UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

CRISTINA SALDIVIA SIRACUSA

CARACTERIZAÇÃO DOS ASPECTOS DEMOGRÁFICOS E CLINICOPATOLÓGICOS DO CARCINOMA ESPINOCELULAR ORAL INCIPIENTE

DEMOGRAPHIC AND CLINICOPATHOLOGICAL CHARACTERIZATION OF INCIPIENT ORAL SQUAMOUS CELL CARCINOMA

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DEMOGRAPHIC AND CLINICOPATHOLOGICAL CHARACTERIZATION OF INCIPIENT ORAL SQUAMOUS CELL CARCINOMA

Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestra em Estomatopatologia, na Área de Estomatologia.

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Orientador: Prof. Dr. Alan Roger dos Santos Silva

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“Too often I have to remind myself the walls of my comfort zone are not only transparent; they are actually imaginary.
I can cross through them at any time.”

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RESUMO

Introdução: O carcinoma espinocelular oral (CEC) é uma neoplasia maligna que tem origem no epitélio de revestimento da cavidade oral, e é a forma mais frequente de câncer oral, representando um problema de saúde de alta relevância a nível mundial. O carcinoma espinocelular oral incipiente (CECi) é representado por o CEC *in situ* e o CEC microinvasivo (CECmi), que caracterizam as fases mais precoces da doença. Uma vez que o prognóstico dos pacientes com CEC está estreitamente associado ao momento do diagnóstico, os métodos para melhorar o diagnóstico precoce são vitais. **Objetivo:** A presente dissertação teve como finalidade caracterizar o CECi por meio de dois objetivos principais: 1) Avaliar padrões histopatológicos de CEC microinvasivo através de uma revisão sistemática da literatura e 2) Descrever aspectos demográficos e clinicopatológicos de uma coorte sul-americana de pacientes com CECi. **Materiais e Métodos:** 1) Foram realizadas pesquisas nas principais bases de dados sem restrição de período, e foram obtidas 2.024 publicações em inglês, espanhol e português. Após a triagem e elegibilidade, foram selecionados 4 estudos. O risco de viés foi avaliado utilizando o Joanna Briggs Institute Critical Appraisal Checklist. Foi realizada uma síntese descritiva; 2) Foi realizado um estudo transversal, observacional e internacional para avaliar as características demográficas e clinicopatológicas do CECi a partir de uma amostra constituída por pacientes de 6 instituições sul-americanas. Foi realizada uma análise estatística utilizando os testes qui-quadrado ou teste exato de Fisher para avaliar associação entre o diagnóstico histopatológico e variáveis clinicodemográficas. **Resultados:** 1) Todas as 4 publicações incluídas foram retrospectivas, e reportaram um total de 116 pacientes com CEC microinvasivo, com predominância masculina (1.6:1) e uma idade média de 55.9 anos. Os principais parâmetros considerados para a microinvasão foram espessura do tumor (TT) (intervalo 4-10mm) e a profundidade de invasão (DOI) (intervalo 0.02-5mm). A definição, valores de corte e avaliação das características microscópicas não foram padronizadas. Outras medidas relevantes, tais como invasão perineural ou linfovascular e padrão de *front* invasivo mal foram descritas, e as características citológicas/arquitetônicas não foram discutidas; 2) Cento e sete pacientes dentro do espectro histopatológico do CECi foram incluídos nesta amostra. Cinquenta e oito (54.2%) eram homens com uma idade média de 60.69 anos. Quarenta e nove (45.8%) e 39 (36.5%) pacientes tinham antecedentes de consumo de tabaco e álcool, respectivamente. Clinicamente, a maioria das lesões eram placas (n=88, 82.2%), com ≥ 2 cm de extensão (n=77, 72%), afetando a borda lateral de língua (n=59, 55.1%) e o palato mole (n=13, 12.1%) com uma aparência mista (leucoeritroplásica). Oitenta e duas (76.7%) lesões eram predominantemente brancas e 25 (23.3%) predominantemente vermelhas. **Conclusões:** 1) TT e DOI são atualmente os principais critérios histopatológicos utilizados para definir o CECmi. No entanto, os resultados desta revisão sistemática mostraram ausência de parâmetros quantitativos padronizados para realizar o diagnóstico de CEC microinvasivo. Por conseguinte, estudos para padronizar e validar as características histopatológicas são centrais. 2) Segundo nosso conhecimento, esta é até agora a maior coorte de pacientes com CECi já investigada com propriedade para aprimorar a performance do diagnóstico por inspeção clínica, caracterizando os aspectos clínicos mais frequentes desse estágio da doença, contribuindo com estratégias de prevenção secundária do câncer de boca.

Palavras-chave: Neoplasias de boca; diagnóstico; carcinoma espinocelular; microinvasão; carcinoma in-situ.

ABSTRACT

Introduction: Oral squamous cell carcinoma (OSCC) is a malignant neoplasm that originates in the oral cavity lining epithelium, and it is the most frequent form of oral cancer, representing a major health problem worldwide. Incipient oral squamous cell carcinoma (OSCCi) is represented by *in-situ* and microinvasive OSCC (OSCCmi), which characterize the earliest stages of this disease. As the prognosis of OSCC patients is tightly associated with the time of diagnosis, methods to improve early diagnosis are vital. **Objective:** The present dissertation aimed to characterize OSCCi through two main objectives: 1) Assess patterns of histopathological outcomes reported in OSCCmi cases through a systematic review of the literature; 2) To describe demographic and clinicopathological aspects of a South-American cohort of OSCCi patients. **Materials and Methods:** 1) An online search in major databases was performed without period restriction, and 2,024 publications in English, Spanish and Portuguese were obtained. After screening and eligibility, 4 studies were selected. The risk of bias was assessed using Joanna Briggs Institute Critical Appraisal Checklist. A descriptive synthesis was conducted; 2) A cross-sectional, observational, international study was performed to assess demographic and clinicopathological characteristics of OSCCi from 6 South-American institutions. A statistical analysis was performed using Chi-square or Fisher's exact tests to assess association between histopathological diagnosis and clinicodemographic variables. **Results:** 1) All 4 publications included were retrospective, reporting a total of 116 OSCCmi patients, with a male predominance (1.6:1) and a mean age of 55.9 years. The main parameters considered for microinvasion were tumor thickness (TT) (range 4-10mm) and depth of invasion (DOI) (range 0,02-5mm). Definition, cut-off values, and assessment of microscopic features were not standardized. Other relevant measures such as perineural or lymphovascular invasion and pattern of invasive front were barely described, and cytological/architectural characteristics were not discussed; 2) One-hundred and seven patients within the histopathological spectrum of OSCCi were included in this sample. Fifty-eight (54.2%) patients were men with a mean age of 60.69 years. Forty-nine (45.8%) and thirty-nine (36.5%) patients had history of tobacco and alcohol use, respectively. Clinically, most of the lesions were plaques (n=88, 82.2%), ≥ 2 cm in extension (n=77, 72%), affecting the lateral tongue (n=59, 55.1%), and soft palate (n=13, 12.1%) with a mixed (with and red) appearance. Eighty-two (76.7%) lesions were predominantly white and 25 (23.3%) predominantly red. **Conclusions: 1)** TT and DOI are currently the primary histopathological criteria used to define OSCCmi. Nonetheless, the outcomes of this systematic review showed the absence of standardized quantitative parameters to render the diagnosis of microinvasive OSCC. Therefore, additional studies aiming to standardize histopathological features to diagnose OSCCmi are paramount. 2) To date, this seems to be the largest cohort of OSCCi patients, which raises awareness of clinicians' inspection acuteness by demonstrating the most frequent clinical aspects of OSCCi, potentially improving oral cancer secondary prevention strategies.

Keywords: Mouth neoplasms; diagnosis; oral squamous cell carcinoma; microinvasive; carcinoma in-situ.

SUMÁRIO

1 INTRODUÇÃO

O carcinoma espinocelular oral (CEC) é uma neoplasia maligna que tem origem no epitélio de revestimento da cavidade oral (Chainani-Wu et al., 2015), onde é o tipo mais frequente de câncer, representando mais de 90% dos tumores malignos (El-Naggar AK, 2017).

Segundo o GLOBOCAN, o câncer do lábio e da cavidade oral tem uma taxa mundial de incidência estandardizada por idade (*age standarized rate*, ASR) de incidência de 6,0 para homens e 2,3 para mulheres por cada 100.000 indivíduos, e uma ASR mundial de mortalidade de 2,8 e 1,0, respetivamente (Sung et al., 2021). Em 2020, foram reportados 377.700 novos casos desta doença (Sung et al., 2021). Estes resultados posicionam o câncer da cavidade oral em geral como o 16º câncer mais comum a nível mundial (Bouvard et al., 2022). Neste contexto, devem ser feitas considerações específicas sobre regiões geográficas quando se avalia a epidemiologia do câncer oral, uma vez que as taxas entre países variam muito (Perdomo et al., 2016; El-Naggar AK, 2017). Alguns países da América Latina e do Caribe estão particularmente caracterizados por elevadas taxas de incidência, tais como Brasil, Cuba, Uruguai, e Porto Rico (Warnakulasuriya & Kerr, 2021; Kolegova et al., 2022).

No Brasil, observam-se as taxas de incidência mais elevadas na região para ambos os sexos (19,7 para homens e 5,0 para mulheres, por cada 100.000 indivíduos) (Warnakulasuriya & Kerr, 2021). Particularmente para homens, essa cifra representa até três vezes mais do que outros países da América Central e do Sul, e como consequência, o câncer da cavidade oral representa o quinto câncer mais comum na população masculina brasileira (de Mattos Camargo Grossmann et al., 2021). Além disso, o limitado acesso à saúde e a prática crônica de hábitos de risco são alguns fundamentos que podem explicar a relatada associação da prevalência CEC oral com o status socioeconômico (Ford & Farah, 2013), sendo que tem sido exposto que taxas de incidência mais elevadas e diagnósticos tardios ocorrem em grupos populacionais mais desfavorecidos (Perdomo et al., 2016; Vinícius et al., 2022).

A etiologia do câncer da cavidade oral é multifatorial. Uma vasta gama de fatores genéticos, ambientais e comportamentais contribuem para o risco da doença (Bouvard et al., 2022). O principal fator de risco para o CEC é o uso de tabaco (El-Naggar AK, 2017; Shrestha et al., 2020). Esta relação é comprovada amplamente em estudos passados e recentes, evidenciando que existe uma associação diretamente proporcional às doses de consumo (Andrade et al., 2015; Bouvard et al., 2022). Culturalmente, o uso de tabaco em

apresentação para ser fumado é mais comum no Ocidente, enquanto áreas como o Sudeste Asiático e as ilhas do Pacífico Ocidental, onde a incidência de câncer oral é elevada, estão habituados ao uso de tabaco sem fumaça (Shrestha et al., 2020; Chamoli et al., 2021; Bouvard et al., 2022). O consumo de álcool é também um fator de risco relevante (Hashibe et al., 2007; Chamoli et al., 2021), sobretudo, na sua ação sinérgica com o consumo de álcool. O aumento do risco de CEC em pacientes com consumo de tabaco e álcool é amplamente conhecido (Hashibe et al., 2007). No caso do lábio, a etiologia se relaciona com a radiação ultravioleta, principalmente pela exposição solar ocupacional (Vinícius et al., 2022) em populações com a pele mais clara.

O diagnóstico do câncer bucal é feito pela avaliação histopatológica, que é o padrão ouro atual (Seoane Lestón & Diz Dios, 2010; Gattuso et al., 2022; Riccardi et al., 2022). Os achados microscópicos desta doença são representados de acordo com o grau de diferenciação por ninhos, cordões e ilhas de células epiteliais atípicas invadindo o tecido conjuntivo subjacente (El-Naggar AK, 2017).

As opções de tratamento mais comuns do CEC incluem cirurgia (envolvendo o tumor primário e as zonas de drenagem cervicais) e radioterapia como primeira linha e radioterapia, quimioterapia e imunoterapia (essa ainda reservada para pacientes sob tratamento paliativo), separadamente ou em combinação, ditadas pelo estadiamento clínico da doença e infraestrutura humana e física da instituição que realizará o tratamento (Zanoni et al., 2019; Chamoli et al., 2021).

Tem sido amplamente relatado que o estágio de desenvolvimento do CEC no momento do diagnóstico está fortemente associado a fatores prognósticos, como taxas de sobrevida e opções de tratamento (Goy et al., 2009; Carolina et al., 2017; Bray et al., 2018; Nagao & Warnakulasuriya, 2020; Kolegova et al., 2022). Atualmente, o diagnóstico do CEC ocorre em grande parte em etapas tardias (Goy et al., 2009; Seoane et al., 2012; Carolina et al., 2017; Bray et al., 2018; Nagao & Warnakulasuriya, 2020). No Brasil, estudos mostram que o CEC é geralmente diagnosticado nos estádios III/IV (Carolina et al., 2017; Kolegova et al., 2022). Essas fases envolvem tumores locais avançados e expansão metastática regional (e mais raramente à distância), e, portanto, muitas vezes requerem cirurgias mutilantes e abordagens de tratamento combinadas, comprometendo funções orais fundamentais como alimentação, fala e interação social, e influenciando negativamente a sobrevida global e a qualidade de vida dos pacientes (Zanoni et al., 2019; Chamoli et al., 2021; de Mattos Camargo Grossmann et al., 2021).

Atualmente, de acordo com a definição do American Joint Committee on Cancer da classificação TNM para câncer de cavidade oral (MB Amin, SB Edge, FL Greene, 2017), o CEC *in situ* e os tumores T1 (<2cm de espessura tumoral (TT, do inglês *Tumour Thickness*) e <5mm de profundidade de invasão (DOI, do inglês *Depth of Invasion*) representam as fases mais incipientes do CEC oral. Nesse sentido, o carcinoma espinocelular oral microinvasivo (CECmi) é uma manifestação em fase incipiente (Sridharan et al, 2017) que teoricamente se enquadra na classificação de T1. No entanto, alcançar precisão no processo diagnóstico de microinvasão envolve várias dificuldades (Bean et al., 2011) e atualmente existe escassa literatura relativa a critérios objetivos/definitivos para o CECmi em contraste com a mesma doença em outras localizações do corpo (Sridharan et al, 2017; Bhatla et al., 2021).

Embora a pesquisa sobre biomarcadores na saliva, sangue e outros esteja em aumento, os métodos atuais de prevenção secundária do CEC se encontram principalmente no exame clínico oral em populações de alto risco (Bouvard et al., 2022). Isto envolve o *screening* por inspeção visual sistemática e palpação da mucosa da cavidade oral e das regiões externas da face e pescoço, que demonstraram resultados suficientemente fortes de sensibilidade e especificidade para serem considerados um método eficaz para diagnóstico de desordens orais potencialmente malignas (DOPM) e CEC (Walsh et al., 2021; González-Moles et al., 2022). Contudo, um dos principais inconvenientes do *screening* por métodos visuais é a falta de conhecimento dos dentistas sobre o reconhecimento dos sinais e sintomas do câncer oral em etapas mais iniciais, o que afeta significativamente a eficácia dos programas de rastreio (Kujan et al., 2009; González-Moles et al., 2022).

Dessa forma, entende-se que o diagnóstico dos estágios incipientes do CEC ajuda na prevenção das complicações da própria doença e aquelas associadas a o seu tratamento, consequentemente reduzindo taxas de mortalidade e morbidade associadas (Chamoli et al., 2021). Uma vez que o CEC *in situ* e o CECmi são entendidos como fases iniciais, espera-se que o diagnóstico nessa fase seja associado a uma maior sobrevida quando comparado com tumores mais profundamente invasivos. No entanto, a identificação clínica e histopatológica de lesões iniciais como a CECmi e CEC in-situ representa uma dificuldade real para profissionais clínicos no campo da Odontologia e da Medicina (Bolesina et al., 2016), especialmente porque as definições atuais não abrangem de forma realista as nuances encontradas nas análises físicas e microscópicas.

As lesões avançadas de CEC geralmente apresentam-se clinicamente como ulcerações exuberantes ou nódulos fixos aos tecidos adjacentes; em contraste, os CEC incipientes (CECi) são lesões assintomáticas que não envolvem tecidos profundos e muitas vezes apresentam aparência sutil (Bolesina et al., 2016). Assim, no processo de rastreio do CEC, as manifestações clínicas inócuas representam um desafio adicional para a detecção prematura, resultando em dificuldades diagnósticas e diagnóstico tardio (Bolesina et al., 2016).

Portanto, compreendendo que os pacientes podem alcançar melhores resultados no diagnóstico precoce de CEC, e estando conscientes de que o carcinoma *in situ* e o carcinoma microinvasivo representam as manifestações mais incipientes do CEC, é crucial produzir evidência científica relevante acerca de suas apresentações clínicas para auxiliar as ferramentas de exames físico e microscópicos que impulsem a performance da prevenção primária e secundária. Com essa finalidade, os principais objetivos dessa dissertação foram: 1) Avaliar os parâmetros microscópicos existentes para o diagnóstico do CECmi) e 2) Caracterizar aspectos demográficos e clinicopatológicos de uma grande coorte internacional de pacientes com CECi, contribuindo eventualmente com exames mais eficientes para diagnóstico precoce do câncer oral. A seguir, são apresentados os dois capítulos da presente dissertação na forma de artigo científico.

2 ARTIGOS

2.1- Saldivia-Siracusa C, Araújo AL, González-Arriagada WA, Nava FJ, Hunter KD, Lopes MA, Vargas PA, Santos-Silva AR. *Histopathological parameters reported in microinvasive oral squamous cell carcinoma: a systematic review*. Med Oral Patol Oral Cir Bucal. 2022 Dec 24:25675. doi: 10.4317/medoral.25675. Epub ahead of print. PMID: 36565223.

HISTOPATHOLOGICAL PARAMETERS REPORTED IN MICROINVASIVE ORAL SQUAMOUS CELL CARCINOMA: A SYSTEMATIC REVIEW

Short running title: Histopathological parameters in microinvasive OSCC.

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ABSTRACT

BACKGROUND: Microinvasive oral squamous cell carcinoma (OSCCmi) is an incipient stage of oral cancer. Through this systematic review, we aim to assess patterns of histopathological outcomes reported in OSCCmi cases. **MATERIAL AND METHODS:** An online search in major databases was performed without period restriction, and 2,024 publications in English, Spanish and Portuguese were obtained. After screening and eligibility, 4 studies were selected. The risk of bias was assessed using Joanna Briggs Institute Critical Appraisal Checklist. A descriptive synthesis was conducted. **RESULTS:** All 4 publications included were retrospective, reporting a total of 116 OSCCmi patients, with a male predominance (1.6:1) and a mean age of 55.9 years. The main parameters considered for microinvasion were tumor thickness (TT) (range 4-10mm) and depth of invasion (DOI) (range 0,02-5mm). Definition, cut-off values, and assessment of microscopic features were not standardized. Other relevant measures such as perineural or lymphovascular invasion and pattern of invasive front were barely described, and cytological/architectural characteristics were not discussed. **CONCLUSIONS:** TT and DOI are currently the primary histopathological criteria used to define OSCCmi. Nonetheless, the outcomes of this systematic review showed the absence of standardized quantitative parameters to render the diagnosis of microinvasive OSCC. Therefore, additional studies aiming to standardize histopathological features to diagnose OSCCmi are paramount.

Keywords: Microinvasive, microinvasion, oral squamous cell carcinoma, oral cancer, histopathological profile, systematic review.

INTRODUCTION:

Oral squamous cell carcinoma (OSCC) is a malignant epithelial neoplasm with a high prevalence that tends to be diagnosed in advanced stages (1). In Brazil, studies show that OSCC is commonly diagnosed at stages III–IV (1,2). It has been widely reported that OSCC stage at the time of diagnosis is strongly associated with crucial prognostic factors, such as survival rates and treatment options, resulting in alterations in quality-of-life and survival (2,3). This suggests early diagnosis of incipient OSCC as a must to reduce mortality and comorbidities associated with this disease.

Microinvasive oral squamous cell carcinoma (OSCCmi) is an early-stage form of OSCC (4). While frankly invasive OSCC tends to be a straightforward diagnosis, there is scarce literature regarding objective definitive criteria for microinvasive squamous cell carcinoma of the oral cavity in contrast to the same entity arising in other localizations of the body (4), such as cervix (5). The usual definition of OSCCmi states microinvasion as "confined to superficial stroma or lamina propria" (6). It has also been defined as "confined to the papillary lamina propria defined by the depth of the rete processes, and superficially invasive if the tumor remains confined to the reticular (deep) lamina propria, not yet involving the submucosal tissues mentioned above" (7). However, as stated concerning other localizations, there are problems in diagnostic precision even though the concept of microinvasion initially seems obvious (8).

Since OSCCmi is understood as an incipient malignant disease, it is expected to be associated with better survival in patients diagnosed with this form of OSCC rather than more deeply invasive tumors. Nonetheless, clinical, and histopathological identification of initial lesions such as OSCCmi represents a real challenge for oral and maxillofacial pathologists, especially as current definitions do not realistically encompass the nuances encountered on microscopic analysis.

With this systematic review, we aim to assess existing evidence regarding the histopathological features of microinvasive oral squamous cell carcinoma (OSCCmi).

MATERIALS AND METHODS:

Study Design, protocol, and registration

After an initial exploratory literature review, no similar reviews regarding our topic of interest were identified. Therefore, a systematic review of the literature was carried out based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (9) and was registered in the International Prospective Register of Systematic

Reviews (PROSPERO) database (CRD42022323251). The review question was: “Which are the histopathological criteria used to diagnose micro-invasion on oral squamous cell carcinoma patients?”, and the objective was to identify and document the prevalence of histopathological criteria used to diagnose microinvasive oral squamous cell carcinoma on hematoxylin-eosin (HE) stained, formalin-fixed paraffin-embedded (FFPE) samples.

Eligibility Criteria

Articles were included if they met all the following criteria: (a) OSCC with described characteristics on histopathological diagnosis compatible with the definition of microinvasion identified on HE in FFPE tissues according to the AJCC: “confined to superficial stroma or lamina propria”; (b) description of diagnostic criteria on microinvasion; and (c) cross-sectional studies, case-control studies, cohort studies, clinical trials, and case series published in the English, Spanish or Portuguese language.

The exclusion criteria were (a) publications unrelated to the topic of the review; (b) lesions outside of oral and maxillofacial complex; (c) types of publications such as non-human studies (animal or in vitro research), reviews, conference papers, letters, book chapters, surveys, news, retracted articles, double publication (keeping only the most recent one) and publications without full-text availability; (d) insufficient or unclear reported data on histopathological analysis; and (e) frankly invasive T1-T2 OSCC tumors**.**

Information sources and search strategy

Electronic databases (PubMed, Embase, SCOPUS, Web of Science, LILACS, and Cochrane Library) were selected to perform a search on February 18th, 2022, without period restriction, with the aim of identifying articles potentially relevant to this study.

A manual search was also performed in Google Scholar, ProQuest, and reference lists of included articles to detect any eligible articles that may not have been retrieved by the electronic search strategy. The search strategy is presented in Supplementary file 1. The search and selection of the articles were carried out by two authors (CSS and ALDA).

Study selection and data collection process

Following the initial search, two reviewers independently conducted the selection process. Rayyan QCRI was used as a reference manager to exclude duplicates, identify relevant articles according to the reading of title and abstract, perform screening and eligibility of full-text articles congruent with the predefined inclusion/exclusion criteria, as well as recording primary reasons for exclusion. Every step of this process was registered on a flowchart

according to the PRISMA guidelines. Disagreements were solved firstly by discussion and then by consulting a third author. Then, data extraction was conducted by the primary researcher and revised by a second author. The selected articles were scrutinized to extract the following main data: author(s), year of publication, country, objective, study design, eligibility criteria, the total number of cases, OSCCmi cases, age (mean), age (range), gender, tumor localization, clinical appearance, histopathological criteria, treatment, follow-up period, survival, recurrence, metastasis, and method chosen for statistical analysis if used. Qualitative and quantitative data was tabulated and processed in Microsoft Excel®.

Risk of individual bias (quality) assessment

To facilitate the assessment of possible risk of bias for each study, we collected information using The Joanna Briggs Institute Critical Appraisal tool (10). An individual checklist with multiple domains was made describing the procedures undertaken for each study. A judgment of the risk of bias was performed from the extracted information according to the amount of positive or negative domains obtained after evaluation. Subsequently, every investigation was rated by two independent authors as "low", "moderate" or "high" risk. Studies were considered with a low risk of bias if 0-49% of domains were classified as 'Yes'; moderate risk was represented by 50-75% 'Yes' score and high risk of bias was assigned to studies with 76-100% 'Yes' score. Disagreements were solved initially by discussion between the two authors, and then by referring to a third one if needed.

Data synthesis and statistical analysis

We expected to identify the histopathological profile to diagnose an oral squamous cell carcinoma as "micro-invasive". All results were interpreted according to the information extracted from the included studies. The level of consistency of obtained data was completely associated with the information available. Common extracted data found was categorized into groups for further comparison and analysis. Specific data from each study was also tabulated and considered for further separate description and discussion if relevant to the aim of our study.

A narrative descriptive synthesis covering the studies' findings is provided.

RESULTS:

Study selection

A flowchart according to PRISMA guidelines is presented illustrating the selection process (Fig.1). Using the selected search strategy, 2,024 records published between 1962 and 2022 were initially identified in all databases. 981 duplicate records were excluded, with 1,043 remaining for assessment. After screening by title and abstract, 44 reports were sought for retrieval, and 43 full text articles were assessed for eligibility. Consequently, 42 articles were excluded as they did not meet the eligibility criteria. In addition, 323 records were identified through other methods such as Google Scholar, ProQuest and manual retrieval via reference lists of selected articles. 52 duplicates were removed, and 271 were sought for retrieval and eligibility. Due to incompatibility with the predefined criteria, 268 reports were disqualified. Finally, a total of 4 studies were included (4,6,11,12).

Description of individual studies

Table 1 summarizes the main clinical and epidemiological data of the included studies. All the papers included were retrospective studies written in English, published between the years 2011 and 2020. Three of the publications were performed in Europe (Italy (6)), United Kingdom (11), and Ireland (12)) and one in Asia (India (4)). Of the four studies included, two of them analyzed clinical features of OSCCmi samples (4,6), one also analyzed histopathological features (4), one aimed to determine a method of DOI measurement (11), and one recorded the frequency of prognostic pathologic features in early OSCC (12). Correlations of obtained results to local recurrence and node positivity were assessed in different studies. The definition of "microinvasion" was diverse.

Altogether, these studies included a total of 408 patients, 116 of which were considered "microinvasive". The largest reported population was from India with 200 patients (4); however, the biggest "microinvasive" group belongs to Ireland, with 41 patients (12), followed by Italy (32 patients) (6), India (29 patients) (4) and United Kingdom (14 patients) (11). The mean age was 55.9 years, ranging between 20 to 92 years. One study did not report the average age (12). Regarding gender distribution, a male prevalence was noted. One study did not report gender distribution (4). According to the available information, 145 patients were male and 92 females, resulting in a 1.6:1 male:female ratio. All studies reported localization and the most affected site was tongue (91 lesions), followed by buccal mucosa (33 lesions), floor of mouth (23 lesions), soft palate/retromolar (8 lesions), gum/alveolar ridge/hard palate (7 lesions), vestibule (4 lesions), commissure (1 lesion) and 3 lesions in other unspecified sites. Two studies stated clinical appearance (4,6), with most of the lesions being patches (18 lesions), followed

by ulcers (16 lesions), plaques (9 lesions), erosions (7 lesions), verrucous lesions (5 lesions), growths (5 lesions) and a node (1 lesion).

Table 2 summarizes the main histopathological parameters used to diagnose OSCCmi, which were Depth of Invasion (DOI) and Tumor Thickness (TT), with findings reported in 3 (4,11,12) and 2 studies (6,12) respectively. In the 2 studies that used this parameter to determine microinvasion (6,12), OSCCmi lesions ranged in TT from 0.13 to 10mm with a mean thickness of 5.5 mm. Cut-off values used were 4 mm (6) and 10 mm (12). Respecting DOI, lesions ranged from 0.03 to 5 mm with a mean depth of 3 mm. Cut-off values used were 0.5 mm (11) and 5 mm (12). This parameter was also used to determine microinvasion in those studies. No study described nor determined architectural or cytologic findings of the affected epithelium. Parameters such as worst pattern of invasion (WPOI), perineural invasion (PNI), lymphovascular invasion (LVI), differentiation and dysplasia grade were each evaluated in only one article (12).

Surgical excision was the primary treatment modality, reported in 2 studies (6,11). Information regarding regional recurrence was stated in these mentioned studies, with a total of 5 cases of recurrence. Neck dissection was reported in 2 studies (6,12) and performed in 77 patients. Data on survival was evaluated in only one article (6). Follow-up time was heterogeneously stated in 3 studies (6,11,12). One study accompanied their patients for at least 7 years (12), one did it for more than five years (11), and one did it for a mean of 5.3 years (6).

A quantitative synthesis could not be performed as the included studies' present unsuitable quantitative data and are not sufficiently homogenous in terms of design, variables, and results to conduct a meta-analysis.

Risk of bias within studies

3 publications were categorized as having an overall moderate risk and 1 as high risk of bias, as clear information was not informed (Table 2). Detailed explanatory information about evaluation of bias risk is available in Supplementary file 2.

DISCUSSION:

OSCCmi is an incipient malignant disease of the oral mucosa. Several terms had been proposed to talk about very early presentations of OSCC, like superficially invasive OSCC (13) and small and thin OSCC (12). Microinvasion is considered as beyond the epithelial basement membrane, extending into the superficial adjacent stroma as small nests or islands (14). There are various articles citing the difficulty in diagnosing these micro-invasive tumors in various parts of the body (14,15). At present time, AJCC does not define OSCCmi as a separate entity, opposed to other anatomic regions; in the breast, microinvasive carcinoma is defined as "an invasive carcinoma with no focus measuring >1 mm", and it has even been stated through this classification that "the clinical impact of multifocal microinvasive disease is not well understood at this time". This uncertainty could be theoretically extrapolated to the oral cavity.

Besides multiple definitions, histopathological criteria have also been poorly reported. As evidenced by the very small number of studies obtained through this systematic review, there is limited data about OSCCmi, impeding objective analysis of prevalence and incidence. In 2012, Haberland *et al* described clinical and histopathological features of 12 OSCCmi cases and reported them through a conference abstract (13); they obtained an equal sex distribution and an average age of 53 years in this population which presented tumors predominantly in lateral border of the tongue. As a result of this present investigation, we have demonstrated a slight male predominance and a mean age of 56 years. These epidemiological results are consistent with the profile of patients affected by conventional OSCC across the world (1,2,16,17). Furthermore, there are no defined histopathological criteria for identification of microinvasion, and studies assessing microscopic profile or describing findings of this entity are currently minimally recorded and remarkably heterogeneous. Regarding architectural and cytologic features, bulky outgrowth of the epithelial rete pegs and ductal changes were described in 1963 as supportive, but not pathognomonic, histopathological differences in distinguishing carcinoma in situ from micro-invasive carcinoma by Shedd *et al* (18). Nevertheless, these were never verified in subsequent studies. In our study, analysis of other histopathological characteristics was challenging as there was insufficient and variable data. Grade of dysplasia was reported only in one study (4), and none presented details of the cytologic and/or architectural characteristics of the epithelium. In comparison, cervical microinvasive lesions are studied according to their specific histopathological characteristics, such as nuclear, stroma, and architectural findings (8). As identification of breaches in the

basement membrane are helpful, but difficult to identify, previous authors have described the difficulties in early invasion diagnosis to absence of tangible criteria, forcing the professional to rely on judgement and experience (19). This is one of the main reasons why OSCCmi deserves further study (20).

Our results reflect that the microscopic measurement of TT and DOI has been considered a parameter to diagnose OSCCmi. TT considers both exophytic and infiltrative component of the tumor, and it is measured from the highest and most superficial point of the lesion to the deepest point of infiltration (21). In regards of DOI, this measure is usually estimated as the perpendicular distance from the basement membrane region to the deepest point of the tumor front, and it is used to assess the infiltrative component of a malignancy (21). The consensus among pathologists on the maximum dimension in microinvasive OSCCs is limited. We proved this situation for TT and DOI, as designation of cut-off values in each study for both units was merely arbitrary, and no robust evidence was found to support these decisions. Two included studies measured TT (6,12) and three studies used DOI (4,11,12). Also, the variability and frequent lack of clarity in these investigations and in the literature regarding the exact definitions of TT vs DOI is an important issue, particularly when establishing reference points on the epithelium to determine stated dimensions (22). Calculating these measurements is often more theoretical than practical because of the limited thickness of healthy epithelium (22), if any, particularly in incisional biopsies, information which majority of studies do not clarity through their methodology. Localization and tissue disposition would be also a factor to consider because of anatomical variations that could result in a DOI underestimation (23). On this matter, the possible relevance of morphological findings as another factor must be noted in association with TT and DOI; we consider it useful to report differences in atrophic versus exophytic or verrucous lesions, since it is expected that the thickness of these lesions would vary, and therefore this discrepancy could impact the wide ranges found in TT and DOI. Unfortunately, in this review only one article reported 5 verrucous lesions (4) and 5 nodular lesions (6), so the data were unfortunately too sparse to determine their significance. Microinvasion in verrucous lesions is also a topic that has not been widely studied.

Amit-Byatnal *et al* assessed these parameters (TT and DOI) both manually and automatically by an image analysis software and using two different reference points to test variations resulting from these discrepancies, and they attained similar results with non-

significant variations (11). Studies have described higher tumor aggressiveness in early lesions with DOI between 3 and 5 mm (12). Considering that the TNM 8th edition staging has taken depth into account by including 5 mm as the cut off between pT1 and pT2 (12,24) and bearing in mind possible uses of DOI and TT to assess outcomes in patients with OSCCmi, it is extremely relevant to conduct research involving this subpopulation of OSCCmi patients aiming to reach consensus in terms of objective definition and measurements to categorize this disease separately.

Many other features have been associated with predicting a more adverse outcome of OSCC, such as WPOI (25) and PNI (26). However, parameters such as histological differentiation, WPOI, PNI, LVI, and dysplasia were evaluated in just one paper (12). Some studies also highlight possible relevance of lymphocytic or inflammatory stromal response (19,23,25,26). Heavy inflammatory infiltrate has been described as hampering factor for interpretation of invasion, mostly because it can lead to confusion differentiating between reactive epithelial atypia and oral epithelial dysplasia (27,28) and also because it can hinder basal membrane assessment, resulting a false positive on microinvasion of basal cells. Haberland *et al* reported a moderate to severe lichenoid band-like lymphocytic response in 7 of 12 cases of OSCCmi (13). However, this feature was not analyzed in any of the papers included, and so the relevance of this ascertainment is to be further explored.

Early stages of invasion are critical in terms of diagnosis and prognosis. The obtained data was scarce as expected, since microinvasion has been reported to have a lower incidence of metastatic spread and head and neck surgeons usually do not reoperate these cases. More research would also be interesting to back up this situation.

Finally, it is worth noting the significance of alternative methods that could assist in objective analysis (29), and in this matter, machine learning methods may improve the diagnostic process by the development of artificial intelligence models able to recognize existing patterns imperceptible on routine microscopic evaluation (30).

The limitations of this review must be discussed. First, we experienced difficulties previously reported by Pentenero *et al* (6), because the evidence in the literature regarding "early OSCC" mostly refers as T1/T2 cases; hence, during the eligibility phase of the selection process numerous reports assessed were excluded since the sample would analyze these two groups without distinction. For this reason, it is assumed that incipient microinvasive cases in these samples were not considered on this systematic review as there was no way to extract

desired data from the analyses. Also, the divergence in the results of the included studies is not only due to low sampling, but also to methodological differences previously mentioned, such as definitions, diagnostic criteria, and measurements of parameters like DOI and TT, which have been evidenced as critical to diagnose OSCCmi, as well as methods of its detection. As authors consider OSCCmi starting from different principles, there is a tendency to obtain different results even in similar populations or similar objective studies. Consequently, it is expected that the heterogeneity of the included studies could have influenced our results, particularly since data was, in most cases, not comparable.

CONCLUSION:

OSCCmi is an under-reported incipient malignant entity with a male prevalence that commonly involves tongue and buccal mucosa and has primarily been determined by measures of TT and DOI. Histopathological parameters are not standardized and vary greatly among the evidence available. Characteristics such as cytoarchitectural changes, WPOI, PNI, LVI, and grade of dysplasia have not been considered relevant to the diagnosis of OSCCmi, and there are minimal data about these features and their relation to diagnosis, recurrence, and survival. Thus, there is difficulty in standardizing diagnostic criteria for OSCCmi. This systematic review has highlighted a lack of evidence and absence of agreement concerning histopathological specific parameters to assist proper diagnosis. Consequently, only a few studies were conducted focusing on this population. The significance of a proper histopathological profile assigning defined objective measures such as TT and DOI with distinct established cut-off values is highlighted in this study, as early diagnosed diseases are associated with increased favorable outcomes. By means of this study, we emphasize the need for research concerning this entity. The expansion of this line of research would favor the correct diagnosis of incipient lesions, contributing to a consensus and facilitating microscopic analysis, consequently increasing the number of patients that can be diagnosed prematurely.

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Informed consent

For this type of study informed consent is not required.

Conflict of interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethics

This article does not contain any studies with human participants or animals performed by any of the authors.

Data availability

Data supporting the findings of this study is available on the supplementary material and from the corresponding author upon reasonable request.

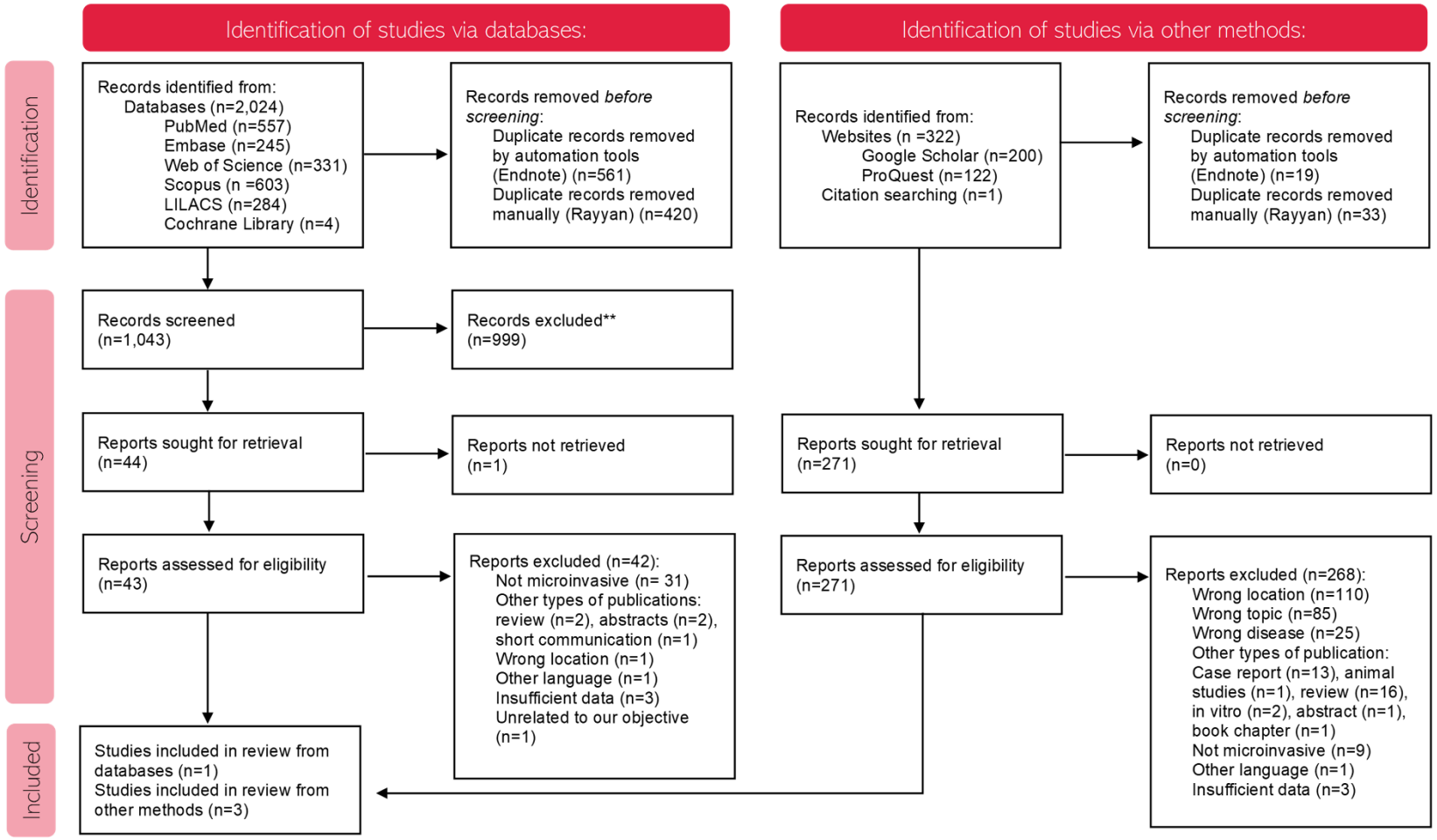
Author contributions

CSS and AR-SS: Conceptualization; CSS and ALDA: Data curation; CSS and ALDA Formal analysis; AR-SS and CSS: Funding acquisition; CSS, ALDA, WAGA, FJTN, MAL, KDH, PAV, AR-SS: Investigation; CSS, ALDA, ARR-SS: Methodology; WAGA, FJTN, MAL, KDH, PAV, AR-SS: Supervision; WAGA, FJTN, MAL, KDH, PAV, AR-SS: Validation; WAGA, FJTN, MAL, KDH, PAV, AR-SS: Visualization; CSS and ALDA: Roles/Writing - original draft; WAGA, FJTN, MAL, KDH, PAV, AR-SS: Writing - review & editing.

List of abbreviations:

OSCC – Oral squamous cell carcinoma.
OSCCmi – Microinvasive oral squamous cell carcinoma.
PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-Analyses.
HE - Hematoxylin-eosin.
FFPE - Formalin-fixed paraffin-embedded.
TT – Tumor thickness.
DOI – Depth of invasion.
WPOI - Worst pattern of invasion.
PNI - Perineural invasion.

Fig. 1 - Flowchart describing literature search and overall included studies according to PRISMA guideline (2021 update).



*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).
**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71.

Table 1 - Baseline clinical and epidemiological characteristics of the included studies.

Author, year and country	Patients (n)	OSCCmi patients (n)	Gender (M/F)	Age mean (range)	Clinical appearance	Site	Follow-up time (years)	Regional recurrence	Neck dissection	5-year DSS rate
Pentenero M *et al* (6). Italy, 2011.	99	32	56/43	64.2 (30-92)	Erosion: 7 (21,9%), Plaques: 9 (28,1%), Patch: 4 (12,5%), Ulcer: 6 (18,8%), Verrucous lesion: 5 (15,6%), Node: 1 (3,1%)	Tongue: 15 (46,9%), Buccal mucosa: 7 (21,9%), Floor of mouth: 7 (21,9%), Other: 3 (9,4%)	5,3 (0,3-13,3)	1 (3,1%)	6 (6,06%)	96% (p=0,550).
Amit-Byatnal A *et al* (11). UK, 2015.	14	14	9/5	53,9 (20-78)	NI	Tongue: 14 (100%)	5+	4 (28,57%)	NI	NI
Sridharan G *et al* (4). India, 2017.	200	29	21/8	49,6 (24-76)	Patch: 14 (48,28%), Ulcer: 10 (34,48%), Growth: 5 (17,24%)	Buccal mucosa: 22 (75.86%), Vestibule: 4 (13.79%), Tongue: 1 (3,45%), Floor of mouth: 1 (3,45%), Commissure: 1 (3,45%)	NI	NI	NI	NI
Mohammed Nur M *et al* (12). Ireland, 2020.	95	41	59/36	-	NI	Tongue: 61 (64,21%), Floor of mouth: 15 (15,79%), Gum/alveolar ridge/hard palate: 7 (7,37%), Soft palate/retromolar: 8 (8,42%), Buccal mucosa: 4 (4,21%)	Minimum of 7 years	NI	71 (74,7%)	NI

NI: No information; DSS: disease-specific survival.

Table 2 – Histopathological parameters used to diagnose OSCCmi on the included studies.

Author, year and country	Principal criteria to diagnose as microinvasive	TT Cut-off value	TT Mean (range)	DOI Cut-off value	DOI Mean (range)
Pentenero M *et al* (6). Italy, 2011.	Invasive squamous cell carcinoma with TT <4mm.	4mm	NI (0,13-1,3mm)	NI	NI
Amit-Byatnal A *et al* (11). UK, 2015.	Invasive squamous cell carcinoma that extends into the stroma by < 0.5 mm, from the adjacent non-neoplastic epithelial basement membrane.	NI	NI	0,5mm	0,356 mm (0,05-0,67 mm)
Sridharan G *et al* (4). India, 2017.	Lesions with suspected basement membrane breach and presence of dense inflammatory infiltrate.	NI	NI	NI	NI (0,03 ± 0,01 mm)
Mohammed Nur M *et al* (12). Ireland, 2020.	Defined and separated two groups: "small" (TT ≤ 10mm) and "thin" (DOI ≤5mm) OSCC.	10mm	11mm (1,2-20mm)	5mm	2,6mm (0,5-10mm)

NI: No information; DOI: Depth of invasion; TT: Tumor thickness.

Table 3 - Overall appraisal of risk of bias assessment for the 4 included studies according to The Joanna Briggs Institute Critical Appraisal tool for Cross Sectional Studies.

SELECTED STUDIES	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	% YES	RISK OF BIAS
Amit-Byatnal *et al*	✓	X	✓	X	✓	✓	✓	✓	75%	Moderate
Pentenero *et al*	✓	X	✓	X	X	X	✓	✓	50%	Moderate
Mohammed Nur *et al*	✓	✓	✓	X	X	X	✓	✓	62,5%	Moderate
Sridharan *et al*	✓	X	✓	X	X	X	✓	N/A	42,8%	High

Q1-Q8: Questions from Joanna Briggs Institute (JBI) risk assessment checklist.
✓ indicates yes, X indicates no, '?' indicates unclear, N/A indicates not applicable.
Ranked as follows: High: 0 - 49% yes; Moderate: 50-75% yes; Low: 76-100% yes scores.

SUPPLEMENTARY MATERIAL

Supplementary file 1 – Search strategy**.**
Search terms:

#1
(Neoplasm[MeSH Terms] OR "Cancer" OR "Malignancy" OR "Malignant Neoplasm" OR "Carcinoma" OR “squamous cell carcinoma” OR "carcinoma, squamous cell"[MeSH Terms] OR "carcinomas, squamous cell" OR "carcinomas, epidermoid" OR "carcinoma, epidermoid" OR "carcinoma, squamous" OR "carcinomas, squamous" OR "Precancerous conditions"[Mesh] OR “precancerous” OR "pre-cancer" OR “precancer” OR “preneoplastic” OR "pre-neoplastic" OR "precursor lesion" OR "precursor lesions" OR "potentially malignant disorder" OR "potentially malignant disorders" OR "potentially malignant lesion" OR "potentially malignant lesions" OR “premalignant” OR "pre malignant" OR” premalignancy” OR "pre malignancy" OR “premalignancies” OR "pre malignancies" OR "Carcinoma in Situ"[Mesh] OR "Carcinoma in situ" OR "in situ carcinoma" OR "Carcinomas in situ" OR "in situ carcinomas" OR "preinvasive carcinoma" OR "preinvasive carcinomas" OR "pre-invasive carcinoma" OR "pre-invasive carcinomas" OR "intraepithelial carcinoma" OR "intraepithelial carcinomas" OR "intraepithelial neoplasm" OR "intraepithelial neoplasms" OR "intraepithelial neoplasia" OR "intraepithelial neoplasias" OR "intraepithelial cancer") AND ("Mouth"[Mesh Terms] OR "mouth" OR "mouths" OR "oral" OR "lip"[MeSH Terms] OR "lip" OR "lips" OR "tongue"[MeSH Terms] OR "tongue" OR "Mouth Mucosa"[MeSH Terms] OR "Mouth Mucosa" OR "buccal" OR "Palate"[MeSH Terms] OR "palate" OR "palates" OR "Mouth Floor"[MeSH Terms] OR "Mouth Floor" OR "cheek mucosa" OR "alveolar" OR "Gingiva"[MeSH Terms] OR "gingiva" OR "gum" OR "gums" OR "interdental papilla" OR "interdental papillae" OR "commissure" OR "maxillary tuberosity" OR "uvula" OR "uvular")
#2
("Histopathological criteria" OR "histopathological features" OR "histopathological characteristics" OR "histopathological findings" OR "microscopic criteria" OR "microscopic features" OR "microscopic characteristics" OR "microscopic findings" OR "pathologic criteria" OR "pathologic features" OR "pathologic characteristics" OR "pathologic findings" OR "grading system" OR "grading systems" OR "dysplasia grading" OR "Histopathologic criteria" OR "histopathologic features" OR "histopathologic characteristics" OR "histopathologic findings" OR "microscopical criteria" OR "microscopical features" OR "microscopical characteristics" OR "microscopical findings" OR "pathological criteria" OR "pathological features" OR "pathological characteristics" OR "pathological findings")
#3
("microinvasive" OR "microinvasion" OR "micro-invasive" OR "micro-invasion" OR "micro invasion" OR "micro invasive" OR "minimally invasive" OR "early-stage" OR "early stage" OR "early" OR "superficial" OR "superficially invasive" OR "incipient" OR "T1" OR "pT1" OR "emerging" OR "emergent")

Search strategy:

Database	Search strategy (Search date: February 18th, 2022)	Results
PubMed	(((Neoplasm[MeSH Terms] OR "Cancer" OR "Malignancy" OR "Malignant Neoplasm" OR "Carcinoma" OR “squamous cell carcinoma” OR "carcinoma, squamous cell"[MeSH Terms] OR "carcinomas, squamous cell" OR "carcinomas, epidermoid" OR "carcinoma, epidermoid" OR "carcinoma, squamous" OR "carcinomas, squamous" OR "Precancerous conditions"[Mesh] OR “precancerous” OR "pre-cancer" OR “precancer” OR “preneoplastic” OR "pre-neoplastic" OR "precursor lesion" OR "precursor lesions" OR "potentially malignant disorder" OR "potentially malignant disorders" OR "potentially malignant lesion" OR "potentially malignant lesions" OR “premalignant” OR "pre malignant" OR” premalignancy” OR "pre malignancy" OR “premalignancies” OR "pre malignancies" OR "Carcinoma in Situ"[Mesh] OR "Carcinoma in situ" OR "in situ carcinoma" OR "Carcinomas in situ" OR "in situ carcinomas" OR "preinvasive carcinoma" OR "preinvasive carcinomas" OR "pre-invasive carcinoma" OR "pre-invasive carcinomas" OR "intraepithelial carcinoma" OR "intraepithelial carcinomas" OR "intraepithelial neoplasm" OR "intraepithelial neoplasms" OR "intraepithelial neoplasia" OR "intraepithelial neoplasias" OR "intraepithelial cancer") AND ("Mouth"[Mesh Terms] OR "mouth" OR "mouths" OR "oral" OR "lip"[MeSH Terms] OR "lip" OR "lips" OR "tongue"[MeSH Terms] OR "tongue" OR "Mouth Mucosa"[MeSH Terms] OR "Mouth Mucosa" OR "buccal" OR "Palate"[MeSH Terms] OR "palate" OR "palates" OR "Mouth Floor"[MeSH Terms] OR "Mouth Floor" OR "cheek mucosa" OR "alveolar" OR "Gingiva"[MeSH Terms] OR "gingiva" OR "gum" OR "gums" OR "interdental papilla" OR "interdental papillae" OR "commissure" OR "maxillary tuberosity" OR "uvula" OR "uvular")) AND ("Histopathological criteria" OR "histopathological features" OR "histopathological characteristics" OR "histopathological findings" OR "microscopic criteria" OR "microscopic features" OR "microscopic characteristics" OR "microscopic findings" OR "pathologic criteria" OR "pathologic features" OR "pathologic characteristics" OR "pathologic findings" OR "grading system" OR "grading systems" OR "dysplasia grading" OR "Histopathologic criteria" OR "histopathologic features" OR "histopathologic characteristics" OR "histopathologic findings" OR "microscopical criteria" OR "microscopical features" OR "microscopical characteristics" OR "microscopical findings" OR "pathological criteria" OR "pathological features" OR "pathological characteristics" OR "pathological findings")) AND ("microinvasive" OR	557

	"microinvasion" OR "micro-invasive" OR "micro-invasion" OR "micro invasion" OR "micro invasive" OR "minimally invasive" OR "early-stage" OR "early stage" OR "early" OR "superficial" OR "superficially invasive" OR "incipient" OR "T1" OR "pT1" OR "emerging" OR "emergent")	
Embase	('neoplasm'/de OR 'cancer'/de OR 'malignancy'/de OR 'malignant neoplasm'/de OR 'carcinoma'/de OR 'squamous cell carcinoma'/de OR 'carcinoma, squamous cell'/de OR 'carcinomas, squamous cell' OR 'carcinomas, epidermoid' OR 'carcinoma, epidermoid' OR 'carcinoma, squamous' OR 'carcinomas, squamous' OR 'precancerous conditions'/de OR 'precancerous' OR 'pre-cancer'/de OR 'precancer'/de OR 'preneoplastic' OR 'pre-neoplastic' OR 'precursor lesion' OR 'precursor lesions' OR 'potentially malignant disorder'/de OR 'potentially malignant disorders' OR 'potentially malignant lesion' OR 'potentially malignant lesions' OR 'premalignant' OR 'pre malignant' OR 'premalignancy'/de OR 'pre malignancy'/de OR 'premalignancies'/de OR 'pre malignancies'/de OR 'carcinoma in situ'/de OR 'in situ carcinoma'/de OR 'carcinomas in situ' OR 'in situ carcinomas' OR 'preinvasive carcinoma'/de OR 'preinvasive carcinomas' OR 'pre-invasive carcinoma' OR 'pre-invasive carcinomas' OR 'intraepithelial carcinoma'/de OR 'intraepithelial carcinomas' OR 'intraepithelial neoplasm'/de OR 'intraepithelial neoplasms' OR 'intraepithelial neoplasia'/de OR 'intraepithelial neoplasias' OR 'intraepithelial cancer') AND ('mouth'/de OR 'mouths' OR 'oral' OR 'lip'/de OR 'lips'/de OR 'tongue'/de OR 'mouth mucosa'/de OR 'buccal' OR 'palate'/de OR 'palates' OR 'mouth floor'/de OR 'cheek mucosa'/de OR 'alveolar' OR 'gingiva'/de OR 'gum'/de OR 'gums' OR 'interdental papilla'/de OR 'interdental papillae'/de OR 'commissure' OR 'maxillary tuberosity'/de OR 'uvula'/de OR 'uvular') AND ('histopathological criteria' OR 'histopathological features' OR 'histopathological characteristics' OR 'histopathological findings' OR 'microscopic criteria' OR 'microscopic features' OR 'microscopic characteristics' OR 'microscopic findings' OR 'pathologic criteria' OR 'pathologic features' OR 'pathologic characteristics' OR 'pathologic findings' OR 'grading system'/de OR 'grading systems' OR 'dysplasia grading' OR 'histopathologic criteria' OR 'histopathologic features' OR 'histopathologic characteristics' OR 'histopathologic findings' OR 'microscopical criteria' OR 'microscopical features' OR 'microscopical characteristics' OR 'microscopical findings' OR 'pathological criteria' OR 'pathological features' OR 'pathological characteristics' OR 'pathological findings') AND ('microinvasive' OR 'microinvasion' OR 'micro-invasive' OR 'micro-invasion' OR 'micro invasion' OR 'micro invasive' OR 'minimally invasive' OR 'early-stage' OR 'early stage' OR	245

	'early' OR 'superficial' OR 'superficially invasive' OR 'incipient' OR 't1' OR 'pt1' OR 'emerging' OR 'emergent')	
Web of Science	TEMA: ("Neoplasm" OR "Cancer" OR "Malignancy" OR "Malignant Neoplasm" OR "Carcinoma" OR “squamous cell carcinoma” OR "carcinoma, squamous cell" OR "carcinomas, squamous cell" OR "carcinomas, epidermoid" OR "carcinoma, epidermoid" OR "carcinoma, squamous" OR "carcinomas, squamous" OR "Precancerous conditions" OR “precancerous” OR "pre-cancer" OR “precancer” OR “preneoplastic” OR "pre-neoplastic" OR "precursor lesion" OR "precursor lesions" OR "potentially malignant disorder" OR "potentially malignant disorders" OR "potentially malignant lesion" OR "potentially malignant lesions" OR “premalignant” OR "pre malignant" OR” premalignancy” OR "pre malignancy" OR “premalignancies” OR "pre malignancies" OR "Carcinoma in Situ" OR "in situ carcinoma" OR "Carcinomas in situ" OR "in situ carcinomas" OR "preinvasive carcinoma" OR "preinvasive carcinomas" OR "pre-invasive carcinoma" OR "pre-invasive carcinomas" OR "intraepithelial carcinoma" OR "intraepithelial carcinomas" OR "intraepithelial neoplasm" OR "intraepithelial neoplasms" OR "intraepithelial neoplasia" OR "intraepithelial neoplasias" OR "intraepithelial cancer") AND TEMA: ("Mouth" OR "mouth" OR "mouths" OR "oral" OR "lip" OR "lips" OR "tongue" OR "Mouth Mucosa" OR "buccal" OR "palate" OR "palates" OR "Mouth Floor" OR "cheek mucosa" OR "alveolar" OR "gingiva" OR "gum" OR "gums" OR "interdental papilla" OR "interdental papillae" OR "commissure" OR "maxillary tuberosity" OR "uvula" OR "uvular") AND TEMA: ("Histopathological criteria" OR "histopathological features" OR "histopathological characteristics" OR "histopathological findings" OR "microscopic criteria" OR "microscopic features" OR "microscopic characteristics" OR "microscopic findings" OR "pathologic criteria" OR "pathologic features" OR "pathologic characteristics" OR "pathologic findings" OR "grading system" OR "grading systems" OR "dysplasia grading" OR "Histopathologic criteria" OR "histopathologic features" OR "histopathologic characteristics" OR "histopathologic findings" OR "microscopical criteria" OR "microscopical features" OR "microscopical characteristics" OR "microscopical findings" OR "pathological criteria" OR "pathological features" OR "pathological characteristics" OR "pathological findings") AND TEMA: ("microinvasive" OR "microinvasion" OR "micro-invasive" OR "micro-invasion" OR "micro invasion" OR "micro invasive" OR "minimally invasive" OR "early-stage" OR "early stage" OR "early" OR "superficial" OR "superficially invasive" OR "incipient" OR "T1" OR "pT1" OR "emerging" OR "emergent")	331

LILACS	("Neoplasm" OR "Cancer" OR "Malignancy" OR "Malignant Neoplasm" OR "Carcinoma" OR “squamous cell carcinoma” OR "carcinoma, squamous cell" OR "carcinomas, squamous cell" OR "carcinomas, epidermoid" OR "carcinoma, epidermoid" OR "carcinoma, squamous" OR "carcinomas, squamous" OR "Precancerous conditions" OR “precancerous” OR "pre-cancer" OR “precancer” OR “preneoplastic” OR "pre-neoplastic" OR "precursor lesion" OR "precursor lesions" OR "potentially malignant disorder" OR "potentially malignant disorders" OR "potentially malignant lesion" OR "potentially malignant lesions" OR “premalignant” OR "pre malignant" OR” premalignancy” OR "pre malignancy" OR “premalignancies” OR "pre malignancies" OR "Carcinoma in Situ" OR "in situ carcinoma" OR "Carcinomas in situ" OR "in situ carcinomas" OR "preinvasive carcinoma" OR "preinvasive carcinomas" OR "pre-invasive carcinoma" OR "pre-invasive carcinomas" OR "intraepithelial carcinoma" OR "intraepithelial carcinomas" OR "intraepithelial neoplasm" OR "intraepithelial neoplasms" OR "intraepithelial neoplasia" OR "intraepithelial neoplasias" OR "intraepithelial cancer") AND ("Mouth" OR "mouth" OR "mouths" OR "oral" OR "lip" OR "lips" OR "tongue" OR "Mouth Mucosa" OR "buccal" OR "palate" OR "palates" OR "Mouth Floor" OR "cheek mucosa" OR "alveolar" OR "gingiva" OR "gum" OR "gums" OR "interdental papilla" OR "interdental papillae" OR "commissure" OR "maxillary tuberosity" OR "uvula" OR "uvular") AND("Histopathological criteria" OR "histopathological features" OR "histopathological characteristics" OR "histopathological findings" OR "microscopic criteria" OR "microscopic features" OR "microscopic characteristics" OR "microscopic findings" OR "pathologic criteria" OR "pathologic features" OR "pathologic characteristics" OR "pathologic findings" OR "grading system" OR "grading systems" OR "dysplasia grading" OR "Histopathologic criteria" OR "histopathologic features" OR "histopathologic characteristics" OR "histopathologic findings" OR "microscopical criteria" OR "microscopical features" OR "microscopical characteristics" OR "microscopical findings" OR "pathological criteria" OR "pathological features" OR "pathological characteristics" OR "pathological findings") AND ("microinvasive" OR "microinvasion" OR "micro-invasive" OR "micro-invasion" OR "micro invasion" OR "micro invasive" OR "minimally invasive" OR "early-stage" OR "early stage" OR "early" OR "superficial" OR "superficially invasive" OR "incipient" OR "T1" OR "pT1" OR "emerging" OR "emergent")	284
SCOPUS	TITLE-ABS-KEY("Neoplasm" OR "Cancer" OR "Malignancy" OR "Malignant Neoplasm" OR "Carcinoma" OR “squamous cell carcinoma” OR "carcinoma, squamous	603

	cell" OR "carcinomas, squamous cell" OR "carcinomas, epidermoid" OR "carcinoma, epidermoid" OR "carcinoma, squamous" OR "carcinomas, squamous" OR "Precancerous conditions" OR “precancerous” OR "pre-cancer" OR “precancer” OR “preneoplastic” OR "pre-neoplastic" OR "precursor lesion" OR "precursor lesions" OR "potentially malignant disorder" OR "potentially malignant disorders" OR "potentially malignant lesion" OR "potentially malignant lesions" OR “premalignant” OR "pre malignant" OR” premalignancy” OR "pre malignancy" OR “premalignancies” OR "pre malignancies" OR "Carcinoma in Situ" OR "in situ carcinoma" OR "Carcinomas in situ" OR "in situ carcinomas" OR "preinvasive carcinoma" OR "preinvasive carcinomas" OR "pre-invasive carcinoma" OR "pre-invasive carcinomas" OR "intraepithelial carcinoma" OR "intraepithelial carcinomas" OR "intraepithelial neoplasm" OR "intraepithelial neoplasms" OR "intraepithelial neoplasia" OR "intraepithelial neoplasias" OR "intraepithelial cancer") AND TITLE-ABS-KEY("Mouth" OR "mouth" OR "mouths" OR "oral" OR "lip" OR "lips" OR "tongue" OR "Mouth Mucosa" OR "buccal" OR "palate" OR "palates" OR "Mouth Floor" OR "cheek mucosa" OR "alveolar" OR "gingiva" OR "gum" OR "gums" OR "interdental papilla" OR "interdental papillae" OR "commissure" OR "maxillary tuberosity" OR "uvula" OR "uvular") AND TITLE-ABS-KEY("Histopathological criteria" OR "histopathological features" OR "histopathological characteristics" OR "histopathological findings" OR "microscopic criteria" OR "microscopic features" OR "microscopic characteristics" OR "microscopic findings" OR "pathologic criteria" OR "pathologic features" OR "pathologic characteristics" OR "pathologic findings" OR "grading system" OR "grading systems" OR "dysplasia grading" OR "Histopathologic criteria" OR "histopathologic features" OR "histopathologic characteristics" OR "histopathologic findings" OR "microscopical criteria" OR "microscopical features" OR "microscopical characteristics" OR "microscopical findings" OR "pathological criteria" OR "pathological features" OR "pathological characteristics" OR "pathological findings") AND TITLE-ABS-KEY("microinvasive" OR "microinvasion" OR "micro-invasive" OR "micro-invasion" OR "micro invasion" OR "micro invasive" OR "minimally invasive" OR "early-stage" OR "early stage" OR "early" OR "superficial" OR "superficially invasive" OR "incipient" OR "T1" OR "pT1" OR "emerging" OR "emergent")	
Cochrane Library	"Neoplasm" OR "Cancer" OR "Malignancy" OR "Malignant Neoplasm" OR "Carcinoma" OR “squamous cell carcinoma” OR "carcinoma, squamous cell" OR "carcinomas, squamous cell" OR "carcinomas, epidermoid" OR "carcinoma,	4

	epidermoid" OR "carcinoma, squamous" OR "carcinomas, squamous" OR "Precancerous conditions" OR “precancerous” OR "pre-cancer" OR “precancer” OR “preneoplastic” OR "pre-neoplastic" OR "precursor lesion" OR "precursor lesions" OR "potentially malignant disorder" OR "potentially malignant disorders" OR "potentially malignant lesion" OR "potentially malignant lesions" OR “premalignant” OR "pre malignant" OR” premalignancy” OR "pre malignancy" OR “premalignancies” OR "pre malignancies" OR "Carcinoma in Situ" OR "in situ carcinoma" OR "Carcinomas in situ" OR "in situ carcinomas" OR "preinvasive carcinoma" OR "preinvasive carcinomas" OR "pre-invasive carcinoma" OR "pre-invasive carcinomas" OR "intraepithelial carcinoma" OR "intraepithelial carcinomas" OR "intraepithelial neoplasm" OR "intraepithelial neoplasms" OR "intraepithelial neoplasia" OR "intraepithelial neoplasias" OR "intraepithelial cancer" in Title Abstract Keyword AND "Mouth" OR "mouth" OR "mouths" OR "oral" OR "lip" OR "lips" OR "tongue" OR "Mouth Mucosa" OR "buccal" OR "palate" OR "palates" OR "Mouth Floor" OR "cheek mucosa" OR "alveolar" OR "gingiva" OR "gum" OR "gums" OR "interdental papilla" OR "interdental papillae" OR "commissure" OR "maxillary tuberosity" OR "uvula" OR "uvular" in Title Abstract Keyword AND "Histopathological criteria" OR "histopathological features" OR "histopathological characteristics" OR "histopathological findings" OR "microscopic criteria" OR "microscopic features" OR "microscopic characteristics" OR "microscopic findings" OR "pathologic criteria" OR "pathologic features" OR "pathologic characteristics" OR "pathologic findings" OR "grading system" OR "grading systems" OR "dysplasia grading" OR "Histopathologic criteria" OR "histopathologic features" OR "histopathologic characteristics" OR "histopathologic findings" OR "microscopical criteria" OR "microscopical features" OR "microscopical characteristics" OR "microscopical findings" OR "pathological criteria" OR "pathological features" OR "pathological characteristics" OR "pathological findings" in Title Abstract Keyword AND "microinvasive" OR "microinvasion" OR "micro-invasive" OR "micro-invasion" OR "micro invasion" OR "micro invasive" OR "minimally invasive" OR "early-stage" OR "early stage" OR "early" OR "superficial" OR "superficially invasive" OR "incipient" OR "T1" OR "pT1" OR "emerging" OR "emergent" in Title Abstract Keyword	
	Grey Literature	
Google Scholar	("oral cancer" OR "oral squamous cell carcinoma") AND ("microscopic" OR "histopathologic" OR "pathologic") AND ("microinvasion" OR "microinvasive" "early" OR "incipient"	200

	OR "minimally invasive" OR "T1" OR "superficial" OR "pT1" OR "emerging")	
ProQuest	TI,AB("Neoplasm" OR "Cancer" OR "Malignancy" OR "Malignant Neoplasm" OR "Carcinoma" OR “squamous cell carcinoma” OR "carcinoma, squamous cell" OR "carcinomas, squamous cell" OR "carcinomas, epidermoid" OR "carcinoma, epidermoid" OR "carcinoma, squamous" OR "carcinomas, squamous" OR "Precancerous conditions" OR “precancerous” OR "pre-cancer" OR “precancer” OR “preneoplastic” OR "pre-neoplastic" OR "precursor lesion" OR "precursor lesions" OR "potentially malignant disorder" OR "potentially malignant disorders" OR "potentially malignant lesion" OR "potentially malignant lesions" OR “premalignant” OR "pre malignant" OR” premalignancy” OR "pre malignancy" OR “premalignancies” OR "pre malignancies" OR "Carcinoma in Situ" OR "in situ carcinoma" OR "Carcinomas in situ" OR "in situ carcinomas" OR "preinvasive carcinoma" OR "preinvasive carcinomas" OR "pre-invasive carcinoma" OR "pre-invasive carcinomas" OR "intraepithelial carcinoma" OR "intraepithelial carcinomas" OR "intraepithelial neoplasm" OR "intraepithelial neoplasms" OR "intraepithelial neoplasia" OR "intraepithelial neoplasias" OR "intraepithelial cancer”) AND TI,AB("Mouth" OR "mouth" OR "mouths" OR "oral" OR "lip" OR "lips" OR "tongue" OR "Mouth Mucosa" OR "buccal" OR "palate" OR "palates" OR "Mouth Floor" OR "cheek mucosa" OR "alveolar" OR "gingiva" OR "gum" OR "gums" OR "interdental papilla" OR "interdental papillae" OR "commissure" OR "maxillary tuberosity" OR "uvula" OR "uvular") AND TI,AB("Histopathological criteria" OR "histopathological features" OR "histopathological characteristics" OR "histopathological findings" OR "microscopic criteria" OR "microscopic features" OR "microscopic characteristics" OR "microscopic findings" OR "pathologic criteria" OR "pathologic features" OR "pathologic characteristics" OR "pathologic findings" OR "grading system" OR "grading systems" OR "dysplasia grading" OR "Histopathologic criteria" OR "histopathologic features" OR "histopathologic characteristics" OR "histopathologic findings" OR "microscopical criteria" OR "microscopical features" OR "microscopical characteristics" OR "microscopical findings" OR "pathological criteria" OR "pathological features" OR "pathological characteristics" OR "pathological findings") AND TI,AB("microinvasive" OR "microinvasion" OR "micro-invasive" OR "micro-invasion" OR "micro invasion" OR "micro invasive" OR "minimally invasive" OR "early-stage" OR "early stage" OR "early" OR "superficial" OR "superficially invasive" OR "incipient" OR "T1" OR "pT1" OR "emerging" OR "emergent")	122

Citation searching		1
TOTAL		2,347

Supplementary file 2 – Critical appraisal tool for use in JBI Systematic Reviews: Checklist for analytical cross-sectional studies (10).

CHECKLIST FOR ANALYTICAL CROSS-SECTIONAL STUDIES
Critical Appraisal tools for use in JBI Systematic Reviews

INTRODUCTION

JBI is an JBI is an international research organisation based in the Faculty of Health and Medical Sciences at the University of Adelaide, South Australia. JBI develops and delivers unique evidence-based information, software, education and training designed to improve healthcare practice and health outcomes. With over 70 Collaborating Entities, servicing over 90 countries, JBI is a recognised global leader in evidence-based healthcare.

JBI Systematic Reviews

The core of evidence synthesis is the systematic review of literature of a particular intervention, condition or issue. The systematic review is essentially an analysis of the available literature (that is, evidence) and a judgment of the effectiveness or otherwise of a practice, involving a series of complex steps. JBI takes a particular view on what counts as evidence and the methods utilised to synthesise those different types of evidence. In line with this broader view of evidence, JBI has developed theories, methodologies and rigorous processes for the critical appraisal and synthesis of these diverse forms of evidence in order to aid in clinical decision-making in healthcare. There now exists JBI guidance for conducting reviews of effectiveness research, qualitative research, prevalence/incidence, etiology/risk, economic evaluations, text/opinion, diagnostic test accuracy, mixed-methods, umbrella reviews and scoping reviews. Further information regarding JBI systematic reviews can be found in the JBI Evidence Synthesis Manual.

JBI Critical Appraisal Tools

All systematic reviews incorporate a process of critique or appraisal of the research evidence. The purpose of this appraisal is to assess the methodological quality of a study and to determine the extent to which a study has addressed the possibility of bias in its design, conduct and analysis. All papers selected for inclusion in the systematic review (that is – those that meet the inclusion criteria described in the protocol) need to be subjected to rigorous appraisal by two critical appraisers. The results of this appraisal can then be used to inform synthesis and interpretation of the results of the study. JBI Critical appraisal tools have been developed by the JBI and collaborators and approved by the JBI Scientific Committee following extensive peer review. Although designed for use in systematic reviews, JBI critical appraisal tools can also be used when creating Critically Appraised Topics (CAT), in journal clubs and as an educational tool.

JBI CRITICAL APPRAISAL CHECKLIST FOR ANALYTICAL CROSS-SECTIONAL STUDIES

Date 10-05-2022

Author: Aditi Amit-Byatnal Year: 2015 Record Number: 1

	Yes	No	Unclear	Not applicable
1. Were the criteria for inclusion in the sample clearly defined?	X	☐	☐	☐
2. Were the study subjects and the setting described in detail?	☐	X	☐	☐
3. Was the exposure measured in a valid and reliable way?	X	☐	☐	☐
4. Were objective, standard criteria used for measurement of the condition?	☐	X	☐	☐
5. Were confounding factors identified?	X	☐	☐	☐
6. Were strategies to deal with confounding factors stated?	X	☐	☐	☐
7. Were the outcomes measured in a valid and reliable way?	X	☐	☐	☐
8. Was appropriate statistical analysis used?	X	☐	☐	☐

Overall appraisal: Include X Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

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_____
_____

JBI CRITICAL APPRAISAL CHECKLIST FOR ANALYTICAL CROSS-SECTIONAL STUDIES

Date 10-05-2022

Author: M Pentenero Year: 2010 Record Number: 2

	Yes	No	Unclear	Not applicable
1. Were the criteria for inclusion in the sample clearly defined?	X	☐	☐	☐
2. Were the study subjects and the setting described in detail?	☐	X	☐	☐
3. Was the exposure measured in a valid and reliable way?	X	☐	☐	☐
4. Were objective, standard criteria used for measurement of the condition?	☐	X	☐	☐
5. Were confounding factors identified?	☐	X	☐	☐
6. Were strategies to deal with confounding factors stated?	☐	X	☐	☐
7. Were the outcomes measured in a valid and reliable way?	X	☐	☐	☐
8. Was appropriate statistical analysis used?	X	☐	☐	☐

Overall appraisal: Include X Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

_____
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_____
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JBI CRITICAL APPRAISAL CHECKLIST FOR ANALYTICAL CROSS-SECTIONAL STUDIES

Date 10-05-2022

Author: Mutaz Mohammed Nur Year: 2020 Record Number: 3

	Yes	No	Unclear	Not applicable
1. Were the criteria for inclusion in the sample clearly defined?	X	☐	☐	☐
2. Were the study subjects and the setting described in detail?	X	☐	☐	☐
3. Was the exposure measured in a valid and reliable way?	X	☐	☐	☐
4. Were objective, standard criteria used for measurement of the condition?	☐	X	☐	☐
5. Were confounding factors identified?	☐	X	☐	☐
6. Were strategies to deal with confounding factors stated?	☐	X	☐	☐
7. Were the outcomes measured in a valid and reliable way?	X	☐	☐	☐
8. Was appropriate statistical analysis used?	X	☐	☐	☐

Overall appraisal: Include X Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

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JBI CRITICAL APPRAISAL CHECKLIST FOR ANALYTICAL CROSS-SECTIONAL STUDIES

Date 10-05-2022

Author: Gokul Sridharan Year: 2017 Record Number: 4

	Yes	No	Unclear	Not applicable
1. Were the criteria for inclusion in the sample clearly defined?	X	☐	☐	☐
2. Were the study subjects and the setting described in detail?	☐	X	☐	☐
3. Was the exposure measured in a valid and reliable way?	X	☐	☐	☐
4. Were objective, standard criteria used for measurement of the condition?	☐	X	☐	☐
5. Were confounding factors identified?	☐	X	☐	☐
6. Were strategies to deal with confounding factors stated?	☐	X	☐	☐
7. Were the outcomes measured in a valid and reliable way?	X	☐	☐	☐
8. Was appropriate statistical analysis used?	☐	☐	☐	X

Overall appraisal: Include X Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

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EXPLANATION OF ANALYTICAL CROSS-SECTIONAL STUDIES CRITICAL APPRAISAL

How to cite: Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfetcu R, Currie M, Qureshi R, Mattis P, Lisy K, Mu P-F. Chapter 7: Systematic reviews of etiology and risk. In: Aromataris E, Munn Z (Editors). *JBI Manual for Evidence Synthesis.* JBI, 2020. Available from https://synthesismanual.jbi.global

Analytical cross sectional studies Critical Appraisal Tool

Answers: Yes, No, Unclear or Not/Applicable

1. Were the criteria for inclusion in the sample clearly defined?

The authors should provide clear inclusion and exclusion criteria that they developed prior to recruitment of the study participants. The inclusion/exclusion criteria should be specified (e.g., risk, stage of disease progression) with sufficient detail and all the necessary information critical to the study.

2. Were the study subjects and the setting described in detail?

The study sample should be described in sufficient detail so that other researchers can determine if it is comparable to the population of interest to them. The authors should provide a clear description of the population from which the study participants were selected or recruited, including demographics, location, and time period.

3. Was the exposure measured in a valid and reliable way?

The study should clearly describe the method of measurement of exposure. Assessing validity requires that a 'gold standard' is available to which the measure can be compared. The validity of exposure measurement usually relates to whether a current measure is appropriate or whether a measure of past exposure is needed.

Reliability refers to the processes included in an epidemiological study to check repeatability of measurements of the exposures. These usually include intra-observer reliability and inter-observer reliability.

4. Were objective, standard criteria used for measurement of the condition?

It is useful to determine if patients were included in the study based on either a specified diagnosis or definition. This is more likely to decrease the risk of bias. Characteristics are another useful approach to matching groups, and studies that did not use specified diagnostic methods or definitions should provide evidence on matching by key characteristics

5. Were confounding factors identified?

Confounding has occurred where the estimated intervention exposure effect is biased by the presence of some difference between the comparison groups (apart from the exposure investigated/of interest). Typical confounders include baseline characteristics, prognostic factors, or concomitant exposures (e.g. smoking). A confounder is a difference between the comparison groups and it influences the direction of the study results. A high quality study at the level of cohort design will identify the potential confounders and measure

them (where possible). This is difficult for studies where behavioral, attitudinal or lifestyle factors may impact on the results.

6. Were strategies to deal with confounding factors stated?

Strategies to deal with effects of confounding factors may be dealt within the study design or in data analysis. By matching or stratifying sampling of participants, effects of confounding factors can be adjusted for. When dealing with adjustment in data analysis, assess the statistics used in the study. Most will be some form of multivariate regression analysis to account for the confounding factors measured.

7. Were the outcomes measured in a valid and reliable way?

Read the methods section of the paper. If for e.g. lung cancer is assessed based on existing definitions or diagnostic criteria, then the answer to this question is likely to be yes. If lung cancer is assessed using observer reported, or self-reported scales, the risk of over- or under-reporting is increased, and objectivity is compromised. Importantly, determine if the measurement tools used were validated instruments as this has a significant impact on outcome assessment validity.

Having established the objectivity of the outcome measurement (e.g. lung cancer) instrument, it's important to establish how the measurement was conducted. Were those involved in collecting data trained or educated in the use of the instrument/s? (e.g. radiographers). If there was more than one data collector, were they similar in terms of level of education, clinical or research experience, or level of responsibility in the piece of research being appraised?

8. Was appropriate statistical analysis used?

As with any consideration of statistical analysis, consideration should be given to whether there was a more appropriate alternate statistical method that could have been used. The methods section should be detailed enough for reviewers to identify which analytical techniques were used (in particular, regression or stratification) and how specific confounders were measured.

For studies utilizing regression analysis, it is useful to identify if the study identified which variables were included and how they related to the outcome. If stratification was the analytical approach used, were the strata of analysis defined by the specified variables? Additionally, it is also important to assess the appropriateness of the analytical strategy in terms of the assumptions associated with the approach as differing methods of analysis are based on differing assumptions about the data and how it will respond.

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2.2

DEMOGRAPHIC AND CLINICOPATHOLOGICAL ASPECTS OF INCIPIENT ORAL SQUAMOUS CELL CARCINOMA: A MULTICENTRIC CROSS-SECTIONAL INTERNATIONAL STUDY

Running title: Demographic and clinicopathological aspects of incipient OSCC

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ABSTRACT

Introduction: Incipient oral squamous cell carcinoma (OSCCi) can have an innocuous clinical appearance, challenging professionals at the secondary cancer prevention level. **Objective:** To describe demographic and clinicopathological aspects of a South-American cohort of OSCCi patients. **Materials and Methods:** A cross-sectional, observational, international study was performed to assess demographic and clinicopathological characteristics of OSCCi from 6 South-American institutions. **Results:** One-hundred and seven patients within the histopathological spectrum of OSCCi (*in-situ* and microinvasive OSCC) were included in this sample. Fifty-eight (54.2%) patients were men with a total mean age of 60.95 years. Forty-nine (45.8%) and thirty-nine (36.5%) patients had history of tobacco and alcohol use, respectively. Clinically, most of the lesions were plaques (82.2%), ≥ 2 cm in extension (72%), affecting the lateral tongue (55.1%), and soft palate (12.1%) with a mixed (with and red) appearance. Eighty-two (76.7%) lesions were predominantly white and 25 (23.3%) predominantly red. **Conclusions:** To our knowledge, this is the largest cohort of OSCCi patients, which raises awareness of clinicians' inspection acuteness by demonstrating the most frequent clinical aspects of OSCCi, potentially improving oral cancer secondary prevention strategies.

Keywords: Oral cancer; diagnosis; oral squamous cell carcinoma; microinvasive; in-situ.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a malignant neoplasm that originates in the oral cavity lining epithelium (Chainani-Wu et al., 2015), and it is, by far, the most frequent form of oral cancer (El-Naggar AK, 2017). According to GLOBOCAN, lip and oral cavity cancer have an incidence age-standardized rate per 100,000 individuals of 6.0 for males and 2.3 for females, and a mortality age standardized rate of 2.8 and 1.0, respectively (Sung et al., 2021). These results position oral cavity cancer overall as the 16th most common cancer worldwide (Bouvard et al., 2022), with some countries of Latin America and the Caribbean particularly characterized by high incidence rates, such as Brazil, Uruguay, and Puerto Rico (Warnakulasuriya & Kerr, 2021). The prognosis of OSCC patients is tightly associated with the time of diagnosis, and as a reflection, several studies have shown that diagnostic delays impact OSCC mortality outcomes (Goy et al., 2009; Nagao & Warnakulasuriya, 2020; Seoane et al., 2012).

Currently, according to The American Joint Committee on Cancer definition of TNM grading for the oral cavity, *in-situ* OSCC and T1 tumours (<2cm TT, <5mm DOI) represent the earliest stages of OSCC (MB Amin, SB Edge, FL Greene, 2017). OSCC advanced lesions usually present as exuberant ulcerations and/or nodules fixed to adjacent tissues (Bagan et al., 2010); in contrast, incipient OSCC (OSCCi) are asymptomatic lesions that do not involve deep tissues and often present a subtle appearance (Warnakulasuriya et al., 2021). Thus, in the process of oral cancer screening, innocuous clinical manifestations represent an additional challenge for premature detection, resulting in diagnostic pitfalls and delayed diagnosis (Saku et al., 2014).

Present methods of secondary prevention focus mainly on clinical oral examination in high-risk population (Bouvard et al., 2022). This involves screening by systematic visual inspection and palpation of the oral cavity mucosa and the external facial and neck regions, which have shown strong enough sensitivity and specificity results to be consider an effective method for oral potentially malignant disorders (OPMD) and OSCC diagnosis (González-Moles et al., 2022; Walsh et al., 2021). However, one of its main drawbacks is the lack of acquaintance of dental professionals regarding the recognition of OSCCi signs and symptoms, which significantly affects the efficacy of screening programs (González-Moles et al., 2022; Kujan et al., 2009).

Understanding that clinicians can achieve better results on early diagnosis based on deep knowledge of clinical findings to be recognized through systematic visual

examination, and being aware that carcinoma *in-situ* and microinvasive carcinoma represent the most initial manifestations of OSCC, the present study aimed to characterize demographic and clinicopathological aspects of a large international cohort of patients with OSCCi eventually contributing with useful clinical evidence for premature oral cancer manifestations.

PATIENTS AND METHODS

This study was performed in accordance with the Helsinki Declaration and was approved by Piracicaba Dental School Ethical Committee, under protocol 45545121.1.3002.5432. Informed consent was obtained from all patients. STROBE guidelines for observational studies were followed to report this research (Vandenbroucke et al., 2007).

For this cross-sectional observational study, oral cavity squamous cell carcinomas with histopathological diagnoses of *in-situ* OSCC or microinvasive OSCC were grouped as OSCCi. Cases from the files of the Laboratory of Oral Pathology and Oral Medicine departments of Piracicaba Dental School of the University of Campinas (Piracicaba, Brazil), AC Camargo Cancer Center (São Paulo, Brazil), Cordoba National University (Córdoba, Argentina), Federal University of Rio de Janeiro (Rio de Janeiro, Brazil), Venezuela Central University (Caracas, Venezuela), and Valparaiso University (Valparaíso, Chile) received throughout their entire period of operation were collected from January 2021 to December 2022. To target the diagnosis of interest, histopathological records with a diagnosis of “leukoplakia”, “oral epithelial dysplasia”, “oral squamous cell carcinoma”, “microinvasive oral squamous cell carcinoma”, “*in-situ* oral squamous cell carcinoma”, and “superficially invasive oral squamous cell carcinoma” were initially retrieved. The sample size was determined by the number of available cases.

Eligibility criteria

The following inclusion criteria were applied: OSCCi lesions with a) diagnostic biopsy of *in-situ* squamous cell carcinoma or microinvasive squamous cell carcinoma (<5mm DOI); b) high-resolution corresponding clinical images.

Exclusion criteria were as follows: a) Lip, pharynx and perioral skin lesions; b) verrucous proliferative leukoplakia or oral lichen planus clinical diagnosis; c) recurrent OSCC lesions; d) post-biopsy clinical images; e) bad quality images.

To address potential sources of selection bias, cases with histopathological diagnosis of interest with clinical appearance compatible with conventional oral squamous cell carcinoma were also excluded, as this was considered as a probable indication of a non-representative biopsy.

A representative clinical photograph of each lesion was individually evaluated by two experienced oral medicine specialists (CSS and ALDA) blinded to the histopathological diagnosis. The following clinical variables were determined and their methods of assessment were established as described by the corresponding cited authors: classification according to anatomic site, localization (Pentenero et al., 2011), size (<2cm and ≥2cm, modified from Brandizzi et al (Brandizzi et al., 2008), primary or secondary lesion (Behnaz et al., 2019; Linton, 2011), OPMD classification (Warnakulasuriya et al., 2021), ulceration (White et al., 2004), distribution (Monteiro et al., 2017), and colour predominance. Localization was classified as anterior and posterior, distinguished using the premolar area as a reference point as reported by Pentenero et al (Pentenero et al., 2011), and lesions located at midline, involving both left and right sides. Distribution parameters were adapted from Monteiro et al (Monteiro et al., 2017); lesions affecting only one anatomic region (i.e, tongue or palate or buccal mucosa) and lesions affecting two or more anatomic areas (i.e tongue and floor of mouth or buccal mucosa and palate) were categorized separately. Histopathological evaluation to confirm diagnosis was performed either by one researcher at the local of origin or by two authors (CSS and ALDA) at the research's main location (FOP-UNICAMP). Disagreements were resolved by consensus between both parties.

Clinicopathological data including sex, age, tobacco and alcohol use, time of evolution, presence of other OPDM lesions, type of biopsy, clinical hypothesis, and histopathological diagnosis were obtained by reviewing medical records. When evaluating habits, tobacco and alcohol were assessed as dichotomous variables (positive/negative). The consumption of at least one alcohol unit per day (1 unit = 8–10 g of ethanol = 1 glass of wine = ¼ l of beer = 1 measure of liqueur) was considered a positive drinking habit (Pentenero et al., 2011).

Data Synthesis and Statistical Analysis

A narrative descriptive synthesis was provided, using values of mean, median, range, and frequency percentages. Relationships between variables were assessed by using Chi-square or Fisher's exact tests as appropriate. Evaluation of variables with missing data was performed following a listwise deletion approach A P-value of ≤0.05 was considered significant. All analyses were performed using SPSS version 25 (SPSS Inc., Chicago, USA).

RESULTS

Five hundred and fifty-three cases were initially retrieved from all Oral Pathology and Oral Medicine services. After application of eligibility criteria, a total of 107 patients were included. Overall, 92 (63.6%) cases were retrieved from Brazilian institutions (UNICAMP, ACCCC, and UFRJ), 11 (10.3%) from Argentina (UNC), 3 (2.8%) from Venezuela (UCV), and 1 (0.9%) from Chile (UV).

Table 1 shows a summary of patients' demographic features. The mean age was 60.69 years (range 23-92 years) and 58 (54.2%) patients were men. Fifty-seven of them were 60 (53.3%) years or older. Forty-nine patients (45.8%) were current or former smokers and 46 (43%) were nondrinkers. Fifty cases (46.7%) had information on the time of evolution, with a mean of 13.7 months (range 1-144 months).

Of 107 patients, 20 (18.7%) had more than one lesion with the clinical diagnosis of OPMD. Excisional biopsy was performed as the initial approach in 13 (12.1%) cases. Concerning histopathological diagnosis, there were 75 (70.1%) *in-situ* OSCC cases followed by 32 (29.9%) microinvasive OSCC cases (Figure 1). Remarkably, 68 (63.6%) cases did not have clinical hypotheses of malignancy.

Table 2 shows a summary of the clinical features of 107 cases assessed. The lateral border of the tongue was the main affected anatomic site, with 59 (55.1%) cases, followed 13 (12.1%) cases involving the soft palate, 12 (11.2%) cases on the floor of the mouth, 8 (7.5%) on the buccal mucosa, 3 (2.8%) cases each affecting alveolar ridge and retromolar trigone, respectively, and lastly 2 (1.9%) cases in the gingival area. Overall, 77 (72%) lesions were 2 cm or larger and 53 (49.5%) appeared on the right side over 49 (45.8%) on the left side and 5 (4.7%) with midline involvement. Figure 2 shows representative clinical images of included cases.

When assessing clinical features, OSCCi were predominantly plaques, represented by 88 cases (82.2%), 73 (68.2%) of which corresponded to non-homogeneous speckled leukoplakia (Figure 3). Thirteen (12.1%) lesions were considered erythroplakia, 7 (6.5%) of which presented a non-homogeneous surface that exhibits very scant white areas. Altogether, 82 (76.7%) lesions were predominantly white (Figure 4) and 25 (23.3%) were predominantly red (Figure 5). A total of 40 (37.4%) cases had some extent of ulceration within the lesion, but only 1 (0.9%) lesion was identified as a single solitary ulcer, and yet all of them had a superficial fibrin membrane and none had necrotic centre or indurated borders. Only 14 (13.1%) cases were big enough to comprise more than one anatomic region.

Pearson's chi-square test was used to compare *in-situ* OSCC and microinvasive OSCC in relation to sex (p=0.883), age (p=0.984), size (p=0.354), and colour predominance (p=0.208), showing no dependence between the aforementioned variables and histopathological diagnosis. Likewise, Fisher's exact test showed no association between histopathological diagnosis and tobacco (p= 0.621) or alcohol (p= 0.296) use, anatomic site (p=0.412), location (p=0.214), primary lesion (p=0.180), OMPD classification (p= 0.227) or distribution (p=0.347). After our analysis, we did not find statistically significant evidence to demonstrate differences in this sample between *in-situ* OSCC and microinvasive OSCC subgroups regarding the assessed demographic and clinical variables.

DISCUSSION

Through this study, we characterized demographic and clinicopathological aspects of a multicentric South American cohort of OSCCi patients. To our knowledge, this is the largest experienced publication in this context, followed by Pentenero et al (Pentenero et al., 2011), which described a case series of 99 Italian-based patients with diagnosis of microinvasive oral squamous cell carcinoma, evaluating clinical features.

Our study showed a discrete preponderance in the older masculine population, as slightly more than half of our sample was represented by men over 60 years. This is in agreement with data from international literature, that extensively reports OSCC as a disease that affects primarily older male adults (Abati et al., 2020; Ford & Farah, 2013; Ho et al., 2008; McCullough et al., 2010). The reasoning behind this fact has been related to increased exposure to alcohol consumption and tobacco use, widely established OSCC

risk factors (Bagnardi et al., 2015; Conway et al., 2018; Nagao & Warnakulasuriya, 2020; Sung et al., 2021). Our findings from this group of patients in relation to habits partially contrast with current knowledge, because most of our cases are indeed represented by former or current smokers, but mainly nondrinkers. We interpret this data as a particularity of our sample and not as a relevant factor within the etiopathology of initial lesions, especially considering the missing data about habits on approximately 20.6% of the sample, which was not available for collection.

Notably, 45.8% of OSCCi patients were women, which is an interesting fact to consider in future OSCCi studies, as this distribution regarding sex is similar to what was reported by Pentenero et al, who found that 43% of their sample of stage I microinvasive OSCC was also represented by women. Also, an important part of our sample is represented by patients somewhat younger than expected, and this fact can be associated with the natural biological course of the disease, since the OSCC lesions included in this study are in initial phases, and therefore, they would be expected to be found in younger patients.

As for time of evolution, patients reported an average of 13.7 months, ranging from 1 month to 12 years. Interestingly, the median time of evolution of microinvasive OSCC was twice as high as *in-situ* OSCC. However, the reliability of this data to estimate the actual course of the disease should be questioned by physicians (Yildirimyan, 2021), as the cumulative effect of mutations during cancer development depends on a progressive sequence of non-clinically evident events that happens over time (Jolly & van Loo, 2018). Additionally, patients with a higher health awareness could be more vigilant of any noticeable changes, while others could exhibit modest variations that could not be recognized as concerning by a non-trained individual.

Size of OSCC can vary. Through this study, we describe the majority of cases as larger than 2 cm of extension (72%), even involving multiple anatomic regions (13.1%). Some authors agree with this result (Brandizzi et al., 2008). While early OSCC has been associated with smaller dimensions (Bagan et al., 2010; Shedd, 1965.), this represented a minority of 28% of cases in our sample. Size is not to be interpreted as a *sine qua non* feature, as in our analysis, we noted some cases of extensive white plaques with predominately smooth surface to represent OSCCi, which could be unexpected.

Past and recent evidence supports biopsies for histopathological examination as the gold standard for diagnosis of oral cancer (Avon SL, 2012; Gattuso et al., 2022; Riccardi

et al., 2022; Lestón & Dios, 2010). We reported 57 biopsies (53.3%) to be incisional. The proportion of underdiagnosis for OSCC and OPMD from incisional biopsies was studied by Pentenero et al (Pentenero et al., 2003), who showed that the incisional approach appears to be reliable for early OSCC diagnosis. Nonetheless, some difficulties regarding size of specimen or site for biopsy were noted. Likewise, by multivariate analysis, Lee et al (Lee et al., 2007) exposed OSCC underdiagnosis on non-homogeneous oral leukoplakias, reaffirming the relevance of choosing the proper area to biopsy and opting for multiple regions, if needed. Inversely, an excisional biopsy was performed in 13 cases (12.1%). The discrepancy in this decision could be related to a lack of experience of the professional in identifying a lesion with higher risk of malignancy, but also to the subtlety of clinical features, that could difficult certainty about clinical hypotheses. However, opting by an excisional approach on a malignant lesion also represents a problem, because if resection is not done properly, it can lead to treatment failure associated with poor pathological margin control, especially when using electrosurgical techniques (Park, 2016).

Most of our OSCCi sample is composed by *in-situ* carcinoma cases (70.1%). Other observational studies assessing early OSCC have been performed with different populations, such as microinvasive carcinoma (which already encompass various definitions, and therefore, different eligibility criteria) (Amit-Byatnal et al., 2015; Mohammed et al., 2021; Pentenero et al., 2011; Sridharan et al, 2017) or T1-T2 cases (Bundgaard et al., 2002; Elseragy et al., 2019). Despite tumours T1 and T2 being deemed as early diagnosis, this group of OSCC patients still show an almost 20% mortality rate (Almangush et al., 2015). This strengthens the notion that diagnosis at T1-T2 stages is yet not early enough and that there is still room for improvement in the current approaches to oral cancer diagnosis. González-Moles et al recently elaborated on the difficulty to conceptualize oral lesions as "early carcinoma" (González-Moles et al., 2022), since there is a broad spectrum of variables that affect cancer development and, therefore, classifications and gradings hardly achieve a definition that can be uniformly applied. We see these nuances not only with TNM grading regarding tumour size, as formerly explained (González-Moles et al., 2022), but also in oral epithelial dysplasia classification (McCullough et al., 2010) and oral microinvasive OSCC definition, which we have previously described as an under-reported malignancy that currently lacks measurement parameters (Saldivia-Siracusa et al., 2022). This absence of standardization can explain

the low amount of microinvasive OSCC. Hence, we consider this a valid reason to evaluate *in-situ* and microinvasive carcinomas as a group of incipient lesions.

Uchiyama et al (Uchiyama et al., 2021) systematically reviewed the literature to find association between anatomic site and clinical outcomes of OSCC related to metastases, reporting the tongue as the primary location for OSCC (25-40%), followed by the floor of mouth (15-20%) and buccal mucosa and gingiva (10%). Mohammed Nur et al (Mohammed Nur et al., 2021) also reported the tongue, floor of mouth and soft palate/retromolar trigone as the main sites for early OSCC. We report a similar anatomic distribution, with a predilection for the tongue (61.7%), followed by the soft palate (12.1%), and then floor of mouth (11.2%) and buccal mucosa (7.5%), which is mostly consistent with those statements.

Superficially invasive carcinomas clinically resemble OPMD, particularly non-homogeneous leukoplakia and erythroplakia (Warnakulasuriya et al., 2021). In our study, 73 (68.2%) cases presented as mixed lesions with both red and white areas. This is not uncommon; non-homogeneous leukoplakia or OPMD with speckled appearance had been reported to exhibit microinvasive OSCC following a biopsy at baseline detection of a OPMD lesion (Madden et al., 2015). Twenty-five included cases (23.3%) were predominantly red. The relevance regarding red or atrophic areas within these lesions was also indicated by Mashberg (Mashberg, 2000). Yet, we also address the importance of our results finding 13 homogeneous leukoplakias (12.1%) representing OSCCi. Since OPMD can, in some cases, be tackled through a “watch-and-wait” approach, recognition of clinical concerns to adopt a biopsy tactic is crucial. Lee et al confirms this in their study, highlighting clinical features a critical factor to aim for unexpected malignancy (Lee et al., 2007).

When assessing OSCCi by histopathological diagnosis, we were unable to find statistical evidence to establish association between any clinical or demographic variables. Therefore, we cannot statistically prove that the frequency of the variables within *in-situ* OSCC and microinvasive groups is not due to chance. In this sense, in our study, these two diagnoses had comparable findings. This is not to say that dissimilarities would not be found in the future. As we performed an observational study, particularities of this sample such as low statistical power because of insufficient population and subjective evaluation could affect statistical results. Further larger research is required in order to expand knowledge in this matter, especially at molecular level.

Diagnosis of oral cancer encompasses a series of factors related to the patient, the professional, the healthcare system, and the disease itself. As for the patients' end, awareness and conscientization of the disease represent an important aspect to enhance, because, aside of screening programs, they must seek professional attention for early diagnosis to be made in the first place. Frequently, patients with initial-stage oral cancer present with only vague symptoms and minimal physical findings (Villa et al., 2011), which reduces the chances of patients seeking premature evaluation, and therefore, hinders primary prevention. At the professional's end, improvements in making a diagnosis may be sought through continuous learning, careful examination, building expertise and confidence of professionals in detecting incipient OSCC, and developing reliable adjunctive tools to improve findings and accuracy of a visual oral exam. As previously stated by Essat et al (Essat et al., 2022), conventional oral visualization as a diagnostic method has limitations, because the evaluation of clinical findings is subjective and depends on the clinician's expertise. Only more in-depth training programs for examiners can improve this aspect (González-Moles et al., 2022). Nonetheless, when using established standards, it has been endorsed that visual screening by dentists in primary care or extended healthcare facilities can accurately identify OSCC and/or OPMDs (Warnakulasuriya et al., 2015). The efforts to characterize clinical aspects of a group of incipient malignant lesions, as suggested here, broadens the opportunity to boost premature identification practices as well as to decrease the burden of oral cancer (McCullough et al., 2010; Varela-Centelles, 2022).

Finally, subjectivity is a major concern in the assessment of potentially malignant and incipient malignant oral lesions, in both clinical and histological aspects, and it is intrinsic to human evaluation. Today, there is colossal potential for Artificial Intelligence (AI) uses in this field to aid in the diagnosis, since it can be used to create image analysis tools useful for the interpretation and classification of visual data, deduction of novel insights into disease biology, and diagnostic support (Moxley-Wyles et al., 2020), assisting conventional practices and quickly adapting to new information. Application of this and other modern adjunctive methods to minimize error in appraisal would be of great value.

LIMITATIONS

This study presents some limitations. Being a retrospective study, results are affected by multiple factors that cannot be adjusted to avoid specific biases, such as

incomplete medical records and unstandardized treatment decisions, conditions that impact the available data. Also, we recognize that the use of mostly incisional biopsies can also confound the obtained results as some of the included cases could be already frankly malignant in other not biopsied areas. Similarly, the subjective nature of dysplasia evaluation must be also noted. However, we aim to highlight the importance of visual characteristics of discrete lesions which in most instances would not be considered malignant, especially by general dentists or oral clinicians not specialized in Oral Medicine, so we consider this objective is still achieved through this analysis.

CONCLUSIONS

In essence, we present a series of OSCCi cases, represented by lesions diagnosed as *in-situ* and microinvasive OSCC, that usually appear as mixed plaques or erosions on the lateral border of the tongue and soft palate of men in their sixth decade of life or older, with past or current habits of tobacco use. The findings of the present study display subtle clinical presentations of oral cancer and are useful to raise awareness on clinicians' visual acuteness when performing systematic visual examination, assisting in primary and secondary prevention. Advanced tools focused on image pattern recognition of these entities could be valuable to further improve this issue.

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TABLES

Table 1. Summary of demographic characteristics of included patients.

Sample	***Total (%)***	***In-situ OSCC cases (%)***	***Microinvasive OSCC cases (%)***	***p value***
Histopathological diagnosis	107 (100)	75 (70.1)	32 (29.9)	
Sex				0.883[a]
Female	49 (45.8)	34 (31.8)	15 (14)	
Male	58 (54.2)	41 (38.3)	17 (15.9)	
Age (years)				0.984[a]
Mean	60.69	59.60	63.21	

Range	23-92	23-80	34-92	
<60 years	50 (46.7)	35 (32.7)	15 (14)	
≥60 years	57 (53.3)	40 (37.4)	17 (15.9)	
Risk factors				
Tobacco				0.621[b]
Yes	32 (29.9)	22 (20.6)	10 (9.3)	
Former smoker	17 (15.9)	14 (13.1)	3 (2.8)	
No	36 (33.6)	27 (25.2)	9 (8.4)	
No information	22 (20.6)	12 (11.2)	10 (9.3)	
Alcohol				0.296[b]
Yes	22 (20.6)	16 (15)	6 (5.6)	
Former drinker	17 (15.9)	15 (14)	2 (1.9)	
No	46 (43)	31 (29)	15 (14)	
No information	22 (20.6)	13 (12.1)	9 (8.4)	
Time of evolution (months)				
Median	13.7	11.05	21.42	
Range	1-144	1-60	1-144	
Information available	50 (46.7)	36 (33.6)	14 (13.1)	
No information	57 (53.3)	39 (36.4)	18 (16.8)	
Type of biopsy				
Incisional	57 (53.3)	41 (38.3)	16 (15)	
Excisional	13 (12.1)	10 (9.3)	3 (2.8)	
No information	37 (34.6)	24 (22.4)	13 (12.1)	

[a]Pearson's chi-square test double-sided p value; [b]Fisher's exact test double-sided p value.

Table 2. Summary of clinical features of 107 lesions assessed.

Clinical features	***Total (%)***	***In-situ OSCC cases (%)***	***Microinvasive OSCC cases (%)***	***p value***
	107 (100)	75 (70.1)	32 (29.9)	
Anatomic site				0.412[b]
Tongue	66 (61.7)	46 (43)	20 (18.7)	
Lateral border	59 (55.1)	42 (39.3)	17 (15.9)	
Ventral	5 (4.7)	3 (2.8)	2 (1.9)	
Dorsum	2 (1.9)	1 (0.9)	1 (0.9)	
Buccal mucosa	8 (7.5)	5 (4.7)	3 (2.8)	
Anterior*	4 (3.7)	3 (2.8)	1 (0.9)	
Posterior*	2 (1.8)	1 (0.9)	1 (0.9)	
Both	6 (5.4)	4 (3.7)	2 (1.8)	
Floor of the mouth	12 (11.2)	10 (9.3)	2 (1.9)	
Anterior	10 (9.1)	8 (7.3)	2 (1.8)	
Posterior	2 (1.8)	2 (1.8)	0 (0)	
Soft palate	13 (12.1)	10 (9.3)	3 (2.8)	
Anterior	6 (5.6)	4 (3.7)	2 (1.9)	
Posterior	5 (4.7)	5 (4.7)	0 (0)	
Both	2 (1.9)	1 (0.9)	1 (0.9)	
Alveolar ridge	3 (2.8)	2 (1.9)	1 (0.9)	
Posteroinferior	2 (1.9)	1 (0.9)	1 (0.9)	
Anterosuperior	1 (0.9)	1 (0.9)	0 (0)	
Gingiva	2 (1.9)	0 (0)	2 (1.9)	
Anteroinferior	1 (0.9)	0 (0)	1 (0.9)	
Anterosuperior	1 (0.9)	0 (0)	1 (0.9)	
Retromolar trigone	3 (2.8)	2 (1.9)	1 (0.9)	
Location				0.214[b]
Right	53 (49.5)	36 (33.6)	17 (15.9)	
Left	49 (45.8)	37 (34.6)	12 (11.2)	
Midline**	5 (4.7)	2 (1.9)	3 (2.8)	
Size				0.354[a]
≥2cm	77 (72)	52 (48.6)	25 (23.4)	
<2cm	30 (28)	23 (21.5)	7 (6.5)	
Primary lesion				0.180[b]
Plaque	88 (82.2)	64 (59.8)	24 (22.4)	
Erosion	18 (16.8)	11 (10.3)	7 (6.5)	
Ulcer	1 (0.9)	0 (0)	1 (0.9)	
OPMD				0.227[b]
Leukoplakia	93 (86.9)	67 (62.6)	26 (24.3)	
Homogeneous	13 (12.1)	8 (7.5)	5 (4.7)	
Nonhomogeneous, speckled	73 (68.2)	50 (46.7)	23 (21.5)	
Non-homogeneous, verrucous	8 (7.5)	7 (6.5)	1 (0.9)	
Non-homogeneous, nodular	7 (6.5)	6 (5.6)	1 (0.9)	
Erythroplakia	13 (12.1)	8 (7.5)	5 (4.7)	
Homogeneous	5 (4.7)	3 (2.8)	2 (1.9)	
Non-homogeneous	7 (6.5)	4 (3.7)	3 (2.8)	
Does not apply (ulcer)	1 (0.9)	0 (0)	1 (0.9)	

Distribution				0.347[b]
Unifocal	93 (86.9)	67 (62.6)	26 (24.3)	
Multifocal	14 (13.1)	8 (7.5)	6 (5.6)	
Color predominance				0.208[a]
White	82 (76.7)	60 (56.1)	22 (10.6)	
Red	25 (23.3)	15 (14)	10 (9.3)	

*Anterior/posterior: premolar area used as a reference point; **Bilateral: midline lesions involving right and left side; [a] Pearson's chi-square test double-sided p value; [b] Fisher's exact test double-sided p value.

Figure 1. Histopathological examples of OSCCi (Hematoxylin and eosin, Whole Slide Image, 20x). a) In-situ OSCC on a 72-year-old man with an erythroplakia involving buccal mucosa; c) Microinvasive OSCC of a 49-year-old woman presenting an erythroplakia on lateral border of the tongue.

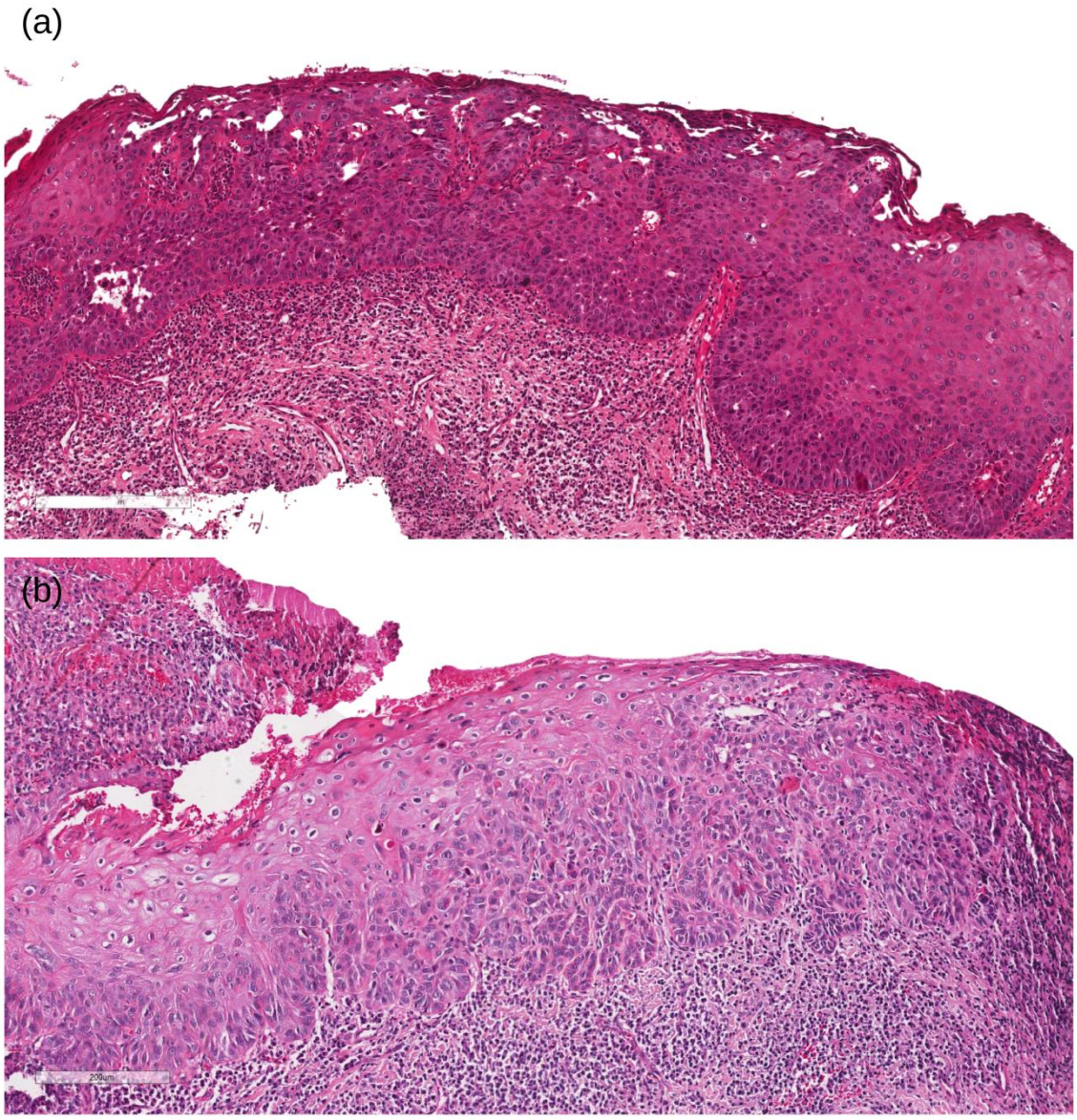
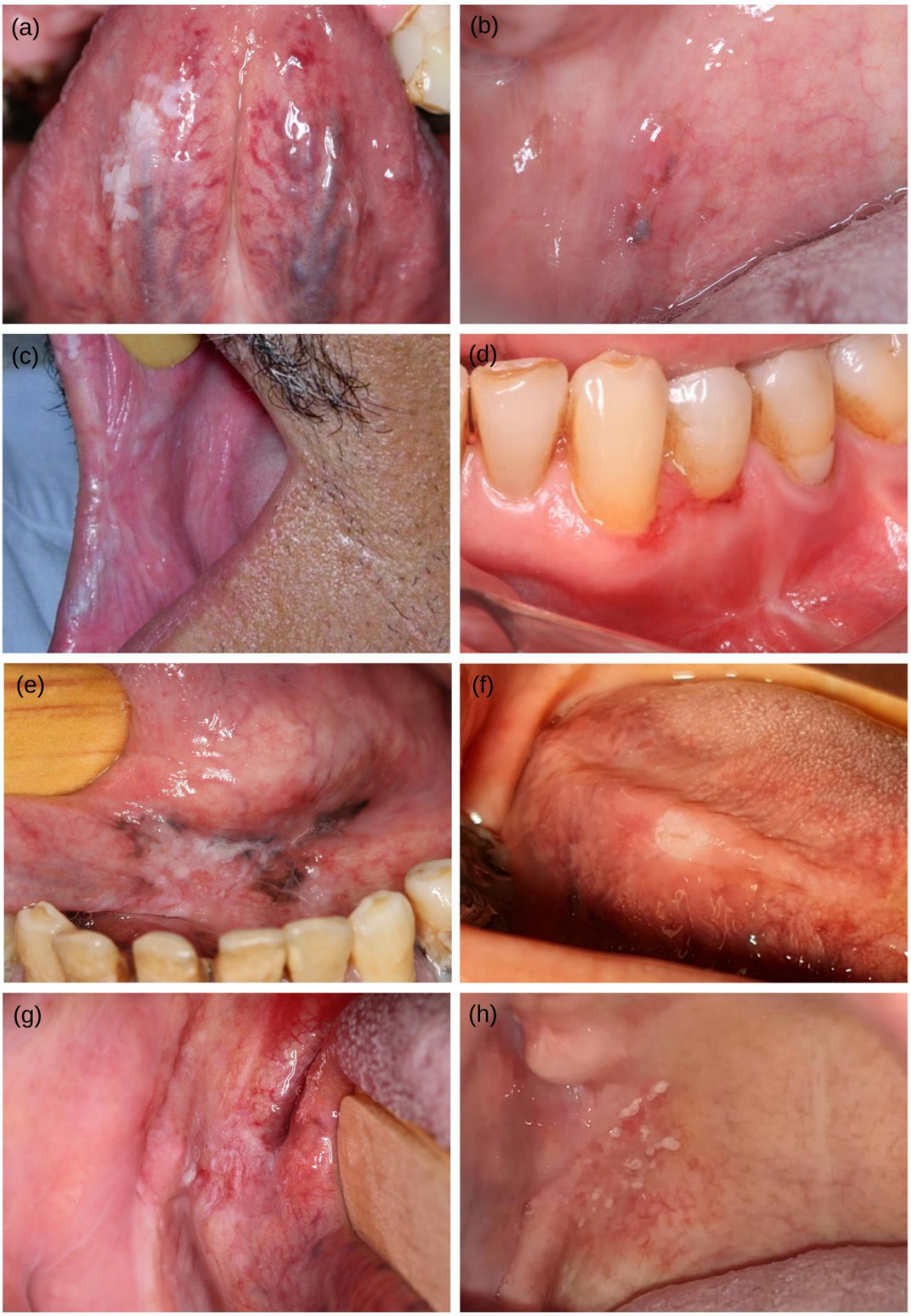


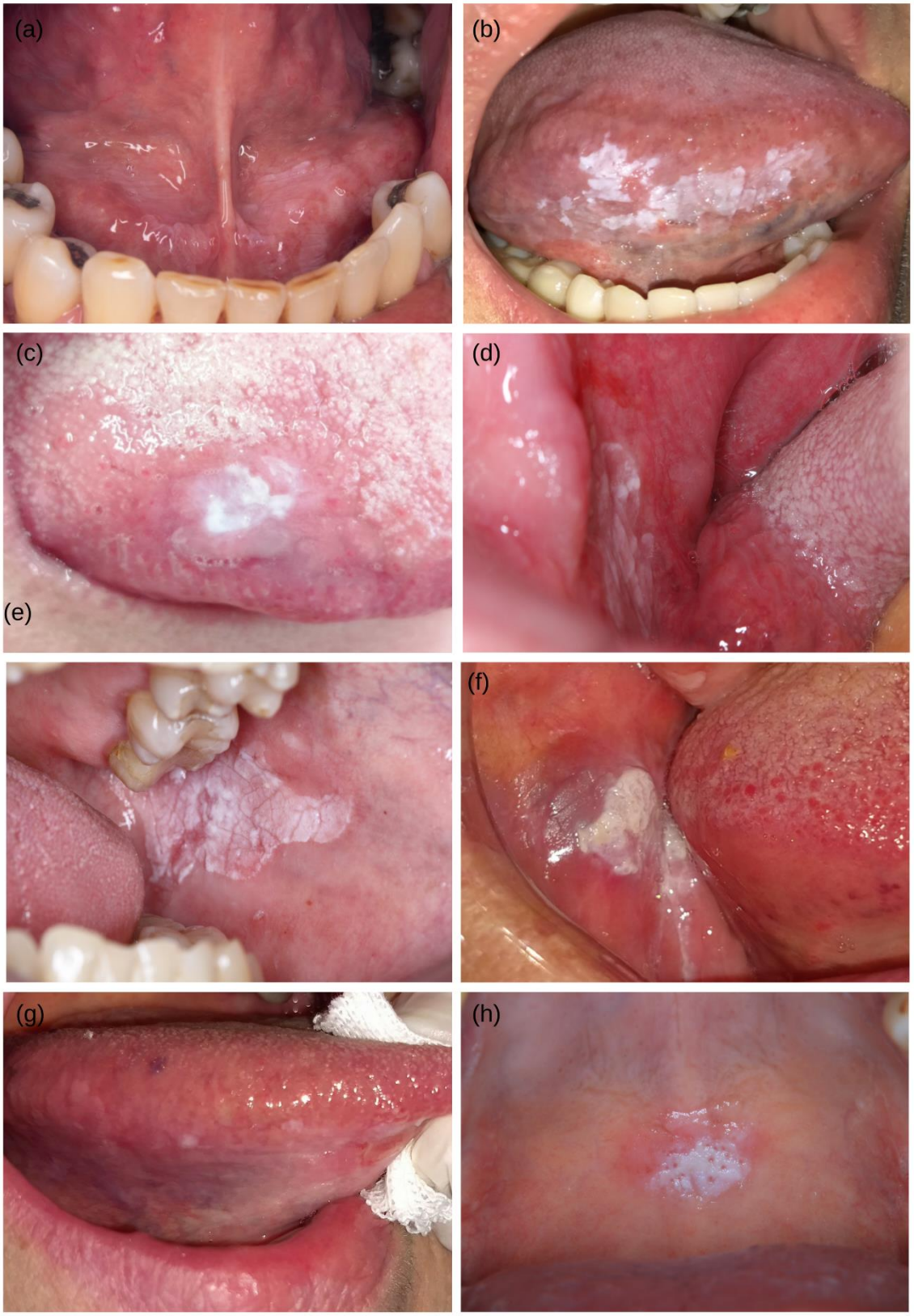
Figure 2. Incipient oral squamous cell carcinoma cases with subtle clinical presentation.



a) Homogeneous leukoplakia of ventral tongue, removed through excisional biopsy resulting on a microinvasive OSCC; b) Erythroleukoplakia of the soft palate of a 58-year-

old treated through excisional biopsy, showing *in-situ* OSCC; c) Mixed leukoerythroplakia of the buccal mucosa with diagnosis of *in-situ* OSCC; d) Microinvasive OSCC noticed as a small ulcer without indurated borders, affecting marginal gingiva; e) Pigmented non homogeneous speckled leukoplakia on left lateral border of tongue, with diagnosis of microinvasive OSCC: f) Nodular leukoplakia of lateral border of tongue with diagnosis of *in-situ* OSCC; g) Non homogeneous plaque situated on retromolar trigone, diagnosed as *in-situ* OSCC. Due to posterior localization and smaller size, the identification of this lesion could easily be overlooked by the patient or during a careless visual examination; h) 52-year-old with a diagnosis of microinvasive OSCC presenting as a non-homogeneous speckled leukoplakia comprising right side of the soft palate.

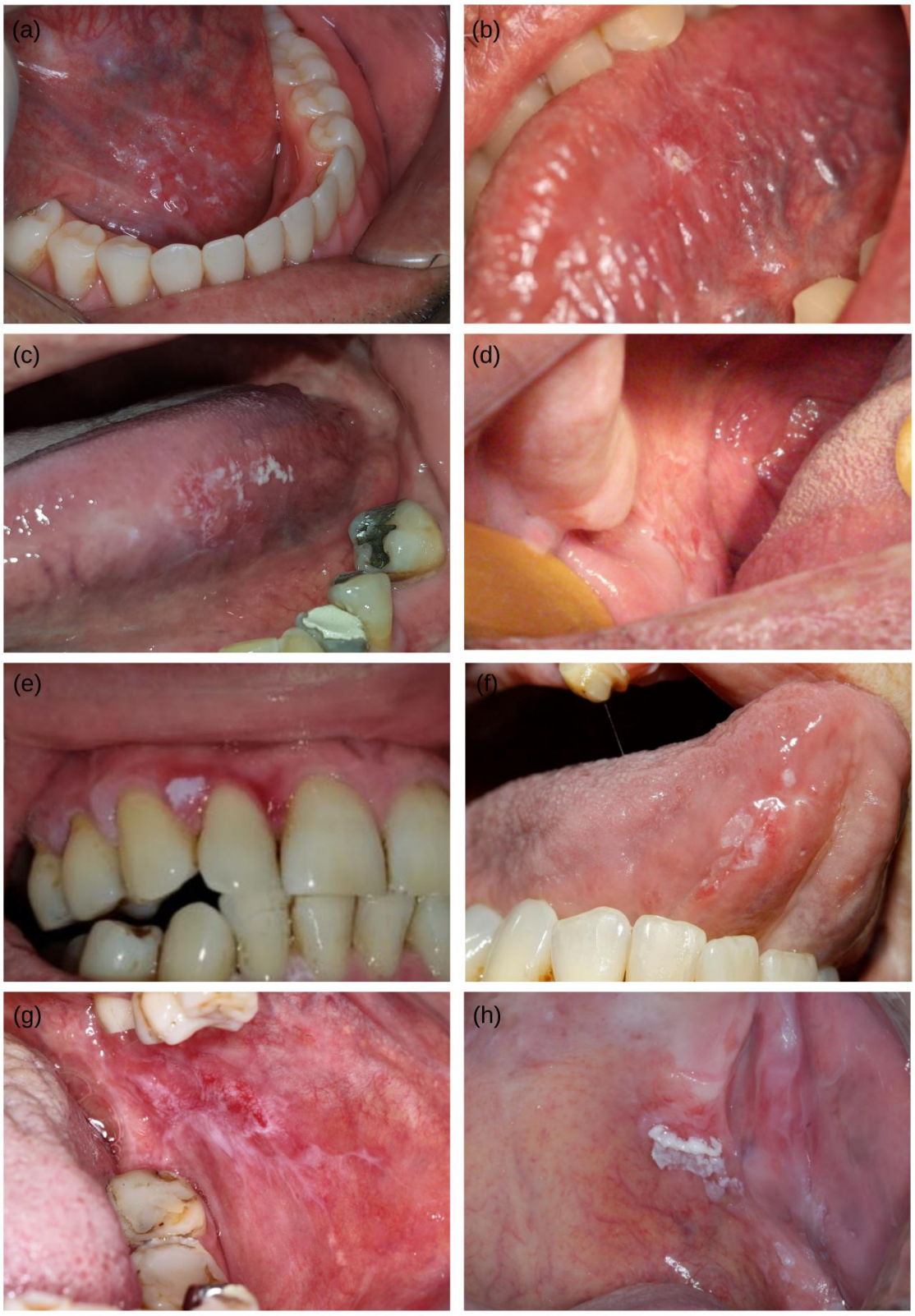
Figure 3. Clinical presentation of incipient OSCC: white predominance.



a)Thin homogeneous leukoplakia with bilateral on the floor of the mouth of a 54-year-old, diagnosed as microinvasive OSCC; b) Leukoplakia of the right lateral border of the

tongue, resulting in *in-situ* OSCC on a 45-year-old woman; c) In-situ OSCC presenting as a <2cm heterogeneous leukoplakia on the right side of the tongue, assessed by excisional biopsy; d) Well delimited, fissured leukoplakia on a 59-year-old male, biopsy revealed a microinvasive OSCC; e) Extensive, fissured leukoplakia involving left buccal mucosa of a 59-year-old male, resulting in *in-situ* OSCC; f) Right buccal mucosa affected by an *in-situ* OSCC observed as a non-homogeneous leukoplakia: g) *In-situ* OSCC presenting as a predominantly homogeneous leukoplakia on the right lateral border of the tongue of a 60-year-old woman; h) Small, mixed, predominantly white lesion approached at first instance by excisional biopsy, with the clinical hypothesis of frictional keratosis and final diagnosis of *in-situ* OSCC.

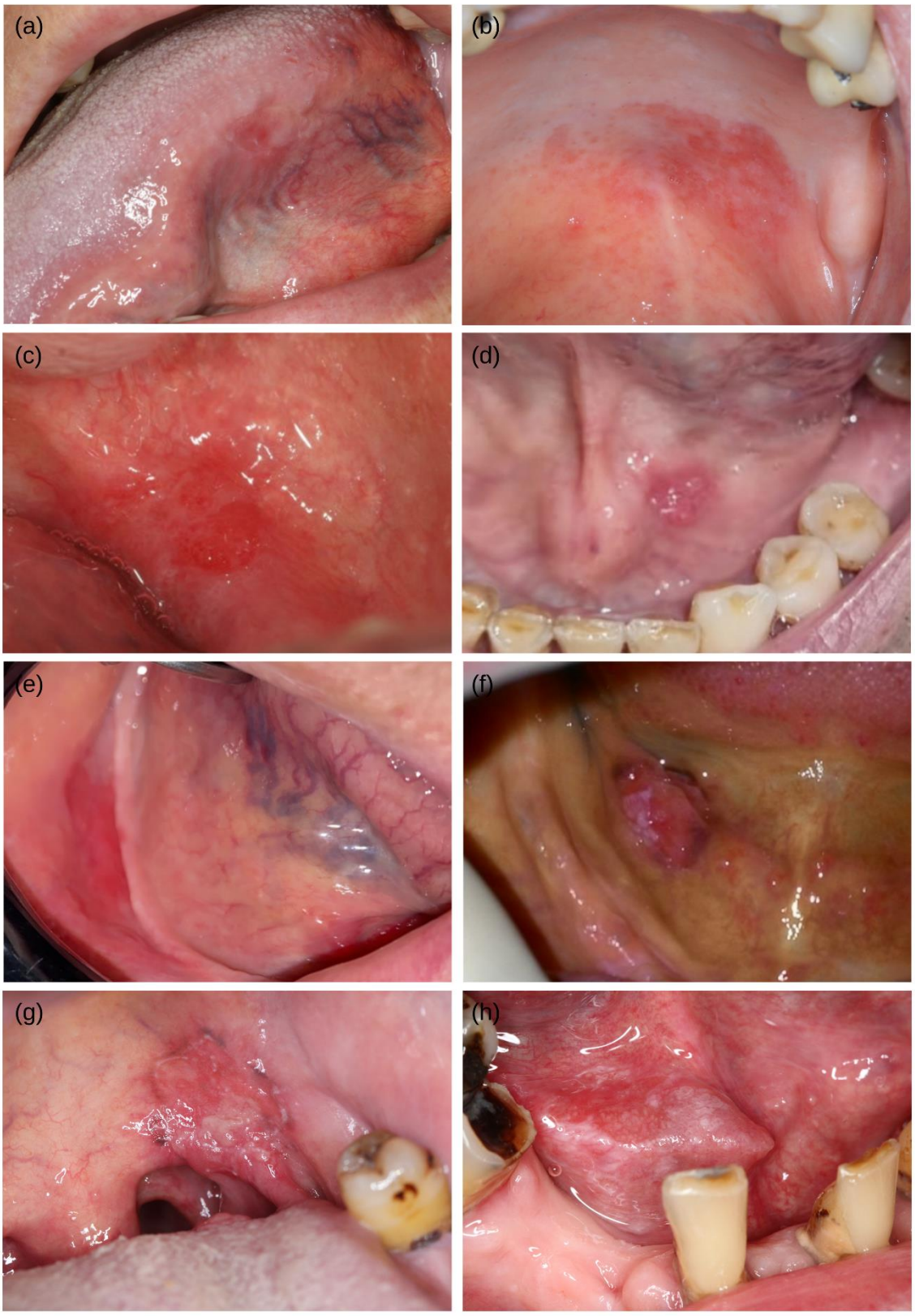
Figure 4. Clinical presentation of incipient OSCC: mixed lesions.



a) Speckled leukoplakia located on the anterior floor of the mouth, diagnosed with a microinvasive OSCC; b) Small mixed lesion with white nodular area and erytrhoplastic surroundings on the left lateral border of the tongue of a 57-year-old, resulting in an *in-*

situ OSCC; c) *In-situ* OSCC of the left lateral border of tongue of a 70-year-old male patient presenting as a non-homogeneous speckled leukoplakia with clinical hypothesis of lichenoid reaction; d) Non-homogeneous speckled leukoplakia with erosive areas with *in-situ* OSCC diagnosis, affecting the soft palate of a 66-year-old man; e) 53-year-old man presented an heterogeneous plaque involving anterosuperior gingiva that was sent with clinical hypotheses of frictional keratosis or leukoplakia, resulting in a microinvasive OSCC; f) Small leukoerytrhoplakia located on the ventral tongue of a 48-year-old woman, with clinical hypothesis of oral lichen planus and final diagnosis of *in-situ* OSCC; g) Microinvasive OSCC as a leukoerytrhoplakia in the left buccal mucosa of a 78-year-old male; h) Mostly white, mixed plaque of the palate, approached by an excisional biopsy which resulted in an *in-situ* OSCC.

Figure 5. Clinical presentation of incipient OSCC: red predominance.



a) 68-year-old female patient showing a small, well-defined erythroplakia located on the left lateral border of the tongue, consistent with *in-situ* OSCC; b) Erythematous lesion on the palate of a 77-year-old former smoker; which resulted in a microinvasive OSCC; c) *In-situ* OSCC of a 62-year-old male presenting as a <2 cm erythroplakia on left retromolar trigone; d) <2cm erythroplakia on the floor of the mouth of a 65-year-old man. Incisional biopsy showed microinvasive OSCC; e) Extensive, homogeneous red lesion of a 74-year-old with clinical hypotheses of pemphigus or pemphigoid. Histopathology demonstrated an microinvasive OSCCC; f) *In-situ* OSCC of a 58-year-old woman with a mixed, mostly red lesion on the floor of mouth; g) 56-year-old man with an ulcerated red lesion on the left portion of the soft palate and tonsillar pillar, with *in-situ* OSCC diagnosis; h) Anterior floor of mouth affected by an erythroplastic lesion on a 60-year-old man, histopathologic analysis resulted on *in-situ* OSCC.

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3 DISCUSSÃO

Nesta dissertação, foram caracterizados aspectos demográficos e clinicopatológicos do carcinoma espinocelular oral incipiente (CECi). Estudos com objetivos semelhantes foram realizados por meio da análise de amostras de carcinoma espinocelular oral (CEC) em etapas iniciais. A título de exemplo, Pentenero et al. descreveram características clínicas de uma série de 99 pacientes italianos com diagnóstico de carcinoma espinocelular oral microinvasivo (CECmi) (Pentenero et al., 2011); Sridharan et al. reavaliaram amostras histopatológicas de casos de leucoplasia, CEC e fibrose submucosa oral para encontrar e medir potenciais achados subdiagnosticados de microinvasão (Sridharan et al, 2017); e Mohammed Nur et al avaliaram amostras de CEC categorizado como "pequeno e delgado" para aferir o valor prognóstico de achados histopatológicos (Mohammed et al., 2021). O presente estudo destaca-se porque, por meio da nossa abordagem, conseguimos obter evidências para esclarecer e expandir o conhecimento dos dentistas, estomatologistas, patologistas orais e cirurgiões de cabeça e pescoço, entre outros profissionais da saúde, sobre o CECi, realizando uma revisão sistemática para avaliar critérios diagnósticos microscópicos para o diagnóstico do CECmi e descrevendo uma coorte sul-americana multicêntrica de pacientes com CECi que é, de acordo com o nosso conhecimento da literatura atual, o maior estudo já publicado nesse campo.

Como discutido anteriormente, há grande relevância no estudo de doenças malignas incipientes, no entanto, existe um espectro de variáveis subjetivas que desempenham um papel no diagnóstico e que podem impactar o prognóstico da doença (González-Moles et al., 2022). A subjetividade na classificação das displasias orais, por exemplo, tem sido uma fonte de controversa persistente entre a comunidade da patologia oral. Nesse sentido, achados microscópicos como a microinvasão entram neste espaço de difícil compreensão (Speight, 2007; Sanjai et al., 2017) e os resultados provenientes dessa dissertação demostraram heterogeneidade nos critérios histopatológicos atuais para a identificação da microinvasão no CECmi, dos quais a espessura tumoral (TT, do inglês *tumour thickness*) e a profundidade de invasão (DOI, do inglês *depth of invasion*) foram os mais usados, com valores que variam entre 4 a 10mm para TT e 0.02 a 5 mm para DOI, respectivamente. Assim, foi possível validar a hipótese de que existe ausência na padronização desses parâmetros necessários, impedindo uma análise objetiva e induzindo profissionais a confiar no julgamento subjetivo e na experiência adquirida individualmente (Barnes et al., 2005; Speight, 2007). Esta ausência de padronização pode

explicar a baixa quantidade de CECmi no nosso estudo primário, sendo que apenas 32 (29,9%) dos casos incluídos tiveram o diagnóstico mencionado. Assim, esse fato constitui uma das principais razões pelas quais esse tópico merece um estudo mais aprofundado (Heffner, 2002). Dessa forma, estudos com o objetivo de revelar possíveis vantagens do diagnóstico nessas fases são necessários e representam uma lacuna de conhecimento na literatura atual, que potencialmente poderá revelar utilidades de grande interesse para a redução das consequências associadas aos atuais métodos de tratamento, como feito em mama (Strang et al., 2020; Xu et al., 2022) ou cervix (Nicol et al., 2019; Hartman et al., 2021).

Os estudos que analisam os métodos de prevenção secundária do CEC oral têm deixado claro que o treinamento de inspeção visual para os profissionais de saúde oral é fundamental para ajudar a reduzir as taxas de incidência e mortalidade dessa doença, com foco em pacientes de alto risco (Kujan et al., 2009; Warnakulasuriya et al., 2015; Nagao & Warnakulasuriya, 2020; Warnakulasuriya & Kerr, 2021). Nesse cenário, também vale a pena destacar a relevância da teoria da transição epitélio-mesênquima (EMT) no CEC oral (Kalluri & Weinberg, 2009; de Lima et al., 2017), pois ela poderia representar um processo molecular visualmente indetectável no exame histopatológico convencional. Assim, várias alterações já poderiam estar ocorrendo em doenças aparentemente sutis na clínica ou de difícil interpretação histopatológica, o que ressalta ainda mais a relevância do diagnóstico precoce.

Diante disso, mediante o nosso estudo observacional, fornecemos resultados detalhados sobre uma coorte internacional sul-americana e multicêntrica de CECi, os quais são proveitosos para integrar conhecimentos sobre informações relevantes no contexto desses pacientes, por exemplo, o fato de pacientes masculinos em idades adultas e o uso de tabaco como fator de risco serem a população afetada. Além disso, providenciamos uma representação visual e descritiva abrangente dos aspectos clínicos comuns da doença, destacando características como a alta incidência também em mulheres, heterogeneidade na cor e superfície de lesões que usualmente são placas ou erosões localizadas em áreas de alto risco como língua, palato mole e assoalho da boca. Essa evidência tem potencial para servir como material de ensino e enriquecimento da literatura de grande utilidade no treinamento necessário para calibrar clínicos a identificar o câncer oral precocemente durante o exame visual oral adequado, entendendo que tem sido demostrado que clínicos como dentistas ou médicos gerais podem sentir incerteza

quanto ao seu conhecimento sobre câncer oral e técnicas de exame oral (Nicotera et al., 2004; Wade et al., 2009; Ford & Farah, 2013).

Finalmente, a informação apresentada neste trabalho renova evidências de como a subjetividade é uma grande limitação na avaliação de CECi, tanto em aspectos clínicos como histopatológicos, deixando uma grande margem para diagnósticos baseados meramente na opinião empírica humana. Nesse sentido, vale ressaltar a importância de novos métodos que possam auxiliar na análise objetiva, como o desenvolvimento de tecnologia baseada em métodos de aprendizado de máquina que possam reduzir essas dificuldades, auxiliando no diagnóstico e na previsão prognóstica para auxiliar no reconhecimento de padrões na análise de imagens clínicas e histopatológicas do CEC oral (Araújo et al., 2023; Souza et al., 2023).

4 CONCLUSÃO

Em resumo, com base nas informações obtidas através dos dois estudos apresentados nesta dissertação, podemos concluir que:

- TT e DOI são atualmente os principais critérios histopatológicos utilizados para definir o CECmi, contudo, existe grande heterogeneidade na definição dos parâmetros quantitativos para relatar microinvasão no CEC oral.

- O CECi é representado por uma miríade de lesões de aparência clínica sutil como placas mistas (leucoeritroplásicas) ou erosões, sobretudo, na borda lateral da língua e no palato mole dos homens na sexta década de vida, com histórico de tabagismo.

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ANE XOS

Anexo 1 – Dispensa de aprovação do Comitê de Ética em Pesquisa

UNICAMP - FACULDADE DE ODONTOLOGIA DE PIRACICABA DA UNIVERSIDADE DE CAMPINAS - FOP/UNICAMP

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: ASPECTOS CLÍNICOS E HISTOPATOLÓGICOS DO CARCINOMA ESPINOCELULAR ORAL IN SITU E CARCINOMA ESPINOCELULAR ORAL MICROINVASIVO: UM ESTUDO OBSERVACIONAL.

Pesquisador: Cristina Saldivia Siracusa

Área Temática:

Versão: 4

CAAE: 45545121.1.0000.5418

Instituição Proponente: Faculdade de Odontologia de Piracicaba - Unicamp

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 5.187.270

Apresentação do Projeto:

O parecer inicial é elaborado com base na transcrição editada do conteúdo do registro do protocolo na Plataforma Brasil e dos arquivos anexados à Plataforma Brasil. Os pareceres de retorno, emendas e notificações são elaborados a partir dos dados e arquivos da última versão apresentada.

Trata-se de SOLICITAÇÃO DE EMENDA (E1) AO PROTOCOLO originalmente aprovado em 13/10/2021 para inclusão de pesquisador e inclusão de centros coparticipantes no registro do protocolo na PB. O parecer foi atualizado de acordo com a documentação apresentada. A solicitação está detalhadamente descrita ao final do parecer.

A EQUIPE DE PESQUISA citada na capa do projeto de pesquisa inclui CRISTINA SALDIVIA SIRACUSA (Cirurgiã Dentista, Mestranda no PPG em Estomatopatologia da FOP/UNICAMP, Pesquisadora responsável), ALAN ROGER DOS SANTOS SILVA (Cirurgião Dentista, Docente da área de Semiologia da FOP/UNICAMP), ANNA LUÍZA DAMACENO ARAÚJO (Cirurgiã Dentista, Doutoranda no PPG em Estomatopatologia da FOP/UNICAMP), PABLO AGUSTIN VARGAS (Cirurgião Dentista, Docente da área de Patologia da FOP/UNICAMP), MÁRCIO AJUDARTE LOPES (Cirurgião Dentista, Docente da área de Semiologia da FOP/UNICAMP), FÁBIO DE ABREU ALVES (Cirurgião Dentista, Diretor do

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Continuação do Parecer: 5.187.270

Justificativa de Ausência	2Parecer_TCLE.pdf	12/10/2021 13:21:57	Cristina Saldivia Siracusa	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PIRACICABA, 27 de Dezembro de 2021

Assinado por:
jacks jorge junior
(Coordenador(a))

Anexo 2 – Situação do Projeto na Plataforma Brasil (Print do sistema online)

DETALHAR PROJETO DE PESQUISA

DADOS DA VERSÃO DO PROJETO DE PESQUISA

Título da Pesquisa: ASPECTOS CLÍNICOS E HISTOPATOLÓGICOS DO CARCINOMA ESPINOCELULAR ORAL IN SITU E CARCINOMA ESPINOCELULAR ORAL MICROINVASIVO: UM ESTUDO OBSERVACIONAL.
Pesquisador Responsável: Cristina Saldivia Siracusa
Área Temática:
Versão: 4
CAAE: 45545121.1.0000.5418
Submetido em: 22/12/2021
Instituição Proponente: Faculdade de Odontologia de Piracicaba - Unicamp
Situação da Versão do Projeto: Aprovado
Localização atual da Versão do Projeto: Pesquisador Responsável
Patrocinador Principal: Financiamento Próprio

PLATAFORMA BRASIL – COORDENADOR – PLATAFORMA BRASIL

Comprovante de Recepção: PB_COMPROVANTE_RECEPCAO_1869897

DOCUMENTOS DO PROJETO DE PESQUISA

- Versão Atual Aprovada (E1) - Versão 4
  - Documentos de Centros Coparticipantes (E1)
    - Faculdade de Odontologia da Universidade
    - Fundação Antônio Prudente - A.C. Camarg
      - Pareceres
    - UFRJ - Hospital Universitário Clementino F
      - Pareceres
  - Pendência Documental (E1) - Versão 4
    - Documentos do Projeto
      - Comprovante de Recepção - Submissã
      - Declaração de Instituição e Infraestrutu
      - Declaração de Manuseio Material Biológ
      - Declaração de Pesquisadores - Submis
      - Folha de Rosto - Submissão 2
      - Informações Básicas do Projeto - Subm
      - Outros - Submissão 2
      - Parecer Anterior - Submissão 2
      - Projeto Detalhado / Brochura Investigad
      - TCLE / Termos de Assentimento / Justif
    - Apreciação 2 - UNICAMP - Faculdade de C
- Projeto Completo

Tipo de Documento	Situação	Arquivo	Postagem	Ações

LISTA DE APRECIAÇÕES DO PROJETO

Apreciação	Pesquisador Responsável	Versão	Submissão	Modificação	Situação	Exclusiva do Centro Coord.	Ações
E1	Cristina Saldivia Siracusa	4	22/12/2021	27/12/2021	Aprovado	Não	
PO	Cristina Saldivia Siracusa	3	13/10/2021	14/10/2021	Aprovado	Não	

P	45545121.1.0000.5418	4	Cristina Saldivia Siracusa	5418 - UNICAMP - Faculdade de Odontologia de Piracicaba da Universidade de Campinas - FOP/UNICAMP		PO	E1	Aprovado	
Pc	45545121.1.3003.5257	1	Cristina Saldivia Siracusa	5257 - UFRJ - Hospital Universitário Clementino Fraga Filho da Universidade Federal do Rio de Janeiro / HUCFF-UFRJ		E1	POc	Aprovado	
Pc	45545121.1.3002.5432	2	Cristina Saldivia Siracusa	5432 - Fundação Antônio Prudente - A.C. Camargo Cancer Center		E1	POc	Aprovado	

Anexo 3 – Documento de aceite do artigo 1 (Print do sistema online)

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Journal section: Oral Cancer and Potentially malignant disorders
Publication Types: Review

doi:10.4317/medoral.25675

Histopathological parameters reported in microinvasive oral squamous cell carcinoma: a systematic review

Cristina Saldivia-Siracusa [1], Anna Luíza Damaceno Araújo [2], Wilfredo Alejandro González-Arriagada [3], Francisco Javier Tejeda Nava [4], Keith D Hunter [5], Marcio Ajudarte Lopes [6], Pablo Agustin Vargas [7], Alan Roger Santos-Silva [8]

Anexo 4 – Comprovação de condição OPEN ACCESS da revista

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Anexo 5 – Relatório de similaridade Turnitin

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