additional research to elucidate the mechanisms of action of PBM and establish safe treatment protocols.

Keywords: Photobiomodulation Therapy, Cell, Atypical Squamous, Head and Neck Neoplasms

INTRODUCTION

Head and neck squamous cell carcinoma (SCC) presents remarkable biological heterogeneity, characterized by a diversity of molecular and phenotypic subtypes [1, 2]. This intrinsic variability significantly influences tumor behavior, response to treatment, and patient prognosis. Accurate molecular characterization of tumors is essential to identify prognostic and response to therapy, allowing patient stratification and treatment personalization, with the aim of optimizing results and improving quality of life [3, 4]. Oncological treatments for head and neck SCC, often combining surgery, chemotherapy (CT), and radiotherapy (RT), expose patients to structural losses and a series of adverse effects. The functional and structural consequences resulting from these treatments can significantly compromise quality of life, affecting swallowing, communication, and psychological well-being [5, 6].

The heterogeneity of survival rates highlights the importance of careful planning for early rehabilitation, including strategies such as photobiomodulation (PBM) [7-11]. PBM, through low-level laser therapy, has demonstrated therapeutic potential for recovery from the adverse effects of cancer treatment, such as oral mucositis, xerostomia, dysgeusia, and had also improved muscle performance [12, 13]. Its therapeutic effects occur through the interaction of light with mitochondrial chromophores, triggering a cascade of molecular events that include the activation of cellular signaling pathways, modulation of calcium transport, and optimization of mitochondrial respiration, accelerating tissue regeneration [14-16]. However, there is scarce evidence to date to support definitive clinical recommendations for the use of PBM, since there are no clinical guidelines supporting the safety of PBM use [17, 18].

The available studies generate a confounding bias since the experiments are performed under different conditions, with a limited number of parameters, doses, and strains, as well as with few applications of PBM [19]. There is evidence that demonstrates an increase in cell proliferation and viability in SCC when analyzing in vitro behavior [20]. In addition, the aggressiveness of the cell phenotype and the increase in the expression of proteins involved in cell proliferation, survival, and invasion suggest that PBM, although promoting beneficial clinical effects, can potentiate tumor progression [20]. Nonetheless, PBM seems to promote a biphasic effect on cells. Under exposure to a single application, SCC cells showed an increase in viability, reducing this same parameter when subjected to a greater number of daily applications [21].

PBM has been widely investigated as a resource for tissue regeneration in cases of mucositis [22]. However, the scarcity of studies exploring the interaction between PBM and head and neck SCC (HNSCC), considering specific parameters and applications for the rehabilitation of stomatognathic functions, limits its clinical application in this context. Thus, the present study aims to contribute to the advancement of knowledge in this area, evaluating in vitro the effect of different parameters and doses of PBM on the proliferation and viability of HNSCC cell lines. The results obtained may provide support for the development of safe and effective PBM protocols, aiming to optimize oral rehabilitation in patients with head and neck cancer.

METHODS

This is a quantitative, descriptive, comparative *in vitro* experimental study. Approved by the Research Committee of the location where it was carried out.

Cell culture

Cells from the CAL27 (low-invasive OSCC – ATCC® CRL-2095 TM) and SCC09 (high-invasive OSCC – ATCC® CRL-1629 TM) cell lines were used. The temperature was maintained in culture at 37° C with 5% CO_2, and Dulbecco's modified Eagle's (DMEM, Gibco BRL) supplemented with glucose, 10% fetal bovine serum (FBS) (Gibco BRL), kanamycin 0.5 mg/mL or 1% penicillin/streptomycin (Invitrogen) was used as the cell culture medium. Passages were performed with trypsin/EDTA (0.25% w/v and 0.53 mM, respectively). When they reached confluence, the cells were trypsinized and plated at $1x10^2$ cells per well in 24-well plates, with the cells distributed in intercalated wells. Five experiments were conducted for each lineage, each of which was analyzed in triplicate.

Prototype creation

In order to control the dispersion of light into the wells close to the irradiated area, a prototype was developed with the help of engineering (figure 1). The accessory was created to be adapted to a 24-well culture plate and filled the empty wells, thus reducing the intervention bias. The device was CNC machined in nylon polymer with the exact measurements to fill the wells.

The device was also designed to maintain a fixed distance (20 mm) between the emitting source (pen) and the cell culture well, allowing the light radiation to cover an area of 1 cm^2 (figure 2). In addition, the plates were placed on a black surface to attenuate the reflection of the irradiation by the bottom of the culture plates.

<<< figure 1>>>

<<< figure 2>>>

Experimental design

For each experiment, cells were seeded, and the culture medium was changed every 2 days. The following experimental groups were established (figure 2):

- Control (C): cells were only monitored, and the culture medium was changed for maintenance.
- *In Vitro* Intervention: PBM was applied once a day, for 5 consecutive days, maintaining the application at the same time in the afternoon. Experiments were performed with two different wavelengths (658nm and 830nm). For each wavelength, 3 different intensities

were used: 1J, 3J, and 6J, as described in figure 1, totaling 4 wells for each intensity in each experiment.

- o 3 LASER 658 nm plates for proliferation analysis with 12 cultured wells;
- o 3 LASER 658 nm plates for viability analysis with 12 cultured wells;
- o 3 LASER 830 nm plates for proliferation analysis with 12 cultured wells;
- o 3 LASER 830 nm plates for viability analysis with 12 cultured wells;

After the application of PBM, the cells remained at rest for 24 hours before processing for analysis.

<<< figure 3 >>>

Irradiation with PBM

The HTM® Fluence device and the Infrared (IR) LASER pens of 830 nm and power of 200 mW (ME04725A) and Red (V) 658 nm and power of 100 mW (ME04722A) were used in the experiments. The dose-response curve, for each irradiation mode, was composed of the following irradiation fluence rates (irradiated energy density): 1J, 3J, and 6J per cm² (Table 1). After application, the plates were placed back in the incubator where they remained until the end of the fifth application, when the viability or cell proliferation experiments were performed.

<<< table 1 >>>

Viability assessment

Cell viability was measured using the MTT ([3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium] bromide) experiment. A total of 10^3 cells were used per well in 24-well plates. 50 μL of culture medium was removed from the plates and 50 μL of MTT (5 mg/ml PBS, pH 7.2) was added. The cells were incubated for 4 h at 37 °C and then the culture medium was removed from the plates and 100 μL of DMSO was added per well. All contents were transferred to a 96-well plate for analysis and two wells were kept only with DMSO to evaluate the blank group. Cell viability was quantified using a spectrophotometer at 540 nm.

Cell proliferation assessment

Cell proliferation was assessed using the Sulforhodamine B Assay (SRB) in 24-well plates with 3 replicates of each group, with a density of 10^3 cells per well. In the first 24 hours after the cells were plated, T0 was assessed.

Before performing the assay, the cell culture medium was replaced by a medium without the addition of SBF, following an adaptation of the original protocol, to avoid a false increase in cell density values due the proteins present in SBF that could be fixed together with the cells [23]. The cells were then fixed with 100 μL of 50% trichloroacetic acid (50% TCA) and incubated for 1 h at 4ºC.

The cells were then washed four times with running distilled water and dried in an airflow. Subsequently, 200 μL of sulforhodamine B solution (0.4% in 1% acetic acid) was added per well, and incubation was maintained at room temperature for 25 min. The plates were washed four times with 200 µL of 1% acetic acid to eliminate excess dye and, finally, solubilized with 200 µL of 10 mM Tris and incubated shaking for 10 min at room temperature. The contents were transferred to a 96-well plate for analysis in a spectrophotometer at an absorbance of 570 nm.

Statistical analysis

One-way or two-way ANOVA, followed by Tukey's or Sidak's multiple comparison tests, were used to analyze the results performed in the Prisma 8 software. The data are expressed as mean ± standard deviation (SD) and represent at least three independent experiments. The hypothesis test was adjusted to consider the probability of alpha error of $p < 0.05$.

RESULTS

Cell Proliferation (SRB)

There was no significant difference in cell proliferation between the experimental and control groups, regardless of the type of cell line (SCC09 or CAL 27), wavelength (658nm or 830nm), or irradiation dose (1J, 3J, or 6J) (Figure 4).

<<< figure 4 >>>

Cell Viability (MTT)

The analysis of cell viability revealed no significant differences between the experimental and control groups, regardless of the type of cell line, wavelength, or irradiation dose.

<<< figure 5 >>>

DISCUSSION

The results obtained in this study did not show any interaction between PBM and the viability and proliferation of the two SCC cell lines (CAL27 and SCC09). None of the two different wavelengths (658 nm and 830 nm) at the three intensities (1J, 3J, and 6J) tested changed the cell count after five consecutive days of irradiation. Not even the mitochondrial metabolic rate (succinate dehydrogenase), assessed in the MTT test, had significant modification. These data suggest that the proliferation rate and mitochondrial activity of those two neoplastic cell lines are not influenced by PBM, since after 5 days of experiment, no signs of inhibition or stimulation were observed when compared to non-irradiated cells.

This research innovates by evaluating the effects of PBM on different oral SCC cell lines, using a experimental protocol that includes varying dosages, wavelength, and the use of a prototype that minimizes light reverberation on a flat surface (Figure 1). Unlike most studies in literature, which focus on a single application of PBM, this study simulates clinical treatment conditions, with multiple sessions, contributing to the optimization of treatment protocols and to a better understanding of the mechanisms of action of PBM. Our study is a first step towards understanding the effects of PBM on tumor cells biology, to support clinical studies related to the safety of PBM through or after oncological treatments.

A systematic review [24] highlighted a significant gap in knowledge regarding the safety of PBM use. Although the literature has a considerable number of studies, most of these focus on the efficacy of the therapy, with few specifically dedicated to evaluating its long-term safety. Among the 27 studies included in the review, only four [25-28] performed a follow-up of at least 24 months with the primary objective of evaluating the safety of PBM use in oncology. Although other studies reported the absence of adverse events during treatment, they did not have the appropriate methodological design for a rigorous evaluation of long-term safety. Based on this systematic review, the data available in the literature are poor and the safety of this technology needs to be studied more effectively [24].

There is high heterogeneity in the follow-up period of patients in the available studies, extending from 10 days to 41.3 months. Most studies limited the follow-up to the period of oncological treatment [25, 26, 29]. However, some studies followed up the patients for longer periods, exceeding two years post-treatment, with divergent results [27]. One study reported a higher rate of tumor recurrence in the group treated with laser [28], while others did not show significant differences between the groups [25-27, 29].

Brandão *et al.* [26] performed a retrospective analysis of 152 patients with advanced stage (III and IV) tumors who received PBM as standard treatment for oral mucositis, observing no negative effects of PBM on survival outcomes, development of new tumors, and local and distant recurrence [26]. However, the retrospective nature of the studies [26, 30] and the heterogeneity of the population limit the strength of the conclusions.

Another study [25] evaluated preliminary clinical evidence suggesting that PBM may modulate tumor response in patients with head and neck cancer. Analysis of overall survival showed a trend toward improvement in the PBM-treated group, with a rate of 57.4% compared with 40.4% in the control group [25]. This is possibly associated with the influence of PBM on the prevention and recovery of OM, which may have impacted swallowing and nutrition, as well as the individual's tolerance to treatment.

Although prevention of mucositis is a possible mechanism, ensuring completion of treatment and nutrition, modulation of the tumor microenvironment, and interaction with radiochemotherapy may also be involved. This hypothesis was raised based on studies that found inhibition of cell viability and proliferation [31, 32]. The antiproliferative effect of PBM has been investigated, and preclinical studies suggest the hypothesis that modulating the cell cycle, inducing arrest in specific phases, such as the S phase, and inhibiting DNA replication may influence the tumor microenvironment [31-34]. Our results do not allow us to identify any inhibitory effect with the daily application of PBM.

Tumor heterogeneity, as highlighted by Sonis [18], raises significant concerns regarding the safety of PBM application in cancer treatment. Variability in cellular response to PBM may result in adverse effects, especially in subgroups of patients with tumors that present specific molecular characteristics. The absence of a complete safety profile and the possibility of off-target effects reinforce the need for this study model. The response to PBM is not uniform among different tumor types, highlighting the need for additional *in vivo* studies and the personalization of therapy based on the molecular and genomic characterization of malignancies before treatment.

When interacting with biological tissues, PBM promotes the absorption of photons by chromophores, triggering a series of biochemical reactions that can stimulate or inhibit cells and result in therapeutic effects such as modulation of inflammation and stimulation of cell proliferation, regardless the cell type – tumor or healthy cells. In terms of safe use of the resource, the standardized approach, with protocols that are not individualized, for the application of PBM in oncology is not the most appropriate and, in terms of safety, direct application in tumor cells is questionable [18].

In addition to observing the effects on the proliferation rate, it is important to elucidate possible changes in the cellular signaling pathways involved in tumor progression, which may be influenced by PBM. Sperandio *et al.* [20], evaluating the Akt/mTOR pathway, demonstrated that PBM induces a more aggressive phenotype in oral SCC cells and oral dysplastic cells. The regulation of proteins such as pAkt, pS6, and cyclin D1, associated with cell proliferation and invasion, corroborates the hypothesis that PBM may promote tumor progression. These results suggest that PBM, by modulating the Akt/mTOR pathway, may significantly influence the biological behavior of oral neoplastic cells. Thus, although PBM has therapeutic applications, its use in patients with dysplastic or malignant oral lesions should be considered with caution, due to its potential to increase tumor growth aggressiveness.

Rhee *et al.* [35] demonstrated that a wavelength of 650 nm and a dose of 30 J/cm² exacerbates tumor growth in an orthotopic model of anaplastic thyroid carcinoma in mice. Immunohistochemistry revealed a significant increase in the expression of HIF-1α and p-Akt in tumor cells, suggesting activation of the Akt/HIF-1α pathway. Concomitantly, a decrease in TGF-β1 expression was observed. These results indicate that PBM, by negatively modulating the TGF-β1 pathway and activating the Akt/HIF-1α pathway, promotes a tumor microenvironment more conducive to cell proliferation and resistance to apoptosis in anaplastic thyroid carcinoma cells.

Regarding the effect of PBM on cell viability, studies are controversial. While Pinheiro *et al.* [36] did not observe an increase in cell viability after daily application with doses ranging from 0.04 to 4.8 J/cm² in the H.Ep.2 cell line, Al-Watban *et al.* [37] reported both proliferative and inhibitory effects, depending on the dose and cell line. In the latter study, doses between 60 and 180 mJ/cm² promoted cell proliferation, while higher doses (420-600 mJ/cm²) showed an inhibitory effect in EMT-6 and RIF-1 cell lines. In the SCC25 cell line, Schalch *et al.* [32] observed a decrease in cell viability and increased apoptosis after three days of treatment with PBM, suggesting an antitumor effect *in vitro*. The discrepancy in the results can be attributed to several factors, such as differences in cell lines, dosimetric parameters, and methodologies used.

Sperandio *et al.* [20] observed that 2 J/cm2 was able to increase the expression of cyclin D1 in the SCC9 cell line 24 h and 72 h after irradiation, as well as increase the expression of phosphorylated S6 protein, suggesting that PBM may contribute to the aggressiveness of tumor cells. Our study did not identify significant changes in cell viability and proliferation; however, additional analyses are necessary to better understand the metabolism of light in tumor cells.

In the study by Schalch *et al.*, a reduction in mitochondrial activity was found in laser-irradiated SCC9 cells using 11 different dosimetric parameters. The results demonstrated significant heterogeneity in the effects of PBM on the cell viability of SCC9 and HeLa cell lines. The application of different dosages (1, 3, 5, 6, 10, and 20 J/cm²) and exposure times (1, 2, 4, and 72 hours) with 660 and 780 nm resulted in variable cellular responses, including inhibition of proliferation, absence of effect and, in some cases, even growth stimulation [32]. However, unlike the other parameters employed, the 660 nm laser with 30 mW and 2 J/cm2 did not lead to a significant reduction compared to non-irradiated cells (control) in the MTT test 1 day after irradiation. These data are in line with our findings, where the 658 nm wavelength did not demonstrate a significant effect regardless of the cell line. This diversity of results, combined with the complexity of the *in vitro* models and the variability inherent to each cell line, highlights the need for standardization of PBM protocols to ensure the reproducibility of the results and the clinical translation of the findings.

Ibarra *et al.* emphasize that PBM, depending on the dose and frequency of application, can induce biphasic effects on cell viability. While a single application promoted an increase in viability (with 3 J/cm^2), daily applications with a higher dose induced a cytotoxic effect. However, the self-renewal capacity of CTCs, assessed by the expression of the CD44 and ESA markers, was not significantly altered by PBM. Interestingly, the frequency of sphere formation and the expression of the BMI1 gene, markers associated with the maintenance of the stem cell phenotype and therapeutic resistance, were reduced after daily applications with a higher dose (6 J/cm^2)[21].

The application of PBM in patients with HNC undergoing oncological treatment raises crucial questions about its safety. Current evidence does not allow a definitive conclusion about the impact of PBM on tumor progression. Given this scenario, health professionals must inform patients about the nature of this therapy and the potential associated risks, especially considering the divergence of data on its influence on tumor response. It is not possible, with the existing data, to consider PBM a risk-free technique, as well as that it can cause harm. The nature of experimental models, such as *in vitro* studies, does not represent the complexity of *in vivo* tumor microenvironment and the host immune response that plays crucial roles in tumor progression, which makes it difficult to translate these results into daily clinical practice.

CONCLUSION

PBM has found increasingly broad applications in the health field, being used in several areas such as physiotherapy, nursing, dentistry, and speech-language pathology. In the treatment of HNC, multidisciplinary rehabilitation, often aided by technologies, has proven to be essential. However, the use of PBM in different specialties, although can promote benefits, requires caution, since the physiological changes that occur in the head and neck region can be the target of multiple interventions, increasing the risk of overdose and adverse effects. Furthermore, *in vitro* studies, although fundamental for the understanding of the cellular and molecular mechanisms associated with tumor biology, are only the first step towards a complete understanding of the effects of PBM on tumor cells *in vivo*.

The results suggest that, *in vitro*, the SCC09 and CAL 27 cell lines show low potential for interaction with PBM. There was no significant effect on the proliferation rate or cell viability, regardless of the tested wavelengths (630 nm and 830 nm) and intensities (1 J, 3 J, and 6 J). In summary, our findings enhance the understanding of PBM effects on these head and neck cancer cell lines. However, further studies are needed to examine other types of neoplastic cells, as well as the adjacent non tumoral cells. Additionally, the evaluation of PBM's interaction with cultured cells *in vitro* is limited by the absence of a tumor microenvironment. These methodological constraints decrease the predictability of the experimental model, but they also motivate continued research to gather information that could aid in assessing the safety of PBM use in areas potentially affected by neoplastic cells.

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TABLES:

Table 1: Characteristics of the different modes of electromagnetic radiation in the study

	Mode 1	**Mode 2**
Wave-length (λ = nm)	658 ± 10%	830 ± 10%
Average LASER power (mW)	100 ± 20%	150 ± 20%
Electromagnetic spectrum	Visible red	Infrared
Mode	Continuous	Continuous
Beam area (mm^2)	12,566	12,566
Exit spot diameter (cm^2)	0.03	0.03
Beam divergence (degrees)	8	12
Radiation emission mode	Continuous	Continuous
Dose/cm^2	1 J, 3 J, 6 J	1 J, 3 J, 6 J

J: joule; nm: nanometers; mm: millimeters

FIGURES

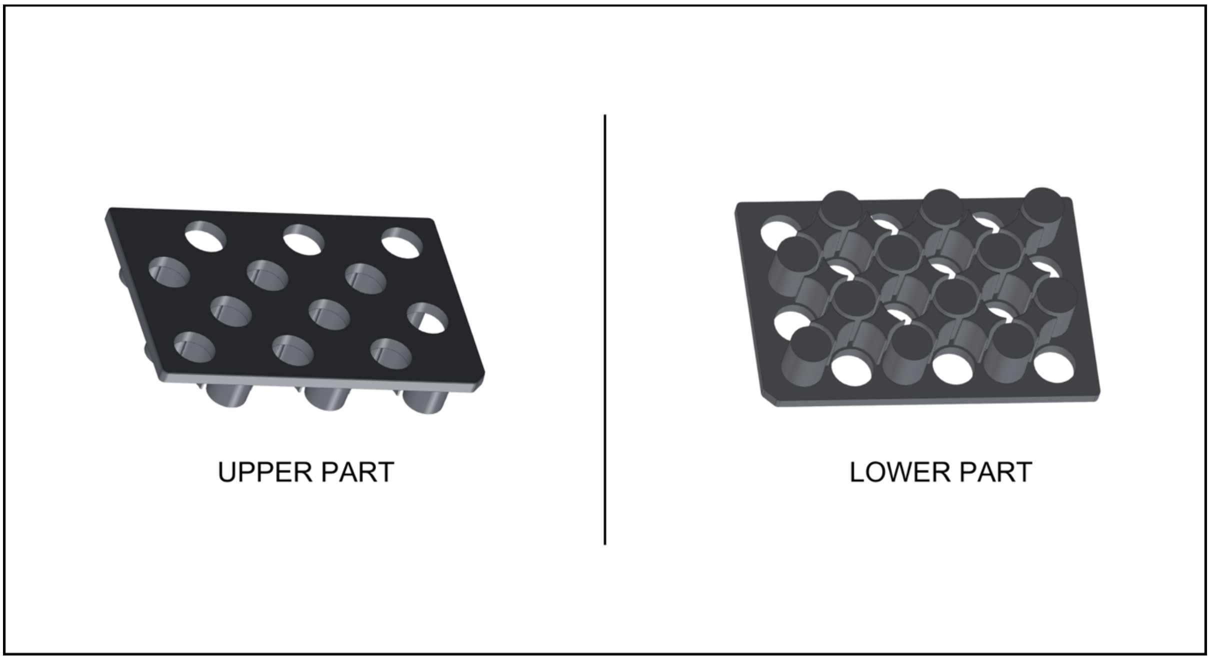

Fig 1 Prototype created for adaptation to the 24-well culture plate

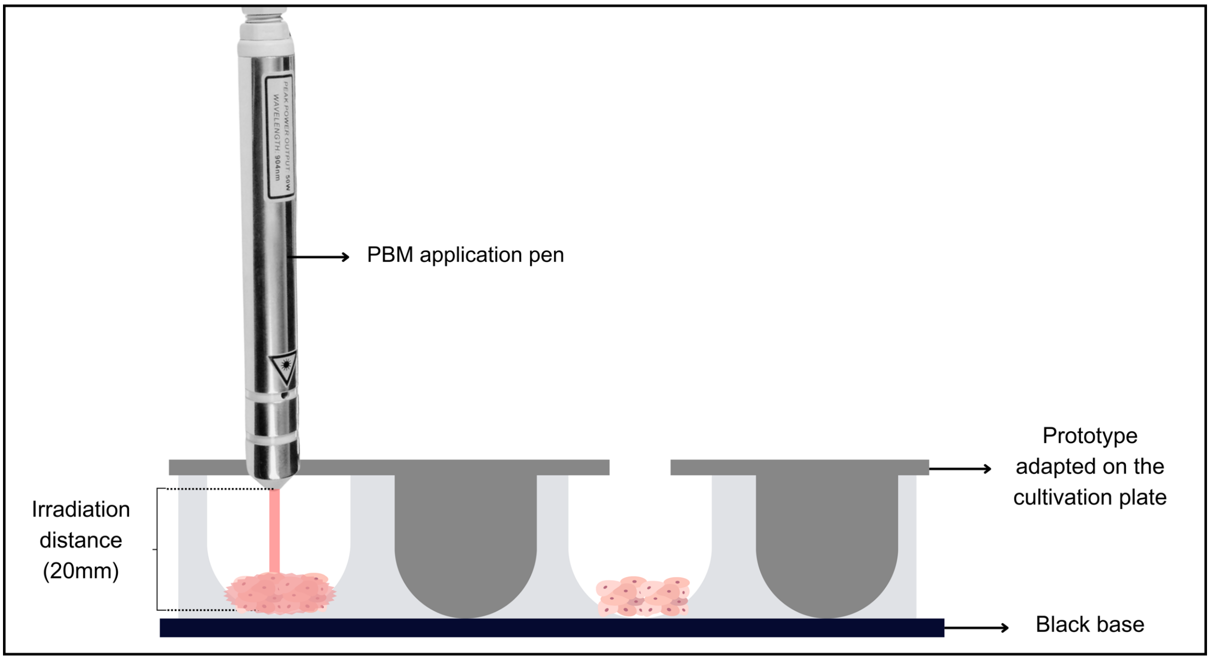

Fig 2 PBM application scheme with the adaptation of the prototype on the cultivation plate.

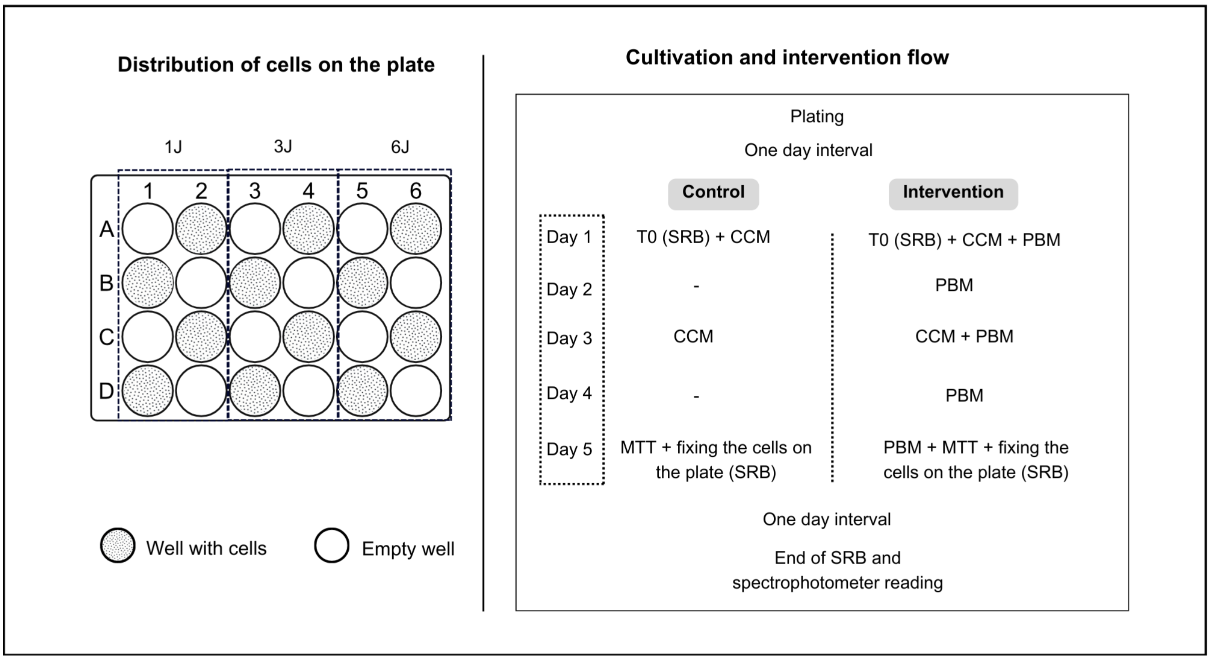

Fig 3 Division of the cultivation plate according to the PBM application doses and cultivation and intervention flow. J: Joule; T0: zero time; SRB: Sulforhodamine; MTT: ([3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium] bromide; CCM: change of culture medium; PBM: photobiomodulation; - : without intervention.

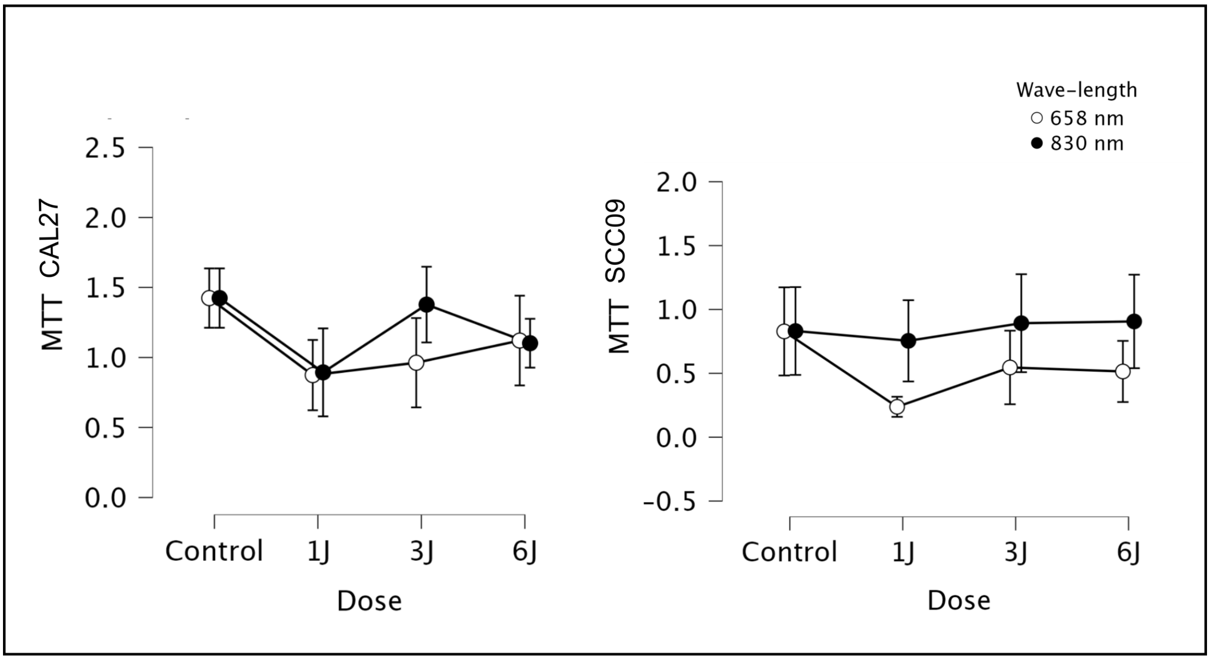

Fig 4 Analysis of cell viability in CAL27 and SCC09 cell lines after irradiation with 658nm and 830nm LASER. Data are reported as mean ± SD, and each graph is representative of at least three independent experiments. Statistical significance was tested by a two-way ANOVA and represents $p < 0.05$ respectively, between the treated sample and the untreated sample used as control.

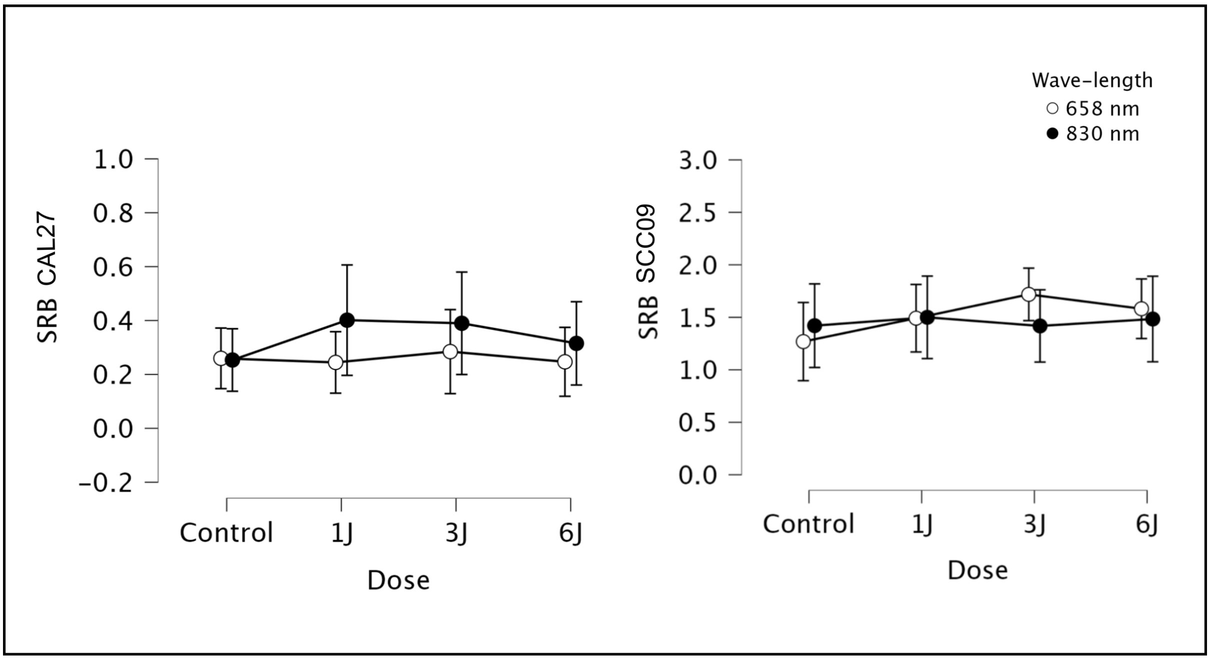

Fig 5 Analysis of proliferation in CAL27 and SCC09 cell lines after irradiation with 658nm and 830nm LASER. Data are reported as mean ± SD, and each graph is representative of at least three independent experiments. Statistical significance was tested by a two-way ANOVA and represents $p < 0.05$ respectively, between the treated sample and the untreated sample used as control.

6 CONCLUSÃO GERAL

Os recursos como a eletroestimulação e a fotobiomodulação são técnicas que podem potencializar a reabilitação em Fononcologia visando acelerar o processo terapêutico. São recursos complexos e que mantém algumas lacunas cujas respostas estão sendo construídas por uma base sólida de evidência científica.

Independente da tecnologia, esta deve se somar às demais estratégias cotidianas para promover ganhos em caráter complementar como identificado em nossos estudos. Reflexões acerca das indicações, momento de iniciar, parâmetros, modo de aplicação são válidas e necessitam de continuidade dos experimentos para que, em breve, seja possível auxiliar na tomada de decisão do fonoaudiólogo.

Quanto à FBM, nenhum dos estudos existentes foi desenhado de forma adequada para testar o momento ideal de início e, portanto, as condições clínicas do paciente permanecem sendo o requisito obrigatório para a indicação e início do uso no CCP. No quesito segurança, até o momento, não se pode expandir, para todos os pacientes, sem hesitar, a aplicação da FBM em regiões onde estão presentes células com displasia ou mesmo tumorais independente da finalidade. Como discutido na revisão de literatura desta tese, a terapia com luz interage em nível sistêmico, molecular e genético, promovendo benefícios clínicos, porém sem descartar um possível efeito sobre as células cancerígenas. Essa permanece uma questão crítica que ainda carece de uma resposta definitiva.

7 IMPACTOS DO TRABALHO

A presente pesquisa, ao investigar a aplicação da eletroestimulação e da fotobiomodulação na reabilitação de pacientes com câncer de cabeça e pescoço, considerando também a avaliação da segurança *in vitro*, apresenta um potencial significativo para gerar impactos positivos em diversas esferas da saúde.

A identificação de protocolos seguros e eficazes para o uso dessas tecnologias pode contribuir significativamente para a melhoria da qualidade de vida dos pacientes, reduzindo sintomas como disfagia, disfonia e dor, e aumentando sua independência funcional. A avaliação da segurança *in vitro* é fundamental para entender os efeitos associados ao uso dessas terapias no nível celular, fornecendo subsídios para estudos *in vivo* para a identificação de possíveis efeitos adversos e a otimização dos protocolos de tratamento.

O aprofundamento do conhecimento sobre os mecanismos de ação dessas tecnologias pode permitir a personalização dos tratamentos, considerando as características individuais de cada paciente. Além disso, a reabilitação fonoaudiológica, associada ao uso dessas tecnologias, pode auxiliar na recuperação das funções estomatognáticas. Ao participar ativamente do processo de reabilitação e ter acesso a novas tecnologias, os pacientes podem se sentir mais empoderados e capazes de lidar com os desafios impostos pela doença.

A otimização dos protocolos de tratamento e a redução de complicações podem levar à diminuição dos custos com saúde, tanto para os pacientes quanto para os sistemas de saúde. A recuperação mais rápida das funções comprometidas pela doença pode permitir que os pacientes retornem mais rapidamente às suas atividades cotidianas e profissionais, aumentando a produtividade e gerando benefícios econômicos para a sociedade.

A divulgação dos resultados da pesquisa pode contribuir para a disseminação do conhecimento científico e podem levar à atualização dos protocolos clínicos, garantindo que os profissionais da saúde utilizem as melhores práticas disponíveis. O conhecimento gerado pode contribuir para a formação de profissionais mais qualificados para atuar na área da reabilitação fonoaudiológica, com conhecimento sobre as novas tecnologias e seus benefícios. A presente pesquisa possui um potencial significativo para gerar impactos positivos na vida de pacientes com câncer de cabeça e pescoço, além de contribuir para o avanço da ciência e da tecnologia na área da reabilitação fonoaudiológica. Os resultados obtidos podem servir como base para futuras pesquisas e para o desenvolvimento de novas terapias mais eficazes e seguras.

9 ANEXOS

ANEXO A

Registro Comissão de Pesquisa da UFCSPA

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA EDUCAÇÃO

UFCSPA

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE
COMISSÃO DE PESQUISA

Atestado

Atestamos que o projeto de pesquisa intitulado *"Efeitos da fotobiomodulação sobre a patofisiologia de linhagens de carcinoma de células escamosas da cavidade oral"* está registrado na Comissão de Pesquisa da Universidade Federal de Ciências da Saúde de Porto Alegre com o número 065/2019, sob responsabilidade de Gisele Branchini.

Salientamos que este registro **não autoriza o pesquisador a coletar ou analisar dados oriundos de sujeitos de pesquisa**.

Salientamos ainda que este registro **não garante a concessão de recursos financeiros por parte da UFCSPA a este projeto de pesquisa**.

Porto Alegre, 20 de maio de 2019.

Dinara Jaqueline Moura
Coordenadora
Comissão de Pesquisa
UFCSPA

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