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IGOR FELIPE PEREIRA LIMA

USO DA AUTOFLUORESCÊNCIA E DE SONDAS FLUORESCENTES COMO POTENCIAL FERRAMENTA DE DETECÇÃO PRECOCE DO CÂNCER BUCAL: UMA REVISÃO SISTEMÁTICA

Porto Alegre
2021

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Dissertação apresentada como requisito parcial para a obtenção do título de Mestre em Odontologia, área de concentração Patologia Bucal, ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul.

Orientador: Prof. Dr. Marcelo Lazzaron Lamers

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_____

Prof. Dr. Marcelo Lazzaron Lamers - UFRGS

_____

Prof. Dr. Manoela Domingues Martins - UFRGS

_____

Prof. Dr. José Antônio Poli Figueiredo - UFRGS

_____

Prof. Dr. Felipe Rodrigues de Matos - UFS

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Antecedentes e Justificativa

OMS - Organização Mundial de Saúde

CEC - Carcinoma Espinocelular Oral

DNA - Ácido desoxirribonucleico

RNA - Ácido ribonucleico

HPV - Papiloma Vírus Humano

EMT - Transição epitélio-mesênquima

TME - Microambiente tumoral

Artigo científico

OSCC - Oral Squamous Cell Carcinoma

PCR - Polymerase Chain Reaction

PRISMA - Preferred Reporting Items for Systematic Review and Meta-Analyses

DeCS - Health Sciences Descriptors

MeSH - Medical Subject Headings

Emtree - Embase Subject Headings

RESUMO

O carcinoma espinocelular oral (CEC) é uma das neoplasias mais frequentes da cavidade bucal. Contudo, a maioria dos pacientes é diagnosticada em estágios avançados, minimizando a chance de cura, piorando a sobrevida e dificultando o tratamento a ser realizado. Assim, a detecção precoce das lesões de CEC é fundamental para o diagnóstico em estágios iniciais. Diversos métodos não invasivos têm sido propostos para este fim e alguns deles utilizam o mecanismo da autofluorescência tecidual e aplicação de sondas fluorescentes. Assim, o objetivo do presente trabalho foi realizar uma revisão sistemática acerca do uso da autofluorescência e de sondas fluorescentes como potencial ferramenta de detecção precoce do câncer bucal. Quatro bases de dados (PubMed, Embase, Web of Science e Scopus) foram utilizadas como fonte de busca, no qual foram incluídos estudos que usaram autofluorescência e sondas fluorescentes para detecção do câncer bucal. O estudo foi registrado no PROSPERO sob número CRD42020169535. Quarenta e cinco estudos foram selecionados para a revisão sistemática. O VELscope foi o dispositivo mais utilizado para autofluorescência (sensibilidade 33% a 100% e especificidade 12% a 88%) e se mostrou eficaz na distinção de mucosa bucal normal e neoplásica. Por outro lado, a adição de sondas fluorescentes no tecido neoplásico se baseia no princípio do acúmulo de moléculas fluorescentes nas células tumorais que, após uma reação fotodinâmica, permite a distinção de tecido normal e tumoral. Observamos que as sondas fluorescentes, especialmente o 5-ácido aminolevulínico (sensibilidade de 90% a 100% e especificidade de 51% a 96%), também são estratégias promissoras na detecção precoce do CEC. Diante disso, a avaliação da autofluorescência tecidual e a aplicação de sondas fluorescentes devem ser encorajadas, pois permitem a distinção da mucosa bucal normal e alterada, auxiliando o cirurgião-dentista em sua rotina clínica na triagem de pacientes e, consequentemente, aumentando as chances do diagnóstico do CEC em estágios iniciais.

Palavras-chave: Corantes Fluorescentes; Diagnóstico; Detecção Precoce de Câncer; Fluorescência; Neoplasias bucais.

ABSTRACT

Oral squamous cell carcinoma (CPB) is one of the most frequent neoplasms of the oral cavity. However, most patients are diagnosed in advanced stages, minimizing the chance of cure, worsening survival and making treatment more difficult. Thus, the early detection of CPB lesions is essential for early diagnosis. Several non-invasive methods have been proposed for this purpose and some of them use the mechanism of tissue autofluorescence and application of fluorescent probes. Thus, the objective of the present study was to carry out a systematic review about the use of autofluorescence and fluorescent probes as a potential tool for early detection of oral cancer. Four databases (PubMed, Embase, Web of Science and Scopus) were used as a search source, which included studies that used autofluorescence and fluorescent probes to detect oral cancer. The study was registered with PROSPERO under number CRD42020169535. Forty-five studies were selected for systematic review. The VELscope was the most used device for autofluorescence (sensitivity 33% to 100% and specificity 12% to 88%) and was effective in distinguishing between normal and neoplastic oral mucosa. On the other hand, the addition of fluorescent probes to neoplastic tissue is based on the principle of accumulation of fluorescent molecules in tumor cells, which, after a photodynamic reaction, allows the distinction between normal and tumor tissue. We observed that fluorescent probes, especially 5-aminolevulinic acid (sensitivity from 90% to 100% and specificity from 51% to 96%), are also promising strategies in the early detection of CPB. Therefore, the evaluation of tissue autofluorescence and the application of fluorescent tubes should be encouraged, as they allow the distinction between normal and altered oral mucosa, assisting the dental surgeon in his clinical routine in screening patients and, consequently, increasing the chances of diagnosis of CPB in early stages.

Keywords: Diagnosis; Early Detection of Cancer; Fluorescence; Fluorescent Dyes; Mouth Neoplasms.

SUMÁRIO

1 ANTECEDENTES E JUSTIFICATIVA

O câncer é uma doença que envolve a transformação de células normais em malignas. Esse processo é mediado por alterações genéticas e influenciado por uma série de fatores conhecidos como "*hallmarks of cancer*". Tais marcadores envolvem sinais de crescimento, resistência à apoptose e supressores de crescimento, indução à angiogênese, mitoses descontroladas, invasão tecidual, metástase, desregulação do metabolismo energético e evasão da resposta imunológica (CREE, 2011; HANNAHAN; WEINBERG, 2011).

Os dados sobre o câncer no mundo são alarmantes. A Organização Mundial da Saúde (OMS) estima que quase 10 milhões de pessoas morreram de câncer em 2018 em todo o mundo e cerca de 300.000 novos casos são diagnosticados a cada ano na faixa etária de 0 a 19 anos (WORLD HEALTH ORGANIZATION, 2018). Economicamente, a OMS estima que se gasta, anualmente, U$ 1,16 trilhões com tratamentos para o câncer no mundo (WORLD HEALTH ORGANIZATION, 2018).

1.1 Carcinoma Espinocelular Oral

O carcinoma espinocelular oral (CEC) é a neoplasia epitelial maligna mais comum da cavidade bucal. Apresenta-se com diferentes graus de diferenciação que pode acometer a região do vermelhão dos lábios, assoalho de boca, língua, mucosa bucal, superfícies gengivais, trígono retromolar, palato duro e palato mole (HUANG *et al*., 2010). Esta neoplasia possui caráter altamente invasivo e com potencial de metástase (AL-AFIFI *et al*., 2019), o que dificulta o tratamento, uma vez que grande parte dos pacientes não buscam atendimento de imediato e, quando buscam, são diagnosticados com estágio avançado da doença (LLEWELLYN; JOHNSON; WARNAKULASURIYA, 2004).

1.2 Aspectos epidemiológicos

O CEC atinge milhões de pessoas ao redor do mundo (LI et al., 2019), sendo considerado um problema de saúde pública global (RADHAKRISHNAN *et al*., 2012). O câncer bucal é o sexto tipo de câncer mais comum, possuindo uma taxa de sobrevida de 5 anos que não melhorou nos últimos 30 anos, permanecendo em torno de 50% (DOS REIS et al., 2008; CALIXTO *et al*., 2014),

sendo que a maioria desses diagnósticos são realizados em estágio III e IV (WORLD HEALTH ORGANIZATION, 2018).

As taxas de incidência e mortalidade variam entre os países, sendo influenciada por diversos fatores (WORLD HEALTH ORGANIZATION, 2018). Os últimos dados divulgados pela OMS revelaram uma incidência estimada em 354.864 casos de câncer de boca em 2018 no mundo. O resultado é ainda mais alarmante quando analisada a distribuição entre os continentes. Dos 354.864 casos, 221.046 (63,5%) são encontrados somente na Ásia, enquanto a América Latina e Caribe concentram 5,7% dos casos. Se comparado entre homens e mulheres, observa-se que a taxa é maior em homens, representando 246.420 (69,44%) dos casos. De maneira semelhante, a taxa de mortalidade foi de 177.384 em 2018, dos quais 119.693 (67,47%) foram representados por homens (WORLD HEALTH ORGANIZATION, 2018). Para 2020, dados da GLOBOCAN estimaram cerca de 377.713 casos de câncer bucal, com uma mortalidade estimada em 177.757 (IARC, 2020).

A população brasileira é uma das maiores do mundo e a preocupação com o desenvolvimento do câncer bucal deve ser levada a sério. Dados divulgados pelo Instituto Nacional do Câncer (BRASIL, 2018) revelaram as estimativas de câncer bucal para 2018, dos quais 11.200 novos casos são estimados em homens, enquanto 3.500 são estimados em mulheres. O Rio Grande do Sul é um dos estados com maior risco para o desenvolvimento de câncer bucal, possuindo uma taxa estimada de 15,72 casos para cada 100.000 homens e 3,80 para cada 100.000 mulheres. Esta taxa fica atrás apenas dos estados do Rio de Janeiro e Paraná (BRASIL, 2018)

1.3 Etiologia e fatores de risco

O CEC possui etiologia multifatorial, sendo que fatores de risco como tabagismo e alcoolismo, possuem importante associação com o seu desenvolvimento (BEYNON *et al*., 2018). O tabaco é um forte agente etiológico do CEC por promover danos à estrutura do DNA. As nitrosaminas, como a N-Nitrosonornicotina, são exemplos de agentes carcinógenos presentes no tabaco que aumentam a metilação do DNA (RADHAKRISHNAN *et al*., 2012). Além disso, a temperatura provocada pelo cigarro aceso promove alterações na mucosa bucal, especialmente aqueles que ficam em contato direto de forma

prolongada (FREITAS *et al*., 2016). O risco de desenvolvimento do CEC por tabaco é dependente da dose (BEYNON *et al*., 2018), tanto que indivíduos fumantes possuem um risco relativo de 3,43 de desenvolver CEC do que indivíduos não fumantes (CHI; DAY; NEVILLE, 2015).

Apesar do álcool também estar sendo apontado como um agente etiológico para o desenvolvimento de CEC, isso ainda não está claro. Estudos têm relatado que isso é ainda mais nocivo quando o indivíduo também é tabagista, uma vez que tal associação resulta em efeito sinérgico (RIBEIRO *et al*., 2017). O álcool promove o aumento da permeabilidade da mucosa, causando atrofia epitelial que facilita a penetração dos agentes carcinogênicos presentes no tabaco (DU *et al*., 2000), bem como atua nas alterações da expressão gênica, superexpressando microRNAs envolvidos no processo de progressão tumoral (SAAD *et al*., 2015). O acetaldeído, principal metabólito do álcool, causa danos à estrutura do DNA, interferindo na síntese e reparo do material genético (KUMAR *et al*., 2016). Carrard *et al*. (2012) sugeriram que os danos causados pelo álcool na mucosa oral são cumulativos e o aumento da proliferação celular ocorre em virtude do desequilíbrio bioquímico prolongado. Além disso, os efeitos sistêmicos do álcool, como lesões hepáticas, podem reduzir a capacidade do organismo de desintoxicar compostos prejudiciais, potencializando os efeitos nocivos dos componentes do tabaco (RADHAKRISHNAN *et al*., 2012). Contudo, a atuação do álcool independente do tabaco ainda é pouco elucidada na literatura, uma vez que os estudos encontram dificuldade de analisar pacientes que bebem, mas não fumam (KUMAR *et al*., 2016).

A exposição crônica à radiação ultravioleta sem a devida proteção constitui um importante fator de risco para o câncer de boca, especialmente no lábio (DAHER; PEREIRA; OLIVEIRA, 2008). A radiação ultravioleta promove alterações no ciclo celular por meio de espécies reativas de oxigênio, induzindo mutações no DNA (CLYDESDALE; DANDIE; MULLER, 2001; BOTA *et al*., 2017). Além disso, outros fatores de risco têm sido propostos na literatura científica, como a infecção pelo Papiloma Vírus Humano (HPV), higiene bucal, fatores genéticos e dieta (GUHA *et al*., 2007; GOLDENBERG, 2002). O HPV é um vírus fortemente associado ao desenvolvimento de CEC de orofaringe. Alguns tipos de HPV, considerados de alto risco, como os HPV’s 16, 18, 31, 33,

35 e 39 possuem capacidade de alterar o ciclo celular, inativando genes supressores de tumor, como a p53 e a pRb (KUMAR *et al*., 2016).

1.4 Alterações genéticas e epigenéticas envolvidas na progressão tumoral

A transição epitélio-mesênquima (EMT) foi identificada como uma das alterações mais importantes para a progressão tumoral (RUSCETTI *et al*., 2015). A EMT é um processo pelo qual as células epiteliais adquirem características fenotípicas de células mesenquimais que permitem metástases a distância pelo seu potencial migratório e invasivo (RUSCETTI *et al*., 2015). A indução da EMT permite que as células epiteliais percam as junções célula-célula, degradem a membrana basal através de enzimas e permite a migração individual e/ou coletiva das células tumorais (KREBS *et al*., 2017). Um aspecto marcante do processo de EMT é a redução da expressão da E-caderina, uma importante proteína presente na junção célula-célula, mediado por fatores de transcrição, como Snail, Twist e Zeb (NIETO *et al*., 2016). Em contrapartida, há o aumento da expressão de N-caderina e vimentina, proteínas características de células mesenquimais (SMITH *et al*., 2013). Tais alterações genéticas e epigenéticas ocorrem em um ambiente patológico que favorece a progressão tumoral (DEL PRETE *et al*., 2017).

1.5 Microambiente tumoral

O microambiente tumoral (TME) tem sido apontado como um dos fatores mais importantes envolvidos na tumorigênese (ARNETH, 2019). O TME é um ambiente patologicamente ativo formado por células tumorais e não-tumorais, abrangendo células do sistema imunológico, fibroblastos, vasos sanguíneos, matriz extracelular e moléculas de sinalização (DEL PRETE *et al*., 2017). Trata-se, portanto, de um ambiente com metabolismo reprogramado e com alterações na síntese de proteínas associadas ao reparo (DITTMER; LEYH, 2015). As interações destes componentes estimulam a proliferação das células tumorais (HANAHAN; WEINBERG, 2011) e favorecem a sua invasão a tecidos vizinhos (ARNETH, 2019).

As células endoteliais oferecem suporte nutricional para o crescimento e desenvolvimento do tumor por meio das ramificações dos vasos angiogênicos (HANAHAN; WEINBERG, 2011). A formação de novos vasos garante oxigênio,

nutrientes e excreção de metabólitos para as células tumorais, favorecendo a progressão do tumor (ZUAZO-GAZTELU; CASANOVAS, 2018). A ativação do processo angiogênico pode ser realizada por qualquer célula componente do microambiente tumoral, aumentando a capacidade de desenvolvimento do tumor (DE PALMA *et al*., 2017). Esse processo produz células-tronco tumorais que possuem a característica de gerar resistência contra eventos citotóxicos a partir de alterações genéticas e epigenéticas (CHEN *et al*., 2015).

O processo inflamatório associado ao tumor constitui uma barreira para a vigilância imunológica (BAYNE *et al*., 2012). As células do sistema imunológico – especialmente os macrófagos – atuam suprimindo as respostas antitumorais. Dependendo do estímulo, os macrófagos podem se polarizar no perfil M1 (pró-inflamatório e antitumoral) ou M2 (anti-inflamatório), podendo ser considerados importantes para o prognóstico dos pacientes com CEC (ALVES; DIEL; LAMERS, 2017). Adicionalmente, os fibroblastos desempenham atividade pró-tumoral ao permitir que as células tumorais migrem da localização do tumor primário para a corrente sanguínea e, com isso, promover metástases (ARNETH, 2019). Estudos mostraram que os fibroblastos associados ao câncer (CAFs) promovem um ambiente de matriz extracelular mais rígido, de modo a aumentar a permeabilidade para as células tumorais (PANKOVA *et al*., 2016; ERDOGAN *et al*., 2017). Além disso, os CAFs expressam altos níveis extracelulares de ligantes para quimiocinas – como o CXCL, CCL2 e CCL8 – e fatores de crescimento, de modo que seja possível remodelar a atividade das células tumorais (FEIG *et al*., 2013).

Outros grupos de células do sistema imunológico desempenham importantes funções no TME. Por exemplo, as células supressoras derivadas de mieloide, como os neutrófilos e as células dendríticas, inibem a resposta imunológica adaptativa por meio da ativação de inúmeras quimiocinas, como o fator estimulador de colônia de granulócitos-macrófagos (BAYNE *et al*., 2012). Aliado a isso, este grupo celular também estimula a angiogênese através da secreção de fator de crescimento endotelial vascular (VEGF), fator de crescimento de fibroblastos (FGF) e fator de crescimento transformador β (TGF-β), contribuindo, dessa forma, para a progressão tumoral (MOTZ; COUKOS, 2011).

A matriz extracelular (MEC) consiste numa rede de macromoléculas e por componentes que influenciam a comunicação celular (KORNEEV *et al*., 2017). Tais componentes incluem fatores de crescimento – como as integrinas – que facilitam a comunicação celular com o TME, bem como podem alterar suas propriedades físicas e de composição para promover a migração das células tumorais (WALKER *et al*., 2018). A velocidade de migração será mediada pelo gradiente de adesão (ARNETH, 2019).

Uma vez que as células tumorais migram e invadem locais diferentes do tumor primário, estabelece-se a metástase, um importante evento responsável por mais de 90% da mortalidade por câncer. Por isso a importância de impedir esse processo (VALASTYAN; WEINBERG, 2011). A metástase passa por diversos estágios, desde invasão local até a recolonização ectópica (CHEN *et al.*, 2015). O TME do local primário exerce impacto direto na cascata metastática por meio de sinalização parácrina, gerando um sistema de retransmissão quimiotática. A angiogênese induzida pela expressão de VEFG auxilia na dispersão do tumor (BRANCO-PRICE *et al*., 2012). Além disso, o TME cria um nicho receptivo para a chegada das células tumorais (CHEN *et al.*, 2015).

Não obstante, algumas terapias antineoplásicas sofrem com a resistência provocada por fatores estromais derivados do TME (CHEN *et al.*, 2015). Isso é resultado de um TME estruturado e organizado por meio de fatores extrínsecos celulares, como citocinas e fatores de crescimento que reduzem o estresse oxidativo e aumentam a estabilidade genômica das células-tronco tumorais para resistir aos efeitos terapêuticos (VISVADER; LINDEMAN *et al*., 2008). Assim, compreender como as interações ocorrem no TME para promover a progressão do tumor é fundamental para o direcionamento de novas estratégias terapêuticas e manejo dos pacientes com câncer (ARNETH, 2019).

1.6 Características clínicas

A língua é o sítio mais afetado da cavidade bucal, seguido da mucosa labial, gengiva, palato e mucosa alveolar (DHANUTHAI *et al*., 2018). Clinicamente, as lesões de CEC são, geralmente, precedidas por uma placa branca (leucoplasia), vermelha (eritroplasia) ou mista (leucoeritroplasia) (RADHAKRISHNAN *et al*., 2012). Tais alterações de mucosa podem, ou não, se transformar em CEC e, por isso, são denominadas desordens potencialmente

malignas, um grupo de lesões com risco variável de transformação maligna (CHI; DAY; NEVILLE, 2015).

As leucoplasias são definidas como placas brancas que não podem ser caracterizadas clinicamente ou patologicamente como outra doença (CHI; DAY; NEVILLE, 2015) e possuem potencial de transformação maligna (BROUNS *et al*., 2014). Pode ocorrer em qualquer lugar da cavidade bucal e, normalmente, é assintomática, com risco de transformação maligna anual entre 2% e 3% (CARRARD; WAAL, 2018). A leucoplasia verrucosa proliferativa é uma forma agressiva da leucoplasia caracterizada por lesões multifocais, de superfície rugosa/verrucosa e de crescimento lento (CAPELLA *et al*., 2017). É uma variante de alto risco representada por placas brancas não homogêneas com alto potencial de transformação maligna (ABATI *et al*., 2020).

Por outro lado, as eritroplasias são lesões vermelhas que não podem ser diagnosticadas clinicamente ou patologicamente como qualquer outra patologia (CHI; DAY; NEVILLE, 2015). São menos frequentes do que as leucoplasias, contudo, possuem alta capacidade de transformação maligna, sendo, muitas vezes, observada displasia epitelial ou carcinoma *in situ* (CHI; DAY; NEVILLE, 2015). Clinicamente, as eritroplasias podem apresentar superfície áspera e granulosa (CHI; DAY; NEVILLE, 2015). Em geral, são lesões assintomáticas, embora alguns pacientes se queixem de dor e queimação (VILLA; VILLA; ABATI, 2011).

Além disso, as lesões de CEC podem apresentar padrão crescimento endofítico ou exofítico. O padrão de crescimento exofítico é caracterizado por uma lesão nodular com superfície ulcerada, bordos irregulares e endurecidos (RADHAKRISHNAN *et al*., 2012). Por outro lado, o crescimento endofítico é representado por uma área central deprimida, ulcerada e irregular, com borda laminada, que resulta da invasão do tumor, sendo considerada o aspecto clássico das lesões de CEC (RADHAKRISHNAN *et al*., 2012). Tais lesões ulceradas normalmente não cicatrizam, permanecendo por algum tempo nesta condição e causando, em alguns casos, dor e sangramento (BAGAN; SARRION; JIMENEZ, 2010). As lesões em lábio normalmente são representadas por uma ulceração com superfície crostosa endurecida. O indivíduo pode manifestar outros sintomas, como aumento dos linfonodos cervicais, febre e disfagia, por exemplo. (CHI; DAY; NEVILLE, 2015).

1.7 Características histopatológicas

Microscopicamente é possível observar o tecido epitelial invadindo o tecido conjuntivo subjacente e formando ilhas e cordões, alcançando vasos sanguíneos e canais linfáticos (NEVILLE, 2004). A hiperceratose pode estar presente na superfície e a ulceração é um achado comum (NATARAJAN; EISENBERG, 2011). As anormalidades citológicas mais encontradas incluem hipercromatismo, pleomorfismo celular e nuclear, disceratose, acantose e mitoses frequentes. Além disso, presença de necrose e inflamação crônica também são frequentemente encontradas em lâminas histológicas de CEC (NATARAJAN; EISENBERG, 2011).

O CEC pode ser graduado em quatro níveis, baseado em sua característica histológica. O carcinoma de grau I apresenta um grau de ceratinização elevado, pouco polimorfismo nuclear, com padrão de invasão bem delineado e discreto infiltrado linfoplasmocitário. O carcinoma grau II é caracterizado por moderada ceratinização e polimorfismo nuclear, padrão de infiltração em cordões ou bandas e moderado infiltrado linfoplasmocitário. Por outro lado, o carcinoma grau III apresenta mínima ceratinização, abundante polimorfismo nuclear, com padrão de invasão com pequenos grupos ou cordões de células invasivas e leve infiltrado linfoplasmocitário. Por fim, o carcinoma grau IV é caracterizado por ausência de ceratinização, polimorfismo nuclear extremo, padrão de invasão com dissociação celular marcada e difundida em pequenos grupos e/ou em células individuais e ausência de infiltrado linfoplasmocitário (BRYNE *et al*., 1992).

Além disso, a OMS classifica o CEC histologicamente baseado nos níveis de diferenciação celular (BARNES *et al*., 2005). CECs bem diferenciados apresentam células escamosas dispostas em ilhas, com pérolas de queratina. Estas células são acidófilas e apresentam núcleos picnóticos, lembrando a arquitetura tecidual padrão do epitélio escamoso (BARNES *et al*., 2005; BUNGET *et al*., 2018). CECs moderamente diferenciados exibem ilhas de células epiteliais atípicas com núcleos de tamanhos e formas variados, sendo caracterizados por pleomorfismos celulares. Além disso, é possível observar invasão do estroma com raras pontes intercelulares e alta atividade mitótica

(BARNES *et al*., 2005). Por fim, CECs pouco diferenciados são caracterizados pelo predomínio de células imaturas, mitoses típicas e atípicas, com arquitetura e disposição completamente diferentes do epitélio normal (BARNES *et al*., 2005).

1.8 Diagnóstico

O diagnóstico precoce do CEC é fundamental para se obter um bom prognóstico dos pacientes (SHIN *et al*., 2010). A biópsia do tecido é considerada o padrão-ouro no diagnóstico do CEC. Este espécime sofrerá os processos histológicos habituais para permitir a análise morfológica dos tecidos e a sua relação com as estruturas adjacentes (NATARAJAN; EISENBERG, 2011). Contudo, a biópsia pode ser considerada uma limitação técnica para alguns profissionais, somado ao fato de que lesões extensas podem dificultar a técnica, pois é necessário selecionar as áreas mais representativas das lesões, tendo em vista que diferentes locais podem apresentar diferentes características histopatológicas, podendo induzir, dessa forma, ao erro no diagnóstico (EPSTEIN *et al*., 2002). Uma técnica alternativa inclui a biópsia líquida, que é um método de diagnóstico que vem ganhando destaque na comunidade científica por ser menos invasivo que a biópsia convencional (LOUSADA-FERNANDEZ *et al*., 2018). Este método baseia-se na extração de fluidos corporais, como sangue, urina, saliva para detectar as células tumorais circulantes, DNA e RNA tumoral circulantes, além de proteínas e exossomos (SIRAVEGNA *et al*., 2017; PENG *et al*., 2017). Contudo, este método deve ser associado à biópsia do local primário, exceto quando não há lesão clinicamente evidente (BARNES *et al*., 2005).

1.9 Autofluorescência e sondas fluorescentes

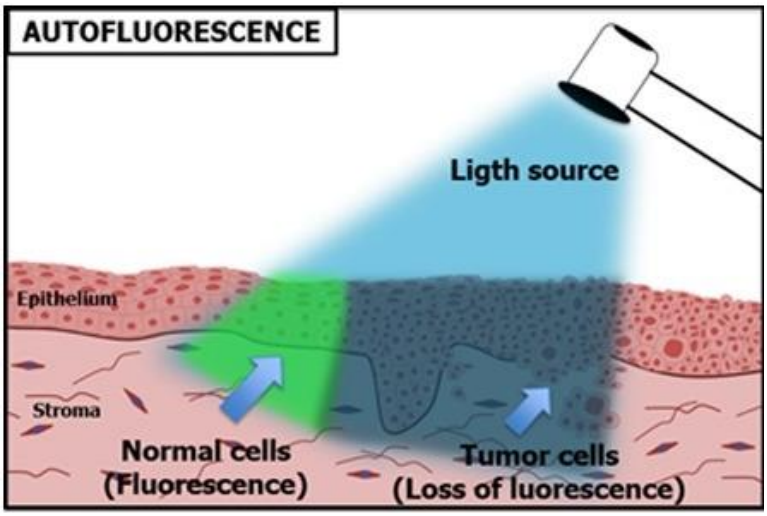
Além da biópsia, outros métodos para facilitar a detecção precoce têm sido propostos, como o azul de toluidina, um corante catiônico que supostamente se liga aos tecidos em proliferação, corando os ácidos nucleicos das células que estão em divisão (NATARAJAN; EISENBERG, 2011).

Com a descoberta de que os tecidos bucais contêm fluoróforos endógenos, dispositivos portáteis foram projetados para visualizar a fluorescência desses tecidos (SWEENY *et al*., 2011). Tais fluoróforos – como nicotinamida adenina dinucleotídeo (NADH), flavina adenina dinucleotídeo

(FAD), colágeno e elastina – absorvem a luz exógena e reemitem num comprimento de onda maior, exibindo fluorescência e podendo ser útil na detecção precoce do CEC (SHIN *et al*., 2010). Cada fluoróforo endógeno está associado a um comprimento de onda específico. Por exemplo, ao excitá-los a um comprimento de onda entre 375 e 440 nm, o tecido alterado apresentará uma faixa espectral escura devido à perda da capacidade de emissão de fluorescência causada pela alteração na concentração dos fluoróforos endógenos, enquanto a mucosa normal apresentará uma coloração verde pálida (NAGI *et al.*, 2016). Assim, a autofluorescência pode melhorar a capacidade de distinguir a mucosa normal do tecido neoplásico devido a emissão de sinal de fluorescência nos tecidos em diferentes comprimentos de ondas, sem a necessidade de agentes fluorescentes adicionais (NAGI *et al.*, 2016).

No câncer bucal, as células tumorais alteram a concentração dos fluoróforos nos tecidos, promovendo modificações nas propriedades espectrais da mucosa, favorecendo, assim, a sua percepção por meio da autofluorescência (Figura 1) (BALASUBRAMANIAM *et al*., 2015). Estudos que usam a autofluorescência para diagnosticar câncer bucal e de pele em pacientes demonstram que esta metodologia ajuda na detecção precoce (TAMOŠIŪNAS *et al*., 2020; HUANG *et al*., 2017). Contudo, é preciso que novos estudos sejam conduzidos a fim de estabelecer a sensibilidade e especificidade desses métodos alternativos de diagnóstico.

Figura 1 – Princípio de funcionamento da autofluorescência. A alteração celular provocada pelo processo tumorigênicos provoca alterações nas concentrações dos fluoróforos endógenos, levando a alterações de cor da mucosa. Uma fonte de excitação é direcionada ao tecido alterado para estimular a visualização direta dessas alterações. Como resultado, as células tumorais apresentarão cor escura, representando a perda de autofluorescência provocada pelas alterações nas concentrações dos fluoróforos endógenos. Por outro lado, as células não alteradas exibem aspecto verde pálido em virtude da concentração normal de fluoróforos endógenos (LIMA *et al.*, 2020).



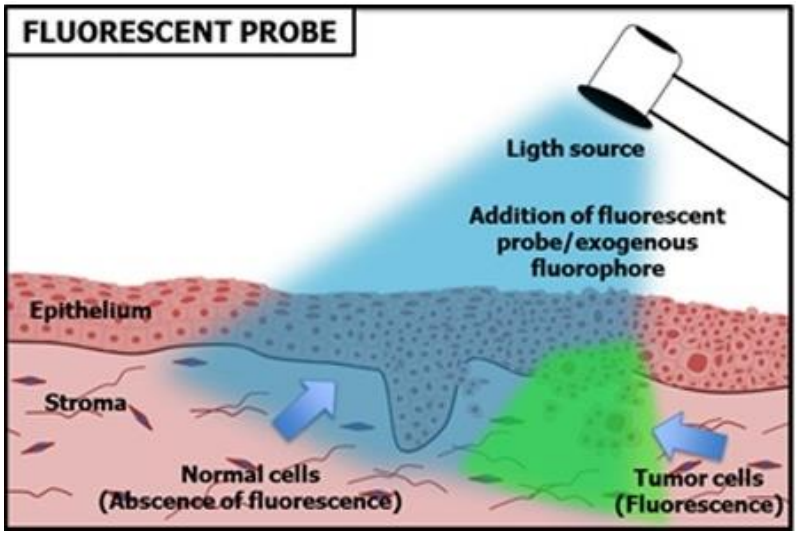
Uma dessas técnicas utiliza sondas fluorescentes no tecido neoplásico com o objetivo de observar alterações espectrais na mucosa produzidas pelas células tumorais (XIAO *et al*., 2018). As sondas são facilmente aplicadas – topicamente ou pulverizadas – sobre a mucosa e, após aguardar um tempo específico, tornam-se fluorescentes a partir do seu acúmulo nas células tumorais (Figura 2) (NAKAMURA *et al*., 2017). Para visualizá-las, é preciso excitá-las com auxílio de uma fonte de luz (Figura 2) (JENDZELOVSKA *et al*., 2016).

Várias sondas fluorescentes têm sido propostas para este fim, como o 5-ácido aminolevulínico (5-ALA), hipericina, γ-*glutamyl hydroxymethyl rhodamine green*, rodamina 610, rodamina 6G, porifirina, apresentado sensibilidades e especificidades variadas. O uso do 5-ALA parece ser o método mais promissor, uma vez que as células tumorais o metabolizam em protoporfirina IX (PpIX), um subproduto fluorescente (KATO *et al*., 2020). Com isso, as células tumorais acumulam seletivamente a PpIX em virtude do aumento da atividade da porfobilinogênio desaminase, redução da atividade da ferrocelatase e alteração

da atividade enzimática dessas células (MINAMIKAWA *et al*., 2016). Desse modo, a proposta deste método é avaliar a intensidade de fluorescência da PpIX (MINAMIKAWA *et al*., 2016). No entanto, um dos principais desafios durante a aplicação do 5-ALA é reduzir a autofluorescência tecidual, pois esta funciona como uma fonte de ruído na medição da PpIX e alguns métodos têm sido propostos para isso, como imagem de proporção de cor RGB (KONDO *et al*., 2014) ou detecção de pico espectral (VALDES *et al*., 2011). Apesar disso, a grande vantagem desta técnica é a sua alta sensibilidade, aliado à sua característica não-invasiva, tornando-a uma excelente opção para viabilizar o diagnóstico precoce de pacientes com CEC, melhorando o prognóstico e aumentando a sobrevida (HUSSEIN *et al*., 2018).

Diante do exposto, é preciso realizar o diagnóstico em estágios iniciais, uma vez que o atraso dificulta o tratamento, o torna mais oneroso e piora o prognóstico dos pacientes, reduzindo, dessa forma, a chance de cura. Com isso, utilizar de meios não invasivos como sondas fluorescentes e do mecanismo de autofluorescência pode ser uma estratégia promissora na detecção precoce do câncer bucal.

Figura 2 – Princípio de funcionamento das sondas fluorescentes. Ao adicionar as sondas fluorescentes no tecido alterado, as células tumorais acumulam essas moléculas em seu interior em virtude do seu elevado metabolismo. Uma fonte de luz é direcionada ao tecido com um comprimento de onda específico para servir de fonte de excitação dos fluoróforos. Como resultado, as células tumorais reemitem tal luz em um novo comprimento de onda, sendo possível detectar alterações as espectrais da mucosa alterada (LIMA *et al.*, 2020).



2 OBJETIVOS

2.1 Objetivo geral

- Avaliar, através de uma revisão sistemática da literatura, a eficácia do uso da autofluorescência e sondas fluorescentes como potencial meio de detecção precoce do câncer bucal a partir da detecção seletiva da fluorescência dos tecidos.

2.2 Objetivos específicos

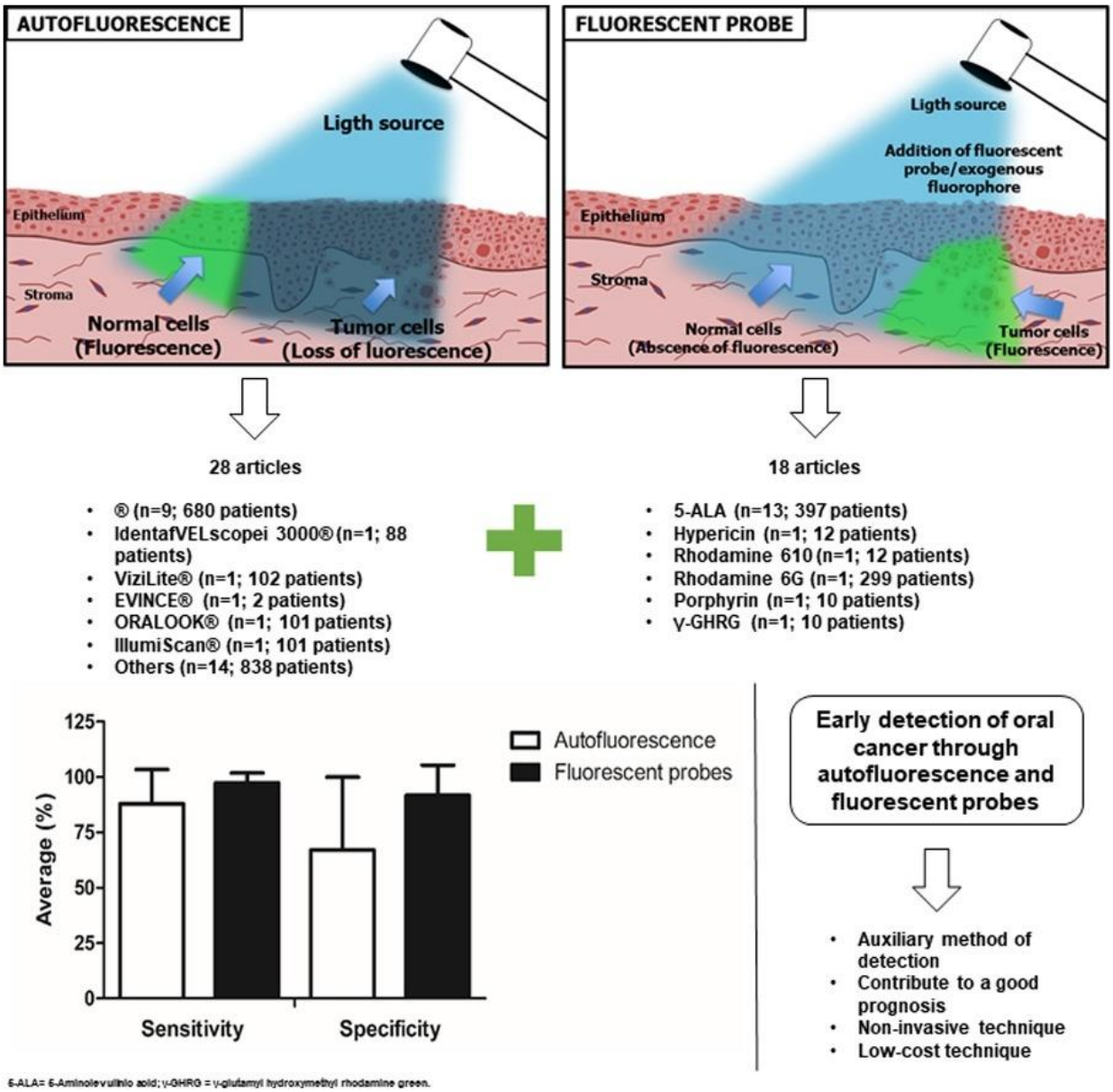
- Avaliar o poder discriminatório da autofluorescência e sondas fluorescentes na detecção precoce do câncer bucal.

3 ARTIGO CIENTÍFICO

Artigo científico publicado na *Photodiagnosis and Photodynamic Therapy* (fator de impacto: 2,894; DOI: 10.1016/j.pdpdt.2020.102073).

A sobrevida dos pacientes com CEC está diretamente relacionada ao estágio do tumor no momento do diagnóstico. Neste cenário, um dos principais desafios é realizar a detecção precoce, tendo em vista que a maioria dos pacientes é diagnosticada em estágios avançados, reduzindo a chance de cura e dificultando o tratamento. Diante disso, o presente estudo trata-se de uma revisão sistemática que avaliou a capacidade das sondas fluorescentes e do mecanismo de autofluorescência em detectar lesões de CEC a fim de auxiliar o cirurgião-dentista em sua rotina clínica. Observamos que, apesar da grande variabilidade entre sensibilidades e especificidades, ambos métodos demonstraram grande poder de detectar as lesões de CEC, podendo ser usado como uma ferramenta de auxílio e triagem de pacientes.

3.1 Graphical abstract



3.2 Artigo científico

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Use of autofluorescence and fluorescent probes as a potential diagnostic tool for oral cancer: A systematic review

Igor Felipe Pereira Lima [a], Luiza Meurer Brand [b], José Antônio Poli de Figueiredo [c], Liviu Steier [d], Marcelo Lazzaron Lamers [c,*]

[a] Department of Oral Pathology, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[b] Academic in Dentistry, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[c] Department of Morphological Sciences, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[d] Division of Restorative Dentistry, Penn Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA

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ABSTRACT

Introduction: The prognosis of patients with Oral squamous cell carcinoma (OSCC) are directly related to the stage of development of the tumor at the time of diagnosis, but it is estimated an average delay in diagnosis of 2–5 months. New non-invasive techniques for the early diagnosis of OSCC are being developed, such as methodologies to detect spectral changes of tumor cells. We conducted a systematic review to analyze the potential use of autofluorescence and/or fluorescent probes for OSCC diagnosis.

Material and Methods: Four databases (PubMed, Scopus, Embase and Web of Science) were used as research sources. Protocol was registered with PROSPERO. It was included studies that evaluated tissue autofluorescence and/or used fluorescent probes as a method of diagnosing and/or treatment of oral cancer in humans.

Results: Forty-five studies were selected for this systematic review, of which 28 dealt only with autofluorescence, 18 on fluorescent probes and 1 evaluated both methods. The VELscope® was the most used device for autofluorescence, exhibiting sensitivity (33%–100%) and specificity (12%–88.6%). 5-Aminolevulinic acid (5-ALA) was the most used fluorescent probe, exhibiting high sensitivity (90%–100%) and specificity (51.3%–96%). Hypericin, rhodamine 6 G, rhodamine 610, porphyrin and γ-glutamyl hydroxymethyl rhodamine green have also been reported.

Conclusion: Thus, the autofluorescence and fluorescent probes can provide an accurate diagnosis of oral cancer, assisting the dentist during daily clinical activity, but it is not yet possible to suggest that this method may replace histopathological examination.

1. Introduction

Oral squamous cell carcinoma (OSCC) is the most prevalent malignant neoplasm in the oral cavity, representing approximately 90 % of head and neck tumors [1]. The cure and survival are directly related to the stage of development of the tumor at the time of diagnosis. Thus, an early detection allows a minimally invasive approach, improving the prognosis [2]. Besides advances in the diagnostic process, the low 5-year survival rate (~50 %) for patients with OSCC has not improved in the past decades [3].

Histopathological examination is considered the gold standard in the diagnosis of oral cancer. However, biopsy may be a technical limitation for some professionals while extensive lesions may complicate the technique. This is because it is necessary to select the most representative areas since different locations have different histopathological characteristics that can lead to error in diagnosis [4]. Also, biopsy can have negative psychological effects on patients, such as anticipatory emotional stress, which makes many of them to be reluctant to perform the exam due to fear of the procedure [5], preventing the early detection of OSCC. It is estimated that the average delay in diagnosis is 2–5 months, enough time for the evolution of the pathological process [6] contributing to a worse prognosis [7].

Alternative diagnostic techniques have been used, such as exfoliative cytology and Polymerase Chain Reaction (PCR), but they encounter low

* Corresponding author at: Department of Morphological Sciences, Institute of Basic Health Science, Federal University of Rio Grande do Sul, Rua Sarmento Leite, 500, subsolo, Porto Alegre, Rio Grande do Sul, Brazil.
E-mail address: marcelo.lamers@ufrgs.br (M.L. Lamers).

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sensitivity [8] and high cost [9], respectively. Thus, new non-invasive techniques for the early diagnosis of OSCC are being developed, such as methodologies that use spectral changes in the mucosa produced by tumor cells [8]. Autofluorescence can improve the ability to distinguish normal mucosa from neoplastic tissue due to fluorescence signal emission in tissues at different wavelengths, without additional fluorescent agents [10–12]. Also, in order to increase the sensitivity of the diagnosis, it is also possible to use fluorescent probes that make the healthy or the neoplastic tissue fluorescent when exposed to a light source with a specific wavelength, thereby allow the visualization of lesions and detection of the margins [13]. For instance, 5-aminolevulinic (5-ALA), hypericin, γ-glutamyl hydroxymethyl rhodamine green, rhodamine and porphyrin probes are studied for their ability to differentiate normal from neoplastic tissue in patients with OSCC [14–16].

Since tissue spectral changes can be an important strategy for the early detection of OSCC, the aim of the present systematic review was to analyze the potential use of autofluorescence and fluorescent probes for the diagnosis of OSCC. Herein, we observed that autofluorescence and fluorescent probes have a good discriminating power between oral cancer and normal mucosa.

2. Materials and methods

2.1. Protocol and registration

This systematic review was performed following the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement and the Cochrane guidelines. The systematic review protocol was registered at PROSPERO database CRD42020169535.

2.2. Study design

This is a systematic review that aimed to answer the following guiding question PICOS: "Can autofluorescence and/or fluorescent probes (**Intervention**) be used as an accurate tool in the diagnosis (**Outcome**) of oral cancer (**Population**) when compared to biopsy (**Comparator**) considering only clinical studies (**Study design**)?".

2.3. Eligibility criteria

It was included studies that evaluated tissue autofluorescence and used fluorescent probes as a method of diagnosing and/or treatment of oral cancer in humans, without restriction on year, language or publication status (*in press*).

The following works were excluded: 1) studies not related to the theme; 2) animal studies; 3) *in vitro* studies; 4) review studies; 5) letters to the editor or editorials, conference summaries, personal opinions, books and / or book chapters.

Table 1
Search strategies in the databases.

Databases	Search strategies (May, 2020)	Results
PubMed http://www.ncbi.nlm.nih.gov/pubmed	("Fluorescence" OR" Fluorescent Dyes" OR "Fluorescent Agents" OR "Fluorochromes" OR "Fluorophore" OR "Fluorescent Probes") AND ("Oral Cancer" OR "Mouth Neoplasms" OR "Mouth Cancer" OR "Oral Neoplasms")	862
Scopus https://www.scopus.com/	("Fluorescence" OR" Fluorescent Dyes" OR "Fluorescent Agents" OR "Fluorochromes" OR "Fluorophore" OR "Fluorescent Probes") AND ("Oral Cancer" OR "Mouth Neoplasms" OR "Mouth Cancer" OR "Oral Neoplasms")	1172
Embase https://www.embase.com/	('fluorescent dyes'/exp OR 'fluorescent dyes' OR 'fluorescent agents' OR 'fluorochromes' OR 'fluorophore'/exp OR 'fluorophore' OR 'fluorescent probes'/exp OR 'fluorescent probes') AND ('oral cancer'/exp OR 'oral cancer' OR 'mouth neoplasms'/exp OR 'mouth neoplasms' OR 'mouth cancer'/exp OR 'mouth cancer' OR 'oral neoplasm')	796
Web Of Science http://apps.webofknowledge.com/	("Fluorescence" OR" Fluorescent Dyes" OR "Fluorescent Agents" OR "Fluorochromes" OR "Fluorophore" OR "Fluorescent Probes") AND ("Oral Cancer" OR "Mouth Neoplasms" OR "Mouth Cancer" OR "Oral Neoplasms")	520
TOTAL		3350

2.4. Sources of information and search

Two reviewers (IFPL and LMB) independently searched the electronic databases. The descriptors were researched in DeCS (Health Sciences Descriptors), MeSH (Medical Subject Headings) and Emtree (Embase Subject Headings) and the boolean operators "AND" and "OR" were used in order to potentialize the search strategy (Table 1). The results obtained were exported to the EndNote Basic/Online software (Thomson Reuters, Toronto, Canada), desktop version, and the duplicates were removed.

2.5. Study selection

The selection of studies was carried out in three distinct stages. The first step consisted of a systematic analysis of the titles of the records by two reviewers (IFPL and LMB). The articles that had the title according to the objective of this systematic review were selected for the second stage. At this stage, the same reviewers analyzed the abstracts of the articles and applied the inclusion/exclusion criteria. Studies that did not have available abstracts were evaluated in the third stage.

In the third and last stage, the articles were analyzed in full (full text). When the two reviewers disagreed about the study's eligibility, a third and fourth reviewers (MLL and JAPF) was consulted to make a final decision. Studies that did not meet the criteria were registered separately, clarifying the reasons for exclusion.

2.6. Process of data collection and extraction

Two authors (IFPL and LMB) performed data extraction in specific spreadsheets for this purpose. The following information was extracted from the eligible studies: author and year, sample (n), age (average), type of sample, comparisons, device used, excitation source, incubation time, fluorescent probe, fluorescence, white light *vs* biopsy *vs* fluorescence, tissue with higher fluorescence intensity, maximum fluorescence intensity, medium fluorescence spectrum, sensitivity and specificity.

As a calibration exercise, the reviewers (IFPL and LMB) discussed the eligibility criteria and applied them to a sample of 20 % of the retrieved studies to determine the agreement between examiners. After reaching an adequate level of agreement ($\kappa = 0.81-0.85$), the reviewers read all the studies independently. The divergences were resolved by consensus and supervision of the third and fourth reviewers (MLL and JAPF). An email was sent to authors whose studies contained insufficient data or information that would prevent them from summarizing and making comparisons with data from other eligible articles, so that additional information could be provided.

2.7. Risk of individual Bias of the studies

The risk of individual study bias was assessed by two independent authors (IFPL and LMB) with the aid of the "The Joanna Briggs Institute Critical Appraisal tools for use in JBI Systematic Reviews Checklist for Diagnostic Test Accuracy Studies". The risk of bias was ranked as ***High*** when the study reached up to 49 % of "yes" score, ***Moderate*** when the study reached from 50 % to 69 % of "yes" score, and ***Low*** when the study reached over 70 % of "yes" score (Table 3).

3. Results

3.1. Selection of studies

The search in the PUBMED, Web of Science, Embase and Scopus database resulted in 3350 articles. After removing the duplicate records, 2097 proceeded to analyze the titles. Of these, only 285 were considered potentially eligible. After abstract reading, it remained 44 articles in which the full text was analyzed. All references of the selected manuscripts (total of 1273 references) were carefully analyzed to find articles not captured in the main search strategy, of which one has been selected, totaling, therefore, 45 articles (Fig. 1).

3.2. Study characteristics

The publication date of the articles ranged from 1996 to 2019. Of the 45 studies evaluated, 11 articles used patients as a sample, while 5 studies used only biopsy and 29 studies included patient and biopsies samples. The mean age ranged from 49 to 76 years. The examination of the mucosa with white light (conventional), fluorescence and autofluorescence associated with biopsy were the methods used for comparisons (Table 2).

To direct the light, the device most reported was the endoscope [13,17–28]. The VELscope® (Apteryx Imaging, Akron OH, USA) was used in 9 articles [8,29–36], while a fiber optic probe and Identafi® (StarDental-DentalEZ, Lancaster, PA, USA) was evaluated in 5 [37–41] and 1 studies [42], respectively. Other devices were also described, as a photographic camera [43,44], oblique telescope [45], spectrograph [46], portable LIAF [47,48], Storz liquid light guide [49], spectrometer [37], EVINCE® [50], ORALOOK® [51], IllumiScan® [51] and Night Owl LB983 [52]. Four studies [53–56] used devices developed by themselves. One article [8] compared two diagnostic methods: VELscope® and Vizilite®. Only 1 study [57] did not inform which device was used (Table 2).

As for the excitation source, the xenon lamp and the diode laser were the most used with 15 and 7 studies, respectively. Of the 15 articles that used the xenon lamp, 2 used only violet light [19,20], 10 associated white and violet light [13,17,18,21–23,25–27,44], 1 white and blue light [49] and 2 did not inform which light was used [41,46]. Other sources of excitation have been reported, as blue light [29,31–34,51,

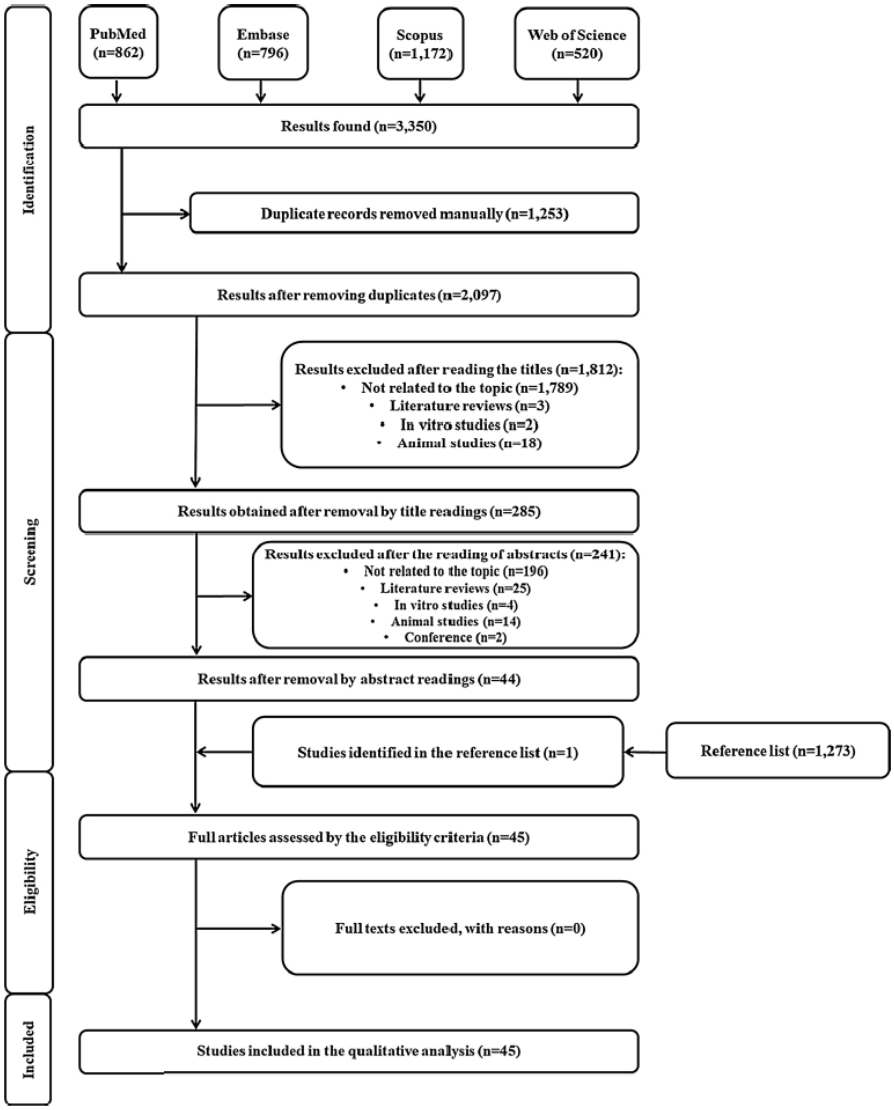

Fig. 1. Flowchart showing the different steps for the selection of articles included in this systematic review.

Table 2
Summary of the main characteristics of the eligible studies.

Authors/ Year	Sample	Age and SD (mean)	Sample type	Comparation	Device	Excitation source	Probe administration	Incubation time	Fluorescent probe (concentration)	Analysis of fluorescence images
Betz et al., 1999[17]	1 patient (healthy) 30 patients (oral cancer)	58.8 (SD: 8.8)	Patient (*in vivo*)	White light x Fluorescence	Endoscope	Xenon arc lamp (white and violet light) (375–440 nm)	–	–	Autofluorescence	Optical multichannel analyzer SigmaPlot 4.0 Software
Betz et al., 2002[18]	85 patients 214 biopsies	58.1 (SD: 9.47)	Patient (*in vivo*) and biopsy	White light x Fluorescence x Biopsy	Endoscope	Xenon arc lamp (white and violet light) (375–440 nm)	Topical	1 to 4 h	Autofluorescence and 5-ALA	Spectrophotometer SigmaPlot 4.0 Software
Onisawa et al., 2003[43]	55 patients	63.6 (–)	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	Photographic camera with SC-48 Fuji Filter	Ultraviolet flash lamp (360 nm)	–	–	Autofluorescence	HPLC
de Veld et al., 2005[46]	70 patients (healthy) 16 patients (oral lesions)	50 (healthy) (SD: 15) 57 (oral lesions) (SD: 13)	Patient (*in vivo*)	–	Spectrograph	Xenon arc lamp (365–450 nm)	–	–	Autofluorescence	–
Svistun et al, 2004[44]	2 biopsies	–	Biopsy	White light x Fluorescence	Bandpass filters and Photographic camera (SLR Canon EOS 630)	Xenon arc lamp (white and violet light) (350–440 nm)	–	–	Autofluorescence	–
Poh et al., 2006[57]	20 patients 122 biopsies	58 (–)	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	–	Violet/blue light (–)	–	–	Autofluorescence	–
Lane et al., 2006[56]	50 biopsies 44 patients	–	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	Developed by authors	Blue light (400–460 nm)	–	–	Autofluorescence	–
Jayanthi et al., 2009[47]	15 (healthy) 34 (oral cancer)	–	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	Portable LIAF with fiber-optic probe	Diode Laser (404 nm)	–	–	Autofluorescence	–
Jayaprakash et al., 2009[49]	189 biopsies	–	Biopsy	White light x Fluorescence	Storz liquid light guide	Xenon arc lamp (white and blue light) (405 nm)	–	–	Autofluorescence	–
Mehrotra et al., 2010[8]	102 biopsies (ViziLite) 156 biopsies (VELscope)	39 (ViziLite) (–) 41 (VELscope) (–)	Patient (*in vivo*) and biopsy	White light x Fluorescence	ViziLite® VELscope®	Violet light (–)	–	–	Autofluorescence	–
Koch et al., 2010[29]	78 patients	61.7 (–)	Patient (*in vivo*) and biopsy	White light x Fluorescence x Biopsy	VELscope®	Blue light (400 nm)	–	–	Autofluorescence	–
Paderni et al., 2011[30]	175 patients	60.38 (SD: 12.26)	Patient (*in vivo*) and biopsy	White light x Fluorescence x Biopsy	VELscope®	–	–	–	Autofluorescence	–
Sweeny et al., 2011[42]	88 patients	64 (–)	Patient (*in vivo*) and biopsy	White light x Fluorescence x Biopsy	Identafi® 3000	Violet light (–)	–	–	Autofluorescence	–
Petrussi et al., 2014[31]	49 patients 56 biopsies	56.7 (–)	Patient (*in vivo*) and biopsy	White light x Fluorescence x Biopsy	VELscope®	Blue light (–)	–	–	Autofluorescence	–

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Table 2 (*continued*)

Authors/ Year	Sample	Age and SD (mean)	Sample type	Comparation	Device	Excitation source	Probe administration	Incubation time	Fluorescent probe (concentration)	Analysis of fluorescence images
Francisco et al., 2014[58]	28 patients (healthy) 28 patients (oral cancer)	60.9 (–)	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	Spectrometer	Diode Laser (406 nm)	–	–	Autofluorescence	MATLAB
Francisco et al., 2014b[37]	30 patients (healthy) 56 patients (oral cancer)	58.5 (–)	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	Fiber-optic probe	Diode Laser (violet and green light) (406 nm)	–	–	Autofluorescence	WEKA Software Naïve Bayes,k-nearest neighbor (kNN) and a decision tree C4.5 (J48)
Scheer et al., 2015[32]	41 patients	–	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	VELscope®	Blue light (400–460 nm)	–	–	Autofluorescence	–
Yan et al., 2017[48]	31 biopsies	Male: 54.9 (SD: 11.73) Female: 76 (SD: 0)	Biopsy	–	Portable LIAF	LED (350–425 nm)	–	–	Autofluorescence	–
Ganga et al., 2017[33]	23 patients	–	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	VELscope®	Blue light (375–440 nm)	–	–	Autofluorescence	–
Amirchaghmaghi et al., 2017[34]	50 patients 45 biopsies	52.3 (SD: 14.8)	Patient (*in vivo*) and biopsy	White light x Fluorescence x Biopsy	VELscope®	Blue light (400–460 nm)	–	–	Autofluorescence	–
Adil et al., 2017[35]	90 patients	22–70 (–)	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	VELscope®	Blue/Violet light (400–460 nm)	–	–	Autofluorescence	–
Bagri-Manjrekar et al., 2018[53]	10 patients (healthy) 40 patients (suspected oral cancer)	49.08 (SD: 12.73)	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	Developed by authors	Ultraviolet light (360 a 410 nm)	–	–	Autofluorescence	–
Canjau et al., 2018[36]	18 patients	–	Patient (*in vivo*) and biopsy	White light x Fluorescence x Biopsy	VELscope®	Blue/Violet light (400–460 nm)	–	–	Autofluorescence	–
Huang et al., 2018[54]	77 biopsies (healthy) 49 biopsies (oral cancer)	59	Biopsy	–	Portable device developed by authors	LED (375–460 nm)	–	–	Autofluorescence	Iterative closest point algorithm
Patil et al., 2018[38]	133 patients (healthy) 92 patients (oral cancer)	–	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	Fiber-optic probe	CW He-Cd Laser (325 nm)	–	–	Autofluorescence	GRAMS/AI Software
Kumar et al., 2019[55]	36 patients (healthy) 67 patients (oral cancer)	48 (SD: 11)	Patient (*in vivo*)	–	Device developed by authors	Diode Laser (405 nm)	–	–	Autofluorescence	Mahalanobis method Ocean Optics Spectra Suit Software
Morikawa et al., 2019[51]	101 (oral cancer)	65.2 (–)	Patient (*in vivo*)	–	ORALOOK® and IllumiScan®	Blue light (520–560 nm)	–	–	Autofluorescence	ImageJ 1.5
Simonato et al., 2019[50]	2	–	Patient (*in vivo*)	White light x Fluorescence x Biopsy	EVINCE®	–	–	–	Autofluorescence	–
Leunig et al., 1996[20]	16 patients	60 (–)	Patient (*in vivo*)	–	Endoscope	Xenon arc lamp (violet light) (375–440 nm)	Topical	–	5-ALA (0,4%)	Optical multichannel analyzer

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Table 2 (*continued*)

Authors/ Year	Sample	Age and SD (mean)	Sample type	Comparation	Device	Excitation source	Probe administration	Incubation time	Fluorescent probe (concentration)	Analysis of fluorescence images
Leunig et al., 1996b[19]	11 patients	59.9 (–)	Patient (*in vivo*)	–	Endoscope	Xenon arc lamp (violet light) (375–440 nm)	Topical	1–3 hours	5-ALA (0,4%)	Optical multichannel analyzer
Betz et al., 1998[23]	33 patients	57.1 (–)	Patient (*in vivo*) and biopsy	White light x Fluorescence x Biopsy	Endoscope	Xenon arc lamp (white and violet light) (375–440 nm)	Topical	1–3 hours	5-ALA (0,4%)	Optical multichannel analyzer
Zenk et al., 1999[24]	56 patients	53.6 (–)	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	Endoscope	Argon laser (380 a 440 nm)	Topical	3 h	5-ALA (0,4%)	Optical multichannel analyzer
Leunig et al., 2000[21]	58 patients	57.7 (–)	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	Endoscope	Xenon arc lamp (white and violet light) (375–440 nm)	Topical	1,5 to2 hours	5-ALA (0,4%)	Optical multichannel analyzer
Zheng et al., 2002[25]	16 patients 27 biopsies	59 (–)	Patient (*in vivo*) and biopsy	White light x Fluorescence x Biopsy	Endoscope	Xenon arc lamp (white and violet light) (375–440 nm)	Topical	1,5 to 2 h	5-ALA (0,4%)	–
Zheng et al., 2002b[26]	28 patients 70 biopsies	58 (–)	Patient (*in vivo*) and biopsy	White light x Fluorescence x Biopsy	Endoscope	Xenon arc lamp (white and violet light) (370–450 nm)	Topical	1,5 to 2 h	5-ALA (0,4%)	–
Leunig et al., 2002[22]	126 patients 454 biopsies	–	Patient (*in vivo*) and biopsy	White light x Fluorescence x Biopsy	Endoscope	Xenon arc lamp (white light) (375–440 nm)	Topical and systemic	1,5 to 3 h	5-ALA (0,4 e 0,6%)	Optical multichannel analyzer
Jiang et al., 2003[59]	–	–	Patient (*in vivo*)	White light x Fluorescence	–	Diode Laser (–)	Topical	1 h	5-ALA	–
Wang et al., 2003[45]	–	–	Patient (*in vivo*)	–	Oblique telescope	Diode Laser (white and violet light) (410 nm)	Topical	1 h	5-ALA	LabVIEW 6.1 Software
Zheng et al., 2004[27]	49 patients 118 biopsies	60 (–)	Patient (*in vivo*) and biopsy	White light x Fluorescence x Biopsy	Endoscope	Xenon arc lamp (white and violet light) (380–450 nm)	Topical	1,5 to 2 h	5-ALA (0,4%)	–
Jiang et al., 2011[28]	4 patients	–	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	Endoscope	Diode Laser (violet light) (410 nm)	Topical	–	5-ALA (005 mL/cm^2)	RGB method
Thong et al., 2009[13]	12 patients	59.3 (SD: 13.5)	Patient (*in vivo*)	White light x Fluorescence	Endoscope	Xenon arc lamp (white and violet light) (380–440 nm)	Topical	1,5 to 4 h	Hypericin (100 mL)	–
Gillenwater et al., 1998[39]	8 patients (healthy) 12 patients (com lesões bucais)	–	Patient (*in vivo*)	White light x Fluorescence x Biopsy	Fiber-optic probe	Nitrogen-pumped dye lasers (337, 365 and 410 nm)	–	–	Rhodamine 610	–
Chaturvedi et al., 2010[40]	97 patients 299 biopsies	50 (SD: 10)	Patient (*in vivo*) and biopsy	–	Fiber-optic probe	Nitrogen laser (10 Hz)	–	–	Rhodamine 6 G (2 mg/L)	MRDF SMLR
Shimane et al., 2016[52]	10 biopsies	67.6 (–)	Biopsy	White light x Fluorescence	Night Owl LB983 (BERT-HOLD Japan, Toky	Fluorescent lamp (–)	Topical	10 min	γ-glutamyl hydroxymethyl rhodamine green	IndiGO Software
Ebenezar et al., 2012[41]	15 patients (healthy)	27 (healthy) (–)	Patient (*in vivo*) and biopsy	Fluorescence x Biopsy	Optical fiberprobe attached to Fluoromax-2	Xenon arc lamp (440–470 nm)	–	–	Porphyrin	Data Max Software

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Table 2 (*continued*)

Authors/ Year	Sample	Age and SD (mean)	Sample type	Comparation	Device	Excitation source	Probe administration	Incubation time	Fluorescent probe (concentration)	Analysis of fluorescence images
	10 patients (oral cancer)	52 (oral cancer) (–)			spectrofluorometer (ISA JobinYvon-Spex, Edison, NJ)					

Note: (–) not informed by authors; SD: standard deviation; 5-ALA: 5-aminolevulinic Acid; HPLC: High-Performance Liquid Chromatography; MRDF: Maximum Representation and Discrimination Feature; SMLR: Sparse Multinomial Logistic Regression; LIAF: portable LED Induced autofluorescence.

56], violet light [8,42], blue/violet laser [35,36,57], LED [48,54], ultraviolet flash lamp [43], nitrogen laser [40], Nitrogen -pumped dye lasers [39], argon laser [24], ultraviolet light [53], fluorescent lamp [52] and CW He-Cd Laser [38]. Only 1 study [30] did not report which source of excitation was used (Table 2).

As for the outcome, 27 evaluated only autofluorescence, 17 studies used only fluorescent probes, while 1 study associated fluorescent probes and autofluorescence. Of the 18 studies that evaluated fluorescent probes, 5-ALA was the most used, present in 13 studies. The hypericin, γ-glutamyl hydroxymethyl rhodamine green, porphyrin, rhodamine 6 G and rhodamine 610 were also reported, used in 1 study each. For the administration of the probe, 14 studies used the topical application, while only one study reported the administration by topical and systemic application. Another 3 articles [39–41] did not report form of administration. The probe incubation time ranged from 10 min (minimum time) to 4 h (maximum time) (Table 2).

The multichannel optical analyzer was used in 7 studies [17,19–24] for image analysis, while the SigmaPlot Software was used in 2 studies [17,18], followed by the spectrophotometer [18], HPLC [43], Software LabVIEW 6.1 [45], MRDF / SMLR [40], RGB method [28], Data Max software [41], MATLAB [58], WEKA software [37], indiGO software [52], iterative closest point algorithm [54], GRAMS / AI software [38] and Mahalanobis method [55] (Table 2).

3.3. Risk of bias of studies

Twenty-five studies had a low risk of bias, followed by fourteen studies with a moderate risk of bias and six studies (with a high risk of bias. Studies that did not apply any diagnostic threshold received the score "Not applicable" in item 5, following the recommendations of the "Joanna Briggs Institute Critical Appraisal Checklist for Diagnostic Test Accuracy Studies" (Table 3).

3.4. Individual results of the studies

In general, of the 28 studies that evaluated autofluorescence (n = 1190 patients), only 2 articles [8,44] diagnosed oral squamous cell carcinoma in 100 % of the sample (n = 3/1037). In the remaining studies, the diagnostic rate ranged from 0% to 96.9 %. VELscope® was evaluated in 9 studies [8,29–36](n = 680/1037), with sensitivity ranging from 33.3 % (n = 41) to 100 % (n = 35), while specificity varied from 12 % to 88.6 %. ViziLite® system was analyzed in only 1 study [8] (n = 102/1037) and showed a sensitivity of 0% and a specificity of 75 %. Identafi 3000® was evaluated in 1 article [42] (n = 88/1037) and showed sensitivity and specificity, which was 50 % and 81 %, respectively. The EVINCE® device was evaluated in only 1 article [50] and obtained a sensitivity of 100 % and specificity of 90.8 %. ORALOOK® and IllumiScan® devices were evaluated in 1 study [51], although the authors did not provide the sensitivity and specificity of each device separately. (Table 4).

Of the 18 studies that used fluorescent probes, 13 used 5-ALA (n = 397 patients). Of these 13 studies, OSCC was diagnosed in 100 % (174/397) of the sample in 5 studies [18–21,23]. The diagnosis rate varied from 827% (n = 52) to 94.6 % (n = 56) in the other 9 studies [22, 24–28,45,59]. Neoplastic tissue showed higher fluorescence compared the normal mucosa. Maximum fluorescence intensity was reached 1–3 hours after the application of 5-ALA. The sensitivity of this fluorescent probe ranged from 90 % to 100 %, while the specificity ranged from 51.3%–96% (Table 5).

Hypericin was used as a fluorescent probe in only 1 study [13] (n = 12 patients) and allowed to differentiate normal mucosa from oral cancer in 100 % of the patients. Neoplastic tissue revealed higher intensities of fluorescence compared with the normal tissue. These authors did not inform the time until the maximum fluorescence intensity after the application of the probe. The sensitivity was 100 %, while the specificity ranged from 75 % to 100 % (Table 5).

Table 3
Risk of Bias Assessed by the Joanna Briggs Institute Critical Appraisal Checklist for Diagnostic Test Accuracy Studies.

Author, year	Q.1	Q.2	Q.3	Q.4	Q.5	Q.6	Q.7	Q.8	Q.9	Q.10	% Yes/Risk
Zenk et al., 1999[24]	√	√	√	√	NA	√	U	√	√	√	80 % (Low)
Zheng et al., 2002[25]	√	–	√	–	√	√	–	√	√	√	70 % (Low)
Zheng et al., 2002b[26]	√	–	√	–	√	√	–	√	√	√	70 % (Low)
Svistun et al., 2004[44]	–	√	√	√	NA	√	–	√	√	√	70 % (Low)
Zheng et al., 2004[27]	–	–	√	–	√	√	√	√	√	√	70 % (Low)
de Veld et al., 2005[46]	√	√	√	√	√	√	–	√	√	√	90 % (Low)
Jayanthi et al., 2009[47]	–	–	√	–	√	√	√	√	√	√	70 % (Low)
Jayaprakash et al., 2009[49]	√	–	√	–	NA	√	√	√	√	√	70 % (Low)
Mehrotra et al., 2010[6]	√	–	√	√	NA	√	–	√	√	√	70 % (Low)
Chaturvedi et al., 2010[40]	√	√	–	√	NA	√	–	√	√	√	70 % (Low)
Paderni et al., 2011[30]	√	√	√	√	NA	√	–	√	√	√	80 % (Low)
Jiang et al., 2011[28]	–	–	√	√	√	√	–	√	√	√	70 % (Low)
Sweeny et al., 2011[42]	√	–	√	√	NA	√	–	√	√	√	70 % (Low)
Koch et al., 2011[29]	√	√	√	√	NA	√	–	√	√	√	80 % (Low)
Petruzzi et al., 2014[31]	√	√	U	√	NA	√	–	√	√	√	70 % (Low)
Francisco et al., 2014[58]	–	√	√	√	NA	√	–	√	√	√	70 % (Low)
Francisco et al., 2014b[37]	√	√	√	√	NA	√	–	√	√	√	80 % (Low)
Scheer et al., 2015[32]	√	–	√	√	NA	√	–	√	√	√	70 % (Low)
Shimane et al., 2016[52]	√	√	√	√	NA	√	–	√	√	√	80 % (Low)
Amirchaghmaghi et al., 2017[34]	–	√	√	√	NA	√	–	√	√	√	70 % (Low)
Ganga et al., 2017[33]	√	√	√	√	NA	√	–	√	√	√	80 % (Low)
Patil et al., 2018[38]	√	–	√	–	√	√	U	√	√	√	70 % (Low)
Canjau et al., 2018[36]	√	U	√	√	NA	√	√	√	√	√	80 % (Low)
Bagri-Manjrekar et al., 2018[53]	√	–	√	√	NA	√	U	√	√	√	70 % (Low)
Simonato et al., 2019[50]	√	√	√	√	NA	√	–	√	√	√	80 % (Low)
Leunig et al., 1996[20]	–	–	√	–	NA	√	√	U	√	√	Moderate (50 %)
Leunig et al., 1996b[19]	–	–	√	–	NA	√	√	U	√	√	50 % (Moderate)
Betz et al., 2002[18]	–	–	√	–	NA	√	√	U	√	√	50 % (Moderate)
Leunig et al., 2002[22]	–	–	√	–	NA	√	–	√	√	√	50 % (Moderate)
Onizawa et al., 2003[45]	–	–		–	–	√	–	√	√	√	50 % (Moderate)
Poh et al., 2006[57]	√	–	√	–	NA	√	–	√	√	√	60 % (Moderate)
Lane et al., 2006[56]	√	–	√	–	NA	√	–	√	√	√	60 % (Moderate)
Thong et al., 2009[13]	–	–	√	–	NA	√	√	√	√	–	50 % (Moderate)
Ebenezar et al., 2012[41]	–	–	√	√	NA	√	–	√	–	√	50 % (Moderate)
Adil et al., 2017[35]	–	√	–	√	NA	√	–	√	√	√	60 % (Moderate)
Yan et al., 2017[48]	–	–	√	–	√	√	U	√	√	√	60 % (Moderate)
Huang et al., 2018[54]	–	–	√	–	√	√	U	√	√	√	60 % (Moderate)

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Table 3 (*continued*)

Author, year	Q.1	Q.2	Q.3	Q.4	Q.5	Q.6	Q.7	Q.8	Q.9	Q.10	% Yes/Risk
Kumar et al., 2019[55]	–	–	√	–	NA	√	–	√	√	√	50 % (Moderate)
Morikawa et al., 2019[51]	√	–	√	–	NA	√	–	√	√	√	60 % (Moderate)
Betz et al., 1998[23]	–	–	U	–	NA	√	√	U	√	–	40 % (High)
Gillenwater et al., 1998[39]	–	–	–	–	NA	√	√	U	√	√	40 % (High)
Betz et al., 1999[17]	–	–	U	–	NA	√	√	U	√	–	30 % (High)
Leunig et al. 2000[21]	–	√	U	√	NA	√	–	U	√	–	40 % (High)
Wang et al., 2003[45]	–	U	U	NA	NA	√	NA	NA	NA	U	10 % (High)
Jiang et al., 2003[59]	–	U	U	NA	NA	√	NA	NA	NA	U	10 % (High)

Q.1: Was a consecutive or random sample of patients enrolled?; Q.2: Was a case control design avoided?; Q.3: Did the study avoid inappropriate exclusions?; Q.4: Were the index test results interpreted without knowledge of the results of the reference standard?; Q.5: If a threshold was used, was it pre-specified?; Q.6: Is the reference standard likely to correctly classify the target condition?; Q.7: Were the reference standard results interpreted without knowledge of the results of the index test?; Q.8: Was there an appropriate interval between index test and reference standard?; Q.9: Did all patients receive the same reference standard?; Q.10: Were all patients included in the analysis?; √ = Yes; – = No; U = Unclear; NA = Not applicable.

Rhodamine 610 was used in only 1 study [39] (n = 12 patients), and the wavelength of 337 nm detected 100 % of oral cancer, while 410 nm diagnosed 53.8 % (n = 7/12). Fluorescence findings were compatible with the histopathological diagnosis in the entire sample. The authors did not mention which tissue had higher intensity of fluorescence, as well as the time until the maximum fluorescence intensity after the application. The sensitivity and specificity at 337 nm was 88 % and 100 %, respectively, against 76.5 % and 100 % of the wavelength at 410 nm (Table 5).

Rhodamine 6 G was used in only 1 study [40] (n = 299 patients) and OSCC was diagnosed in 82.2 % of the sample (246/299). The fluorescence findings were compatible with histopathology. However, authors did not show which tissue had higher fluorescence intensity, the time until the maximum fluorescence intensity after the application, or the sensitivity or specificity of the probe used (Table 5).

Porphyrin was used in only 1 study [41] (n = 10 patients) and allowed the diagnosis of 100 % of oral cancer that correlated with histopathology. The tumor tissue showed higher fluorescence intensity than surrounding oral mucosa. The authors did not inform the time until the maximum fluorescence intensity after the application of the probe and the sensitivity and specificity were 100 % (Table 5).

γ-glutamyl hydroxymethyl rhodamine green was used in only 1 study [52] (n = 10 patients) and allowed the diagnosis of the entire sample with OSCC. The fluorescence findings also correlated to histopathology and the neoplastic tissue revealed higher fluorescence intensity than oral mucosa. Maximum fluorescence intensity was reached after 30 min of incubation. The authors did not report the sensitivity and specificity (Table 5).

4. Discussion

Oral squamous cell carcinoma is a malignant epithelial neoplasia with invasive behavior and can affect the vermilion region of the lips, floor of the mouth, tongue, oral mucosa, gingival surfaces, retromolar trine, hard palate and soft palate [1]. The mortality rate has increased due to the late diagnosis, since patients seek care when the disease is in an advanced stage [7].

Thus, early diagnosis is essential for a good prognosis [60]. However, biopsy, considered the gold standard for the diagnosis of oral cancer, is invasive and many patients are reluctant to perform. In addition, complete resection of tumor tissues is still considered a major challenge in oncological surgery, since tumor cells can migrate to different tissues, allowing the recurrence of cancer and failure to treat these patients [61, 62].

In addition to histopathological examination, other methods have been used to diagnose oral cancer, such as cytology and molecular biology techniques. Cytology is a simple and non-invasive technique that allows the analysis of epithelial cells after scraping the surface of oral mucosa [63]. However, it is an unreliable method, since access to the deep cell layers is not possible [8]. Alternatively, the deregulation of gene expression as well as microRNAs in oral cancer has been a potential biomarker of this pathology, which can be evaluated using real-time PCR [64]. Despite the PCR high sensitivity, it is necessary to identify specific targets, which makes an expensive method not accessible for most population. Thereby, the use of fluorescent probes can be a valuable strategy to enable early diagnosis and potentially be used as a guide to improve surgical precision [65] as well as to avoid unnecessary biopsies [66]. Therefore, the autofluorescence tissue properties as well as the use of probes can accelerate the diagnostic process and help the clinical dentist to perform the appropriate treatment.

Autofluorescence is a phenomenon which cells excited by a light source - with a specific wavelength - emit light at a different wavelength from an endogenous fluorophore, as collagen and elastin [67]. In oral cancer, tumor cells alter the concentration of fluorophores in tissues, promoting changes in the spectral properties of the mucosa and, thus, allow their perception through autofluorescence. The dentist uses a portable device to direct the fluorescent light that works as a source of excitation. This method is simple and quick to track changes in mucosa, which can be applied in daily clinical practice [66].

In this systematic review, 28 studies evaluated autofluorescence, which VELscope® was the most used device, present in 9 articles [8, 29–36]. VELscope® sends a light at a wavelength of 400–460 nm, which results in the excitation of endogenous fluorophores of the healthy oral mucosa and the emission of a green light. The neoplastic mucosa will exhibit a dark appearance, because of a change in the quantity and quality of natural fluorophores in tumor cells [66]. A recent systematic review [68] revealed that VELscope® device is excellent for screening, but not for distinguishing a benign, malignant or simple acute inflammation. Similarly, we observed that the sensitivity of VELscope® ranged from 33.3%–100%, while the specificity varied from 12 % to 88.6, indicating a high amplitude in the rate of diagnosis of oral cancer. Identafi 3000® is another diagnostic tool for detecting autofluorescence from multispectral reflectance. For this, the device uses 3 lights at different wavelengths: white, violet and amber. Excitation with violet

Table 4
Summary of the main results of the eligible studies (autofluorescence).

Authors/ Year	Patients detected with oral cancer / total sample	Comparation	Tissue with higher fluorescence intensity	Sensitivity	Specificity
Betz et al., 1999[17]	20/30 (666%)	–	Normal tissue	–	–
Betz et al., 2002[18]	31/41 (874%)	Autofluorescence was compatible with biopsy in 87.8 % of cases	Normal tissue	White light 992% Autofluorescence 878%	White light 429% Autofluorescence 564%
Onizawa et al., 2003[43]	50/55 (909%)	There was no significant difference in degrees of fluorescence clinically	–	–	–
de Veld et al., 2005[46]	Autofluorescence allowed to distinguish normal tissue from oral cancer (–)	Three lesions were classified incorrectly by autofluorescence	Tumor	94 %	94 %
Svistun et al, 2004[44]	2/2 (100 %)	The autofluorescence results were compatible with the histopathological findings	–	White light 87 % Autofluorescence 94 %	White light 67 % Autofluorescence 78 %
Poh et al., 2006[57]	32/33 (969%)	The autofluorescence results were compatible with the histopathological findings	Normal tissue	–	–
Lane et al., 2006[56]	33/33 (100 %)	The autofluorescence results were compatible with the histopathological findings	–	100 %	100 %
Jayanthi et al., 2009[47]	Autofluorescence allowed to distinguish normal tissue from oral cancer (–)	–	Tumor	–	–
Jayaprakash et al., 2009[49]	Autofluorescence allowed to distinguish normal tissue from oral cancer (–)	–	–	Autofluorescence 72 % White light 52 % White light + Autofluorescence 83 %	Autofluorescence 50 % White light 70 % White light + Autofluorescence 38 %
Mehrotra et al., 2010[9]	ViziLite 0/1 (0%) VELscope 1/1 (100 %)	Biopsy showed 1 patient with oral cancer, but ViziLite was unable to detect VELscope was able to diagnose the patient with oral cancer.	–	ViziLite 0% VELscope 100 %	ViziLite 0% VELscope 100 %
Koch et al., 2010[29]	The autofluorescence test did not obtain satisfactory results to differentiate benign lesions from oral cancer (–)	–	–	White light 966% Autofluorescence 93 %	White light 958% Autofluorescence 13–17%
Paderni et al., 2011[30]	27/28 (964%)	The autofluorescence results were compatible with the histopathological findings	Normal tissue	–	–
Sweeny et al., 2011[42]	2/4 (50 %)	4 patients were diagnosed with SCC by histopathological examination, but autofluorescence detected 2	–	White light 50 % Autofluorescence 50 %	White light 98 % Autofluorescence 81 %
Petruzzi et al., 2014[31]	A autofluorescência apresentou bons resultados para triagem do CCE (–)	The diagnostic agreement with histopathology was 27.8 %	–	70 %	57.69 %
Francisco et al., 2014[50]	The examination found high intensity in the autofluorescence of patients with oral cancer (–)	–	–	–	–
Francisco et al., 2014b[37]	The autofluorescence exam differentiate the normal from the	–	–	885%	938%
Scheer et al., 2015[32]	33/41 (804%)	The autofluorescence results were compatible with the histopathological findings	–	333%	886%
Yan et al., 2017[48]	Autofluorescence revealed accuracy in the diagnosis of oral cancer (–)	–	–	846%	764%
Ganga et al., 2017[33]	17/23 (739%)	The VELscope autofluorescence findings were compatible with histopathology	–	809%	662%
Amirchaghmaghi et al., 2017[34]	8/9 (888%)	The VELscope autofluorescence findings were compatible with histopathology	–	White light 81 % VELscope 90 %	White light 67 % VELscope 15 %
Adil et al., 2017[35]	31/34 (911%)	The VELscope autofluorescence findings were compatible with histopathology	–	100 %	67 %
			–	–	–

(continued on next page)

Table 4 (*continued*)

Authors/ Year	Patients detected with oral cancer / total sample	Comparation	Tissue with higher fluorescence intensity	Sensitivity	Specificity
Bagri-Manjrekar et al., 2018[55]	26/31 (838%)	The autofluorescence profile showed a statistically significant correlation (P = 0.0001) with the histopathological diagnosis			
Canjau et al., 2018[56]	16/17 (94,11 %)	16 positive biopsies for malignant lesions were confirmed by VELscope. Only 1 biopsy positive for carcinoma was not in accordance with the device	–	944%	100 %
Huang et al., 2018[54]	Autofluorescence demonstrated high sensitivity for detecting oral cancer (–)	–	–	974%	898%
Patil et al., 2018[30]	Autofluorescence showed higher spectra in oral cancer than in normal mucosa (–)	–	Tumor	–	–
Kumar et al., 2019[55]	Autofluorescence was more identified in patients with SCC than in healthy ones (–)	–	Tumor	71–98%	91–100%
Morikawa et al., 2019[51]	Nonuniform FVL was observed, and the boundaries of FVL were unclear. The FVL rate was 98 %.	–	–	98 %	432%
Simonato et al., 2019[50]	–	–	–	White light 100 % Autofluorescence 100 %	White light 90.4 % Autofluorescence 90.8 %

Note: (–) not informed by authors.

light results in a blue light emission for the normal mucosa and a dark appearance for the tumor tissue, allowing the distinction between normal and altered mucosa [69]. In our review, the study that evaluated Identafi 3000® [42] observed loss of tissue autofluorescence, presenting the sensitivity (50 %) and specificity (81 %). These results corroborate Lalla et al. [70] findings, that observed excellent visibility in 342 patients with oral lesions using this device, when compared with conventional clinical examination. However, one question is whether autofluorescence can be used as a method of early diagnosis, as it is possible that initial lesions do not cause significant spectral changes in the mucosa that can be viewed as loss of autofluorescence.

Fluorescent probes are increasingly been used as adjuvant tools for differentiation of healthy and tumor tissues. A recent study [71] showed promising results about its potential use in measuring DNA damage and repair. Once applied topically or sprayed on the mucosa, the probes become fluorescent from specific local conditions, as temperature, endogenous enzyme activity of the tumor microenvironment or pH [72]. There are several types of fluorescent probes available on the market, but in the present systematic review, only 6 were used for OSCC detection: 5-ALA, hypericin, γ-glutamyl hydroxymethyl rhodamine green, rhodamine 610, rhodamine 6 G and porphyrin.

Of the 18 studies that used fluorescent probes, 13 used 5-ALA, showing high diagnostic power, with sensitivity ranging from 95 % to 100 %. These results corroborate Kamp et al. [73] findings that analyzed the impact of 5-ALA in 84 patients with brain metastases and observed a significant correlation between tumor progression and fluorescence by 5-ALA. Similarly, 5-ALA has been widely studied in other tumors, as bladder cancer [74] and glioma [75]. 5-ALA is a natural amino acid precursor of hemoglobin and its application induces biosynthesis of protoporphyrin IX (PpIX) in mitochondria. Since tumor cells use the anaerobic glycolysis pathway to produce ATP - not the tricarboxylic acid cycle or the electron transport chain – there is an inactivation of ferrocelatase that results in inhibition of PpIX catabolism [74] and accumulation of PpIX in tumor cells. Once PpIX is photosensitive and can be excited at specific wavelengths - red light (600–740 nm) and green light (450–580 nm) - emitting fluorescence that can be used for cancer screening [74,76]. Herein, it was observed that 5-ALA can be considered a promising probe for OSCC detection, since it is a non-invasive strategy with good discriminatory power.

With a similar mechanism, hypericin has also been used as a fluorescent probe for visualizing oral cancer. It is a secondary metabolite of plants and has capacity for photodynamic diagnosis. When applied topically, hypericin accumulates in tumor cells, increasing the contrast between normal and neoplastic tissue after excitation by a light source [77]. In the present systematic review, this fluorescent probe was used in 1 article [13], which revealed high sensitivity (100 %) and specificity (75%–100%), allowing to differentiate normal tissue from oral cancer. However, the sample number was low. Hypericin has also been studied in bladder cancer [78,79], and has shown higher fluorescence intensity in tumor cells than in normal tissue, so less malignant lesions are neglected. In addition, Ritz et al. [80] observed, after injecting hypericin intravenously, greater fluorescence intensity in glioma tumor cells and demonstrated high sensitivity (91%–94) and specificity (90%–100%). Therefore, these results suggest that hypericin is a valuable promising alternative in the early diagnosis of oral cancer.

Aminopeptidases are overexpressed in many tumors including OSCC, with increased activity when compared to normal tissue [37]. Among the probes for aminopeptidases, the topical application of γ-glutamyl hydroxymethyl rhodamine green allows the detection of the enzymatic activity of γ-glutamyltranspeptidase (GGT), a highly active transpeptidase in some tumors [52]. The cleavage of γ-glutamyl hydroxymethyl rhodamine green by GGT generates green fluorescence that can be detected [81]. This probe is used to visualize lesions intraoperatively, in order to accurately determine surgical margins of ovarian cancer [81, 82]. Herein, one study [52] showed the potential use as a diagnostic tool for OSCC since it made the distinction between normal and tumor tissue in all patients (n = 10). Similarly, Shinden [83] showed that it is possible to perform a rapid diagnosis of lymph node metastases of several subtypes of breast cancer.

Rhodamines 6 G and 610 are part of the group of fluorescent probes with photostability and high quantum yield at different pH values [84]. It penetrates tumor cells quickly due to the negative potential of the plasma membrane and the cationic π system that allows them to accumulate in the mitochondria [85]. Additionally, rhodamines have been studied as a potential antitumor agent, since they can depolarize the mitochondrial membrane [86] leading to apoptosis. In our study, 2

Table 5
Summary of the main results of the eligible studies (fluorescent probes).

Authors/ Year	Patients detected with oral cancer / total sample	Comparation	Tissue with higher fluorescence intensity	Time elapsed to maximum fluorescence intensity	Sensitivity	Specificity
Leunig et al., 1996[20]	16/16 (100 %)	The fluorescence results were compatible with the histopathological findings	Tumor	1–2 hours after application of 5-ALA	–	–
Leunig et al., 1996b[19]	11/11 (100 %)	–	Tumor	1–2 hours after application of 5-ALA	–	–
Betz et al., 1998[23]	33/33 (100 %)	The fluorescence results were compatible with the histopathological findings	Tumor	2 h after application of 5-ALA	–	–
Zenk et al., 1999[24]	53/56 (946%)	–	–	3 h after application of 5-ALA	–	96 %
Leunig et al., 2000[21]	58/58 (100 %)	The fluorescence results were compatible with the histopathological findings	Tumor	1,5 h after application of 5-ALA	99 %	60 %
Betz et al., 2002[18]	56/56 (100 %)	The fluorescence and white light results were compatible with the histopathological findings	Tumor	–	White light 992% Fluorescence 100 %	White light 429% Fluorescence 513%
Zheng et al., 2002[25]	Neoplastic tissue showed higher fluorescence compared the normal mucosa. (–)	–	Tumor	–	–	–
Zheng et al., 2002b[26]	23/26 (884%)	The fluorescence results were compatible with the histopathological findings	Tumor	–	90 %	90 %
Leunig et al., 2002[22]	Rinse: 119/119 (100 %) Inhalation: 44/47 (936%) Oral administration: 43/52 (827%)	The fluorescence results were compatible with the histopathological findings. The rate of false-positive biopsies was lower with fluorescence test	Tumor	1 h after topical application and 3 h after systemic application of 5-ALA	Fluorescence 100 % White light 986%	–
Jiang et al., 2003[59]	Fluorescence can be detected in patients with oral cancer (–)	–	Tumor	1 h after application of 5-ALA	–	–
Wang et al., 2003[45]	Fluorescence can be detected in patients with oral cancer (–)	–	Tumor	–	–	–
Zheng et al., 2004[27]	Tumor tissues were identified using fluorescence (–)	–	Tumor	–	98 %	96 %
Jiang et al., 2011[28]	Fluorescence, in the R / G ratio, showed better contrast between normal and neoplastic tissue (–)	–	Tumor	–	–	–
Thong et al., 2009[13]	It was possible to distinguish normal tissue from oral cancer by fluorescence (–)	–	Tumor	–	R/B ratio 100 % R/G ratio 100 %	R/B ratio 100 % R/G ratio 75 %
Gillenwater et al., 1998[39]	337 nm: 13/13 (100 %) 410 nm: 7/13 (538%)	13 cases were confirmed histopathologically with oral cancer. The white light exam diagnosed 12. The fluorescence examination at 337 nm identified abnormalities in all.	–	–	337 nm: 88 % 410 nm: 765%	337 nm: 100 % 410 nm: 100 %
Chaturvedi et al., 2010[40]	246/299 (822%)	The fluorescence results were compatible with the histopathological findings	–	–	98 %	100 %
Shimane et al., 2016[52]	10/10 (100 %)	The fluorescence results were compatible with the histopathological findings	Tumor	30 min after application of g-Glu-HMRG	–	–
Ebenezar et al., 2012[41]	10/10 (100 %)	The fluorescence results were compatible with the histopathological findings	Tumor	–	100 %	100 %

Note: (–) not informed by authors; 5-ALA: 5-aminolevulinic Acid; R/B: Red-Blue; R/G: Red-Green.

articles [39,40] showed promising results for rhodamines as fluorescent probes. Rhodamine 6 G allowed to diagnose oral cancer in 82.2 % (n = 246/299) of the sample, while Rhodamine 610 was able to correctly diagnose the entire sample (100 %; n = 13) when excited at a wavelength of 337 nm. Rhodamine 6 G, for example, has already been used to diagnose colon cancer, revealing important differences between normal and neoplastic tissue [87]. Thus, rhodamines 6 G and 610 may represent a good alternative diagnostic method.

One potential pitfall for the use of fluorescence images for diagnosis is to stablish intensity thresholds to differentiate the healthy from tumor tissue. Visual analysis is a subjective method, as it depends on the sensitivity of the clinician who is examining. In order to minimize possible measurement errors, software has been used to quantify fluorescence images. In the present systematic review there was no standardization in the measurement of fluorescence. Software such as SigmaPlot, WEKA, GRAMS / AI, Ocean Optics Spectra Suit, LabVIEW, indiGO and DataMax have been reported. In fact, software analysis is an interesting alternative for spectroscopic analysis of fluorescence, which

can increase the accuracy of diagnosis, since it is based on specific algorithms for quantification of fluorescent points.

The standardization of fluorescent probes protocols is a challenge for advancing the diagnosis of oral cancer. We observed different methodologies and protocols, especially in the wavelength used and excitation font, which makes it difficult to analyze which method is the most appropriate for each situation. Therefore, we suggest the standardization of the protocols for the application of fluorescent probes so that more studies can be developed along the same lines to obtain similar results and allow progress in the diagnosis. In addition, many studies have used a limited number of patients, making it difficult to interpret the external validity of this diagnostic method. Thus, we suggest conducting studies with larger samples.

Another interesting topic is the evaluation of the impact and economic viability of using this methodology in the daily routine of the clinical dentist. The evaluation of tissue autofluorescence from a specific device is a low-cost alternative and can be easily applied in the clinical routine. The addition of fluorescent probes is important, as it increases the sensitivity of the diagnosis, being cheaper than performing a PCR, for example. Thus, fluorescent probes can be characterized as a non-invasive, low-cost technique with high diagnostic power for oral cancer. That way, we believe that fluorescent probes are promising strategies for the diagnosis of oral cancer and can be used in line with the histopathological exam. This systematic review has strengths, as it used a broad search strategy in important databases, applying a careful analysis of eligible articles, without restriction of year, language or publication status.

5. Conclusion

The autofluorescence and fluorescent probes can provide an accurate diagnosis of oral cancer, assisting the dentist during daily clinical activity, but it is not yet possible to suggest that this method may replace histopathological examination.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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4 CONSIDERAÇÕES FINAIS

O uso das sondas fluorescentes e a autofluorescência das células tumorais permitem a detecção precoce do CEC e isso pode auxiliar o dentista em sua rotina clínica. A facilidade de aplicação das sondas fluorescentes e o simples uso de dispositivos portáteis na visualização da fluorescência tecidual auxiliaria na localização de uma área representativa do tumor para realização da biópsia e, com isso, permitiria o diagnóstico em estágios iniciais, aumentado a chance de cura e a sobrevida dos pacientes. Assim, acreditamos ser importante investir em métodos alternativos de diagnóstico.

A presente dissertação demonstrou que:

- A autofluorescência e o ácido 5-aminolevulínico apresentam alta sensibilidade e especificidade
- O ácido 5-aminolevulínico foi a sonda fluorescente mais utilizada
- Autofluorescência e sondas fluorescentes podem ser usadas para a detecção precoce do câncer bucal

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