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AVALIAÇÃO DE MICRONÚCLEOS NA MUCOSA ORAL DE PACIENTES COM DESORDENS POTENCIALMENTE MALIGNAS EXPOSTOS A AGENTES CARCINOGÊNICOS: UMA REVISÃO SISTEMÁTICA E META-ANÁLISE

Manaus - AM
2021

RAFAELA COSTA FREIRE

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Dissertação apresentada ao Programa de Pós-graduação em Odontologia da Universidade Federal do Amazonas como requisito parcial para obtenção do título de Mestre em Odontologia.

Orientadora: Dra. Juliana Vianna Pereira

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Manaus, 15 de setembro de 2021.

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RESUMO

O teste do micronúcleo é uma abordagem citogenética minimamente invasiva para avaliar a genotoxicidade em células epiteliais. Essa abordagem tem sido utilizada para avaliar a frequência de micronúcleos em pacientes com desordens potencialmente malignas (DPM), considerando que essas doenças podem preceder o carcinoma epidermóide oral. Este estudo teve como objetivo avaliar a frequência de micronúcleos (MNF) e células micronucleadas (MNC) em pacientes com DPM expostos a agentes carcinogênicos. Com base nas diretrizes do PRISMA, foi realizada uma revisão sistemática (PROSPERO (CRD42020222509). Uma busca eletrônica foi realizada em junho de 2021 e incluiu estudos observacionais. Estudos que investigaram pacientes com OPMD (leucoplasia - LKP, eritroplasia - ETP, líquen plano oral - LPO, fibrose submucosa oral - OSMF e queilite actínica) expostos ao tabaco ou substâncias relacionadas ao tabaco foram incluídos como elegibilidade. Dezoito estudos foram incluídos na análise qualitativa, dos quais treze foram incluídos nas meta-análises. Um total de 995 indivíduos com DMP foram incluídos. A OSMF foi a DPM mais estudada, seguida pela LKP e OLP. A MNF foi maior em pacientes com LKP ($p < 0,00001$) e com OSMF em comparação com mastigadores de tabaco sem lesões ($p = 0,003$) e não mastigadores ($p = 0,005$), quando o corante era específico para DNA. Quando o corante era inespecífico, a contagem de MN também era maior nos DPMs comparados a não mastigadores, incluindo LKP ($p < 0,00001$) e OSMF (($p = 0,02$). A frequência de MNC foi maior em pacientes com OSMF usando noz de areca ($p < 0,00001$) ou mistura de tabaco em comparação com não mastigadores ($p = 0,03$), independentemente da especificidade do corante. Conclui-se que pacientes que consomem substâncias relacionadas ao tabaco e noz de areca apresentam aumento significativo na contagem de micronúcleos em OLK e OSMF quando comparados a não mastigadores.

Palavras-chave: Desordens potencialmente malignas, Tabaco, Testes de micronúcleo, Revisão sistemática; Meta-análise.

ABSTRACT

Micronucleus test is a minimally invasive cytogenetic approach for assessing genotoxicity in epithelial cells. This approach has been used to assess the frequency of micronuclei in patients with oral potentially malignant disorders (OPMD), considering that these disorders may precede oral squamous cell carcinoma. This study aimed to evaluate the frequency of micronuclei (MNF) and micronucleated cells (MNC) in patients with OPMD exposed to carcinogenic agents. Based on the guideline of PRISMA a systematic review was performed (PROSPERO (CRD42020222509). An electronic search was carried out in June 2021 and included observational studies. Studies that investigated patients with OPMD (leukoplakia - LKP, erythroplakia - ETP, oral lichen planus - OLP, oral fibrous submucosa OSMF, and actinic cheilitis) exposed to tobacco and tobacco-related substances were included as eligibility. Eighteen studies were included in the qualitative analysis of which thirteen were included in the meta-analyses. A total of 995 individuals with DMP were included. OSMF was the most studied DPM, followed by LKP and OLP. MNF was higher in patients with LKP ($p<0.00001$) and OSMF compared to chewers without lesions ($p=0.003$) and non-chewers ($p=0.005$) when the dye was specific for DNA. When the dye is nonspecific, the MN count was also higher in OPMDs compered to non-chewers, including LKP ($p<0.00001$) and OSMF (($p=0.02$). MNC was higher in patients with OSMF using areca nut ($p<0.00001$) or tobacco mix compared to non-chewers ($p=0.03$), regardless of the specificity of the dye. t is concluded that patients who consume tobacco-related substances and areca nut have a significant increase in micronucleus counts in OLK and OSMF when compared to non-chewers.

Keywords: Oral potentially malignant disorders, Tobacco, Micronucleus Tests, Systematic review; Meta-analysis.

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LISTA DE ABREVIATURAS

CONSORT	Consolidated Standart of Reporting Trials
DPM	Desordem Potencialmente Maligna
ETP	Eritroplasia
FAO	Faculdade de Odontologia
GRADE	Grading of Recommendation, assessment, development, and evaluation
JBI	Joanna Briggs Institute
LPO	Líquen Plano Oral
LKP	Leucoplasia
MMG	May-Grünwald-Giemsa
MN	Micronúcleos
MNC	Célula micronucleada
MNF	Frequência de micronúcleos
OPMD	Oral potentially malignant disorders
OSCC	Oral squamous cell carcinoma
OSMF	Fibrose submucosa oral
PAP	Papanicolau
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
RCT	Randomized Clinical Trial
UFAM	Universidade Federal do Amazonas

LISTA DE SÍMBOLOS

%	Percentual
=	Igual
<	Menor que
>	Maior que
≤	Menor ou igual a
≥	Maior ou igual a
®	Marca registrada

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1 INTRODUÇÃO

A literatura tem reportado o aumento da incidência de câncer em todo mundo (ANNERTZ; ANDERSON; PALMÉR *et al.*, 2012; BRAY; FERLAY; SOERJOMATARAM *et al.*, 2018). A última estimativa global, em 2018, supôs 354.864 novos casos de câncer e 177.384 mortes por câncer de lábio e cavidade oral (BRAY; FERLAY; SOERJOMATARAM *et al.*, 2018). Dentre as lesões malignas que acometem a cavidade bucal, o carcinoma de células escamosas compreende mais de 90% dos casos (PAI; WESTRA, 2009). Além disso, são crescentes os casos em pacientes jovens, com idade inferior a 45 anos (HUSSEIN; HELDER; DE VISSCHER *et al.*, 2017).

Vários fatores de risco estão relacionados ao o desenvolvimento do carcinoma de células escamosas na cavidade bucal, destacando-se o tabaco, uso abusivo de álcool e o uso de tabaco sem fumaça (WARNAKULASURIYA, 2011). Este último, especificamente em países asiáticos (KUMAR; DEBNATH; ISMAIL *et al.*, 2015).

Os carcinomas de células escamosas podem ser precedidos por uma das desordens potencialmente malignas (DPM), que compreendem um grupo de lesões que apresentam risco aumentado de transformação maligna (WARNAKULASURIYA; JOHNSON; VAN DER WAAL, 2007). Diversas lesões são listadas como DPM, incluindo leucoplasia, leucoplasia verrucosa proliferativa, eritroplasia, lesões palatinas em fumantes reversos, fibrose submucosa oral, queilite actínica, líquen plano oral e lúpus eritematoso, além da disceratose congênita, lesão liquenóide e doença de enxerto oral versus hospedeiro, acrescentados recentemente pela Organização mundial de Saúde (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR *et al.*, 2020; WHO, 2019).

Apesar do risco aumentado de transformação maligna, muitas dessas lesões não progridem para um carcinoma, tornando desafiador a presunção prognóstica das mesmas (WARNAKULASURIYA; JOHNSON; VAN DER WAAL, 2007). Nesse sentido, a busca por biomarcadores para o diagnóstico, estadiamento, monitoramento e prognóstico do câncer e das DPMs estão em constante investigação, visando ao diagnóstico precoce, com consequente aumento da taxa de sobrevida e melhor qualidade de vida ao paciente (PAI; WESTRA, 2009; SANGLE; BIJJARAGI; SHAH *et al.*, 2016), visto que na maioria dos casos, o diagnóstico do câncer bucal é tardio e impacta negativamente na sobrevida (MURPHY; GALLOWAY; HANDORF *et al.*, 2016; RUTKOWSKA; HNITECKA; NAHAJOWSKI *et al.*, 2020).

Diferentes métodos estão disponíveis para o diagnóstico do câncer bucal e das DPMs. Informações obtidas na anamnese, dados do exame clínico e confirmação histopatológica de alterações epiteliais são fundamentais para o diagnóstico. A citologia esfoliativa pode ser realizada em triagens em massa, para auxiliar no processo diagnóstico até que uma biópsia possa ser realizada (LINGEN; ABT; AGRAWAL *et al.*, 2017). Considerando-se as alterações genotípicas que ocorrem no processo de malignidade, a citologia esfoliativa permite visualização das alterações celulares (PALVE; TUPKARI, 2008). Dentre essas alterações, há a formação de micronúcleos (MN), que são fragmentos citoplasmáticos de DNA que surgem em decorrência da exposição do epitélio a agentes carcinogênicos. Por ser uma abordagem citogenética minimamente invasiva, o ensaio de MN tem sido utilizado como um biomarcador de dano genômico e considerado bom indicador de prognóstico (PALVE; TUPKARI, 2008), utilizado desde 1937 (HALDER; CHAKRABORTY; MANDAL *et al.*, 2003).

O ensaio de MNs vem sendo utilizado para avaliar a frequência de MNs (MNF) (GUPTA; GUPTA; AGARWAL, 2019; JOSHI; VERMA; GAUTAM *et al.*, 2011; JYOTI; KHAN; AFZAL *et al.*, 2013; KATARKAR; MUKHERJEE; KHAN *et al.*, 2014) e de células micronucleadas (MNC) (ANILA; KAVERI; NAIKMASUR, 2011; DESAI; GHAISAS; JAKHI *et al.*, 1996; KAYAL; TRIVEDI; DAVE *et al.*, 1993) em pacientes com DPMs, especialmente em países asiáticos em que o consumo de tabaco, além dos hábitos de mascar betel e/ou noz de areca são amplamente prevalentes nas populações resultando em uma maior prevalência de DPMs (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR *et al.*, 2020).

Apesar da literatura ter investigado a aplicação de testes de genotoxicidade em diferentes lesões e condições bucais, incluindo as DPMs e câncer bucal (BOLOGNESI; BRUZZONE; CEPPI *et al.*, 2021), além das buscas por evidências dos danos citogenéticos em pacientes com hábito de mascar tabaco, não há revisões sistemáticas que avaliem pacientes com DPM expostos a carcinógenos que considerem a especificidade do corante utilizada na citologia, o número de células incluídas na contagem de MNs e pacientes controles com e sem exposição aos carcinógenos.

Dessa forma, o objetivo desse estudo foi realizar uma revisão sistemática e meta-análise sobre a avaliação de micronúcleos na mucosa oral de pacientes com DPMs expostos a agentes carcinogênicos.

2 REVISÃO DA LITERATURA

2.1 Desordens potencialmente malignas

As desordens potencialmente malignas constituem um importante grupo de lesões da mucosa bucal que podem preceder o diagnóstico do carcinoma de células escamosas (WARNAKULASURIYA; JOHNSON; VAN DER WAAL, 2007). Muitas DPMs não evoluem para o câncer, entretanto, essas condições têm maior probabilidade de progressão para um carcinoma do que uma mucosa clinicamente normal (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR *et al.*, 2020). A taxa de transformação maligna das DPMs é de 7,9%, variando entre as diversas desordens que compõem esse grupo de lesões (IOCCA; SOLLECITO; ALAWI *et al.*, 2020).

O termo atual "desordens potencialmente malignas" foi aprimorado ao longo dos anos e engloba as antigas terminologias "pré-câncer", "lesões precursoras epiteliais", "pré-maligna", "pré-cancerosa" e "lesão intra-epitelial", além de incluir lesões e condições, agora combinadas como "desordens", considerando a exposição à carcinógenos ambientais, suscetível em qualquer indivíduo (JOHNSON, 2020; WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR *et al.*, 2020).

Assim como a nomenclatura, o grupo de lesões que compõe as DPMs também sofreu alterações com o passar dos anos, considerando as evidências sobre o risco aumentado de transformações malignas dessas condições. Atualmente, o grupo é composto por: leucoplasia, leucoplasia verrucosa proliferativa, eritroplasia, lesões palatinas em fumantes reversos, fibrose submucosa oral, queilite actínica, líquen plano oral, lúpus eritematoso, disceratose congênita, lesão liquenóide e doença de enxerto oral versus hospedeiro (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR *et al.*, 2020; WHO, 2019).

Apesar do grupo de DPMs ser amplo, algumas dessas lesões são mais comuns do que outras e a prevalência difere entre as populações (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR *et al.*, 2020). A prevalência global é de 4,47%, sendo os homens mais frequentemente afetados (59,99%) (MELLO; MIGUEL; DUTRA *et al.*, 2018). Além disso, as DPMs são estatisticamente mais prevalentes em consumidores de noz de areca e tabaco em comparação com não consumidores. Entretanto, associação com nível socioeconômico não foi observada (KUMAR; DEBNATH; ISMAIL *et al.*, 2015)

A leucoplasia, eritroplasia, fibrose submuocosa oral, líquen plano oral e a queilite actínica são as condições mais estudadas (MELLO; MIGUEL; DUTRA *et al.*, 2018).

2.1.1 Leucoplasia

A leucoplasia foi definida pela Organização Mundial da Saúde como "Uma mancha ou placa branca que não pode ser caracterizada clínica ou patologicamente como qualquer outra doença (KRAMER; LUCAS; PINDBORG *et al.*, 1978). Atualmente é caracterizada como "Uma placa predominantemente branca de risco questionável tendo excluído (outras) doenças ou distúrbios conhecidos que não trazem risco aumentado de câncer" (WARNAKULASURIYA; JOHNSON; VAN DER WAAL, 2007). Enfatiza-se que o termo "leucoplasia" é clínico e que a lesão não apresenta histologia específica (WARNAKULASURIYA; JOHNSON; VAN DER WAAL, 2007). A leucoplasia é de longe a DPM mais estudada na cavidade bucal (MELLO; MIGUEL; DUTRA *et al.*, 2018; WARNAKULASURIYA; JOHNSON; VAN DER WAAL, 2007; WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR *et al.*, 2020).

Clinicamente pode ser classificada em homogênea, quando é uniformemente branca, plana, fina, lisa, podendo apresentar rachaduras superficiais e não pode ser removida por raspagem; heterogênea, com variadas apresentações clínicas: salpicada (eritroleucoplásica), nodular (com projeções polipoides) e verrucosa (superfície enrugada). A leucoplasia verrucosa proliferativa, atualmente é considerada uma desordem distinta, caracterizada por ser progressiva, persistente e irreversível e multifocal, com maior probabilidade para evolução para o câncer em comparação com as demais DPM (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR *et al.*, 2020).

Uma recente revisão sistemática constatou uma prevalência de 4,11% em adultos (MELLO; MIGUEL; DUTRA *et al.*, 2018), superior ao reportado previamente (1,49%) (PETTI, 2003), provavelmente por incluir estudos realizados em centros de diagnóstico. A lesão geralmente ocorre em homens de meia idade e, em 70% dos casos a mucosa bucal, vermelhão do lábio ou gengiva são os locais de maior ocorrência. Além disso, a lesão está presente, usualmente, 5 anos antes de evoluir para o carcinoma de lesões escamosas, em casos em que ocorreram transformação maligna (Mortazavi et al., 2014). A frequência de leucoplasia oral é baixa em pacientes jovens, representando 9,2% dos casos (ROZA; KOWALSKI; WILLIAM *et al.*, 2021).

Apesar do risco de transformação maligna, correspondente a 9,5% (IOCCA; SOLLECITO; ALAWI *et al.*, 2020), na maioria dos casos a lesão permanece estável ou

regride, dificultando o prognóstico (BOUQUOT; SPEIGHT; FARTHING, 2006). A displasia epitelial foi reportada em apenas 1,8% dos casos dos estudos incluídos em uma revisão sistemática de prevalência de leucoplasia oral (MELLO; MIGUEL; DUTRA *et al.*, 2018).

Nos países asiáticos a prevalência da leucoplasia oral é de 7,77% (MELLO; MIGUEL; DUTRA *et al.*, 2018). Nesses países, as pesquisas também buscam correlacionar as leucoplasias com a prevalência significativamente aumentada de MN na mucosa bucal quando comparada à mucosa normal de pacientes que não estão expostos a carcinógenos como o tabaco (GUPTA; GUPTA; AGARWAL, 2019; KATARKAR; MUKHERJEE; KHAN *et al.*, 2014; KOHLI; AHUJA; MEHENDIRATTA *et al.*, 2017; MAHIMKAR; SAMANT; KANNAN *et al.*, 2010; SINGAM; MAJUMDAR; UPPALA *et al.*, 2019).

2.1.2 Eritroplasia

Eritroplasia foi descrita inicialmente por Queyrat, em 1911, como uma lesão pré-cancerosa de característica aveludada, coloração vermelha brilhante na glande do pênis (REICHART; PHILIPSEN, 2005). Atualmente é caracterizada por uma mancha, predominantemente vermelha que não pode ser caracterizada clínica ou patologicamente como qualquer outra doença definível. A área afetada é nitidamente demarcada, podendo ser plana ou deprimida (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR *et al.*, 2020). A nomenclatura já foi considerada inadequada, considerando-se que, diferentemente da leucoplasia, não há formação de placa, observando-se de fato uma área deprimida em relação à mucosa circundante (CAWSON RA; LANGDON JD; JW., 1996). Diferentemente de outras condições eritematosas, como líquen plano, lúpus eritematoso e candidíase eritematosa, as eritroplasias são lesões solitárias (VAN DER WAAL, 2010). Por outro lado, podem se assemelhar com alterações vasculares (REICHART; PHILIPSEN, 2005).

Poucos estudos reportam a prevalência da eritroplaisa, sendo a maioria dos dados disponíveis de populações com hábitos específicos ou dados hospitalares (HOLMSTRUP, 2018). Sua prevalência é baixa, 0.17%, segundo dados de uma revisão sistemática (MELLO; MIGUEL; DUTRA *et al.*, 2018), reportada como a DPM menos prevalente (KUMAR; DEBNATH; ISMAIL *et al.*, 2015). No entanto, a displasia epitelial grave ou carcinoma *in situ* são frequentes (MELLO; MIGUEL; DUTRA *et al.*, 2018), podendo a transformação maligna ocorrer entre 14 e 50% dos casos (REICHART;

PHILIPSEN, 2005). Atualmente a taxa de transformação maligna evidenciada é de 33,1% (IOCCA; SOLLECITO; ALAWI *et al.*, 2020). Considerando que uma proporção significativa dos casos de eritroplasia evoluirá para malignidade, a lesão deve ser acompanhada clinicamente em curtos intervalos de tempo (HOLMSTRUP, 2018).

A lesão é mais comum em homens e a mucosa jugal e palatina são os sítios mais prevalentes. Assim como a leucoplasia, o hábito de mascar ou fumar tabaco, mascar betel com ou sem tabaco e o consumo de álcool, são os fatores etiológicos relacionados à essa desordem (HOLMSTRUP, 2018).

2.1.3 Fibrose submucosa oral

A fibrose submucosa oral é considerada uma doença crônica e insidiosa da mucosa oral, resultante da perda da fibroelasticidade da lâmina própria e consequente fibrose da mucosa oral e atrofia epitelial (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR *et al.*, 2020). Os pacientes acometidos podem relatar sensação de queimação e intolerância à alimentos picantes. Os sinais são mucosa pálida e coriácea, além de perda de papilas linguais (KERR; WARNAKULASURIYA; MIGHELL *et al.*, 2011). A mucosa torna-se ainda esbranquiçada e, o enrijecimento da mucosa oral incluindo lábios, bochechas e palato mole, leva a limitação da abertura da boca (TILAKARATNE; EKANAYAKA; WARNAKULASURIYA, 2016).

A fibrose submucosa oral é predominantemente encontrada na população do sul e sudeste asiático, em função do consumo da noz de areca. Entretanto, a suscetibilidade genética também deve ser considerada, uma vez que apenas 1 a 2% dos mascadores de noz de areca desenvolvem a doença (TILAKARATNE; EKANAYAKA; WARNAKULASURIYA, 2016). Um revisão sistemática mostrou que o consumo de noz de areca foi reportado em 30% dos estudos incluídos, seguido do uso de álcool e tabaco (22%) (KERR; WARNAKULASURIYA; MIGHELL *et al.*, 2011). A prevalência atual é de 4,96%, concentrada na população asiática (MELLO; MIGUEL; DUTRA *et al.*, 2018), Na Índia, as mulheres são mais acometidas do que os homens, nas demais regiões, é mais comum entre os homens na faixa etária entre 20 a 40 anos (SHIH; WANG; SHIEH *et al.*, 2019).

A correlação do estadiamento clínico e histopatológico foi considerada altamente significativa, sugerindo que o indivíduo com fibrose submucosa oral clinicamente avançado apresentava fibrose extensa histologicamente (BIRADAR; MUNDE;

BIRADAR *et al.*, 2018). A taxa de transformação maligna é de 5,2%, evidenciada recentemente em uma revisão sistemática (IOCCA; SOLLECITO; ALAWI *et al.*, 2020).

2.1.4 Líquen plano oral

O Líquen plano oral é uma desordem inflamatória crônica de etiologia desconhecida com recidivas e remissões características, exibindo lesões reticulares brancas, acompanhadas ou não de áreas atróficas, erosivas e ulcerativas e / ou em placa. As lesões são frequentemente bilateralmente simétricas, podendo causar dor e desconforto, principalmente quando erosivo (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR *et al.*, 2020; WHO, 2019).

Embora a etiologia do líquen plano oral ainda seja incerta, há evidências de que se trata de uma doença imunológica complexa mediada por células citotóxicas dirigidas contra queratinócitos basilares e resultando em degeneração vacuolar e lise das células basais (CRINCOLI; DI BISCEGLIE; SCIVETTI *et al.*, 2011). Dessa forma, a correlação com fatores de risco comuns para o desenvolvimento do carcinoma de células escamosas não é bem definida na literatura. Acredita-se que o infiltrado inflamatório nas lesões de linquen plano oral é reduzido na exposição ao tabaco, alterando a vigilância imunológica (ALRASHDAN; ANGEL; CIRILLO *et al.*, 2016).

A prevalência global do líquen plano oral é de 1,01%, sendo mais elevada na Europa (1,43%) e reduzida n Índia (0,49%), onde as ceratoses associadas ao tabaco parecem mascarar a lesão, consequentemente atenuando a prevalência (GONZÁLEZ-MOLES; WARNAKULASURIYA; GONZÁLEZ-RUIZ *et al.*, 2021). A taxa de transformação maligna é de 1,4% (IOCCA; SOLLECITO; ALAWI *et al.*, 2020). A desordem é pouco prevalente em pacientes jovens (0,02%) quando comparada aos idosos (1,92%), além de aumentar progressivamente após os 40 anos (GONZÁLEZ-MOLES; WARNAKULASURIYA; GONZÁLEZ-RUIZ *et al.*, 2021).

2.1.5 Queilite actínica

A queilite actínica é uma desordem causada pelos danos do sol nos lábios, mais comumente na borda do vermelhão do lábio inferior, com uma apresentação variável de áreas atróficas, erosivas e placas brancas (WARNAKULASURIYA; KUJAN; AGUIRRE-URIZAR *et al.*, 2020). A proliferação e diferenciação anormais dos queratinócitos são induzidos pela radiação ultravioleta, após a exposição crônica (WHO, 2019).

Homens de idade avançada, pele clara que vivem em regiões tropicais, com excessiva exposição à radiação ultravioleta, além de fumantes devem ser considerados de risco quanto a transformação maligna (DANCYGER; HEARD; HUANG *et al.*, 2018). A idade média de ocorrência da queilite actínica é de 54,3 anos (MELLO; MELO; MODOLO *et al.*, 2019). Interessantemente, 60% dos casos dos pacientes afetados, usam tabaco em alguma forma de apresentação (MARKOPOULOS; ALBANIDOU-FARMAKI; KAYAVIS, 2004).

No noroeste da Espanha a prevalência reportada foi de 31,3% (RODRÍGUEZ-BLANCO; FLÓREZ; PAREDES-SUÁREZ *et al.*, 2018). Entretanto, a prevalência global é de 15,32% (MELLO; MIGUEL; DUTRA *et al.*, 2018).

Uma revisão sistemática evidenciou que em 60,5% dos casos de queilite actínica apresentam algum grau de displasia epitelial e, 25% dos diagnósticos clínicos são histologicamente carcinoma de células escamosas de lábio (MELLO; MELO; MODOLO *et al.*, 2019).

2.2 Carcinógenos associados às desordens potencialmente malignas

Diversos fatores genéticos e ambientais estão relacionados à etiologia das DPM (IOCCA; SOLLECITO; ALAWI *et al.*, 2020). O consumo de álcool, tabaco e o hábito de mascar noz de areca são frequentemente associados com a maioria das DPMs (RIMAL; SHRESTHA; MAHARJAN *et al.*, 2019). Em acréscimo, a má-nutrição, comum entre os grandes consumidores de álcool, tabaco e betel, contribui significativamente (AMARASINGHE; USGODAARACHCHI; KUMARAARACHCHI *et al.*, 2013). Além desses, o papel do Papilomavírus humano (HPV) no desenvolvimento das DPMs e do câncer oral continua em debate (TANG; MENEZES; BAETEN *et al.*, 2020).

Apesar de compartilharem os mesmos fatores de risco, um estudo reportou que apesar de 68% dos indivíduos conhecerem do risco associado do tabaco e câncer bucal e, 93% desconheciam os fatores de risco relacionados às DPM, além de desconhecerem essas lesões (KADASHETTI; SHIVAKUMAR; CHOUDHARY *et al.*, 2020).

Estudos buscam associar os agentes carcinógenos com cada DPMs especificamente. Há consenso de que a leucoplasia está associada ao tabagismo, consumo excessivo de álcool e uso de noz de areca (VILLA; WOO, 2017), este último, especialmente em países asiáticos, que apresentam, consequentemente, maior prevalência dessa desordem (MELLO; MIGUEL; DUTRA *et al.*, 2018). O consumo de noz de areca está associada à fibrose submucosa oral (KERR; WARNAKULASURIYA; MIGHELL *et al.*, 2011),

também com prevalência concentrada nos países asiáticos (MELLO; MIGUEL; DUTRA *et al.*, 2018). Diferentemente, o líquen plano oral parece não apresentar associação com agentes ambientais (ALRASHDAN; ANGEL; CIRILLO *et al.*, 2016) e a queilite actínica tem predileção para indivíduos com exposição crônica à radiação ultravioleta (DANCYGER; HEARD; HUANG *et al.*, 2018).

Relevantemente, uma parcela substancial da população (10%) consome noz de areca, sendo o hábito endêmico em todo o subcontinente indiano, em grandes partes do sul da Ásia e na Melanésia. A noz de areca pode ser utilizada de forma isolada, com tabaco, além da mistura com diversos outros ingredientes, incluindo o tabaco, em uma mistura conhecida como betel. Dentre os componentes que compõem o betel estão a noz de areca; folhas, caule, flores e vagem da videira *Piper betle*; hidróxido de cálcio (*Lime*), obtido de conchas e corais; extrato da árvore de Acácia (*Catechu*); folhas de tabaco; especiarias e adoçantes (GUPTA; WARNAKULASURIYA, 2002). Quando a noz de areca é preparada em formulações comerciais, é denominada *Pan masala* e, esta quando contém tabaco, é conhecida como *Gutka* (GUPTA; WARNAKULASURIYA, 2002).

O principal agente carcinógeno do betel são os alcalóides e polifenóis que podem estar associados aos cânceres de boca e faringe (CHEN; MAHMOOD; MARIOTTINI *et al.*, 2017).

As evidências atuais são convincentes de que o tabaco sem fumaça (mascado), consumido frequentemente como componente do betel e o uso do betel, mesmo que sem o tabaco, são fatores de risco fortes e independentes para câncer de boca. Porém, estudos com melhor separação dos tipos de tabaco e formas de uso e estudos com poder suficiente para quantificar as relações dose-resposta ainda são necessários (GUPTA; JOHNSON, 2014).

2.3 Micronúcleos

O teste de micronúcleos (MN) é um dos métodos citogenéticos para avaliar a genotoxicidade de carcinógenos em células epiteliais (NADERI; FARHADI; SARSHAR, 2012), uma vez que as alterações celulares que ocorrem no processo de malignidade, são possíveis de serem visualizadas com a citologia esfoliativa (PALVE; TUPKARI, 2008).

A técnica é minimamente invasiva e tem sido utilizada como um biomarcador da exposição de vários agentes genotóxicos e sua correlação com o risco de câncer (BOLOGNESI; BONASSI; KNASMUELLER *et al.*, 2015; BOLOGNESI;

BRUZZONE; CEPPI *et al.*, 2021). Dentre os estudos que usam o ensaio de MN, 53% estão relacionadas ao câncer oral, de cabeça e pescoço e DPMs, sendo, portanto, uma técnica com utilidade potencial no rastreamento e no acompanhamento das DPMs (BOLOGNESI; BONASSI; KNASMUELLER *et al.*, 2015).

A presença de MNs em células esfoliadas, indicam perda ou fragmentação cromossômica que ocorre nos estágios iniciais da divisão celular. Geralmente um MN por célula são encontrados, no entanto, quantidade superior podem ser observada (THOMAS; HOLLAND; BOLOGNESI *et al.*, 2009). São caracterizados por serem redondos ou ovais, com mesmo formato, textura e intensidade de coloração que o núcleo principal, variando em diâmetro de 1/3 a 1/16 do mesmo. A frequência de MN em pacientes saudáveis é praticamente nula, numa proporção de 0,30 a 1,70 a cada 1000 células (HOLLAND; BOLOGNESI; KIRSCH-VOLDERS *et al.*, 2008).

Esta abordagem tem sido usada para avaliar a frequência de micronúcleos (MNF) (MNF) (GUPTA; GUPTA; AGARWAL, 2019; JOSHI; VERMA; GAUTAM *et al.*, 2011; JYOTI; KHAN; AFZAL *et al.*, 2013; KATARKAR; MUKHERJEE; KHAN *et al.*, 2014) e de células micronucleadas (MNC) (ANILA; KAVERI; NAIKMASUR, 2011; DESAI; GHAISAS; JAKHI *et al.*, 1996; KAYAL; TRIVEDI; DAVE *et al.*, 1993) Em pacientes com DPMs.

O uso da coloração e do número de células incluídas na contagem de MNs variam bastante entre as pesquisas. Desde que a técnica foi inicialmente utilizada na cavidade bucal (STICH; CURTIS; PARIDA, 1982), várias estudos tem sido realizados com colorações DNA-específicas (Feulgen, Acridine orange, fluorescent stain 4′,6′-diamidino-2-phenylindole - DAPI) (DAVE, 1990; HORNBY, 1989; JOSHI; VERMA; GAUTAM *et al.*, 2011; JYOTI; KHAN; AFZAL *et al.*, 2013; KATARKAR; MUKHERJEE; KHAN *et al.*, 2014; KAYAL; TRIVEDI; DAVE *et al.*, 1993; PELLICIOLI; VISIOLI; FERREIRA *et al.*, 2011; SINGAM; MAJUMDAR; UPPALA *et al.*, 2019; STICH; ROSIN; HORNBY *et al.*, 1988; TRIVEDI, 1991). Entretanto, colorações não específicas também sido utilizadas (Giemsa, May Grunwald Giemsa - MGG, Papanicolau - PAP) (ANILA; KAVERI; NAIKMASUR, 2011; DESAI; GHAISAS; JAKHI *et al.*, 1996; DOSI; GUPTA; HAZARI *et al.*, 2016; GUPTA; GUPTA; AGARWAL, 2019; MAHIMKAR; SAMANT; KANNAN *et al.*, 2010; SHAH; MANJUNATHA; SHAH *et al.*, 2015; WAGH; RAVAL; AIYER *et al.*, 2019). As colorações não específicas podem refletir em um maior número de MN incluídos na contagem (BONASSI; COSKUN; CEPPI *et al.*, 2011; RIBEIRO, 2019), influenciando

diretamente na sensibilidade da contagem (KOHLI; AHUJA; MEHENDIRATTA *et al.*, 2017).

Quanto ao número de células incluídas na contagem, a recomendação atual é de que 2000 células por indivíduos sejam consideradas (FENECH; CHANG; KIRSCH-VOLDERS *et al.*, 2003). Entretanto, a contagem de 1000 células é frequentemente adotada (DAVE, 1990; DESAI; GHAISAS; JAKHI *et al.*, 1996; GUPTA; GUPTA; AGARWAL, 2019; JOSHI; VERMA; GAUTAM *et al.*, 2011; KATARKAR; MUKHERJEE; KHAN *et al.*, 2014; KAYAL; TRIVEDI; DAVE *et al.*, 1993; KOHLI; AHUJA; MEHENDIRATTA *et al.*, 2017; PELLICIOLI; VISIOLI; FERREIRA *et al.*, 2011; SHAH; MANJUNATHA; SHAH *et al.*, 2015; TRIVEDI, 1991; WAGH; RAVAL; AIYER *et al.*, 2019).

3 OBJETIVOS

3.1 Geral

Realizar uma revisão sistemática e meta-análise sobre a avaliação de micronúcleos na mucosa oral de pacientes com DPMs expostos a agentes carcinogênicos.

3.1 Específicos

Avaliar a frequência de micronúcleos (MNF) e células micronucleadas (MNC) em pacientes com desordens potencialmente malignas (leucoplasia, eritroplasia, fibrose submucosa oral, linquen plano e queilite actínica) expostos a agentes carcinogênicos (tabaco e substâncias relacionadas e noz de areca) comparados à pacientes controles.

4 MÉTODOS

4.1. Protocolo e Registro

Este protocolo de estudo foi registrado na base de dados da PROSPERO (International Prospective Register of Systematic Reviews) sob o número CRD42020222509, e seguiu as recomendações da declaração PRISMA para o relatório desta revisão sistemática

4.2. Critérios de Elegibilidade

Foram incluídos estudos que investigaram a MNF e de MNC em pacientes que apresentavam desordens potencialmente malignas, em comparação a pacientes com mucosa íntegra e pacientes controles, a partir da exposição à carcinógenos. Os critérios de inclusão foram baseados na estratégia PECO:

P - População: pacientes com diagnóstico de desordem potencialmente maligna: (leucoplasia, eritroplasia, eritroleucoplasia, línquen plano, fibrose submucosa oral, e queilite acítnica)

E - Exposição: exposição à carcinógenos (tabaco e substâncias relacionadas e noz de areca)

C - Comparação: pacientes expostos e não expostos a agentes carcinogênicos sem o diagnóstico de DPM

O - *Outcome* (Resultado): frequência de micronúcleos e de células micronucleadas

S - Study Design (Desenho do estudo): estudos observacionais

Pergunta: Existe diferença na MNF e de MNC em pacientes com DPM expostos a agentes carcinogênicos (tabaco e substâncias relacionadas e noz de areca), comparados à pacientes expostos e não expostos a agentes carcinogênicos sem o diagnóstico de DPM?

Os critérios de exclusão envolveram: (1) Revisões, cartas ao editor, resumos de conferências, opiniões pessoais, capítulos de livros; (2) Estudos que não avaliaram OPMD ou com dados não individualizados para OPMD; (3) A citologia não investigou MN ou estudos que avaliam a genotoxicidade por uma técnica diferente de MN; (4) Estudos que não relataram exposição a carcinógenos; (5) Cópia do texto completo não disponível; (6) Amostras duplicadas; (7) Desenho do estudo; Estudos descritivos (relatos

de casos e séries de casos); (8) Restrição de idioma (aqueles que não estão em inglês, português ou espanhol).

4.3. Estratégia de busca

As estratégias de busca foram realizadas em junho de 2021, em cinco bases de dados eletrônicas: PubMed, SCOPUS, Web of Science, Embase e LILACS. A literatura cinzenta foi incluída e englobou ProQuest Dissertations & Theses Global. As referências duplicadas foram excluídas pelo software gerenciador de referências (EndNote®, Thomson Reuters). Posteriormente, uma análise da lista de referências dos artigos selecionados foi realizada manualmente. A estratégia de pesquisa completa é apresentada na Tabela Suplementar S1.

4.4. Seleção dos estudos

A seleção dos estudos foi realizada de forma independente por dois autores (RCF e JVP). Em caso de desacordo, um terceiro autor (TNLK) foi consultado. Considerando os critérios de elegibilidade foram avaliados primeiramente os títulos e resumos e, posteriormente, todos os textos completos dos artigos selecionados.

4.5. Coleta de dados

Para todos os estudos incluídos, a extração de dados englobou as seguintes informações: (a) autores, ano de publicação e país; (b) desenho do estudo; (c) tamanho da amostra e sexo dos participantes; (d) idade dos pacientes; (e) recrutamento; (f) tipo de hábito; (g) frequência do hábito; (h) local da mastigação; (i) número de células (j) coloração; (k) contagem de MN; (l) conclusões. A coleta de dados foi realizada de forma independente por dois autores (RCF e JVP). Em caso de desacordo, um terceiro autor foi consultado (TNLK). Quando necessário, por falta de dados, os autores foram contatados por e-mail.

4.6 Risco de viés em estudos individuais

Os mesmos dois revisores (RCF e JVP) avaliaram o risco de viés de forma independente usando as ferramentas de avaliação crítica do Joanna Briggs Institute (JBI) para estudos de prevalência (MUNN; MOOLA; LISY *et al.*, 2015). Os estudos foram pontuados em cada item com “sim”, “não”, “pouco claro” e “não aplicável” e qualquer discordância foi resolvida por consenso. Os estudos foram categorizados como: (a) baixo

risco de viés, se os estudos atingissem mais de 70% de escores de “sim”; (b) risco moderado de viés, se os escores “sim” estivessem entre 50% e 69%; e (c) alto risco de viés, se as pontuações “sim” fossem abaixo de 49%.

Como critérios para risco de viés foram considerados estudos que não apresentavam a descrição completa na metodologia do estudo, que incluía o tipo de estudo, tamanho, características (sexo e idade) e forma de recrutamento da amostra, tipo, frequência e sítio de mastigação do hábito carcinogênico, inclusão de uma contagem mínima de 1.000 células para avaliação de micronúcleos e uso de coloração específica, além do método estatístico adequado.

4.7 Medidas sumárias

O desfecho primário foi MNF e MNC em pacientes com diagnóstico de OPMD, considerando-se a média e desvio padrão. Foram considerados diferentes tipos de agentes de exposição à base de tabaco e substâncias relacionadas, além de noz de areca.

4.8 Síntese dos resultados

As meta-análises foram realizadas com estudos que apresentaram média e desvio padrão nas contagens de MNF e MNC. Apenas os resultados da pesquisa que contaram pelo menos 1000 células foram considerados. A diferença média de MNF e MNC, de acordo com o tipo de corante utilizado (específico para DNA ou não específico), entre pacientes com DPMs e controles foi avaliada por meio de meta-análise de acordo com as Diretrizes Cochrane (HIGGINS JPT, 2019). Os gráficos de floresta, como parte da meta-análise, com média, desvio padrão e IC de 95% determinados em um nível de significância de 5%, foram construídos usando Review Manager® 5.4 (RevMan 5.4, The Nordic Cochrane Centre, Copenhagen, Dinamarca). A heterogeneidade foi determinada por índices de inconsistência (I2), onde um valor superior a 50% foi considerado um indicador de heterogeneidade substancial entre os estudos. Uma meta-análise de efeito aleatório foi usada.

4.9 Análise de evidências cumulativas

O instrumento *Grading of Recommendation, Assessment, Development, and Evaluation* (GRADE)(BALSHEM; HELFAND; SCHÜNEMANN *et al.*, 2011) foi usado para avaliar a certeza das evidências e a força das recomendações. A avaliação foi baseada

no desenho do estudo, risco de viés, inconsistência, imprecisão e outras considerações, como viés de publicação e magnitude do efeito. Também foi considerado o tamanho da amostra e o efeito absoluto resultante da meta-análise. A certeza da evidência foi classificada como alta, moderada, baixa ou muito baixa.

5 ARTIGO

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Micronuclei in the oral mucosa of patients with potentially malignant disorders exposed to carcinogenic agents: a systematic review and meta-analysis

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ABSTRACT

Objectives: Evaluate the frequency of micronuclei (MNF) and micronucleated cells (MNC) in patients with oral potentially malignant disorders (OPMD) exposed to carcinogenic agents. **Study Design**: Based on the guideline of PRISMA a systematic review was performed (PROSPERO (CRD42020222509). An electronic search was carried out in June 2021 and included observational studies. Studies that investigated patients with OPMD (leukoplakia - LKP, erythroplakia - ETP, oral lichen planus - OLP, oral fibrous submucosa OSMF, and actinic cheilitis) exposed to tobacco and tobacco-related substances were included as eligibility. **Results**: Eighteen studies were included in the qualitative analysis of which thirteen were included in the meta-analyses. OSMF was the most studied OPMD. MNF was higher in patients with LKP ($p<0.00001$) and OSMF compared to patients exposed to carcinogens without OPMD ($p=0.003$) and patients not exposed to carcinogens ($p=0.005$) when the dye was specific for DNA. When the dye is nonspecific, the MN count was also higher in OPMDs compered to non-chewers, including LKP ($p<0.00001$) and OSMF (($p=0.02$). MNC was higher in patients with OSMF using areca nut ($p<0.00001$) or tobacco mix compared to unexposed patients ($p=0.03$), regardless of the specificity of the dye. **Conclusion:** Patients who consume tobacco-related substances and areca nut have a significant increase in micronucleus counts in OLK and OSMF when compared to unexposed patients.

Keywords: Oral potentially malignant disorders, Tobacco, Micronucleus Tests, Systematic review; Meta-analysis.

INTRODUCTION

Oral Potentially Malignant Disorders (OPMD) comprise the mucosal diseases that may precede oral squamous cell carcinoma (OSCC)[1, 2]. Several lesions are listed as OPMD, including leukoplakia (LKP), erythroplakia (ETP), palatal lesions in reverse smokers, oral submucous fibrosis (OSMF), actinic keratosis, oral lichen planus (OLP) and lupus erythematosus, in addition to dyskeratosis congenital, oral lichenoid lesion, and oral graft versus host disease , recently added by the World Health Organization[2, 3].

A systematic review including LKP, ETP, OSMF, and actinic cheilitis showed an overall prevalence of OPMD of 4.47% [4]. However, some OPMD are more common than others, depending on the population, due to cultural risk factors[3]. This fact is quite observed in India, where tobacco, tobacco-related substances and areca nut consumption are frequent, resulting in a prevalence of OPMD of 13.7% and a predominance of OSMF[5].

In 2018, the estimate on the global burden of cancer worldwide (GLOBOCAN 2018) by the International Agency for Research on Cancer was 354,864 new cancer cases and 177,384 deaths for cancer of the lip and oral cavity[6]. A scoping review assessed the prevalence of oral cancer in low- and middle-income countries. The oral mucosa was the most common location for oral cancer, and it was associated with exposure to chewing tobacco[7]. However, predicting the risk of malignant transformation of OPMD is a great challenge[3]. Micronucleus test is a minimally invasive cytogenetic approach for assessing genotoxicity in epithelial cells[8]. The technique is used for screening chemicals for chromosome-breaking effects[9]. The micronucleus results from chromosomal fragments that arise during cell division, and they represent 1/3 to 1/5 of the size of the nucleus[10].

This approach has been used to assess the frequency of micronuclei (MNF)[11-14] and of micronucleated cells (MNC)[15-17] in patients with OPMD. Thus, this study aimed

to systematically review the literature and assess the frequency of micronuclei and micronucleated cells in patients with OPMD exposed to tobacco and derivatives compared to control patients.

MATERIAL AND METHODS

Protocol and registration

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA Statement) guideline[18], and it was recorded in the International Prospective Registry of Systematic Reviews (PROSPERO) database (CRD42020222509).

Eligibility criteria

The research question was: Is there a difference in MNF and MNC in OPMD patients exposed to carcinogens (tobacco and related substances and areca nut) compared to patients exposed and not exposed to carcinogens without a diagnosis of OPMD? The inclusion criteria were based on the PECOS approach (Population, Exposure, Comparator, Outcome, and Studies). Observational studies evaluating the MNF and MNC in patients with OPMD (leukoplakia - LKP, erythroplakia - ETP, oral lichen planus - OLP, oral fibrous submucosa OSMF, and actinic cheilitis) exposed to carcinogenic agents (tobacco and related substances, and areca nut) were included. Controls were patients not exposed to carcinogens and patients exposed to carcinogens without the presence of OPMD.

Exclusion criteria involved: (1) Reviews, letters to the editor, conference abstracts, personal opinions, book chapters; (2) Studies that did not assess OPMD or with non-individualized data for OPMD; (3) Cytology did not investigate MN or studies that

assess genotoxicity by a technique other than MN; (4) Studies that did not report exposure to carcinogens; (5) Full text copy not available; (6) Duplicated samples ; (7) Study design; Descriptive studies (case reports and case series); (8) Language restriction (those not in English, Portuguese or Spanish)

Search strategy and selection of studies

Search strategies were done in June 2021 and developed in five electronic databases: PubMed, SCOPUS, Web of Science, Embase, and LILACS. The gray literature was included and encompassed ProQuest Dissertations & Theses Global. Duplicated references were excluded by reference manager software (EndNote®, Thomson Reuters). Therefore, a subsequent analysis of the reference list of selected articles was carried out manually. The full search strategy is presented in Supplementary Table S1.

The studies' selection was performed independently by two authors (RCF and JVP). In case of disagreement, a third author (TNLK) was consulted. First, titles and abstracts were evaluated regarding inclusion criteria. Then, all the full text of the selected articles were evaluated according to the exclusion criteria.

Data collection process

The following data were collected from the included studies: (a) authors, year of publication and country; (b) study design; (c) sample size and gender of participants; (d) patients age; (e) recruitment; (f) type of habit; (g) frequency of habit; (h) site of chewing; (i) number of cells (j) stain; (k) MN counting; (l) conclusions. The data collection was performed independently by two authors (RCF and JVP). In case of any disagreements, a third author made the final decision (TNLK). When necessary for missing data, the

authors were contacted by e-mail.

Risk of bias in individual studies

The same two reviewers (RCF and JVP) assessed the risk of bias independently by using The Joanna Briggs Institute (JBI) Critical Appraisal tools for Prevalence studies[19]. The studies were scored each item with "yes", "no", "unclear" and "not applicable" and any disagreement between them was resolved by consensus. Studies were categorized as: (a) low risk of bias, if studies reached more than 70% scores of "yes"; (b) moderate risk of bias, if "yes" scores were between 50% and 69%; and (c) high risk of bias, if "yes" scores were below 49%.

Summary measures

The primary outcome was MNF and MNC in patients diagnosed with OPMD. It was considered mean and standard deviation. Different types of exposure agents made with tobacco and its derivates and areca nut were considered.

Synthesis of results

Meta-analyses were performed with studies that presented mean and standard deviation in MNF and MNC counts. Only search results that counted at least 1000 cells were considered in the quantitative analysis. The mean difference in MNF and MNC, according to the type of dye used (DNA-specific or non-specific), between patients with OPMD and controls was assessed through a meta-analysis according to Cochrane Guidelines[20]. The forest plots as part of the meta-analysis, with the mean, standard deviation and 95% CI determined at a significance level of 5% were constructed using Review Manager® 5.4 (RevMan 5.4, The Nordic Cochrane Centre, Copenhagen, Denmark). Heterogeneity was determined by inconsistency indexes (I^2), where a value

greater than 50% was considered an indicator of substantial heterogeneity between studies. A random-effect meta-analysis was used.

Confidence in cumulative evidence

The Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) instrument[21] was used to evaluate the certainty of evidence and the strength of recommendations. The assessment was based on study design, risk of bias, inconsistency, indirectness, imprecision, and other considerations such as publication bias, and effect magnitude. It was also considered the sample size and the absolute effect resulted from meta-analysis. The certainty of evidence was scored as high, moderate, low, or very low.

RESULTS

Studies Selection

One thousand ninety-one records were identified through research in databases and additional literature included 129 studies. After duplicated studies removal, 832 studies remained, and 112 articles were selected for full-text review. The gray literature and articles from the reference resulted in eight additional studies for full-text review. Ninety-seven studies from databases and five records identified via other methods were excluded (Supplementary Table S2). A total of 18 studies[11-17, 22-32] were selected for qualitative synthesis and 13 studies[11-17, 22, 23, 25, 26, 31, 32] for quantitative evaluation. Figure 1 presents the PRISMA flow chart of the identified, screened, and included articles.

Study Characteristics

Most studies were published between 2010 and 2019, totalizing thirteen articles[11-15, 23, 25-29, 32]. Two studies were published before 1990[24, 30], and four studies were published

between 1990 and 1996[16, 17, 22, 31]. Regarding the study design, only two where case-control [15, 23], while the remaining studies were cross-sectional studies.

In total, 995 individuals with OPMD (LKP, ETP, OSMF, OLP) were included. The control group (patients not exposed to carcinogens and patients exposed to carcinogens without the presence of OPMD) totalized 1028 subjects. Most participants were male in both groups, although some studies did not report gender. The age of patients with OPMD ranged from 12 to 84 years, and 15 to 84 years for controls. Only one study has not been carried out in the Indian population[27].

Tobacco use, used alone or in association, was reported in most cases, in addition to the consumption of areca nut. Among tobacco-related substances, gutkha, pan masala, mava, betel nut, betel quid, kheni, khaini, slaked lime, and the betel leaf were listed. The MNC frequency was assessed in 10 investigations [16, 17, 22, 24, 26, 27, 29-32], another 5 studies assessed MNF[11, 12, 14, 25, 28], and 3 others researches investigated both situations[13, 15, 23].

The use of DNA-specific stains (Feulgen, Acridine orange, fluorescent stain 4′,6′-diamidino-2-phenylindole - DAPI) for micronucleus evaluation was reported in most cases[12-14, 17, 22, 24, 27, 29-31]. However, an expressive number of researchers used non-specific dyes (Giemsa, May Grunwald Giemsa - MGG, Papanicolaou - PAP)[11, 15, 16, 23, 26, 28, 32]. Only two studies made comparisons between specific and non-specific dyes[11, 25].

Regarding the number of cells included in the micronucleus count, most studies used at least 1000 cells. We highlight the works that counted more than 1000 cells[13, 23, 26] and those that counted a lower number[24, 29, 30].

The main characteristics of the 18 included studies are described in Table I.

Risk of bias within studies

Most studies had a low risk of bias[12-14, 16, 17, 22-24, 27, 30-32] and six researches had moderate risk[11, 15, 25, 26, 28, 29]. Many studies did not report whether participants were properly sampled or whether the sample size was adequate. In some situations, the population was not described in detail, or it was not clear whether the exposure was properly measured (Supplementary Table S3).

Results of individual studies

OSMF was the most studied OPMD[11-17, 22, 25, 28, 31, 32]. The results showed an increase in micronuclei in patients with OSMF compared to individuals not exposed to carcinogenic agents: OSMF: 1.5±0.6, control: 1.1±0.2, $p<0.01$[11]; OSMF: 1.18±0.18, control: 0.35±0.03, $p<0.0001$[12]; MNF-OSMF/Guthka: 34.4±1.79, control: 4.36±0.27, $p<0.05$, MNC- OSMF/Guthka: 19.8± 0.69, control: 4.20± 0.27, $p<0.05$[13]; OSMF/mix-chewers: 6.24±2.68, control: 2.14±1.17, $p< 0.0001$[14]; MNF-OSMF/mix chewers: 1.9395 ± 1.4327, control: 0.4208 ± 0.2435, p=0.00, MNC-OSMF/mix chewers: 1.7160 ±1.4177, control: 0.3930 ± 0.2013, p=0.0002[15]; OSMF/mix chewers: 11.6 ± 0.03, control: 1.9 ± 0.03, $p<0.01$[16]; OSMF/mava: 7.05±0.75, OSMF/areca nut: 6.3±0.79, control: 1.90 ±0.19, $p<0.001$[17]; OSMF/areca nut x control: Pap stain p<001, Feulgen Stain p=0.001, MGG satin p=0.02[25]; OSMF/mix chewers: 5.3±1.79, Control: 1±1.09[28]; OSMF/mix chewers: 0.730±0.072, control: 0.190±0.19, $p<0.001$[31]; OSMF/Tobacco: 28.6±1.53, control: 11.4±1.04, $p<0.05$[32]. Furthermore, the MNC frequency was three times higher in the group of chewers of areca nut alone (0.730±0.078, $p<0.001$) or areca nut plus tobacco (0.753±0.097, $p<0.001$) than the healthy controls (0.193±0.022)[22]. On the other hand, it can present similar results to normal chewers: OSMF/mava: 7.05±0.75, OSMF areca nut: 6.3±0.79, normal-mava-chewers: 6.9±0.54, normal areca nut chewers: 7.30±0.85,[17]; OSMF/areca nut: 0.730±0.078, OSMF/mix chewers: 0.753±0.097, normal chewers:

0.730±0.085[22]; OSMF/tobacco + areca nut + lime: 0.730±0.072, normal-chewers: .690±0.054[31].

The second most reported OPMD was LKP[11, 14, 23-27, 29, 30, 32]. Studies showed similar MN count results between chewers with LKP and normal chewers: LKP/tobacco: 23.46±13.49, normal-chewers:14.84±9.04, p=0.1105[23]; LKP/mix chewers: 3.60±1.22, normal-chewers: 4.10±1.54[24]; LKP/mix chewers: 3.69± 1.22, normal-chewers: 4.10±1.54[30]; LKP/Tobacco: 18.1±0.71, normal-chewers: 18.2±1.26[32]. However, patients with this lesion had more MN than healthy control patients: LKP: 2.3 ±0.3, control:1.1±0.2, p<0.01[11]; LPK: 7.89±3.59, control: 2.14±1.17, p< 0.0001[14]; LKP/areca nut x control: Pap stain p<001, Feulgen Stain p=0.001, MGG satin p=0.02[25]; LKP: 5.70 ± 4.50, control: 2.99±1.74, p=0.02[26]; LKP = 5.1±1.619, control = 1±0.617, p<0.001[29].

The OLP was found in three publications[11, 14, 16]. Patients had higher MN counts than healthy patients: OLP: 1.7±0.5, control:1.1±0.2, p<0.01[11]; OLP: 5.0±2.76, control: 2.14±1.17, p< 0.0001[14]; OLP: 11.7 ± 0.14, control: 1.9 ± 0.03, p<0.01[16] and lower counts than patients with OSCC (p<0.0001)[14].

Synthesis of Results

The meta-analyses were performed based on the results of observational studies that evaluated MNF and MNC in patients with OPMDs. The quantitative synthesis was performed subdividing the studies according to the outcome (MNC or MNF), substance (tobacco or areca nut), OPMD (OSMF of LKP), control sample (patients exposed to carcinogens without OPMD and patients not exposed to carcinogens), and DNA dye (specific or non-specific). Thirteen studies reported comparable data and were included in the quantitative synthesis[11-17, 22, 23, 25, 26, 31, 32].

MNF assessed by specific DNA dye in tobacco users was assessed by 3 meta-

analyses. The meta-analysis of patients with LKP compared to non-chewers demonstrated a significant mean difference (MD) of 0.57 (95%CI: 0.48, 0.66; p<0.00001) with low heterogeneity (I^2= 0%), demonstrating greater MNF in LKP group (Figure 2A). Similarly, patients with OSMF also demonstrated larger mean MNF compared to non-chewers, with a significant mean difference of 0.91 (95%CI: 0.28, 1.55; p=0.005) (Figure 2B). Compared to chewers without lesions, OSMF patients also demonstrated higher MNF, with a significant mean difference of 0.75 (95%CI: 0.26, 1.24; p=0.003) (Figure 2C). Despite the significancy of the results, considerable inconsistency was observed in both meta-analysis, with 100% and 99%, respectively.

When using nonspecific dye, it was demonstrated that patients with LKP compared to chewers without lesions presented no significant mean difference on MNF, with an inconsistency of 69% (mean difference: 0.34, 95% CI: -0.43, 1.10; p=0.39) (Figure 3A). On the other hand, when compared to non-chewers, LKP patients presented a greater mean MNF with a significant mean difference of 0.98 (95% CI: 0.63, 1.33; p <0.00001; I^2= 80%) (Figure 3B). Similarly, patients with OSMF also presented greater mean MNF when compared to non-chewers, with a mean difference of 1.19 (95% CI: 0.21, 2.17; p=0.02) (Figure 3C).

The assessment of MNC using specific dyes resulted in 4 meta-analyses. Two of them compared users of Areca nut who had OSMF compared to chewers without lesions and non-chewers. The first comparison did not result in a significant mean difference (MD: -0.05, 95%CI: -0.15, 0.04; p =0.28; I^2= 80%) (Figure 4A) while the second analysis demonstrated a significant mean difference of 0.49 although high inconsistency was also found (95%CI: 0.39, 0.58; p>0.00001; I^2 = 87%) (Figure 4B), respectively. Regarding users of tobacco and tobacco-related substances, patients with OSMF compared to chewers without lesions did not present significant mean difference (MD: 0.11, 95%CI:

-0.08, 0.30; p=0.27; I^2= 99%) (Figure 5A), while compared to non-chewers, a significant mean difference of 0.35 was observed (95%CI: 0.03, 0.68; p=0.03; I^2= 100%) (Figure 5B).

When the dye used was nonspecific, tobacco users with LKP did not differ in the frequency of MNC compared to chewers without lesion (MD: 1.18, 95%CI: -0.72 4.08; p=0.42; I^2= 71%) and non-chewers (MD: 0.51, 95%CI: -0.22, 1.25; p=0.17) (Figures 6A and 6B, respectively). For tobacco users with OSMF, on the other hand, they had more MNC when compared to non-chewers (MD: 1.00, 95%CI: 0.80, 1.20; p<0.00001, ; I^2=18%) (Figure 6C).

Confidence in cumulative evidence

The certainty of evidence for outcomes assessed by the GRADE system was high only for the MNF assessed by specific DNA dye comparing LKP patients to non-chewers, meaning that further research is very unlikely to change the confidence in the estimate of effect. Conversely, very low certainty of evidence was demonstrated for MNC non-specific DNA dye (LKP vs Non-chewers and OSMF vs Chewers without lesion) and for MNC specific DNA dye (OSMF vs Tobacco chewers without lesion), which means that any estimate of effect is very uncertain. The remaining outcomes were graded as moderate or low certainty of evidence, demonstrating that further research is likely or very likely to have an important impact on the confidence in the estimate of effect and may change the estimate (Supplementary Tables S4 and S5).

DISCUSSION

This systematic review evaluated the frequency of micronuclei and micronucleated cells in patients with oral potentially malignant disorders exposed to

tobacco and tobacco-related substances. In 2015, a systematic review was carried out on the clinical application of the genotoxicity assay. The results highlighted that 53% of the literature analyzed applied the test to oral, head and neck cancer, and premalignant oral diseases[8]. Similarly, the literature was revised in 2019 considering the smokeless tobacco habit and DNA damage, however without identifying the OPMD[33]. As a differential, the present study used the meta-analysis approach considering patients exposed to carcinogens and who had some OPMD, in addition to dye specificity, number of cells included in the micronucleus count, and controls with and without exposure to carcinogens.

Tobacco and areca nut consumption is reported as an important etiological factor in the development of potentially malignant oral disorders[5]. Especially in India, a wide variety of products and mixtures containing tobacco as the main constituent and are used without combustion orally or nasally. In these formulations, except for those containing areca nut that is a knowledge carcinogen[22], the source of carcinogens is tobacco[34].

Predicting the malignant transformation potential of an OPMD is an important challenge, considering that although patients diagnosed with OPMD are more susceptible to cancer development, many of these lesions may not progress to carcinoma[3]. However, there is agreement that the presence of OPMD can serve as an alert[1].

Although many lesions are listed as OPMD, the studies included in this systematic review evaluated LKP, OSMF, and OLP in patients exposed to tobacco and tobacco-related substances, in addition to areca nut. LKP is the most studied OPMD worldwide[3] and it is described as a white plaque with an increased risk of cancer, whose diagnosis excludes other white lesions[1, 2]. OSMF is characterized by the initial loss of lamina propria fibroelasticity, evolving to submucosal fibrosis of the oral cavity, together with epithelial atrophy[3]. This chronic disease is common in the oral mucosa of patients

consuming tobacco, tobacco-related substances, and areca nut, especially in India[5]. OLP, on the other hand, is defined as a chronic inflammatory disorder of unknown etiology, characterized by bilaterally presenting white reticular lesions, accompanied or not by atrophic, erosive, ulcerative, and/or plaque areas[2, 3]. Its diagnosis is uncertain and malignant transformation rates are still underestimated[35].

OSMF was the most common OPMD among individuals exposed to tobacco and tobacco-related substances and areca nut[11-17, 22, 25, 28, 31, 32], followed by LPK[11, 14, 23-27, 29, 30, 32] and OLP[11, 14, 16]. However, the qualitative analysis showed heterogeneity in relation to dye specificity, the number of cells per individual included in the count, comparisons with healthy controls and those chewers without lesions, in addition to counting MNC and MNF in a specific number of cells. Due to the wide variety of methodologies, thirteen meta-analyses were performed.

The quantitative analysis showed that MNF was higher in patients with LKP[14, 25], and OSMF[12, 13] compared to patients exposed to carcinogens without OPMD and patients not exposed to carcinogens when the dye is specific for DNA. When the dye is nonspecific, the MN count was also higher in OPMDs compered to patients not exposed to carcinogens, including LKP[11, 25, 32], and OSMF[11, 15, 32]. MNC was most frequently in patients with OSMF using areca nut[17, 22] or tobacco mix[13, 17, 22, 31] compared to patients not exposed to carcinogens, regardless of the specificity of the dye[15, 16].

On the other hand, there is no evidence that MNC, evaluated by DNA-specific dyes, can be increased in areca nut users with OSMF and those users who do not have the lesion[17, 22]. The non-specific DNA dye also did not show higher MNC counts between patients with LKP and patients exposed to carcinogens without OPMD[23, 26] and patients not exposed to carcinogens,[16, 26]. Just as there was no evidence of MNF between LKP and patients exposed to carcinogens without OPMD [23, 25, 32].

It is noteworthy that the micronucleus assay in oral lesions as a biomarker of genomic damage is not a recent approach[22, 24, 30, 31]. Over the years, the use of the technique has varied widely among the studies included. One of the relevant points is the use of dyes. Since the technique began to be used in the oral cavity in the early1980s[36], many studies have been carried out with the Feulgen dye. DNA-specific dyes (Feulgen, Acridine orange, fluorescent stain 4′,6′-diamidino-2-phenylindole - DAPI) were the most used among the included studies[12-14, 17, 22, 24, 27, 29-31]. The use of a non-specific DNA dye may suggest a large number of micronuclei during counting[37, 38], as reported in one of the studies[25]. This happens since the use of non-specific dye, in addition to staining MN, can also mark other nuclear alterations, further to keratin granules and bacterial contamination, resulting in an overcount[38].

The number of cells evaluated is also a point of discussion. Although the HUman MicroNucleus (HUMN) collaborative program suggests unifying the MN counting to 2000 cells per volunteer [39], counting 1000 cells per individual is still quite common and was the most used among the analyzed studies[11, 12, 14, 16, 17, 22, 25, 27, 28, 31, 32].

The results showed a significant increase in micronucleus counts in the oral mucosa of patients with LKP and OSMF compared to patients not exposed to carcinogens, including users of tobacco-related substances and areca nut. However, the data must be analyzed with caution, considering that the MNF and MNC analyzes were performed on 1000 exfoliated cells of the oral mucosa. In addition, some of the included studies have a moderate risk of bias. Another point to be highlighted refers to the high variety of methodological approaches among the included studies, resulting in few studies included in each of the meta-analyses, which impacted the quality of evidence. Further observational research using DNA-specific stains and counting 2000 cells per volunteer is still needed.

CONCLUSION

Current evidence has shown that patients who consume tobacco-related substances and areca nut have a significant increase in micronucleus counts in OLK and OSMF when compared to non-chewers.

Considerando o consume de tabaco e substâncias relaciondas e areca nut, a fibrose submucosa oral foi a lesão mais recorrente e, consequentemente, mais estudada

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Supplementary Tables S1, S2, S3 S4 and S5; available at [URL/link*]

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FIGURES LEGENDS

Figure 1- Flow diagram of literature search and selection criteria adapted from PRISMA (Page et al., 2020)

Figure 2 - Forest plot of MNF with DNA-specific dyes: A. Mean difference between LKP (tobacco-related substances) and non-chewers; B. Mean difference between OSMF (tobacco-related substances) and non-chewers; C. Mean difference between OSMF (tobacco-related substances) and chewers without lesion.

Figure 3 - Forest plot of mean difference on MNF with non-specific dyes. A. Mean difference between LKP (tobacco-related substances) and chewers without lesion; B. Mean difference between LKP (tobacco-related substances) and non-chewers; C. Mean difference between OSMF (tobacco-related substances) and non-chewers.

Figure 4 - Forest plot of mean difference on MNC with DNA-specific dyes. A. Mean difference between OSMF (areca nut chewers) and chewers without lesion; B. Mean difference between OSMF (areca nut chewers) and non-chewers.

Figure 5 - Forest plot of mean difference on MNC with DNA-specific dyes. Mean difference between OSMF (tobacco-related substances) and chewers without lesion; B. Mean difference between OSMF (tobacco-related substances) and non-chewers.

Figure 6 - Forest plot of mean difference on MNC with non-specific dyes. A. Mean difference between LKP (tobacco-related substances) and chewers without lesion; B. Mean difference between LKP (tobacco-related substances) and non-chewers; C. Mean difference between OSMF (tobacco-related substances) and non-chewers.

Supplementary Table S1. Search strategies

Data base	Query (June 21, 2021)	Items found
PubMed http://www.ncbi.nlm.nih.gov/pubmed	"Leukoplakia"[All Fields] OR "Oral Leukoplakia"[All Fields] OR "Erythroplasia"[All Fields] OR "erythroleukoplakia"[All Fields] OR "erythroleukoplakias"[All Fields] OR "Cheilitis"[All Fields] OR "Actinic Cheilitis"[All Fields] OR "Oral Submucous Fibrosis"[All Fields] OR "lichen planus oral"[All Fields] OR "Oral Lichen Planus"[All Fields] OR "Lichen Planus"[All Fields] OR "Precancerous Conditions"[All Fields] OR "mouth disease"[All Fields] AND "micronuclei"[All Fields] OR "micronucleus"[All Fields]	95
LILACS http://lilacs.bvsalud.org/	("Leukoplakia" OR "Oral Leukoplakia" OR "Erythroplasia" OR erythroleukoplakia OR "Cheilitis" OR "Actinic Cheilitis" OR "Oral Submucous Fibrosis" OR "Lichen Planus, Oral" OR "Oral Lichen Planus" OR "Lichen Planus" OR "precancerous conditions" OR "mouth disease") AND (micronuclei OR micronucleus) AND (db☹"LILACS"))	47
Embase https://www.embase.com	('leukoplakia'/exp OR 'leukoplakia' OR 'oral leukoplakia'/exp OR 'oral leukoplakia' OR 'erythroplasia'/exp OR 'erythroplasia' OR 'erythroleukoplakia'/exp OR erythroleukoplakia OR 'cheilitis'/exp OR 'cheilitis' OR 'actinic cheilitis'/exp OR 'actinic cheilitis' OR 'oral submucous fibrosis'/exp OR 'oral submucous fibrosis' OR 'lichen planus, oral'/exp OR 'lichen planus, oral' OR 'oral lichen planus'/exp OR 'oral lichen planus' OR 'lichen planus'/exp OR 'lichen planus' OR 'precancer'/exp OR 'precancer' OR 'erythroplasia of queyrat'/exp OR 'erythroplasia of queyrat' OR 'mouth disease'/exp OR 'mouth disease') AND ('micronuclei'/exp OR micronuclei OR 'micronucleus'/exp OR micronucleus) AND [embase]/lim	241
Scopus http://www.scopus.com/	("Leukoplakia" OR "Oral Leukoplakia" OR "Erythroplasia" OR erythroleukoplakia OR "Cheilitis" OR "Actinic Cheilitis" OR "Oral Submucous Fibrosis" OR "Lichen Planus, Oral" OR "Oral Lichen Planus" OR "Lichen Planus" OR "Precancerous Conditions" OR "mouth disease") AND (micronuclei OR micronucleus) AND (LIMIT-TO (DOCTYPE , "ar"))	604

Web of Science **http://apps.webofknowledge.com/**	((((((((((("Leukoplakia" OR "Oral Leukoplakia") OR "Erythroplasia") OR erythroleukoplakia) OR "Cheilitis") OR "Actinic Cheilitis") OR "Oral Submucous Fibrosis") OR "Lichen Planus, Oral") OR "Oral Lichen Planus") OR "Lichen Planus") OR "Precancerous Conditions") OR "mouth disease") AND (micronuclei OR micronucleus))	104

Table 1 - Summary of descriptive characteristics of included studies (n= 18)

Author, year, country	Study design	Sample size (M/F)	Age (years)	Recruitment	Type of habit	Frequency of habit	Site of chewing	Number of cells	Stain	MN counting	Conclusion
Anila et al., 2011[15] India	Case-control	Total N = 40 Chewers with OSMF = 20 (18/2) (Guthka chewers =18 / Areca nut chewers = 2) Control = 20 (18/2)	26.7 in both groups	Patients of the Outpatient Department of Oral medicine and radiology, SDM Dental College and Hospital, Dharwad.	Guthka (areca nut, catechu, cardamom, lime, chewing tobacco and artificial flavors) Areca nut only	Average chews/day = 11-20 pouches (for 1 to 10 years)	Right side and left side of buccal mucosa.	1000	PAP stain and counterstai ned with Harris he- matoxylin.	MN frequency OSMF: = 1.9395 ± 1.4327 Control: 0.4208 ± 0.2435 P=0.00 MN Cell OSMF: 1.7160 ±1.4177 Control: 0.3930 ± 0.2013 p=0.0002	There is an increase in micronuclei in patients with OSMF compared to healthy individual. The micronucleus test can be used as an early indicator of genotoxicity in oral submucosal fibrosis.
Dave, 1990[22] India	Cross-sectional	Total N = 75 Normal-chewers (normal mucosa) = 35 (25/10) OSMF = 25 (21/4)	Control: 17-60 Areca nut: Normal chewers: 27-78 OSMF: 12-65	Not informed	Only areca nut or combination (areca nut, lime and tobacco) or pan masala	Pan masala: 6000 to 8000 mg/day.	The region where the chew was usually placed.	1000	Feulgen + fast green	MN cell Control:0.193±0. 022 Areca nut (p< 0.001)	The MN cells frequency in the group of individuals either chewing areca nut alone or areca nut plus tobacco, showed three

		Control (no habit) = 15 (8/7)	Combination: Normal chewers: 21-56 OSMF: 17-60 Pan masala normal chewres: 22-55							Normal chewers: 0.730±0.085 OSMF: 0.730±0.078 Combination (p< 0.001) Normal chewers: 0.740±0.064 OSMF: 0.753±0.097 Pan masala normal chewers (p< 0.001) 0.700±0.051	times higher values than the controls.
Desai et al., 1996[16] India	Cross-sectional	Total N =120 OSMF = 68 (55/13)	15-65 years	Patients attending at Nair Dental College, Bombay, India	OSMF: Mawa n=4 Pan-masala n=21 Betel Nut n=9 Betel Nut + Tobacco n=4 Betel Quid + Tobacco n=2 Mixed n=18 LKP	Not informed	Not informed	1000	Giemsa	MN cells OSMF = 11.6 ± 0.03 LKP: 10.8 ± 0.10 OLP: 11.7 ± 0.14 Control = 1.9 ± 0.03	The number of oral precancerous lesions such as OSMF, LKP and OLP are associated with increased numbers of MN in oral exfoliated cells.

		LKP= 18 (18/0)			Betel Nut + Tobacco n=3 Betel Quid + Tobacco n=5 Tobacco n=6 Mixed n=4					(p<.001)
		OLP = 14 (3/11)			OLP Betel Nut + Tobacco n=1 Betel Quid + Tobacco n=2 Tobacco n=2 Mixed n=1					
		Control (no habit) = 20			None n=8					
Dosi et al., 2016[23] India	Case-control	Total N = 20 LKP = 10 Control (consumption of tobacco without oral lesions) = 10	37.8± 12.8 35.3±10.58	Department of Oral Medicine and Radiology, Dr. D. Y. Patil Dental College and Hospital, Nerul, Navi Mumbai, Maharashtra.	Tobacco chewing + smoking n=3 Tobacco chewing + drinking n=7 Tobacco Smoking + drinking n=4 Tobacco chewing + smoking + drinking n=3 Tobacco chewing + smoking n=1 Tobacco chewing + drinking n=1	Not informed	Right side and left side of buccal mucosa.	3000	Giemsa	MN frequency LKP: 23.46 ± 13.49 Control = 14.84 ± 9.04 p=0.1105 MN cells LKP: 9.08±4.14 Control: 5.90±3.28 p=0.073	The number of micronucleated cells is high in patients with significant consumption of tobacco, in the presence or absence of leukoplakia.

					Tobacco Smoking + drinking n=2 Tobacco chewing + smoking + drinking n=0						
Gupta et al., 2019[11] India	Cross-sectional	Total N = 150 OSMF = 40 (40/0) OLP = 40 (16/24) LKP = 40 (37/3) Control (healthy patients without oral lesions) = 30(15/15)	28.7±11.2 39.4±14.3 45.4±13.3 34.7±10.3	Not informed	Smoking habits non specifiy	Not informed	Not informed	1000	Giemsa	MN frequency OSMF = 1.5±0.6 OLP: 1.7±0.5 LKP: 2.3 ±0.3 Control:1.1±0.2 P<0.01	The frequency of MN among the OPMDs, together or separately, was statistically higher than the controls.
Hornby, 1989[24] India	Cross-sectional *	Total N = 60 LKP: 30 Normal chewers: 30	Not informed	East Indians in Kerala, India	Tobacco/Betel Quid Chewers	9.1 dips per day at 20.3 min per dip and	Oral mucosa or gum	300	Feulgen and Fast green	MN cell LKP: 3.60±1.22 Normal mucosa: 4.10±1.54	The frequency of MNC was elevated in areas of oral LKP and in normal-appearing mucosa of betel quid chewers

Joshi et al., 2011[12] India	Cross-sectional	Total N = 262 Chewers = 81 Chewers with OSMF = 20 Non-chewers = 161	 36.5± 1.3 33.7±2.7 32.7± 0.9	Government Dental College and Hospital, Ahmedabad, India	areca nut, panmasala (plain and gutkha), mawa (a mixture of sun-cured unflavoured tobacco, dried pieces of areca nut, and lime) and tobacco with lime (kheni)	≤5 quids/day ≥10	Buccal mucosal cells	1000	Feulgen	MN frequency Chewers = 0.68± 0.09 P<0.0001 compared with non-chewers Chewers with OSMF = 1.18 ±0.18 P<0.0001 compare with non-chewers and P<0.05 compared with chewers Non-chewers = 0.35±0.03	Any mixture containing areca nut and tobacco have genotoxic and cytotoxic potential indicating higher oral diseases among chewers as indicated by all the OSMF subjects which were chewers.
Jyoti et al., 2013[13] India	Cross-sectional	Total N = 75 OSMF (chewing gutkha along with smoking) = 25 Guthka chewers = 25 Control (healthy patients) = 25	30.5±1.98 31.5± 2.60 34.0±2.56	Outpatient Department of the Ziauddin Ahmed Dental College and Hospital, Aligarh, India.	Gutkha	Not informed	Mucosa of the cheeks	2000	Acridine orange	MN frequency OSMF: 34.4±1.79 Guthka chewers: 14.4±0.73 Control: 4.36±0.27 P<0.05 MN cells OSMF: 19.8± 0.69 Gutcha chewers: 12.6± 0.51 Control: 4.20± 0.27	Chewing gutkha along with smoking is more dangerous for human health as it hastens the incidence of OSMF.
Katarkar et al., 2014[14] India	Cross-sectional	Total N = 260		Outpatient department of Dr R. Ahmed Dental College and	areca, khaini, cigarettes or multiple habit	Pack years: Areca (OLP:171.0; LKP: 16.1; OSMF:	one or both cheeks in the control group, and	1000	DAPI (Fluorescent stain 4′,6′-diamidino-2phenylind	MN frequency Control:	A progressive increase in MN formation was noted in precancerous

		OLP = 52 (22/30) LKP = 51 (38/13) OSMF = 51 (31/20) OSCC = 54 (33/21) Control (healthy patients) = 52 (19/33)	37.65±11.40 45.5±15.36 31.37±10.16 49.18±10.54 29.43±6.74	Hospital in Kolkata, India.		245.82; OSCC: 170.44); Cigarette (OLP: 232.85; LKP: 332.3; OSMF: 240.0; OSCC: 390.88); Khaini (OLP: 0; LKP:124.09; OSFM: 96.63; OSCC: 101.45)	in the area with lesion in the cases groups.		ole)	2.14±1.17 With habit OLP: 5.0±2.76 LPK: 7.89±3.59 OSMF: 6.24±2.68 OSCC: 18.08±3.52 p = 0.0009 (LPK compared with the other two conditions)	conditions while OSCC presented the highest MN frequency compared with controls.
Kayal et al., 1993[17] India	Cross-sectional	Total N = 76 OSMF mava group = 21 (20/1) OSMF arena nut group = 10 (7/3)	25-65 32-65	Subjects from the two North-eastern states of India and from the two Western states of India were included.	Área nut and mava chewing	2-15 per day 0.5-5 per day 2-11 per day	Not informed	1000	Feulgen and fast green	MN cells OSMF mava: 7.05±0.75 Control mava: 6.9±0.54 OSMF areca nut: 6.3±0.79 Control:7.30±0.85	The incidence of MNCs in OSMF patients is significantly higher than that observed in the corresponding control groups; however, it did not differ from that observed in 'normal' chewers with Areca nut or mava chewing habits.

		Control (chewers without oral lesions) Mava =20 (19/1) Areca nut =10 (5/5) Control (no habit) = 15 (8/7)	21-56 27-28 20-25			1-5 per day				No habit: 1.90 ±0.19	
Kohli et al., 2017[25] India	Cross-sectional	Total N = 200 G1: healthy patients with normal oral mucosa) = 50 G2: tobacco chewers without LKP = 50 G3: Tobacco chewers with LKP = 50 G4 areca nut chewers with OSMF = 50	30-60	I.T.S Dental College, Hospital and Research Centre, Centre, Greater Noida, India,	Tobacco chewers and areca nut chewers	Not informed	Not informed	1000	PAP, MGG (May-Grünwald-Giemsa), Feulgen	MN frequency Pap stain G1: 1.42±1.10 G2: 1.78±0.737 G3: 2.42±0.992 G4: 2.88±1.06 Feulgen Stain G1: 0.24±0.431 G2: 0.48±0.505 G3: 0.78±0.465 G4: 0.78±0.465 MGG satin G1: 1.00±0.926 G2: 1.38±0.725 G3: 1.92±0.944 G4: 1.90±0.839	A significant increase in the count of MN was observed from normal to OPMDs.
Mahimkar et al., 2010[26] India	Cross-sectional	Total N = 167 LKP = 66 (60/6) Control habit-control = 62 (60/2) Control: habit-free = 39 (39/0)	39 ± 13	Not informed	Exclusive chewers, exclusive smokers and mixed habits	Not informed	Both cheeks	2000	Giemsa	MN cell: LKP: 5.70 ± 4.50 Habit-control: 4.00 ± 3.38	A stepwise increase in the percentage of micronucleated cells was observed from habit-free individuals to

			39 ± 13 35 ± 10							Habit-free: 2.99 ± 1.74 P=0.002	habit-control group and from habit-control to LKP group.
Pellicioli et al., 2011[27] Brazil	Cross-sectional	Total N = 40 Alcohol/ tobacco group = 10 10/0 LKP = 12 12/0 OSCC = 8 8/0 Control (non smokers /stopped smoking > 10 years, who consumed, on average, less than one alcoholic drink per day) = 10 (10/0)	Median: 50 62 56.5 50	School of Dentistry of Universidade Federal do Rio Grande do Sul; Department of Oral Medicine of Hospital de Clínicas de Porto Alegre; Porto Alegre Water and Sewerage Department, in the city of Porto Alegre, southern Brazil.	Filtered cigarettes, alcoholic drink	Alcohol/tobacco group: at least 20 filtered cigarettes / day, for at least 1 year, or > 10 filtered cigarettes for more than 10 years. Alcohol consumption: one alcoholic drink per day for at least 1 year.	For control and alcohol/tobacco groups: lower lip, tongue border, and floor of the mouth. For LKP and OSCC groups: mucosa contralateral and adjacent to the lesions.	1000	Feulgen	MN cells Median and Interquartile Range Values: Control: 0 (0–1) Alcohol/tobacco: 0 (0–1) LKP: 1 (0–2) P= 0.0016 (control x LKP) p=0.0048 (Alcohol/tobacco x LKP) p= 0.0462 (control x OSCC) p= 0.0879 (Alcohol/tobacco x OSCC)	A progressive increase in MN frequency was observed in patients with OPMDs and OSCC.
Shah et al., 2015[28]	Cross-sectional	Total N = 90 OSMF = 30 (26/4)	OSMF: 38.97 (20>60)	Not informed	Tobacco or tobacco related produtcs	Not informed	Not informed	1000	Pap	MN frequency	MN assay can be used as an easy and consistent marker for

India		OSCC = 30 (24/6) Control (non-smokers) = 30 (15/15)	OSCC: 45.47 (20>60) Control: 42.53 (20> 60)							Mean ± SD OSMF: 5.3±1.79 OSCC: 10.57±2.10 Control: 1±1.09	genotoxic evaluation in higher risk groups and can be used for better treatment evaluation and prognosis in cases of OSMF and OSCC.
Singam et al., 2019[29] India	Cross-sectional	Total N = 60 LKP = 20 OSCC = 20 Control (healthy patients) = 20	Not informed	GITAM Dental College and Hospital, Rushikonda, Visakhapatnam and Peripheral Cancer Hospitals in and around Visakhapatnam	Tobacco and tobacco-related substances	Not informed	Buccal mucosa	100	Fluorescent stain DAPI (4′, 6′-diamidino-2 phenylindole)	MN cells LKP = 5.1±1.619 OSCC= 10.1±2.315 Control = 1±0.617 P <0.001	MN cells were seen twice as often in OSCC when compared to patients with OPMD.
Stich et al., 1988[30] India	Cross-sectional) *	Total N = 30 LKP = 30 Normal-chewers = 30 (same patients, adjacent areas to LKP)	48.8±12.9	Fishermen living along a coastal strip near Trivandrum, Kerala (India)	Simple betel quid consisting of areca nut (Areca catechu L.), tobacco (mainly stems) (Nicotiana tabacum L.), slaked lime (calcium hydroxide) from marine shells, and betel leaf (Piper betle L.).	17.2±9.6 quids per day for 24.1 ± 14.3 years	In the lesion and adjacent to it	500	Feulgen and fast green	MN cells LKP: 3.69± 1.22 control = 4.10 ±1.54	Micronucleated cells in areas of leukoplakia and areas of normal mucosa were similar.

Trivedi, 1991[31] India	Cross-sectional	Total N = 136 Normal Tobacco uses (without oral lesions) = 48: snuff (2/11); tobacco + lime (14/1); tobacco + areca nut + lime (19/1) OSMF = 20 (19/1) tobacco + areca nut + lime) OSCC = 48: snuff (0/20); tobacco + lime (6/4); tobacco + areca nut + lime (13/5) Control (healthy patients) = 20 (10/10)	Normal users: 21-80 OSMF: 17-60 OSCC: 22-80 Control: 17-60	Not informed	Snuff, tobacco + lime and tobacco + areca nut + lime	Gram tobacco/day Snuff Normal users: 4.04±0.804 OSCC: 2.65±0.187 Tobacco + lime: Normal users: 2.25±0.375 OSCC: 1.43±0.169 Tobacco + areca nut + lime: Normal users: 3.29±0.415 OSMF: 2.69±0.344 OSCC: 3.03± 0.677	Region where the tobacco was usally placed in the mouth.	1000	Feulgen and Fast green	MN cells Control= 0.190±0.19 Snuff Normal users: 0.56±0.037 OSCC: 0.136±0.006 Tobacco + lime Normal users: 0.59±0.046 OSCC: 0.140±0.05 Tobacco + areca nut + lime Normal users 0.690±0.054 OSMF: 0.730±0.072 OSCC: 0.153± 0.009 p<0.001	The frequency of MNC in exfoliated buccal mucosa of normal tobacco consumers (snuff or tobacco with lime or tobacco with areca nut and lime) were significantly higher compared to those of control individuals.

Wagh et al., 2019[32] India	Cross-sectional	Total N = 420 (280/140) G1 - Control: no tobacco habit with no obvious oral lesion = 60 G2 - Tobacco habit with no obvious oral lesion = 60 G3: OSMF = 60 G4: LKP = 60 G5: melanoplakia = 60 G6: ETP = 60 G7: OSCC = 60	Mean: 44 (17-84)	Department of Otorhinolaryngology and Head Neck surgery at Medical College and Sir Sayajirao General Hospital, Baroda, Gujarat	Tobacco	Not informed	Both sides of cheek	1000	PAP	MN frequency G1: 11.4±1.04 G2: 18.2±1.26 G3: 28.6±1.53 G4: 18.1±0.71 G5: 17.7±0.69 G6: 32.3±2.39 G7: 48.8±2.75 $p<0.05$	The mean micronuclei index was significantly higher in those using tobacco, for longer duration and with frequent tobacco use. The mean micronuclei index can be used as a potential screening tool of genotoxic damage and biomarker for epithelial carcinogenesis.

ETP: Erythroplakia; LKP: Leukoplakia; MN: Micronucleus; OLP: Oral lichen planus; OPMDs: oral potentially malignant disorders; OSCC: oral squamous cell carcinoma; OSMF: oral submucous fibrosis; PAP: Papanicolau

* baseline placebo data from an RCT (Randomized Clinical Trial)

Supplementary Table S2. Excluded studies and reasons for exclusion (databases n=97, Records identified via other methods = 5)

Author, year	Reasons for exclusion
Abbas; Ahmed, 2013	2
Adhikari; De, 2013	1
Adhvaryu et al., 1991	7
Ahad et al., 2020	2
Bakshi, 1998	2
Balachandar et al, 2008	2
Balraj et al, 2020	2
Benner et al, 1994	4
Benner et al, 1994	4
Bhavasar et al, 2011	2
Bloching, 2003	8
Bonassi; Fenech, 2019	1
Buajeeb et al., 2008	4
Buajeeb et al., 2007	4
Burzlaff et al., 2007	3
Cao et al., 2011	8
Carvalho et al., 2002	2
Casartelli et al., 2000	5
Chadha et al, 2011	2
Chandirasekar et al, 2014	2
Chandirasekar et al, 2019	2
Chandirasekar et al, 2013	2
Chatterjee et al, 2009	2
Christobher et al, 2016	2
Das Graças Alnonso de Oliveira et al., 2014	2
Dash et al, 2018	2
Dave et al, 1991	2
Dave et al, 1992	6
Delfino et al, 2002	4
Devi 2011	4
Dindgire 2012	5
El-Setouhy et al, 2008	2
Ergun et al, 2010	1
Ergun et al, 2009	2
Fareed et al, 2011	2
Feliciano et al, 2011	1
Francielli De Oliveira et al., 2011	2
Gabriel et al, 2006	2
Giri et al., 2021	2
Grover et al, 2012	2
Gupta et al, 2019	4
Gupta et al, 2014	1
Halder, 2004	2
Jaiswal et al, 2018	1
Jaitley et al, 2015	3
Kamath et al, 2014	2
Kamboj; Mahajan, 2006	4
Kaveri; Anila, 2011	1
Kiran et al, 2018	4
Lee et al, 2000	7
Li et al., 1999	4
Li et al, 1998	8
Liede et al, 1998	3
Liu et al, 2015	3
Liu et al, 2017	3
Mainali et al, 2015	2

Mohanta et al, 2015	2
Motgi et al, 2014	2
Mukherjee et al, 2011	4
Nadaf et al, 2014	4
Nersesyan et al, 2006	2
Ogenyi et al, 2019	2
Ozkul et al, 1997	2
Palaskar et al, 2010	2
Parmar, 2004	2
Piyathilake et al, 1995	2
Prasad et al, 1995	7
Pratheepa et al, 2012	2
Pratheepa et al, 2008	7
Rajabi-Moghaddam et al, 2020	2
Ramirez; Saldanha, 2002	2
Rana et al, 2017	2
Ranjbar et al, 2018	4
Reis et al, 2006	2
Ribeiro, 2008	1
Roberts, 1997	2
Sánchez-Siles et al, 2014	4
Sanchez-Siles et al, 2011	4
Sangle et al, 2016	2
Saran et al, 2008	2
Saruhanoğlu et al, 2014	4
Sivasankari et al, 2016	2
Stich et al, 1989	1
Stich et al, 1988	3
Stich et al, 1991	7
Stich et al, 1992	2
Stich; Rosin, 1983	2
Stich et al, 1986	2
Stich et al, 1984	2
Stich et al, 1982	2
Stich et al, 1984	2
Suarez-Alpire; Ribeiro, 2019	1
Suhas et al, 2004	2
Sun et al, 2000	8
Teja et al, 2014	3
Tolbert et al, 1991	2
Trivedi et al, 1993	5
Vidyalakshmi et al, 2016	4
Weber et al, 2010	1
Wu et al, 2004	2
Zaridze et al, 1985	2
Zoller et al, 1996	8

Reasons for exclusion:

(1) Reviews, letters to the editor, conference abstracts, opinions

personal, book chapters (n = 11);

(2) Studies that did not assess PMD or with non-individualized data for PMD (n = 50);

(3) Cytology did not investigate MN or studies that assess genotoxicity by a technique other than MN (n =8);

(4) Studies that do not report exposure to carcinogens (n = 15);

(5) Full text copy not available (n = 2);

(6) Duplicate samples (n=2);

(7) Study design; Descriptive studies (case reports and case series) (n = 3);

(8) Language restriction (n = 5).

Supplementary Table S3 – Risk of Bias assessed by the Joanna Briggs Institute Critical Appraisal checklist for prevalence studies for use in JBI Systematic Reviews. Risk of bias was categorized as High when the study reaches up to 49% score "yes", Moderate when the study reached 50% to 69% score "yes", and Low when the study reached more than 70% score "yes".

Authors, year	Q.1	Q.2	Q.3	Q.4	Q.5	Q.6	Q.7	Q.8	Q.9	% Yes	risk
Anila et al., 2011[15]	Y	N	N	Y	Y	U	Y	Y	Y	66,67%	M
Dave, 1990[22]	Y	N	U	Y	Y	Y	Y	Y	Y	77,77%	L
Desai et al., 1996[16]	Y	N	U	Y	Y	Y	Y	Y	Y	77,77%	L
Dosi et al., 2016[23]	Y	N	N	Y	Y	Y	Y	Y	Y	77,77%	L
Gupta et al., 2019[11]	Y	N	N	N	Y	Y	N	Y	Y	55,55%	M
Hornby, 1989[24]	Y	Y	U	Y	Y	Y	N	Y	Y	77,77%	L
Joshi et al., 2011[12]	Y	Y	N	Y	Y	N	Y	Y	Y	77,77%	L
Jyoti et al., 2013[13]	Y	Y	N	Y	Y	N	Y	Y	Y	77,77%	L
Kartakar et al., 2014[14]	Y	Y	U	Y	Y	Y	Y	Y	Y	88,89%	L
Kayal et al., 1993[17]	Y	Y	U	Y	Y	Y	Y	Y	Y	88,89%	L
Kohli et al., 2017[25]	Y	Y	U	N	Y	U	Y	Y	Y	66,67%	M
Mahimkar et al., 2010[26]	Y	Y	U	N	Y	U	Y	Y	Y	66,67%	M
Pellicioli et al., 2011[27]	Y	N	N	Y	Y	Y	Y	Y	Y	77,77%	L
Shah et al., 2015[28]	U	N	U	N	Y	Y	Y	Y	Y	55,55%	M
Singam et al., 2019[29]	Y	N	U	N	Y	Y	N	Y	Y	55,55%	M
Stich et al., 1988[30]	Y	Y	U	Y	Y	Y	N	Y	Y	77,77%	L
Trivedi, 1991[31]	Y	Y	U	Y	Y	Y	Y	Y	Y	88,89%	L

Wagh et al., 2019[32]	Y	Y	U	Y	Y	N	Y	Y	Y	77,77%	L

Q1. Was the sample frame appropriate to address the target population? Q2. Were study participants sampled in an appropriate way? Q3. Was the sample size adequate? Q4. Were the study subjects and the setting described in detail? Q5. Was the data analysis conducted with sufficient coverage of the identified sample? Q6 Were valid methods used for the identification of the condition? Q7. Was the condition measured in a standard, reliable way for all participants? Q8. Was there appropriate statistical analysis? Q9. Was the response rate adequate, and if not, was the low response rate managed appropriately?
Y - Yes; N- No; U – Unclear, NA – Not applicable; H – High, M – Moderate; L – Low.

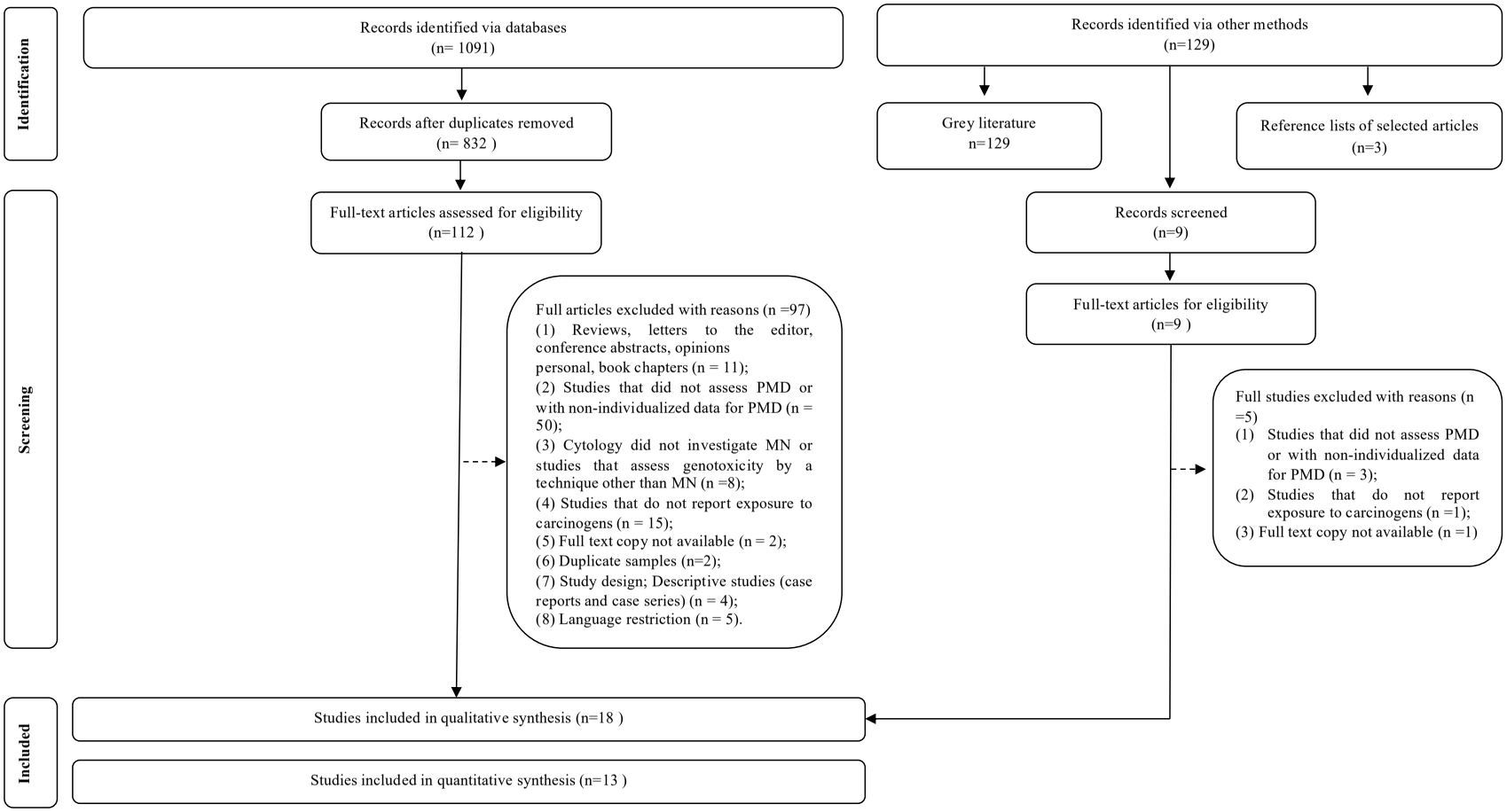

Figure 1- Flow diagram of literature search and selection criteria adapted from PRISMA (Page et al., 2020)

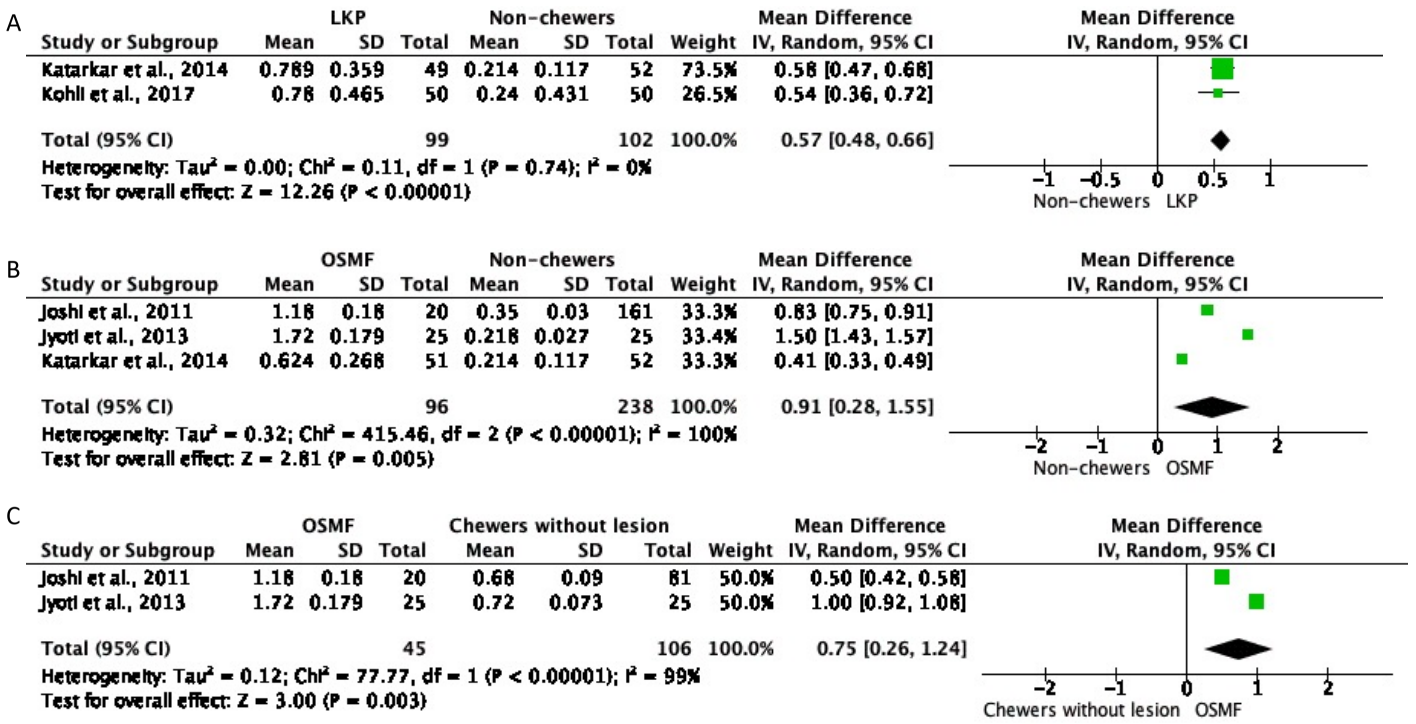

Figure 2 - Forest plot of MNF with DNA-specific dyes: A. Mean difference between LKP (tobacco-related substances) and non-chewers; B. Mean difference between OSMF (tobacco-related substances) and non-chewers; C. Mean difference between OSMF (tobacco-related substances) and chewers without lesion.

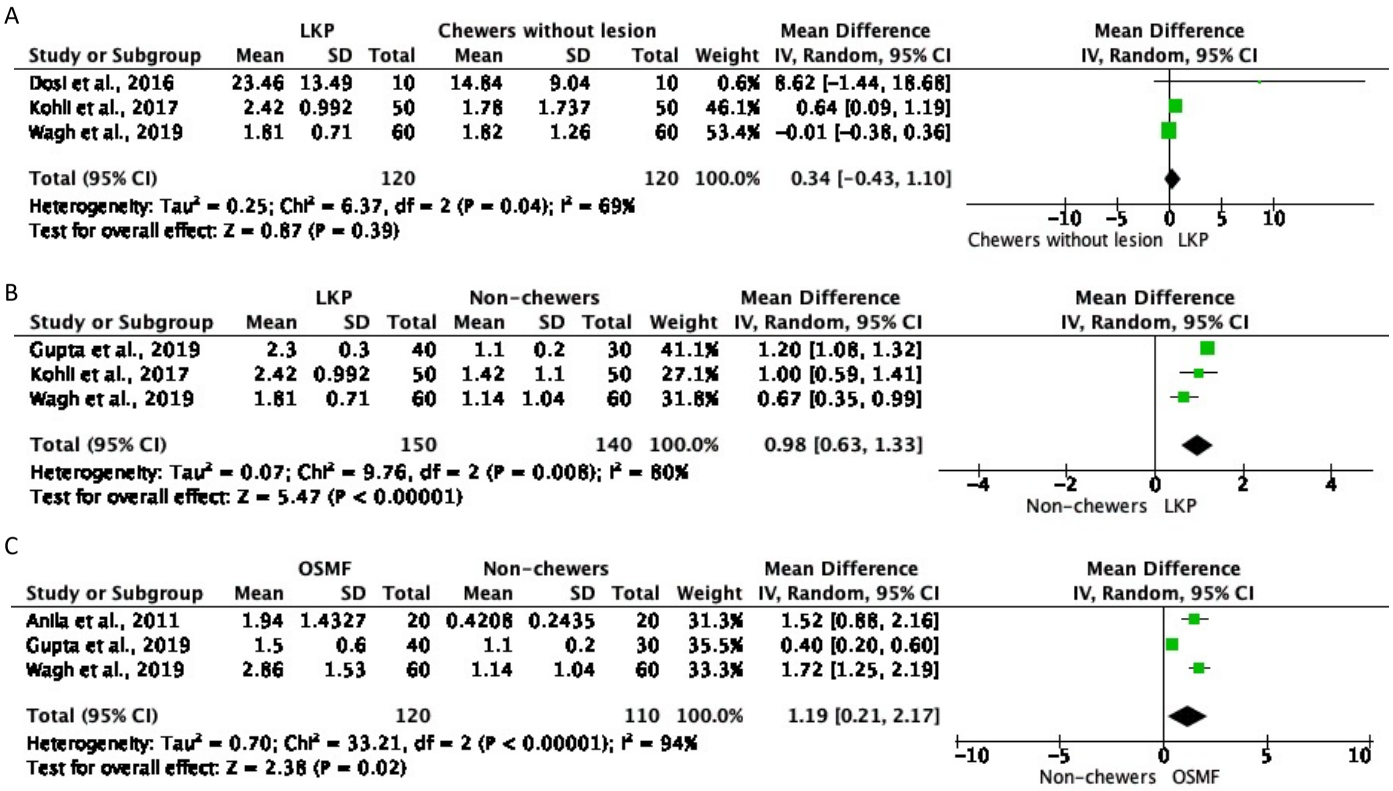

Figure 3 - Forest plot of mean difference on MNF with non-specific dyes. A. Mean difference between LKP (tobacco-related substances) and chewers without lesion; B. Mean difference between LKP (tobacco-related substances) and non-chewers; C. Mean difference between OSMF (tobacco-related substances) and non-chewers.

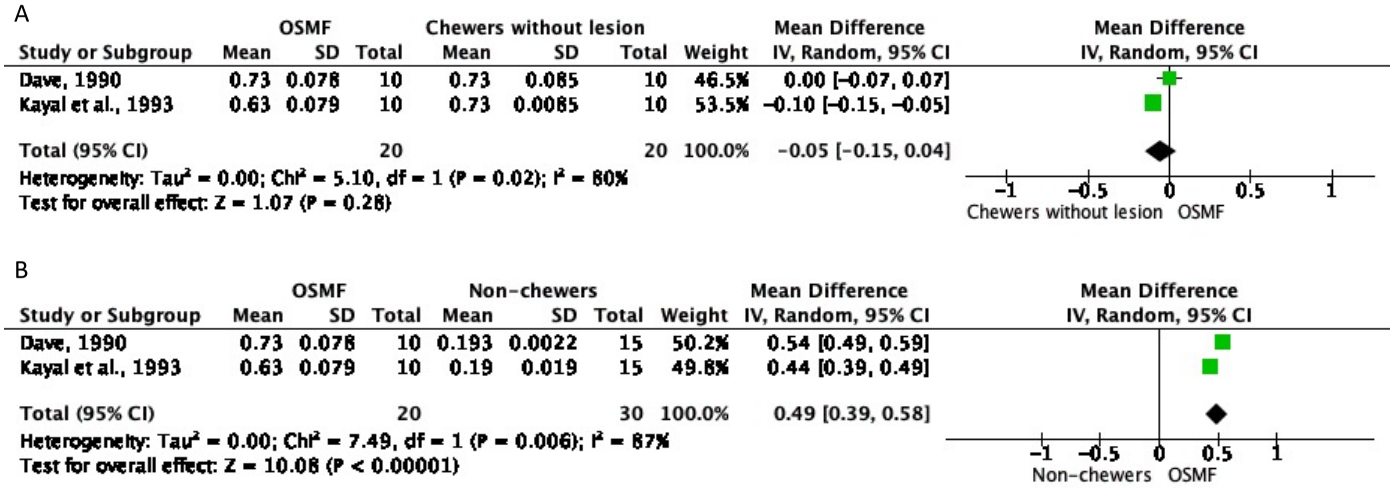

Figure 4 - Forest plot of mean difference on MNC with DNA-specific dyes. A. Mean difference between OSMF (areca nut chewers) and chewers without lesion; B. Mean difference between OSMF (areca nut chewers) and non-chewers.

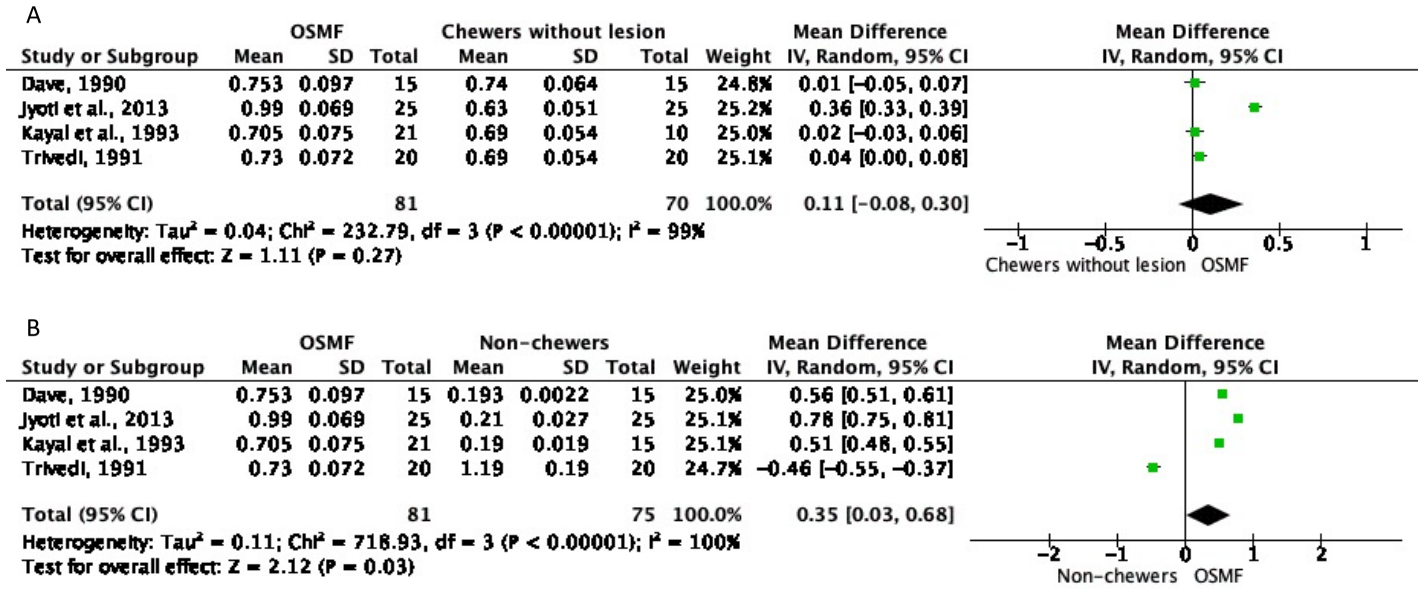

Figure 5 - Forest plot of mean difference on MNC with DNA-specific dyes. Mean difference between OSMF (tobacco-related substances) and chewers without lesion; B. Mean difference between OSMF (tobacco-related substances) and non-chewers.

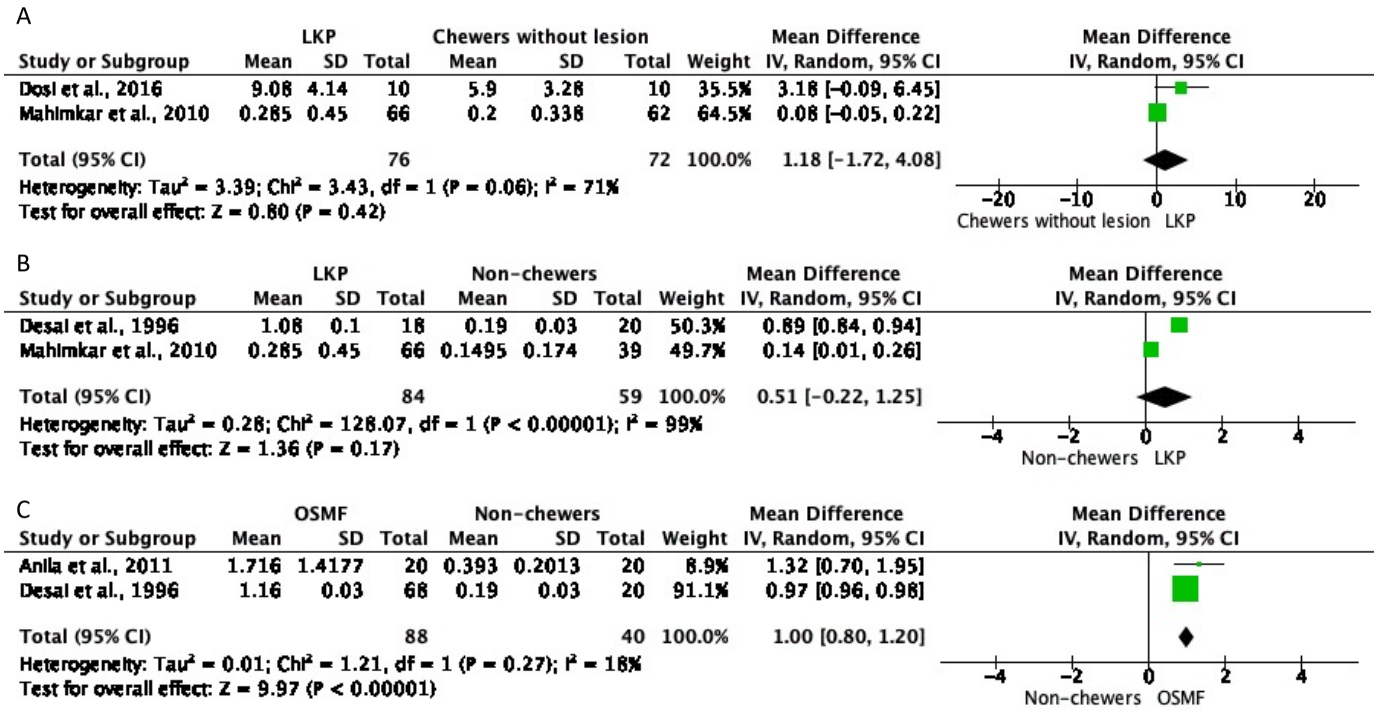

Figure 6 - Forest plot of mean difference on MNC with non-specific dyes. A. Mean difference between LKP (tobacco-related substances) and chewers without lesion; B. Mean difference between LKP (tobacco-related substances) and non-chewers; C. Mean difference between OSMF (tobacco-related substances) and non-chewers.

Supplementary Table S4 - Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) instrument.

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	MNC 1000 non-specific DNA dye	placebo	Relative (95% CI)	Absolute (95% CI)		
MNC non-specific DNA dye – OSMF vs Non-chewers												
2	observational studies	not serious	not serious	not serious	not serious	strong association	88	40	-	MD **1 higher** (0.8 higher to 1.2 higher)	⨁⨁⨁◯ MODERATE	CRITICAL
MNC non-specific DNA dye – LKP vs Non-chewers												
2	observational studies	not serious	serious [a]	not serious	serious [b]	strong association	84	59	-	MD **0.51 higher** (0.22 lower to 1.25 higher)	⨁◯◯◯ VERY LOW	IMPORTANT
MNC non-specific DNA dye – OSMF vs Chewers without lesion												
2	observational studies	not serious	serious [c]	not serious	serious [b]	strong association	76	72	-	MD **1.18 higher** (1.72 lower to 4.08 higher)	⨁◯◯◯ VERY LOW	IMPORTANT
MNC specific DNA dye – OSMF vs Non-chewers (tobacco)												
4	observational studies	not serious	serious [a]	not serious	not serious	very strong association	81	75	-	MD **0.35 higher** (0.03 higher to 0.68 higher)	⨁⨁⨁◯ MODERATE	CRITICAL
MNC specific DNA dye – OSMF vs Chewers without lesion (tobacco)												
4	observational studies	not serious	serious [a]	not serious	serious [b]	strong association	81	70	-	MD **0.11 higher** (0.08 lower to 0.3 higher)	⨁◯◯◯ VERY LOW	IMPORTANT
MNC specific DNA dye – OSMF vs Non-chewers (areca nut)												
2	observational studies	not serious	serious [a]	not serious	not serious	very strong association	20	30	-	MD **0.49 higher** (0.39 higher to 0.58 higher)	⨁⨁⨁◯ MODERATE	CRITICAL
MNC specific DNA dye – OSMF vs Chewers without lesion (areca nut)												
2	observational studies	not serious	serious [a]	not serious	not serious	strong association	20	20	-	MD **0.05 lower** (0.15 lower to 0.04 higher)	⨁⨁◯◯ LOW	IMPORTANT

CI: Confidence interval; **MD:** Mean difference. Explanations: a. Considerable heterogeneity; b. Large CI; c. Substantial heterogeneity

Supplementary Table S5 - Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) instrument.

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	MNF 1000 non-specific DNA dye	placebo	Relative (95% CI)	Absolute (95% CI)		
MNF non-specific DNA dye – OSMF vs Non-chewers												
3	observational studies	not serious	serious [a]	not serious	not serious	very strong association	120	110	-	MD **1.19 higher** (0.21 higher to 2.17 higher)	⊕⊕⊕◯ MODERATE	CRITICAL
MNF non-specific DNA dye – LKP vs Chewers without lesion												
3	observational studies	not serious	serious [b]	not serious	not serious	strong association	120	120	-	MD **0.34 higher** (0.43 lower to 1.1 higher)	⊕⊕◯◯ LOW	IMPORTANT
MNF non-specific DNA dye – LKP vs Non-chewers												
3	observational studies	not serious	serious [a]	not serious	not serious	very strong association	150	140	-	MD **0.98 higher** (0.63 higher to 1.33 higher)	⊕⊕⊕◯ MODERATE	CRITICAL
MNF specific DNA dye – OSMF vs Non-chewers												
3	observational studies	not serious	serious [a]	not serious	serious [b]	very strong association	96	238	-	MD **0.91 higher** (0.28 higher to 1.55 higher)	⊕⊕◯◯ LOW	CRITICAL
MNF specific DNA dye – OSMF vs chewers without lesion												
2	observational studies	not serious	serious [a]	not serious	serious [b]	very strong association	45	106	-	MD **0.75 higher** (0.26 higher to 1.24 higher)	⊕⊕◯◯ LOW	CRITICAL
MNF specific DNA dye – LKP vs Non-chewers												
2	observational studies	not serious	not serious	not serious	not serious	very strong association	99	102	-	MD **0.57 higher** (0.48 higher to 0.66 higher)	⊕⊕⊕⊕ HIGH	CRITICAL

CI: Confidence interval; **MD:** Mean difference. Explanations: a. Considerable heterogeneity; b. Substantial heterogeneity

6 CONCLUSÕES

- Os resultados dessa revisão sistemática e mostraram um aumento significativo na contagem de micronúcleos na mucosa oral de pacientes com leucoplasia e fibrose submucosa oral em usuários de substâncias relacionadas ao tabaco e noz de areca em comparação com pacientes não usuários.
- A certeza da evidência para os resultados avaliados pelo sistema GRADE foi alta apenas para o MNF avaliado por um corante de DNA específico comparando pacientes com leucoplasia a não mastigadores.
- Uma certeza de evidência muito baixa foi demonstrada para corante de DNA não específico de MNC, considerando pacientes com leucoplasia comparados a controle não expostos à carcinógenos e pacientes com fibrose submucosa oral comparados a controles expostos, porém sem lesão; e para corante de DNA específico de MNC (fibrose submucosa oral comparados a pacientes expostos aos carcinógenos sem lesão).

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