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Relación entre los comportamientos de riesgo para la salud, los desórdenes orales potencialmente malignos y el estado de salud oral

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TESIS DOCTORAL

RELACIÓN ENTRE LOS COMPORTAMIENTOS DE RIESGO PARA LA SALUD, LOS DESÓRDENES ORALES POTENCIALMENTE MALIGNOS Y EL ESTADO DE SALUD ORAL

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LISTA DE ABREVIATURAS

AUC:	Área bajo la curva
AUD:	Trastorno por consumo de alcohol
CAGE:	C: Cut down; A: Annoyed; G: Guilty; E: Eye opener
CAST:	Cannabis Abuse Screening Test
CC:	Coeficiente de correlación
CCC:	Cáncer de cabeza y cuello
CHC:	Candidiasis hiperplásica crónica
CO:	Candidosis oral
COCE:	Carcinoma oral de células escamosas
CpG:	Citosina fosfo Guanina
DAST:	Drug Abuse Screening Test
DE:	Displasia epitelial
DS:	Desviación estándar
EE:	Error estándar
EO:	Eritroplasia oral
HR:	Hazard Ratio
IARC:	Agencia Internacional para la Investigación del Cáncer
IC:	Intervalo de confianza
ICA:	Índice cintura altura
IHQ:	Inmunohistoquímica

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IMC:	Índice de masa corporal
LO:	Leucoplasia oral
LPO:	Liquen plano oral
LVP:	Leucoplasia verrugosa proliferativa
MESH:	Medical Subject Headings
MGMT:	Metil guanina metil transferasa
MLH1:	MutL homólogo 1
MMR:	Mismatch repair
ODS:	Objetivos de Desarrollo Sostenible
OMS:	Organización Mundial de la Salud
OPMD:	Desorden oral potencialmente maligno
OR:	Odds Ratio
OSMF:	Fibrosis submucosa oral
PAS:	Periodic Acid–Schiff
TM:	Transformación Maligna
TCGA:	The Cancer Genome Atlas
RoB:	Risk of Bias
ROC:	Receiver Operating Characteristic
RR:	Risks Ratio
VPH:	Virus Papiloma Humano
QUIPS:	QUality In Prognosis Studies
XI:	Xerostomia Inventory

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RESUMEN

Introducción: Esta tesis doctoral aborda diversos aspectos relacionados con la salud oral, como el consumo de alcohol y otras drogas entre estudiantes de odontología y medicina, la evaluación de la xerostomía y su relación con la calidad de vida en los pacientes adultos mayores y el estudio de diferentes desórdenes orales potencialmente malignos (OPMDs) y su potencial de transformación maligna.

Objetivo: Proporcionar una comprensión integral del riesgo para la salud de factores como el consumo de alcohol y otras sustancias, el papel de la xerostomía en la salud general y la calidad de vida, la transformación maligna de distintas lesiones orales potencialmente malignas y la expresión de biomarcadores en OPMDs y en el carcinoma oral de células escamosas (COCE).

Material y método: El diseño metodológico se caracterizó por la diversidad de enfoques, desde estudios transversales hasta revisiones sistemáticas con metaanálisis. Se emplearon diferentes métodos y herramientas, como cuestionarios validados, análisis de datos de diversas fuentes, pirosecuenciación y análisis estadísticos especializados para abordar las complejidades de cada aspecto investigado en esta tesis doctoral. El primer conjunto de estudios (artículos 1 y 2) fueron de diseño transversal y se examinó a través de cuestionarios validados el consumo de alcohol y sustancias psicoactivas en estudiantes de odontología y medicina, así como las percepciones del riesgo y los conocimientos y actitudes asociados. Un segundo conjunto de estudios (artículos 3 y 4) también de diseño transversal, analizaron la asociación entre la xerostomía y diversos factores de salud. Se utilizaron cuestionarios específicos y análisis estadísticos que incluyeron Kruskal-Wallis, ANOVA y regresión logística multinomial. El tercer grupo de investigaciones (artículos 5 y 6) fueron revisiones sistemáticas con metaanálisis que abordaron la transformación maligna de la eritroplasia oral y la candidiasis hiperplasia crónica. El cuarto conjunto de estudios estuvo compuesto por los artículos 7 y 8. El séptimo artículo evaluó los niveles de metilación de MLH1 y MGMT en pacientes con COCE y OPMDs, mediante pirosequenciación, dividiendo las muestras en subgrupos según su evolución clínica. Además, se integraron datos del proyecto The Cancer Genome Atlas (TCGA) para obtener una visión más amplia de la metilación en la carcinogénesis oral. El octavo estudio fue una revisión sistemática con metaanálisis sobre la expresión de $p16^{INK4a}$ en OPMDs.

Resultados: El primer grupo de investigaciones "Trastornos relacionados con sustancias y su impacto en estudiantes de medicina y odontología" subrayan la importancia de abordar el consumo de alcohol y drogas entre estos estudiantes, mejorando su

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conocimiento y actitudes, y promoviendo programas de formación específicos para abordar este problema en ambos grados. Los resultados revelaron una alarmante prevalencia de consumo de alcohol, y se encontró que las mujeres tenían un riesgo significativamente mayor de padecer trastorno por consumo de alcohol (AUD) (OR=2,63, IC 95% 1,55-4,45, p < 0,001). A pesar de la alta prevalencia de AUD, se detectó entre los estudiantes un conocimiento insuficiente sobre el alcohol y sus efectos, y se destacaron diferencias en el conocimiento entre los estudiantes de odontología y medicina. Aunque muchos estudiantes sentían que debían identificar casos de AUD en sus pacientes, creían que no tenían las competencias necesarias. El consumo de drogas aumentó con la edad y avanzó en el ciclo académico (p < 0,001), con el segundo ciclo presentando un mayor consumo que el primero en el modelo univariado (OR = 1,77, IC 95% 1,27-2,48, p = 0,001) y en el modelo ajustado (OR = 1,86, IC 95% 1,32 -2,62, p < 0,001). Los estudiantes tenían percepciones variables sobre el riesgo de las sustancias, con menos consideración del riesgo para el alcohol. Sin embargo, la mayoría de los estudiantes indicaron que estaban dispuestos a ayudar a sus pacientes a dejar de consumir estas sustancias. Estos hallazgos plantean la urgencia de implementar programas de capacitación que aborden estas cuestiones, diferenciados por género, y resaltan la responsabilidad de los futuros profesionales de la salud.

El segundo grupo de artículos “Xerostomía y su impacto en la salud oral y general” se adentran en el fenómeno de la xerostomía (evaluada mediante el Xerostomia Inventory) y su relación con enfermedades sistémicas, condiciones psicológicas y la obesidad. Se documenta una prevalencia del 30,7% de xerostomía en la población estudiada, y la regresión logística mostró que el mayor OR de xerostomía se asoció a pacientes con mala autopercepción de salud, (OR=6,31, IC 95% 2,89-13,80, p < 0,001). En el modelo ajustado por movilidad dental, enfermedades óseas o respiratorias y consumo de ansiolíticos y/o antidepresivos, el OR fue de 3,46 (IC 95% 1,47-8,18, p = 0,005). En el estudio realizado en 800 adultos mayores, se observó una fuerte relación con la mala salud general y oral, con un riesgo más alto para quienes necesitaban ayuda con las tareas domésticas y para las mujeres. La regresión logística mostró que el OR más alto para xerostomía se observó entre adultos con mala salud general (2,81; IC 95% 1,8–4,3; p <0,001) y para el modelo ajustado el OR seguía siendo significativo (2,18; IC 95% 1,4–3,4; p = 0,001). Aquellos que necesitaron ayuda con las tareas del hogar tuvieron un OR 2,16 mayor (IC 95% 1,4–3,4; p = 0,001) y 1,69 (IC 95% 1,1–2,7; p = 0,03) en el modelo ajustado. Estos resultados subrayan la importancia de una evaluación temprana y un seguimiento regular de la xerostomía, dado su impacto en la calidad de vida y su relación con el estado de salud general y oral.

El tercer grupo de estudios “Tasa de transformación maligna (TM) de OPMDs” se enfocan en el potencial de TM de la eritroplasia oral (EO) y la candidiasis hiperplasia crónica (CHC). La EO presentó una tasa de TM del 19,9% (IC 95% −1,6 a 41,4; I^2 = 91,74%, p = 0,00001), destacando la displasia epitelial (DE) como un factor pronóstico relevante, aunque no se identificaron características clínicas específicas asociadas al riesgo de malignidad. La CHC presentó una tasa global de transformación maligna del 12,1% (IC 95% 4,1-19,8 %, I^2

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= 78,27%, p = 0,002). En ambos casos, se enfatiza la necesidad de estudios prospectivos y criterios de diagnóstico más claros para comprender completamente el riesgo de malignidad de estas afecciones orales.

En el último grupo de investigaciones “Biomarcadores en OPMDs Y COCE” se investigaron diferentes aspectos moleculares del COCE y los OPMDs. En el artículo 7 los resultados no mostraron diferencias significativas en los niveles de metilación entre los grupos y sugirieron una ligera mayor supervivencia estimada para los casos con metilación de MGMT. Sin embargo, no se encontraron correlaciones sólidas entre la metilación génica y las características clínico-patológicas o la supervivencia en nuestra cohorte institucional. El octavo estudio, centrado en la expresión inmunohistoquímica de $p16^{INK4a}$ en OPMDs y su asociación con el riesgo de TM, reveló un riesgo de malignización duplicado en casos con sobreexpresión de $p16^{INK4a}$ (RR = 2,01, IC 95% 1,36-2,96, I^2 = 0%) lo que sugiere que la evaluación de $p16^{INK4a}$ podría complementar la graduación de displasia y mejorar el pronóstico de los OPMDs. Esta técnica inmunohistoquímica resulta prometedora como herramienta adicional en la evaluación pronóstica de rutina en los OPMDs.

Conclusiones: Estas investigaciones resaltan la necesidad de un enfoque multidisciplinario en la odontología y la medicina para abordar las cuestiones de salud oral. Además, enfatizan la importancia de la prevención, la detección temprana y el seguimiento, y señalan el potencial de marcadores biológicos. Estos resultados abren nuevas perspectivas para la prevención y el manejo de la patología oral, contribuyendo a una atención más integral y efectiva.

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RESUMO

Introdución: Esta tese de doutoramento aborda diversos aspectos relacionados coa saúde oral, como o consumo de alcol e outras drogas entre estudantes de odontoloxía e medicina, a avaliación da xerostomía e a súa relación coa calidade de vida nos pacientes adultos maiores e o estudo de diferentes desordes orais potencialmente malignos (OPMDs) e o seu potencial de transformación maligna.

Obxectivo: Proporcionar unha comprensión integral do risco para a saúde de factores como o consumo de alcol e outras substancias, o papel da xerostomía na saúde xeral e na calidade de vida, a transformación maligna de distintas lesións orais potencialmente malignas e a expresión de biomarcadores en OPMDs e no carcinoma oral de células escamosas (COCE).

Material e método: O deseño metodolóxico caracterizouse pola diversidade de enfoques, desde estudos transversais ata revisións sistemáticas con metaanálises. Empregáronse diferentes métodos e ferramentas, como cuestionarios validados, análise de datos de diversas fontes, pirosecuenciación e análises estatísticas especializadas para abordar as complexidades de cada aspecto investigado nesta tese de doutoramento. O primeiro conxunto de estudos (artigos 1 e 2) foron de deseño transversal e examinouse a través de cuestionarios validados o consumo de alcol e substancias psicoactivas en estudantes de odontoloxía e medicina, así como as percepcións do risco e coñecementos e actitudes asociadas. Un segundo conxunto de estudos (artigos 3 e 4) tamén de deseño transversal, analizaron a asociación entre a xerostomía e diversos factores de saúde. Empregáronse cuestionarios específicos e análises estatísticas que incluíron Kruskal-Wallis, ANOVA e regresión loxística multinomial. O terceiro grupo de investigacións (artigos 5 e 6) foron revisións sistemáticas con metaanálises que abordaron a transformación maligna da eritroplasia oral e a candidíase hiperplasia crónica. O cuarto conxunto de estudos estivo composto polos artigos 7 e 8. O sétimo artigo avaliou os niveis de metilación de MLH1 e MGMT en pacientes con COCE e OPMDs mediante pirosecuenciación, dividindo as mostras en subgrupos segundo a súa evolución clínica. Ademais, integráronse datos do proxecto The Cancer Genome Atlas (TCGA) para obter unha visión máis ampla da metilación na carcinoxénese oral. O oitavo estudo foi unha revisión sistemática con metaanálise sobre a expresión de $p16^{INK4a}$ en OPMDs.

Resultados: O primeiro grupo de investigacións "Trastornos relacionados con substancias e o seu impacto en estudantes de medicina e odontoloxía" subliña a importancia de abordar o consumo de alcol e drogas entre estes estudantes, mellorando o seu

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coñecemento e actitudes, e promovendo programas de formación específicos para abordar este problema en ambos os graos. Os resultados revelaron unha prevalencia alarmante de consumo de alcol, e atopouse que as mulleres tiñan un risco significativamente maior de padecer trastorno por consumo de alcol (AUD) (OR=2,63, IC 95% 1,55-4,45, p < 0,001). A pesar da alta prevalencia de AUD, detectouse entre os estudantes un coñecemento insuficiente sobre o alcol e os seus efectos, e destacáronse diferenzas no coñecemento entre os estudantes de odontoloxía e medicina. Aínda que moitos estudantes sentían que debían identificar casos de AUD nos seus pacientes, crían que non tiñan as competencias necesarias. O consumo de drogas aumentou coa idade e avanzou no ciclo académico (p < 0,001), co segundo ciclo presentando un maior consumo que o primeiro no modelo univariado (OR = 1,77, IC 95% 1,27-2,48, p = 0,001) e no modelo axustado (OR = 1,86, IC 95% 1,32 -2,62, p < 0,001). Os estudantes tiñan percepcións variables sobre o risco das substancias, con menos consideración do risco para o alcol. Sen embargo, a maioría dos estudantes indicaron que estaban dispostos a axudar aos seus pacientes a deixar de consumir estas substancias. Estes achados plantexan a urxencia de implementar programas de formación que aborden estas cuestións, diferenciados por xénero, e resaltan a responsabilidade dos futuros profesionais da saúde.

O segundo conxunto de artigos "Xerostomía e o seu impacto na saúde oral e xeral" afondan o fenómeno da xerostomía (avaliada mediante o Xerostomia Inventory) e a súa relación con enfermidades sistémicas, condicións psicolóxicas e a obesidade. Documentase unha prevalencia do 30,7% de xerostomía na poboación estudada, e a regresión loxística mostrou que o maior OR de xerostomía asociouse a pacientes con mala autopercepción de saúde,(OR= 6,31, IC 95% 2,89-13,80, p < 0,001). No modelo axustado por mobilidade dental, enfermidades óseas ou respiratorias e consumo de ansiolíticos e/ou antidepresivos, o OR foi de 3,46 (IC 95% 1,47-8,18, p = 0,005). No estudo realizado en 800 adultos maiores, observouse unha forte relación coa mala saúde xeral e oral, cun risco máis alto para aqueles que precisaban axuda coas tarefas domésticas e para as mulleres. A regresión loxística mostrou que o OR máis alto para xerostomía observouse entre adultos con mala saúde xeral (2,81; IC 95% 1,8–4,3; p <0,001) e para o modelo axustado o OR seguía sendo significativo (2,18; IC 95% 1,4–3,4; p = 0,001). Aqueles que precisaron axuda coas tarefas do fogar tiveron un OR 2,16 maior (IC 95% 1,4–3,4; p = 0,001) e 1,69 (IC 95% 1,1–2,7; p = 0,03) no modelo axustado. Estes resultados subliñan a importancia dunha avaliación temperá e un seguimento regular da xerostomía, dado o seu impacto na calidade de vida e a súa relación co estado de saúde xeral e oral.

O terceiro conxunto de estudos "Taxa de transformación maligna (TM) de OPMDs" enfócanse no potencial de TM da eritroplasia oral (EO) e a candidíase hiperplasia crónica (CHC). A EO presentou unha taxa de TM do 19,9% (IC 95% −1,6 a 41,4; I2 = 91,74%, p = 0,00001), destacando a displasia epitelial (DE) como un factor pronóstico relevante, aínda que non se identificaron características clínicas específicas asociadas ao risco de malignidade. A CHC presentou unha taxa global de transformación maligna do 12,1% (IC

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95% 4,1-19,8 %, I2 = 78,27%, p = 0,002). En ambos os casos, enfatízase a necesidade de estudos prospectivos e criterios de diagnóstico máis claros para comprender completamente o risco de malignidade destas afeccións orais.

No último conxunto de investigacións "Biomarcadores en OPMDs e COCE" investigáronse diferentes aspectos moleculares do COCE e os OPMDs. No artigo 7 os resultados non mostraron diferenzas significativas nos niveis de metilación entre os grupos e suxeriron unha lixeira maior supervivencia estimada para os casos con metilación de MGMT. Sen embargo, non se encontraron correlacións sólidas entre a metilación xénica e as características clínico-patolóxicas ou a supervivencia na nosa cohorte institucional. O oitavo estudo, centrado na expresión inmunohistoquímica de $p16^{INK4a}$ en OPMDs e a súa asociación co risco de TM, revelou un risco de malignización duplicado en casos con sobreexpresión de $p16^{INK4a}$ (RR = 2,01, IC 95% 1,36-2,96, I2 = 0%) o que suxire que a avaliación de $p16^{INK4a}$ podería complementar a graduación de displasia e mellorar o pronóstico dos OPMDs. Esta técnica inmunohistoquímica resulta prometedora como ferramenta adicional na avaliación pronóstica de rutina nos OPMDs.

Conclusións: Estas investigacións resaltan a necesidade dun enfoque multidisciplinario na odontoloxía e a medicina para abordar as cuestións de saúde oral. Ademais, enfatizan a importancia da prevención, a detección temperá e o seguimento, e sinalan o potencial de marcadores biolóxicos. Estes resultados abren novas perspectivas para a prevención e o manexo da patoloxía oral, contribuíndo a unha atención máis integral e efectiva.

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ABSTRACT

Introduction: This doctoral thesis addresses various aspects related to oral health, such as the consumption of alcohol and drug consumption among dentistry and medical students, the assesment of xerostomia and its relationship with quality of life in older adult patients, and the study of different oral potentially malignant disorders (OPMDs) and their potential of malignant transformation.

Aim: To provide a comprehensive understanding of the health risk associated with factors such as alcohol and other substance use, the role of xerostomia on general health and quality of life, malignant transformation of different oral potentially malignant lesions, and biomarker expression in OPMDs and oral squamous cell carcinoma (OSCC).

Material and Methods: The methodological design was characterized by diverse approaches, ranging from cross-sectional studies to systematic reviews with meta-analyses. Various methods and tools, such as validated questionnaires, data analysis from various sources, pyrosequencing, and specialized statistical analyses, were employed to address the complexities of each aspect investigated in this dissertation. The first set of studies (articles 1 and 2) employed a cross-sectional design, examining alcohol and psychoactive substance consumption among dentistry and medical students, along with perceptions of risk and associated knowledge and attitudes using validated questionnaires. A second set of studies (articles 3 and 4) also of cross-sectional design, analyzed the association between xerostomia and various health factors. Specific questionnaires and statistical analyses, including Kruskal-Wallis, ANOVA, and multinomial logistic regression, were used. The third group of investigations (articles 5 and 6) consisted of systematic reviews and meta-analyses addressing the malignant transformation of oral erythroplakia and chronic hyperplastic candidiasis. The fourth set of studies consisted of articles 7 and 8. The seventh article evaluated MLH1 and MGMT methylation levels in patients with OSCC and OPMDs using pyrosequencing, categorizing samples into subgroups based on their clinical evolution. Additionally, data from The Cancer Genome Atlas (TCGA) project were integrated to obtain a broader view of methylation in oral carcinogenesis. The eighth study was a systematic review with a meta-analysis on the expression of $p16^{INK4a}$ in OPMDs.

Results: The first set of research, "Substance-related disorders and their impact on medical and dental Students," underline the importance of addressing alcohol and drug consumption among dentistry and medical students. The findings highlight the need for enhancing knowledge and attitudes and promoting specific training programs to address this issue in both degrees. The results revealed an alarming prevalence of alcohol consumption,

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with women having a significantly higher risk of alcohol use disorder (AUD) (OR=2.63, 95% CI 1.55-4.45, $p < 0.001$). Despite the high prevalence of AUD, students demonstrated insufficient knowledge about alcohol and its effects, with notable differences in knowledge between dentistry and medical students. Although many students felt competent to identify cases of AUD in their patients, they believed they lacked the necessary skills. Drug consumption increased with age and progressed in the academic cycle ($p < 0.001$), with the second cycle showing higher consumption than the first in both univariate (OR = 1.77, 95% CI 1.27-2.48, $p = 0.001$) and adjusted models (OR = 1.86, 95% CI 1.32-2.62, $p < 0.001$). Students had varying perceptions of substance risks, with less consideration for alcohol. However, most students expressed willingness to assist their patients in quitting drug consumption. These findings underscore the urgency of implementing training programs addressing these issues, differentiated by gender, and highlight the responsibility of future healthcare professionals.

The second group of articles, "Xerostomia and its impact on oral and general health," delves into the phenomenon of xerostomia (evaluated through the Xerostomia Inventory) and its relationship with systemic diseases, psychological conditions, and obesity. A prevalence of 30.7% of xerostomia was documented, with logistic regression showing that the highest OR for xerostomia was associated with patients with poor self-perceived health (OR=6.31, 95% CI 2.89-13.80, $p < 0.001$). In the model adjusted for tooth mobility, bone or respiratory diseases, and anxiolytic and antidepressant use, the OR was 3.46 (95% CI 1.47-8.18, $p = 0.005$). In the study of 800 older adults, a strong association with poor general and oral health was observed, with higher risk for those needing assistance with household tasks and for women. Logistic regression showed that the highest OR for xerostomia was among adults with poor general health (2.81; 95% CI 1.8–4.3; $p <0.001$), and for the adjusted model the OR was still significant (2.18; 95% CI 1.4–3.4; $p = 0.001$). Those needing help with household tasks had a 2.16 higher OR (95% CI 1.4–3.4; $p = 0.001$), and 1.69 (95% CI 1.1–2.7; $p = 0.03$) in the adjusted model. These results underscore the importance of early assessment and regular monitoring of xerostomia, given its impact on quality of life and its association with overall oral and general health.

The third set of studies, "Malignant transformation rate of OPMDs," focuses on the potential of malignant transformation of oral erythroplakia (OE) and chronic hyperplastic candidiasis (CHC). OE exhibited a malignant development rate of 19.9% (95% CI -1.6 to 41.4; $I^2 = 91.74\%$, $p = 0.00001$), highlighting epithelial dysplasia as a relevant prognostic factor, although no specific clinical characteristics associated with malignancy risk were identified. CHC showed an overall malignant transformation rate of 12.1% (95% CI 4.1-19.8%, $I^2 = 78.27\%$, $p = 0.002$). Both cases emphasize the need for prospective studies and clearer diagnostic criteria to fully understand the risk of malignancy of these oral conditions.

In the last group of investigations, "Biomarkers in OPMDs and OSCC", different molecular aspects of OSCC and OPMDs were explored. In paper 7 the results showed no significant differences in methylation levels between groups and suggested slightly higher

estimated survival for cases with MGMT methylation. However, no solid correlations were found between gene methylation and clinicopathological characteristics or survival in our institutional cohort. The eighth study, focused on the immunohistochemical expression of $p16^{INK4a}$ in OPMDs and its association with risk of malignant transformation, revealed a doubled malignancy risk in cases with $p16^{INK4a}$ overexpression (RR = 2.01, 95% CI 1.36-2.96, I^2 = 0%), suggesting that $p16^{INK4a}$ assessment could complement dysplasia grading and improve the prognosis of OPMDs. This immunohistochemical technique shows promise as an additional tool in routine prognostic assessment of OPMDs.

Conclusions: These investigations underscore the need for a multidisciplinary approach in dentistry and medicine to address oral health issues. Furthermore, they emphasize the importance of prevention, early detection and follow-up, highlighting the potential of biological markers. These findings open new perspectives for the prevention and management of oral pathology, contributing to a more comprehensive and effective healthcare approach.

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1 INTRODUCCIÓN

1.1 TRASTORNOS RELACIONADOS CON SUSTANCIAS Y SU IMPACTO EN ESTUDIANTES DE MEDICINA Y ODONTOLOGÍA

La detección temprana del cáncer oral es una tarea en la que los profesionales de la salud desempeñan un papel crucial (1). Estos profesionales pueden brindar intervenciones breves en el primer nivel de atención. Sin embargo, es importante destacar que, hasta la fecha, la literatura existente no ha buscado optimizar o recopilar información específica sobre el trastorno por consumo de alcohol o estrategias de prevención de alcohol en este subgrupo, mientras que en los últimos tiempos se ha puesto énfasis en otros factores de riesgo modificables relevantes para la carcinogénesis oral (como la infección por VPH) (2). Es imperativo abordar esta problemática en consonancia con los objetivos de desarrollo sostenible establecidos por la Agenda 2030 de las Naciones Unidas (3) .

En este contexto, el Objetivo de Desarrollo Sostenible (ODS) 3 de la Agenda 2030 cobra especial relevancia, ya que tiene como objetivo "Garantizar una vida sana y promover el bienestar de todos a todas las edades". En particular, la meta 3.5 de este ODS destaca la necesidad de "Fortalecer la prevención y el tratamiento del abuso de sustancias adictivas, incluido el uso indebido de estupefacientes y el consumo nocivo de alcohol". Esta dimensión global resalta la importancia de abordar de manera integral el consumo de alcohol, un factor de riesgo reconocido para el cáncer oral.

Según el "Informe sobre el estado mundial del alcohol y la salud", publicado por la OMS en 2018 (4), el uso nocivo del alcohol provocó alrededor de 3 millones de muertes en todo el mundo ese año. La Unión Europea presenta la tasa de consumo de alcohol más alta del mundo, con un promedio de 10,2 litros de alcohol puro por persona al año. El alcohol ha sido clasificado como carcinógeno del Grupo 1 por la Agencia Internacional para la Investigación del Cáncer (IARC); y en particular, se ha detectado una fuerte relación entre el consumo de alcohol y el cáncer de cabeza y cuello (CCC). Cabe destacar que cada unidad de alcohol consumida aumenta la incidencia de CCC en 1 por 1000 (5). Diferentes estudios in vivo e in vitro han demostrado la influencia del consumo de alcohol en el cáncer oral y en la transición de OPMDs a cáncer oral (6). Desde la perspectiva de la salud pública, varios grupos de profesionales de la salud han desarrollado intervenciones que tienen como objetivo reducir el consumo de alcohol y así mismo este problema también ha sido abordado en algunos proyectos de detección de CCC (7).

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Los profesionales sanitarios también desempeñan un papel fundamental en la labor de asesoramiento, por ello, sus actitudes frente a esta problemática resultan decisivas a la hora de aconsejar a sus pacientes que dejen de consumirlas. El consumo de drogas a nivel mundial está aumentando, tanto en términos generales como en términos de proporción de la población mundial que consume estas sustancias. Alrededor de 269 millones de personas consumieron drogas en al menos una ocasión en 2018, es decir, el 5,3 % de la población mundial de 15 a 64 años (8). Este fenómeno tiene serias implicaciones sociales y de salud, y es fundamental que los profesionales de la salud desempeñen un papel activo en su prevención y abordaje, alineándose con el espíritu de la Agenda 2030.

A lo largo de las décadas se han realizado numerosos estudios que han analizado el consumo de sustancias entre los estudiantes de medicina (9,10). Estos futuros trabajadores de la salud desempeñarán un papel crucial en la detección del consumo de sustancias y en el asesoramiento a sus pacientes, por lo que es fundamental que sean conscientes de las consecuencias negativas que el consumo de estas sustancias puede tener en la salud (11). A pesar de que se considera el entorno dental un lugar útil para identificar problemas relacionados con el consumo de sustancias (12,13), diversos estudios han concluido que existe un importante desconocimiento del papel que pueden jugar los odontólogos a la hora de abordar este tema (14-16).

1.2 XEROSTOMÍA Y SU IMPACTO EN LA SALUD ORAL Y GENERAL

El patrón de estilo de vida de una persona, incluyendo el consumo de alcohol, tabaco y otras drogas, es un factor clave en la aparición de xerostomía (o la sensación de boca seca) (17). La sensación subjetiva (xerostomía) y la medición objetiva (hiposialia) no siempre coinciden (18), la sensación de sequedad oral no implica intrínsecamente una cantidad reducida de saliva, ya que algunos pacientes diagnosticados de hiposalivación no perciben esta sensación. La hiposalivación se mide mediante sialometría (19), y para evaluar la gravedad de la sensación de sequedad bucal se recurre a cuestionarios como el Xerostomia Inventory (XI) (20). La dieta desempeña un papel relevante, y asegurar el consumo diario de frutas y verduras frescas y la ingesta de líquidos resulta esencial para la rehidratación y el mantenimiento de la salud bucodental (21), lo que reduce los síntomas de xerostomía (22). La obesidad, por su parte, se ha establecido como un factor de riesgo importante para una variedad de enfermedades, y se ha relacionado con la xerostomía y otros problemas orales, como caries, periodontitis y pérdida de dientes (23,24). La presencia de problemas bucodentales se ha asociado a una peor autopercepción de la calidad de vida (25). Además, se ha evidenciado que la mala salud oral aumenta el riesgo de ciertas condiciones sistémicas, como neumonía por aspiración, trastornos cardiovasculares y diabetes, entre otras (26).

Por otra parte, la población mundial está envejeciendo a un ritmo mucho más acelerado que nunca, y se estima que entre 2015 y 2050, la proporción de la población mundial mayor de 60 años aumentará del 12 al 22 % (27). La xerostomía afecta principalmente a las personas

mayores y puede deberse a una variedad de etiologías subyacentes (28). Los trastornos por consumo de alcohol en la población geriátrica son preocupantes, y se sabe que éste puede causar deshidratación y atrofia de las glándulas salivales, contribuyendo a la xerostomía (29-31). Además, estudios preliminares han demostrado que fumar a largo plazo se asocia significativamente con la hiposalivación (32). La relación entre la nutrición y la sequedad bucal en personas mayores no ha sido completamente estudiada, pero se ha vinculado la dieta mediterránea con una menor probabilidad de padecer síndrome de Sjögren primario (33). Un metaanálisis reciente encontró una asociación positiva entre la mala salud general y una baja calidad de vida relacionada con la salud oral en adultos mayores (34), sin embargo, en un mapa sistemático de revisiones que examinaron el conocimiento actual sobre el estado de salud bucal y el cuidado dental de las personas mayores, se concluyó que existe una necesidad urgente de investigación en la mayoría de los dominios de la odontología geriátrica (35).

1.3 TASA DE TRANSFORMACIÓN MALIGNA (TM) DE OPMDS

La neoplasia más común y mortal en la región de la cabeza y el cuello sigue siendo el carcinoma oral de células escamosas (COCE) (36) y la necesidad de abordar este problema de salud global es evidente. A pesar de los avances en la investigación y las terapias durante las últimas décadas, la supervivencia específica del COCE ha mejorado solo marginalmente (37). La detección tardía en etapas avanzadas es un problema común, lo que resulta en un pronóstico desfavorable, similar al observado hace medio siglo. De hecho, en la actualidad, la supervivencia a cinco años en relación con el cáncer oral se estima en alrededor del 50%.

La carcinogénesis del COCE implica una mutación del ADN, cuyo origen es multifactorial, en el que intervienen factores relacionados con el estilo de vida, la genética, el medio ambiente y los agentes infecciosos. La Figura 1 muestra los principales factores de riesgo del COCE (38).

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Figura 1. Principales factores de riesgo de COCE. Tomada de: Pérez Jardón A, Arroyo E, Gándara Vila P, Pérez-Sayáns M. Risk Factors of Oral Cancer: Identification and Mechanism of Prevention. Handbook of Cancer and Immunology: Springer; 2023 Apr. p. 1-29.

A pesar de tener una etiología multifactorial, el estilo de vida es el principal factor de riesgo reportado del COCE (39). Actualmente, la mayoría están relacionados con el tabaco y el alcohol, que tienen un efecto sinérgico. La IARC ha considerado estos dos hábitos como factores de riesgo suficientemente probados para el COCE, además del cáncer de amígdalas y faringe. De hecho, tres de cada cuatro tumores malignos de cabeza y cuello se atribuyen al consumo de tabaco y alcohol (40). Asimismo, estas sustancias actúan como factores de riesgo de OMPDs (41). Se sabe que el alcohol es un factor de riesgo independiente para OPMDs, independientemente de la bebida o el patrón de consumo (42) y que su consumo conjunto con el tabaco tiene un efecto negativo, acumulativo y exponencial.

En 1972, la OMS propuso una clasificación de enfermedades que probablemente progresen hasta convertirse en cáncer y definió los términos “lesión precancerosa” y “condición precancerosa”. En 2005, esta organización sugirió el término “lesiones precursoras” para referirse a los cambios en el epitelio que tienen más probabilidades de progresar a COCE (43). Como la interpretación del término “precáncer” o “precursor” podría resultar confusa, un grupo de expertos en el Workshop celebrado en Londres sugirió el término desórdenes potencialmente malignos (44). En 2020, los expertos reafirmaron el término OPMD y dieron la siguiente definición: “Los OPMD representan “campos” de tejido

con apariencia clínica más o menos distintiva en la evaluación inicial, y en los que se ha documentado que una proporción dentro de cada categoría clínica ha desarrollado posteriormente cáncer durante el seguimiento" (45). En la Tabla 1 se muestran las diversas clasificaciones históricamente relevantes propuestas.

Tabla 1. Clasificaciones propuestas de lesiones orales potencialmente malignas.

1972	Lesion precancerosa	Leucoplasia (LO) Eritroplasia (EO) Lesiones palatinas en fumadores invertidos
	Condición precancerosa	Fibrosis submucosa oral (OSMF) Queratosis actínica Liquen plano oral (LPO) Lupus eritematoso discoide
2005	Lesiones precursoras epiteliales	LO EO
	Condición Precancerosa	LPO Lupus eritematoso discoide Queratosis actínica Deficiencia de hierro Xerodermia pigmentoso Sífilis OSMF
2007	Desórdenes potencialmente malignos	LO EO Lesiones palatinas en fumadores invertidos OSMF Queratosis actínica LPO Lupus eritematoso discoide Trastornos hereditarios con mayor riesgo (disqueratosis congénita, epidermólisis ampollosa)
2020	Desórdenes orales potencialmente malignos (OPMD)	LO Leucoplasia verrugosa proliferativa (LVP) EO OSMF LPO Queratosis actínica / queilitis actínica Lesiones palatinas en fumadores invertidos Lupus eritematoso oral Disqueratosis congénita Lesión liquenoide oral Enfermedad de injerto oral contra huésped

Tomada de: Pérez Jardón A, Arroyo E, Gándara Vila P, Pérez-Sayáns M. Risk Factors of Oral Cancer: Identification and Mechanism of Prevention. Handbook of Cancer and Immunology: Springer; 2023 Apr. p. 1-29.

Se ha informado que la prevalencia mundial de este grupo de trastornos oscila entre el 2,6 % y el 4,1 %, con notables variaciones geográficas (46) . Varios informes han estimado una tasa de transformación maligna (TM) del 7,9% al 12,1% con una gran variabilidad entre los subtipos de OPMDs (47,48) . El manejo clínico de pacientes diagnosticados con OPMDs resulta complejo, ya que todavía se carece de los medios para predecir con precisión el riesgo de TM de estos.

Dentro del contexto del diagnóstico y pronóstico de OPMDs, es importante destacar que la eritroplasia oral (EO) es un desorden oral potencialmente maligno raro, pero de alto

riesgo que ha sido objeto de múltiples controversias desde sus informes iniciales (49-52). La EO se ha considerado clásicamente como la OPMD con mayor tasa de TM (45,48,53,54). Según el último consenso convocado por el Centro Colaborador de la OMS para el Cáncer Oral (45), la eritroplasia representa la contraparte roja de la leucoplasia oral (LO), y se define como "una mancha predominantemente rojo fuego que no se puede caracterizar clínica o patológicamente como cualquier otra enfermedad definible." En la Figura 2 se muestra una EO en la lengua.

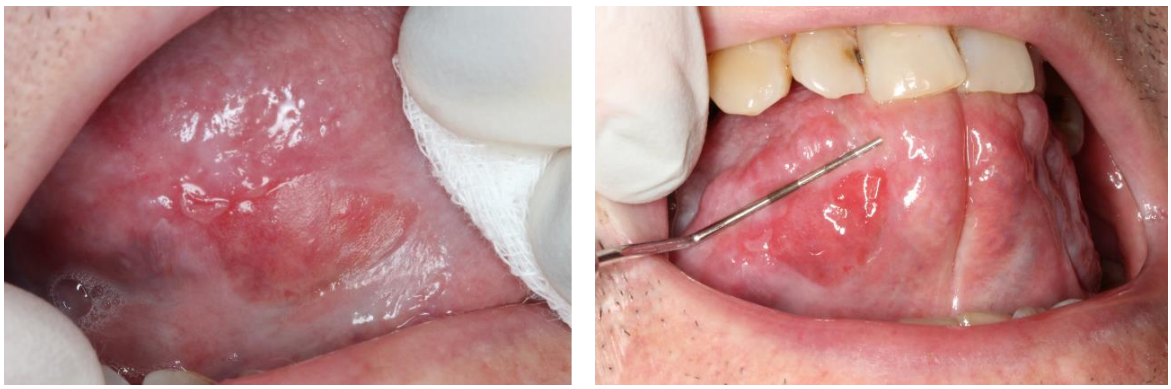


Figura 2. Eritroplasia en cara ventral de lengua. Realizadas en el máster de Medicina Oral, Cirugía Oral e Implantología (USC).

Debido a su apariencia clínica, es muy importante realizar un buen diagnóstico diferencial con otras OPMDs como la eritroleucoplasia (Figura 3) y las formas rojas (atróficas y erosivas) del LPO (Figura 4), así como con otros "trastornos rojos" de la mucosa oral, como la candidiasis y las erupciones fijas medicamentosas (45,53,55).



Figura 3. Eritroleucoplasias en dos pacientes varones. Realizadas en el máster de Medicina Oral, Cirugía Oral e Implantología (USC).

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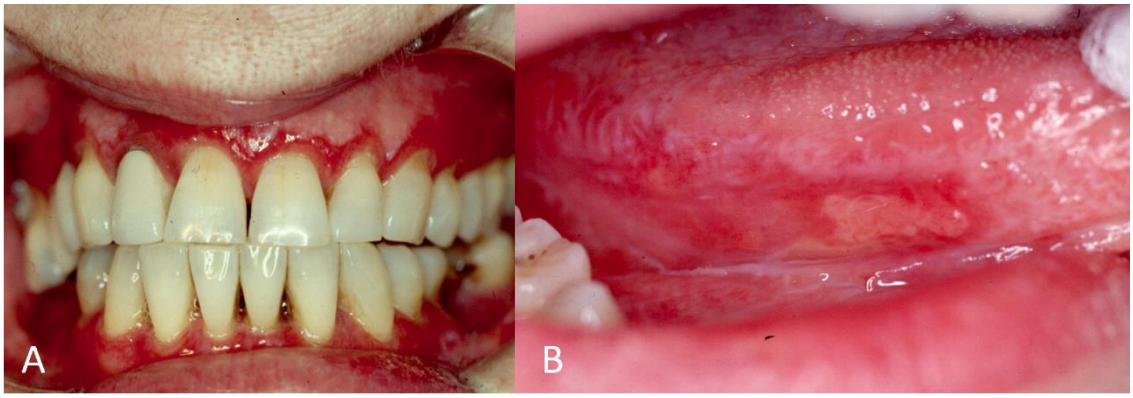


Figura 4. Liquen plano oral. A) gingivitis descamativa y B) lesión erosiva en borde lateral de lengua. Realizadas en el máster de Medicina Oral, Cirugía Oral e Implantología (USC).

Desafortunadamente, no se dispone de suficientes datos sobre los diferentes factores clínico-patológicos que pueden influir en la TM de la EO, como la edad y el sexo de los pacientes, el consumo de tabaco, alcohol o betel, la ubicación de las lesiones y la presencia de displasia epitelial (DE).

En el último informe de consenso de expertos, se describen también los "trastornos con pruebas epidemiológicas limitadas o insuficiente evidencia de potencial maligno", ya que la bibliografía hasta el momento hacía referencia a otros tres trastornos que probablemente estén asociados a una mayor frecuencia de cáncer oral: la epidermólisis bullosa, la candidosis hiperplásica crónica y la hiperplasia verrugosa exofítica (o hiperplasia verrugosa). Por el momento, no se recomienda la inclusión en el grupo de OPMD de las dos últimas (45).

La candidiasis hiperplásica crónica (CHC) es un subtipo de candidosis oral, causada por especies de *Candida*, un grupo relevante de levaduras sexuales diploides eucariotas. Aunque estas levaduras son comensales inofensivos en la microbiota oral de individuos sanos (con una incidencia entre el 35 y el 80%) (56), en ocasiones pueden adoptar un comportamiento patógeno, contribuyendo a la aparición de un grupo de patologías comúnmente denominadas candidiasis oral o candidosis (CO) (57). La patogénesis de las CO está impulsada por la elevada producción de factores de virulencia y el posterior deterioro de las respuestas inmunes del huésped (58).

CHC se considera un término clínico-patológico, que alberga una lesión blanca intraoral generada por una infección fúngica persistente, comúnmente *Candida albicans* (59). Se han descrito dos presentaciones clínicas de esta entidad: una forma homogénea caracterizada por una placa blanca aislada, adherente y gruesa y una forma nodular o moteada que surge con múltiples nódulos blancos sobre un fondo eritematoso. Estas lesiones aparecen comúnmente en la zona retrocomisural, el dorso de la lengua y el paladar de los usuarios de prótesis (56,58,59).

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A pesar de que la incidencia reportada de CHC es baja entre las CO (alrededor del 1,61 %) (60), su diagnóstico diferencial puede ser complejo debido a la similitud de sus manifestaciones clínicas con enfermedades más comunes, como la eritroleucoplasia y el LPO (61) (Figuras 3 y 4). El diagnóstico diferencial de CHC es complejo y requiere tiempo, ya que implica histopatología para excluir la atipia celular y combinar el uso de cultivos de laboratorio para la identificación de hongos (62). Más del 15 % de las CHC presentan características histopatológicas de DE y tasas de TM más altas que las formas clásicas de LO que carecen de infección por *Candida spp.* (58,63).

Otro problema radica en la nomenclatura, ya que desde la primera descripción de esta entidad por Cawson y Lehner en 1968, el término ha sido considerado sinónimo de "leucoplasia candidiásica" (64); sin embargo, el papel de la CO en la LO sigue siendo motivo de controversia (59). Por último, y lo que es más importante, todavía se debate la TM de la CHC.

En cuanto a las estrategias de prevención de CHC, la evidencia es limitada. Se recomienda a los pacientes abstenerse de fumar o mascar tabaco, mejorar la higiene bucodental y retirar la prótesis durante la noche. Problemas sistémicos como la anemia, avitaminosis y deficiencias en el sistema inmunológico también pueden contribuir al desarrollo de este tipo de CO (57). Para su tratamiento se ha usado tratamiento médico (en forma de terapia antifúngica o la aplicación tópica de retinoides, bleomicina o betacarotenos) y métodos quirúrgicos (cirugía con bisturí, terapia con láser o criocirugía) (59).

La LO se define clásicamente como "una placa blanca con un riesgo cuestionable de cáncer que sólo puede diagnosticarse una vez que se han descartado otras condiciones específicas" (59). *Candida spp*., particularmente *albicans*, pueden promover la oncogénesis mediante mecanismos como subproductos cancerígenos, mimetismo molecular o desencadenamiento de la cascada de inflamación (65). Los hallazgos histopatológicos de DE en la primera biopsia de cualquier lesión oral son un fuerte predictor de futura TM (66). Sin embargo, las características de la DE no son exclusivas de las lesiones premalignas, y dichos patrones histológicos pueden ser reactivos en lugar de preneoplásicos (67). El Centro Colaborador de la OMS para el Cáncer Oral señaló que la definición de LO es ad excludendum, lo que descarta causas específicas como la infección por *Candida spp.* En este contexto, CHC per se ya no se define como un OPMD (45).

En la Figura 5 podemos ver a un paciente varón de 71 años con CHC en múltiples localizaciones que fue diagnosticado y tratado en el Máster de Medicina Oral, Cirugía Oral e Implantología de la USC.

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Figura 5. Paciente varón con CHC en múltiples localizaciones. Realizadas en el máster de Medicina Oral, Cirugía Oral e Implantología (USC).

Se le realizó una biopsia en la zona retrocomisural izquierda, donde se observó acantosis, paraqueratosis (Figura 6A) e infiltrado inflamatorio crónico en el conectivo (Figura 6B). La tinción PAS fue positiva, mostrando pseudohifas y esporas que penetran en el interior del epitelio (Figura 6C).

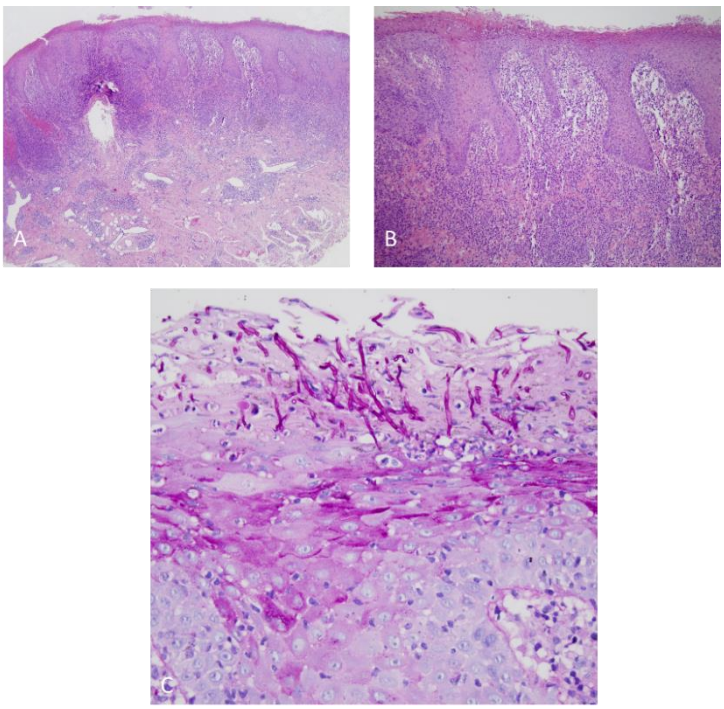


Figura 6. Histopatología de la lesión retrocomisural izquierda del paciente de la Figura 5. A, B) Tinción con hematoxilina-eosina, C) tinción PAS. Realizadas en el Hospital Clínico Universitario de Santiago de Compostela.

En la Figura 7 podemos ver el mismo paciente tras el tratamiento antifúngico con Fluconazol 50 mg/ 12 h durante 3 semanas.



Figura 7. Paciente de la Figura 5 tras instalar tratamiento médico con antifúngico. Realizadas en el máster de Medicina Oral, Cirugía Oral e Implantología (USC).

1.4 BIOMARCADORES EN OPMDS Y COCE

El COCE puede estar precedido por cambios moleculares que corrompen las vías del sistema inmunológico y que no pueden verse mediante una evaluación histopatológica; por lo tanto, se puede subestimar el comportamiento clínico y el pronóstico de estas lesiones. La utilización de biomarcadores podría mejorar la detección de cambios no perceptibles en el análisis histopatológico, lo que podría aumentar la efectividad en la identificación de pacientes con mayor riesgo de desarrollar COCE (68).

Es sabido que la acumulación de alteraciones genéticas puede desencadenar el desarrollo de OPMDs y carcinomas (48). Las alteraciones genéticas se consideran cambios irreversibles en la secuencia de ADN, que resultan en la activación del oncogén o la desactivación de los genes supresores de tumores (69). Por otro lado, las alteraciones epigenéticas resultan en cambios reversibles y hereditarios que no afectan la secuencia de ADN (70). La metilación del ADN es un mecanismo epigenético en el que un grupo metilo se transfiere a la posición C5 de la citosina para formar 5-metilcitosina (71). La metilación del ADN suele tener lugar en las citosinas que preceden a una guanina o, alternativamente, puede tener lugar en los sitios CpG. Esta modificación epigenética es esencial para silenciar el elemento retroviral y regular la expresión génica para tejidos específicos, y también para la impronta genómica y la inactivación del cromosoma X (72).

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La reparación de desajustes (MMR, por sus siglas en inglés mismatch repair) es uno de los principales sistemas de reparación del ADN que se relaciona con el sistema bacteriano homólogo MutLS (proteínas MutS y MutL humanas) (71). El gen humano MLH1 (mutL homólogo 1) desempeña un papel clave en la reparación de errores de duplicación del ADN y en la preservación de la estabilidad genómica. Por otro lado, la metil guanina metil transferasa (MGMT) es una proteína específica de reparación de daños en el ADN que desempeña un papel crucial en el mantenimiento de la fisiología celular normal y la estabilidad genómica (70).

MHL1 es uno de los genes de reparación del ADN comúnmente relacionado con el síndrome de Lynch y asociado a mutaciones de la línea germinal. Los pacientes con mutaciones en el gen MLH1 a menudo desarrollan cáncer colorrectal a una edad temprana (73). Diferentes estudios han demostrado que la metilación de MLH1 se produce principalmente en los estadios iniciales de la carcinogénesis oral, aunque también puede aparecer, en menor medida, en estadios más avanzados (74,75). Se ha sugerido una posible influencia del tabaquismo en la metilación de MLH1, proceso que podría ser reversible (76). La metilación de MGMT es un biomarcador bien descrito en varios tumores sólidos que clínicamente se usa en el glioblastoma. MGMT se considera un biomarcador de buen pronóstico ya que su propia expresión puede silenciarse, lo que resulta en la muerte celular, particularmente mediante la inducción de la apoptosis en las células cancerosas (77).

Como hemos comentado anteriormente, hoy en día, la evaluación del riesgo de oncogénesis en los OPMDs se centra principalmente en la minuciosa revisión de la presencia y clasificación de la DE en una biopsia (66). No obstante, esta intervención presenta ciertos desafíos, incluyendo la variabilidad inter e intraexaminador (78,79). Además, es ampliamente conocido que no todas las displasias se convertirán en neoplasias malignas y, por otro lado, las lesiones no displásicas también pueden eventualmente progresar a carcinomas (67) . Con la evolución de la biología molecular , ha surgido un creciente interés en definir y caracterizar los factores moleculares que impulsan la TM de estos trastornos (80). Este contexto exige la búsqueda de nuevos biomarcadores, con el objetivo de orientar las terapias desde una perspectiva biológica y molecular. Estos enfoques pueden ser capaces de predecir de qué tejido puede surgir un tumor (81) . De acuerdo con la teoría de varios pasos de la cancerización de campo, la historia natural del COCE parece desarrollarse gradualmente a través de precursores de transición que conducen a un cambio progresivo hacia fenotipos cancerígenos (82,83) . Este proceso se debe a la acumulación de alteraciones genéticas y epigenéticas (83-85). Se ha observado desregulación en proteínas como p53 y podoplanina en pacientes con OPMD, aunque con patrones inconsistentes y heterogeneidad, lo que ha limitado su aplicación clínica (80) .

En este contexto, el ciclo celular (fenómeno biológico en el que una célula replica su contenido generando dos células genéticamente idénticas) en células eucariotas está controlado básicamente por ciclinas , quinasas dependientes de ciclina (CDK) e inhibidores de CDK (86). En oncología molecular , estas entidades se clasifican como genes supresores

de tumores debido a su capacidad de inhibir la proliferación celular . Existen principalmente dos familias de inhibidores de CDK: la familia CIP/KIP y la familia INK4 (87) . $P16^{INK4a}$, que pertenece a la familia INK4 , desempeña un papel fundamental como regulador negativo de progresión del ciclo celular mediante la regulación de las funciones de la proteína retinoblastoma. La inactivación de $p16^{INK4a}$ se ha descrito clásicamente como un evento temprano en COCE (66,88) . Se ha planteado la hipótesis de que la evaluación de $p16^{INK4a}$ podría resultar útil como marcador predictivo para evaluar el potencial maligno de los OPMDs (80). Sin embargo, los estudios realizados han presentado importantes inconvenientes, como el diseño transversal o el tamaño reducido de muestra. Por lo tanto, es fundamental validar de manera concluyente la capacidad de las alteraciones de CDKN2A/$p16^{INK4a}$ para predecir la progresión maligna de los OPMDs.

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2 HIPÓTESIS Y OBJETIVOS

La **Hipótesis** de esta Tesis Doctoral se fundamenta en la premisa de que la población en España presenta comportamientos de riesgo para la salud que afectan a la salud oral y sistémica. Se postula que estas actitudes se asocian con el deterioro del estado de salud oral, una mayor prevalencia de xerostomía y un incremento significativo del riesgo de malignización de los OPMDs.

Por ello, el **objetivo principal** de esta Tesis Doctoral es investigar diversos aspectos relacionados con la salud oral, como el consumo de alcohol y drogas entre estudiantes de odontología y medicina, la evaluación de la xerostomía en una muestra autonómica y estatal, la transformación maligna de desórdenes orales potencialmente malignos y la expresión de biomarcadores en OPMDs y COCE.

Como objetivos secundarios (se presentan consecutivamente en el orden de los artículos incluidos)

1. Analizar el consumo de alcohol de los estudiantes de odontología y medicina de la Universidad de Santiago de Compostela (USC), así como su conocimiento sobre los efectos de dicho consumo y estudiar la etapa de cambio en el que se encuentran con respecto a ayudar a los pacientes a dejar de consumir alcohol.

2. Evaluar el uso de sustancias psicoactivas y las percepciones de riesgo entre estudiantes de odontología y medicina de la USC, así como estudiar sus actitudes y conocimientos y evaluar su motivación a la hora de ayudar a sus pacientes a dejar de consumir estas sustancias.

3. Estudiar la asociación entre variables de salud oral y general e indicadores de obesidad con la sensación de boca seca o xerostomía evaluada con el XI.

4. Analizar la asociación entre la xerostomía y los comportamientos de riesgo para la salud, la salud general y bucodental y la calidad de vida relacionada con la salud bucodental en una muestra representativa de adultos mayores de 65 años.

5. Estimar la tasa de transformación maligna de la eritroplasia oral e identificar los factores de riesgo asociados.

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6. Evaluar la evidencia actual sobre la tasa de malignización de la Candidiasis hiperplásica crónica y determinar las variables que tienen mayor influencia en el desarrollo del cáncer.

7. Cuantificar los niveles de metilación de MHL1 y MGMT en COCE y OPMDs para determinar su significado pronóstico y clínico-patológico.

8. Disertar la evidencia publicada sobre la expresión de $p16^{INK4a}$ como biomarcador predictivo para la transformación maligna de OPMDs.

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3 HERRAMIENTAS METODOLÓGICAS

Se describen detalladamente los materiales y métodos utilizados en los ocho artículos que forman parte del compendio de la Tesis Doctoral.

3.1 TRASTORNOS RELACIONADOS CON SUSTANCIAS Y SU IMPACTO EN ESTUDIANTES DE MEDICINA Y ODONTOLOGÍA

3.1.1 Artículo 1. Counselling toward reducing alcohol use, knowledge about its morbidity and personal consumption among students of medical and dental courses in north-western Spain.

3.1.1.1 Diseño del estudio

Este estudio transversal se realizó en la Facultad de Medicina y Odontología de la Universidad de Santiago de Compostela (España) siguiendo las pautas STROBE para estudios observacionales (89). El comité de bioética de la USC dio su consentimiento para su ejecución (Anexo 1). Se invitó a participar a todos los estudiantes de odontología de primero a quinto y posgrado, y a los estudiantes de medicina de primero y último año. Las encuestas impresas se distribuyeron en horario lectivo y la participación fue anónima y voluntaria. La encuesta también se entregó a través de un formulario de Google que se envió a los estudiantes que no pudieron asistir a las clases presenciales.

3.1.1.2 Cálculo del tamaño muestral

Para calcular el tamaño de muestra mínimo requerido se utilizó Epidat 4.2 (SERGAS, Galicia, España) con un intervalo de confianza (IC) del 95% y un margen de error del 5%. El cálculo del tamaño de la muestra se basó en el número de estudiantes matriculados en ese momento (n=225 en Odontología y n=700 en Medicina). Con base en este número, el tamaño de muestra mínimo requerido para este estudio fue de 275, y finalmente se incluyeron 500 encuestas.

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3.1.1.3 Validación del instrumento

Se probó una encuesta piloto en una muestra de 50 estudiantes seleccionados al azar para evaluar la confiabilidad y exhaustividad de la encuesta, así como el tiempo necesario para completarla. El tiempo promedio para completar la encuesta fue de siete minutos. Estos 50 casos piloto fueron posteriormente descartados y se recolectó la muestra final. Se utilizó la prueba alfa de Cronbach para determinar la confiabilidad final de la prueba. Se utilizó la estratificación en bloques temáticos y el valor medio ponderado de esta encuesta fue de 0,74. Los datos permitieron asegurar una confiabilidad moderada.

3.1.1.4 Cuestionario

La encuesta se dividió en seis secciones:

(I) Datos sociodemográficos (edad, género, facultad, año de estudio, y ciclo). Los alumnos de 1° y 2° año se agruparon en el 1° ciclo; los de 3°, 4° y 5° año se agruparon en el 2° ciclo, y los estudiantes de posgrado, doctorado en odontología y 6° año (adicionales, con características muy específicas y prácticas médicas) se agruparon en el grupo de "posgrado".

(II) Consumo individual de alcohol. Se utilizó la versión validada del AUDIT-C (aumentando el punto de corte en una unidad, por lo que aumentó su valor predictivo, llevándolo a 4 en hombres y 5 en mujeres) (90) y el test CAGE, validado por Mayfield en 1974 (91).

(III) Facilidad con la que tratan temas sensibles durante la práctica clínica. Se utilizó la escala Likert de 5 puntos con las preguntas sobre actitud y confianza descritas por Ntouva et al. (92).

(IV) Conocimiento sobre la morbilidad relacionada con el alcohol. Combinó dos preguntas de opción múltiple y ocho preguntas de respuesta única sobre el conocimiento del alcohol según lo descrito por Miller et al. (93).

(V) Conocimiento de las estrategias de prevención del consumo de alcohol. Se utilizó la escala descrita en la sección III.

(VI) Etapas de cambio. Esta sección se basó en el modelo transteórico diseñado por Prochaska y DiClemente, y que los autores adaptaron a partir de una adaptación previa de Lorenzo-Pouso et al. (2). La versión adaptada buscó evaluar la disposición de los estudiantes de medicina y odontología para brindar consejos a los pacientes sobre el abandono del alcohol. Este último apartado nos permitió dividir a los alumnos en cuatro grupos: precontemplación, contemplación, acción y cambio.

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3.1.1.5 Variables y datos recogidos

Datos demográficos: edad, género, facultad, año de estudio, ciclo.

Consumo de alcohol: AUDIT-C, Sumatorio AUDIT-C, Consumo de Riesgo (Alto/Bajo), CAGE (por sus siglas en inglés, C: Cut down; A: Annoyed; G: Guilty; E: Eye opener, que haría referencia en español a los términos: Reducir; Molesto; Culpable; Revelador), Sumatorio CAGE. Actitudes (Escala Likert).

Conocimientos sobre alcohol (test) y su morbilidad en patologías orales y sistémicas (opción múltiple). Niveles de confianza (escala Likert). Calificaciones ponderadas (0-10) y agrupadas (suspenso, aprobado, bien, notable y sobresaliente).

Etapas de cambio.

3.1.1.6 Análisis estadístico

Los datos se recopilaron en una base de datos específicamente diseñada y se ingresaron manualmente con control repetido para evitar errores. Estos datos se analizaron estadísticamente con el software SPSS v.24.0 (IBM, Statistics, NY, EE. UU.). Las variables categóricas se describieron mediante frecuencias y porcentajes, y las variables cuantitativas mediante media y desviación estándar (DS). De acuerdo con el teorema del límite central, se consideró la distribución de las variables cuantitativas normal dado el gran tamaño de la muestra. Se establecieron tablas de contingencia para estudiar las relaciones entre las variables categóricas mediante la prueba de chi-cuadrado. Se utilizó estadística paramétrica para estudiar el efecto de las variables cuantitativas sobre las variables cualitativas, realizando la prueba ANOVA con corrección post hoc de Bonferroni para comparaciones con más de dos elementos. Se analizó la correlación pareada de variables cuantitativas mediante el coeficiente de correlación (CC) de Pearson. Se realizó un análisis de regresión logística binomial para verificar el riesgo de AUD. El nivel de significación se fijó en $p \leq 0,05$.

3.1.2 Artículo 2. Drug use, risk perceptions and attitudes towards drug use amongst medical and dentistry students.

3.1.2.1 Diseño del estudio

Se realizó un estudio descriptivo que fue aprobado por el Comité de Bioética de la USC (Anexo 1). Se invitó a participar a todos los estudiantes de odontología de primero a quinto año y de posgrado, y a los estudiantes de medicina de primero a sexto año. Los instrumentos de evaluación se administraron en formato en línea utilizando Microsoft Forms

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y fueron distribuidos por la autoridad académica a través de un enlace o código zappa. Los participantes tuvieron libre acceso al cuestionario entre octubre de 2020 y enero de 2021. La participación fue anónima y voluntaria, cumpliendo con los requisitos éticos y de protección de datos.

3.1.2.2 Cálculo del tamaño muestral

La estimación del tamaño muestral se hizo con base en la diferencia de las proporciones esperadas entre ciclos académicos. Así, para una proporción esperada del 10% en la población 1 (2° ciclo) y del 5% en la población 2 (1° ciclo), para una razón de tamaño de muestra de 1, un nivel de confianza del 95% y una potencia del 80%, el tamaño de muestra requerido fue de 435 en cada grupo de población, (con un total de 870 participantes). Este cálculo se realizó utilizando Epidat 4.2 (SERGAS, Galicia, España).

3.1.2.3 Validación del instrumento

Se realizó un estudio piloto con una muestra de 50 estudiantes del mismo modo que se describió en el apartado 3.1.1.3. El tiempo promedio para terminar la evaluación fue de siete minutos. Se utilizó la prueba alfa de Cronbach para determinar la confiabilidad final de la prueba. Se utilizó la estratificación en bloques temáticos y el valor promedio ponderado para este estudio fue de 0,86. Estos datos permitieron asegurar una alta confiabilidad.

3.1.2.4 Cuestionario

El cuestionario se dividió en ocho secciones:

(I) Datos sociodemográficos: edad, género, facultad y año de estudios. Los alumnos se agruparon por ciclos como se detalla en el apartado 3.1.1.4.

(II) Consumo de tabaco (sí/no), edad de inicio del consumo de tabaco y número de cigarrillos al día.

(III) Drug Abuse Screening Test (DAST), diseñado por Skinner en 1982 (94) y validado en español (95), evalúa el consumo de sustancias y las consecuencias derivadas de dicho consumo. Esta prueba consta de 20 ítems y una puntuación de 5 o más se considera consumo problemático.

(IV) Cannabis Abuse Screening Test (CAST), un cuestionario de seis ítems utilizado para detectar patrones problemáticos de consumo de cannabis y trastorno por consumo de cannabis (CUD), proporcionando una puntuación total que oscila entre 0 y 6. En el caso de que un encuestado obtenga una puntuación de 4 o más, se considera que tiene un consumo problemático (96,97),

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(V) Evaluación de la frecuencia de consumo de sustancias psicoactivas. Adaptación de los ítems utilizados en el estudio EDADES (98) para evaluar la prevalencia del consumo de diferentes drogas psicoactivas (hipnosedantes, opioides, cannabis, cocaína, éxtasis, GHB, anfetaminas, metanfetaminas, alucinógenos, setas mágicas, heroína e inhalables volátiles), mediante el uso de cuatro indicadores temporales: al menos una vez en mi vida, en los últimos 12 meses, en los 30 días anteriores a la encuesta y todos los días en los 30 días anteriores a la encuesta.

(VI) Evaluación de la percepción de riesgos asociados al consumo de drogas. Adaptación de los ítems utilizados en el estudio publicado por el Plan Nacional sobre Drogas de España (99) para evaluar la percepción de riesgos asociados al consumo de drogas. Se analizaron los siguientes comportamientos: fumar un paquete de cigarrillos al día, consumo habitual de marihuana, consumo de éxtasis los fines de semana, consumo de LSD una vez al mes, consumo de dos bebidas alcohólicas al día y consumo de más de cuatro bebidas alcohólicas en cada ocasión. La escala utilizada fue: 1 sin riesgo a 4 alto riesgo.

(VII) Valoración de las actitudes y conocimientos de los profesionales sanitarios a la hora de aconsejar a sus pacientes para que dejen de consumir drogas. Adaptación de la valoración del alcohol descrita por Ntouva et al. (92). Se utilizó la escala de Likert (1 muy en desacuerdo - 5 muy de acuerdo). Se valoraron las aptitudes: “Siempre pregunto sobre el uso de sustancias estupefacientes cuando hago la historia clínica de un paciente”, “Siento que es parte de mi trabajo identificar a los pacientes que usan sustancias y asesorarlos” y los conocimientos: “Puedo dar correctamente consejos a mis pacientes sobre el uso de diferentes drogas y sus efectos en la salud”, “Estoy familiarizado con las herramientas de detección de uso de drogas”.

(VIII) Valoración de la motivación de los estudiantes para dar consejos a sus pacientes sobre el abandono del consumo de drogas. Elaboramos los ítems siguiendo la evaluación de los estadios de cambio según el modelo transteórico de Prochaska y DiClemente, posteriormente adaptado por Daley (100) : “No brindo asistencia a mis pacientes para ayudarlos a dejar las drogas y no tengo la intención de hacerlo” (precontemplación); “No les brindo asistencia a mis pacientes para que dejen las drogas, pero he pensado en hacerlo” (contemplación); “Proporciono asistencia a mis pacientes para ayudarlos a dejar las drogas de vez en cuando” (acción); “A mis pacientes habituales les brindo asistencia para que dejen de consumir drogas” (mantenimiento). Los estudiantes de odontología de 1° a 2° año, y los estudiantes de medicina de 1° a 3° año no realizan actividad clínica, por lo que se les preguntó si tenían intención de realizar dicha actividad cuando atendiesen a los pacientes.

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3.1.2.5 Análisis estadístico

Se recogieron y analizaron los datos como se explica en el apartado 3.1.1.6. Se describieron de la misma forma las variables categóricas y las cuantitativas. Se utilizaron las pruebas de chi-cuadrado, Kruskal-Wallis, ANOVA y la corrección post hoc de Bonferroni así como el CC de Pearson según lo detallado en el apartado 3.1.1.6. Se calculó el índice Kappa para las preguntas de los cuestionarios CAST y DAST. Se construyeron modelos de regresión logística binomial y multinomial univariados y ajustados para determinar el Odds ratio (OR) para el consumo de drogas y para la etapa de cambio en relación con las covariables: género, ciclo y facultad. El nivel de significación se estableció en $p \leq 0,05$.

3.2. XEROSTOMÍA Y SU IMPACTO EN LA SALUD ORAL Y GENERAL

3.2.1 Artículo 3. Association between xerostomia, oral and general health, and obesity in adults. A cross-sectional pilot study.

3.2.1.1 Diseño del estudio

Este estudio transversal cumplió con los principios éticos de la Declaración de Helsinki de 2013 y fue aprobado por el Comité Ético de Galicia como parte del proyecto de investigación que analiza la asociación entre salud general y oral. Los datos se recopilaron de forma anónima desde octubre de 2019 hasta febrero de 2020 de acuerdo con las recomendaciones de las guías STROBE para estudios observacionales (101). Todos los participantes se ofrecieron libremente a participar y se obtuvo el consentimiento informado antes de su inclusión en el estudio. Los datos fueron anónimos y confidenciales de acuerdo con las Leyes de Protección de Datos.

3.2.1.2 Cuestionario

Se diseñó un cuestionario en formularios de Google, compuesto por 36 preguntas con formato de respuesta binaria o múltiple con opciones cerradas, que fue leído en voz alta por el entrevistador para ayudar en la comprensión y en el uso de la tecnología cuando el encuestado lo solicitó, o para aclarar cualquier duda. El cuestionario se dividió en 5 apartados:

(I) Datos sociodemográficos: lugar de distribución del cuestionario, edad, género, nivel académico, hábito tabáquico.

(II) Salud oral: movilidad dental, pérdida de dientes, prótesis/implantes, sangrado gingival, dolor mandibular y sensación de boca seca.

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(III) Salud General: hipertensión, diabetes, colesterol, trastornos degenerativos óseos, cardíacos, gastrointestinales y neurológicos, consumo de ansiolíticos y/o antidepresivos, ejercicio físico, salud general autopercibida, consumo de frutas y verduras frescas.

(IV) Sobrepeso y obesidad: altura, peso y diámetro de la circunferencia de la cintura.

(V) Xerostomía: Ítems: (1) Bebo líquidos para ayudar a tragar las comidas; (2) Mi boca se siente seca cuando como; (3) Me levanto en la noche a beber líquido; (4) Mi boca se siente seca; (5) Me cuesta comer alimentos secos; (6) Chupo caramelos para aliviar la sequedad bucal; (7) Tengo dificultades para tragar algunas comidas; (8) La piel de mi cara se siente seca; (9) Mis ojos se sienten secos; (10) Mis labios se sienten secos; (11) El interior de mi nariz se siente seco.

3.2.1.3 Población muestral, criterios de inclusión y exclusión

Los datos se recopilaron en un área diversa y extensa de la Comunidad Autónoma de Galicia. La encuesta se realizó a pie de calle, en salas de espera de centros de salud y hospitales (entre trabajadores de la salud no odontólogos), farmacias y trabajadores del sector servicios (en supermercados, tiendas, panaderías, etc.). La muestra estuvo compuesta por 355 adultos de 50 años o más, que fueron seleccionados aleatoriamente de la población general de Galicia.

Criterios de inclusión: adultos de 50 años o más que se ofrecieron como voluntarios para participar en este estudio.

Criterios de exclusión: menores de 50 años, madres embarazadas.

3.2.1.4 Variables y datos recogidos

- Xerostomia Inventory (XI): El XI es un instrumento de 11 ítems con un formato de respuesta graduada de 5 puntos para evaluar los aspectos de gravedad de la xerostomía medidos en un continuo. La versión en español (102) del instrumento diseñado en 1999 (103), tiene un rango de puntuación de 0 a 44 puntos, donde los valores altos indican síntomas severos de xerostomía. Los rangos de valores establecidos para cada grado fueron los siguientes: 0-11: sin xerostomía, 12-22 xerostomía leve, 23-33 xerostomía moderada y 34-44 xerostomía severa.

- IMC: El cálculo del índice de masa corporal se realiza mediante la siguiente fórmula: peso corporal en kilogramos dividido por la altura en metros al cuadrado. Según este cálculo, la OMS ha clasificado el estado nutricional de la siguiente manera: Delgadez muy severa ≤ 15; Delgadez severa 15-15,9; Delgadez 16-18,4; Peso saludable 18,5-24,9; Sobrepeso 25-29,9; Obesidad moderada 30-34,9; Obesidad severa 35-39,9 y Obesidad mórbida ≥ 40. Esta

fórmula presenta algunas limitaciones al no evaluar la distribución de la grasa corporal, por ello se utilizó también el índice cintura altura.

- ICA: El índice cintura-altura se define como la circunferencia de la cintura de una persona dividida por la altura, ambas en centímetros. Se ha observado que el ICA es un indicador más simple y predictivo que el IMC (104). Los valores críticos varían según la edad y el sexo, siendo de 0,6 para mayores de 50 años. Se observaron diferencias de género significativas entre hombres y mujeres en la distribución ICA: extremadamente delgado (hombres y mujeres <0,34); sano delgado (hombres 0,35 a 0,42; mujeres 0,35 a 0,41); sanos (hombres 0,43 a 0,52; mujeres 0,42 a 0,48); sobrepeso (hombres 0,53 a 0,57; mujeres 0,49 a 0,53); mucho sobrepeso (hombres 0,58 a 0,62; mujeres 0,54 a 0,57); obesidad mórbida (hombres > 0,63; mujeres > 0,58).

3.2.1.5 Análisis estadístico

Los datos se recopilaron y analizaron como se describe en el apartado 3.1.1.6. Del mismo modo se describieron las variables categóricas y cuantitativas, así como se utilizaron las pruebas ya descritas (chi-cuadrado, Kruskal-Wallis, ANOVA y corrección post hoc de Bonferroni y CC de Pearson). Para evaluar la eficacia diagnóstica del XI, se utilizaron curvas ROC para determinar la sensibilidad, la especificidad y el área bajo la curva (AUC). Para determinar el riesgo (OR) de xerostomía se utilizaron tanto la regresión logística multinomial univariada como la ajustada. El nivel de significación fue $p \leq 0,05$.

3.2.2 Artículo 4. Xerostomia, the perception of general and oral health and health risk behaviours in people over 65 years of age.

3.2.2.1 Diseño de estudio

Los datos se obtuvieron de la Encuesta SEGER (Sociedad Española de Gerodontología) de 2020, una encuesta nacional que sigue las pautas STROBE para estudios observacionales (101).

La población incluyó a hombres y mujeres mayores de 65 años, con una representación significativa en toda España. Se realizó un muestreo aleatorio simple a nivel nacional, con estratificación proporcional por área geográfica. Los datos se recogieron de forma anónima, y el estudio estuvo exento de requerir la aprobación ética por parte del Comité de Bioética de la USC.

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3.2.2.2 Cuestionario

Se realizó una encuesta telefónica asistida por ordenador utilizando un cuestionario estructurado, con una duración de aproximadamente 16 minutos. Este cuestionario constó de cinco secciones que incluyeron información relacionada con: (I) Datos sociodemográficos; (II) Salud general y oral; (III) Problemas orales durante 2019; (IV) Consumo de tabaco y alcohol y (V) Hábitos alimentarios.

3.2.2.3 Variables recogidas

Para evaluar la sensación de boca seca se utilizó la versión española validada (102) del Xerostomia Inventory (XI), diseñado en 1999 (103). Los rangos de valores asociados con cada grado fueron los descritos en el apartado 3.2.1.4 (105).

En relación con las conductas de riesgo para la salud, se valoró el consumo de tabaco (pasado o actual, número de cigarrillos fumados y, en el caso de los exfumadores, años desde que dejaron de fumar), así como el consumo de alcohol (tipo de bebidas alcohólicas y frecuencia) y los hábitos dietéticos como frecuencia de consumo de verduras, legumbres, frutas, carnes blancas (pollo, conejo, pavo), carnes rojas (ternera, cerdo, cordero) y agua consumida diariamente.

En relación con la salud general y bucodental, se valoraron las preocupaciones sobre el propio estado de salud general y bucodental y la autopercepción de la salud general y bucodental siguiendo una escala tipo Likert de 5 puntos. En cuanto a la calidad de vida relacionada con la salud oral, se evaluó en cuanto a problemas bucales durante el año 2019.

Las variables elegibles para su inclusión en el modelo, “salud general” y “salud oral”, se dicotomizaron, considerando “mala o muy mala” como mala y “aceptable, buena o muy buena” como buena, para la regresión logística binaria multinivel.

3.2.2.4 Análisis estadístico

Se recogieron y analizaron los datos como se describe en el apartado 3.1.1.6. Se describieron las variables categóricas y las cuantitativas y se utilizaron las pruebas de chi-cuadrado, Kruskal-Wallis, ANOVA y la corrección post hoc de Bonferroni según lo detallado en el apartado 3.1.1.6. Se utilizó la regresión logística multinomial univariante y ajustada para determinar la Odds Ratio (OR) de xerostomía. Se construyó un gráfico acíclico dirigido (DAG, por sus siglas en inglés, directed acyclic graph) utilizando la versión 0.2.7 del paquete R ggdag (R Core Team, 2022). Este gráfico proporciona una representación visual de las relaciones causales entre el conjunto de variables involucradas en la regresión logística multinomial ajustada. El nivel de significación se estableció en $p \leq 0,05$.

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3.3 TASA DE TM DE OPMDS

3.3.1 Artículo 5. Critical update, systematic review, and meta-analysis of oral erythroplakia as an oral potentially malignant disorder.

3.3.1.1 Protocolo y registro

Esta revisión sistemática y metaanálisis cumple con las pautas PRISMA (106), (Figura 8) y MOOSE (107). El protocolo de revisión se registró en PROSPERO (CRD42022299026) para minimizar el riesgo de sesgo y mejorar la transparencia, precisión e integridad. Se creó un grupo de revisión ad hoc con especialistas en medicina oral de la Unidad de Medicina Oral de la USC y la Unidad de Medicina y Patología Oral de la Universidad del País Vasco.

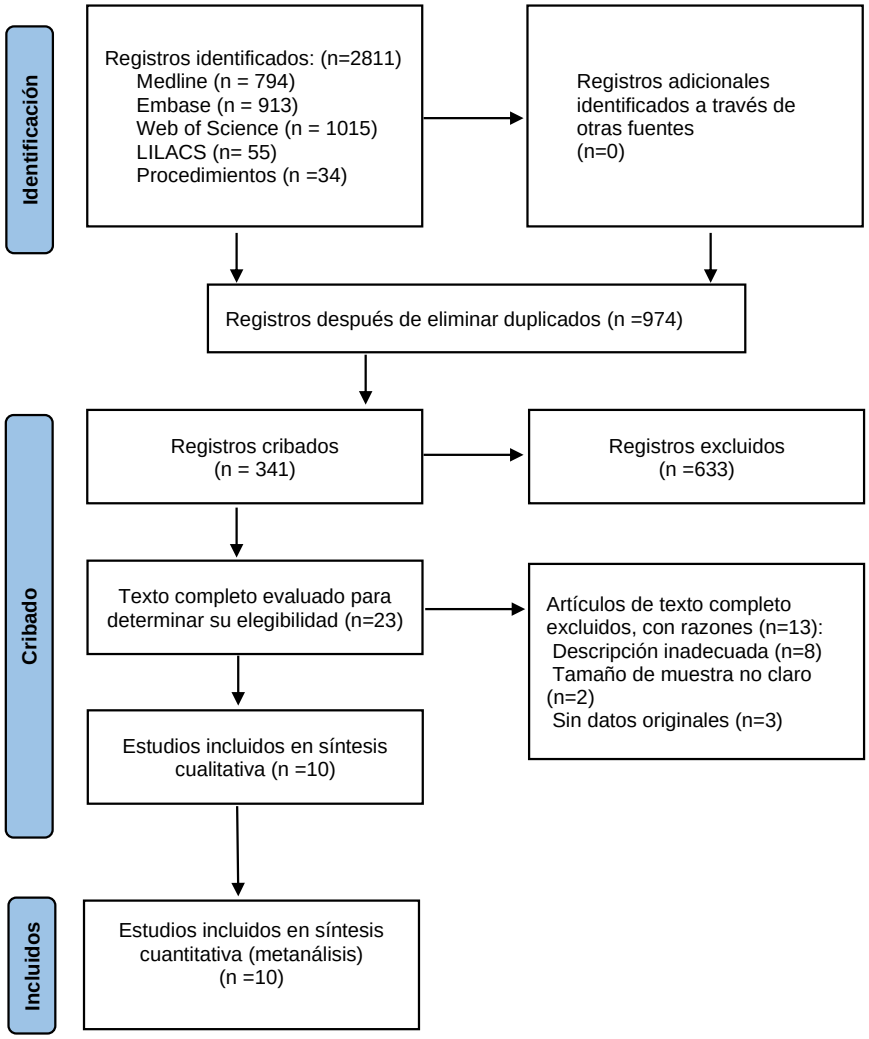


Figura 8. Diagrama de flujo PRISMA. Síntesis del análisis bibliográfico.

3.3.1.2 Pregunta de investigación

Esta revisión fue diseñada para responder las siguientes preguntas: (1) ¿cuál es la transformación maligna (TM) general de EO? y (2) ¿cuáles son los factores de riesgo que contribuyen a la TM de EO? Se utilizó el acrónimo PECOS de la siguiente manera: (1) pacientes con EO (población), que desarrollaron COCE (resultado); (2) pacientes con EO (población), con factores clínico-patológicos específicos (exposición), en comparación con pacientes no expuestos a esos factores clínico-patológicos (comparación), y determinar su relación con la TM de EO (resultado). Solo se incluyeron estudios observacionales longitudinales (tipo de estudios).

3.3.1.3 Estrategia de búsqueda

Las búsquedas electrónicas se realizaron en MEDLINE a través de PubMed, EMBASE, Web of Science, Scopus, la base de datos bibliográfica regional de la OMS LILACS y las bases de datos Conference Proceedings Citation Index. Las búsquedas combinaron términos tesauros (p. ej., MeSH y EMTREE) y términos libres para maximizar la sensibilidad. Los algoritmos utilizados en cada base de datos contenían las siguientes palabras clave: “erythroplakia,” “erythroplasia,” “malignant transformation,” “malignant development,” “malignization,” “carcinogenesis,” “oral cancer,” “oral squamous cell carcinoma,” “mouth neoplasm,” and “prognosis”. Estas palabras fueron sometidas a adaptación sintáctica para cada base de datos. Se realizaron búsquedas en todas las bases de datos desde el inicio hasta febrero de 2022 (límite superior).

Se realizó una segunda búsqueda, introduciendo cada palabra clave de forma no estructurada para verificar si se recuperaran todos los artículos sobre el tema. También se revisaron exhaustivamente los artículos potencialmente relevantes con los que cualquiera de los autores ya estaba familiarizado y las listas de referencias de los artículos recuperados. Todas las referencias se gestionaron utilizando Mendeley v.1.19.9 (Elsevier, Ámsterdam, Países Bajos).

3.3.1.4 Criterios de elegibilidad

Los criterios de elegibilidad de los estudios incluidos en el análisis cualitativo y cuantitativo fueron los siguientes: (I) artículos de investigación originales publicados en inglés, (II) estudios observacionales longitudinales y (III) estudios que analizaron la TM de EO.

Los criterios de exclusión fueron los siguientes: (I) cartas al editor, informes de casos, resúmenes de congresos, ensayos clínicos, etc.; (II) revisiones sistemáticas y metaanálisis previos; (III) estudios que informaron sobre pacientes con diagnóstico de eritroleucoplasia;

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(IV) estudios sin un diagnóstico clínico-patológico completo de EO; y (V) estudios que no informaron datos específicos sobre la EO.

3.3.1.5 Selección y extracción de datos

Los artículos fueron seleccionados en dos fases por dos autores, primero mediante la selección de títulos y resúmenes que aparentemente cumplían con los criterios de inclusión, y luego mediante la lectura de los textos completos para evaluar su inclusión final. Durante el ejercicio de calibración, los revisores discutieron a fondo los criterios y los aplicaron a una muestra del 50 % de los estudios recuperados para determinar el acuerdo entre examinadores. Después de lograr un acuerdo adecuado (puntuación $\kappa = 0{,}78$), los revisores leyeron todos los estudios de forma independiente. Cualquier discrepancia entre los investigadores fue resuelta por un tercer o cuarto investigador ciego a la hipótesis del estudio.

La información fue recuperada utilizando una hoja de extracción ad hoc. Los datos registrados incluyeron: primer autor y año de publicación, país, diseño del estudio, tamaño de la muestra, sexo y edad de los pacientes, consumo de tabaco/alcohol/betel, número y localización de EO, datos histopatológicos, período de seguimiento, número de casos de TM y tiempo hasta TM.

3.3.1.6 Evaluación de calidad y riesgo de sesgo

El riesgo de sesgo (RoB) se evaluó mediante la herramienta QUality In Prognosis Studies-QUIPS, respaldada por el Grupo Cochrane de Métodos de Pronóstico para estudios de pronóstico. QUIPS considera los siguientes dominios: (1) participación en el estudio, (2) abandono del estudio, (3) medición de factores pronósticos, (4) medición de resultados, (5) factores de confusión del estudio y (6) análisis e informes estadísticos (108). RoB se calificó como bajo, moderado o alto para cada dominio. Cada ítem calificado como alto agregó 3 puntos, mientras que cuando se calificó como moderado 2 y como bajo 1 punto a la evaluación de la calidad general de cada estudio. Los estudios se clasificaron como de alta calidad cuando la puntuación general fue superior a 13. RoB fue evaluado por dos autores y las discrepancias fueron resueltas por todos los autores participantes por consenso.

3.3.1.7 Análisis estadístico

En el metaanálisis principal, se calculó la prevalencia de eventos de TM y la del total de casos de EO dividiendo el número de eventos por el tamaño de la muestra del estudio. Luego, se ponderó la prevalencia logarítmica específica del estudio por el inverso de su varianza para calcular una prevalencia agrupada. Para los estudios recuperados, se aplicó el intervalo de Clopper-Pearson para estimar los IC del 95%. Las proporciones agrupadas combinadas se estimaron con un modelo de efectos aleatorios (método de DerSimonian y

Laird). Se consideró un metaanálisis adicional previamente planificado para reconocer el efecto de los factores clínico-patológicos en la TM de la EO, pero se descartó debido a la notificación deficiente de los datos primarios. Por lo tanto, se optó por realizar un metaanálisis de prevalencia con los grados de DE/presencia de COCE en la biopsia inicial en el momento del diagnóstico.

Para el análisis de heterogeneidad estadística, se calcularon los parámetros de la prueba Cochran Q (χ^2) y Higgins I^2. La prueba Q de Cochran $p < 0,1$ se consideró significativa para asumir heterogeneidad aparente. Se consideró que los puntos de corte del estadístico Higgins I^2 del 25 %, 50 % y 75 % indicaban una heterogeneidad baja, moderada y alta, respectivamente (109).

Se evaluó el sesgo de publicación, primero de forma visual mediante gráficos de embudo y luego, mediante la prueba propuesta por Egger et al. (110), realizando una regresión lineal de las estimaciones del efecto sobre sus errores estándar, ponderando por 1/ [varianza de la estimación del efecto], considerando significativa una $p_{Egger} < 0,1$). Se utilizó el paquete de software R gratuito Metafor (v.3.6.2; https://www.r-project.org) para todos los análisis estadísticos, así como para el trazado de figuras con comandos escritos por el usuario. El nivel de significación considerado en todos los análisis estadísticos fue del 5% ($p < 0,05$).

3.3.2 Artículo 6. Oral Chronic Hyperplastic Candidiasis and Its Potential Risk of Malignant Transformation: A Systematic Review and Prevalence Meta-Analysis.

3.3.2.1 Protocolo y registro

Esta revisión se elaboró de acuerdo con el método de notificación preferido para revisiones sistemáticas (PRISMA) (111) (Figura 9) y se registró prospectivamente en la base de datos PROSPERO (ID: CRD42022319572).

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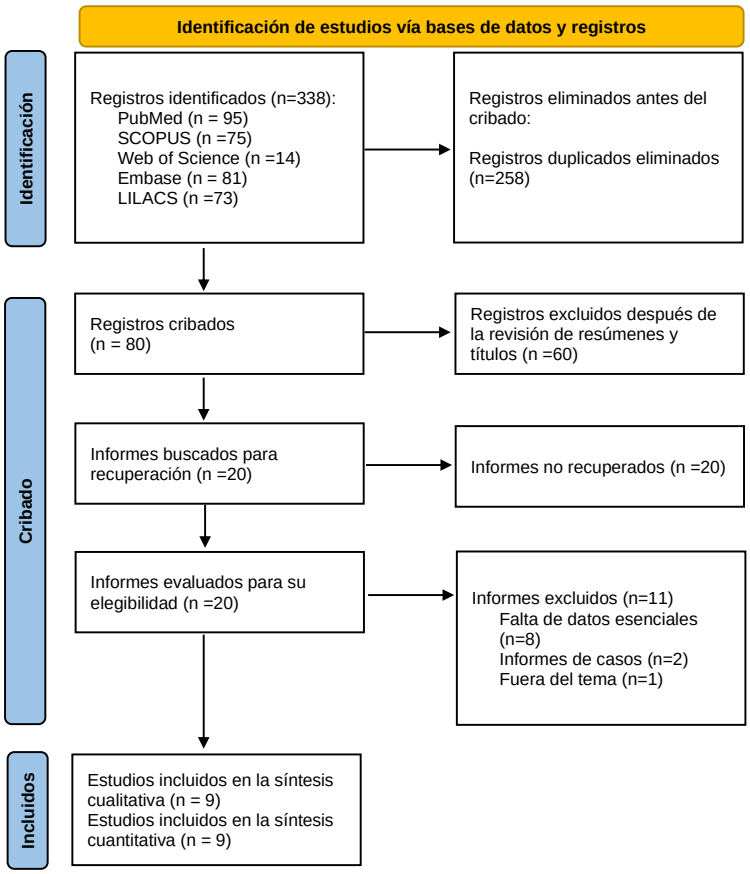


Figura 9. Diagrama de flujo PRISMA de los procesos de búsqueda.

3.3.2.2 Estrategia de búsqueda

Se analizaron las bases de datos de Medline a través de PubMed, Embase, Web of Science, Scopus y LILACS en busca de registros publicados desde el inicio hasta el 22 de junio de 2022 (límite superior). Nuestra estrategia de búsqueda y sintaxis se conceptualizaron combinando términos tesauros (p. ej., MeSH o EMTREE) con palabras libres. Para Medline se utilizó la siguiente sintaxis tanto en MeSH como en los términos libres: "Candidiasis, Chronic Mucocutaneous" [MeSH] or "Chronic Hyperplastic Candidiasis" [All Fields] or "Candidal leukoplakia" [All Fields] or "Candida leukoplakia" [All Fields]) and (malign* or premalignant* or "potentially malignant disorder" or "precancer" or "cancer" [All Fields] or "Carcinoma, Squamous Cell" [MeSH] or "squamous cell carcinoma" [All Fields] or "oscc" [All Fields] or "transformation" [All Fields] or "risk" [All Fields] or "progression" [All Fields].

El algoritmo antes mencionado fue convenientemente reformulado para cada base de datos, como se puede ver con más detalle en la Tabla 2. Además, para verificar si se recuperaron todos los informes relevantes relacionados con el tema en estudio, se realizó una búsqueda manual complementaria con los términos: Candida, oral cancer, and malignant transformation en forma no estructurada.

Tabla 2. Estrategia de búsqueda en bases de datos en el artículo 6.

Base de datos	Búsqueda
PubMed	(“Candidiasis, Chronic Mucocutaneous” [Mesh] or “Chronic Hyperplastic Candidiasis” [All Fields] or “candidal leukoplakia” [All Fields] or “Candida leukoplakia” [All Fields]) and (malign* or premalign* or “potentially malignant disorder” or “precancer” or “cancer” [All Fields] or “Carcinoma, Squamous Cell” [Mesh] or “squamous cell carcinoma” [All Fields] or “oscc” [All Fields] or “transformation” [All Fields] or “risk” [All Fields] or “progression” [All Fields]).
Scopus	TITLE-ABS (“Chronic Hyperplastic Candidiasis” OR “Chronic Mucocutaneous Candidiasis” OR “candidal leukoplakia” OR “candida leukoplakia”) AND TITLE-ABS (“malignant” OR “malignant” OR “evolution” OR “evolves” OR “evolve” OR “progress” OR “progresses” OR “progression” OR “transformation” OR “prognosis” OR “prognostic” OR “prognoses”)
Web of Science	TS = (“Chronic hyperplastic candidiasis” OR “CHC”) AND TS = (“malignant” OR “malignant” OR “evolution” OR “evolves” OR “evolve” OR “progress” OR “progresses” OR “progression” OR “transformation” OR “prognosis” OR “prognostic” OR “prognoses”) AND article type: articles
LILACS	tw:(“Chronic hyperplastic candidiasis” OR “candidiasis hiperplasica cronica“ OR “candidiase hiperplasica“) AND tw:(“malignant” OR “malignent” OR “maligna” OR “malignas” OR “maligno” OR “malignos” OR “evolution” OR “evolução” OR “evolución” OR “progression” OR “progressão” OR “progressión” OR “avance” OR “avanço” OR “transformation” OR “transformação” OR “transformación” OR “prognosis” OR “prognostic” OR “prognoses” OR “prognóstico” OR “pronóstico” OR “prognósticos” OR “pronósticos”)
EMBASE	(‘Chronic hyperplastic candidiasis’) AND (‘malignant’ OR ‘malignent’ OR ‘evolution’ OR ‘evolves’ OR ‘evolve’ OR ‘progress’ OR ‘progresses’ OR ‘progression’ OR ‘transformation’ OR ‘prognosis’ OR ‘prognostic’ OR ‘prognoses’)

3.3.2.3 Criterios de elegibilidad

Los criterios de inclusión fueron los siguientes: (I) Diseño del estudio: estudios observacionales con un diseño prospectivo y retrospectivo (estudios de cohortes, estudios de casos y controles o series de casos); (II) Participantes: estudios en humanos de cualquier sexo o edad con diagnóstico de CHC; (III) Intervención: Diagnóstico histopatológico comprobado de *Candida spp*. o con enfoques de cultivo/moleculares; (IV) Resultado: incidencia de TM en la muestra estudiada.

Entre los criterios de exclusión se encontraban: (I) Informes de casos, revisiones, artículos de posición y opiniones de los autores; (II) Estudios que no definieron con precisión el diagnóstico de CHC tanto clínica como histopatológicamente; (III) Estudios que omitieron la metodología de detección de *Candida spp*.; (IV) Estudios que no proporcionaron la tasa de TM de CHC o que proporcionaron datos insuficientes para realizar estimaciones; (V) Informes que incluyeron menos de 3 casos de CHC.

Se incluyó cualquier artículo relevante de acuerdo con los criterios antes mencionados sin restricción en términos de idioma o fechas de publicación. También se exploraron las referencias de los estudios incluidos para complementar la estrategia de búsqueda. Además, se estableció contacto personal vía e-mail con los investigadores para rastrear más publicaciones o datos relevantes no incluidos en sus artículos. Este enfoque minimizó el riesgo de introducir un sesgo de selección y mejoró la transparencia, precisión e integridad de la metodología empleada (111).

3.3.2.4 Selección y extracción de datos

En la primera etapa, los títulos y resúmenes de los informes recuperados se examinaron sobre la base de los criterios de inclusión/exclusión utilizando la plataforma Rayyan QCRI. Posteriormente, todos los estudios seleccionados que se consideraron adecuados de acuerdo con estos criterios se sometieron a una evaluación de texto completo y luego se tomó una decisión final. Se crearon hojas de cálculo de Excel para recopilar información sobre este proceso de toma de decisiones. Se aplicó el coeficiente kappa de Cohen (κ) para cuantificar el acuerdo interno de los revisores en el proceso de selección de texto completo; este coeficiente se calculó con un paquete estadístico Epidat 4.2 (Sergas, Galicia, España).

La selección de estudios fue realizada por dos operadores independientes con experiencia en medicina y patología oral. En los casos de desacuerdos entre estos revisores, se resolvieron en reuniones conjuntas realizadas con la participación de todos los autores. Un tercer revisor, ciego a la hipótesis del estudio, realizó un análisis de concordancia intraevaluador calculando un valor para κ.

Los datos fueron extraídos con un formulario específico y mejorados mediante pruebas piloto antes de su implementación. Se recopiló información sobre el nombre del autor, la fecha de publicación, el país de origen, el diseño del estudio, el tamaño de la población, las tasas de TM, los períodos de reclutamiento/seguimiento, el método de detección de *Candida spp.*, características histopatológicas, topografía de las lesiones, sexo, edad, tabaquismo y consumo de alcohol entre los individuos. Cuando faltaban datos sobre un artículo en la literatura primaria, se intentó contactar a los autores correspondientes para incorporar la información que faltaba.

Dos revisores fueron los responsables de la extracción de datos. Este proceso fue revisado exhaustivamente más tarde por un tercer participante. Cualquier desacuerdo se resolvió mediante una cuidadosa discusión.

3.3.2.5 Evaluación de calidad y riesgo de sesgo

El RoB se estableció utilizando una herramienta desarrollada para revisiones sistemáticas y prevalencia de metaanálisis desarrollada por el Instituto Joanna Briggs (JBI), conocida como la Lista de verificación crítica de prevalencia de JBI (112). Esta lista de verificación evalúa el RoB de los estudios a través de preguntas cerradas: (1) ¿Fue adecuado el marco de la muestra para abordar la población objetivo?, (2) ¿Se seleccionaron adecuadamente los participantes?, (3) ¿Fue el tamaño de la muestra adecuado para el objetivo del estudio?, (4) ¿Se describieron correctamente los participantes y el entorno?, (5) ¿Se realizó el análisis de datos con suficiente cobertura?, (6) ¿Se aplicaron métodos válidos para la identificación del resultado?, (7) ¿Se midió el resultado de una manera estándar y confiable? (8) ¿Se realizó un análisis estadístico adecuado? (9) ¿Fue adecuada la tasa de

respuesta? ¿O al menos se manejó adecuadamente la baja tasa de respuesta? Los estudios se clasificaron como "alto RoB " si el 49% de los ítems se obtuvieron un "sí"; como "RoB intermedio" si un rango entre el 50% y el 69% de las preguntas puntuaron "sí"; y como "RoB bajo" si ≥ 70% de las preguntas puntuaron "sí". https://jbi.global/sites/default/files/2019-05/JBI_Critical_Appraisal-Checklist_for_Prevalence_Studies2017_0.pdf, consultado el 9 de sepitembre de 2022.

3.3.2.6 Análisis estadístico

Las proporciones específicas del estudio se calcularon con la porción de casos de CHC informados con TM actuando como numeradores y el tamaño de la cohorte de CHC como denominadores. Luego se estimaron los IC del 95% para los estudios recuperados con un método asintótico simple con una corrección de continuidad para evitar aberraciones o artefactos matemáticos (113). Posteriormente, se ponderaron las prevalencias logarítmicas específicas del estudio por el inverso de su varianza para lograr valores de prevalencia agrupados con sus IC del 95% correspondientes. Los metaanálisis se realizaron con el método de DerSimonian y Laird, que se debió a la lógica dictada por cierto grado de heterogeneidad entre los estudios. Se generaron diagramas de bosque para mostrar gráficamente las proporciones específicas del estudio como proporciones agrupadas derivadas del modelo de efectos aleatorios. También se realizó análisis de sensibilidad, evaluando la influencia individual que ejerce cada estudio en la estimación de las tasas agrupadas; este enfoque se aplicó para verificar la confiabilidad de los resultados combinados y detectar estudios con resultados aberrantes, teniendo en cuenta el constructo matemático (114). También se desarrolló una metarregresión de efectos mixtos para verificar la variación dentro del estudio y la variación entre estudios para confrontar una covariable específica (año de publicación) con el resultado principal en estudio (es decir, la tasa de TM).

Para evaluar la heterogeneidad entre estudios se utilizaron las pruebas Q Cochran e I^2 descritas en el apartado 3.3.1.7. Posteriormente, planeamos explorar el origen de la heterogeneidad entre los estratos restringiendo el análisis a los subgrupos definidos por las características específicas del estudio, como el origen del estudio y la evaluación del RoB (RoB alto versus moderado y bajo).

El sesgo de publicación o los “small study effects” se evaluaron visualmente utilizando el gráfico de embudo de Begger, pero también formalmente a través de la prueba descrita por Egger et al. (110). El análisis de los datos se realizó con el software libre R (v.3.4.4; https://www.r-project.org) en particular con el paquete Metafor. Se consideró un p-valor inferior al 5 % estadísticamente significativo.

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3.4. BIOMARCADORES EN OPMDS Y COCE

3.4.1. Artículo 7. DNA Methylation by Bisulfite Next-Generation Sequencing for MLH1 and MGMT in Oral Squamous Cell Carcinomas and Potentially Malignant Disorders: An Integrative Analysis towards Field Cancerization.

3.4.1.1 Pacientes y Muestras

Para este estudio, se seleccionaron ochenta y cinco muestras de pacientes diagnosticados con COCE. Las muestras se dividieron de la siguiente manera: (1) pacientes diagnosticados con COCE (grupo compuesto por 16 muestras), (2) pacientes con OPDM que desarrollaron COCE en la misma ubicación (grupo de transformación compuesto por 47 muestras) y (3) pacientes con OPDM que desarrollaron COCE en una ubicación diferente (grupo simultáneo compuesto por 22 muestras). Las muestras se obtuvieron de la Unidad de Medicina Oral de la Universidad de Santiago de Compostela.

Los pacientes incluidos en este estudio firmaron el consentimiento informado y, posteriormente, todas las muestras fueron procesadas en el Servicio de Anatomía Patológica del Complejo Hospitalario Universitario de Santiago de Compostela (CHUS). Este proyecto fue aprobado por el Comité Ético de Investigación Clínica de Galicia. Se siguieron los principios de la Declaración de Helsinki y sus posteriores modificaciones. Las directrices STROBE se utilizaron como referencia para el informe de datos (101).

3.4.1.2 Estudio de Metilación mediante Pirosecuenciación MLH1 y MGMT

Para determinar el porcentaje de metilación de las muestras estudiadas en los genes MLH1 y MGMT se realizó un análisis de pirosecuenciación. Las muestras se incluyeron inicialmente en parafina. El ADN total fue extraído y tratado con bisulfito, utilizando el Epitect Fast DNA Bisulfite Kit (Qiagen ®, Hilden, Alemania), y siguiendo el protocolo recomendado por el fabricante. La concentración de las muestras se midió con NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, EE. UU.), y todas las muestras tuvieron 260/280 valores de entre 1,7 y 2. Una vez transformado, se extrajo el ADN y se utilizó el kit Therascreen MGMT Pyro (Qiagen ®) luego se usó para realizar PCR y pirosecuenciación para la detección de MGMT metilada. En cuanto a MLH1, la PCR se realizó con el Pyromark PCR kit 200 (Qiagen ®) con una concentración no superior a 100 ng/microlitro. La pirosecuenciación se realizó con Pyromark Q24 CPG MLH1 (4 × 24) (Qiagen ®). Todas las PCR se realizaron en Agilent Technologies Surecycler 8800, y la pirosecuenciación se realizó en la estación de trabajo Pyromark Q24 y Pyromark Q24 (Qiagen ®), utilizando Pyromark Q24 2.0.7 para calcular y analizar los resultados.

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3.4.1.3 Cuantificación de metilación

Para cada muestra, se calculó el promedio de las cuatro y cinco islas CpG respectivamente, y el resultado se consideró positivo (es decir, metilado) si el valor estaba entre 11 y 15 % (según lo indicado por Qiagen®). En aquellos casos en los que el resultado era incierto, la isla CpG y el pirograma correspondiente se comprobaron por separado.

3.4.1.4 Variables y datos recopilados

Las variables cualitativas determinadas fueron las siguientes: grupo de estudio (COCE, transformación y simultáneo), sexo (masculino o femenino), área (encía, lengua, mucosa bucal, paladar duro, suelo de boca, trígono retromolar, mucosa labial, paladar blando o amígdala), trastorno inicial y progresión posterior (LO sin displasia, LO de bajo grado, LO de alto grado, LPO, papiloma, carcinoma verrugoso, úlcera inespecífica o COCE), exitus y metilación cualitativa de MLH1 y MGMT (sí > 11 %, no < 11% o pobremente 11-15%).

3.4.1.5 Selección de muestra de cohorte TCGA y descarga de datos

Como cohorte de validación en este estudio, se utilizaron datos de la base de datos The Cancer Genomic Atlas (TCGA), en particular con respecto al carcinoma de células escamosas de cabeza y cuello (115). La consulta estaba restringida a los COCE primarios. Los datos de las muestras se recuperaron del GDAC Broad Institute (https://gdac.broadinstitute.org/ , consultado el 15 de marzo de 2022). Después de esta recopilación, los datos fueron catalogados y verificados manualmente en función del sitio primario de aparición del tumor para excluir a los pacientes sin COCE, como se mencionó anteriormente. Después de la filtración, quedaron 342 casos que fueron descargados directamente del servidor e ingresados al cBioPortal for Cancer Genomics (http://www.cbioportal.org, consultado el 17 de marzo de 2022). A continuación, se recopilaron los datos de metilación para MGMT y MLH1 del TCGA derivados del ensayo Human Methylation-450 Bead Chip. Para los genes con múltiples islas CpG, como en el caso de MGMT, solo se eligieron para análisis posteriores los datos de metilación de la sonda con la correlación negativa más fuerte entre la señal de metilación y la expresión génica. Con el fin de identificar el estado de metilación dicotomizado (es decir, metilado o no) para la cohorte TCGA, se realizó un clustering k-means usando valores beta para sitios CpG seleccionados según lo informado inicialmente por el programa TCGA (115).

3.4.1.6 Análisis estadístico

Los datos se recopilaron y analizaron como se describe en el apartado 3.1.1.6. Del mismo modo se describieron las variables categóricas y cuantitativas, así como se utilizaron tablas de contingencia y la prueba chi-cuadrado. Para describir las diferencias de medias se

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utilizó la prueba t de Student o el análisis de varianza de una vía ANOVA, según la distribución de los datos. Se crearon gráficos de supervivencia basadas en curvas de Kaplan-Meier y sus correspondientes log-rank. Para realizar este análisis se utilizó el software SPSS v.24.0 (IBM, Statistics, NY, EE. UU.) para Windows.

Todos los datos sin procesar obtenidos de la base de datos TCGA se ingresaron en el software R v.4.1.0 y su paquete JMV para realizar análisis estadísticos de correlación con la supervivencia global (en meses) tanto para para la metilación de MGMT como para la de MLH1 en la cohorte. Además, se realizó un análisis de correlación para comprobar la exclusividad mutua de la metilación de ambos genes. Para lograr exhaustividad, se presentaron ambos tipos de estimaciones de correlación (es decir, las pruebas de correlación de Spearman y Pearson), aunque se consideró que el coeficiente de Spearman era la figura más apropiada según los constructores de las bases de datos. Se realizaron modelos de regresión lineal múltiple para evaluar más formalmente los resultados. Por último, teniendo en cuenta el análisis post hoc de nuestra cohorte institucional, optamos por realizar un análisis bioinformático para determinar las vías de neoplasias implicadas en la metilación de MGMT y MLH1. La generación de gráficos se realizó utilizando el servicio implementado en la aplicación en cBioPortal. El nivel de significanción establecido para los resultados fue $p \leq 0,05$.

3.4.2. Artículo 8. Predictive value of CDKN2A/p16^{INK4a} expression in the malignant transformation of oral potentially malignant disorders: Systematic review and meta-analysis.

3.4.2.1 Protocolo y registro

Esta revisión sistemática y metaanálisis se realizó siguiendo las pautas PRISMA-P (116) (Figura 10), y se registró en PROSPERO (ID: CRD42022355931).

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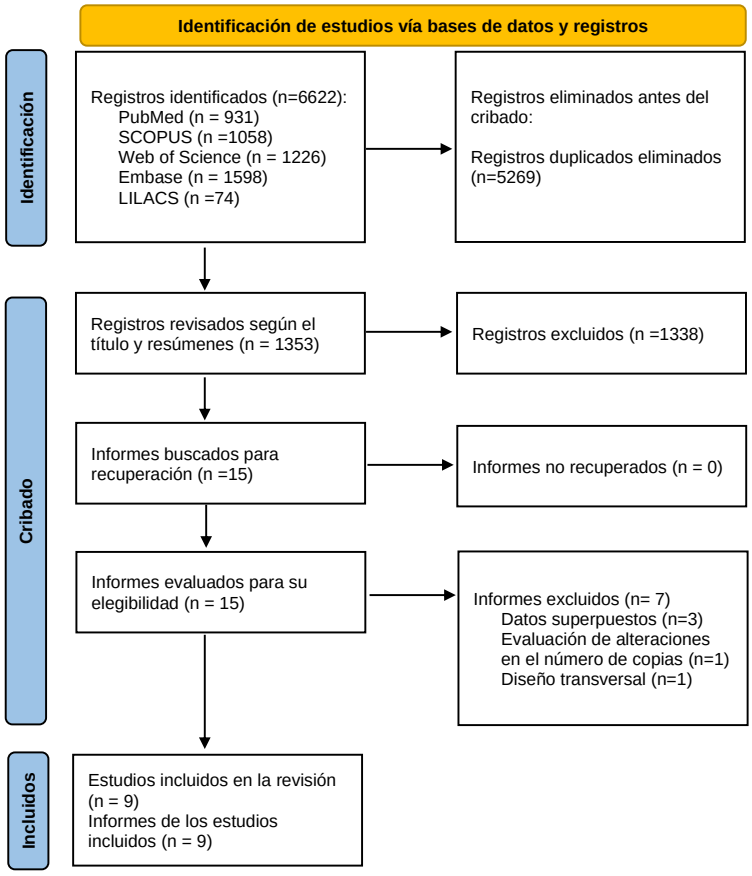


Figura 10. Diagrama de flujo PRISMA de los procesos de búsqueda.

3.4.2.2 Pregunta de investigación

La pregunta planteada fue: ¿Cuál es la importancia de la expresión de CDKN2A/p16^{INK4a} en la predicción del riesgo de TM en pacientes con OPMDs?

La pregunta fue abordada utilizando la metodología PICO: (1) P (población): Pacientes diagnosticados con OPMDs (con o sin DE). (2) I (intervención): Evaluación de CDKN2A/p16^{INK4a} mediante métodos objetivos y cuantitativos, en muestras de biopsia de la población en estudio. (3) C (comparación): El grupo de control estará representado por el grupo de pacientes de la población de estudio con baja expresión de CDKN2A/p16^{INK4a}. (4) Las diferencias en la expresión de CDKN2A/p16^{INK4a} se clasificarán como sobreexpresión para el grupo de exposición y baja expresión para el grupo de control, según el valor de corte elegido por los autores. (5) O (resultado): progresión a COCE.

3.4.2.3 Estrategia de búsqueda

Dos de los autores realizaron búsquedas de forma independiente en PubMed a través de Medline, Scopus, EMBASE, Web of Science y LILACS desde su inicio hasta diciembre de 2022 sin restricciones de idioma. Se agruparon una combinación de términos tesauros específicos para cada base de datos y palabras de texto libre a través de operadores booleanos para construir nuestra estrategia de búsqueda. La siguiente sintaxis se aplicó en PubMed: "Tumor Suppressor

Protein p16"[MeSH Terms] OR " Genes, p16"[MeSH Terms] OR "p16"[All Fields] OR "CDKN2A"[All Fields]) AND ("mouth"[MeSH Terms] OR "mouth"[All Fields] OR "oral"[All Fields] OR oropharyn*[All Fields]) AND ("carcinoma, squamous cell"[MeSH Terms] OR ("carcinoma"[All Fields] AND "squamous"[All Fields] AND "cell"[All Fields]) OR "squamous cell carcinoma"[All Fields] OR "dysplasia"[All Fields] OR "potentially malignant disorders"[All Fields] OR premalign* [All Fields] OR precancer* [All Fields] OR "leukoplakia"[All Fields] OR "erythroplakia"[All Fields] OR "lichen planus"[All Fields] OR "submucous fibrosis"[All Fields]) AND ("transformation"[All Fields] OR "progression"[All Fields] OR "risk"[All Fields]). La sintaxis se modificó y optimizó convenientemente para cada base de datos (Tabla 3). Para maximizar la sensibilidad, las referencias de las revisiones relacionadas y las de los estudios incluidos se verificaron exhaustivamente para complementar la búsqueda manual en la base de datos.

Tabla 3. Sintaxis para la estrategia de búsqueda en cada base de datos en el artículo 8.

Base de datos	Búsqueda
Pubmed	("Tumor Suppressor Protein p16"[MeSH Terms] OR " Genes, p16"[MeSH Terms] OR "p16"[All Fields] OR "CDKN2A"[All Fields]) AND ("mouth"[MeSH Terms] OR "mouth"[All Fields] OR "oral"[All Fields] OR oropharyn*[All Fields]) AND ("carcinoma, squamous cell"[MeSH Terms] OR ("carcinoma"[All Fields] AND "squamous"[All Fields] AND "cell"[All Fields]) OR "squamous cell carcinoma"[All Fields] OR "dysplasia"[All Fields] OR "potentially malignant disorders"[All Fields] OR premalign*[All Fields] OR precancer*[All Fields] OR "leukoplakia"[All Fields] OR "erythroplakia"[All Fields] OR "lichen planus"[All Fields] OR "submucous fibrosis"[All Fields]) AND ("transformation"[All Fields] OR "progression"[All Fields] OR "risk"[All Fields]).
Scopus	TITLE-ABS-KEY(("p16" OR " CDKN2A") AND ("mouth" OR "oral" OR "oropharyn*") AND ("squamous cell carcinoma") AND ("transformation" OR "progression" OR "risk"))
Embase	('protein p16'/exp OR 'p16 signaling'/exp OR 'p16' OR 'CDKN2A') AND ('mouth' OR 'oral' OR 'oropharyn*') AND ('squamous cell carcinoma'/exp OR 'squamous cell carcinoma' OR ('squamous' AND ('cell'/exp OR 'cell') AND ('carcinoma'/exp OR 'carcinoma')) OR 'dysplasia' OR 'potentially malignant disorders' OR 'premalign*' OR 'precancer'/exp OR 'precancer*' OR 'leukoplakia'/exp OR 'leukoplakia' OR 'erythroplakia' OR 'erythroplakia' OR 'lichen planus'/exp OR 'lichen planus' OR 'submucous fibrosis') AND ('transformation' OR 'progression' OR 'risk')
Web of science	TS=(p16 OR CDKN2A) AND TS=(mouth OR oral OR oropharyn*) AND TS=("squamous cell carcinoma") AND TS=(transformation OR progression OR risk)
LILACS	tw:(precancerous OR "pre-cancer" OR precancer OR "precursor lesion" OR "precursor lesions" OR premalignan$ OR "pre malignant" OR "pre malignancy" OR "pre malignancies" OR leukoplakia OR Leukoplakias OR leukoplakic OR keratosis OR keratoses OR leukokeratosis OR leukokeratosis OR erythroplasia OR erythroplasias OR erythroplakia OR erythroplakias OR "Carcinoma in situ" OR "in situ carcinoma" OR "preinvasive carcinoma" OR "preinvasive carcinomas" OR "pre-invasive carcinoma" OR "pre-invasive carcinomas" OR "intraepithelial carcinoma" OR "intraepithelial carcinomas" OR "intraepithelial neoplasm" OR "intraepithelial neoplasms" OR "intraepithelial neoplasia" OR "intraepithelial neoplasias" OR "intraepithelial cancer" OR "Submucous Fibrosis" OR cheilitis OR "actinic cheilosis" OR "condições pré-cancerizáveis" OR "condiciones precancerosas" OR precancerizáv$ OR precanceroza$ OR "lesões epiteliais precursoras" OR "lesiones precursoras epiteliales" OR "lesiones percursoras de malignidade" OR "pré-cancer" OR precáncer OR "lesões precursoras" OR "lesiones percursoras" OR pré-maligna OR premaligna$ OR "pré-malignas" OR leucoplasia$ OR keratosis OR ceratose$ OR queratose$ OR keratoses OR leucoceratose$ OR leucoqueratose$ OR eritroplasia$ OR "carcinoma pré-invasivo" OR "carcinoma preinvasiva" OR "carcinoma intra-epitelial" OR "carcinoma intra-epitelial" OR "neoplasia intra-epitelial" OR "neoplasia intraepitelial" OR "fibromatose submucosa oral" OR "fibromatosis submucosa oral" OR "fibromatoses submucosas orais" OR queilite OR queilose) AND tw:("malignant" OR "malignent" OR "maligna" OR "malignas" OR "maligno" OR "malignos" OR "evolution" OR "evolução" OR "evolución" OR "progression" OR "progressão" OR "progresión" OR "avance" OR "avanço" OR "transformation" OR "transformação" OR "transformación" OR "prognosis" OR "prognostic" OR "prognoses" OR "prognóstico" OR "pronóstico" OR "prognósticos" OR "pronósticos")

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3.4.2.4 Criterios de elegibilidad

Para ser elegibles para su inclusión, los estudios tenían que: (I) evaluar las alteraciones en la expresión de la proteína CDKN2A/p16^{INK4a} mediante inmunohistoquímica (IHQ) en biopsias de OPMDs, (II) correlacionarlas con su transformación maligna, incluidos datos sobre la TM en estudios con un diseño longitudinal, y (III) proporcionar el HR o RR con sus correspondientes IC del 95% como medida de asociación, o al menos datos suficientes para su cálculo. En el caso de resultados derivados de una población de estudio superpuesta, se priorizó el informe que proporcionara más datos.

Los criterios de exclusión fueron: (I) estudios que incluyeran muestras heterogéneas con trastornos potencialmente malignos que no pertenecen a la cavidad oral, (II) estudios retirados, artículos de revisión, informes de casos, comentarios, resúmenes de reuniones científicas, cartas o capítulos de libros, (III) estudios basados en animales o modelos in vitro, (IV) evaluación de alteraciones del gen CDKN2A y (V) estudios transversales.

3.4.2.5 Selección y extracción de datos

En un proceso de dos pasos, los observadores evaluaron de forma independiente los informes recuperados. Primero, se verificaron los títulos y resúmenes seleccionados en la búsqueda inicial. Luego, aquellos que aparentemente cumplían con los criterios establecidos fueron leídos exhaustivamente para llegar a una decisión sobre su inclusión o exclusión final. La fiabilidad entre evaluadores se evaluó mediante el estadístico κ. Finalmente se obtuvo κ = 0,92. Los desacuerdos entre los revisores se resolvieron cuidadosamente mediante discusión o con la inclusión de un tercer participante. Toda la gestión de datos se realizó con Mendeley Desktop v1.19.8 (Elsevier, Londres, Reino Unido).

Tres autores extrajeron por separado los datos de cada registro admitido en la selección final utilizando una hoja de prueba piloto, con el software Excel v.2021 (Microsoft. Redmond, WA, EE. UU.). De cada informe incluido, se extrajeron los siguientes datos: primer autor, año de publicación, país y continente del estudio, tamaño de la muestra, sistema y clasificación de la DE, tipo de OPMD, período de reclutamiento, tiempo de seguimiento, evaluación de la alteración CDKN2A/p16^{INK4a}, presencia/ausencia de VPH y método de evaluación, metodología aplicada, frecuencia de alteración, medida del efecto y su ajuste. Para informes de IHQ, también se recogieron el punto de corte para positividad, el anticuerpo anti-P16^{INK4a} y el patrón de IHQ celular utilizado. Las diferencias en los niveles de expresión de CDKN2A/p16^{INK4a} se categorizaron como positivas o negativas según el valor de corte predefinido de cada documento principal. Se investigó el valor RR de un estudio obteniendo una respuesta que permitió la inclusión (117).

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3.4.2.6 Evaluación de calidad y riesgo de sesgo

Se implementó la herramienta QUIPS para evaluar el RoB de los estudios incluidos (118). Esta herramienta consta de seis dominios: (1) Muestras: a) Estudio de cohorte (retrospectivo o prospectivo) con una población de estudio bien definida; b) Se explicó el tratamiento médico aplicado a los pacientes. Los autores explicaron si todos los pacientes recibieron el mismo tratamiento o no; (2) Datos clínicos de la cohorte: Se proporcionaron los datos clínicos básicos como edad, sexo, estadio clínico y grado histopatológico; (3) Inmunohistoquímica: protocolo de tinción bien descrito o referido al artículo original; (4) Pronóstico: Los criterios de valoración de supervivencia analizados estaban bien definidos; (5) Estadística: a) Se describió bien el punto de corte, que se utiliza para dividir los casos en grupos de riesgo; b) se proporcionó el efecto estimado que describe la relación entre el biomarcador evaluado y el resultado; c) Se realizó un análisis estadístico adecuado (p. ej., modelo de regresión de Cox) para ajustar la estimación del efecto del biomarcador para factores pronósticos conocidos; (6) Factor pronóstico clásico: Se informó el valor pronóstico de otros factores pronósticos clásicos y su relación con el factor estudiado.

El RoB para cada elemento se asignó como bajo o alto. La evaluación de RoB fue realizada de forma independiente por seis autores, con cada dominio calificado por un mínimo de dos autores. Dos participantes realizaron RoB y los desacuerdos se resolvieron por consenso. También se asignó una calificación general a los estudios individuales con fines estadísticos (es decir, para explorar la posible influencia de la calidad/riesgo de sesgo en las estimaciones agrupadas).

3.4.2.7 Análisis estadístico

Los Risk Ratios (RR) y sus correspondientes IC del 95 % se obtuvieron directamente de los estudios primarios como medida para evaluar la relación entre la sobreexpresión de CDKN2A/P16^{INK4a} y la TM de los OPMD. Se recuperaron tanto los RR multivariados como univariados pero, cuando estaban disponibles, se eligieron los multivariados para un análisis adicional. Cuando no se informaron datos de RR, los HR se tomaron como una aproximación de esta medida (119). En nuestro análisis, los RR específicos del estudio se ponderaron por el inverso de su varianza para calcular los RR agrupados con sus IC del 95 %. Los análisis agrupados se obtuvieron utilizando el método de Mantel-Haenszel y el método de DerSimonian y Laird. En nuestro cálculo, la alta heterogeneidad justificó el uso de modelos de varianza inversa. Por otro lado, se aplicaron modelos de efectos fijos en escenarios de heterogeneidad no significativa. Se generaron diagramas de bosque para mostrar gráficamente los resultados.

La heterogeneidad se evaluó con la prueba Q de Cochran, utilizando $p < 0,05$ como umbral significativo. También se utilizó el índice de Higgins I^2 con los puntos de corte descritos en el apartado 3.3.1.7 (109). Posteriormente, se estudió el origen de la

heterogeneidad mediante análisis de subgrupos teniendo en cuenta las características predefinidas relevantes del estudio (es decir, tipo de OPMD, continente, anticuerpo anti-$P16^{INK4a}$, punto de corte, ajuste, patrón inmunohistoquímico, evaluación de la calidad). Se construyó un diagrama de Galbraith para evaluar la contribución relativa de cada estudio individual a la heterogeneidad global de manera más formal. También se utilizó este método para comprobar la posible presencia de valores atípicos estadísticos (120).

El sesgo de publicación también se evaluó mediante gráficos de embudo. Estadísticamente se utilizó la prueba de regresión propuesta por Egger, así como la prueba de correlación de rangos de Begg y Mazumdar. También se utilizó el método de "trim-and-fill" propuesto por Duval et al. (121). Se emplearon Reviewer Manager 5.4 (Colaboración Cochrane, Copenhague, Dinamarca) y Stata 16.1 (Stata Corp, College Station, TX, EE. UU.) para la gestión de datos. Se consideró significativo un p-valor < 5%.

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4 DISCUSIÓN

4.1 TRASTORNOS RELACIONADOS CON SUSTANCIAS Y SU IMPACTO EN ESTUDIANTES DE MEDICINA Y ODONTOLOGÍA

Los comportamientos de riesgo para la salud, como el consumo de alcohol y drogas, son una preocupación relevante en la formación de futuros profesionales de la salud, como son los estudiantes de medicina y odontología. El primer paso para abordar este problema es comprender la magnitud del consumo y sus consecuencias. Los estudios 1 y 2 (cuyos datos descriptivos pueden observarse en las Tablas 4 y 5) proporcionan una visión crítica de esta cuestión en la USC.

Tabla 4. Datos descriptivos de la muestra del primer artículo.

Variables		N (%)
Género	Masculino	134 (26,8)
	Femenino	361 (72,2)
	Otro	5 (1)
Facultad	Odontología	167 (33,4)
	Medicina	333 (66,6)
Año de estudio	1	259 (51,8)
	2	37 (7,4)
	3	39 (7,8)
	4	34 (6,8)
	5	78 (15,6)
	6	35 (7)
	Posgrado	18 (3,6)
Ciclo	Primer ciclo	335 (67)
	Segundo ciclo	112 (22,4)
	Posgrado	53 (10,6)
CAGE	Test negativo	228 (45,7)
	Bebedor social	121 (24,2)
	Alto riesgo	98 (19,6)
	Consumo nocivo de alcohol	48 (9,6)
	Dependencia de bebidas alcohólicas	4 (0,8)
AUDIT-C	Riesgo bajo	70 (14,1)
	Alto riesgo	428 (85,9)
Etapa de cambio	Precontemplación	12 (2,6)
	Contemplación	100 (21,6)
	Acción	82(17,7)
	Cambio	270 (58,2)
Conocimiento de patología general	Suspenso	265 (53)
	Aprobado	127 (25,4)
	Bien	0
	Notable	89 (17,8)
	Sobresaliente	19 (3,8)
Conocimientos de patología oral	Suspenso	325 (65)
	Aprobado	86 (17,2)
	Bien	0
	Notable	66 (13,2)
	Sobresaliente	23 (4,6)

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Tabla 5. Datos descriptivos del segundo artículo.

Variables categóricas		N (%)
Datos demográficos		
Género	Hombre	248 (25,8)
	Mujer	702 (73,1)
	Otro	10 (1)
Facultad	Odontología	222 (23,1)
	Medicina	737 (76,9)
Curso	Primero	224 (23,3)
	Segundo	283 (29,4)
	Tercero	133 (13,8)
	Cuarto	83 (8,6)
	Quinto	98 (10,2)
	Sexto	108 (11,2)
	Posgrado	31 (3,2)
Ciclo	Primer ciclo	507 (52,8)
	Segundo ciclo	314 (32,7)
	Posgrado	139 (14,5)
Consumo tabaco	No	810 (84,5)
	Sí	149 (15,5)
CAST	Puntuación 0-1	861 (89,5)
	Puntuación 2-3	43 (4,5)
	Consumo problemático	58 (6)
DAST-20	Consumo no problemático	747 (77,7)
	Consumo problemático	214 (22,3)
Percepción riesgo		
Fumar 1 paquete cigarros al día	No peligrosa	9 (0,9)
	Poco peligrosa	47 (4,9)
	Peligro moderado	323 (33,7)
	Muy peligrosa	580 (60,5)
Fumar marihuana regularmente	No peligrosa	6 (0,6)
	Poco peligrosa	75 (7,8)
	Peligro moderado	430 (44,9)
	Muy peligrosa	447 (46,7)
Consumir éxtasis el fin de semana	No peligrosa	3 (0,3)
	Poco peligrosa	14 (1,5)
	Peligro moderado	201 (21,1)
	Muy peligrosa	736 (77,1)
Consumir LSD una vez al mes	No peligrosa	10 (1)
	Poco peligrosa	66 (6,9)
	Peligro moderado	284 (29,8)
	Muy peligrosa	593 (62,2)

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Variables categóricas		**N (%)**
2 bebidas alcohólicas al día	No peligrosa	35 (3,7)
	Poco peligrosa	281 (29,4)
	Peligro moderado	447 (46,7)
	Muy peligrosa	194 (20,3)
4 bebidas alcohólicas/ocasión	No peligrosa	37 (3,9)
	Poco peligrosa	266 (27,7)
	Peligro moderado	403 (42)
	Muy peligrosa	253 (26,4)
Actitudes y conocimientos		
Pregunta sobre el consumo de estupefacientes en la historia clínica	1	34 (4)
	2	58 (6,9)
	3	148 (17,6)
	4	170 (20,2)
	5	431 (51,2)
Identifica consumidores y asesora	1	9 (1)
	2	32 (3,6)
	3	89 (10,1)
	4	236 (26,8)
	5	516 (58,5)
Aconseja sobre consumo y efectos	1	31 (3,6)
	2	107 (12,3)
	3	236 (27,1)
	4	199 (22,8)
	5	298 (34,2)
Conoce herramientas screening	1	264 (30)
	2	246 (27,9)
	3	212 (24,1)
	4	91 (10,3)
	5	68 (7,7)
Etapa de cambio		
Etapa de cambio	Precontemplación	17 (2,3)
	Contemplación	156 (20,7)
	Acción	81 (10,8)
	Mantenimiento	498 (66,2)
Variables cuantitativas		
	Media (DS)	Mínimo-Máximo
Sumatorio DAST-20	3,14 (2,15)	0-15
Sumatorio CAST	0,17 (0,72)	0-6
Edad inicio tabaco	16.70 (2.05) años	12-24 años
N cigarros/día	4.04 (3.91)	0-20
Gramos cannabis/mes	4.91 (7.53)	0,5-35

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4.1.1 Consumo

Los artículos 1 y 2 revelaron estadísticas preocupantes, con una proporción significativa de estudiantes que padecen trastorno por consumo de alcohol y consumen drogas, especialmente a medida que avanzan en sus ciclos académicos. Los resultados destacaron que el 75,5% de los bebedores de riesgo de nuestra muestra eran mujeres, frente al 24,1% de los hombres, lo que se consideró una diferencia estadísticamente significativa (p <0,001) (Tabla 6). El género femenino constituyó un factor de riesgo independiente para AUD (OR=2,63, IC 95% 1,55-4,45, p <0,001).

Tabla 6. Análisis de riesgo de consumo según género, facultad, año de estudio y ciclo del artículo 1.

		Bebedor de riesgo N (%)			
		Bajo riesgo	Alto riesgo	Total	p-valor
Género‡	Masculino	31 (44,3)	103 (24,1)	134 (26,9)	<0,001**
	Femenino	37 (52,9)	323 (75,5)	360 (72,3)	
	Otro	2 (2,9)	2 (0,5)	4 (0,8)	
Facultad†	Odontología	26 (37,1)	141 (32,9)	167 (33,5)	0,490
	Medicina	44 (62,9)	287 (67,1)	331 (66,5)	
Año de estudio‡	1	38 (54,3)	220 (51,4)	258 (51,8)	0,437
	2	7 (10)	30 (7)	37 (7,4)	
	3	2 (2,9)	37 (8,6)	39 (7,8)	
	4	7 (10)	27 (6,3)	34 (6,8)	
	5	11 (15,7)	66 (15,4)	77 (15,5)	
	6	4 (5,7)	31 (7,2)	35 (7)	
	Postgrado	1 (1,4)	17 (4)	18 (3,6)	
Ciclo‡	Primer ciclo	47 (67,1)	287 (67,1)	334 (67,1)	0,505
	Segundo ciclo	18 (25,7)	93 (21,7)	111 (22,3)	
	Postgrado	5 (7,1)	48 (11,2)	53 (10,6)	

p-valor de Chi-cuadrado† y prueba ANOVA‡.

Esta alta prevalencia de consumo de alcohol también ha sido reportada entre estudiantes de ciencias de la salud en España por Rabanales Sotos et al. (122). Nuestros resultados son comparables con los datos recogidos en Europa: Irlanda (65,2% de hombres y 67,3% de mujeres presentaron AUD) (123) y Reino Unido (86,1% consumidores y más de la mitad presentaron AUD) (124) ; incluso a nivel mundial en Túnez el 52,5% de los estudiantes presentó AUD (125) , en Ecuador el 49,73% de los hombres y 23,80% de las mujeres reportaron AUD (126), y Australia (casi el 25% presenta consumo de alcohol de riesgo, mayor en el caso de los hombres) (127). Sin embargo, al comparar en profundidad los resultados, observamos que los datos sobre la prevalencia del consumo “de riesgo” son heterogéneos debido a la variabilidad de las definiciones utilizadas por los autores, las

herramientas de medición y los umbrales establecidos. En nuestra opinión, se deben establecer protocolos y pautas estándar globales para determinar el riesgo de AUD.

En el segundo artículo el 22,3% de los participantes presentó un consumo problemático de drogas, siendo el cannabis la sustancia más consumida. El cannabis es la tercera droga más consumida en todo el mundo, después del alcohol y el tabaco, y se estima que 192 millones de personas consumieron cannabis en 2018 (8). En nuestra encuesta, el 45% de los participantes había probado cannabis al menos una vez, aunque cabe señalar que los estudiantes de odontología y medicina tienen características diferentes en cuanto a carga de trabajo y contenido curricular. Se trata de un porcentaje superior a los resultados de un metaanálisis reciente en el que esta cifra representaba el 31,4% a nivel mundial (128). Entre estos estudiantes, el consumo de drogas varió y aumentó con la edad según lo evaluado por las pruebas DAST y CAST, observándose un consumo más problemático a medida que avanza el ciclo académico ($p < 0,001$). Los participantes del segundo ciclo presentaron mayor consumo que los del primer ciclo, en el modelo univariado (OR = 1,77, IC 95% 1,27-2,48, $p = 0,001$) y en el modelo ajustado (OR = 1,86, IC 95% 1,32 -2,62, $p < 0,001$). Respecto al CAST, el consumo no problemático en el primer ciclo vs. el tercer ciclo presentó un OR = 8,69 (IC 95% 4,50-16,78, $p < 0,001$) y para el consumo de bajo riesgo presentó un OR = 15,18 (IC 95% 1,83- 14.68). En nuestro estudio, la importancia del curso académico fue un descubrimiento novedoso. Esto podría proporcionar una base para establecer el punto en el que deben tener lugar las intervenciones para prevenir los riesgos crecientes resultantes del uso de sustancias.

4.1.2 Actitudes y percepciones, nivel de confianza y conocimientos

A pesar de que el consumo de estas sustancias entre estudiantes de medicina y odontología es común, se observó que a menudo carecen de conocimientos sobre las consecuencias de su consumo, tal y como se muestra en las Figuras 11,12 y 13, lo cual puede afectar directamente al ODS 4 (Garantizar una educación inclusiva, equitativa y de calidad para todos).

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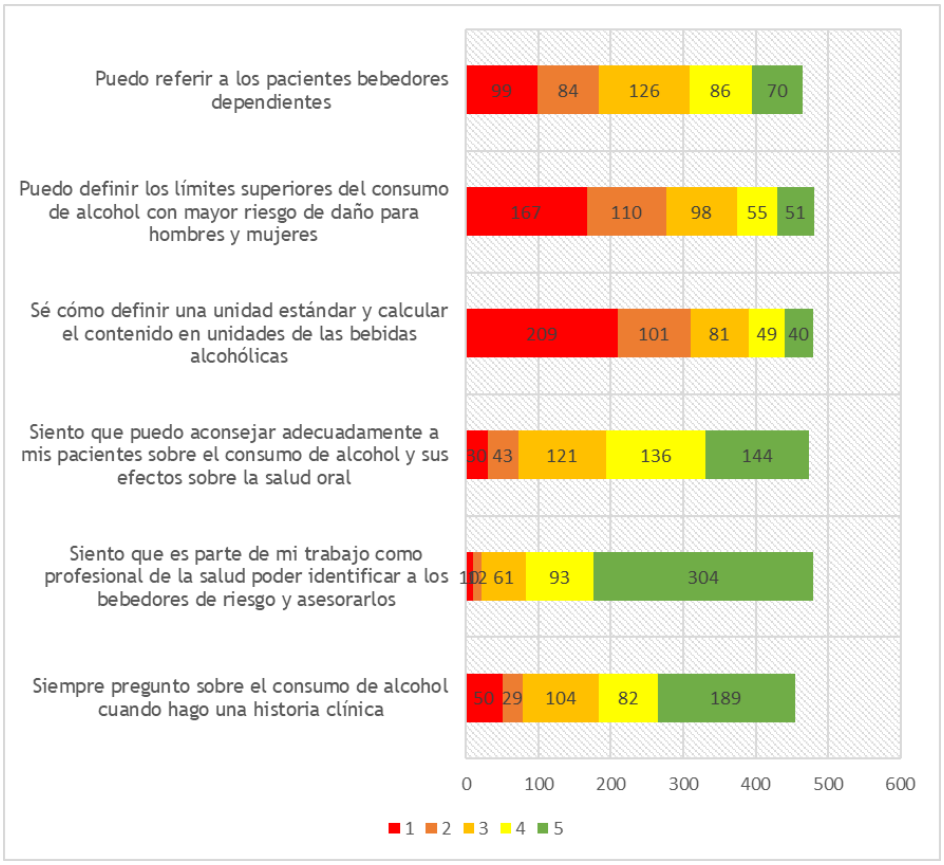


Figura 11. Actitudes y percepciones. 1 totalmente en desacuerdo - 5 totalmente de acuerdo.

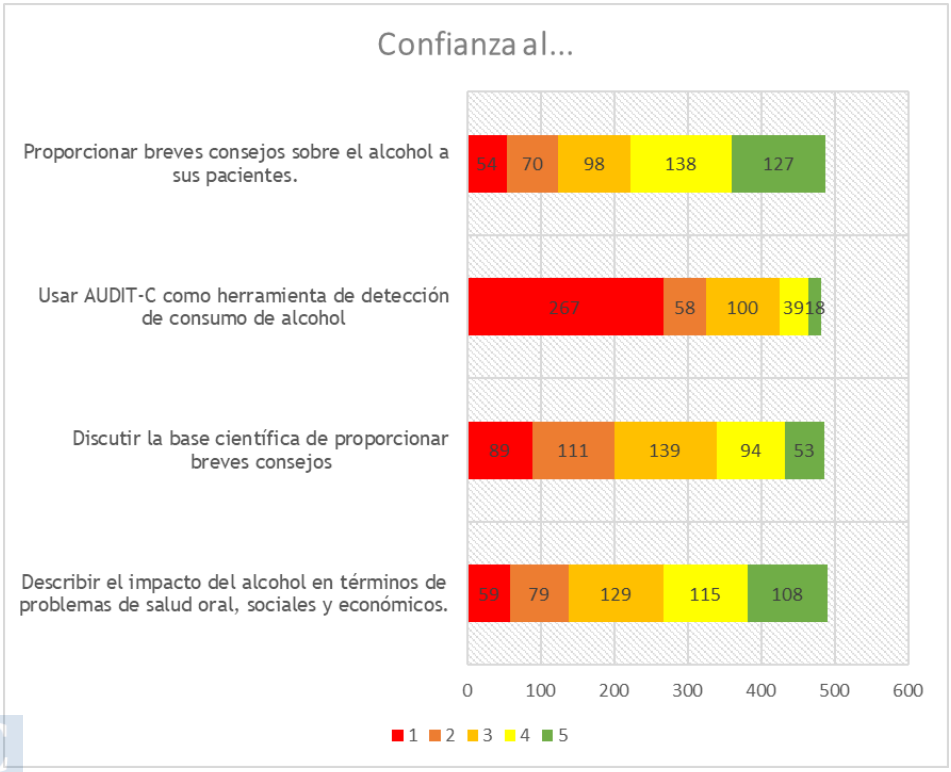


Figura 12. Nivel de confianza. 1 totalmente en desacuerdo - 5 totalmente de acuerdo.

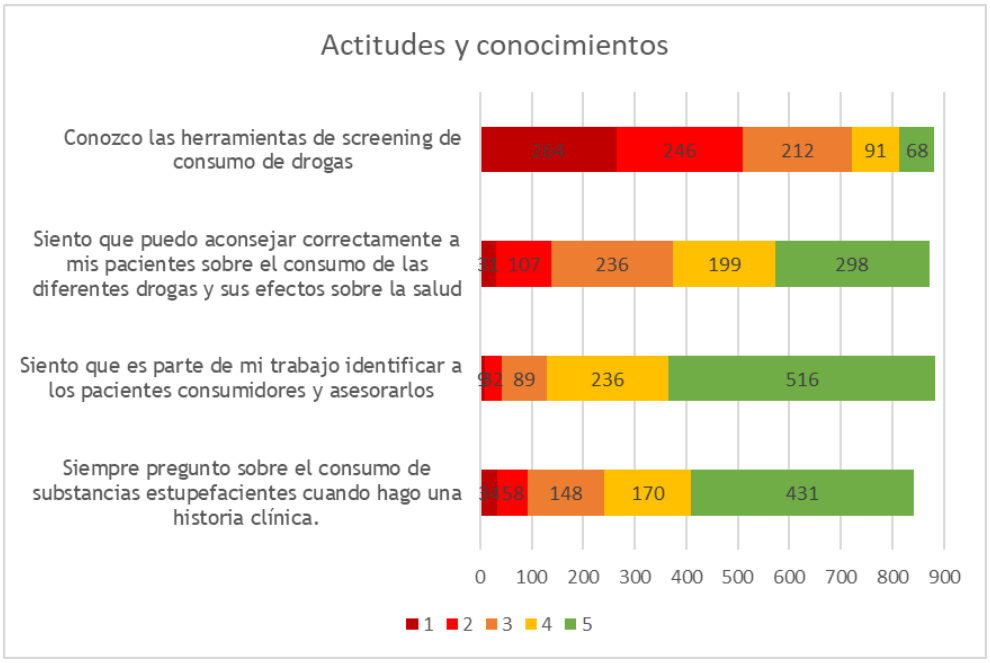


Figura 13. Actitudes y conocimientos de los profesionales sanitarios.

En nuestro estudio, el 85,3% de los estudiantes (87,5% en medicina y 77,8% en odontología) cree que identificar y asesorar a los consumidores de drogas forma parte de su trabajo. Esta cifra es superior a la observada en un estudio realizado a odontólogos, en el que se determinó que sólo la mitad de ellos (53,9%) consideraba que dichas tareas forman parte de su trabajo (14). Priyadarshini et al. también observaron que los dentistas estaban de acuerdo en que debían identificar a los pacientes que consumían drogas (15). Goel et al. (16) afirmaron que la mayoría de los odontólogos mostraron una actitud positiva hacia dichos pacientes y coincidieron en que los usuarios problemáticos deben ser identificados y tratados en el ambiente odontológico. El 71,4% de los estudiantes pregunta o es consciente de que debe preguntar sobre el consumo de sustancias al realizar la historia clínica de sus pacientes, porcentaje similar al observado por Parish et al. (77,2%) (14). Sin embargo, sólo el 18% afirmó conocer las herramientas de detección del consumo de drogas, cifra que coincide con los estudios realizados por otros autores, en los que se ha determinado que a pesar de que los odontólogos conocen la importancia de detectar el consumo de drogas, no cuentan con los conocimientos específicos, la formación clínica y las herramientas necesarias para impulsar esas estrategias de intervención (15,16).

4.1.3 Percepción del riesgo

La falta de conciencia sobre los riesgos asociados con el consumo de alcohol y drogas entre estos futuros profesionales resulta preocupante, tal y como se observa en la Figura 14.

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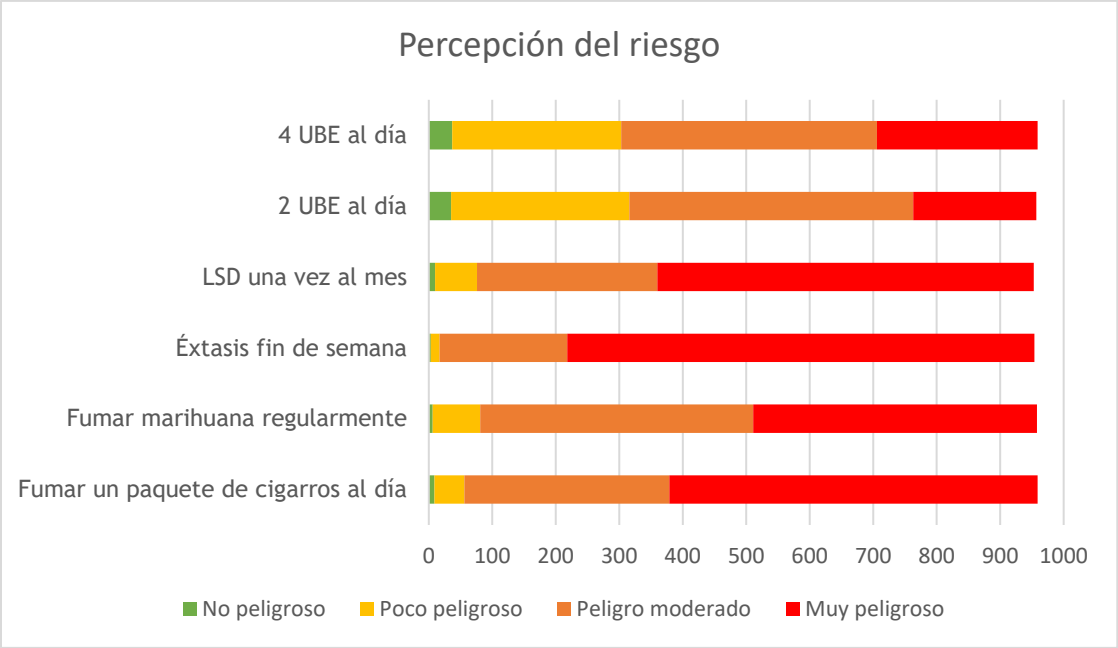


Figura 14. Riesgo percibido asociado al consumo de drogas.

El 46,7% consideró consumir marihuana de forma habitual como muy peligroso y el 60,5% afirmó que fumar una cajetilla de cigarrillos representa una conducta muy peligrosa. Nuestros hallazgos muestran que pocos participantes consideraron el consumo excesivo de alcohol y cannabis como un comportamiento peligroso, como también se observó en el estudio de Calafat et al. (99). Tal y como Kilmer et al. (129) sugirieron, la percepción de riesgo puede ser un factor relevante con respecto a los participantes que inician el consumo de sustancias.

4.1.4 Conocimientos

En general, el conocimiento de los participantes sobre la morbilidad del alcohol fue bajo. Nuestros resultados fueron similares a los del estudio publicado por Wang et al. en 2020 (130) en el que la mayoría de los estudiantes de salud en Mongolia demostraron un conocimiento muy limitado sobre los daños causados por el alcohol. En cuanto a la patología sistémica, los conocimientos de más de la mitad de los estudiantes de ambas facultades se consideraron no satisfactorios, sin embargo, los resultados mostraron mejores niveles de conocimiento entre los participantes en los últimos años de sus estudios ($p < 0,001$). Los estudiantes de odontología demostraron mayores deficiencias en su conocimiento de patología general, mientras que los estudiantes de medicina mostraron peor conocimiento de patología oral ($p < 0,001$). En la Figura 15 pueden observarse los datos relacionados con las materias específicas de cada campo. Las principales patologías que los estudiantes asociaron con el consumo de alcohol fueron las neuropatías (n=399), seguidas de cáncer oral (n=365) y caries (n=343).

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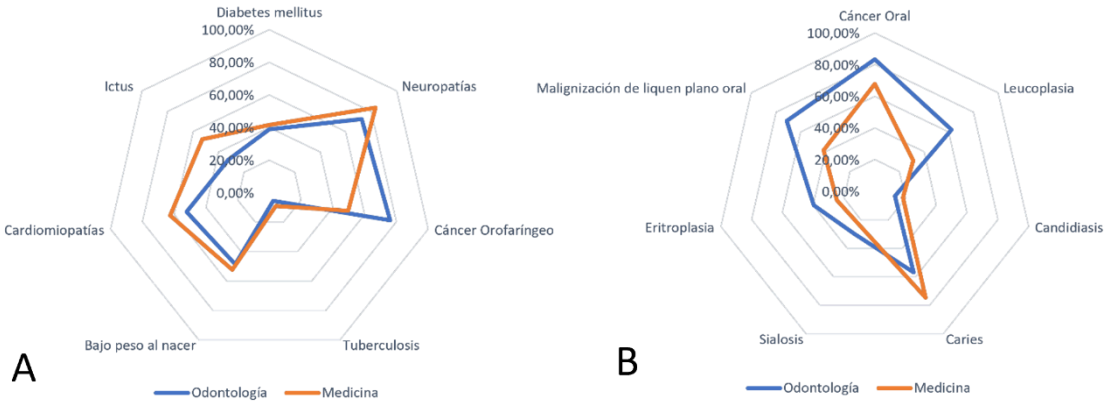


Figura 15. Diagrama de araña sobre el conocimiento de la morbilidad alcohólica: A) Patología general, B) Patología oral.

4.1.5 Etapa de cambio

En relación con la etapa de cambio en la que se encuentran a la hora de brindar asistencia a los pacientes, según lo descrito por Daley et al. (28), la mayoría de nuestros encuestados en el primer artículo se encontraban en una etapa de cambio en ese momento (58,2%), encontrándose en esta situación un porcentaje significativamente mayor de estudiantes de medicina que de odontología (63,3 % vs. 49,1 %, p <0,001). Ramos y Perkins estudiaron los estadios de cambio en relación con el fomento del abandono del hábito y recomendaron adaptar las estrategias al estadio de cambio en el que se encontraban, sugiriendo utilizar técnicas cognitivas, afectivas y de evaluación en los estadios iniciales (precontemplación y contemplación) para que puedan progresar a través de las etapas (131).

En el segundo artículo observamos que el 66,2% ayuda a sus pacientes consumidores a dejar de consumir, teniendo las mujeres más probabilidad de estar en esta etapa (68,6% vs. 59,9% p = 0,012). Los estudiantes de odontología se encontraban en etapas más tempranas que los estudiantes de medicina. Hasta donde sabemos, no se han realizado más estudios que aborden este tema.

Los estudiantes de medicina y odontología no solo son modelos a seguir para sus pacientes, sino que también pueden desempeñar un papel fundamental en la prevención y el asesoramiento sobre el uso de sustancias nocivas. Esto enfatiza la necesidad de incorporar programas de capacitación específicos por género que aborden de manera efectiva estos problemas y preparen a los estudiantes para enfrentar y prevenir el consumo de sustancias tóxicas en sus pacientes, contribuyendo así a la consecución del ODS 3 (3).

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4.2 XEROSTOMÍA Y SU IMPACTO EN LA SALUD ORAL Y GENERAL

La xerostomía es un fenómeno común, especialmente en la población geriátrica. Los artículos 3 y 4 destacan tanto su prevalencia como los factores asociados a este síntoma.

4.2.1. Comunidad autónoma de Galicia

Los resultados de este estudio revelan que el 30,7% de los participantes experimentaron xerostomía, similar a la reportada por otros autores, que la sitúan entre el 20 y el 30% (132). Los datos descriptivos completos pueden observarse en la Tabla 7.

Tabla 7. Datos descriptivos del artículo 3.

Variables		N	%	Xerostomia Inventory					P
				No	Leve	Moderada	Severa	Xeros total	
Sección I: Datos sociodemográficos									
Punto de muestreo	Calle	262	75,1	193 (73,7)	59 (22,5)	8 (3,1)	2 (0,8)	69 (26,4)	0,012
	Pacientes institucionalizados	35	10	18 (51,4)	10 (28,6)	7 (20)	0 (0)	17 (48,6)	
	Personal de farmacias	7	2	3 (42,9)	3 (42,9)	1 (14,3)	0 (0)	4 (57,1)	
	Personal de servicios	5	1,4	3 (60)	2 (40)	0 (0)	0 (0)	2 (40)	
	Personal sanitario no odontológico	33	9,5	21 (63,6)	10 (30,3)	2 (6,1)	0 (0)	12 (36,4)	
	Centros de salud	7	2	4 (57,1)	2 (28,6)	0 (0)	1 (14,3)	3 (42,9)	
Sexo	Hombre	125	35,3	93 (74,4)	30 (24)	2 (1,6)	0 (0)	32 (25,6)	0,072
	Mujer	229	64,7	153 (66,8)	57 (24,9)	15 (6,6)	4 (1,7)	76 (33,2)	
Edad	De 50 a 64 años	269	75,8	195 (72,5)	65 (24,2)	7 (2,6)	2 (0,7)	74 (27,5)	0,001
	De 65 a 79 años	62	17,5	40 (64,5)	13 (21)	8 (12,9)	1 (1,6)	22 (35,5)	
	Más del 80	24	6,8	11 (45,8)	9 (37,5)	3 (12,5)	1 (4,2)	13 (54,2)	
Nivel de estudios	Primaria	86	24,2	51 (59,3)	26 (30,2)	7 (8,1)	2 (2,3)	35 (40,6)	0,378
	Secundaria	39	11	31 (79,5)	7 (17,9)	1 (2,6)	0 (0)	8 (20,5)	
	Formación profesional	51	14,4	43 (84,3)	6 (11,8)	0 (0)	2 (3,9)	8 (15,7)	
	Título universitario	179	50,4	121 (67,6)	48 (26,8)	10 (5,6)	0 (0)	58 (32,4)	
Hábito tabáquico	Nunca he fumado	173	48,7	117 (67,6)	45 (26)	9 (5,2)	2 (1,2)	56 (32,4)	0,517
	Sí, soy fumador	59	16,6	37 (62,7)	21 (35,6)	1 (1,7)	0 (0)	22 (37,3)	
	Soy ex-fumador	123	34,6	92 (74,8)	21 (17,1)	8 (6,5)	2 (1,6)	31 (25,2)	

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Variables		N	%	Xerostomia Inventory					P
				No	Leve	Moderada	Severa	Xeros total	
Sección II: Salud bucodental									
Movilidad dental	No	193	54,4	148 (76,7)	40 (20,7)	5 (2,6)	0 (0)	45 (23,3)	0,001
	Sí	162	45,6	98 (60,5)	47 (29)	13 (8)	4 (2,5)	64 (39,5)	
Espacio edéntulo	No	141	39,8	98 (69,5)	33 (23,4)	8 (5,7)	2 (1,4)	43 (30,5)	0,926
	Sí	213	60,2	147 (69)	54 (25,4)	10 (4,7)	2 (0,9)	66 (31)	
Sangrado gingival	No	254	71,8	183 (72)	58 (22,8)	10 (3,9)	3 (1,2)	71 (27,9)	0,213
	Sí	100	28,2	62 (62)	29 (29)	8 (8)	1 (1)	38 (38)	
Dolor mandibular	No	279	78,8	198 (71)	67 (24)	11 (3,9)	3 (1,1)	81 (29)	0,245
	Sí	75	21,2	47 (62,7)	20 (26,7)	7 (9,3)	1 (1,3)	28 (37,3)	
Boca seca	No	236	66,9	204 (86,4)	30 (12,7)	2 (0,8)	0 (0)	32 (13,5)	<0,001
	Sí	117	33,1	41 (35)	56 (47,9)	16 (13,7)	4 (3,4)	76 (65)	
Exodoncias	Ninguna	94	26,5	65 (69,1)	24 (25,5)	4 (4,3)	1 (1,1)	29 (30,9)	0,836
	De 1 a 4	173	48,7	122 (70,5)	41 (23,7)	9 (5,2)	1 (0,6)	51 (29,5)	
	De 5 a 10	54	15,2	37 (68,5)	12 (22,2)	4 (7,4)	1 (1,9)	17 (31,5)	
	La mayoría	25	7	16 (64)	8 (32)	1 (4)	0 (0)	9 (36)	
	Todos los dientes	9	2,5	6 (66,7)	2 (22,2)	0 (0)	1 (11,1)	3 (33,3)	
Sección III: Salud general									
Hipertensión arterial	No	276	77,7	199 (72,1)	68 (24,6)	7 (2,5)	2 (0,7)	77 (27,9)	<0,001
	Sí	79	22,3	47 (59,5)	19 (24,1)	11 (13,9)	2 (2,5)	32 (40,5)	
Diabetes	No	324	91,3	227 (70,1)	81 (25)	13 (4)	3 (0,9)	97 (29,9)	0,017
	Sí	31	8,7	19 (61,3)	6 (19,4)	5 (16,1)	1 (3,2)	12 (38,7)	
Colesterol	No	239	67,3	167 (69,9)	60 (25,1)	8 (3,3)	4 (1,7)	72 (30,1)	0,096
	Sí	116	32,7	79 (68,1)	27 (23,3)	10 (8,6)	0 (0)	37 (31,9)	
Enfermedad ósea degenerativa	No	251	70,7	185 (73,7)	54 (21,5)	9 (3,6)	3 (1,2)	66 (26,3)	0,026
	Sí	104	29,3	61 (58,7)	33 (31,7)	9 (8,7)	1 (1)	43 (41,4)	
Enfermedad cardíaca	No	317	89,5	225 (71)	78 (24,6)	11 (3,5)	3 (0,9)	92 (29)	<0,001
	Sí	37	10,5	20 (54,1)	9 (24,3)	7 (18,9)	1 (2,7)	17 (45,9)	
Enfermedad respiratoria	No	326	91,8	233 (71,5)	77 (23,6)	14 (4,3)	2 (0,6)	93 (28,5)	<0,001
	Sí	29	8,2	13 (44,8)	10 (34,5)	4 (13,8)	2 (6,9)	16 (55,2)	
Enfermedad gastrointestinal	No	302	85,6	218 (72,2)	69 (22,8)	12 (4)	3 (1)	84 (27,8)	0,011
	Sí	51	14,4	26 (51)	18 (35,3)	6 (11,7)	1 (2)	25 (49)	

Se encontró un coeficiente de correlación entre la pregunta directa ¿tiene usted la boca seca? y la prueba XI de CC=0,525, p <0,001. La pregunta de boca seca, tomando el XI como "estándar de oro", mostró una sensibilidad diagnóstica del 70,37 %, y una especificidad del 83,27 % (índice de Youden=0,54, AUC=0,768, p < 0,001) (Figura 16).

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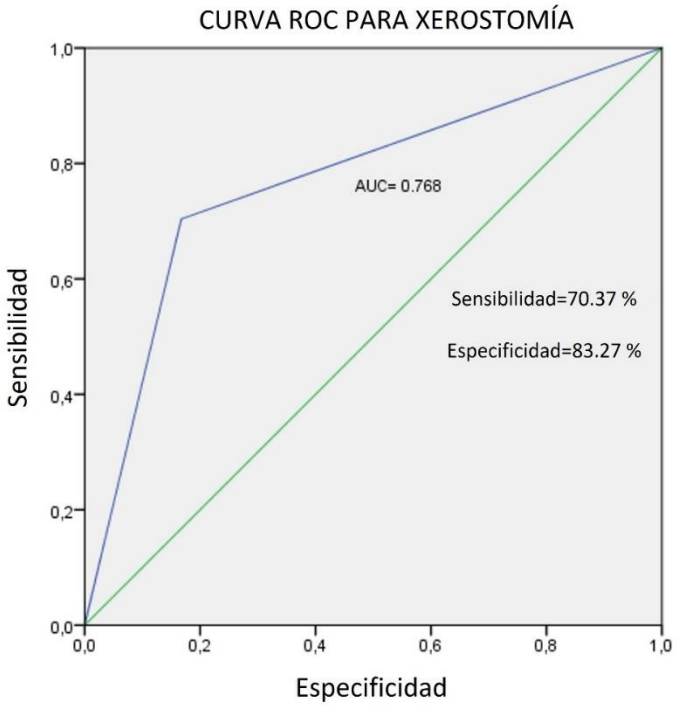


Figura 16. Curva ROC para el rendimiento diagnóstico de la pregunta sobre la boca seca, tomando el XI como "patrón de oro".

También se destaca la fuerte relación entre la xerostomía y la percepción de la salud general y oral. La regresión logística (Tabla 8) mostró que el mayor OR de xerostomía se asoció a los pacientes con mala salud autopercibida, 6,31 (IC 95% 2,89-13,80, p < 0,001). En el modelo ajustado por movilidad dental, enfermedades óseas o respiratorias y consumo de ansiolíticos y/o antidepresivos, el OR fue de 3,46 (IC 95% 1,47-8,18, p = 0,005).

Tabla 8. Análisis de regresión logística univariante y ajustado para determinar el OR para xerostomía en el artículo 3.

Covariable	Xerostomía basada en el XI			
	Univariante (OR IC 95%)	p-valor	OR ajustado (IC 95%)	p-valor
Movilidad dental Positivo frente a negativo	2,15 (1,35-3,39)	0,001	1,77 (1,07-2,90)	0,024
Enfermedad ósea Positivo frente a negativo	1,98 (1,22-3,19)	0,006	1,49 (0,87-2,55)	0,146
Enfermedades respiratorias Positivo frente a negativo	3,08 (1,42-6,66)	0,004	2,62 (1,11-6,17)	0,028
Enfermedades digestivas Positivo frente a negativo	2,49 (1,36-4,56)	0,003	2,05 (1,05-3,99)	0,035
Ansiolíticos Positivo frente a negativo	3,33 (1,96-5,66)	<0,001	2,26 (1,25-4,08)	0,007
Salud autopercibida Bueno frente a malo	6,31 (2,89-13,80)	<0,001	3,46 (1,47-8,18)	0,005

El análisis de regresión logística mostró una asociación entre la sensación de boca seca y varias enfermedades sistémicas y trastornos psicológicos. En los encuestados que tomaban medicación ansiolítica y/o antidepresiva, el riesgo de xerostomía fue 3,33 mayor en el modelo univariante y 2,26 en el modelo ajustado. En cuanto a la salud general, se observó xerostomía en el 40,5% de los pacientes con hipertensión arterial, lo que puede deberse a su influencia en la calidad y cantidad de la saliva (133). Además, se observó una mayor prevalencia de xerostomía en los encuestados diabéticos, lo que coincide con los hallazgos de López-Pintor et al. (134). En una investigación realizada en Suecia se estudió la asociación entre xerostomía y obesidad, encontrando una asociación en el análisis de los datos en bruto; no obstante, esta asociación desapareció con los factores de confusión ajustados (135). En nuestro estudio no se observaron diferencias significativas entre el IMC y el ICA en relación con la xerostomía, pero sí entre la pérdida de dientes y el IMC ($p = 0,025$) y el ICA ($p = 0,003$). Los resultados coinciden con los hallazgos del metaanálisis publicado en 2016, donde la pérdida de dientes fue 1,49 veces mayor en los obesos, con una posible bidireccionalidad entre ambas variables (24).

La asociación mutua y compleja entre la patología oral y las enfermedades sistémicas está bien documentada en la literatura. Tavares et al. observaron una correlación entre ambas, en la que la salud oral afecta a la salud general y viceversa (136); sin embargo, los datos disponibles hasta la fecha no son suficientes para establecer una asociación concluyente (18). Por ello, el objetivo de este estudio fue obtener una primera instantánea de la asociación entre xerostomía y salud oral y sistémica y proporcionar las primeras evidencias relacionadas con este tema en España.

No debería descartarse la posibilidad de que la xerostomía no sea solo un síntoma aislado, sino que potencialmente revele un deterioro más amplio en el bienestar de los adultos mayores. Los profesionales de la salud deben considerar la xerostomía como una señal de alerta para evaluar y abordar problemas de salud más amplios.

Entre las principales limitaciones de este estudio se encuentra que los datos se circunscriben a una sola comunidad autónoma en España, de modo que no es totalmente representativo epidemiológicamente a nivel nacional, por ese motivo se desarrolló el cuarto artículo.

4.2.2 Adultos mayores de 65 años

En el cuarto artículo se profundiza más en los factores de riesgo específicos relacionados con la xerostomía en adultos mayores. Este estudio se basó en datos del Estudio SEGER 2020 y expande los conocimientos sobre las asociaciones entre la xerostomía y otros indicadores de salud. Los datos descriptivos completos pueden observarse en la Tabla A1 y la distribución geográfica de los participantes en la Figura 17.

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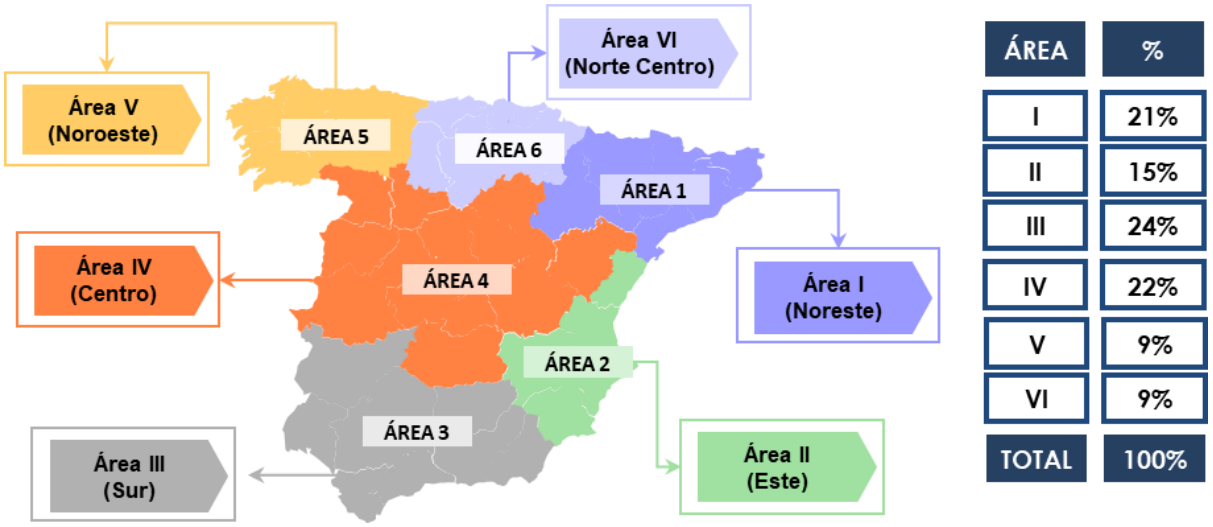


Figura 17. Distribución geográfica de los participantes.

Como es sabido, la incidencia y prevalencia de la xerostomía es mayor en las mujeres (132) y al igual que en el anterior estudio realizado en Galicia, se observó una mayor percepción de xerostomía entre las mujeres en comparación con los hombres (34,8% vs. 24%; p = 0,003). Las implicaciones clínicas de esta asociación son sustanciales y destacan la necesidad de abordar de manera diferenciada la xerostomía en adultos mayores, considerando las características específicas de género.

Además, se destacó la influencia negativa de una percepción deficiente de la salud general y oral en la prevalencia de la xerostomía. La regresión logística (Tabla 9) mostró que el OR más alto para xerostomía se observó entre adultos con mala salud general (2,81; IC 95% 1,8-4,3; p <0,001) y para el modelo ajustado el OR seguía siendo significativo (2,18; IC 95% 1,4-3,4; p = 0,001). Aquellos que necesitaron ayuda con las tareas del hogar tuvieron un OR 2,16 mayor (IC 95% 1,4-3,4; p = 0,001) y 1,69 (IC 95% 1,1-2,7; p = 0,03) en el modelo ajustado.

Tabla 9. Análisis de regresión logística univariado y ajustado para determinar el OR para xerostomía en el artículo 4.

Covariable	Xerostomía basada en el XI			
	Univariante (OR IC 95%)	p-valor	OR Ajustado (IC 95 %)	p- valor
Género				
Mujer vs. hombre	1,68 (1,22-2,32)	0,001	1,41 (0,99-1,99)	0,05
Salud general				
Mala vs. buena	2,81 (1,84-4,28)	< 0,001	2,18 (1,38-3,44)	0,001
Salud oral				
Mala vs. buena	2,63 (1,42-4,84)	0,002	1,79 (0,92-3,49)	0,86
Educación				
Sin educación vs. educación primaria	1,31 (0,65-2,63)	0,44	1,48 (0,71-3,05)	0,28
Sin educación vs. educación superior	0,77 (0,38-1,58)	0,48	1,04 (0,49-2,22)	0,91
Sin educación vs. educación vocacional de primer nivel	1,31 (0,59-2,91)	0,50	1,67 (0,73-3,84)	0,22
Educación y formación profesional vs. educación y formación profesional de nivel superior	0,73 (0,34-1,55)	0,41	0,90 (0,41-1,98)	0,81
Empleo				
Trabajadores por cuenta propia vs. empleados	1,88 (0,94-3,79)	0,76	1,52 (0,73-3,16)	0,26
Autónomos vs. otros	1,11 (0,74-1,68)	0,61	1,15 (0,75-1,76)	0,51
Tareas del hogar				
Requerir ayuda vs. ser autosuficiente	2,16 (1,39-3,37)	0,001	1,69 (1,05-2,74)	0,03

Una de las contribuciones significativas de este estudio es la inclusión de factores de riesgo específicos, como el consumo de alcohol y tabaco, así como hábitos dietéticos. Los resultados no mostraron una relación significativa entre el consumo de alcohol y tabaco y la severidad de la xerostomía. Aunque estos hallazgos pueden parecer sorprendentes, se destaca la importancia de abordar estos factores de riesgo como parte de una estrategia integral de atención a la salud en adultos mayores. El estudio también reveló que los adultos mayores que consumen frutas y vegetales regularmente tenían una percepción más baja de la xerostomía, lo que resalta la importancia de una dieta equilibrada en su prevención.

La relación entre las principales variables estudiadas se ha ilustrado en la Figura 18 mediante un gráfico acíclico dirigido o DAG, donde se puede observar la influencia de la salud general y oral y las conductas de riesgo para la salud.

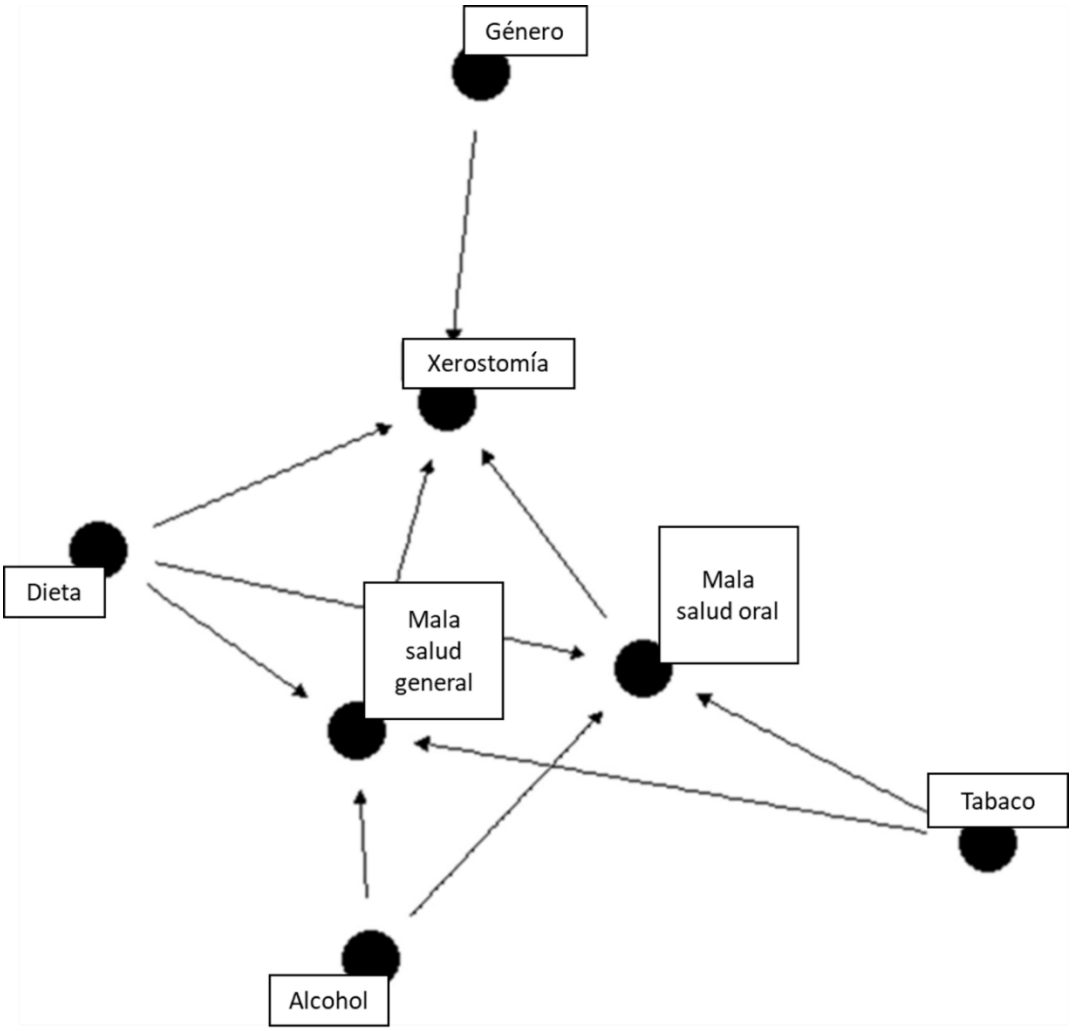


Figura 18. Gráfico DAG (Directed Acyclic Graph).

En conjunto, estos dos estudios destacan la importancia de considerar la salud general, la salud oral y otros factores de riesgo en la evaluación y el tratamiento de la xerostomía en

esta población. La fuerte asociación entre la xerostomía y el estado de salud general y oral de los adultos mayores justifica la necesidad de una evaluación temprana y un seguimiento regular. Aunque se requieren investigaciones futuras para comprender completamente los mecanismos subyacentes, estos hallazgos tienen implicaciones significativas para mejorar la calidad de vida de los adultos mayores y promover una atención de salud más integral y personalizada.

4.3 TASA DE TM DE DESÓRDENES ORALES POTENCIALMENTE MALIGNOS

4.3.1 Eritroplasia oral

En el artículo 5 se estudiaron 441 pacientes con eritroplasia oral, cuyos principales datos clínico-demográficos pueden verse en la Tabla 10. En la biopsia inicial, el 42,8% de las EO ya eran COCEs. La mucosa bucal fue la localización más frecuente de EO, pero el suelo de boca fue el sitio más común de TM. Todos los pacientes que sufrieron TM mostraron DE en la biopsia diagnóstica inicial.

Tabla 10. Principales datos clínico-demográficos de los pacientes con eritroplasia oral incluidos en el estudio. M: mujer, H: hombre

Autor y año	País	Pacientes				Factores de riesgo (*n*)			Seguimiento (años)	Transformación maligna (%)
		n	Género		Edad (años)	Tabaco	Betel	Alcohol		
			M	H						
Shafer y Waldron, 1975	EE. UU.	58	27	31	47 (>50) 11 (<50)	-	-	-	-	-
Nielsen et al. 1996	Dinamarca	10	9	1	-	-	-	-	6,3	0
Qin et al. 1999	EE. UU.	24	11	13	Media: 61,9	15	-	-	-	-
Holmstrup et al. 2006	Dinamarca	15	-	-	-	5	-	-	7,5	6,67
Lapthanasupkul et al. 2007	Tailandia	9	3	6	3 (<50) 6 (>50)	-	-	-	-	-
Feng et al. 2012	China	34	18	16	Media: 58,7	10	-	12	16	50
Queiroz et al. 2014	Brasil	11	7	4	4 (<50) 7 (>50)	9	-	3	-	-
Yang et al. 2015	Taiwan	84	10	74	Media: 54,2	65	57	42	3,83	0
Chuang et al. 2018	Taiwan	188	0	188	Media: 46,0	170	160	120	9	6,9
de Azevedo et al. 2020	Brasil	8	5	3	61-80	1	-	1	-	-
Total		441	90	336	-	275	217	178	Media: 6,66	Media: 12,7

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En los cinco estudios sin periodo de seguimiento de los pacientes (137-141) la TM media de EO fue del 51,10%. Además, el 68,38% de los casos de estos estudios ya eran COCE en el momento del diagnóstico.

La tasa general de TM de EO en el metaanálisis fue del 19,9% (IC 95% −1,6 a 41,4; $I^2 = 91{,}74\%$, p-valor del Q test = 0,00001) como se muestra en la Figura 19A. Se observó una notable asimetría con ausencia de estudios en la parte central del gráfico (Figura 19B); no obstante, la prueba de regresión de Egger desestimó la existencia de sesgo de publicación (p-valor del intercepto = 0,276).

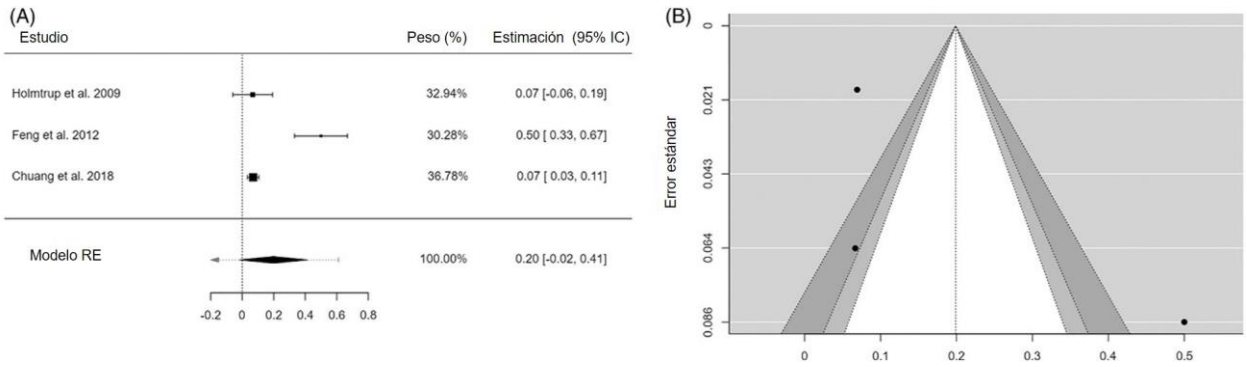


Figura 19. Transformación maligna. (A) Diagrama de bosque que representa el metaanálisis de la tasa de TM de la EO. Modelo RE (de efectos aleatorios) y peso de cada estudio. (B) Gráfico de embudo que evalúa el sesgo de publicación.

En cuanto al riesgo de sesgo, la herramienta QUIPS mostró que el RoB era alto en tres estudios y bajo en siete (Figura 20).

Considerando la definición actual de OPMD: "*cualquier anomalía de la mucosa oral que se asocia con un riesgo estadísticamente mayor de desarrollar cáncer oral*" (45), creemos que algunos aspectos de revisiones anteriores sobre la TM de la OE son algo cuestionables (53,142). La mayoría de los estudios incluyen, al mismo tiempo, casos de EO, eritroleucoplasia y COCE (143-149). Para evitar mayores confusiones, solo seleccionamos para nuestra revisión los casos diagnosticados clínico-patológicamente como EO homogéneas.

Creemos que el seguimiento clínico de los pacientes es necesario para evaluar la verdadera TM de la EO como OPMD. Por lo tanto, cuando consideramos los estudios sin período de seguimiento, logramos una tasa de TM similar a la de revisiones anteriores (44,9% -50%) (53,142). Por el contrario, cuando consideramos los estudios con un período de seguimiento, obtuvimos una tasa de TM "correcta" del 19,9%. Además, el número de COCEs en los estudios sin seguimiento clínico (68,38%) fue dos veces mayor que en todos los estudios (30,99%), y cinco veces mayor que en los estudios con seguimiento clínico (12,7%). Dado que el período de seguimiento ha sido más largo en los estudios con casos de TM (Tabla 10), creemos que los pacientes con OE deben ser seguidos activamente de por vida.

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Exceptuando el estudio de Chuang et al. (150), la distribución por sexo y edad fue similar en pacientes con OE y en aquellos que padecen TM (Tabla 10) (151,152). La mucosa bucal fue la localización más frecuente de EO; lo que podría estar relacionado con algunos hábitos cancerígenos particulares, como el consumo de betel (141,153).



Figura 20. Riesgo de sesgo de los estudios incluidos según QUIPS.

También observamos diferencias interesantes entre la ubicación EO y el lugar donde ocurrió la TM. Algunas áreas poco frecuentes de EO como el suelo de boca, la lengua y el área retromolar son sitios comunes de TM. Por el contrario, las áreas más frecuentes de EO como la mucosa bucal y el labio tienen una tasa baja de TM. Estos resultados sugieren la existencia de localizaciones específicas de la mucosa oral con mayor riesgo de TM de EO. Además, señalan la necesidad de un diagnóstico diferencial clínico e histopatológico

exhaustivo con todas las patologías mucosas clínicamente similares a la EO (45). Realizar una buena correlación clínico-patológica es clave para llegar al diagnóstico final de EO; por lo tanto, es obligatorio obtener una buena biopsia de todas las "placas rojas" orales sospechosas para (1) descartar otros diagnósticos y (2) evaluar la existencia y/o el grado de DE.

Sólo el estudio de Feng et al. (154) valoró la presencia de DE en la biopsia diagnóstica inicial de casos con TM, de los cuales todos presentaron DE (47,1% de alto grado y 52,9% de bajo grado). En nuestra revisión, casi la mitad de las lesiones de EO tenían DE en la primera biopsia, y la presencia de DE fue seis veces mayor que su ausencia (41,2% vs. 7,19%) (Tabla 11). Además, la DE sigue siendo el factor pronóstico más importante en relación con la TM de múltiples OPMDs (155-157).

Tabla 11. Prevalencia combinada, tasa de TM y análisis de subgrupos del diagnóstico histopatológico inicial de EO. (PP= proporción agrupada)

	Tamaño muestral (*n*)		Datos combinados		Heterogeneidad	
	Estudios	Pacientes	(IC I95%)	*p*-valor	p_{het}	I^2 (%)
Transformación maligna						
	3	237	PP = 19,9% (−1,6-41,4)	0,001	0,0001	91,7
Diagnóstico histopatológico inicial						
COCE	9	259	PP = 41,2% (16,8-65,5)	0,001	0,0001	95,2
DE alto riesgo	9	259	PP = 9,17% (29,8-51,4)	0,001	0,0001	82,5
DE bajo riesgo	9	259	PP = 37,4% (20,9-53,9)	0,001	0,0001	87,9
No DE	9	259	PP = 7,19% (2,0-12,4)	0,007	0,0001	75,63

Desafortunadamente, encontramos varias limitaciones durante la realización de esta revisión sistemática y metaanálisis. En primer lugar, se observó un bajo número de estudios observacionales sobre la TM de la EO con un buen periodo de seguimiento clínico de los pacientes. En segundo lugar, se destaca la falta de estandarización y los cambios en los criterios diagnósticos de la EO a lo largo de los años. Esto comprometió la asociación entre la TM de la EO y diferentes características clínico-patológicas.

No pudimos asociar esta TM con ninguna característica clínico-patológica específica, incluida la DE. Sin embargo, creemos que la presencia de DE en la biopsia diagnóstica inicial sigue siendo el principal factor pronóstico en esta OPMD. Se necesitan más estudios observacionales sobre EO, con criterios diagnósticos bien establecidos y un buen seguimiento clínico, para identificar el verdadero riesgo de TM de la EO y los factores de riesgo relacionados.

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4.3.2 Candidiasis hiperplásica crónica

El artículo 6 examina el riesgo de TM en 274 pacientes con CHC. Las características de los estudios incluidos se muestran en las Tablas 12 y 13.

Tabla 12. Principales datos clínico-demográficos de los pacientes con CHC incluidos en el estudio (M: mujer; H: hombre; n : número).

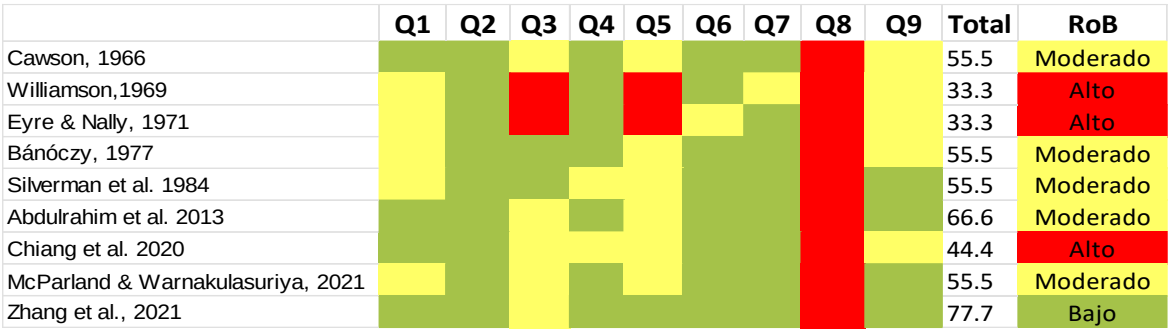
Autor y año	País	Tipo de estudio	Pacientes				Seguimiento (años)	Método de evaluación	Transformación maligna (%)
			n	Género		Edad media (años)			
				M	H				
Cawson, 1966	Reino Unido	Series de casos	15	4	11	50,5	10	Biopsia (P.A.S)	13,3
Williamson, 1969	Reino Unido	Series de casos	2	0	2	59,5	-	Biopsia (P.A.S), cultivo	50
Eyre y Nally. 1971	Reino Unido	Series de casos	3	0	3	55,7	-	Raspado y cultivo	66,6
Bánóczy, 1977	Hungría	Cohorte	70	-	-	-	9,8	Biopsia	28,7
Silverman et al. 1984	EE. UU.	Cohorte	80	-	-	54	7,2	Biopsia (P.A.S), cultivo	4,4
Abdulrahim et al. 2013	Irlanda	Casos-controles	31	14	17	57,8	2	Biopsia	6,5
Chiang et al. 2020	Taiwan	Cohorte	22	-	-	-	5	Biopsia (P.A.S)	13,6
McParland y Warnakulasuriya, 2021	Reino Unido	Descriptivo	3	-	-	-	16	Biopsia (P.A.S)	33,3
Zhang et al. 2021	China	Cohorte	48	13	35	54,9	6	Biopsia (P.A.S)	4,2
Total			274	31	68	55,4	8	-	12,4

Tabla 13. Localización de las lesiones de CHC en el momento del diagnóstico.

Autor y año	Localización							Histopatología			
	Suelo de boca	Retromolar	Encía	Paladar	Tongue	Yugal	Labio	COCE	DE alto grado	DE bajo grado	No DE
Cawson, 1966	0	0	0	4	4	6	1	-	-	-	-
Williamson, 1969	-	-	-	-	-	-	-	1	-	-	1
Eyre y Nally, 1971	-	-	-	-	-	2	3	2	-	-	-
Bánóczy, 1977	-	-	-	-	-	-	-	-	-	-	-
Silverman et al., 1984	-	-	-	-	-	-	-	2	-	-	-
Abdulrahim et al., 2013	0	0	2	3	4	22	-	2	8	18	3
Chiang et al., 2020	-	-	-	-	-	-		3	-	-	-
McParland y Warnakulasuriya, 2021	-	-	-	-	-	-	-	-	-	-	-
Zhang et al., 2021	-	-	-	-	-	-	-	0	1	9	38
Total	-	-	2	7	8	30	4	10	9	27	42

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Estos estudios se publicaron entre 1966 y 2021, la mayoría fueron series de casos o estudios de cohortes retrospectivos, y la población objetivo fue siempre de tipo clínico. No todos los estudios utilizados para el análisis agrupado se realizaron con el mismo vigor, como se muestra en la Figura 21.



	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Total	RoB
Cawson, 1966										55.5	Moderado
Williamson,1969										33.3	Alto
Eyre & Nally, 1971										33.3	Alto
Bánóczy, 1977										55.5	Moderado
Silverman et al. 1984										55.5	Moderado
Abdulrahim et al. 2013										66.6	Moderado
Chiang et al. 2020										44.4	Alto
McParland & Warnakulasuriya, 2021										55.5	Moderado
Zhang et al., 2021										77.7	Bajo

Figura 21. Diagrama de calidad que representa gráficamente el riesgo de sesgo entre los estudios individuales, evaluado mediante la lista de comprobación de valoración crítica del Instituto Joanna Briggs diseñada para revisiones sistemáticas que abordan cuestiones de prevalencia, incidencia acumulada y/o para metaanálisis de proporciones.

Nuestro estudio mostró un riesgo significativo de TM en pacientes con CHC, con una tasa del 12,1% (IC 95% 4,1-19,8 %, I^2 = 78,27%, p-valor de la prueba Q = 0,002) (Figura 22A). Se pudo ver una notable asimetría hacia la derecha en el diagrama del bosque. El sesgo de publicación se evaluó visualmente utilizando el gráfico de embudo de Begger (Figura 22B), y se confirmó también formalmente mediante la prueba descrita por Egger et al. (p_{Egger} = 0,001).

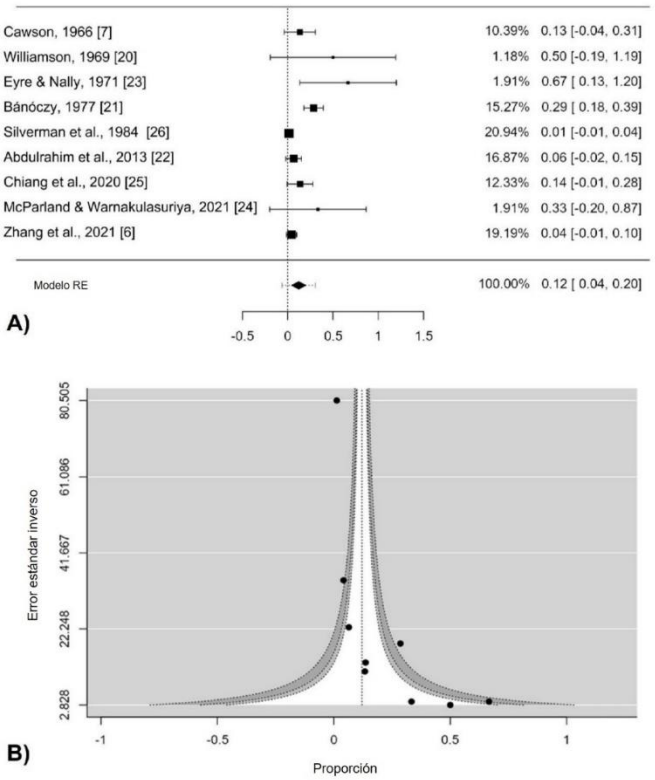


Figura 22.A) Diagrama de bosque. TM general de la candidiasis hiperplásica crónica. Modelo RE (de efectos aleatorios) y peso de cada estudio; B) Gráfico de embudo que evalúa el sesgo de publicación.

En el análisis de sensibilidad, los resultados generales no fluctuaron apreciablemente después de la repetición secuencial del análisis agrupado con la omisión de cada estudio individual (Tabla 14). Se observo una única excepción cuando el estudio de Bánóczy (158) fue omitido la prevalencia bajó al 6,6, lo que puede explicar en parte la alta heterogeneidad.

Tabla 14. Análisis de prevalencia Leave-one-out pooled.

Estudios	Proporción agrupada	IC 95%
General	12,1	4,3-19,8
Excluyendo a Cawson, 1968	12,0	3,7-20,4
Excluyendo a Williamson, 1969	11,6	3,9-19,3
Excluyendo a Eyre y Nally, 1971	10,8	3,4-18,1
Excluyendo a Bánóczy, 1977	6,6	1,1-12,1
Excluyendo a Silverman et al., 1984	15,6	5,9-25,3
Excluyendo a Abdulrahim et al., 2013	13,9	4,6-23,1
Excluyendo a Chiang et al., 2020	12,0	3,6-20,4
Excluyendo a McParland y Warnakulasuriya, 2021	11,7	3,9-19,5
Excluyendo a Zhang et al., 2021	15,6	4,7-26,4

La metaregresión mostró una correlación negativa significativa entre las tasas de TM y el año de publicación de los informes incluidos (p = 0,001), como se muestra en la Figura 23.

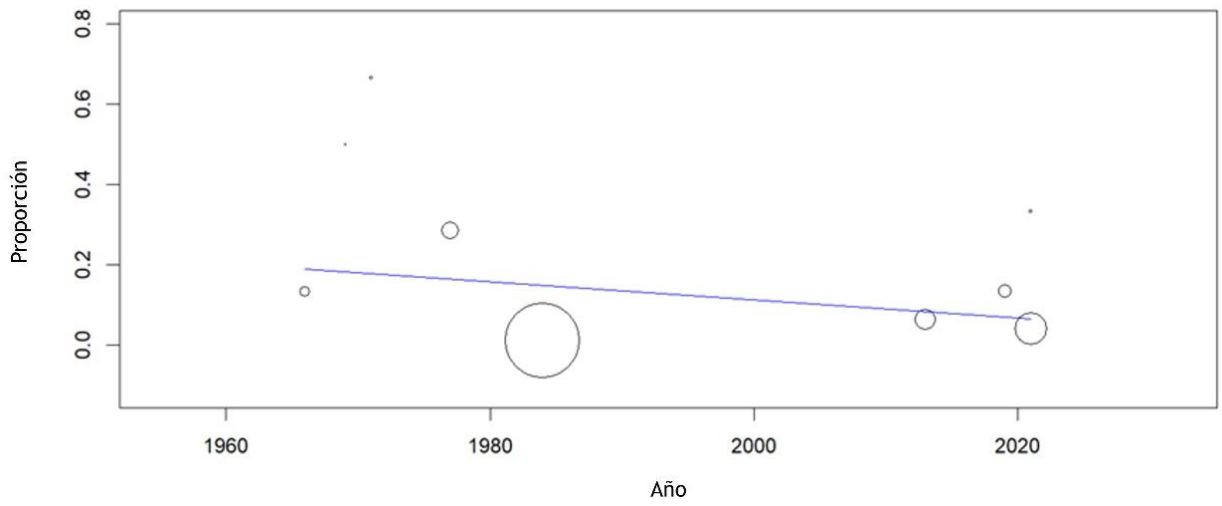


Figura 23. Diagrama de burbujas de metarregresión sobre la tasa de TM de la CHC en función del año de estudio.

Nuestra revisión revela que el potencial de TM de la CHC está infravalorado en la literatura científica actual, ya que algunos autores consideran la CHC como un trastorno de bajo riesgo (159,160), por consiguiente, es crucial realizar un diagnóstico y control tempranos, así como vigilar las características clínico-patológicas que podrían estar implicadas en su malignidad (62).

Consideramos que la infección por *Candida* se subestima como factor de riesgo para el COCE, principalmente porque no se registra en la mayoría de los estudios epidemiológicos que abordan la tasa de TM de condiciones aparentemente relacionadas, como la LO canónica (161). Además, el complejo diagnóstico diferencial y la variedad de nomenclaturas para la CHC en la literatura científica pueden ser responsables de un diagnóstico erróneo de este trastorno y, en consecuencia, una subestimación de su potencial de malignización. Sin embargo, encontramos una diferencia notable entre las tasas de TM de la CHC y la LO (161). Según estudios previos, la tasa de TM de la LO oscila entre el 9,5% y el 9,8% (47,156), mientras que aquí informamos una tasa del 12,1%. La CHC podría entonces cumplir con la definición clásica de una condición precancerosa como "un tejido morfológicamente alterado en el que el cáncer es más probable que ocurra que en su contraparte aparentemente normal" (162). De hecho, en la actualidad existen diferentes teorías sobre los mecanismos de infección por *Candida* relacionados con la carcinogénesis oral. *Candida spp*. puede producir nitrosaminas como la N-nitroso-bencil-metilamina y el acetaldehído, que son compuestos cancerígenos (163,164). Además, durante la infección, hay un aumento en las citoquinas proinflamatorias, lo que conduce a un desequilibrio en los mecanismos de vigilancia inmunológica y cambios en el entorno tisular (165-167). La candidalisina es una toxina citolítica que se ha demostrado que induce las vías NF-kB y MAPK, que a menudo se encuentran desreguladas en el cáncer (168).

La distribución por sexo y edad en nuestro estudio fue similar a las cifras informadas en revisiones narrativas anteriores (es decir, predominantemente hombres de 50 a 60 años) (169). La mucosa bucal fue la ubicación más frecuente de la CHC, como se informa clásicamente, pero otras regiones, como la lengua o el paladar, también pueden verse afectadas (56,58,59). No se pudo informar una ubicación preferencial en la que pueda surgir el COCE debido a la falta de información detallada recuperada de los estudios incluidos. En cuanto a la histopatología, y especialmente en relación con la DE, solo dos estudios informaron su grado (Tabla 12 y Tabla 13) (61,170). Ninguno de estos estudios confirmó que la presencia de DE representara un factor de riesgo para la TM. Por otro lado, varios autores consideran que la infección por *Candida* se correlaciona con grados más pronunciados de DE y, por lo tanto, indirectamente implica un mayor riesgo de malignidad (171). Odell et al. señalaron cómo en la histopatología de la CHC es importante identificar los cambios epiteliales como reactivos o preneoplásicos. Este grupo también destacó que la repetición de la biopsia debería retrasarse 6 semanas después del tratamiento antifúngico teniendo en cuenta el tiempo de renovación del epitelio mucoso oral (64). Después del tratamiento antifúngico sistémico, se ha informado de la resolución de la CHC con una reducción en la DE (172).

Deben destacarse algunas limitaciones en nuestro estudio. Se observó una heterogeneidad estadística significativa entre los estudios, como se esperaba, y se utilizaron estimaciones a priori planificadas con efectos aleatorios para ser más conservadores. Esta situación es extremadamente común en metaanálisis (173). En este sentido, la presencia de

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heterogeneidad debería considerarse más como la regla que como la excepción (174). Los análisis de subgrupos también indicaron que los estudios no asiáticos y de baja calidad tienden a sobrestimar la tasa de TM de la CHC. El análisis de subgrupos, según la evaluación del RoB, mostró que los resultados carecían de robustez y probablemente debilitaron nuestra síntesis de datos de manera moderada. Además, es importante señalar que solo se incluyeron datos de 274 individuos en este metaanálisis. En este sentido, no se pueden obtener conclusiones concluyentes sobre las características contextuales de la cancerización de la CHC para evitar generalizaciones. En segundo lugar, el sesgo de publicación (la tendencia a publicar solo estudios con resultados positivos) puede explicar en parte nuestros resultados. Nuestros métodos carecen de poder estadístico debido a que el número de estudios primarios no alcanza los diez (175). En tercer lugar, se debe considerar el sesgo de información. Este concepto se refiere a errores que se introducen durante la evaluación de la exposición, evento u otras covariables en cada población de estudio específica (176). La falta de consenso con respecto a los criterios diagnósticos de la CHC probablemente introduzca errores de medición y, por lo tanto, diagnósticos erróneos. Además, en términos de exposición a covariables que pueden ejercer un efecto sobre la TM, se encontraron informes heterogéneos sobre factores de riesgo, incluida la histopatología, lo que dificultó análisis más detallados. Este estudio puede tener un sesgo protopático, que se detecta cuando una exposición está influenciada por etapas tempranas (subclínicas) de una patología (177). A pesar de los mecanismos explicados que podrían explicar el potencial carcinogénico de la CHC, otras explicaciones pueden ser plausibles, como la presencia de una causalidad inversa. Esto se debe a la falta de un umbral claro para establecer un diagnóstico diferencial entre la CHC y una LO canónica sin sufrir una infección fúngica durante su curso.

A pesar de las limitaciones mencionadas, la naturaleza robusta del análisis combinado se indica mediante el diagrama de bosque, que demuestra una tasa de TM estadísticamente consistente para la candidiasis hiperplásica crónica, y su robustez se confirma mediante análisis de sensibilidad. Se necesitan estudios de cohortes prospectivos para definir la historia natural de la CHC y una declaración de consenso para aclarar un conjunto adecuado de criterios diagnósticos.

Estos dos metaanálisis subrayan la importancia de abordar de manera individualizada cada patología oral y cada paciente, considerando su contexto clínico y evaluando la presencia de factores de riesgo adicionales. La educación pública sobre los signos y síntomas de estos trastornos es esencial para fomentar la detección temprana por parte de los pacientes y la remisión oportuna a especialistas. Un enfoque multidisciplinario que involucre a especialistas en medicina oral, patólogos, oncólogos y otros profesionales de la salud es esencial para una evaluación completa y un manejo adecuado de los pacientes con OPMDs. Además, se necesita una mayor investigación para comprender mejor los factores que contribuyen a la TM en estas lesiones, lo que podría llevar al desarrollo de mejores estrategias de prevención y tratamiento.

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4.4 BIOMARCADORES EN OPMDS Y COCE

4.4.1 Epigenética y Metilación del ADN en OPMDs

La metilación anormal de genes clave, como MLH1 y MGMT, se ha asociado con la progresión del cáncer oral. Esta comprensión abre nuevas puertas para el desarrollo de biomarcadores epigenéticos que podrían usarse en la identificación temprana y la estratificación del riesgo. Sin embargo, la estandarización de las técnicas de detección y el análisis de datos es un desafío importante en este campo emergente.

La investigación en epigenética y metilación del ADN, como se aborda en el artículo 7, ofrece una visión de los posibles mecanismos moleculares involucrados en la TM de los OPMD. La muestra estuvo compuesta por 16 COCE, 47 lesiones transformadas por OPDM anteriores y 22 lesiones en pacientes con neoplasia simultánea con OPDM en otra localización de la topografía oral. Los datos descriptivos se muestran en la Tabla 15.

Tabla 15. Características de la cohorte institucional y niveles de metilación del artículo 7.

Variables cualitativas			N		%
Grupo de estudio	COCE		16		18,8
	Transformación		47		55,3
	Simultáneo		22		25,9
Género	Mujer		48		56,5
	Hombre		37		43,5
Localización	Encía		13		15,3
	Lengua		27		31,8
	Mucosa yugal		24		28,2
	Paladar duro		2		2,4
	Suelo de boca		2		2,4
	Trígono Retromolar		8		9,4
	Labio		3		3,5
	Paladar blando		3		3,5
	Amígdala		3		3,5
Exitus	No		68		80,0
	Sí		17		20,0
Recaída	No		54		63,5
	Sí		31		36,5
Tratamiento	Solo cirugía		56		65,9
	Radioterapia		22		25,9
	Radioterapia y quimioterapia		7		8,2
Variables cuantitativas	**N**	**Mínimo**	**Máximo**	**Media**	**DS**
Porcentaje MLH1	75	0,4	63,8	8,6	11,5
Porcentaje MGMT	82	1,0	41,0	8,0	9,2
Tiempo hasta malignidad	58	0,4	116,6	33,4	39,4

Se obtuvieron niveles generales de metilación de MLH1 de 8,6 ± 11,5% y 8,1 ± 9,2% para MGMT. Con respecto a MHL1, el COCE presentó el mayor grado de metilación con 9,3 ± 7,3% (IC 95% 5,1-13,6), y con respecto a MGMT, el grupo de malignidad simultánea presentó el mayor grado de metilación con 10 ± 13,5% (IC 95 % 6-10), aunque no se encontraron diferencias significativas entre los grupos (p = 0,934 y p = 0,515, respectivamente). La supervivencia estimada fue mayor para los casos metilados con MGMT (19,1 meses, IC 95% 19,1-19,1) que para los casos no metilados (9,4 meses, IC 95% 6-12,8) (Tabla 16), pero no fue estadísticamente significativa.

Tabla 16. Asociación de la metilación de MLH1 y MGMT con la supervivencia y las recurrencias entre la cohorte.

Kaplan Meier			Meses (media)	IC 95%		p-valor del test Log-Rank
				Límite inferior	Límite superior	
Supervivencia	Metilación de MLH1	No	9,1	5,2	13,1	0,827
		Sí	9,3	1,3	17,3	
	Metilación MGMT	No	9,4	6,0	12,8	0,134
		Sí	19,1	19,1	19,1	
Reaparición	Metilación de MLH1	No	19,7	12,0	27,3	0,137
		Sí	42,4	9,9	74,9	
	Metilación MGMT	No	30,9	19,2	42,6	0,267
		Sí	18,2	9,4	26,9	

Se descartaron restricciones para incluir exclusivamente casos de COCE debido a los bajos niveles de metilación en los genes específicos dentro de la cohorte. En este sentido, se incluyeron todos los carcinomas epidermoides de cabeza y cuello que aportaron datos sobre la cuestión en estudio (n = 504). El análisis de clustering k-means separó claramente cuatro grupos de muestras. Según el análisis de los datos de TCGA disponibles, 495 muestras de tumores estaban hipometiladas, mientras que nueve muestras de tumores estaban hipermetiladas para MGMT. En el caso de MLH1, las cifras fueron bastante similares: solo nueve casos se consideraron hipermetilados según el ensayo Mmethylation-450 Bead Chip. Los niveles no significativos de correlación y análisis de regresión se muestran en la Figura 24 A. Debido a los bajos niveles de hipermetilación, se descartó la intención de realizar un análisis de Kaplan-Meier y se computarizó un análisis de correlación de MGMT y MLH1 versus un análisis de supervivencia general (en meses). El análisis se muestra en la Figura 24B para MLH1 y en la Figura 24C en el caso de MGMT. La hipermetilación de MGMT no se correlacionó con la supervivencia general (coeficiente de correlación de Spearman = −0,05, p = 0,233). De manera similar, la hipermetilación de MLH1 tampoco se correlacionó con la supervivencia general (coeficiente de correlación de Spearman = −0,04, p = 0,319). La Figura 24 muestra el análisis matemático mediante modelos de regresión.

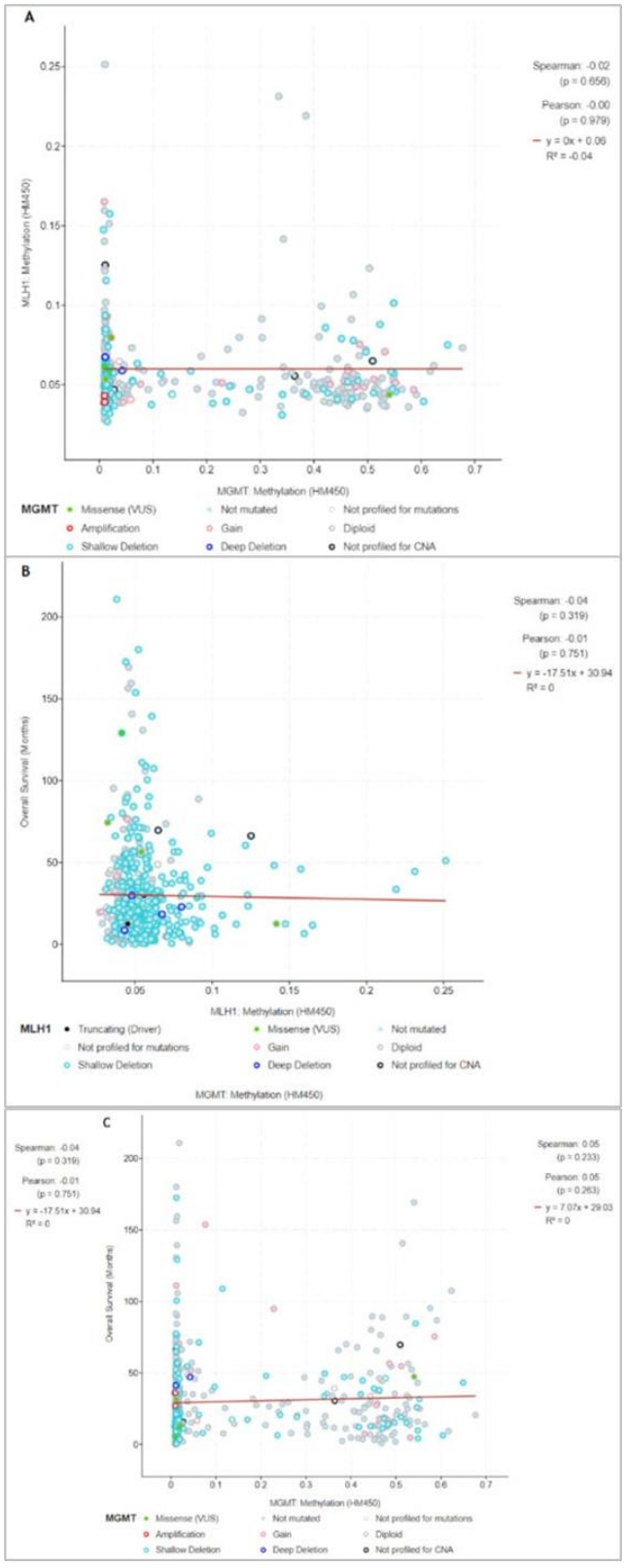


Figura 24. Análisis integrativo con la base de datos TCGA. (A) Modelo de correlación y regresión lineal de metilación de MGMT y MLH1. Correlación lineal de MLH1 (B) y metilación de MGMT (C) con la supervivencia global en meses.

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Al analizar la metilación de ambos genes conjuntamente, indagamos las vías implicadas en la carcinogénesis en la porción de la base de datos TCGA objeto de estudio. La vía más afectada parecía ser la WNT. Estos genes pueden actuar sobre ligandos WNT, afectando a subsistemas como los que implican a genes que desempeñan papeles fundamentales en el complejo receptor dual WNT, SFRP, DKK y AXIN. Al mismo tiempo, este estado coordinado puede inducir la proliferación celular a través de una maquinaria molecular en la que intervienen la familia Groucho/TLE/Grg de correpresores transcripcionales y los factores de transcripción TCF/LEF (Figura 25).

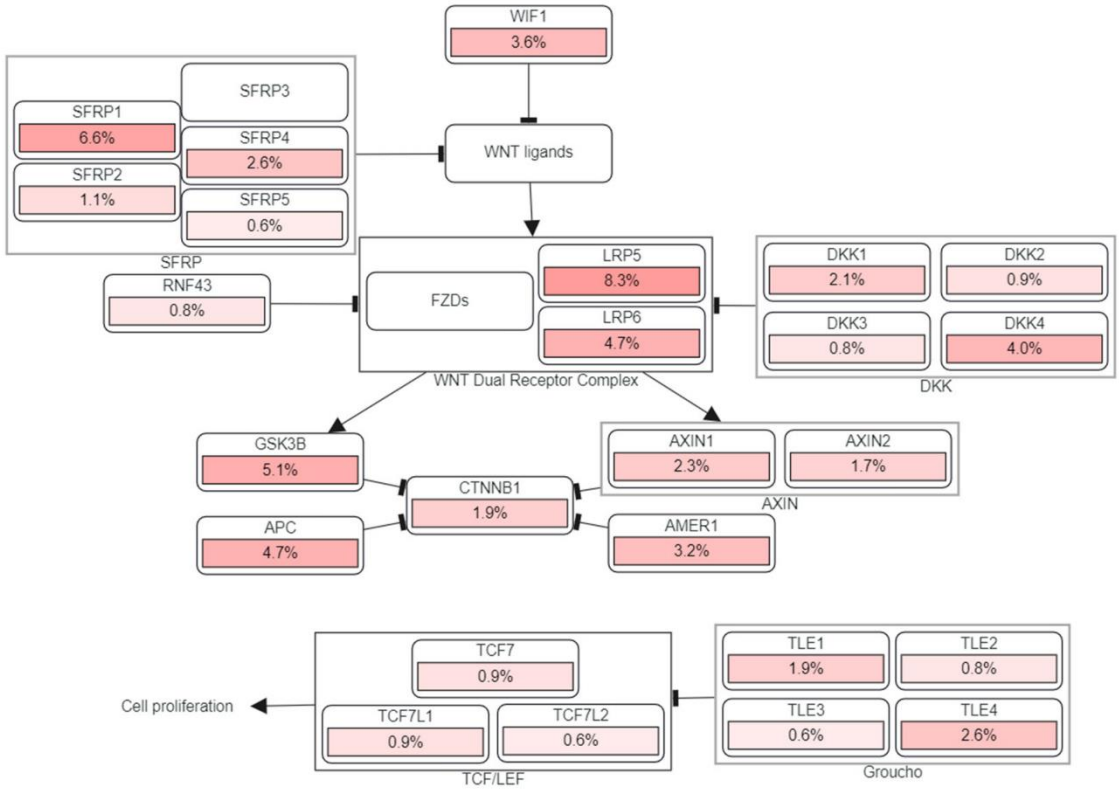


Figura 25. Vías implicadas en la carcinogénesis de cabeza y cuello relacionadas con la metilación de MLH1 y MGMT según la base de datos TCGA.

A pesar de las alteraciones epigenéticas encontradas en los niveles de metilación de COCE y OPMDs y sus correlaciones con los resultados clínicos y la supervivencia en el presente estudio, nuestros resultados son inconsistentes con la literatura previa (178). Estas diferencias reflejan la heterogeneidad de estas enfermedades en su histología y comportamiento clínico, con diferentes etiologías y factores de riesgo, y la conocida especificidad de los patrones de metilación en tejidos y tipos tumorales (179). La prevalencia de la metilación de MGMT y MLH1 detectada en este estudio también es consistente con los datos previamente resumidos para CCC en la base de datos TCGA (180,181).

La determinación de la metilación del promotor de MGMT es de gran interés para los clínicos y los pacientes, dado que en estudios in vitro recientes con líneas celulares, se ha

observado una asociación entre la metilación del promotor de MGMT y el diagnóstico de COCE en comparación con las líneas celulares normales. Además, esta supresión se revirtió después del tratamiento con un agente desmetilante (5-Aza-2'-deoxicitidina) (182), lo cual fue corroborado por un aumento en la expresión génica (180). Sin embargo, el porcentaje de metilación obtenido en nuestras muestras no fue significativo para ninguna característica clínico-patológica o de supervivencia.

El porcentaje de casos de COCE metilados con MGMT fue del 9,2%, porcentaje inferior al obtenido en otros estudios con un tamaño muestral similar. En esos estudios, se obtuvo un porcentaje de metilación del 27%, y en su grupo de control, se obtuvo un porcentaje de metilación del 7% (183). Este equipo también observó que la metilación combinada de los promotores de p16 y MGMT se encontró solo en el grupo de COCE. Metaanálisis recientes también han sugerido que la metilación de MGMT puede estar relacionada con COCEs que metastatizan; sin embargo, estos resultados no revelaron un peor pronóstico en las muestras metiladas (184). En un estudio realizado por Taioli et al. observaron la asociación entre la metilación de MGMT y un peor pronóstico para el COCE (185). Sin embargo, nuestros análisis encontraron una tasa de supervivencia más alta en casos metilados de MGMT (19,1 meses, IC 95% 19,1–19,1) que en casos no metilados (9.4 meses, IC 95% 6–12,8) sin diferencias estadísticamente significativas. Por otro lado, en el presente estudio, la metilación de MGMT fue más marcada en el grupo de malignización simultánea, obteniendo un 10 ± 13,5%. Basándonos en la teoría de la cancerización de campo, los resultados de metilación del ADN pueden reflejar que toda la histología epitelial adyacente a tumores y lesiones premalignas tiene niveles prominentes de metilación de este gen, sugiriendo que la metilación de MGMT es un evento temprano en la carcinogénesis oral (70). No obstante, la metilación de otros genes en neoplasias orales, como APC, DAPK, ECAD, RASSF1, TIMP3 y p16, ha obtenido valores diagnósticos y pronósticos más prometedores (178).

Menos estudios han considerado la metilación del gen MLH1 en el cáncer oral en comparación con el MGMT (186,187). Un estudio encontró que el 100% de las neoplasias orales están afectadas por la metilación de este gen, lo que sugiere que esto ocurre en pacientes en un estadio avanzado de la enfermedad (74). Esto no coincide en absoluto con los resultados obtenidos en este estudio, ni con los obtenidos por Ramírez et al., quienes sugirieron que es un marcador temprano de malignidad en lesiones orales (75).

La fortaleza de nuestro estudio es la población bien caracterizada de los grupos OPMD y COCE, que cuenta con datos epidemiológicos detallados sobre factores de riesgo y también un seguimiento completo. Una limitación obvia del presente estudio es su diseño transversal y el tamaño de muestra reducido. Por otro lado, uno de los objetivos principales de este estudio fue evaluar la metilación temprana en ambos genes en lesiones orales previas a la malignidad y, en particular, su implicación en la cancerización de campo. Sin embargo, la metilación en los grupos de OPDM no fue significativa, ni se asoció con las muestras

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correspondientes de COCE con patrones de metilación mantenidos a lo largo del tiempo. En este sentido, los resultados actuales impiden la generalización de los resultados.

En conclusión, nuestros hallazgos no mostraron una correlación entre la metilación de los genes MGMT y MLH1 y ninguna característica clínico-patológica o supervivencia en nuestra cohorte institucional. Además, nuestros resultados mostraron que la metilación de MLH1 estaba presente principalmente en COCE, mientras que MGMT en OPMD representó una contribución modesta a la cancerización de campo. Hubo una coherencia general con la base de datos TCGA, aunque hay que tener en cuenta que individualmente la metilación de estos genes no ha demostrado significación clínica. Con base en los resultados de este estudio, alentamos el desarrollo de más estudios que analicen la metilación de más genes de forma prospectiva en cohortes más grandes para desentrañar un panel capaz de alcanzar la traducción clínica.

Sin embargo, es esencial destacar que la aplicación clínica de estos hallazgos aún se encuentra en sus primeras etapas y requiere una validación sólida antes de su implementación generalizada. La epigenética está emergiendo como un campo emocionante en la medicina oral y tiene el potencial de revolucionar nuestra comprensión de la TM en la cavidad oral.

4.4.2 $p16^{INK4a}$ como biomarcador en la malignización de OPMDs

La sobreexpresión de $p16^{INK4a}$ se ha asociado con un mayor riesgo de TM, lo que podría influir en la estratificación del riesgo y las decisiones de tratamiento. Sin embargo, la utilidad clínica precisa de $p16^{INK4a}$ como biomarcador sigue siendo un tema de investigación activa, y se requiere una validación más sólida en estudios prospectivos y ensayos clínicos.

El artículo 8 se centra en la sobreexpresión de $p16^{INK4a}$ como posible biomarcador para evaluar el riesgo de TM en OPMDs. Las ocho publicaciones incluidas abarcaron sólo estudios de cohortes retrospectivos. Todos los estudios determinaron la expresión de $p16^{INK4a}$ mediante técnicas IHQ en tejidos incluidos en parafina y fijados con formalina con diferentes puntos de corte para establecer la positividad como una variedad de anticuerpos anti-$p16^{INK4a}$ (Tabla A2).

La evaluación RoB concluyó en 3 estudios (37,5%) con una puntuación alta, mientras que fue baja en 5 (62,5%). La mayoría de los sesgos potenciales surgieron debido a la falta de consideración de cofactores relevantes y al uso de enfoques estadísticos no meticulosos. Los resultados de la evaluación de RoB se presentan en Tabla 17.

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Tabla 17. Evaluación de la calidad mediante la herramienta Quality in Prognosis Studies tool.

Autor	Año	1. Participación en el estudio	2. Deserción de estudios	3. Medición del factor pronóstico	4. Medición de resultados	5. Estudio de confusión	6. Análisis e informes estadísticos	En general
Shah et al.	2007	Bajo	Alto	Alto	Moderado	Alto	Bajo	Alto
Kaur et al.	2013	Bajo	Bajo	Moderado	Bajo	Bajo	Bajo	Bajo
Nankivell et al.	2014	Bajo	Bajo	Bajo	Bajo	Moderado	Bajo	Bajo
Bazarsad et al.	2017	Moderado	Bajo	Bajo	Bajo	Moderado	Alto	Alto
Zhang et al.	2017	Bajo	Bajo	Moderado	Bajo	Bajo	Bajo	Bajo
Upadhyaya et al.	2018	Bajo	Bajo	Bajo	Bajo	Bajo	Alto	Alto
Wu et al.	2019	Bajo	Bajo	Bajo	Bajo	Moderado	Bajo	Bajo
Monteiro et al.	2022	Bajo	Bajo	Moderado	Bajo	Bajo	Bajo	Bajo

El análisis combinado de ocho estudios que evaluaron la sobreexpresión de $P16^{INK4a}$ en OPMDs reveló un riesgo dos veces mayor de progresar a COCE (RR = 2,01, IC del 95% 1,36–2,96). La tasa de heterogeneidad entre estudios fue insignificante ($I^2 = 0\%$), y por ello, se calculó un modelo de efectos fijos. Los RR individuales y agrupados se representan en la Figura 26A. El gráfico de Galbraith mostró que ningún estudio individual podía considerarse un valor atípico importante, en este sentido, la heterogeneidad general no se vio afectada de manera desproporcionada por ningún estudio de nivel primario en nuestro análisis agrupado (Figura 26B).

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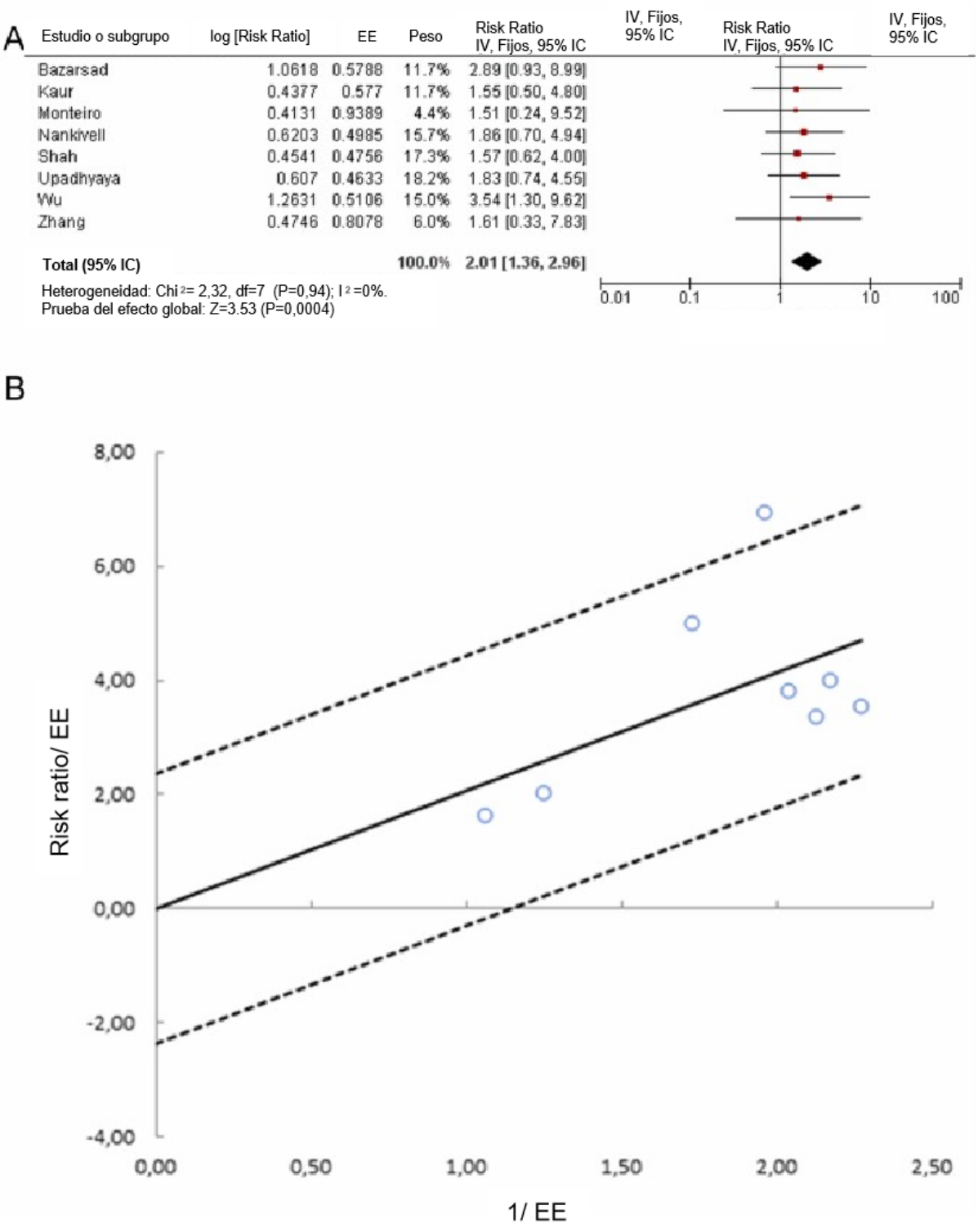


Figura 26. A) Diagrama de bosque que muestra el riesgo de progresión maligna en los OPMDs positivos para la sobreexpresión de $P16^{INK4a}$. B) Diagrama de Galbraith que representa la contribución individual de los estudios a la heterogeneidad global. El eje X representa los cocientes de riesgos individuales estandarizados según los errores estándar siguiendo la siguiente fórmula, y = RR/EE(RR), mientras que el eje Y muestra la precisión (x = 1/EE[RR]). La línea diagonal de regresión se proyecta desde el origen del sistema de coordenadas (es decir, x = 0, y = 0). Los IC aproximados del 95% discurren entre las dos líneas paralelas a ± 2 unidades alrededor de la línea diagonal.

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El gráfico de embudo presenta una ligera asimetría hacia la derecha (Figura 27). Sin embargo, la existencia de sesgo de publicación no fue confirmada por la regresión de Egger (p = 0,977), ni por la prueba de correlación de Begg y Mazumdar (p = 0,229). Sin embargo, la implementación de la metodología de “trim-and-fill” resultó en la adición de un nuevo RR "faltante". La inclusión no alteró sustancialmente nuestro análisis combinado, lo que arrojó una estimación cercana (RR = 1,83; IC del 95 % 1,42 a 2,24). Se realizó otra simulación para evaluar la solidez de nuestra síntesis cuantitativa. Calculamos cuántos informes negativos no publicados (es decir, RR = 1) con tantos casos y controles como el número promedio de los incluidos en nuestros estudios primarios fueron necesarios para revertir nuestra estimación. Nuestra simulación mostró la necesidad de 329 estudios negativos para obtener un RR agrupado igual a la unidad y no significativo.

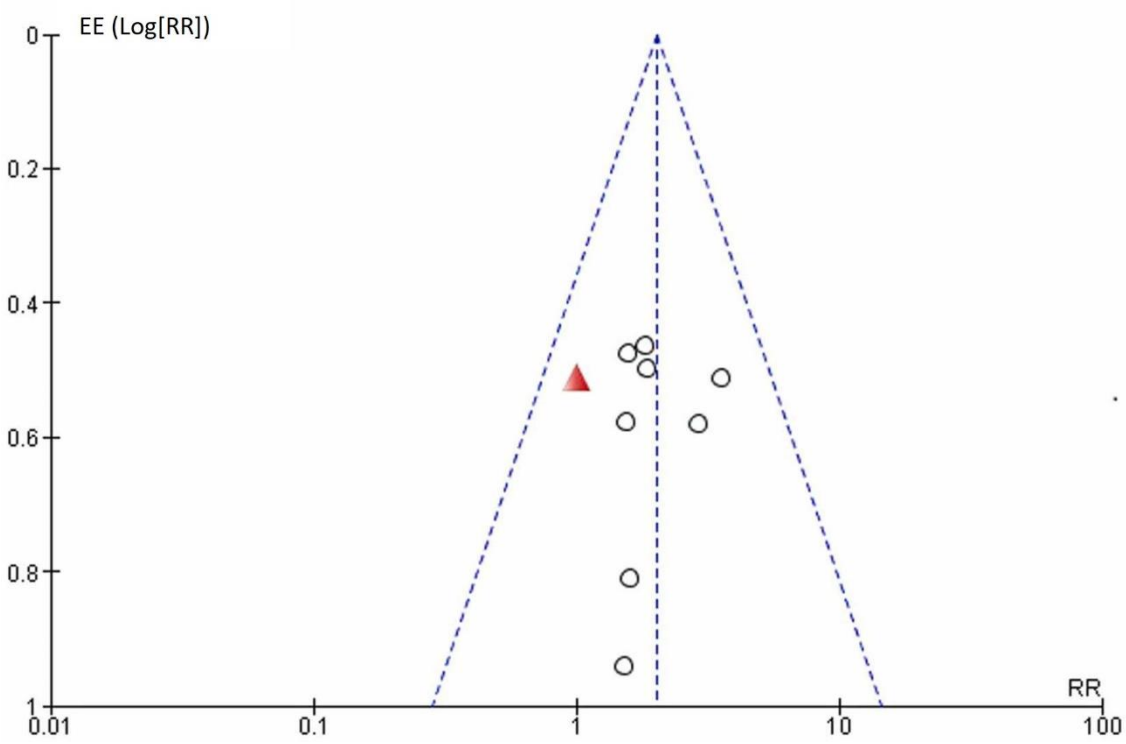


Figura 27. Gráfico de embudo. El log RR de la TM se representa en el eje x, mientras que sus valores EE se representan en el eje y. Las burbujas azules representan los 8 estudios de la literatura primaria y el triángulo representa el "nuevo estudio" agregado por la metodología trim-and-fill.

La progresión desde el epitelio oral normal hacia la neoplasia generalmente ocurre en un proceso gradual (45). La acumulación de alteraciones celulares e histológicas que preceden a su desarrollo acaban manifestándose como lesiones clínicamente visibles en la topografía oral. Estos OPMDs tienen potencial de reversión, así como de TM, dependiendo de factores como el estrés celular y el microentorno tumoral (67). La carcinogénesis oral es un proceso multipaso complejo que requiere variaciones tanto genéticas como epigenéticas. Dentro de la maquinaria molecular del ciclo celular, los genes supresores de tumores como

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P16^{INK4a} desempeñan funciones clave tanto en la oncogénesis como en la transición de lesiones precursoras (88).

Sin embargo, no se ha intentado abordar sistemáticamente la evidencia actual sobre p16^{INK4a} como marcador predictivo de OPMDs. El presente estudio alberga la muestra agrupada más grande analizada hasta la fecha (8 estudios/496 pacientes), representando el primer análisis conjunto que aborda la capacidad pronóstica de las alteraciones de p16^{INK4a} para predecir la TM. En general, este metaanálisis sugiere que la sobreexpresión de p16^{INK4a} en OPMDs está significativamente asociada con un mayor riesgo de TM a COCE con una heterogeneidad insignificante (Figura 26, Tabla 18).

Tabla 18. Análisis de subgrupos. (F= modelo de efectos fijos)

Subgrupos		No. de estudios	Modelo estadístico	Datos agrupados	P	Heterogeneidad		I^2 (%)
				RR (IC del 95%)		Q	P_{het}	
Todos		8	F	2,01 (1,36-2,96)	< 0,001	0,941	0,93	0
Continente	Asia	4	F	1,81 (1,06-3,10)	0,03	0,837	0,84	0
	América	2	F	1,72 (0,84-3,48)	0,135	0,050	0,82	0
	Europa	2	F	1,78 (0,75-4,21)	0,192	0,038	0,84	0
Punto de corte	75%	2	F	1,84 (0,94-3,58	0,071	0,001	0,98	0
	Otro	6	F	1,74 (1,09-2,78)	0,020	0,923	0,96	0
Anticuerpo	SC-166	2	F	1,57 (0,76-3,22)	0,22	0,001	0,98	0
	Otro	6		2,22 (1,40-3,52)	0,001	1,669	0,89	0
Tipo de OPMD	LO	3	F	1,57 (3,29-3,29)	0,23	0,003	0,99	0
	OPMD mixtos	3	F	1,96 (1,09-3,50)	0,023	0,666	0,72	0
	OSMF	1	F	2,89 (0,93-8,99)	0,001			
	LVP	1	F	1,83 (0,74-4,55)	0,658			
Patrón IHQ	Nuclear/ citoplasmático	7	F	2,22 (1,40-3,52)	< 0,001	1,669	0,89	0
	Nuclear	2	F	1,57 (0,76-3,22)	0,22	0,001	0,98	0

Además del valor predictivo atribuible a la sobreexpresión de p16^{INK4a}, una fracción significativa de su valor como predictor de la TM de OPMDs puede deberse a distorsiones derivadas de confusión residual. Se evidenció que algunos de los estudios primarios incluidos en nuestra síntesis de datos no proporcionaron estimaciones ajustadas de RR para factores de confusión relevantes, como la DE o la infección por VPH. No obstante, se sabe que la sobreexpresión de p16^{INK4a} es indicativa de la infección por VPH (188). En este sentido, solo 3 estudios incluidos investigaron la infección por VPH en OPMDs (189-191), con resultados inconclusos. Además, un número creciente de publicaciones señala que la sobreexpresión de p16 no representa de manera inequívoca la presencia real o, lo que es más importante, la transcripción activa de VPH en estos trastornos, ya que otros mecanismos

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relacionados con el ciclo celular y otras vías moleculares pueden contribuir a la tinción positiva de $p16^{INK4a}$ (192,193).

Reconocemos algunas limitaciones de esta revisión sistemática, comenzando por la posible presencia de sesgo de publicación. Los " small study effects " son una explicación poco probable para nuestros resultados debido a las pruebas de asimetría utilizadas, como se interpreta en el gráfico de embudo. Además, enfatizamos que el método trim-and-fill detectó un estudio aparentemente "ausente" (Figura 27). Además, nuestra síntesis cuantitativa logró resultados sólidos con una heterogeneidad casi nula, como se evidencia en el análisis de subgrupos y la interpretación del gráfico de Galbraith (Figura 26, Tabla 18). La aparente ausencia de estos sesgos estadísticos es de suma importancia, ya que, en la literatura oncológica sobre marcadores biológicos con valor pronóstico o predictivo, existe consistentemente una tendencia relevante a encontrar exclusivamente resultados positivos (194).

En nuestros análisis de subgrupos, no pudimos identificar una justificación específica que explicara la heterogeneidad del estudio debido a su grado insignificante en nuestro cálculo global. Específicamente, no se encontraron factores relevantes relacionados con técnicas de IHQ, como el punto de corte, los anticuerpos $p16^{INK4a}$ o los patrones histológicos que podrían mejorar el valor predictivo de este biomarcador. Tampoco se identificó un subtipo específico de OPMD en el cual la evaluación de $p16^{INK4a}$ podría tener un valor predictivo más fuerte para la carcinogénesis; esta suposición puede derivarse de la confusión residual dado que varios informes incluyeron datos agrupados sobre grupos no especificados de OPMDs (Tabla 18).

Si bien este estudio brinda información valiosa sobre el papel de la expresión inmunohistoquímica de $p16^{INK4a}$ en la predicción de la TM de OPMDs, se deben considerar varias limitaciones. En primer lugar, el sesgo de publicación. En segundo lugar, el número de estudios primarios incluidos en el metaanálisis fue relativamente limitado, lo que podría afectar la potencia estadística y la generalización de los hallazgos. Además, la heterogeneidad entre los estudios incluidos no fue significativa, pero las variaciones en el diseño de los estudios, las características de los pacientes y las metodologías podrían haber influido en los resultados generales. Además, aunque el uso de la expresión IHQ de $p16^{INK4a}$ parece prometedor como herramienta pronóstica, su implementación en la práctica clínica rutinaria puede requerir una validación adicional y una estandarización en diferentes laboratorios e instituciones. Por último, es importante señalar que este estudio se centró específicamente en $p16^{INK4a}$ y su asociación con la TM, y no se exploraron exhaustivamente otros biomarcadores o factores que podrían contribuir al pronóstico de OPMDs.

En general, los hallazgos de este estudio tienen implicaciones prácticas de suma importancia para el manejo de los OPMDs. El doble riesgo de TM identificado en el metaanálisis subraya la importancia de considerar la expresión de $p16^{INK4a}$ en la evaluación clínica de pacientes con OPMDs. La incorporación de la IHQ de $p16^{INK4a}$ en el estudio de pronóstico de los OPMDs puede proporcionar a los médicos información valiosa para la estratificación del riesgo y la planificación del tratamiento. Además, la multitud de ventajas

asociadas con el análisis de sobreexpresión de $p16^{INK4a}$, como su disponibilidad, simplicidad y rentabilidad, lo convierten en una adición factible y valiosa a la práctica clínica habitual en la evaluación de OPMDs.

En conclusión, nuestro estudio mostró que $p16^{INK4a}$ puede ser una herramienta complementaria notable para la evaluación de la DE, lo que lleva a una mejor caracterización biológica de la posible progresión a cáncer de OPMDs, complementándola desde una perspectiva molecular. Las investigaciones futuras deberían abordar las lagunas de conocimiento identificadas y proporcionar una comprensión más completa del valor pronóstico de $p16^{INK4a}$ y otros marcadores relacionados en el contexto de OPMDs.

A modo de resumen, la Tabla 19 presenta una síntesis de los hallazgos clave extraídos de los ocho artículos de investigación.

Tabla 19. Síntesis de los hallazgos claves de las distintas investigaciones.

Artículo	Tema Principal	Resultados Clave	Implicaciones
1	Consumo de alcohol, actitudes, percepciones y conocimientos	Alta prevalencia de AUD entre estudiantes de odontología y medicina. Diferencias de género significativas. Deficiencias en el conocimiento sobre el tema.	Necesidad de programas de capacitación específicos, diferenciados por género, para abordar el consumo de alcohol en estudiantes de la salud.
2	Uso de sustancias psicoactivas, actitudes, percepciones y conocimientos	Aumento del uso de drogas con la edad y al avanzar el ciclo académico. Percepción del riesgo varía según la sustancia.	Los programas de prevención temprana del uso de drogas deben enfocarse en los primeros años de estudio.
3	Xerostomía y su relación con la salud general en Galicia	Alta prevalencia de xerostomía, especialmente en mujeres y con la edad. Asociación con enfermedades sistémicas, condiciones psicológicas y trastornos funcionales orales.	Destaca la importancia de la detección temprana y el seguimiento de la xerostomía en pacientes con condiciones sistémicas.
4	Xerostomía en adultos mayores en España	Alta prevalencia de xerostomía en adultos mayores de 65 años. Asociación con la salud general y oral.	Importancia de evaluar y abordar la xerostomía en la atención de adultos mayores para mejorar la calidad de vida y prevenir condiciones orales relacionadas.
5	Tasa de malignización de eritroplasia oral	Tasa general de malignización del 19,9%. La presencia de DE es un factor pronóstico importante.	Es necesaria la evaluación continua y protocolos de seguimiento rigurosos en pacientes con EO.
6	Tasa de malignización de candidiasis hiperplásica crónica	Tasa de malignización del 12,1%. Su potencial de TM se subestima en la literatura actual.	Se necesita una declaración de consenso para aclarar los criterios de diagnósticos de CHC.
7	Metilación de MLH1 y MGMT en COCE	No se observó correlación entre la metilación de MGMT y MLH1 y ninguna característica clínico-patológica o de supervivencia.	Se necesitan investigaciones adicionales para confirmar el impacto de la metilación de MGMT en el pronóstico de COCE.
8	Expresión de $p16^{INK4a}$ en OPMDs	La expresión de $p16^{INK4a}$ se asocia con un riesgo dos veces mayor de malignización en OPMDs.	La utilización de $p16^{INK4a}$ se destaca como marcador para identificar a pacientes con OPMDs en riesgo de progresión a cáncer.

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En conjunto, estos artículos destacan la naturaleza multifacética de la salud oral, desde los comportamientos y conocimientos de los estudiantes hasta las posibles transformaciones malignas y los mecanismos moleculares. Los hallazgos subrayan la importancia de la educación temprana y los programas de capacitación para abordar los trastornos por consumo de sustancias. Además, la prevalencia de la xerostomía y su asociación con la salud en general enfatiza la necesidad de enfoques de atención médica integrales, especialmente en adultos mayores.

La investigación realizada en esta tesis doctoral tiene una conexión directa con el Objetivo de Desarrollo Sostenible 3, que se centra en garantizar una vida saludable y promover el bienestar para todas las edades. Al abordar la relación entre los comportamientos de riesgo, como el consumo de alcohol y sustancias psicoactivas, y la TM de OPMDs, se contribuye a la comprensión de factores que impactan la salud oral y sistémica. También se destaca la importancia de una educación de calidad en el ámbito de la medicina y odontología. Integrar conocimientos sobre los riesgos asociados con el consumo de sustancias tóxicas en los programas educativos de estos grados podría contribuir al ODS 4 de la Agenda 2030, que busca garantizar una educación inclusiva, equitativa y de calidad.

En lo que respecta a los OPMDs, las tasas variables de TM en la EO y el estatus incierto de CHC subrayan la complejidad de estas condiciones. Se necesita más investigación y criterios de diagnóstico en este sentido. La investigación en epigenética y biomarcadores se perfila como una vía prometedora para mejorar la precisión diagnóstica y la estratificación del riesgo en el cáncer oral. En última instancia, la colaboración interdisciplinaria y la investigación continua son esenciales para avanzar en la medicina oral y mejorar la atención al paciente.

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5 CONCLUSIONES

1. A high prevalence of Alcohol Use Disorder is revealed among students, with the female gender identified as an independent risk factor. Despite this, there is a lack of knowledge regarding the harmful effects of alcohol. The need for gender-differentiated training programs focusing on AUD detection is highlighted.

2. The findings indicate an increase in substance use as students progress through their academic cycle. Diverse risk perceptions and underestimation of associated dangers are evident. There is an identified necessity for preventive and educational programs, particularly in the first cycle, to raise awareness about substance abuse.

3. A high prevalence of xerostomia was observed, more frequent in women and related to systemic and oral diseases. These preliminary results suggest the need for guidelines to improve the quality of life of people with xerostomia, especially in older adults.

4. Xerostomia is more prevalent in older adults and is significantly associated with poorer oral and general health. Emphasizing the importance of assessing and managing xerostomia in older adults is crucial for enhancing their oral and overall well-being.

5. The malignant transformation rate of erythroplakia was 19.9%. The presence of epithelial dysplasia in the initial biopsy remains the main prognostic factor. Further observational studies are needed to identify the risk and its related factors in order to design effective preventive and therapeutic programs.

6. Research establishes a malignant transformation rate of 12.1% in CHC. *Candida* infection is highlighted as a possible risk factor for OPMDs, especially in oral leukoplakia. It remains challenging to state whether CHC is an individual OPMD based on the evidence collected in this study. The need for prospective studies and a consensus statement to define the natural history of CHC and establish diagnostic criteria is emphasized.

7. MHL1 and MGMT methylation was not significantly associated with clinical characteristics or survival. Increased MLH1 methylation was observed in oral squamous cell carcinoma compared to OPMD. Prospective studies in larger

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cohorts are urged to analyze gene methylation and understand the prognostic implications.

8. Analyses suggest that $p16^{INK4a}$ expression may predict malignant transformation in OPMDs. Its overexpression is linked to a twofold increased risk of malignancy. Further research is needed to fully validate this biomarker and establish specific criteria for its clinical use.

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Alba Pérez-González, Alejandro I Lorenzo-Pouso, Pilar Gándara-Vila, Andrés Blanco-Carrión, José M Somoza-Martín, Tamara García-Carnicero, Mario Pérez-Sayáns

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Counselling toward reducing alcohol use, knowledge about its morbidity and personal consumption among students of medical and dental courses in north-western Spain

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Abstract

Background: Alcohol use disorder (AUD) is directly linked to high-risk consumption. Healthcare students have a crucial role to play in its prevention and management. The aim of this study is to analyse alcohol consumption, as well as to consider the knowledge and attitudes regarding morbidity, and the stage of change when providing assistance to quit AUD.

Material and Methods: A cross-sectional study was conducted among Dentistry and Medical students using specific and validated questionnaires in an anonymous and voluntary way. Initially, 925 students were invited to participate, of them 500 were reached.

Results: Among them 85.9% suffered from AUD of whom 75% were women ($p<0.001$), and it was considered that the female gender constituted an independent risk factor (OR=2.63, CI 95% 1.55-4.45, $p<0.001$). The majority of the participants did not achieve the pass mark, nonetheless, the results showed improved levels of knowledge among participants in the latter years of their studies ($p<0.001$). Dental students demonstrated greater shortcomings in terms of their knowledge of general pathology, whereas the medical students' knowledge of oral pathologies proved worse ($p<0.001$). Most of students believed that identifying cases of AUD-affected patients falls within their competence, nonetheless, they believed that they do not have the necessary competencies. Among participants 58.2% were in a stage of change regarding AUD attitudes.

Conclusions: The majority of respondents presented AUD. In general, the participants' knowledge about alcohol was low. Reviewing the syllabuses and evaluating the implementation of gender-differentiated training programmes in both degrees would be considered necessary.

Key words: *Alcohol use disorder, healthcare students, addiction, health literacy, behaviours.*

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Introduction

Alcohol use disorder (AUD) refers to a long-term alcohol addiction that is linked to mental and/ or physical problems, and which is characterised by compulsive consumption, loss of control over consumption, and a negative emotional state when not using alcohol (1). According to the "Global status report on alcohol and health", which was published by the WHO in 2018, the harmful use of alcohol resulted in around 3 million deaths (5.3 per cent of all deaths) worldwide that year as well as 132.6 million "disability-adjusted life years" (DALY) -the number of years lost due to illness, disability or premature death-, that is to say, 5.1 per cent of all of the DALYs in that year. The European Union has the highest rate of alcohol consumption in the world (10.2 litres of pure alcohol per person per year).

Alcohol has been classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen; and in particular, a strong relationship has been detected between alcohol consumption and head and neck cancer (HNC). Each unit of alcohol consumed increases the incidence of HNC by 1 per 1000 (2) . Different in vivo and in vitro studies have presented the influence that alcohol consumption has on cancer of the oral cavity and on the transition from potentially malignant oral disorders to oral cancer (3). From the perspective of public health, several groups of health professionals have developed interventions which aim to reduce alcohol consumption and likewise this problem has also been addressed in some HNC detection projects (4).

Oral health professionals can play a crucial role in the early diagnosis of oral cancer, which represents a particularly relevant group (5). These professionals can provide brief interventions at the primary care level. However, there is no literature which seeks to optimise or collect specific information on AUD or alcohol prevention strategies in this subgroup, whereas in recent times, emphasis has been placed on other modifiable risk factors relevant to oral carcinogenesis (such as HPV infection) (6).

The study of alcohol consumption among university students has generated considerable interest throughout the world, and the first research on this topic in industrialised countries was performed in the 1970s and showed the high levels of consumption and the problems associated with this excessive use since the 1980s (7). Subsequently, several epidemiological studies have revealed a high prevalence of alcohol consumption among university students (8). Very limited research has provided information on the prevalence and intensity of alcohol consumption specifically among dentistry and medical students.

Our working hypothesis considered that consumption levels in the faculties of medicine and dentistry are similar to those observed in the general population for the same age group. Therefore, the aims of this study were: 1) to analyse the alcohol consumption of dentistry and medical students at the University of Santiago de Compostela (USC) as well as their knowledge of the effects of said consumption and 2) to study the stage of change which they are in with regards to helping patients to give up alcohol.

Material and Methods

- Design of the study

This cross-sectional study was conducted in the Faculties of Medicine and Dentistry of the University of Santiago de Compostela (Spain) following the STROBE guidelines for observational studies (9). The bioethics committee of the USC gave consent for its execution (Ref. AUC-19-2019). All of the first to the fifth year and postgraduate dentistry students, and the first and final year medical students were invited to participate. Printed surveys were distributed during teaching hours and participation was anonymous and voluntary. The survey was also delivered by means of a Google form which was sent to students who were unable to attend the on-site classes.

- Validation of the instrument

A pilot survey was tested on a sample of 50 randomly selected students in order to assess the reliability and thoroughness of the survey, as well as the time taken to complete it. The average time taken to complete the survey was seven minutes. These 50 pilot cases were subsequently discarded and the final sample was collected. Cronbach's alpha test was used to determine the final reliability of the test. Stratification in thematic blocks was used and the weighted mean value for this survey was 0.74. The data allowed us to ensure moderate reliability.

- Questionnaire

The survey was divided into six sections: (i) Sociodemographic data (age, gender, year of study and faculty), (ii) Individual alcohol consumption, (iii) Ease with which they deal with sensitive issues during clinical practice, (iv) Knowledge about alcohol morbidity, (v) Knowledge of alcohol prevention strategies and (vi) Stages of change. The second section used the validated version of the AUDIT-C (increasing the cut-off point by one unit, which therefore increased its predictive value, bringing it to 4 in men and 5 in women) (10) and the CAGE test, validated by Mayfield in 1974 (11). In sections III and V, the 5-point Likert scale was used with the questions on attitude and confidence described by Ntouva *et al.* (12). Section IV combined two multiple choice questions and eight single-answer questions about alcohol knowledge as described by Miller *et al.* (13). The last section (VI) measured the stages of change. This section was based on the transtheoretical model which was designed by Prochaska & DiClemente, and which the authors had adapted in 2019 based on a previous adaptation by Lorenzo-Pouso *et al.* (6). Although this version looked to analyse professional

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health attitudes in relation to providing advice to patients regarding the HPV infection, whereas the adapted version used for this study looked to evaluate the readiness of medical and dental students to provide advice to patients on giving up alcohol. This last section enabled us to divide students into four groups: pre-contemplation, contemplation, action and change.

- Sample size

In order to calculate the minimum sample size required, we used Epidat 4.2 (SERGAS, Galicia, Spain) with a confidence interval of 95% and a margin of error of 5%. The sample size calculation was based on the number of enrolled students at that time (n=225 in Dentistry) and (n=700 in Medicine). Based on this number, the minimum sample size required for our design was 275, and in the end we included 500 surveys.

- Variables and collected data

Demographic data: age, gender, faculty, year of study, cycle (first and second year students corresponded to the first cycle; third, fourth and fifth year to the second cycle and the postgraduate degrees in dentistry and the sixth year of the medical degree corresponded to postgraduate studies). Alcohol consumption: AUDIT-C, Sum AUDIT-C, Risk Drinking (High/Low), CAGE (C: Cut down; A: Annoyed; G: Guilty; E: Eye opener), Sum CAGE. Attitudes (Likert Scale). Knowledge about alcohol (test) and its morbidity in oral and general pathologies (multiple choice). Trust levels (Likert scale). Stages of change. Weighted (0-10) and grouped grades (fail, pass, good, outstanding).

- Statistical analysis

The data was collected in a specifically designed database and it was manually entered with repeated control in order to avoid errors. This data was statistically analysed using the SPSS v.24.0 software (IBM, Statistics, NY, USA). The categorical variables were described by frequencies and percentages, and the quantitative variables were described by mean and standard deviation. According to the central limit theorem, we considered the distribution of quantitative variables to be normal given the large sample size. Contingency tables were established in order to study the relationships between the categorical variables using the Chi-square test. Parametric statistics were used to study the effect of the quantitative variables on the qualitative variables, performing the ANOVA test with Bonferroni post-hoc correction for comparisons with more than two elements. We analysed the paired correlation of quantitative variables using Pearson's coefficient. Binomial logistic regression analysis was performed in order to verify the risk of AUD. The significance level was set at $p \leq 0.05$.

Results

- Descriptive

The sample consisted of 500 participants with a mean age of 20.7±3.8 years. 134 of the participants were men (26.8%), 361 were women (72.2%) and 5 were self-included in the gender: other (1%). The response rate was 47% in the faculty of medicine and 74.2% in the faculty of dentistry. 420 surveys were handed out on site, of which 394 were returned, and 500 were sent online, with 106 responses. Participation in the virtual survey was lower and mostly female (74.3%). Full descriptive data can be found in Table 1.

- Alcohol consumption

The AUDIT-C showed an average value of (5.72±2.06) with a range of 3-15. By turning this variable into low and high-risk drinkers, we observed that 75.5% of the at-risk drinkers from our sample were women, compared to 24.1% of men, which was considered to be a statistically significant difference ($p<0.001$) (Table 2). However, when using the CAGE test no statistically significant differences regarding consumption were observed. By performing a binomial logistic regression, we verified the role of gender as the only independent factor for suffering from AUD, and in particular women have an accentuated risk (OR=2.63, CI:95% 1.55-4.45, $p<0.001$).

- Knowledge

Overall the participants' knowledge on alcohol morbidity was considered to be low. In terms of general pathology, the knowledge of more than half of the students from both faculties was not considered to be satisfactory. 55.7% of the students from the faculty of dentistry and 51.7% of students from the faculty of medicine failed, and just 1.2% and 5.1% of the students from the respective faculties were awarded the score of outstanding ($p=0.020$). With regards to the cycle, almost 60% of undergraduate students failed compared with 36.6% of the postgraduate students ($p<0.001$). In terms of oral pathology, 67.9% of the male students failed in comparison with 64.3% of the female students ($p=0.044$). In this specific field, 73% of the students from the faculty of medicine failed, in comparison with 49.1% of the students from the faculty of dentistry ($p<0.001$), and more than 80% of students from the first cycle failed compared to 29.5% of students from the second cycle ($p<0.001$). With regards to the quantitative analysis of the students' weighted knowledge, the results were similar to the grouped results and we found significant differences in the students' knowledge of general and oral pathology depending on which academic cycle they were in. With regards to their knowledge about general pathology, the average score for students in the second cycle of 5.58 (CI: 95% 5.24-6.10) was higher than that of the students in the first cycle in which the average of 4.52 represented a fail grade (CI: 95% 4.31-4.72) ($p<0.001$). With regards to knowledge of oral pathology, the average mark for the dentistry students was 5.09, which represented a pass grade (CI: 95% 4.76-5.42) while the average mark

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for medical students was 4.02, representing a fail grade (CI: 95% 3.75-4.27) (p<0.001). We were able to appreciate a greater difference in the students' knowledge of oral pathology when taking their level of study into account, than we did when we made the same comparison when considering their knowledge of general pathology. In the latter the second cycle students achieved higher marks (6.03, CI:95% 5.57-6.49) than the students in the first cycle (3.60, CI:95% 3.40-3.80) (p<0.001). However, these differences were not appreciated in terms of their knowledge of alcohol, which in general was low, and was only minimally acceptable amongst the students from the second cycle and without any statistically significant differences.

Table 1: Descriptive data of the sample.

Variable		Number (%)
Gender	Male	134 (26.8)
	Female	361 (72.2)
	Other	5 (1)
Faculty	Dentistry	167 (33.4)
	Medicine	333 (66.6)
Year of study	1	259 (51.8)
	2	37 (7.4)
	3	39 (7.8)
	4	34 (6.8)
	5	78 (15.6)
	6	35 (7)
	Postgraduate	18 (3.6)
Cycle	First cycle	335 (67)
	Second cycle	112 (22.4)
	Master's degree	53 (10.6)
CAGE	Negative test	228 (45.7)
	Social drinker	121 (24.2)
	High risk	98 (19.6)
	Harmful alcohol use	48 (9.6)
	Dependence on alcoholic beverages	4 (0.8)
AUDIT-C	Low risk	70 (14.1)
	High risk	428 (85.9)
Stages of change	Pre-contemplation	12 (2.6)
	Contemplation	100 (21.6)
	Action	82(17.7)
	Change	270 (58.2)
Knowledge of general pathology	Fail	265 (53)
	Pass	127 (25.4)
	Good	0
	Very good	89 (17.8)
	Outstanding	19 (3.8)
Knowledge of oral pathology	Fail	325 (65)
	Pass	86 (17.2)
	Good	0
	Very good	66 (13.2)
	Outstanding	23 (4.6)
General knowledge on alcohol	Fail	193 (38.6)
	Pass	123 (24.6)
	Good	118 (23.6)
	Very good	65 (13)
	Outstanding	0

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In Fig. 1 is displayed the data related to the specific matters for each field. The main pathologies that the students associated with alcohol consumption were: neuropathies (n=399), followed by oral cancer (n=365) and cavities (n=343). The least associated were: tuberculosis (n=39) and candidiasis (n=89). With regards to their general knowledge about alcohol, no statistically significant differences were observed, with 61.3% of the individuals presenting sufficient knowledge on this topic.

- Stages of change

Broadly speaking, most of the participants were in a stage of change (58.2%) (Table 3). This was the case for 63.3% of the medical students and 49.1 % of the dentistry students (p<0.001). However, with regards to the cycle, 62% of the students in the first cycle were in a stage of change, compared with 56% of students in the second cycle and 40.4% of the master's degree students (p=0.024) respectively.

Table 4 shows the relationship between the scores obtained in the different knowledge areas and the influence of these on the stages of change. Here we can observe that as the students' knowledge of general pathology improves, so too does their stage. As such, students in the pre-contemplation stage achieved an average mark of 3.10, (CI:95% 2.33-3.85) and this increased amongst students in the action (5.05, CI:95% 4.59-5.51, p=0.015) and change stages (5.08, CI:95% 4.81-5.34, p=0.008) with an average pass mark. Statistically significant differences were not observed in the rest of the knowledge areas.

Table 2: Analysis of risk of consumption according to gender, faculty, year of study and cycle.

		At-risk drinker			
		Number (%)			*p* value
		Low risk	High risk	Total	
Gender‡	Male	31 (44.3)	103 (24.1)	134 (26.9)	<0.001**
	Female	37 (52.9)	323 (75.5)	360 (72.3)	
	Other	2 (2.9)	2 (0.5)	4 (0.8)	
Faculty†	Dentistry	26 (37.1)	141(32.9)	167 (33.5)	0.490
	Medicine	44 (62.9)	287 (67.1)	331 (66.5)	
Year of study‡	1	38 (54.3)	220 (51.4)	258 (51.8)	0.437
	2	7 (10)	30 (7)	37 (7.4)	
	3	2 (2.9)	37 (8.6)	39 (7.8)	
	4	7 (10)	27 (6.3)	34 (6.8)	
	5	11 (15.7)	66 (15.4)	77 (15.5)	
	6	4 (5.7)	31 (7.2)	35 (7)	
	7	1 (1.4)	17 (4)	18 (3.6)	
Cycle‡	First cycle	47 (67.1)	287 (67.1)	334 (67.1)	0.505
	Second cycle	18 (25.7)	93 (21.7)	111 (22.3)	
	Master's degree	5 (7.1)	48 (11.2)	53 (10.6)	

p values from Chi-square† and ANOVA test‡.

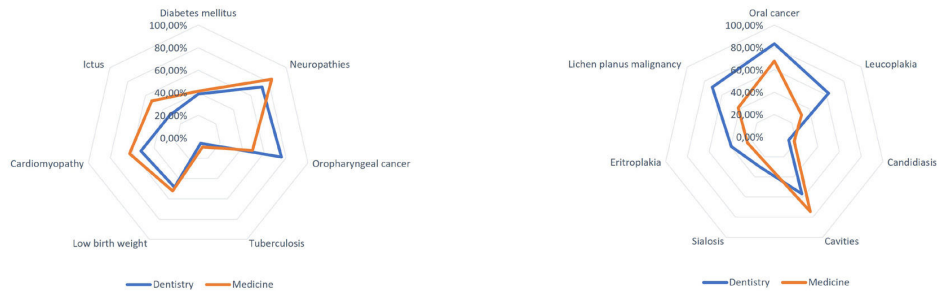


Fig. 1: Spider web diagram on knowledge of alcohol morbidity: A) General pathology, B) Oral pathology.

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Table 3: Distribution of the stages of change according to Prochaska-DiClement model.

Variables		Stages of change Number (%)					*p* value
		Pre-contemplation	Contemplation	Action	Change	Total (%)	
Gender‡	Male	2 (1.6)	31 (24.8)	24 (19.2)	68 (54.4)	125 (100)	0.105
	Female	9 (2.7)	68 (20.3)	58 (17.3)	200 (59.7)	335 (100)	
	Other	1 (25)	1 (25)	-	2 (50)	4 (100)	
Faculty†	Dentistry	7 (4.2)	55 (32.9)	23 (13.8)	82 (49.1)	167 (100)	<0.001**
	Medicine	5 (1.7)	45 (15.2)	59 (19.9)	188 (63.3)	297 (100)	
Cycle‡	First cycle	7 (2.3)	58 (19.1)	50 (16.5)	188 (69.6)	303 (100)	0.024*
	Second cycle	2 (1.8)	30 (27.5)	16 (14.7)	61 (56)	109 (100)	
	Master's degree	3 (5.8)	12 (23.1)	16 (30.8)	21 (40.4)	52 (100)	
AUDIT-C†	Low risk	3 (4.6)	12 (18.5)	10 (15.4)	40 (61.5)	65 (100)	0.601
	High risk	9 (2.3)	88 (22.1)	71 (17.8)	230 (57.8)	398 (100)	
CAGE‡	Negative test	6 (2.9)	43 (21)	33(16.1)	123 (60)	205 (100)	0.521
	Social drinker	-	25 (21.6)	25 (21.6)	66 (56.9)	116 (100)	
	Risky alcohol use	5 (5.3)	18 (19.1)	18 (19.1)	53 (56.4)	94 (100)	
	Harmful alcohol use	1 (2.2)	12 (26.7)	6 (13.3)	26 (57.8)	45 (100)	
	Alcohol dependence	-	2 (50)	-	2 (50)	4 (100)	
	Total (%)	12 (2.6)	100 (21.6)	82 (17.7)	270 (58.2)	464 (100)	

p values from Chi-square† and ANOVA test‡.

Table 4: Analysis of knowledge of alcohol morbidity according to the stage of change.

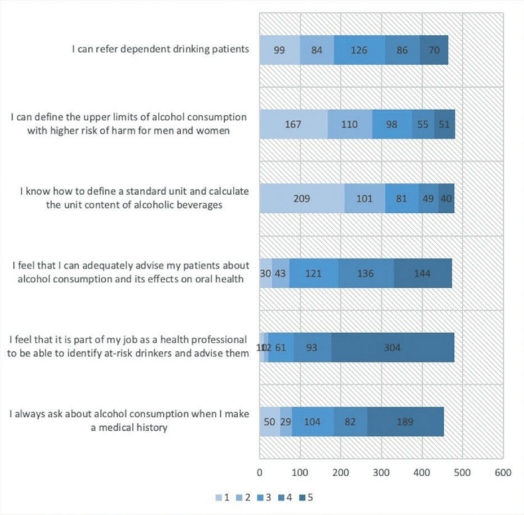
Variable	Covariates	Number (%)	Mean	Standard deviation	95% Confidential interval		*p* value
	E Stage of change				Lower	Upper	
General pathology score†‡	Pre-contemplation	12 (2.59)	3.09	1.19	2.33	3.85	0.015* Pre-contemplation versus Contemplation: 0.022* Pre-contemplation versus Action: 0.015* Pre-contemplation versus Change: 0.008*
	Contemplation	100 (21.55)	4.95	1.90	4.57	5.33	
	Action	82 (17.67)	5.05	2.08	4.59	5.51	
	Change	270 (58.19)	5.07	2.17	4.81	5.34	
Oral pathology score†	Pre-contemplation	12 (2.59)	4.76	1.95	3.51	6.00	0.374
	Contemplation	100 (21.55)	4.82	2.18	4.39	5.26	
	Action	82 (17.67)	4.51	2.31	4.00	5.02	
	Change	270 (58.19)	4.36	2.39	4.07	4.64	
General alcohol score†	Pre-contemplation	12 (2.59)	4.06	1.69	2.98	5.14	0.210
	Contemplation	99 (21.38)	5.16	1.97	4.77	5.55	
	Action	82 (17.71)	4.83	1.64	4.47	5.19	
	Change	270 (58.32)	4.93	1.82	4.71	5.15	

p values from ANOVA test† with Bonferroni correction‡.

- Attitudes

With regards to the students' attitudes, the majority stated that they ask their patients about alcohol consumption when taking their clinical history, and 60.8% completely agreed that identifying AUD and assisting them with this matter forms part of their job. A high percentage of the participants felt that they were able to do so in an adequate manner, however, a high number of the participants were neither able to determine the upper limits of consumption for men and women, nor were they able to calculate the consumption in standard units (only 8% claimed that they definitely knew how to define a standard unit) as we can see in Fig. 2.

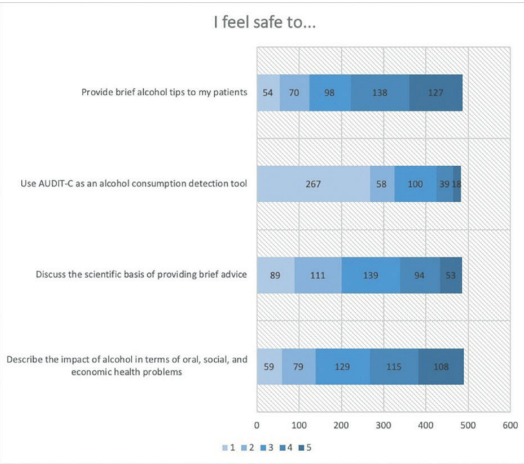
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1 totally disagree – 5 totally agree

Fig. 2: Attitudes and perceptions.

With regard to the participants' levels of confidence (Fig. 3), although a high percentage of the students considered that they were capable of giving advice, very few felt that they were able to discuss the scientific basis of the advice or the effects of alcohol. 41.2% stated that they did not feel at all confident to do so, or that they did not know how to use the AUDIT-C test to detect cases of risk.



1 totally disagree – 5 totally agree

Fig. 3: Level of confidence.

Discussion

Alcohol consumption within our sample proved alarming, as according to the AUDIT-C test, 85.9% of the participants were at-risk drinkers and this high prevalence has also been reported among health science students in Spain by Rabanales Sotos *et al*. (14). Our results are perfectly comparable with the data gathered in Europe: Ireland (65.2% of men and 67.3% of women presented AUD) (15) and UK (86.1% are consumers and more than a half presented AUD) (16); even globally: Tunisia (52.5% of students presented AUD) (17), Ecuador (49.73% of men and 23.80% of women reported AUD) (18), and Australia (almost 25% present risky alcohol use, higher in the case of men) (19). It is worth highlighting that 75.5% of the at-risk drinkers in our study were women. However, when comparing the results in depth, we observed that the data regarding the prevalence of "at-risk" consumption is heterogeneous due to the variability of the definitions used by the authors, the measurement tools and the established thresholds. In our opinion, global standard protocols and guidelines must be established in order to determine the risk of AUD.

On the other hand, we also studied the knowledge of the medical and dentistry students regarding alcohol-related pathologies given that these are fundamental strategies in preventive medicine. The participants' knowledge of oral and general pathologies was considered to be insufficient in both levels, and likewise, significant differences were observed between the cycles. This divergence can be explained by the acquisition of specific knowledge throughout the degree programme. Nevertheless, even in the latter years of study, the scores in general were low. Our results were similar to those of the study published by Wang *et al*. in 2020 (20) in which the majority of health students in Mongolia demonstrated very limited knowledge about the damage caused by alcohol, and this lack of knowledge was greater in occasional and regular drinkers than it was in non-drinkers (74.0% and 75.3% vs 42.1%). Kujan *et al*. studied the knowledge about oral cancer among students in Arabia Saudi and conveyed the need for the undergraduate study syllabus to be reinforced, in particular with regards to education on oral cancer, and specifically its prevention and early detection (21,22).

Therefore, it is clear that initiatives must be introduced in order to reduce alcohol abuse and the damage associated with it. These initiatives must focus on changing social beliefs and standards, as well as looking into the factors that affect the decisions made by young people to consume alcohol (16). These programs must be implemented according to gender (18), focus on the different stages which the students find themselves in, and likewise, they must be implemented in conjunction with effective public policies that can address the problem.

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In recent years, different groups have worked on dental education projects related to the oral pathology, directed both at professionals from the sector and dentistry students (6,21,22). In the USA, a three-week pilot program in which medical students treated patients suffering from mental disorders caused by substance use led to students feeling more confident and at ease when treating these patients and these results were sustained over time (23). In particular, and in relation to alcohol, Farah *et al*. published some of the most relevant references showing the relationship between this addictive behaviour and oral cancer (24).

With regards to attitudes and perceptions, a considerable lack of documentation can be observed. In our sample, the majority of participants were aware that identifying and helping at-risk drinkers is fundamental, however, the results showed that most of them did not feel that they were prepared to do so. In a study conducted among medical students in Lebanon, 38.2% of the students felt that they had received the appropriate training on AUD and just 29.1% showed any interest in working in this field. Around 75% agreed that they must intervene in cases of AUD and more than 80% considered that the prognosis for AUD depends on its proper management (25). In Denmark, the analysis of the perceptions and attitudes of university students showed that these students considered alcohol as an essential part of their lives and most of them agreed that increasing restrictions would be necessary (26). With regards to their attitudes, some of them suggested the need for secondary and tertiary prevention and intervention focused on persons at risk, while others suggested the need for primary universal prevention (27). In relation to the stage of change which they are in when providing patients with assistance, according to Daley *et al*. (28), most of our respondents were in a stage of change at the time (58.2%), with a significantly higher percentage of medical students than dentistry students finding themselves in this situation. As far as we can see, no further studies have been conducted regarding this topic. Diana Ramos *et al*. studied the stages of change in relation to encouraging patients to give up the habit and recommended adapting the strategies to the stage of change which they students were in, suggesting using cognitive, affective and evaluative techniques in the early stages (pre-contemplation and contemplation) in order for them to progress through those stages (29). We consider reinforcing the syllabuses of these degrees to be fundamental since it has been observed that as the student's knowledge of general pathology improves, the stage of change that the students finds themselves in also improves.

A lack of specific literature must be highlighted as one of the most significant limitations in this study. We believe that collaboration between groups could generate a new target for public health and a new path towards more publications. This has been achieved by other researchers who have carried out work on other risk factors in order to integrate this sensitive issues in day-to-day practice, such as Daley *et al*. with HPV (28) or Ramseier *et al*. with tobacco (30), in the case of AUD Miller *et al*. studies are highlighted (13). Likewise, we also believe that, due to the lack of literature on the matter, we should seize the opportunity to take action on what is certainly a matter of urgency.

Conclusions

Most of the respondents presented with AUD, 75% of whom were women. The female gender is an independent risk factor for AUD (OR=2.63). Most of the participants' knowledge of alcohol morbidity was considered to be insufficient. Globally, the participants' level of knowledge was low, although this improved during the most advanced courses, with dentistry students presenting greater gaps in knowledge in terms of general pathology and medical students in terms of oral pathology. Practically all of the students agreed that identifying at-risk drinkers and providing them with assistance with this issue forms part of their work. With regards to their attitudes to AUD, the majority were in a stage of change (58.2%) and we observed that as their knowledge of general pathology improved, so too did the stage that they were in at that precise moment. The syllabuses must be reviewed in terms of their content on alcohol issues, and training programs must be implemented, which should be differentiated by gender in both degrees, and must emphasise morbidity related to alcohol and the knowledge about the tools and strategies for detecting AUD.

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Conflict of interest
Authors certify that there is no conflict of interest.

Ethics
Ethics Committee approval reference AUC-19-2019. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors contributions
A.I.L.P., and M.P.S conceived the ideas; A.P.G., P.G.V., A.B.C, and J.M.S.M: collected the data; M.P.S., A.I.L.P., and A.P.G. analysed the data; A.P.G., and A.I.LP. led the writing.

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ARTÍCULO 2.

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ORIGINAL ARTICLE

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Drug use, risk perceptions and attitudes towards drug use amongst medical and dentistry students

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Abstract

Aims: To analyse the use of psychoactive substances and the risk perceptions amongst odontology and medical students. To study their perceptions, attitudes and knowledge, and to evaluate their motivation when helping their patients to stop using these substances.

Methods: A cross-sectional study was conducted amongst 962 students in Spain, using validated questionnaires on an anonymous basis.

Results: Amongst these students, drug use varies and increases with age as assessed by the DAST and CAST tests, with more problematic use being observed as the academic cycle progresses ($p < .001$). Participants in the 2nd cycle presented higher consumption than those in the 1st cycle, in the univariate model (OR = 1.77, IC 95% 1.27–2.48, $p = .001$) and in the adjusted model (OR = 1.86, IC 95% 1.32–2.62, $p < .001$). Regarding CAST, non-problematic use in the 1st cycle versus the 3rd cycle presented an OR = 8.69 (IC 95% 4.50–16.78, $p < .001$) and for low risk use it presented an OR = 15.18 (IC 95% 1.83–14.68). Only 46.7% considered using marijuana on a regular basis as a high risk, whilst 60.5% stated that smoking a pack of cigarettes represents a high risk. Alcohol was the substance for which the risk perception was lowest. 66.2% are in the maintenance stage "I provide my regular drug-using patients help to give up," with women being more likely to be in this stage ($p = .012$).

Conclusions: High risk of drug use increases after the 1st cycle in Dentistry and in Medicine. Training programmes should be implemented in both degrees, focusing on the 1st years in order to simultaneously prevent drug use amongst students.

KEYWORDS
attitude, drug use, medical and dentistry students, risk perceptions

1 | INTRODUCTION

Drug use worldwide is on the increase, both in general terms, and also in terms of the proportion of the global population that uses these substances. Around 269 million people used drugs on at least one occasion in 2018, that is to say, 5.3% of the global population aged from 15 to 64 years.[1] Substance use has significant social and health consequences, which is why it is so important to continue

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working to reduce its incidence. Health professionals play an important role in this work, and, as such, their attitudes towards drug use prove decisive when it comes to advising their patients to stop using these substances. As a result, numerous studies have analysed substance use amongst medical students since the 1980s.[2,3] As future health workers, it is important that they are aware of the negative consequences that using these substances can have on health, because of the crucial role they will play in detecting drug use and in counselling their patients.[4] Several studies have reported a high prevalence of psychoactive substance use amongst medical[5–9] and nursing[10] students. Colomer-Pérez et al.[11] recently observed that exposure to substances in this group was linked to a greater presence of social and personal problems. A study on dentistry students from the UK, determined that 2.6% males and 0.5% females were regular cannabis users. According to this study, other commonly used substances were ecstasy, LSD, cocaine, inhalants and ketamine.[12] As has been observed with tobacco use, the role of odontology professionals in detecting drug use and in providing subsequent counselling to their patients is fundamental.[13] The dental environment is increasingly recognised as a useful site for detecting multiple problems.[14,15] With regard to the role played by dentists in this field, in other studies they have been asked whether or not they ask their patients about substance use when taking their medical records,[16] whether they agree that identifying patients who use substances forms part of their role as professionals,[16,17] and, what skills they think are required to deal with said patients.[17,18] Said studies have concluded that there is a significant lack of awareness of the relevant role that dentists may play when addressing this issue. When it comes to other medical specialists, a study conducted in Spain observed that resident interns are provided with a considerable amount of advice on this topic, in particular regarding alcohol and tobacco. These studies determined that the frequency with which they are provided with this type of clinical advice depends on the speciality studied, and on certain characteristics of their personal consumption. It has been observed that there is a direct relationship between the use of illegal drugs and the age at which individuals begin to consume alcohol or to smoke.[19]

Previous studies on this group[20] have observed that a high percentage of students in these faculties presented problematic alcohol consumption, and despite being aware that their job requires them to be able to detect problems related to alcohol consumption, nonetheless, they did not consider that they had the necessary skills to do so. Very few studies have actually provided any insight into the attitudes of odontology and medical students, who are the future health workers, regarding drug use.[21] Therefore, studying the use of psychoactive substances amongst this population, as well as determining their risk perception, and their knowledge, attitude, and motivation when helping their patients is essential. Thus, the objectives of this study were (1) to analyse the use of psychoactive substances and the risk perceptions amongst odontology and medical students; (2) to study their perceptions, attitudes and knowledge and (3) to evaluate their motivation when helping their patients to stop using these substances.

2 | METHODS

2.1 | Participants

The final sample was comprised of 962 undergraduate and postgraduate students from the Faculty of Dentistry and the Faculty of Medicine of de University of Santiago de Compostela, of which 73.1% were female, 25.8% male and 1% marked "other," with an average age of 20.90 years (DE = 3.27 years).

2.2 | Sample size calculation

The sample size estimation was done based on the difference in the expected proportions between academic cycles. Thus, for an expected proportion of 10% in population 1 (2nd cycle), and 5% in population 2 (1st cycle), for a sample size ratio of 1, a confidence level of 95% and a power of 80%, the required sample size would be 435 in each population group, making a total of 870 participants. This calculation was done using Epidat 4.2 (SERGAS).

2.3 | Questionnaire

The questionnaire was divided into eight sections: (I) Sociodemographic data are as follows: age, gender, faculty and year. The 1st- and 2nd-year students were grouped together in the 1st cycle; the 3rd, 4th and 5th years were grouped in the 2nd cycle, and the postgraduate, odontology PhD and 6th year students (additional, with very specific characteristics and medical internships) were grouped in the "postgraduate" group. (II) Tobacco consumption (yes/no), age at which they started consuming tobacco and number of cigarettes per day. (III) Drug Abuse Screening Test (DAST), designed by Skinner in 1982[22] and validated in Spanish,[23] which evaluates substance use and the consequences derived from said consumption. This test consists of 20 items, and a score of 5 or over is considered as problematic use. (IV) Cannabis Abuse Screening Test (CAST): a six-item questionnaire used to detect problematic patterns of cannabis use and cannabis use disorder (CUD), providing a total score that ranges from 0 to 6. In the case in which a respondent attains a score of 4 or more, they are considered to have problematic consumption.[24,25] (V) Assessment of the frequency of psychoactive substance use. Adaptation of the items used in the EDADES study[26] to assess the prevalence of the use of different psychoactive drugs (hypnosedatives, opioids, cannabis, cocaine, ecstasy, GHB, amphetamines, methamphetamines, hallucinogens, magic mushrooms, heroin and volatile inhalants), through the use of four time indicators: at least once in my lifetime, in the last 12 months, in the 30 days prior to the survey, and every day in the 30 days prior to the survey. (VI) Assessment of the perception of risks associated with drug use. Adaptation of the items used in the study published by the Spanish National Drugs Plan[27] to assess the perception of risks associated to drug use. We analysed the following behaviours: smoking one pack

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of cigarettes per day, regular use of marijuana, using ecstasy at the weekend, using LSD once a month, drinking two alcoholic beverages per day, and drinking more than four alcoholic beverages in a single sitting. The scale used was: 1 no risk to 4 high risk. (VII) Assessment of the attitudes and knowledge of health professionals when advising their patients to give up drugs. Adaptation of the alcohol assessment described by Ntouva et al.[28] We used the Likert scale (1 strongly disagree–5 strongly agree). Aptitudes: "I always ask about narcotic substance use when taking a patient's medical history," "I feel like it is part of my job to identify patients that use substances and advise them." Knowledge: "I can correctly give advice to my patients about the use of different drugs and their effects on health," "I am familiar with the drug use screening tools." (VIII) Assessment of the students' motivation to give their patients advice about giving up drug use. We prepared the items following the assessment of the stages of change according to Prochaska & DiClemente's transtheoretical model, later adapted by Daley[29]: "I do not provide my patients with assistance to help them to give up drugs and I do not intend to do so" (pre-contemplation); "I do not provide my patients with assistance to help them give up drugs, but I have thought about doing so" (contemplation); "I provide my patients with assistance to help them give up drugs every now and then" (action); "I provide my regular patients with assistance to help them give up drugs" (maintenance). Odontology students from 1st to 2nd year, and medical students from 1st to 3rd year do not undertake clinical activity, for that reason we asked them whether they intended to carry out said activities when they did eventually see patients.

2.4 | Proceedings

We conducted a descriptive study that was approved by the USC Bioethics Committee (Ref. USC-24/2020). All 1st to 5th year and postgraduate dental students, and 1st- to 6th-year medical students were invited to participate. The assessment instruments were administered in an online format using Microsoft Forms, and they were distributed by the academic authority via a link or zappa code. The participants had free access to the questionnaire between October 2020 and January 2021. Participation was anonymous and voluntary, in compliance with the ethical and data protection requirements.

2.5 | Instrument validation

We conducted a pilot study with a sample of 50 students who were randomly selected in order to assess the reliability of the instrument and determine the time needed to complete it. The average time taken to finish the evaluation was7 min. Cronbach's alpha test was used to determine the reliability of the final task. Stratification in thematic blocks was used and the weighted average value for this study was 0.86. This data enabled us to ensure high reliability.

2.6 | Statistical analysis

We collected the data in a database designed ad hoc. The data were statistically analysed using the SPSS v.24.0 software (IBM, Statistics). The categorical variables were described through frequencies and percentages, and the quantitative variables were determined through averages and standard deviation. We studied the relationship between the categorical variables using the chi-square and Kruskal–Wallis tests. To assess the effects that the quantitative variables had on the qualitative variables, we used a parametric statistic for the ANOVA test, and Bonferroni's post hoc correction was used for the multiple comparisons. The degree of correlation of the quantitative variables was analysed using Pearson's correlation coefficient (CC). Kappa index was computed for questions of both CAST and DAST questionnaires. Univariate and adjusted binomial and multinomial logistic regression models were constructed in order to determine the odds ratio (OR) for drug use and for stage of change in relation to the covariates: gender, cycle and faculty. The significance level was established at $p \leq .05$.

3 | RESULTS

3.1 | Data of use

The sample consisted of 962 participants, 15.5% were smokers, and the average age at which they began to smoke was 14.96 years (DE = 5.47). The descriptive data are shown in Table 1. Tables S1 and S2 show the descriptive data for DAST-20 and CAST respectively. The most used substance amongst the participants was cannabis, with 25.4% stating that they had used it once, 11% that they had used it in the last year, and 8.6% that they had used it in the last week. Therefore, it was possible to deduce that 55% of the sample had never used cannabis, compared to a percentage of greater than 92% for the rest of substances, as is shown in Table S3. Exploring cannabis consumption and its relationship with sociodemographic factors in depth, it is observed that there is no relationship between consumption and gender and faculty of origin; however, there are differences in terms of course ($p < .001$), age ($p = .032$) and cycle ($p < .001$). Thus, 18.9% of the first-year students and 28.6% of the second-year students have ever consumed in their lifetime, and 24.7% of the sixth-year students and 23.5% of the second-year students have consumed in the last 7 days. As for the cycle, 61.5% of students in the first cycle have never consumed cannabis, but 43.3% in the second cycle have consumed in the last 12 months. In relation to the mean age, the highest was for the group who have consumed in the last 7 days (21.47 years IC 95% 20.96–21.98) and the lowest for those who have never consumed, 20.62 years (IC 95% 20.30–20.94). In relation to grams of cannabis per month, there were no differences in terms of gender ($p = .262$), or faculty ($p = .312$), although there were differences again in terms of course ($p < .001$) and cycle ($p < .001$). Thus,

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TABLE 1 Descriptive data from the sample.

Categorical variables		N (%)
Demographic data		
Gender	Male	248 (25.8)
	Female	702 (73.1)
	Other	10 (1)
Faculty	Dentistry	222 (23.1)
	Medicine	737 (76.9)
Year	1st	224 (23.3)
	2nd	283 (29.4)
	3rd	133 (13.8)
	4th	83 (8.6)
	5th	98 (10.2)
	6th	108 (11.2)
	Postgraduate	31 (3.2)
Cycle	1st cycle	507 (52.8)
	2nd cycle	314 (32.7)
	Postgraduate	139 (14.5)
Tobacco consumption	No	810 (84.5)
	Yes	149 (15.5)
CAST	Score 0–1	861 (89.5)
	Score 2–3	43 (4.5)
	Problematic use	58 (6)
DAST-20	Non-problematic	747 (77.7)
	Problematic use	214 (22.3)
Risk perception		
1 pack of cigarettes/day	No risk	9 (0.9)
	Low risk	47 (4.9)
	Moderate risk	323 (33.7)
	High risk	580 (60.5)
Regular use of marijuana	No risk	6 (0.6)
	Low risk	75 (7.8)
	Moderate risk	430 (44.9)
	High risk	447 (46.7)
Ecstasy at the weekend	No risk	3 (0.3)
	Low risk	14 (1.5)
	Moderate risk	201 (21.1)
	High risk	736 (77.1)
LSD once a month	No risk	10 (1)
	Low risk	66 (6.9)
	Moderate risk	284 (29.8)
	High risk	593 (62.2)
2 alcoholic beverages/day	No risk	35 (3.7)
	Low risk	281 (29.4)
	Moderate risk	447 (46.7)
	High risk	194 (20.3)
4 alcoholic beverages/occasional	No risk	37 (3.9)
	Low risk	266 (27.7)
	Moderate risk	403 (42)
	High risk	253 (26.4)
Attitudes and behaviour		
Asking about drug use when taking the patient's medical history	Strongly disagree	34 (4)
	Disagree	58 (6.9)
	Undecided	148 (17.6)
	Agree	170 (20.2)
	Strongly agree	431 (51.2)
Identifying drug users	Strongly disagree	9 (1)
	Disagree	32 (3.6)
	Undecided	89 (10.1)
	Agree	236 (26.8)
	Strongly agree	516 (58.5)
Advising about drug use and its effects	Strongly disagree	31 (3.6)
	Disagree	107 (12.3)
	Undecided	236 (27.1)
	Agree	199 (22.8)
	Strongly agree	298 (34.2)
Knowledge of screening tools	Strongly disagree	264 (30)
	Disagree	246 (27.9)
	Undecided	212 (24.1)
	Agree	91 (10.3)
	Strongly agree	68 (7.7)
Stages of change		
Stage of change	Pre-contemplation	17 (2.3)
	Contemplation	156 (20.7)
	Action	81 (10.8)
	Maintenance	498 (66.2)

Quantitative variables	Average (DE)	Min-Max
Sum DAST- 20	3.14 (2.15)	0–15
Sum CAST	0.17 (0.72)	0–6
Start of tobacco consumption (age)	16.70 (2.05) years old	12–24 years old
No. cigarettes/day	4.04 (3.91)	0–20
Grams of cannabis/month	4.91 (7.53)	0.5–35

the highest consumption was for sixth-year students with 9.6 gr (95% CI 5.4–13.8) and the lowest for third-year students with 0.9 gr (95% CI 0.6–1.2). The highest consumption was for students in the third cycle with 8.2 gr (95% CI 4.5–11.8) compared to those in the second cycle with 1.3 gr (95% CI 0.8–3.8). In relation to age and grams of cannabis, there was a low but statistically significant correlation by using non-parametric approach (Spearman's Rho = 0.270) (p = .002).

According to CAST, 6% of the participants presented a problematic use of cannabis (CUD). Hypnosedatives were the 2nd most used substance after cannabis, followed by opioids, magic mushrooms and ecstasy. With regard to cocaine, 98.1% stated that they have never taken it.

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TABLE 2 Comparative analysis from the drug use tests depending on gender, faculty and cycle. Chi-square statistical test.

Variables		N (%) DAST-20			p	N (%) CAST				p
		Non-problematic use	Problematic use	Total		0–1	2–3	Problematic use	Total	
Gender	Male	192 (77.4)	56 (22.6)	248 (25.9)	.642	219 (88.3)	16 (6.5)	13 (5.2)	248 (25.8)	.352
	Female	544 (77.6)	157 (22.4)	701 (73.1)		630 (89.7)	27 (3.8)	45 (6.4)	702 (73.1)	
	Other	9 (90)	1 (10)	10 (1)		10 (100)	0 (0)	0 (0)	10 (1)	
Faculty	Dentistry	181 (81.5)	41 (18.5)	222 (23.2)	.114	199 (89.6)	15 (6.8)	8 (3.6)	222 (23.1)	.045
	Medicine	563 (76.5)	173 (23.5)	736 (76.8)		659 (89.4)	28 (3.8)	50 (6.8)	737 (76.9)	
Cycle	1st cycle	416 (82.2)	90 (17.8)	506 (52.8)	.002	471 (92.9)	20 (3.9)	16 (3.2)	507 (52.8)	<.001
	2nd cycle	227 (72.3)	87 (27.7)	314 (32.7)		286 (91.1)	15 (4.8)	13 (4.1)	314 (32.7)	
	Postgraduate	103 (74.1)	36 (25.9)	139 (14.5)		103 (74.1)	8 (5.8)	28 (20.1)	139 (14.5)	

According to DAST-20, 22.3% of the participants presented a problematic use of substances. When analysing the results from the questionnaires in order to assess the problematic use of psychoactive substances, no significant differences were found in terms of gender. However, significant differences were found in terms of age. According to DAST-20, the average age of the participants who presented non-problematic use was 20.79 years old (DE = 3.4 years, IC 95% 20.5–21 years). That is to say, there was a lower percentage of problematic use amongst participants from the 1st cycle 17.8% ($p = .002$). Cannabis use also varied and according to the CAST assessment it also rose. The average age of the participants who presented problematic use was 20.8 years old (DE = 3.3 years, IC 95% 20.5–21 years), with low risk at 21.6 years old (DE = 3.2 years, IC 95% 20.6–22.6 years) and high risk at 22.5 years old (DE = 2.3 years, IC 95% 21.9–23.1 years), showing significant differences between non-problematic and high-risk use (Bonferroni test, $p < .001$). Problematic use was greater in students in the later academic cycles ($p < .001$) and there was a higher problematic use of cannabis amongst medical students (6.8%) than dentistry students (3.6%) ($p < .01$). The full data are shown in Table 2.

Regarding the efficiency of the different instruments, for the CAST in relation to cannabis use, considering three possible options non-problematic (never or ever in life), low (monthly) and high risk (weekly), the kappa coefficient using Crombach's alpha was 0.391 ($p < .001$). For the ability to detect problematic use using the CAST, the sensitivity was 62.5% and the specificity was 95.9%. For the DAST, in relation to non-prescription drug use (no, non-problematic and yes, problematic use) the kappa coefficient was 0.464 ($p < .001$), sensitivity 94.8% and specificity 68.4%.

3.2 | Risk perception

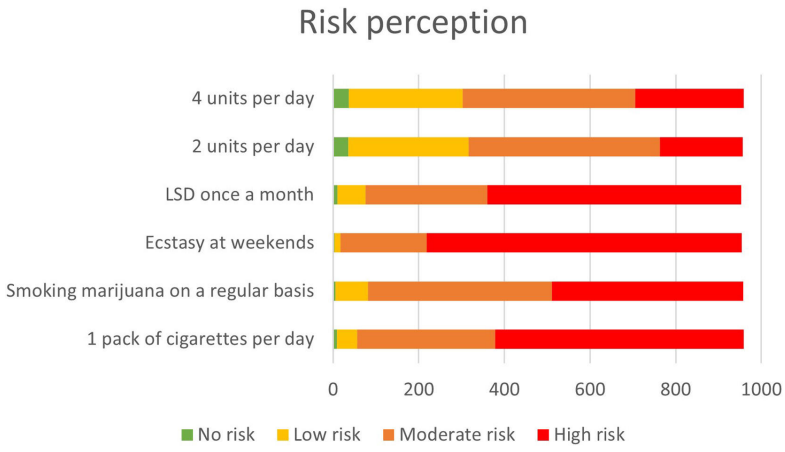
The substances that the participants considered to be a higher risk were ecstasy, when used weekly, followed by LSD, when used monthly. Less than half of the sample (46.7%), considered using marijuana on a regular basis as a high risk, whilst more than half of the participants stated that they believed that smoking a pack of cigarettes represents a high risk (60.5%). Alcohol was the substance for which the risk perception was lowest. Full descriptive data are shown in Table 1 and Figure 1.

3.3 | Attitudes, knowledge and stage of change

With regard to the attitudes and knowledge studied, as shown in Figure 2, the majority of students always ask their patients about these issues when taking their medical history (51.2%) and feel capable of identifying regular users (58.5%). However, only 34.2% advise them about the effects of drugs, and only 7.7% stated that they were familiar with the different screening tools. No significant differences were found in terms of gender regarding any of

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FIGURE 1 Perceived risk associated to drug use



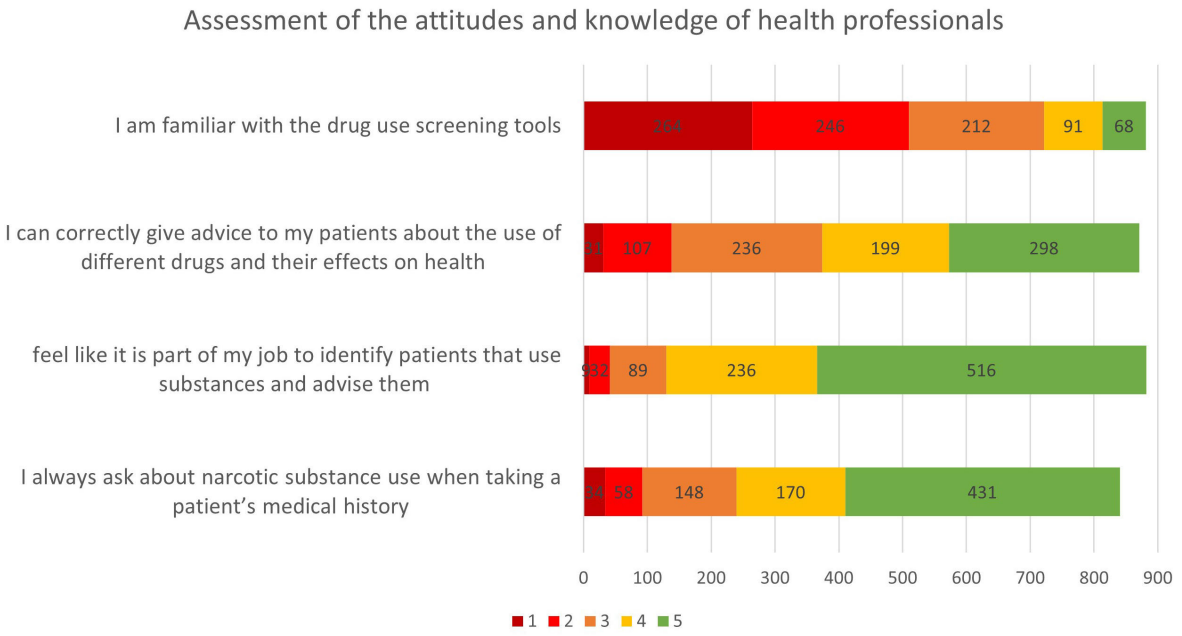


FIGURE 2 Assessment of the attitudes and knowledge of health professionals when advising their patients to give up drugs.

these variables. With regard to the faculty where the participants study, 58.6% of dentistry students always (level 5 Likert scale) ask their patients about drug use when taking their medical history, compared to 48.9% of medical students ($p = .006$), whilst 51.7% of dentistry students feel that it is part of their job to identify and advise drug-using patients, compared to 60.5% of medical students ($p < .001$).

In terms of motivation when it comes to informing patients about drug use, the majority of respondents are in the maintenance stage (66.2%) "I provide my regular drug-using patients help to give up." Regarding gender differences, women are more likely than men to be in this stage (68.6% vs. 59.9% $p = .012$). In terms of the degree, 57.8% of dentistry students stated that they were in this stage, compared to 68.8% of medical students ($p < .001$), and 76.5% of 1st cycle students, compared to 59.7% of 2nd-cycle students and 50.8% of postgraduate students ($p < .001$) (Table 3).

3.4 | Multivariate analysis

Binomial logistic models for DAST-20 were created and multinomial (for CAST and stage of change) univariate models, sequentially adjusted for gender, cycle and faculty models were created to determine the OR.

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TABLE 3 Distribution of the stages of change according to the Prochaska-DiClemente model.

Variable		Stages of change *n* (%)					
		Pre-contemplation	Contemplation	Action	Maintenance	Total	*p*
Gender	Male	5 (2.5)	43 (21.5)	33 (16.5)	119 (59.9)	200 (26.6)	.012
	Female	11 (2)	112 (20.6)	48 (8.8)	374 (68.6)	545 (72.6)	
	Other	0 (0)	1 (16.7)	0 (0)	5 (83.3)	6 (0.8)	
Faculty	Dentistry	9 (4.9)	53 (28.6)	16 (8.6)	107 (57.8)	185 (24.7)	<.001
	Medicine	8 (1.4)	103 (18.2)	65 (11.5)	389 (68.8)	565 (75.3)	
Cycle	1st cycle	8 (2.2)	50 (13.9)	27 (7.5)	276 (76.5)	361 (48.1)	<.001
	2nd cycle	4 (1.5)	73 (27.2)	31 (11.6)	160 (59.7)	268 (35.7)	
	Postgraduate	5 (4.1)	33 (27)	22 (18)	62 (50.8)	122 (16.2)	
DAST-20	Non-problematic	15 (2.6)	116 (20.1)	61 (10.6)	385 (66.7)	577 (76.8)	.574
	Problematic use	2 (1.1)	40 (23)	20 (11.5)	112 (64.4)	174 (23.2)	
CAST	Score 0–1	12 (1.8)	138 (20.7)	69 (10.4)	447 (67.1)	666 (88.6)	.106
	Score 2–3	5 (13.9)	5 (13.9)	3 (8.3)	23 (63.9)	36 (4.8)	
	Problematic use	0 (0)	13 (26)	9 (18)	28 (56)	50 (6.6)	
Total (%)		17 (2.3)	156 (20.7)	81 (10.8)	498 (66.2)	962 (100)	

Note: The comparisons were carried out using the Kruskal–Wallis nonparametric test for ordinal variables.

TABLE 4 Univariate logistics regression analysis to determine the OR for drug use, assessed using DAST-20 and CAST.

Covariate	DAST-20 Problematic			
	Univariate OR (95% CI)	*p*	Adjusted OR (95% CI)	*p*
Gender				
Male vs. Female	1.01 (0.72–1.43)	.955	0.96 (0.67–1.37)	.821
Cycle				
2nd vs. 1st	1.77 (1.27–2.48)	.001	1.86 (1.32–2.62)	<.001
3rd vs. 1st	1.62 (1.04–2.52)	.034	1.60 (1.02–2.52)	.040
Faculty				
Medicine vs. Dentistry	1.36 (0.93–1-98)	.115	1.53 (1.04–2.26)	.032

	CAST not problematic				CAST low-risk			
	Univariate OR (95% CI)	*p*	Adjusted OR (95% CI)	*p*	Univariate OR (95% CI)	*p*	Adjusted OR (95% CI)	*p*
Gender								
Male vs. Female	1.20 (0.64–2.27)	.568	1.55 (0.79–3.07)	.206	2.05 (0.86–4.91)	.107	2.46 (0.99–6.11)	.053
Cycle								
1st vs. 3rd	8.00 (4.17–15.33)	<.001	8.69 (4.50–16.78)	<.001	4.37 (1.57–12.19)	.005	15.18 (1.83–14.68)	.002
2nd vs. 3rd	5.98 (2.98–11.99)	<.001	6.01 (2.98–12.13)	<.001	4.04 (1.37–11.91)	.011	3.96 (1.33–11.83)	.014
Faculty								
Dentistry vs. Medicine	1.89 (0.88–4.05)	.103	1.85 (0.84–4.07)	.124	3.35 (1.26–8.88)	.015	3.12 (1.15–8.44)	.025

Note: The adjusted statistical analysis was carried out by means of a multivariate logistics regression adjusted by gender, cycle and faculty.
Abbreviation: OR, odds ratio.

With regard to consumption assessed through DAST-20, participants in the 2nd cycle presented higher consumption than those in the 1st cycle, both in the univariate model (OR = 1.77, IC 95% 1.27–2.48, $p = .001$) and in the adjusted model (OR = 1.86, IC 95% 1.32–2.62, $p < .001$) (Table 4). Regarding CAST, consumptions with a low and moderate risk were preferential in the 1st cycle, both in the univariate and adjusted model. Thus, non-problematic use in the 1st cycle versus the 3rd cycle presented an OR = 8.69 (IC 95% 4.50–16.78, $p < .001$) and for low-risk use it presented an OR = 15.18 (IC 95% 1.83–14.68). Gender is not expressed as a significant covariate in drug use.

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In terms of the stage of change, dentistry students appear to be at earlier stages than medical students, and in relation to the maintenance stage, in pre-contemplation for the adjusted model, dentistry students presented an OR = 5.06 (IC 95% 1.79–14.27, $p = .002$) and for the contemplation stage, they presented an OR = 1.61 (IC 95% 1.07–2.43, $p = .023$) (Table 5).

4 | DISCUSSION

With regard to drug use, cannabis is the 3rd most used drug worldwide, following alcohol and tobacco, and it was estimated that 192 million people used cannabis in 2018.[1] In our survey, 45% of the surveyed participants had tried cannabis at least once, although it should be noted that participants from dentistry and medicine have quite different characteristics in terms of workload and curriculum content. This is a higher percentage than the results of a recent meta-analysis in which this figure accounted for 31.4%, worldwide.[30] A possible drug-related disorder was observed in 6% of the students, representing a similar percentage to the one obtained by Fond et al.[7] who obtained a 5.7% CUD in their sample of medical students, once the cut-off point had been established at 3 (it is also useful for its detection[31]). With regard to gender differences, Candido et al.[6] observed that the use of different types of drugs, except for depressants, is more common amongst males. Some authors also determined that there is a higher risk of using cannabis and CUD amongst males.[7,30] However, it has also been described that female drug use is different, and they are more likely to suffer from CUD.[32,33] In our sample, no significant differences were found in terms of gender distribution. Thus, as other authors have shown, this could show an increase in substance abuse amongst women.[33]

In the present study, the importance of the academic year was a novel discovery, given that the lowest percentage of drug-related problems was found amongst 1st-cycle students. This could provide a basis for establishing the point at which interventions should take place, as these are necessary in both degrees. It is important for these to take place during the 1st semester in order to prevent the increasing risks resulting from substance usage. With regard to cannabis, its use ranged from being non-problematic, to representing a high risk amongst young people aged 21–22; therefore, it is important to identify the problem amongst young people and set out preventive strategies before that period. Becoña et al.[34] defined a way of better predicting cocaine and ecstasy use amongst young people aged 14–25. Likewise, it was observed that, within this age group, the users of psychostimulants get drunk and develop risk behaviours much more frequently. Thus, specific training and prevention campaigns aimed at this group must be developed.[35]

With regard to perceptions, attitudes and knowledge, 85.3% of the students (87.5% in medicine and 77.8% in dentistry) think that identifying and advising drug users forms part of their job. This figure is higher than the one observed in a study of odontologists, in which it was determined that only half of them (53.9%) considered

TABLE 5 Univariate logistics regression analysis to determine the OR for the stage of change.

	PRE-CONTEMPLATION			CONTEMPLATION			ACTION		
Covariate	Univariate OR (95% CI)	*p*	Adjusted OR (95% CI)	Univariate OR (95% CI)	*p*	Adjusted OR (95% CI)	Univariate OR (95% CI)	*p*	Adjusted OR (95% CI)	*p*
Gender										
Male vs. Female	1.43 (0.49–4.19)	.516	1.11 (0.37–3.37)	1.21 (0.80–1.81)	.366	1.05 (0.69–1.60)	2.16 (1.33–3.52)	.002	1.94 (1.17–3.21)	.010
Cycle										
1st vs. 3rd	0.36 (0.11–1.14)	.081	0.32 (0.09–1.06)	0.34 (0.20–0.57)	<.001	0.35 (0.20–0.59)	0.28 (0.15–0.52)	<.001	0.29 (0.15–0.54)	<.001
2nd vs. 3rd	0.31 (0.81–1.19)	.088	0.23 (0.06–0.91)	0.86 (0.52–1.42)	.550	0.81 (0.48–1.35)	0.55 (0.29–1.02)	.056	0.57 (0.30–1.06)	0.077
Faculty										
Dentistry vs. Medicine	4.09 (1.54–10.86)	.005	5.06 (1.79–14.27)	1.87 (1.26–2.77)	.02	1.61 (1.07–2.43)	0.89 (0.49–1.61)	.711	0.78 (0.43–1.44)	.428

Note: The adjusted statistical analysis was carried out by means of a multivariate logistics regression adjusted by gender, cycle and faculty.
Abbreviation: OR, odds ratio.

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that said tasks form part of their job.[16] Priyadarshini also observed that odontologists agreed that they should identify patients who use drugs.[17] Goel et al.[18] stated that most odontologists showed a positive attitude towards said patients, and they agreed that problematic users must be identified and treated in dental environments. 71.4% of the students ask or are aware that they should ask about substance use when taking their patients' medical histories, a similar percentage to the one obtained by Parish et al.[16] (77.2%) However, only 18% stated that they are familiar with the screening tools for drug use, a figure that concurs with other studies carried out by other authors, in which it has been determined that even though dentists are familiar with the importance of detecting drug use, they do not have the necessary specific knowledge, clinical training and tools to foster those intervention strategies.[17,18]

Our findings show that only a few of the participants considered excessive alcohol and regular cannabis use as a dangerous behaviour, as was also observed in the study by Calafat et al.[27] As Kilmer et al.[36] suggested, risk perception can be a relevant factor with regard the participants initiating substance use. Therefore, highlighting the possible consequences of drug use amongst students that have never used them can lead to continuous abstinence. As far as we know, no specific literature deals with the students´ willingness to help their patients.

With regard to the study limitations, we must firstly highlight its transversality. Furthermore, there are notable academic differences between dental and medical students. Another limitation is the fact that students do not undergo clinical practice until their 3rd year (dentistry students) and 4th year (medical students), although they do acquire preclinical competences; therefore, we assessed their intention. The percentage of participation in these initial years was lower than in the years in which students treat patients, which therefore limits the inferences of this study.

22.3% of the participants presented problematic drug use, with cannabis being the most commonly used substance (6% of them presented suspected Cannabis Use Disorder) followed by hypnosedative drugs, opioids, magic mushrooms and ecstasy. High risk of drug use increases after the 1st cycle. Most of the dentistry and medical students stated that identifying and assessing drug users forms part of their duties, and over half of them stated that they always help patients who are drug users, with a significantly higher percentage of medical students stating this than dentistry students, and especially 1st cycle students. However, most of them were not familiar with the different screening tools that are available in this field.

Therefore, we think that it is essential for the syllabus to be reviewed in terms of its content on drug use, and, likewise, some initiatives must be introduced to reduce its use and the possible associated problems. Training programmes should be implemented in both degrees, focusing on the 1st years in order to simultaneously prevent drug use amongst students.

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CONFLICT OF INTEREST

The authors certify that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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ARTÍCULO 3.

Título: Association between xerostomia, oral and general health, and obesity in adults. A cross-sectional pilot study

Alba Pérez-González, Juan A Suárez-Quintanilla, Eva Otero-Rey, Andrés Blanco-Carrión, Francisco J Gómez-García, Pilar Gándara-Vila, Benjamín Martín-Biedma, Mario Pérez-Sayáns

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Association between xerostomia, oral and general health, and obesity in adults. A cross-sectional pilot study

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Abstract

Background: The objective of this study was to analyse the association between oral and general health variables and obesity indicators with the sensation of dry mouth or xerostomia as evaluated on the Xerostomia Inventory (XI).

Material and Methods: A total of 354 randomly selected subjects participated in this cross-sectional pilot study and completed an anonymous questionnaire. Anthropometric, clinical, and xerostomic variables were evaluated. Kruskal-Wallis, ANOVA and Bonferroni test were used for multiple comparisons. ROC curves and multinomial logistic regression were used to determine the (OR) risk of xerostomia.

Results: A total of 30.7 % of respondents reported xerostomia based on XI. The dry mouth question, the XI taken as a "gold standard", showed a diagnostic sensitivity of 70.37 %, and a specificity of 83.27 % (AUC=0.768, $p<0.001$). Logistical regression showed the highest xerostomia OR was associated to patients with bad self-perceived health, 6.31 (CI 95% 2.89-13.80, $p<0.001$). In the model adjusted for tooth mobility, bone or respiratory diseases, and the consumption of anxiolytics and antidepressants, the OR was 3.46 (CI 95% 1.47-8.18, p=0.005).

Conclusions: a high prevalence of xerostomia was found in this cross-sectional pilot study, which was significantly more frequent in women, and increased with age. Xerostomia was associated to several systemic diseases, psychological conditions, and oral functional disorders such as tooth mobility.

These preliminary results can serve as the basis for developing guidelines for the application of innovative measures designed to improve the quality of life of individuals with xerostomia.

Key words: *Xerostomia, systemic pathology, oral health, obesity, geriatrics.*

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Introduction

Xerostomia is defined as the subjective sensation of a dry mouth and is considered "the illness of modern man". The term is frequently used to refer to a salivary gland dysfunction with decreased salivary secretion, a condition known as hyposialia or hyposalivation. The subjective sensation (xerostomia) and the objective measurement (hyposialia) do not always concur (1). The sensation of oral dryness does not intrinsically involve a diminished quantity of saliva, as some patients diagnosed for hyposalivation do not perceive this sensation. Hyposialia is measured using sialometric (2), and patients are administered the Xerostomia Inventory (XI) to evaluate the severity of the sensation of mouth dryness (3). The incidence and prevalence of xerostomia is higher in women,and increases with age (4). Xerostomia has been associated to an array of chronic pathologies and medications such as hypertension and Angiotensin-converting enzyme (ACE) inhibitor treatment, diuretics, and medication for psychological disorders such as anxiety or depression (5,6). A significant association has been found between medication and xerostomia, (7) with adverse reactions frequently linked to certain drugs, making mouth dryness a pervasive problem among geriatric patients (8). This segment of the population is numerous in Galician society, where according to the National Institute of Statistics the mean age is around 47.48 years.

The person's lifestyle pattern such as alcohol consumption and smoking is a key factor both in the occurrence and in long-term xerostomia given that smokers exhibit salivary gland dysfunction with diminished secretion of aqueous (9). In terms of diet, ensuring the daily consumption of fresh fruit and vegetables and the intake of liquids is vital for rehydration and maintaining oral health (10), given that the correct rehydration of oral mucosa reduces symptoms (11).

Obesity is a major risk factor for an array of diseases,and is related to xerostomia and other oral problems such as caries, periodontitis, and tooth loss (12,13). Dyslipidaemia, arterial hypertension, and hyperglycaemia, together form a cluster of medical conditions disorders broadly referred to as the metabolic syndrome. The underlying causes are multiple and include a sedentary lifestyle, age, genetics, malnutrition, and even masticatory hypofunctionality, as suggested in previous studies of the group, where the number of functional teeth was directly related to abdominal obesity. The increasing prevalence of obesity leads to this metabolic syndrome characterized by abdominal obesity, insulin resistance, hypertension, and dyslipidaemia (14). Previous studies of the group have shown that masticatory hypofunctionality may be associated to this metabolic syndrome, and the number of functional teeth has been directly related to abdominal obesity (15).

The presence of oral problems is associated to a poorer self-perception of quality of life (16). Moreover, bad oral health has been found to raise the risk of certain conditions such as aspiration pneumonia, cardiovascular disorders, diabetes, among others (17), though further research is required in most of the branches of geriatric odontology (18). The objective of this study was to analyse the association between oral and general health variables and obesity indicators with the sensation of dry mouth or xerostomia as evaluated on the XI.

Abbreviations: Body Mass Index (BMI); Waist-to height ratio (WHtR);Xerostomia Inventory (XI).

Material and Methods

- Study design

This cross-sectional study complied with the ethical principles of the Helsinki Declaration 2013, and was approved by the Ethics Committee of Galicia under reference PGU-HD-2018-01, as part of the research project analysing the association between general and oral health. The data were gathered anonymously from October 2019 to February 2020 in accordance with the recommendations of the STROBE guidelines for observational studies (19). All participants freely volunteered and informed consent was obtained prior to inclusion in the study. Respondents were assured their data would remain anonymous and confidential according to the Spanish Data Protection Laws.

- Questionnaire design

A self-report questionnaire was designed in Google forms, consisting of 36 questions with a binary or multiple response format with closed options. The questionnaire was divided into 5 sections referring to (I) sociodemographic data, (II) oral health, (III) general health, (IV) overweightness and/or obesity, (V) xerostomia. The data was gathered using the questionnaire designed in Google Forms, and was read aloud by the interviewer to assist in the understanding and use of technology when requested by the respondent, or to clarify any queries. I) sociodemographic data: location of questionnaire administration, age, sex, academic status, smoking habits. II) Oral health: tooth mobility, tooth loss, dentures/implants, gingival bleeding, mandibular pain, and dry mouth sensation. III) General Health: hypertension, diabetes, cholesterol, degenerative bone, cardiac, gastrointestinal, and neurological disorders, consumption of anxiolytics and/or antidepressants, physical exercise, good self-perceived general health, consumption of fresh fruit and vegetables. IV) Overweightness and obesity: height, weight, and waist circumference diameter. V) Xerostomia: XI. The full questionnaire is available in Supplement 1.

- Sample population, inclusion and exclusion criteria

The data were gathered anonymously from a diverse and widespread area of the Autonomous Community of Galicia, north-western Spain: the survey was con-

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ducted at streets level, in waiting rooms in health centres and hospitals (among non-odontological healthcare workers), chemists, and service sector workers (in supermarkets, shops, bakeries, and so forth). The sample consisted of 355 adults aged 50 years or older, who were randomly selected from the general population of Galicia. Inclusion criteria: adults aged 50 years or older who volunteered to participate in this study. Exclusion criteria: patients under the age of 50 years, pregnant mothers, respondents refusing consent.

- Variables and data

Xerostomia Inventory (XI): The XI is an 11-item instrument with 5-point graded response format to evaluate the severity aspects of xerostomia as measured on a continuum. The Spanish version (20) of the instrument designed in 1999, has a score range from 0 to 44 points, with high values indicating severe xerostomia symptoms. The value ranges for each degree were as follows: 0-11: no xerostomia, 12-22 mild xerostomia, 23-33 moderate xerostomia, and 34-44 severe xerostomia.

- BMI

The body mass index (BMI) is calculated based on the following formula: bodyweight in kilograms divided by height in meters squared. According to this calculation, the World Health Organisation has classified nutritional status as follows: Very severe thinness ≤ 15; Severe thinness 15-15.9; Thinness 16-18.4; Healthy weight 18.5-24.9; Overweight 25-29.9; Moderate obesity 30-34.9; Severe obesity 35-39.9, and Morbid obesity ≥ 40. As this formula is limited in that it fails to evaluate body fat distribution, the waist-to-height ratio (W/Ht) was also used.

- Waist-to-height ratio (WHtR)

The WHtR has been observed to be a simpler and more predictive indicator than the BMI (21). The waist-to-height ratio (WHtR) is defined as a person's waist circumference divided by height, both in centimetres. This index is more predictive of the risk of obesity associated to cardiovascular diseases than the BMI. Critical values vary according to age and sex, being 0.6 for over 50 year-olds. Significant gender differences were observed between men and women in the WHtR distribution: extremely thin (men and women <0.34); healthy thin (men 0.35 to 0.42; women 0.35 to 0.41); healthy (men 0.43 to 0.52; women 0.42 to 0.48); overweight (men 0.53 to 0.57; women 0.49 to 0.53); very overweight (men 0.58 to 0.62; women 0.54 to 0.57); morbid obesity (men > 0.63; women > 0.58).

- Statistical analysis

The data were collected and stored on a database specifically designed for this study. Statistical analyses were performed using the SPSS v.24.0 software package (IBM, Statistics, NY, USA). The categorical variables were expressed in frequencies and percentages, and the quantitative variables in means and standard deviation. According to the central limit theorem, the distribution of the quantitative variables could be considered normal as the sample size was large. Contingency tables were designed to analyse the associations between categorical variables using a chi-squared test, and a Kruskal-Wallis test was used for ordinal-level variables. In order to assess the effects of quantitative variables over qualitative ones, parametric statistics were used for the ANOVA test, and for the Bonferroni post-hoc correction for multiple comparisons. The degree of correlation of certain variables was analysed using Pearson's correlation coefficient (CC). In order to evaluate the diagnostic efficacy of the XI, ROC curves were used to determine sensitivity, specificity, and the area under the curve (AUC). Both univariate and adjusted multinomial logistic regression were used to determine the (OR) risk of xerostomia. The level of significance was $p \leq 0.05$.

Results

The sample consisted of 229 women (64.7 %) and 125 men (35.3%), who were distributed in the following age groups: 269 (75.8%) from 50 to 64 years, 62 (17.5%) from 65 to 79 years, and 24 (6.8%) 80 years or older. The complete data are shown in Table 1. In association to oral health, 45.6 % of respondents had experienced tooth mobility, and 60.2 % lacked dentures or implants. The most prevalent systemic diseases was hypercholesterolemia (32.7 %), followed by degenerative bone conditions (29.3 %), and hypertension in 22.3 % of participants. However, 90.7 % of respondents perceived their general health was good or very good. Moreover, 34.6 % reported no regular physical exercise, and 32.2 % consumed neither fresh fruit nor vegetables daily. The mean BMI was 25.56 ± 4.40, and the WHtR was 0.56 ± 0.10. As for xerostomia, 33.1 % responded Yes to the question 'Do you have a dry mouth sensation?' The mean for the XI test was 9.21 ± 7.70. A total of 69.3 % of respondents reported no xerostomia, 24.5% mild, 5.1 % moderate, and 1.1% severe xerostomia, that is, a total of 30.7 % of those surveyed reported xerostomia. The complete data are shown in Table 2. A correlation coefficient was found between the direct question and the XI test of CC=0.525, $p<0.001$. The dry mouth question, the XI taken as a "gold standard", showed a diagnostic sensitivity of 70.37 %, and a specificity of 83.27 % (Youden index=0.54, AUC=0.768, $p<0.001$) (Fig.1).

According to the XI, (24 %) of men reported mild, and (1.6%) moderate xerostomia, whereas 6.6 % of women reported moderate, and 1.7% severe xerostomia ($p=0.072$). Age was a significant factor, the prevalence of xerostomia was highest in the 80-year-old or older age group, with 54.2 % reporting xerostomia as compared to 35.5 % in the 65-79 year-old group, and 27.5 % in the 50-64 year-old group ($p=0.001$). Patients with tooth mobility reported more xerostomia than patients with no tooth mobility, 39.5 % vs. 23.3 % ($p=0.001$).

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Table 1: Descriptive sample data and the association of the variables with the Xerostomia Inventory.

Variables		N	% No	Xerostomia Inventory					
				Mild	Moderate	Severe	Xeros total	*p*	
Section I: Sociodemographic data									
Sampling point	Street	262	75.1	193 (73.7)	59 (22.5)	8 (3.1)	2 (0.8)	69 (26.4)	0.012
	Institutionalized patients	35	10	18 (51.4)	10 (28.6)	7 (20)	0 (0)	17 (48.6)	
	Chemists	7	2	3 (42.9)	3 (42.9)	1 (14.3)		4 (57.1)	
	Service industry personnel	5	1.4	3 (60)	2 (40)	0 (0)	0 (0)	2 (40)	
	Non-odontologist health-care workers	33	9.5	21 (63.6)	10 (30.3)	2 (6.1)	0 (0)	12 (36.4)	
	Health centres	7	2	4 (57.1)	2 (28.6)	0 (0)	1 (14.3)	3 (42.9)	
Sex	Men	125	35.3	93 (74.4)	30 (24)	2 (1.6)	0 (0)	32 (25.6)	0.072
	Women	229	64.7	153 (66.8)	57 (24.9)	15 (6.6)	4 (1.7)	76 (33.2)	
Age	From 50 to 64	269	75.8	195 (72.5)	65 (24.2)	7 (2.6)	2 (0.7)	74 (27.5)	0.001
	From 65 to 79	62	17.5	40 (64.5)	13 (21)	8 (12.9)	1 (1.6)	22 (35.5)	
	Over 80	24	6.8	11 (45.8)	9 (37.5)	3 (12.5)	1 (4.2)	13 (54.2)	
Academic status	Primary	86	24.2	51 (59.3)	26 (30.2)	7 (8.1)	2 (2.3)	35 (40.6)	0.378
	Secondary	39	11	31 (79.5)	7 (17.9)	1 (2.6)	0 (0)	8 (20.5)	
	Vocational training	51	14.4	43 (84.3)	6 (11.8)	0 (0)	2 (3.9)	8 (15.7)	
	University degree	179	50.4	121 (67.6)	48 (26.8)	10 (5.6)	0 (0)	58 (32.4)	
Smoking habits	Never smoked habitually	173	48.7	117 (67.6)	45 (26)	9 (5.2)	2 (1.2)	56 (32.4)	0.517
	Yes, I am a smoker	59	16.6	37 (62.7)	21 (35.6)	1 (1.7)	0 (0)	22 (37.3)	
	I am an ex-smoker	123	34.6	92 (74.8)	21 (17.1)	8 (6.5)	2 (1.6)	31 (25.2)	
Section II: Oral health									
Tooth mobility	No	193	54.4	148 (76.7)	40 (20.7)	5 (2.6)	0 (0)	45 (23.3)	0.001
	Yes	162	45.6	98 (60.5)	47 (29)	13 (8)	4 (2.5)	64 (39.5)	
Tooth space	No	141	39.8	98 (69.5)	33 (23.4)	8 (5.7)	2 (1.4)	43 (30.5)	0.926
	Yes	213	60.2	147 (69)	54 (25.4)	10 (4.7)	2 (0.9)	66 (31)	
Bleeding gums	No	254	71.8	183 (72)	58 (22.8)	10 (3.9)	3 (1.2)	71 (27.9)	0.213
	Yes	100	28.2	62 (62)	29 (29)	8 (8)	1 (1)	38 (38)	
Jaw pain	No	279	78.8	198 (71)	67 (24)	11 (3.9)	3 (1.1)	81 (29)	0.245
	Yes	75	21.2	47 (62.7)	20 (26.7)	7 (9.3)	1 (1.3)	28 (37.3)	
Dry mouth	No	236	66.9	204 (86.4)	30 (12.7)	2 (0.8)	0 (0)	32 (13.5)	<0.001
	Yes	117	33.1	41 (35)	56 (47.9)	16 (13.7)	4 (3.4)	76 (65)	
Tooth loss/ extractions	None	94	26.5	65 (69.1)	24 (25.5)	4 (4.3)	1 (1.1)	29 (30.9)	0.836
	From 1-4	173	48.7	122 (70.5)	41 (23.7)	9 (5.2)	1 (0.6)	51 (29.5)	
	From 5-10	54	15.2	37 (68.5)	12 (22.2)	4 (7.4)	1 (1.9)	17 (31.5)	
	Most teeth	25	7	16 (64)	8 (32)	1 (4)	0 (0)	9 (36)	
	All teeth	9	2.5	6 (66.7)	2 (22.2)	0 (0)	1 (11.1)	3 (33.3)	
Section III: Health general									
Arterial hypertension	No	276	77.7	199 (72.1)	68 (24.6)	7 (2.5)	2 (0.7)	77 (27.9)	<0.001
	Yes	79	22.3	47 (59.5)	19 (24.1)	11 (13.9)	2 (2.5)	32 (40.5)	
Diabetes	No	324	91.3	227 (70.1)	81 (25)	13 (4)	3 (0.9)	97 (29.9)	0.017
	Yes	31	8.7	19 (61.3)	6 (19.4)	5 (16.1)	1 (3.2)	12 (38.7)	
Cholesterol	No	239	67.3	167 (69.9)	60 (25.1)	8 (3.3)	4 (1.7)	72 (30.1)	0.096
	Yes	116	32.7	79 (68.1)	27 (23.3)	10 (8.6)	0 (0)	37 (31.9)	
Degenerative bone disease	No	251	70.7	185 (73.7)	54 (21.5)	9 (3.6)	3 (1.2)	66 (26.3)	0.026
	Yes	104	29.3	61 (58.7)	33 (31.7)	9 (8.7)	1 (1)	43 (41.4)	
Cardiac Illness	No	317	89.5	225 (71)	78 (24.6)	11 (3.5)	3 (0.9)	92 (29)	<0.001
	Yes	37	10.5	20 (54.1)	9 (24.3)	7 (18.9)	1 (2.7)	17 (45.9)	
Respiratory Illness	No	326	91.8	233 (71.5)	77 (23.6)	14 (4.3)	2 (0.6)	93 (28.5)	<0.001
	Yes	29	8.2	13 (44.8)	10 (34.5)	4 (13.8)	2 (6.9)	16 (55.2)	
Gastrointestinal Illness	No	302	85.6	218 (72.2)	69 (22.8)	12 (4)	3 (1)	84 (27.8)	0.011
	Yes	51	14.4	26 (51)	18 (35.3)	6 (11.7)	1 (2)	25 (49)	

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Table 1 cont.: Descriptive sample data and the association of the variables with the Xerostomia Inventory.

Anxiolytics-antidepressants	No	279	79	209 (74.9)	67 (24)	1 (0.4)	2 (0.7)	70 (25.1)	<0.001
	Yes	74	21	35 (47.3)	20 (27)	17 (23)	2 (2.7)	39 (52.7)	
Neurological illnesses	No	300	84.5	220 (73.3)	73 (24.3)	7 (2.3)	0 (0)	80 (26.6)	<0.001
	Yes	55	15.5	26 (47.3)	14 (25.5)	11 (20)	4 (7.3)	29 (52.8)	
General health self-perception	Bad or very bad	33	9.3	10 (30.3)	12 (36.4)	10 (30.3)	1 (3)	23 (69.7)	<0.001
	Very good or good	322	90.7	236 (73.3)	75 (23.3)	8 (2.5)	3 (0.9)	86 (26.7)	
Physical activity	No	123	34.6	77 (62.6)	39 (31.7)	5 (4.1)	2 (1.6)	46 (37.4)	0.113
	Yes	232	65.4	169 (72.8)	48 (20.7)	13 (5.6)	2 (0.9)	63 (27.2)	
Fruit and vegetables	Not Everyday	118	32.2	76 (64.4)	33 (28)	8 (6.8)	1 (0.8)	42 (35.6)	0.456
	Everyday	237	66.8	170 (71.7)	54 (22.8)	10 (4.2)	3 (1.3)	67 (28.3)	
Section IV: Overweightness and obesity									
BMI	Severely thin	2	0.6	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0.492
	Thin	1	0.3	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	
	Healthy weight	166	48.7	120 (72.3)	34 (20.5)	10 (6)	2 (1.2)	46 (27.7)	
	Overweight	122	35.8	81 (66.4)	34 (27.9)	5 (4.1)	2 (1.6)	41 (33.6)	
	Moderate obesity	39	11.4	26 (66.7)	11 (28.2)	2 (5.1)	0 (0)	13 (33.3)	
	Severe obesity	9	2.6	8 (88.9)	1 (11.1)	0 (0)	0 (0)	1 (11.1)	
	Morbid obesity	2	0.6	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	
WHtR (according to sex)	Extremely thin	3	1	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0.564
	Healthy thin	17	5.6	9 (52.9)	5 (29.4)	2 (11.8)	1 (5.9)	8 (47.1)	
	Healthy	67	22.3	45 (67.2)	19 (28.3)	1 (1.5)	2 (3)	22 (32.8)	
	Overweight	69	22.9	55 (79.7)	12 (17.4)	2 (2.9)	0 (0)	14 (20.3)	
	Very overweight	57	18.9	41 (71.9)	12 (21.1)	4 (7)	0 (0)	16 (28.1)	
	Morbid obesity	88	29.2	61 (69.3)	22 (25)	4 (4.5)	1 (1.1)	27 (30.6)	

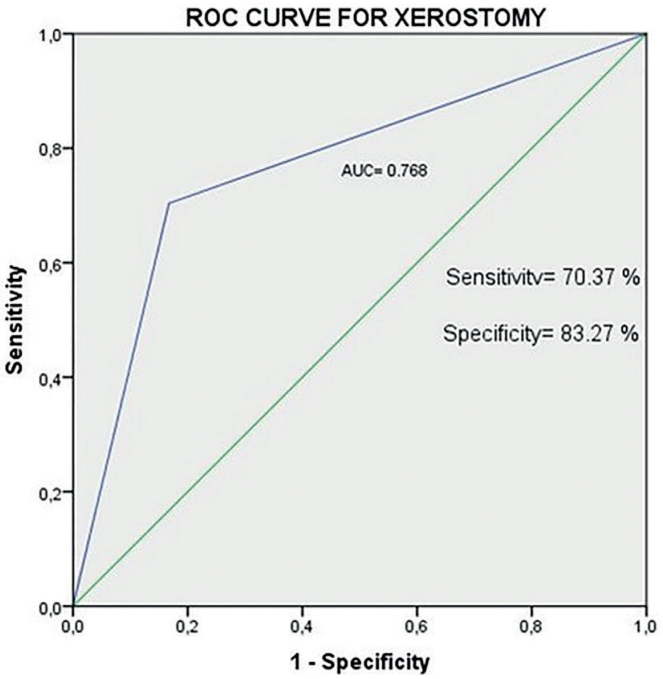


Fig. 1: ROC curve for diagnostic yield of the dry mouth question, taken the XI as a "gold standard": Sensitivity of 70.37 %, and a specificity of 83.27 % (Youden index=0.54, AUC=0.768, $p<0.001$).

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Table 2: Full data of Xerostomia Inventory.

Variables	N (%)				
	Never	Hardly ever	Occasionally	Fairly often	Very often
My mouth feels dry.	153 (43.2)	68 (19.2)	65 (18.4)	35 (9.9)	33 (9.3)
I have difficulty eating dry foods.	261 (73.9)	44 (12.5)	28 (7.9)	13 (3.7)	7 (2)
I get up at night to drink water or other liquids.	202 (57.9)	67 (19.2)	42 (12)	20 (5.7)	18 (5.2)
My mouth feels dry when chewing food.	239 (68.7)	66 (19)	22 (6.3)	14 (4)	7 (2)
I have to drink liquids to swallow food.	194 (56.1)	70 (20.2)	42 (12.1)	26 (7.5)	14 (4)
I have difficulty swallowing food.	257 (74.3)	54 (15.6)	20 (5.8)	10 (2.9)	5 (1.4)
The skin on my face is dry.	133 (38.9)	56 (16.4)	68 (19.9)	42 (12.3)	43 (12.6)
I suck on sweets or lozenges to relieve my dry mouth.	279 (80.4)	35 (10.1)	20 (5.8)	9 (2.6)	4 (1.2)
My eyes are dry.	182 (53.1)	52 (15.2)	51 (14.9)	27 (7.9)	31 (9)
My lips are dry	139 (40.3)	72 (20.9)	62 (18)	40 (11.6)	32 (9.3)
The inside of my nose feels dry.	159 (46.8)	72 (21.2)	55 (16.2)	36 (10.6)	18 (5.3)

Statistically significant more xerostomia was reported in patients with arterial hypertension (p<0.001), diabetes (p=0.017), and bone degenerative (p=0.026), cardiac (p<0.001), pulmonary (p<0.001), or digestive conditions (p=0.011). Moreover, significantly more xerostomia was reported in respondents taking anxiolytic and/or antidepressant medication, with a 52.7 % and 42.7 % reporting a certain degree of xerostomia, respectively. Patients reporting good general health exhibited significantly less xerostomia than patients with bad or very bad general health, 26.8 % vs. 69.7 % (p<0.001). In this study, neither the BMI nor the WHtR were significantly associated to the presence of xerostomia. However, a statistically significant association was found between the WHtR and the BMI with the number of missing teeth i.e., the more the tooth loss the higher the BMI and WHtR. As for the BMI with no tooth loss, the mean was 24.84 ± 5.0 (CI 95% 23.80-25.88), as compared to the BMI with extensive tooth loss with a mean of 27.86 ± 5.72 (CI 95% 25.44-30.28) (p=0.025 Bonferroni test). As for the WHtR with no tooth loss, the mean was 0.53 ± 0.1 (CI 95% 0.51-0.54), in comparison to WHtR with extensive tooth loss with a mean of 0.63 ± 0.1 (CI 95% 0.56-0.69) (p=0.003 Bonferroni test).

Logistical regression showed the highest xerostomia OR was associated to patients with bad self-perceived health, 6.31 (CI 95% 2.89-13.80, p<0.001). In the model adjusted for tooth mobility, bone or respiratory diseases, and the consumption of anxiolytics and antidepressants, the OR was 3.46 (CI 95% 1.47-8.18, p=0.005). Other covariables were statistically significant, but to a lesser extent (Table 3).

Table 3: A univariate logistic regression analysis was performed to determine the univariate OR for xerostomia. The adjusted statistical analysis was undertaken using a gradual multivariate logistic regression adjusted for dental mobility, degenerative bone disease, respiratory disease, OR: Ratio of risk.

Covariate	Xerostomia based on the XI			
	Univariate (OR 95% CI)	*p* value	Adjusted OR (95% CI)	*p* value
Dental mobility Positive vs. negative	2.15 (1.35-3.39)	0.001	1.77 (1.07-2.90)	0.024
Bone disease Positive vs. negative	1.98 (1.22-3.19)	0.006	1.49 (0.87-2.55)	0.146
Respiratory disease Positive vs. negative	3.08 (1.42-6.66)	0.004	2.62 (1.11-6.17)	0.028
Digestive disease Positive vs. negative	2.49 (1.36-4.56)	0.003	2.05 (1.05-3.99)	0.035
Anxiolytics Positive vs. negative	3.33 (1.96-5.66)	<0.001	2.26 (1.25-4.08)	0.007
Self-perceived health Good vs. bad	6.31 (2.89-13.80)	<0.001	3.46 (1.47-8.18)	0.005

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Discussion

In this study the prevalence of xerostomia was 30.7% of the sample, almost within the 20 to 30% range observed by other authors (4), with a higher prevalence in women and the elderly. Though the XI data were not correlated to the objective salivation tests of Hijjaw *et al*. (22), regardless of hyposalivation, xerostomia poses a problem per se, as it had an effect on feeling healthy, with xerostomia observed in 69.9 % of respondents in this study reporting bad or very bad self-perceived health. The logistic regression analysis showed an association between the dry mouth sensation and several systemic diseases and psychological disorders. In respondents taking anxiolytic and/or antidepressant medication, the risk of xerostomia was 3.33 higher in the univariate model, and 2.26 in the adjusted model. As regards general health, xerostomia was observed in 40.5% of arterial hypertension patients, which may be due to its influence on the quality and quantity of saliva (23). Moreover, a higher prevalence of xerostomia was observed in diabetic respondents, which agrees with the findings of López-Pintor *et al*. (24). As for study on the association between xerostomia and obesity in Sweden, an association was found in the analysis of raw data, but it disappeared with adjusted confounding factors (25). In this study no significant differences were observed between the BMI and the WHtR in association to xerostomia, but an association was observed between tooth loss and the BMI (p=0.025), and the WHtR (p=0.003). The results agree with the findings of the meta-analysis published in 2016, where tooth loss was 1.49 times higher in the obese, with a possible bidirectionality between both variables (13).

The mutual and complex association between oral pathology and systemic diseases has been well documented in the literature. Tavares *et al*. observed a correlation between both, with oral health affecting general health and vice versa (26); however, the evidence to date is insufficient to conclusively establish an association (18). Thus, the aim of this study was to obtain an initial snapshot of the association between xerostomia, and oral and systemic health, as well as being the first study to provide data on this association in Spain.

The results of this study corroborated the findings of recent studies that found a xerostomia OR = 5.1 in individuals reporting bad or very bad self-perceived health (27), and substantiated that psychotropic medication is one of the primary causes of the dryness of mouth (6), a view supported by the dryness of mouth sensation OR = 4.74 observed in individuals taking antidepressants. Abdullah *et al*. found patients with systemic pathology presented an OR = 2.80 more xerostomia (28). To our knowledge, this is the first report in the literature describing an association between xerostomia and degenerative bone, cardiac, pulmonary, and digestive illness.

As regards oral pathology, tooth mobility was found to be related to xerostomia (OR of 2.15 in the univariate model, and 1.77 in the adjusted model), but this finding should be validated in a larger sample population. Johansson *et al*. have found an association between xerostomia and several oral problems such as difficulties in chewing, burning and dry mouth sensation, and TMJ pain (27). Regarding the findings of this study and their clinical implications, the results underscore the importance of correctly diagnosing xerostomia, and the efficacy of questionnaires as diagnostic tools (3). The direct dichotomous question proposed on the dryness of mouth sensation could be of diagnostic utility, with a sensitivity of 70.37 %, taking as a gold standard the cut-off point of 11 on the XI. In this particularly vulnerable group, the diagnosis of certain systemic diseases such as bone, cardiac, or digestive pathologies can assist in the preliminary diagnosis of xerostomia. Correct management can reduce the incidence of oral and systemic pathologies, and improve bad self-perceived health. In terms of health organization, it is recommended the odontologist work in collaboration with health professionals in other disciplines such as primary care, traumatology, and cardiology, in order to develop a coordinated and multidisciplinary approach to dental health care programs.

The interpretation of the results of this study are subject to several limitations, given that the data was circumscribed to only one autonomous community in Spain, it is not entirely epidemiologically representative at a national level. Moreover, the cross-sectional design cannot prove causality, that would be ideal for converting into clinical observation, which underscores the needs for prospective longitudinal studies. Notwithstanding, this study has notable strengths, the proper calibration of the researchers, participants, and the selection of diagnostic criteria reasonably. As for the generalization of the results on the basis of the experimental design, the results can be extrapolated to other contexts bearing in mind the inter-territorial homogeneity

In conclusion, a high prevalence of xerostomia was found in this cross-sectional pilot study, which was significantly more frequent in women, and increased with age. Xerostomia was associated to several systemic diseases (such as arterial hypertension, diabetes, and bone, heart, lung, and digestive diseases), psychological conditions, and oral functional disorders such as tooth mobility. These preliminary results can serve as the basis for developing guidelines for the application of innovative measures designed to improve the quality of life of individuals with xerostomia. The comorbidity between xerostomia, deteriorated general health, and oral health disorders should be appraised in the dental management of elderly patients. The diagnosis of associated diseases and their medications can assist the odontologist in the preliminary diagnosis. Further studies are required to validate the conclusions of this study.

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Conflict of interest
Authors certify that there is no conflict of interest.

Ethics
Ethics Committee approval reference PGU-HD-2018-01. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Authors contributions
J.A.S.Q and M.P-S conceived the ideas; A.P.G., E.O.R, A.B.C, P.G.V. and B.M.B designed the study; F.J.G.G. and M.P-S: methodology; A.P.G and J.A.S.Q collected the data; A.B.C., F.J.G.G and M.P-S. analysed the data; M.P-S. : software; A.P.G., J.A.S.Q, E.O.R, and B.M.B writing—original draft preparation; M.P-S, A.B.C, F.J.G.G and P.G.V writing—review and editing; M.P-S.: supervision and project administration; A.P.G, J.A.S.Q, E.O.R, A.B.C, F.J.G.G, P.G.V, B.M.B, M.P-S final approval of the version.

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ARTÍCULO 4.

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RESEARCH

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Xerostomia, the perception of general and oral health and health risk behaviours in people over 65 years of age

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Abstract

Background: This study investigated the association between xerostomia and health risk behaviours, general and oral health and quality of life.

Methods: A cross-sectional study involving 800 adults over 65 years of age residing in Spain using a computer-assisted telephone questionnaire. The severity of xerostomia was assessed through the Xerostomia Inventory (XI). Both univariate and adjusted multinomial logistic regression were used to determine the risk (OR) of xerostomia.

Results: The sample comprised of 492 females (61.5%) and 308 males, with a mean age of 73.7 ± 5.8 years. Some, 30.7% had xerostomia: 25.6% mild, 4.8% moderate and 0.3% severe, the majority being female (34.8% vs 24%; $p = 0.003$). The mean XI was 24.6 ± 6.3 (95% CI 19.2–24.8) for those with poor health, whereas it was 17.4 ± 6.3 (95%CI 16.1–18.6) in those reporting very good health ($p < 0.001$). This difference was also observed in terms of oral health, with the XI mean recorded as 14.7 ± 10.7 for very poor oral health and 6.4 ± 5.4 for those with very good health ($p = 0.002$). Logistic regression showed that the highest OR for xerostomia was observed among adults with poor general health (2.81; 95%CI 1.8–4.3; $p < 0.001$) and for adjusted model the OR was still significant (2.18; 95%CI 1.4–3.4; $p = 0.001$). Those who needed help with household chores had 2.16 higher OR (95%CI 1.4–3.4; $p = 0.001$) and 1.69 (95%CI 1.1–2.7; $p = 0.03$) in the adjusted model. Females had a higher risk of suffering from xerostomia than males.

Conclusion: The strong association between xerostomia and the general and oral health status of older adults justifies the need for early assessment and regular follow-up.

Keywords: Xerostomia, Health, Lifestyle, Oral health, Quality of life

Background

The world's population is ageing at a much faster rate than ever before, and it is estimated that between 2015 and 2050, the proportion of the world's population over 60 years of age will increase from 12 to 22% [1]. Xerostomia (or the feeling of dry mouth) mainly affects older people and may be due to a variety of underlying etiologies [2]. Older people have more comorbidities, meaning that a high proportion are polymedicated. It is estimated that so-called "polypharmacy" affects 40–50% of the older population in high-income countries [3], and it is a well-known cause of hyposalivation and xerostomia [2].

Alcohol use disorders in the geriatric population are considered to be the "invisible epidemic" [4]. The European Union has the highest rate of alcohol consumption in the world, with a seemingly low perception of the associated risks [5]. Alcohol ingestion inhibits the release of

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the antidiuretic hormone, resulting in body dehydration [6], likewise, it also causes salivary gland atrophy and is one of the main causes of sialadenosis [7]. Smoking also has this same effect, and preliminary studies have shown that long-term smoking is significantly associated with hyposalivation [8]. Profound and complex interactions exist between nutrition and oral health [9], however, to our knowledge, no existing studies have considered the relationship between diet in older people and the sensation of dry mouth, although Machowicz et al. [10] associated adherence to a Mediterranean diet with a lower probability of suffering from primary Sjögren's Syndrome.

The relationship between oral and general health has been widely discussed in scientific literature and it is known that poor oral health can increase the risk of certain physical disorders [11]. A meta-analysis published in 2021 found a positive association between poor general health and poor oral health-related quality of life among older adults [12]. In a systematic map of systematic reviews that examined current knowledge about older persons' oral health status and dental care, it is concluded that there is an urgent need for research within most domains in geriatric dentistry [13].

The aim of this study was to analyse the association between xerostomia and health risk behaviours, general and oral health and oral health-related quality of life among a large representative sample of adults over 65 years of age.

Methods

Data came from the 2020 SEGER (Spanish society of Gerodontology) Survey, a national survey following the STROBE guidelines for observational studies [14].

The target population included men and women over the age of 65, with significant representation throughout Spain. Simple random sampling was performed at the national level, with proportional stratification by geographic area. Data were collected anonymously, and the study was granted an exemption from requiring ethics approval by the Bioethics Committee of the University of Santiago de Compostela.

Survey design

A computer-assisted telephone survey (CATI research method: Computer-Assisted Telephone Interviewing) was conducted using a structured questionnaire (interview length: 16 minutes). This questionnaire comprised five sections that includes information related with: Social-demographic data; General and oral health; Oral problems during 2019; Tobacco and alcohol consumption and Dietary habits.

Variables

The Xerostomia Inventory (XI) was used to assess dry mouth sensation, designed in 1999 [15] using the validated Spanish version [16]. The ranges of values associated with each degree were as follows: 0–11: No xerostomia, 12–22: Mild xerostomia, 23–33: Moderate xerostomia, and 34–44: Severe xerostomia [17]. In relation to health risk behaviours, tobacco use has been assessed (former or current, number of cigarettes smoked and, for ex-smokers, years since they gave up smoking) as well as alcohol consumption (type of alcoholic beverages and frequency) and dietary habits (frequency of vegetables, legumes, fruit, white meat (chicken, rabbit, turkey), red meat (beef, pork, lamb) and daily drinking water). In relation to general and oral health, concerns about own general and oral health status and perception of own general and oral health were assessed following a 5-point Likert scale. With regard to oral health-related quality of life, it was assessed in terms of oral problems during 2019.

The variables eligible for inclusion in the model, namely "general health" and "oral health", were split into two by considering "poor or very poor" as poor, and "acceptable, good or very good" as good, for the multilevel binary logistic regression.

Statistical analysis

Contingency tables analysed the associations between categorical variables using a chi-squared test. Parametric statistics were used to describe the differences in the means, using the ANOVA test with Bonferroni post-hoc correction for comparisons with more than two elements. Both univariate and adjusted multinomial logistic regression were used to determine the Odds Ratio (OR) of xerostomia. A Directed Acyclic Graph (DAG) has been constructed by using the version 0.2.7 of the R package ggdag (R Core Team, 2022). It provides a visual representation of causal relationships among the set of variables involved in the adjusted multinomial logistic regression. The data were analyzed with the SPSS v.28.00 (IBM, Madrid, Spain). The significance level was $p \leq 0.05$.

Results

The sample comprised 492 females (61.5%) and 308 males (38.5%) with a mean age of 73.7 years (SD = 5.8). The complete descriptive data are in Table 1 and their territorial distribution is outlined in Fig. 1.

Xerostomia

Some 30.7% of the respondents suffered from xerostomia, most of whom were females (34.8% vs 24% in males) ($p = 0.003$). There were 25.6% with a mild degree of

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Table 1 Sample descriptive data

		N	%	Xerostomia Inventory					
				No	Mild	Moderate	Severe	Xeros total	p
Section I: Social-demographic data									
Gender	Male	308	38.5	234 (76.0)	67 (21.8)	7 (2.3)	0 (0)	74 (24.0)	0.003
	Female	492	61.5	321 (65.2)	138 (28)	31 (6.3)	2 (0.4)	171 (34.8)	
Age	65 to 74 years	460	57.5	321 (69.8)	115 (25.0)	23 (5.0)	1 (0.2)	139 (30.2)	0.944
	Over 74 years	340	42.5	234 (68.8)	90 (26.5)	15 (4.4)	1 (0.3)	106 (31.2)	
Area	North East	158	19.8	109 (69.0)	41 (25.9)	8 (5.1)	0 (0)	49 (31.0)	0.649
	East	117	14.6	82 (70.1)	30 (25.6)	5 (4.3)	0 (0)	35 (29.9)	
	South	120	15.0	84 (70.0)	28 (23.3)	8 (6.7)	0 (0)	36 (30.0)	
	Centre	177	22.1	122 (68.9)	48 (27.1)	6 (3.4)	1 (0.6)	55 (31.1)	
	Northwest	79	9.9	55 (69.6)	24 (30.4)	0 (0)	0 (0)	24 (30.4)	
	North Central	75	9.4	53 (70.7)	16 (21.3)	6 (8.0)	0 (0)	22 (29.3)	
	Islands and autonomous cities	74	9.3	50 (67.6)	18 (24.3)	5 (6.8)	1 (1.4)	24 (32.4)	
Education	Uneducated	43	5.4	30 (69.8)	12 (27.9)	1 (2.3)	0 (0)	13 (30.2)	< 0.001
	Primary Education	287	35.9	183 (63.8)	89 (31.0)	14 (4.9)	1 (0.3)	104 (36.2)	
	Higher Education	227	28.4	170 (74.9)	46 (20.3)	11 (4.8)	0 (0)	57 (25.1)	
	First Level Vocational Education and Training / Secondary Education 1	80	10.0	51 (63.7)	24 (30.0)	5 (6.3)	0 (0)	29 (36.3)	
	Higher-level Vocational Education and Training Secondary Education 2	150	18.8	114 (76.0)	30 (20.0)	6 (4.0)	0 (0)	36 (24.0)	
	Do not know / No answer given	13	1.6	7 (53.8)	4 (30.8)	1 (7.7)	1 (7.7)	6 (46.2)	
Employment	Employed	623	77.9	439 (70.5)	152 (24.4)	31 (5.0)	1 (0.2)	184 (29.5)	0.065
	Self-employed	129	16.1	88 (68.2)	35 (27.1)	6 (4.7)	0 (0)	41 (31.8)	
	Other	48	6.0	28 (58.3)	18 (37.5)	1 (2.1)	1 (2.1)	20 (41.7)	
Retired	No	84	10.5	57 (67.9)	20 (23.8)	7 (8.3)	0 (0)	27 (32.1)	< 0.001
	Yes	710	88.8	495 (69.7)	183 (25.8)	31 (4.4)	1 (0.1)	215 (30.3)	
	Do not know / No answer given	6	0.8	3 (50.0)	2 (33.3)	0 (0)	1 (16.7)	3 (50.0)	
Do you live alone or with someone else?	I live alone	219	27.4	137 (62.6)	72 (32.9)	9 (4.1)	1 (0.5)	82 (37.4)	< 0.001
	I live with someone else	572	71.5	412 (72.0)	131 (22.9)	29 (5.1)	0 (0)	160 (28.0)	
	Do not know / No answer given	9	1.1	6 (66.7)	2 (22.2)	0 (0)	1 (11.1)	3 (33.3)	
Household chores	I require help with household chores	91	11.4	49 (53.8)	34 (37.4)	8 (8.8)	0 (0)	42 (46.2)	< 0.001
	I am self-sufficient	701	87.6	502 (71.6)	168 (24.0)	30 (4.3)	1 (0.1)	199 (28.4)	
	Do not know / No answer given	8	1.0	4 (50.0)	3 (37.5)	0 (0)	1 (12.5)	4 (50.0)	
Section II: General and oral health									
Are you concerned about your general health?	Not at all	144	18.0	124 (86.1)	18 (12.5)	2 (1.4)	0 (0)	20 (13.9)	0.001
	Slightly	128	16.0	85 (66.4)	34 (26.6)	9 (7.0)	0 (0)	43 (33.6)	
	Moderately	148	18.5	108 (73.0)	36 (24.3)	3 (2.0)	1 (0.7)	40 (27.0)	
	Very	224	28.0	140 (62.5)	69 (30.8)	14 (6.3)	1 (0.4)	84 (37.5)	
	Extremely	156	19.5	98 (62.8)	48 (30.8)	10 (6.4)	0 (0)	58 (37.2)	
Are you concerned about your oral health?	Not at all	227	28.4	184 (81.1)	40 (17.6)	3 (1.3)	0 (0)	43 (18.9)	< 0.001
	Slightly	117	14.6	81 (69.2)	28 (23.9)	8 (6.8)	0 (0)	36 (30.8)	
	Moderately	103	12.9	67 (65.0)	32 (31.1)	4 (3.9)	0 (0)	36 (35.0)	
	Very	216	27.0	149 (69.0)	54 (25.0)	12 (5.6)	1 (0.5)	67 (31.0)	
	Extremely	137	17.1	74 (54.0)	51 (37.2)	11 (8.0)	1 (0.7)	63 (46.0)	

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Table 1 (continued)

		N	%	Xerostomia Inventory					
				No	Mild	Moderate	Severe	Xeros total	p
What was your general health status in 2019?	Very Poor	22	2.8	13 (59.1)	7 (31.8)	2 (9.1)	0 (0)	9 (40.9)	< 0.001
	Poor	81	10.1	37 (45.7)	31 (38.3)	12 (14.8)	1 (1.2)	44 (54.3)	
	Acceptable	302	37.8	200 (66.2)	89 (29.5)	13 (4.3)	0 (0)	102 (33.8)	
	Good	293	36.6	222 (75.8)	62 (21.2)	8 (2.7)	1 (0.3)	71 (24.2)	
	Very Good	99	12.4	82 (82.8)	14 (14.1)	3 (3.0)	0 (0)	17 (17.2)	
	Do not know / No answer given	3	0.4	1 (33.3)	2 (66.7)	0 (0)	0 (0)	2 (66.7)	
What was your dental health status in 2019?	Very Poor	9	1.1	5 (55.6)	0 (0)	4 (44.4)	0 (0)	4 (44.4)	< 0.001
	Poor	35	4.4	16 (45.7)	16 (45.7)	3 (8.6)	0 (0)	19 (54.3)	
	Acceptable	346	43.3	225 (65.0)	103 (29.8)	17 (4.9)	1 (0.3)	121 (35.0)	
	Good	331	41.4	246 (74.3)	71 (21.5)	13 (3.9)	1 (0.3)	85 (25.7)	
	Very Good	78	9.8	62 (79.5)	15 (19.2)	1 (1.3)	0 (0)	16 (20.5)	
	Do not know / No answer given	1	0.1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
Section III: Oral problems in 2019									
How often did you experience difficulty in eating in 2019?	Never	594	74.3	446 (75.1)	133 (22.4)	13 (2.2)	2 (0.3)	148 (24.9)	< 0.001
	Rarely	87	10.9	48 (55.2)	31 (35.6)	8 (9.2)	0 (0)	39 (44.8)	
	Occasionally	97	12.1	55 (56.7)	32 (33.0)	10 (10.3)	0 (0)	42 (43.3)	
	Frequently	11	1.4	4 (36.4)	4 (36.4)	3 (27.3)	0 (0)	7 (63.6)	
	Very Frequently	9	1.1	1 (11.1)	4 (44.4)	4 (44.4)	0 (0)	8 (88.9)	
	Do not know / No answer given	2	0.3	1 (50)	1 (50)	0 (0)	0 (0)	1 (50)	
Did you experience tooth or gum pain in 2019?	Never	607	75.9	459 (75.6)	132 (21.7)	15 (2.5)	1 (0.2)	148 (24.4)	< 0.001
	Rarely	79	9.9	42 (53.2)	31 (39.2)	6 (7.6)	0 (0)	37 (46.8)	
	Occasionally	92	11.5	47 (51.1)	34 (37.0)	10 (10.9)	1 (1.1)	45 (48.9)	
	Frequently	13	1.6	3 (23.1)	7 (53.8)	3 (23.1)	0 (0)	10 (76.9)	
	Very Frequently	6	0.8	2 (33.3)	0 (0)	4 (66.7)	0 (0)	4 (66.7)	
	Do not know / No answer given	3	0.4	2 (66.7)	1 (33.3)	0 (0)	0 (0)	1 (33.3)	
Did you experience problems with your teeth, mouth or dentition in 2019?	Never	601	75.1	446 (74.2)	132 (22.0)	21 (3.5)	2 (0.3)	155 (25.8)	< 0.001
	Rarely	57	7.1	39 (68.4)	16 (28.1)	2 (3.5)	0 (0)	18 (31.6)	
	Occasionally	101	12.6	52 (51.5)	43 (42.6)	6 (5.9)	0 (0)	49 (48.5)	
	Frequently	14	1.8	5 (35.7)	5 (35.7)	4 (28.6)	0 (0)	9 (64.3)	
	Very Frequently	12	1.5	3 (25.0)	4 (33.3)	5 (41.7)	0 (0)	9 (75.0)	
	Do not know / No answer given	15	1.9	10 (66.7)	5 (33.3)	0 (0)	0 (0)	5 (33.3)	
Did you ever avoid smiling or talking because of the appearance of your teeth or dentition in 2019?	Never	732	91.5	522 (71.3)	181 (24.7)	28 (3.8)	1 (0.1)	210 (28.7)	< 0.001
	Rarely	12	1.5	8 (66.7)	2 (16.7)	2 (16.7)	0 (0)	4 (33.3)	
	Occasionally	37	4.6	20 (54.1)	14 (37.8)	3 (8.1)	0 (0)	17 (45.9)	
	Frequently	12	1.5	5 (41.7)	6 (50)	1 (8.3)	0 (0)	7 (58.3)	
	Very Frequently	7	0.9	0 (0)	2 (28.6)	4 (57.1)	1 (14.3)	7 (100)	
	Do not know / No answer given	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Section IV: Tobacco and alcohol consumption									
Tobacco consumption	I have never smoked	433	54.1	285 (65.8)	123 (28.4)	23 (5.3)	2 (0.5)	148 (34.2)	0.222
	I am a smoker	71	8.9	57 (80.3)	12 (16.9)	2 (2.8)	0 (0)	14 (19.7)	
	I used to smoke	295	36.9	213 (72.2)	69 (23.4)	13 (4.4)	0 (0)	82 (27.8)	
	Do not know / No answer given	1	0.1	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	

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Table 1 (continued)

		N	%	Xerostomia Inventory					
				No	Mild	Moderate	Severe	Xeros total	p
Cigarettes smoked per day	I do not smoke.	729	91.1	498 (68.3)	193 (26.5)	36 (4.9)	2 (0.3)	231 (31.7)	0.605
	I smoke occasionally, not daily.	4	0.5	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
	1–5	17	2.1	15 (88.2)	1 (5.9)	1 (5.9)	0 (0)	2 (11.8)	
	6–10	20	2.5	18 (90.0)	2 (10.0)	0 (0)	0 (0)	2 (10.0)	
	> 10	29	3.6	20 (69.0)	8 (27.6)	1 (3.4)	0 (0)	9 (31.0)	
	Do not know / No answer given	1	0.1	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	
How many years has it been since you gave up smoking?	None	505	63.1	342 (67.7)	136 (26.9)	25 (5.0)	2 (0.4)	163 (32.3)	0.973
	< 5 years	26	3.3	17 (65.4)	7 (26.9)	2 (7.7)	0 (0)	9 (34.6)	
	5–10 years	38	4.8	26 (68.4)	11 (28.9)	1 (2.6)	0 (0)	12 (31.6)	
	11–15 years	29	3.6	19 (65.5)	9 (31.0)	1 (3.4)	0 (0)	10 (34.5)	
	> 15 years	199	24.9	149 (74.9)	41 (20.6)	9 (4.5)	0 (0)	50 (25.1)	
	Do not know / No answer given	3	0.4	2 (66.7)	1 (33.3)	0 (0)	0 (0)	1 (33.3)	
Alcohol consumption	I have never consumed alcoholic beverages	281	35.1	180 (64.1)	83 (29.5)	17 (6.0)	1 (0.4)	101 (35.9)	0.120
	I occasionally consume alcoholic beverages	375	46.9	273 (72.8)	81 (21.6)	20 (5.3)	1 (0.3)	102 (27.2)	
	I consume alcoholic beverages on a daily basis	140	17.5	100 (71.4)	39 (27.9)	1 (0.7)	0 (0)	40 (28.6)	
	Do not know / No answer given	4	0.5	2 (50)	2 (50)	0 (0)	0 (0)	2 (50)	
Beer	0	754	94.3	522 (69.2)	193 (25.6)	37 (4.9)	2 (0.3)	232 (30.8)	0.967
	1	30	3.8	18 (60.0)	11 (36.7)	1 (3.3)	0 (0)	12 (40.0)	
	2	13	1.6	12 (92.3)	1 (7.7)	0 (0)	0 (0)	1 (7.7)	
	3	1	0.1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
	4	1	0.1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
	> 5	1	0.1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
Wine	0	686	85.8	478 (69.7)	169 (24.6)	37 (5.4)	2 (0.3)	208 (30.3)	0.611
	1	62	7.8	35 (56.5)	26 (41.9)	1 (1.6)	0 (0)	27 (43.5)	
	2	30	3.8	23 (76.7)	7 (23.3)	0 (0)	0 (0)	7 (23.3)	
	3	14	1.8	12 (85.7)	2 (14.3)	0 (0)	0 (0)	2 (14.3)	
	4	5	0.6	4 (80.0)	1 (20.0)	0 (0)	0 (0)	1 (20.0)	
	> 5	3	0.4	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
Spirits	0	784	98.0	543 (69.3)	201 (25.6)	38 (4.8)	2 (0.3)	241 (30.7)	0.976
	1	12	1.5	8 (66.7)	4 (33.3)	0 (0)	0 (0)	4 (33.3)	
	2	2	0.3	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
	3	2	0.3	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
Section V: Dietary habits									
Do you think a proper diet is important for your oral health?	Not Important	30	3.8	23 (76.7)	6 (20.0)	1 (3.3)	0 (0)	7 (23.3)	0.717
	Slightly Important	24	3.0	18 (75.0)	5 (20.8)	1 (4.2)	0 (0)	6 (25.0)	
	Moderately Important	69	8.6	55 (79.7)	11 (15.9)	3 (4.3)	0 (0)	14 (20.3)	
	Important	318	39.8	224 (70.4)	78 (24.5)	15 (4.7)	1 (0.3)	94 (29.6)	
	Very Important	323	40.4	212 (65.6)	96 (29.7)	14 (4.3)	1 (0.3)	111 (34.4)	
	Do not know / No answer given	36	4.5	23 (63.9)	9 (25.0)	4 (11.1)	0 (0)	13 (36.1)	

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Table 1 (continued)

		N	%	Xerostomia Inventory					
				No	Mild	Moderate	Severe	Xeros total	p
How often do you consume white meat?	Never	19	2.4	15 (78.9)	3 (15.8)	1 (5.3)	0 (0)	4 (21.1)	0.500
	Occasionally	114	14.2	86 (75.4)	25 (21.9)	3 (2.6)	0 (0)	28 (24.6)	
	Every four/ five days	226	28.2	149 (65.9)	63 (27.9)	14 (6.2)	0 (0)	77 (34.1)	
	Every two/ three days	413	51.6	284 (68.8)	110 (26.6)	17 (4.1)	2 (0.5)	129 (31.2)	
	Every day	20	2.5	15 (75.0)	2 (10.0)	3 (15.0)	0 (0)	5 (25.0)	
	Do not know / No answer given	8	1.0	6 (75.0)	2 (25.0)	0 (0)	0 (0)	2 (25.0)	
How often do you consume red meat?	Never	84	10.5	58 (69.0)	21 (25.0)	5 (6.0)	0 (0)	26 (31.0)	< 0.001
	Occasionally	264	33.0	176 (66.7)	70 (26.5)	18 (6.8)	0 (0)	88 (33.3)	
	Every four/ five days	261	32.6	178 (68.2)	72 (27.6)	11 (4.2)	0 (0)	83 (31.8)	
	Every two/ three days	175	21.9	131 (74.9)	39 (22.3)	4 (2.3)	1 (0.6)	44 (25.1)	
	Every day	5	0.6	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
	Do not know / No answer given	11	1.4	7 (63.6)	3 (27.3)	0 (0)	1 (9.1)	4 (36.4)	
How often do you consume vegetables?	Never	7	0.9	4 (57.1)	3 (42.9)	0 (0)	0 (0)	3 (42.9)	< 0.001
	Occasionally	28	3.5	20 (71.4)	5 (17.9)	3 (10.7)	0 (0)	8 (28.6)	
	Every four/ five days	51	6.4	33 (64.7)	17 (33.3)	1 (2.0)	0 (0)	18 (35.3)	
	Every two/ three days	257	32.1	175 (68.1)	69 (26.8)	13 (5.1)	0 (0)	82 (31.9)	
	Every day	450	56.3	320 (71.1)	108 (24.0)	21 (4.7)	1 (0.2)	130 (28.9)	
	Do not know / No answer given	7	0.9	3 (42.9)	3 (42.9)	0 (0)	1 (14.3)	4 (57.1)	
How often do you consume fruit?	Never	5	0.6	4 (80.0)	1 (20.0)	0 (0)	0 (0)	1 (20.0)	< 0.001
	Occasionally	26	3.3	19 (73.1)	6 (23.1)	1 (3.8)	0 (0)	7 (26.9)	
	Every four/ five days	8	1.0	7 (87.5)	1 (12.5)	0 (0)	0 (0)	1 (12.5)	
	Every two/ three days	43	5.4	31 (72.1)	8 (18.6)	4 (9.3)	0 (0)	12 (27.9)	
	Every day	712	89.0	491 (69.0)	187 (26.3)	33 (4.6)	1 (0.1)	221 (31.0)	
	Do not know / No answer given	6	0.8	3 (50.0)	2 (33.3)	0 (0)	1 (16.7)	3 (50.0)	
How often do you consume legumes?	Never	9	1.1	4 (44.4)	4 (44.4)	1 (11.1)	0 (0)	5 (55.6)	< 0.001
	Occasionally	90	11.3	65 (72.2)	19 (21.1)	6 (6.7)	0 (0)	25 (27.8)	
	Every four/ five days	217	27.1	147 (67.7)	61 (28.1)	9 (4.1)	0 (0)	70 (32.3)	
	Every two/ three days	418	52.3	296 (70.8)	101 (24.2)	20 (4.8)	1 (0.2)	122 (29.2)	
	Every day	57	7.1	38 (66.7)	17 (29.8)	2 (3.5)	0 (0)	19 (33.3)	
	Do not know / No answer given	9	1.1	5 (55.6)	3 (33.3)	0 (0)	1 (50.0)	4 (44.4)	
How much water or liquids do you drink per day?	Less than half a litre	26	3.3	19 (73.1)	6 (23.1)	1 (3.8)	0 (0)	7 (26.9)	< 0.001
	Half a litre	104	13.0	69 (66.3)	29 (27.9)	6 (5.8)	0 (0)	35 (33.7)	
	One litre	262	32.8	184 (70.2)	65 (24.8)	12 (4.6)	1 (0.4)	78 (29.8)	
	One and a half litres	241	30.1	171 (71.0)	62 (25.7)	8 (3.3)	0 (0)	70 (29.0)	
	Two litres	110	13.8	75 (68.2)	28 (25.5)	7 (6.4)	0 (0)	35 (31.8)	
	Over two litres	49	6.1	32 (65.3)	13 (26.5)	4 (8.2)	0 (0)	17 (34.7)	
	Do not know / No answer given	8	1.0	5 (62.5)	2 (25.0)	0 (0)	1 (12.5)	3 (37.5)	

xerostomia, 4.8% moderate and 0.3% severe. The mean XI score was 8.9 ± 6.8. The mean XI was lower with better overall health, so it was 24.6 ± 6.3 (95% CI 19.2–24.8) for poor health, but 17.4 ± 6.3 (95% CI 16.1–18.6) for very good health ($p < 0.001$ Bonferroni test). This pattern was also observed in terms of oral health, with the XI mean recorded as 14.7 ± 10.7 for very poor oral health versus 6.4 ± 5.4 for very good ($p = 0.002$) (Table 2). The full data for the Xerostomia Inventory are found in Table 3.

General and oral health

There were 74.4% of respondents reporting acceptable or good general health and only 12.9% stated that their health was poor or very poor. The incidence of xerostomia in people with poor general health was 54.3% as

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Fig. 1 Geographical distribution of participants

Table 2 XI mean analysis, depending on general and oral health status, daily cigarette consumption and alcohol consumption

	Covariate	N	Mean	SD	CI 95%		P
					Inferior	Superior	
General health	Very Poor	22	22.0	6.29	19.21	24.79	Anova: < 0.001 Bonferroni: P - A: < 0.001 P - G: < 0.001 P - VG: < 0.001 VP - VG: 0.041
	Poor	81	24.64	7.94	22.89	26.40	
	Acceptable	302	20.21	6.56	19.47	20.95	
	Good	293	18.84	6.18	18.13	19.55	
	Very Good	99	17.37	6.31	16.11	18.63	
	Do not know / No answer given	3	24.00	3.60	15.04	32.96	
Oral health	Very Poor	10	14.70	10.68	7.05	22.35	Anova:< 0.001 Bonferroni: VP - VG:0.002 P - VG:0.001 A - VG: 0.001 G - A: 0.008
	Poor	35	11.86	7.14	9.40	14.31	
	Acceptable	346	9.76	6.91	9.03	10.49	
	Good	331	8.04	6.44	7.34	8.74	
	Very Good	78	6.37	5.44	5.14	7.60	
Tobacco consumption	I have never smoked	433	9.50	7.02	8.84	10.16	Anova: 0.006 Bonferroni: N - S: 0.020
	I am a smoker	71	7.14	5.72	5.79	8.50	
	I used to smoke	295	8.33	6.58	7.58	9.09	
Alcohol consumption	I have never consumed alcoholic beverages	281	20.63	7.11	19.79	21.47	Anova:0.063 Bonferroni: N - D: 0.050
	I occasionally consume alcoholic beverages	375	19.68	6.85	18.99	20.38	
	I consume alcoholic beverages on a daily basis	140	18.81	5.79	17.85	19.78	
	Do not know / No answer given	4	21.00	6.73	10.29	31.71	

Table legend: Anova and Bonferroni test: Very Poor (VP), Poor (P), Acceptable (A), Good (G), Very Good (VG), I have never smoked (N), I am a smoker (S), I used to smoke (E), I have never consumed alcoholic beverages (N), I occasionally consume alcoholic beverages (O), I consume alcoholic beverages on a daily basis (D)

opposed to 17.2% in people with good health ($p < 0.001$). There were 84.7% of respondents reporting acceptable or good oral health, with only 5.5% reporting that their oral health was poor or very poor. Of those who claimed to have poor oral health, 54.3% had xerostomia, unlike 20.5% of those who considered that their oral health is very good ($p < 0.001$). Xerostomia degree was higher among those who expressed greater concerns about their oral health, with an incidence of 46% among those who expressed great concern about this aspect, compared with just 18.9% of those who stated that they were not concerned about their oral health ($p < 0.001$). With

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Table 3 Full XI data. Responses to individual items

	N (%)					
	Never	**Hardly ever**	**Occasionally**	**Fairly often**	**Very often**	**Missing**
My mouth feels dry	337 (42.1)	107 (13.4)	218 (27.3)	80 (10)	57 (7.1)	1 (0.1)
I have difficulty eating dry food	578 (72.3)	73 (9.1)	82 (10.3)	32 (4.0)	25 (3.1)	10 (1.3)
I wake up at night to drink water or other liquids.	499 (62.4)	66 (8.3)	147 (18.4)	39 (4.9)	47 (5.9)	2 (0.3)
My mouth feels dry when I am chewing food.	650 (81.3)	86 (10.8)	43 (5.4)	10 (1.3)	8 (1.0)	3 (0.4)
I need to drink liquids when I am swallowing food.	472 (59.0)	77 (9.6)	149 (18.6)	56 (7.0)	40 (5.0)	6 (0.8)
I have difficulty swallowing certain foods.	699 (87.4)	34 (4.3)	56 (7.0)	6 (0.8)	4 (0.5)	1 (0.1)
The skin on my face is dry.	428 (53.5)	96 (12.0)	130 (16.3)	63 (7.9)	70 (8.8)	13 (1.6)
I need to suck sweets or similar to relieve the dry mouth sensation.	609 (76.1)	47 (5.9)	99 (12.4)	28 (3.5)	15 (1.9)	2 (0.3)
My eyes are dry.	427 (53.4)	77 (9.6)	174 (21.8)	75 (9.4)	42 (5.3)	5 (0.6)
My lips are dry.	418 (52.3)	114 (14.2)	181 (22.6)	47 (5.9)	37 (4.6)	3 (0.4)
The inside of my nose feels dry.	427 (53.4)	77 (9.6)	174 (21.8)	75 (9.4)	42 (5.3)	5 (0.6)

regards to quality of life, 15.9% reported that they had problems with their mouth, teeth or dentition; 14.6% that they had difficulty eating; 13.9% that they experience tooth or gum pain, and 7% that they had avoided smiling or talking because of the appearance of their teeth or dentition. The percentage of xerostomia was higher ($p < 0.001$) the higher the frequency with which oral issues were suffered. In fact, 100% of people who stated that they avoided smiling or talking on a very frequent basis had a certain degree of xerostomia, whereas 28.7% stated that they never avoided said actions ($p < 0.001$).

Health risk behaviours: tobacco, alcohol and dietary habits

There were 54.1% who stated that they had never smoked, while 36.9% declared that they were ex-smokers, with the majority having given up smoking more than 15 years ago, and only 8.9% stated that they currently smoke. The number of non-smokers was higher among women and among those over the age of 74 years ($p < 0.001$).

Of those interviewed, 46.9% stated that they consume alcoholic beverages occasionally, and 17.5% that they do so on a daily basis, mainly wine, consuming around 1 or 2 drinks a day (7.8 and 3.8% respectively). Furthermore, 35.1% stated that they do not consume any alcohol at all, and this percentage was higher among women (47.5% vs 18.2%) ($p < 0.001$). With regards to tobacco and alcohol consumption, no significant differences were observed in terms of xerostomia.

Most of the participants stated that they consume fruit and vegetables on a daily basis (89 and 56.3% respectively), as opposed to their less frequent consumption of meat and legumes (only 0.6% stated that they consume red meat every day and, likewise, this figure was 2.5% for white meat, and 7.1% for legumes). As regards the consumption of red meat, there was a significant difference between men and women ($p < 0.001$), with the latter consuming less red meat. Among those who stated that they never consume vegetables, the percentage of xerostomia was 42.9%, but 28.9% for those who consume said products on a daily basis ($p < 0.001$). The incidence of xerostomia among individuals who stated that they never consume legumes was 55.6% but 29.2% for those who consume legumes every two or three days ($p < 0.001$).

The relation between the main studied variables has been illustrated in Fig. 2 by a DAG. The influence of general and oral health and health risk behaviours can be graphically observed.

Logistic regression analysis

Logistic regression showed that the highest OR for xerostomia was observed among adults with poor general health, who had 2.81 higher odds of suffering from this condition (95%CI 1.8–4.3, $p < 0.001$) than those in good health. In the model adjusted for gender, oral health, education, employment and household chores, the OR was 2.18 (95%CI 1.4–3.4, $p = 0.001$). Those who need help with household chores had 2.16 higher odds of suffering xerostomia (95%CI 1.4–3.4; $p = 0.001$) and the OR was 1.69 (95%CI 1.1–2.7; $p = 0.03$) in the adjusted model. Females had higher odds of suffering from xerostomia than males in both the univariate and multivariate models. Overall, adults with poor oral health had higher odds of suffering from this condition (Table 4).

Discussion

Our findings indicate a strong association between xerostomia (assessed using XI) and general and oral health. We found that the prevalence of xerostomia was 30.6%.

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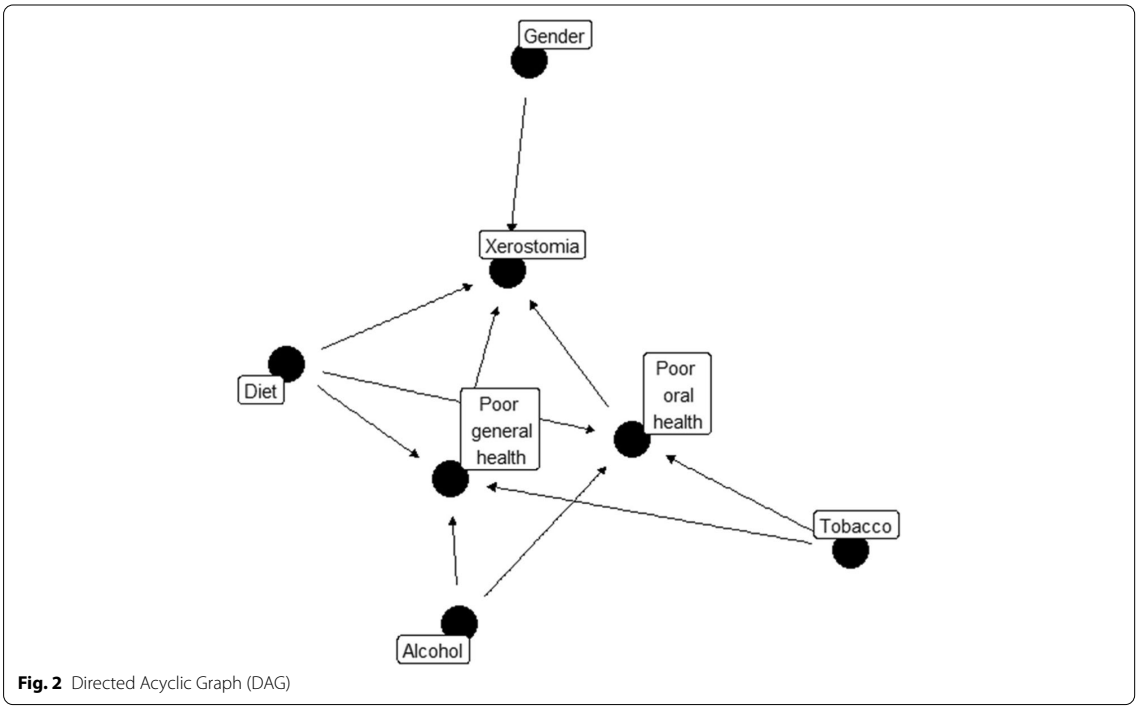


Fig. 2 Directed Acyclic Graph (DAG)

Table 4 A univariate logistic regression analysis was performed to determine the univariate OR *(Odds Ratio)* for xerostomia. The adjusted statistical analysis was performed using multivariate logistic regression corrected for gender, general health and oral health, education, employment, and household chores

	Univariate (OR 95% CI)	*p* value	Adjusted (OR 95% CI)	p value
Gender				
Female vs male	1.68 (1.22–2.32)	0.001	1.41 (0.99–1.99)	0.05
General health				
Poor vs good	2.81 (1.84–4.28)	< 0.001	2.18 (1.38–3.44)	0.001
Oral health				
Poor vs good	2.63 (1.42–4.84)	0.002	1.79 (0.92–3.49)	0.86
Education				
Uneducated vs Primary Education	1.31 (0.65–2.63)	0.44	1.48 (0.71–3.05)	0.28
Uneducated vs Higher Education	0.77 (0.38–1.58)	0.48	1.04 (0.49–2.22)	0.91
Uneducated vs First Level Vocational Education	1.31 (0.59–2.91)	0.50	1.67 (0.73–3.84)	0.22
Uneducated vs Higher-level Vocational Education and Training	0.73 (0.34–1.55)	0.41	0.90 (0.41–1.98)	0.81
Employment				
Self-employed vs employed	1.88 (0.94–3.79)	0.76	1.52 (0.73–3.16)	0.26
Self-employed vs others	1.11 (0.74–1.68)	0.61	1.15 (0.75–1.76)	0.51
Household chores				
Require help vs self-sufficient	2.16 (1.39–3.37)	0.001	1.69 (1.05–2.74)	0.03

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Women had a higher risk of suffering from xerostomia than men. Poor general and oral health have been reported as risk factors for xerostomia. Regarding alcohol and tobacco consumption, the results were quite heterogeneous and we did not observe any variation in relation to xerostomia. A lower perception of xerostomia was observed among those who consume vegetables and legumes on a regular basis.

When interpreting the findings of this study it is important to consider certain limitations. Firstly, given its cross-sectional nature, it is not possible to prove causality, which would be ideal for clinical translation, therefore emphasizing the need for prospective longitudinal studies. Nevertheless, this research has notable strengths, such as a large sample size and the fact that our findings were drawn from a very specific group by age range (over 65 years of age) residing in different areas of Spain.

The prevalence of xerostomia was close to the findings reported by other international organizations, which put it between 20 and 30% [18]. In Australia, an incidence of 26.5% was reported among people over 75 years of age [19]. Among participants aged 20 to 80 years, Nederfors et al., observed a significant difference in incidence (21.3% in men and 27.3% in women) which increased substantially with age [18]. A recent prospective study among younger participants (aged 20–59 years) revealed that general health affects episodes of xerostomia [20]. Although in our study the majority of the respondents claimed to have an acceptable or good oral health status, in the systematic review by Wong et al. [21], the oral hygiene and oral health of older adults was reported to be poor. The explanation for this apparent contradiction could be that self-perceived health is often better than objectively observed health. Furthermore, self-reporting of general and oral health, as well as health risk behaviours may be biased by social desirability, which may lead to inaccurate self-reports and erroneous conclusions. Heberto et al. [22] have observed a high bias in reporting food intake. However, in a study published in 2020 [23], it has been reported that there is no significant association between social desirability bias and general medical beliefs or self-reported health. Methods to decrease this bias include writing and prefacing questions [24], which were designed by gerodontology experts in this study. In terms of the quality of life, as other authors have also observed [25, 26], xerostomia has a significant negative impact on the older population's quality of life.

According to the "Global Status Report on Alcohol and Health" 2018 [27], 43% of the world's population are current drinkers, while in our sample 64.4% reported drinking alcohol occasionally or daily. Wine was the most consumed beverage (14.4% of daily consumers), followed by beer, while only 2.1% stated that they consume spirits every day. This consumption pattern is very different to the one observed worldwide, where 44.8% of all recorded alcohol consumption was in the form of spirits, followed by beer and wine [27]. Several studies have shown that the use of alcohol or alcohol-free mouthwashes does not significantly affect xerostomia [28, 29]. We also did not find any relevant differences in terms of tobacco use and xerostomia severity, although preliminary studies have found that smoking significantly increases symptoms of xerostomia [8]. In studies of younger populations (under 60 years of age), smoking has also been found to increase the likelihood of suffering from regular xerostomia [20]. Xerostomia's relationship with tobacco and alcohol remains unclear [30], however, whether or not these health risk behaviours play a relevant role in the development of xerostomia, there should be avoided as there is evidence that they do play a significant role in the development of oral cancer and other systemic diseases. A healthy diet should be advised and collaboration from doctors and dentists is essential in dietary interventions; indeed, dentists may be the earliest healthcare providers to detect an eating disorder [31]. Dentistry enables older adults to follow a satisfactory diet by restoring dental function and, as oral health improves, there is an opportunity to promote a good diet among this population group.

A cooperative approach involving different healthcare professionals in geriatric caregiving makes it possible to adjust to the individual needs of older patients [32, 33]. In this sense, our study updated and built on the current knowledge on the subject by providing evidence of xerostomia's relationship with general and oral health in Spain. Although, mild cognitive impairment can be assumed in the participants, it was not assessed objectively as it requires a clinical diagnosis aided by a complete medical record, neurological examination, mental status examination and formal neuropsychological testing [34].

Our findings can probably be extrapolated to the rest of Europe. In Norway and Sweden, a dramatically increased incidence of xerostomia has also been reported amongst older patients, which must be taken into account in the clinical management of these individuals. It has also been pointed out that the comorbidity between xerostomia and oral pathologies must not be ignored in older adults [35–37].

In conclusion, we found a strong association between general and oral health with xerostomia in older adults, so this relationship should be taken into account when providing health care to this group. The findings of our study showed the value of focusing on general and oral

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health when detecting xerostomia in older people, as well as periodic assessment of xerostomia in patients with poor health.

Supplementary Information

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Additional file 1. Survey database.

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Authors' contributions
A.B.C and M.P-S conceived the ideas; A.P.J., E.O.R, A.B.C, E.V.O and J.L.L designed the study; JM.M.G and M.P-S: methodology; A.P.J and M.M.P collected the data; A.B.C., M.P-S and J.L.L analysed the data; M.P-S.: software; A.P.J., J.L.L., E.O.R, and M.M.P writing—original draft preparation; M.P-S, A.B.C,, E.V.O, JM.M.G writing—review and editing; M.P-S.: supervision and project administration; A.P.J, M.P-S, M.M.P, E.O.R,, E.V.O, J.L.L, JM.M.G and A.B.C final approval of the version. All authors read and approved the final manuscript.

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Availability of data and materials
The dataset supporting the conclusions of this article is included within the article (and its additional file).

Declarations

Ethics approval and consent to participate
The study has been granted an exemption from requiring ethics approval by the Bioethics Committee of the University of Santiago de Compostela (REF USC-2020). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The survey was conducted over the phone and recorded so that the respondents voluntarily agreed to participate in the study. This consent procedure has been approved by the Bioethics Committee of the University of Santiago de Compostela.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests

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ARTÍCULO 5.

Título: Critical update, systematic review, and meta-analysis of oral erythroplakia as an oral potentially malignant disorder

Alejandro I Lorenzo-Pouso, Irene Lafuente-Ibáñez de Mendoza, Mario Pérez-Sayáns, Alba Pérez-Jardón, Cintia M Chamorro-Petronacci, Andrés Blanco-Carrión, José Manuel Aguirre-Urízar

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REVIEW

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Critical update, systematic review, and meta-analysis of oral erythroplakia as an oral potentially malignant disorder

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Abstract

Background: Oral erythroplakia has been classically considered as the potentially malignant disorder with the highest rate of malignant development into squamous cell carcinoma. This critical systematic review and meta-analysis aim to estimate the malignant development rate of oral erythroplakia and identify the associated risk factors.

Methods: We performed a bibliographic search in PubMed, Scopus, Web of Science, Embase, and LILACS, with keywords "erythroplakia," "erythroplasia," "malignant transformation," "malignant development," "malignization," "carcinogenesis," "oral cancer," "oral squamous cell carcinoma," "mouth neoplasm," and "prognosis." Meta-analysis was conducted using a random-effects model.

Results: Ten observational studies with 441 patients met the inclusion criteria, whose mean malignant development rate was 12.7% and with a mean follow-up period of patients of 6.66 years. In the initial biopsy, 42.8% of oral erythroplakia were already squamous cell carcinoma. The buccal mucosa was the most frequent location of oral erythroplakia, but the floor of the mouth was the most common site of malignant development. All patients who underwent malignant development showed epithelial dysplasia on the initial diagnostic biopsy.

Conclusion: Overall malignant development rate of OE in the meta-analysis was 19.9%. We could not associate any specific clinicopathological feature with the malignant development. The presence of epithelial dysplasia in the initial biopsy remains the worst prognostic factor. Further observational studies on OE are needed, with well-established diagnostic criteria and good clinical follow-up, in order to identify the true risk of malignant development of oral erythroplakia and the related risk factors.

KEYWORDS
malignant development, meta-analysis, mouth neoplasm, oral erythroplakia, oral potentially malignant disorder

Alejandro I. Lorenzo-Pouso and Irene Lafuente-Ibáñez de Mendoza authors have participated equally.

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1 | INTRODUCTION

Oral erythroplakia (OE) is a rare but highly risky oral potentially malignant disorder (OPMD) that has been the subject of multiple controversies since its initial reports.[1–5] OE is classically considered as the OPMD with the highest rate of malignant development (MD).[6–9] According to the last consensus convened by the WHO Collaborating Centre for Oral Cancer,[9] OE represents the red counterpart of oral leukoplakia (OL), and it is defined as "a predominantly fiery red patch that cannot be characterized clinically or pathologically as any other definable disease."

Due to its clinical appearance, it is very important to perform a good differential diagnosis of this pathology with other OPMD like erythroleukoplakia and oral lichen planus, and other "red disorders" of the oral mucosa, such as candidiasis, lupus, and fixed drug eruptions.[6,9,10] Unfortunately, there are not enough data on the different clinicopathological factors that might influence the MD of OE, including age and gender of patients, tobacco, alcohol, or betel consumption, location of lesions, and presence of epithelial dysplasia (ED).

With this background, we designed this critical systematic review and meta-analysis with all the available scientific evidence related to the MD of OE in order to estimate its MD rate and discuss the associated risk factors.

2 | MATERIALS AND METHODS

2.1 | Protocol and registration

This systematic review and meta-analysis comply with PRISMA[11] and MOOSE guidelines.[12] The review protocol was submitted to the PROSPERO register (CRD42022299026) to minimize risk of bias and improve the transparency, precision, and integrity. An ad hoc review group was created with oral medicine specialists of the Oral Medicine Unit from the University of Santiago de Compostela (MPS, AILP, and APJ) and the Oral Medicine and Pathology Unit of the University of the Basque Country (JMAU and ILIM).

2.2 | Focused questions

This review was designed to answer the following questions: (1) what is the overall MD of OE? and (2) what are the risk factors contributing to the MD of OE? We used the PECOS acronym as follows: (1) patients with OE (population), who developed an OSCC (outcome); (2) patients with OE (population), with specific clinicopathological factors (exposure), in comparison with patients unexposed to those clinicopathological factors (comparison), and to determine their link to the MD of OE (outcome). Only longitudinal observational studies (type of studies) were used.

2.3 | Search strategy

Electronic searches were carried out in MEDLINE via PubMed, EMBASE, Web of Science, Scopus, WHO regional bibliographic database LILACS, and the Conference Proceedings Citation Index databases. Searches combined thesaurus (e.g., MeSH and EMTREE) and free terms to maximize sensitivity. The algorithms used in each database contained the following keywords: "erythroplakia," "erythroplasia," "malignant transformation," "malignant development," "malignization," "carcinogenesis," "oral cancer," "oral squamous cell carcinoma," "mouth neoplasm," and "prognosis." These words were subjected to syntax adaptation for each database. All of the databases were searched from inception to February 2022 (upper limit).

We performed a second search, introducing each keyword in an unstructured fashion to check whether every article on the topic was retrieved. Potentially relevant articles that any of the authors were already familiar with and reference lists from the retrieved articles were also comprehensively checked. All retrieved references were managed using Mendeley v.1.19.9 (Elsevier, Amsterdam, The Netherlands).

2.4 | Eligibility criteria

Criteria for eligibility of the studies included in the qualitative and quantitative analysis were as follows: (1) original research articles published in English language, (2) longitudinal observational studies, and (3) studies that analyses the MD of OE. The exclusion criteria were as follows: (1) letters to the editor, case reports, conference abstracts, clinical trials, etc.; (2) previous systematic reviews and meta-analysis; (3) studies which reported on patients with diagnosis of erythroleukoplakia; (4) studies without a complete clinicopathological diagnosis of OE; and (5) studies that did not report specific data on OE.

2.5 | Data selection and extraction

Articles were selected in two phases by two authors (APJ and MPS), first by screening titles and abstracts that apparently meet inclusion criteria, and then by reading the full texts to assess their final inclusion. During the calibration exercise, reviewers thoroughly discussed the criteria and applied them to a sample of 50% of the retrieved studies to determine the inter-examiner agreement. After adequate agreement was achieved (κ score = 0.78), all of the studies were independently read by the reviewers. Any discrepancies between the researchers were resolved by a third or fourth researcher blinded to the study hypothesis (ILIM and JMA).

The information was retrieved by two investigators (APJ and MPS) using a custom-made extraction sheet. The recorded data included: the first author and year of publication, country, study design, sample size, gender and age of patients, tobacco/alcohol/betel consumption, number and location of OE, histopathological data, follow-up period, number of MD cases, and time until MD.

2.6 | Statistical analysis

In the main meta-analysis, we computed the prevalence MD events and that of total OE case by dividing the number of events by the

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sample size of the study. Then, we weighted the study-specific log prevalence by the inverse of their variance to compute a pooled prevalence. For retrieved studies, the Clopper–Pearson interval was applied to estimate 95% confidence intervals (CIs). Combined pooled proportions were estimated with a random-effect model (DerSimonian and Laird method). A further pre-planned meta-analysis to acknowledge the effect of clinicopathological factors on the MD of OE was considered, but this was discarded due to poor reporting of primary data. Therefore, we opted to perform a meta-analysis of prevalence with the degrees of ED/presence of SCC in initial biopsy at diagnosis time-point.

For statistical heterogeneity analysis, Cochran Q (χ^2) and Higgins I^2 test parameters were calculated. Cochran's Q test $p < 0.1$ was considered significant to assume apparent heterogeneity. The Higgins I^2 statistic cut-off points of 25%, 50%, and 75% were considered to indicate low, moderate, and high heterogeneity, respectively.[13]

We assessed publication bias, first visually, using funnel plots, and then, more formally, using the test proposed by Egger et al.[14] (performing a linear regression of the effect estimates on their standard errors, weighting by 1/[variance of the effect estimate], considering a p_{Egger} <0.1 as significant). The Metafor free R software package (v.3.6.2; https://www.r-project.org) was used for all statistical analysis as for figure plotting with user-written commands. The significance level considered in all statistical analyses was 5% ($p < 0.05$).

2.7 | Quality assessment and risk of bias

Risk of bias (RoB) was assessed using the Quality in Prognosis Studies–QUIPS tool, supported by Cochrane Prognosis Methods Group for prognosis studies. QUIPS considers the following domains: (1) study participation, (2) study attrition, (3) prognostic factor measurement, (4) outcome measurement, (5) study confounding, and (6) statistical analysis and reporting.[15] RoB was qualified as low, moderate, or high for each domain. Each item scored as high adds 3 points, while when scored as moderate 2 and as low 1 to overall quality assessment for each study. Studies were categorized as high quality when the overall score was >13. RoB was assessed by two authors (MPS and APJ). Discrepancies between authors were resolved by all participating authors by consensus.

3 | RESULTS

3.1 | Bibliographical research

We identified 974 registers through the aforementioned search, whose abstracts were reviewed for contents relevant to the topic of this study, resulting in 633 exclusions. A total of 341 papers were then retrieved and, after careful consideration, 318 of them were excluded because their information was not useful for this study. The remaining 23 (2.37%) registers were checked according to the inclusion/exclusion criteria set for this investigation.

After the critical analysis of the studies, 10 (1.03%) studies from different geographical areas met the inclusion criteria: USA,[16,17] Denmark,[18,19] Brazil,[20,21] Taiwan,[22,23] China,[24] and Thailand[25] (Figure S1).

3.2 | Malignant development

Only five studies[18,19,22–24] stated the follow-up period of patients. In these, mean MD rate was 12.7%, with a mean follow-up period of 6.66 years (Table 1). In the three studies that reported cases of OE with MD,[19,23,24] mean MD rate was 21.2%, with a mean follow-up period of 8.25 years and a mean time until MD of 3.17 years. Conversely, in the two studies that did not report cases of MD, mean follow-up period was 5.07 years[18,22] (Table 1).

In the five studies without a follow-up period of patients,[16,17,20,21,25] mean MD of OE was 51.10%. Besides, 68.38% of cases of these studies were already SCC at the time of diagnosis.

3.3 | Clinicopathological data

In this review, 90 women (20.4%) and 336 men (79.6%) with OE lesions were analyzed, mostly over 50 years[17,21,22,24,25]; (Table 1). From these, 75.55%, 56.31%, and 79.78% consumed tobacco, alcohol, and betel, respectively[17,19–24] (Table 1).

Two studies[16,22] described patients with more than one OE. The most frequent location of OE was the buccal mucosa (38.5%), followed by the tongue (15.8%), the floor of the mouth (FOM) (12.3%), the gingiva (12.3%), the soft palate (11.2%), the retromolar area (6.9%), and the lip (2.7%) (Table 2).

Regarding the presence of ED, different evaluation systems were used; thus, we divided ED into low-grade ED (previous mild to moderate) and high-grade ED (previous moderate to severe), following the WHO proposal.[26] In the initial biopsy, 47.9% of cases showed ED: 76.6% low-grade ED and 23.4% high-grade ED.[16–22,24] A total of 42.8% of the OE corresponded to SCC at the initial biopsy (Table 3).

Only Feng et al.[24] reported the clinical data of patients who underwent MD: 8 females and 9 males, all of them older than 50 years. Also, 42% and 50% of patients were tobacco and alcohol consumers, respectively.[17,24] The most common area of MD was the FOM (25.6%), followed by the tongue (24.3%), the retromolar area (14.6%), the gingiva (13.4%), the soft palate (13.4%), and the buccal mucosa (8.5%). Only Feng et al.[24] stated that 47.1% of cases with MD showed high-grade ED and 52.9% low-grade ED on the initial diagnostic biopsy.

3.4 | Meta-analysis

In the main meta-analysis, three studies[19,23,24] testing the same hypothesis (prevalence of MD in OE) were included in the quantitative synthesis. The combined MD rate was 19.9% (95% CI = −1.6 to 41.4; $I^2 = 91.74\%$, p-value by Q test = 0.00001) (Figure 1A). A

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TABLE 1 Main clinicodemographic data of patients with oral erythroplakia included in the study

		Patients				Risk factors (*n*)				
			Gender							
Author and year	Country	*n*	F	M	Age (years)	Tabaco	Betel	Alcohol	Follow-up (years)	Malignant development (%)
Shafer and Waldron, 1975	USA	58	27	31	47 (>50) 11 (<50)	–	–	–	–	–
Nielsen et al. 1996	Denmark	10	9	1	–	–	–	–	6.3	0
Qin et al. 1999	USA	24	11	13	Mean: 61.9	15	–	–	–	–
Holmstrup et al. 2006	Denmark	15	–	–	–	5	–	–	7.5	6.67
Lapthanasupkul et al. 2007	Thailand	9	3	6	3 (<50) 6 (>50)	–	–	–	–	–
Feng et al. 2012	China	34	18	16	Mean: 58.7	10	–	12	16	50
Queiroz et al. 2014	Brazil	11	7	4	4 (<50) 7 (>50)	9	–	3	–	–
Yang et al. 2015	Taiwan	84	10	74	Mean: 54.2	65	57	42	3.83	0
Chuang et al. 2018	Taiwan	188	0	188	Mean: 46.0	170	160	120	9	6.9
de Azevedo et al. 2020	Brazil	8	5	3	61–80	1	–	1	–	–
Total		441	90	336	–	275	217	178	6.66 (mean)	12.7 (mean)

Note: F, female; M, male.

remarkable asymmetry with a lack of studies on the middle of the plot was seen (Figure 1B); nonetheless, Egger's regression test neglected the existence of publication bias (*p*-value of the intercept = 0.276).

In the secondary analysis, we performed a pooled analysis on the prevalence of initial histopathological diagnosis: SCC, high-grade ED, low-grade ED, and absence of ED (Table 4). Globally, studies harbored a substantial degree of heterogeneity and no individual study seemed to represent an influential point that increased heterogeneity dramatically. Presence of ED was almost six times higher than absence of ED (41.2% vs. 7.19%). Observation of the asymmetry of the funnel plots (Figure 2) and the statistical tests conducted for the same purpose confirmed the absence of "small-study" effects on the pooled prevalence of the initial diagnosis of SCC ($p_{Egger} = 0.549$) and low-grade ED ($p_{Egger} = 0.685$). The exceptions were high-grade ED ($p_{Egger} = 0.001$) and absence of ED ($p_{Egger} = 0.002$), for which bias could not be ruled out.

3.5 | Risk of bias

The QUIPS tool showed that RoB was high in three studies[16–18] and low in seven (18–24] (Figure S2). The most potential biases were inappropriate statistical analyses and poor assessment of study cofounding. Nevertheless, the quality of some studies was also suboptimal in the remaining four domains.

4 | DISCUSSION

Erythroplakia is a premalignant disorder first described as a raised, velvety red plaque in the male and female genital mucosa.[1,27] The first oral cases were published in the late twentieth century,[28,29] rapidly becoming a matter of controversy.

In 1955, Blau & Hyman[30] made the first review on OE, with lesions on the tongue, the buccal mucosa, and the lip. Later, Shear[31] proposed three clinical forms of OE (homogeneous, erythroleukoplakia, and speckled), with different prognostic aspects; which were later approved by several authors.[10,16] In 2007, "erythroleukoplakia" was considered an OPMD independent of OE,[32] appearing as such in the latest WHO Classification of Head and Neck Tumours.[26] Recently, erythroleukoplakia has been once again reconsidered as a clinical form of nonhomogenous of OL,[9] removing it as an individual OPMD.

Taking into account the current definition of OPMD: "*any oral mucosal abnormality that is associated with a statistically increased risk of developing oral cancer*",[9] we believe that some aspects of previous reviews about the MD of OE are somewhat questionable.[6,33] Most studies include, at the same time, cases of OE, erythroleukoplakia, and SCC.[34–40] Furthermore, MD assessment of OE has been preferably based on Shafer & Waldron's[16] classic study on nonhomogeneous OE, where 91% of cases were already SCC at the time of diagnosis. In order to avoid further confusions, we only selected cases clinicopathologically diagnosed as homogeneous OE for our review.

We think that clinical follow-up of patients is necessary to assess the true MD of OE as an OPMD. Therefore, when we considered the studies without a follow-up period, we achieved a "wrong" MD rate of 50.51%, similar to that of previous reviews (44.9%–50%).[6,33] On the contrary, when we considered the studies with a follow-up period, we obtained a "right" MD rate of 19.9%. Moreover, the number of SCC in the studies without a clinical follow-up (68.38%) is two times bigger than in all the studies (30.99%), and five times bigger than in the studies with a clinical follow-up (12.7%) (Table 1).

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TABLE 2 Location of oral erythroplakia lesions at the time of diagnosis and malignant development

Author and year	Oral erythroplakia (Time of diagnosis)							Oral Carcinoma (Malignant development)						
	FOM	Retromolar	Gingiva	Soft palate	Tongue	Buccal	Lip	FOM	Retromolar	Gingiva	Soft palate	Tongue	Buccal	Lip
Shaffer and Waldron, 1975	19	13	12	8	8	5	0	18	12	9	6	6	2	0
Nielsen et al. 1996	2	0	0	0	2	5	0	1	0	0	0	0	1	0
Qin et al. 1999	5	0	5	9	5	0	0	2	0	2	2	4	0	0
Holmstrup et al. 2006	0	0	3	0	1	11	0	–	–	–	–	–	–	–
Lapthanasupkul et al. 2007	1	0	5	0	0	3	0	–	–	–	–	–	–	–
Feng et al. 2012	0	0	4	5	16	9	0	0	–	–	3	10	4	–
Queiroz et al. 2014	2	1	1	2	3	0	2	–	–	–	–	–	–	–
Yang et al. 2015	2	4	1	2	5	65	5	–	–	–	–	–	–	–
de Azevedo et al. 2020	1	0	1	3	1	2	0	–	–	–	–	–	–	–
Total	32	18	32	29	41	100	7	21	12	11	11	20	7	0

Abbreviation: FOM, floor of the mouth.

All these findings question whether OE is the OPMD with the highest risk of MD, as previously stated.[9,32] We believe that OE should only be considered as an OPMD when (1) there is a clinical follow-up period of patients, and (2) the initial biopsy rules out the presence of an SCC. Since the follow-up period has been longer in the studies with cases of MD (8.25 vs. 5 years) (Table 1), we think patients with OE should be actively followed for life.

Except for the study by Chuang et al.,[23] gender and age distribution were similar in patients with OE and those who suffer MD (Table 1).[41,42] The buccal mucosa has been the most frequent location of OE; which could be related to some particular carcinogenic habits, such as consumption of betel.[22,25] However, the absence of cases with MD in some studies[22,25] suggest that some of these cases may not be true OE.

We also observed interesting differences between the location OE and where MD occurred. Some infrequent areas of OE like the FOM, the tongue, and the retromolar area are common sites of MD (FOM: 25.6%, tongue: 24.3%, and retromolar area: 14.6%). In contrast, more frequent areas of OE like the buccal mucosa and the lip have a low rate of MD (buccal mucosa: 8.5%, lip: 0%). These results suggest the existence of specific locations of the oral mucosa with a bigger risk for MD of OE. Also, they point to the need for a thorough clinical and histopathological differential diagnosis with all mucous pathologies clinically similar to OE, preferably SCC, but also oral lichenoid disease (OLD), erythematous candidiasis, lupus erythematosus, pemphigoid, and so forth.[9] Performing a good clinicopathological correlation is key to reach the final diagnosis of OE; thus, it is mandatory to get a good biopsy of all suspicious oral "red plaques" in order to (1) rule out other diagnoses and (2) assess the existence and/or degree of ED.

In 1972, Shear[31] proposed two histopathological types of OE: a "neoplastic" one, associated with SCC, carcinoma in situ or ED; and an "inflammatory" one, related to *Candida* infection, prosthetic stomatitis, immunological processes, and so forth. In our opinion, this classification is misleading and should not be used. Currently, we cannot consider as an OPMD an oral lesion that is already a SCC in the histopathological analysis; or can all "red plaque-like" lesions that may appear on the oral mucosa be considered as an OE.[9] Indeed, some multifocal cases of OE reviewed on this study,[22] or infected with *Candida spp*., and without ED or low-grade ED, could actually represent other disorders.

The histopathological diagnosis is the gold standard technique for the prognostic assessment of all OPMD, including OE. Different oral mucous pathologies of an inflammatory nature may cause epithelial changes similar to those seen in ED, making the final clinicopathological diagnosis very difficult.[43] Only the study by Feng et al.[24] assesses the presence of ED in the initial diagnostic biopsy of cases with MD, all of which showed ED (47.1% high grade and 52.9% low grade). In our review, almost half of the OE lesions had ED in the first biopsy, and presence of ED was six times higher than absence of ED (41.2% vs. 7.19%) (Table 4). Moreover, ED remains the most important prognostic factor in relation to the MD of

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TABLE 3 Histopathological data of the oral erythroplakia at the time of diagnosis

Author and year	*n*	SCC	High-risk ED	Low-risk ED	No ED
Shaffer and Waldron, 1975	65	59	0	6	0
Nielsen et al. 1996	9	2	0	4	3
Qin et al. 1999	24	10	8	6	0
Holmstrup et al. 2009	15	2	2	10	1
Lapthanasupkul et al. 2007	9	3	0	3	3
Feng et al. 2012	34	0	14	20	0
Queiroz et al. 2014	11	3	5	3	0
Yang et al. 2015	84	23	0	41	20
de Azevedo et al. 2020	8	5	0	2	1
Total	250	107	29	95	28

Abbreviations: ED, epithelial dysplasia; OE, oral erythroplakia; SCC, squamous cell carcinoma.

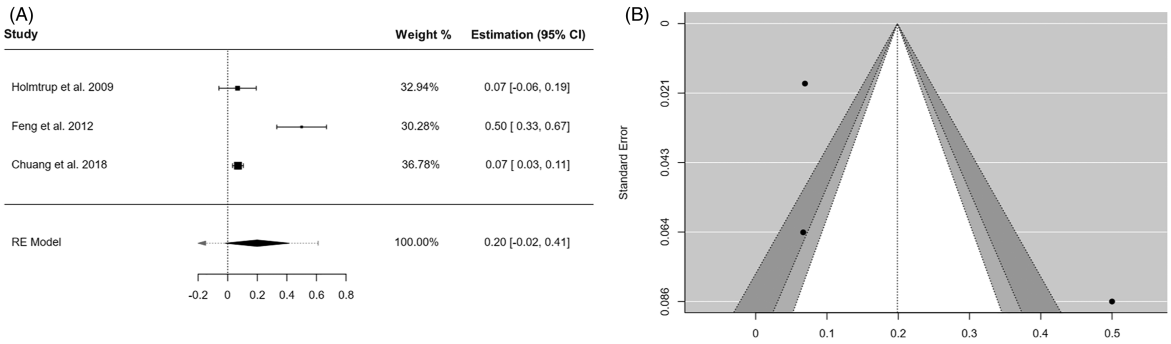


FIGURE 1 Malignant development. (A) Forest plot representing the meta-analysis of the malignant development rate of oral erythroplakia. RE (random-effects) and weight of each study. (B) Funnel plot assessing the publication bias

TABLE 4 Pooled prevalence, MD rate, and subgroup analysis of the initial histopathological diagnosis of OE

	Sample size (*n*)		Pooled data		Heterogeneity	
	Studies	Patients	ES (95% CI)	*p*-value	p_{het}	I^2 (%)
Malignant development						
	3	237	PP = 19.9% (−1.6–41.4)	0.001	0.0001	91.7
Initial histopathological diagnosis						
SCC	9	259	PP = 41.2% (16.8–65.5)	0.001	0.0001	95.2
High-risk ED	9	259	PP = 9.17% (29.8–51.4)	0.001	0.0001	82.5
Low-risk ED	9	259	PP = 37.4% (20.9–53.9)	0.001	0.0001	87.9
No ED	9	259	PP = 7.19% (2.0.12.4)	0.007	0.0001	75.63

Abbreviations: CI, confidence intervals; CIS, carcinoma in situ; ED, epithelial dysplasia: OE, oral erythroplakia; PP, pooled proportion; SCC, squamous cell carcinoma.

multiple OPMD, such as OL, OLD, and proliferative multifocal/verrucous leukoplakia.[44–46]

Unfortunately, we had several limitations during the performance of this systematic review and meta-analysis. First, is the low number of observational studies on the MD of OE, and with good clinical follow-up period of patients; and second, is the lack of standardization and changes in the diagnostic criteria for OE over the years. This jeopardized the association between the MD of OE and different clinicopathological features.

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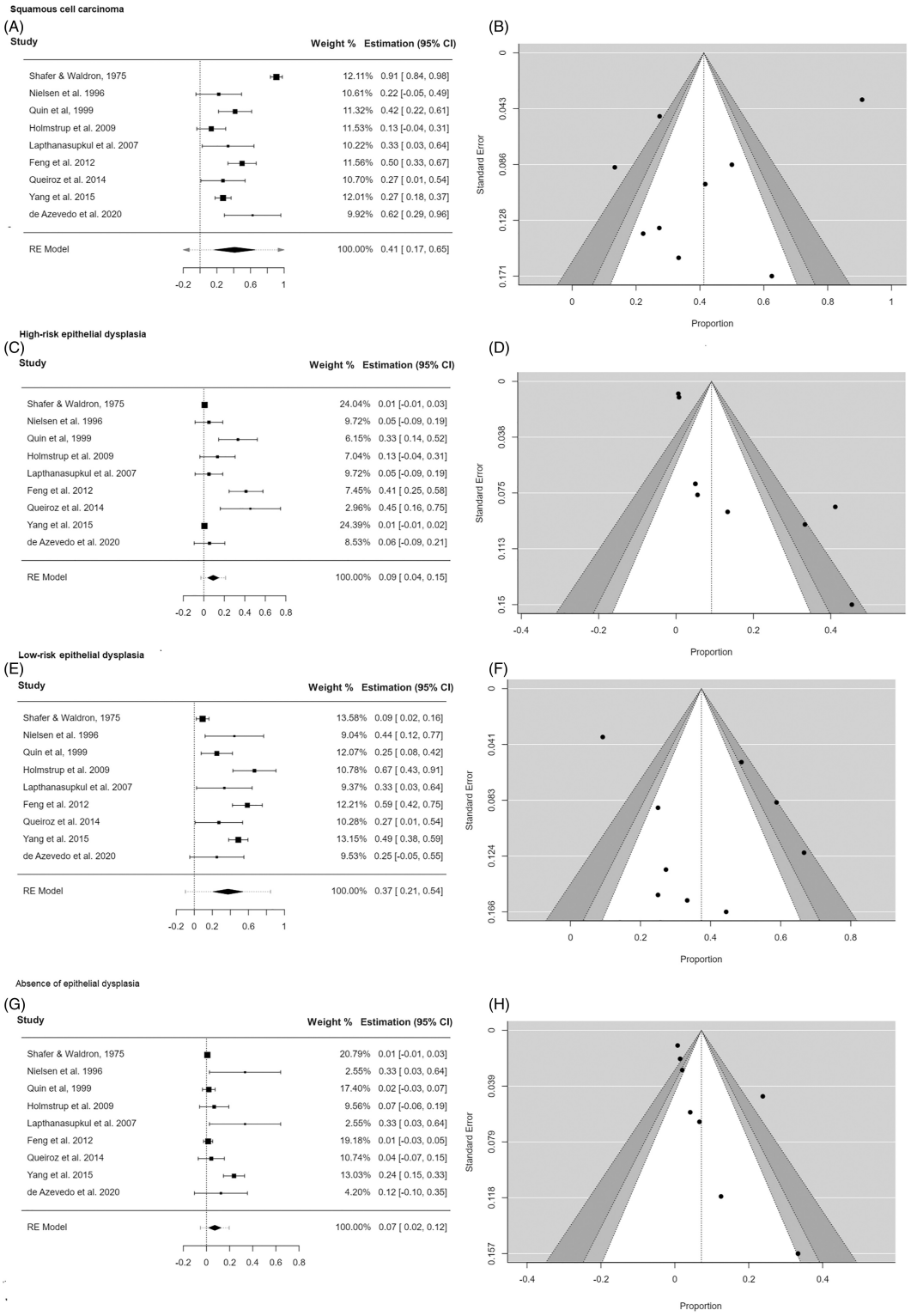


FIGURE 2 Initial histopathological diagnosis. Forest plot of the initial histopathological diagnosis of the oral erythroplakias that suffer malignant development. Funnel plot to assess the publication bias. (A & B): Squamous cell carcinoma; (C & D): High-risk epithelial dysplasia; (E & F): Low-risk epithelial dysplasia; (G & H): Absence of epithelial dysplasia

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5 | CONCLUSIONS

In summary, overall MD rate of OE in this study was 19.9%, significantly lower than previously reported. We were unable to associate this MD with any specific clinicopathological feature, including ED. Nevertheless, we believe the presence of ED in the initial diagnostic biopsy remains the main prognostic factor in this OPMD. Further observational studies are needed, with well-established diagnostic criteria and good clinical follow-up, in order to identify the true risk of MD of OE and its related factors. This way we could design effective preventive and therapeutic programs.

AUTHOR CONTRIBUTIONS

Conceptualization, *data curation*, *formal analysis*, *methodology*, *resources*, *software*, *validation*, *visualization*, *and writing-original draft*: Alejandro Ismael Lorenzo-Pouso. *Conceptualization*, *investigation*, *methodology*, *writing-original draft*, *writing-review & editing*: Irene Lafuente-Ibáñez de Mendoza. *Conceptualization*, *data curation*, *formal analysis*, *methodology*, *project administration*, *resources*, *supervision*, *validation*, *writing-review & editing*: Mario Pérez Sayáns *Data curation*, *investigation*, *methodology*, *writing-original draft*: Alba Pérez-Jardón. *Data curation*, *investigation*, *methodology*, *writing-original draft*: Cintia M. Chamorro-Petronacci. *Data curation*, *investigation*, *methodology*, *supervision*, *writing-original draft*: Andrés Blanco-Carrión. *Conceptualization*, *data curation*, *formal analysis*, *investigation*, *project administration*, *resources*, *supervision*, *validation*, *writing-review & editing*: José Manuel Aguirre-Urizar.

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CONFLICT OF INTEREST

Authors confirm there is no conflict of interest regarding the submission of this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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ARTÍCULO 6.

Título: Oral Chronic Hyperplastic Candidiasis and Its Potential Risk of Malignant Transformation: A Systematic Review and Prevalence Meta-Analysis

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Review

Oral Chronic Hyperplastic Candidiasis and Its Potential Risk of Malignant Transformation: A Systematic Review and Prevalence Meta-Analysis

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Abstract: Chronic hyperplastic candidiasis (CHC) is a prototypical oral lesion caused by chronic *Candida* infection. A major controversy surrounding CHC is whether this oral lesion owns malignant transformation (MT) potential. The aim of the present study was to evaluate current evidence on the MT of CHC and to determine the variables which have the greatest influence on cancer development. Bibliographical searches included PubMed, Embase, Web of Science, Scopus and LILACS. The cohort studies and case series used to investigate the MT of CHC were deemed suitable for inclusion. The quality of the enrolled studies was measured by the Joanna Briggs Institute scale. Moreover, we undertook subgroup analyses, assessed small study effects, and conducted sensitivity analyses. From 338 studies, nine were finally included for qualitative/quantitative analysis. The overall MT rate for CHC across all studies was 12.1% (95% confidential interval, 4.1–19.8%). Subgroup analysis showed that the MT rate increased when pooled analysis was restricted to poor quality studies. It remains complex to affirm whether CHC is an individual and oral, potentially malignant disorder according to the retrieved evidence. Prospective cohort studies to define the natural history of CHC and a consensus statement to clarify a proper set of diagnostic criteria are strongly needed. PROSPERO ID: CRD42022319572.

Keywords: malignant transformation; meta-analysis; mouth neoplasm; *Candida*; chronic hyperplastic candidiasis

1. Introduction

Candida species (spp.) are a relevant group of eukaryotic diploid sexual yeasts involved in the relevant burden of human pathologies. These yeasts are frequently encountered in the oral microbiota of healthy individuals, with an incidence between 35 and 80%, depending on the population sampled [1]. While *Candida* spp. are normally harmless commensals, they can sometimes switch to pathogenic behavior. As a result, they contribute to the appearance of a group of pathologies commonly referred to as oral candidosis or candidiasis (OCs) [2]. OCs pathogenesis is driven by the elevated production of virulence determinants and the subsequent impairment of the host immune responses [3].

Chronic hyperplastic candidosis or candidiasis (CHC) is a subtype of OCs and is considered a clinicopathological term, housing an intraoral white lesion generated by a persisting fungal infection, commonly *Candida albicans* [4]. Two clinical presentations have

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been described for this entity: a homogeneous form characterized by an isolated, adherent, and thick white plaque and a nodular/speckled form arising with multiple white nodules on an erythematous background. CHC commonly appears in the post-commissural buccal mucosa, the upper surface of the tongue, and the velum of denture users [1,3,4]. Although reported CHC incidence is low among OCs (about 1.61%) [5], its differential diagnosis is challenging since its clinical manifestations resemble the presentation of more common diseases, such as oral lichen planus and erythro-leukoplakia [6]. Another issue stands in the nomenclature. Since the first description of this entity by Cawson and Lehner back in 1968, the term has been considered a synonym of "candida leukoplakia" [7]; however, the role of this yeast infection in oral leukoplakia (OL) is still controversial [4]. At last, more importantly, CHC malignant transformation is still debated.

In terms of prevention strategies for CHC, there is poor quality of evidence. Patients should refrain from smoking or chewing tobacco. Some authors algo suggest the relevance of requesting patients to not wear their dentures at night and improve their oral hygiene. Some systemic problems can also contribute to the development of this type of candidiasis, such as anaemia, avitaminosis, and deficiencies in the immune system [2]. Various modalities of treatment for CHC have been used such as medical management in the form of antifungal therapy or the topical application of retinoids, bleomycin, or beta carotenes. On the other side, surgical methods have been used including cold-knife surgery, laser therapy, or cryosurgery [4].

For these reasons, CHC differential diagnosis is complex and time-consuming, requiring histopathology to exclude cellular atypia and combining the use of laboratory test cultures for fungal identification [8]. Indeed, over 15% of CHCs present histopathological features of epithelial dysplasia (ED) and higher malignant development rates than classical forms of OL lacking a *Candida* spp. Infection [3,9]. As is well known, OL is the most frequently encountered and emblematic oral potentially malignant disorder (OPMD). It is classically defined as "a white plaque with a questionable risk of cancer that can only be diagnosed once other specific conditions have been ruled out" [4]. *Candida* spp., particularly *albicans*, are capable of promoting oncogenesis by mechanisms such as carcinogenic by-products, molecular mimicry, or triggering the inflammation cascade [10]. As it is well known, the histopathological findings of ED in the first biopsy of any oral lesion are a strong predictor of future malignant transformation (MT) [11]. However, features of ED are not exclusive to premalignant lesions, and such histologic patterns may be reactive rather than pre-neoplastic [12]. The WHO Collaborating Centre for Oral Cancer noted that the OL definition is ad excludendum, which rules out specific causes such as *Candida* spp. infection. In this scenario, CHC per se is no longer defined as an OPMD [13].

Prompted by the discussed literature, we designed this systematic review and meta-analysis in order to more precisely determine the proportion of MT in the natural history of this particular affection.

2. Materials and Methods

This systematic review protocol was designed and planned in advance by an author (A.I.L.P). The present review was elaborated in accordance with the preferred reporting method for systematic reviews (PRISMA) [14], as prospectively registered at the PROSPERO database (ID: CRD42022319572).

2.1. Inclusion and Exclusion Criteria

The inclusion criteria were as follows: (a) Study design: observational studies with a prospective and retrospective design (i.e., cohort studies, case-control studies, or case series); (b) Participants: studies in humans of any sex or age diagnosis with a CHC; (c) Intervention: Proven histopathological diagnosis of *Candida* spp. or with cultural/or molecular approaches; (d) Outcome: incidence of malignant transformation in the sample studied. Among exclusion criteria were: (a) Case reports, reviews, position papers, and author opinions; (b) Studies that did not accurately define CHC diagnosis both clinical and

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histopathological; (c) Studies that omitted *Candida* spp., detection methodology; (d) Studies that failed to provide the CHC MT rate or provided insufficient data to allow estimations; (e) reports including less than 3 CHC cases.

We included any relevant article according to the aforementioned criteria without restriction in terms of language or publication dates. In addition, we explored the references of included studies to complement our search strategy. Moreover, we established personal contact via e-mail with researchers to trace further publications or relevant data not included in their articles. This approach minimized the risk of introducing selection bias and improved the transparency, accuracy, and integrity of our methodology [14].

2.2. Search Strategy

Medline via PubMed, Embase, Web of Science, Scopus, and LILACS databases were screened for records published from inception to 22 June 2022 (upper limit). Our search strategy and syntax were conceptualized by combining thesaurus terms (e.g., MeSH or EMTREE) with free words. For Medline via PubMed, the following syntax was used both in MeSH and in the free terms: ("Candidiasis, Chronic Mucocutaneous" [MeSH] or "Chronic Hyperplastic Candidiasis" [All Fields] or "Candidal leukoplakia" [All Fields] or "Candida leukoplakia" [All Fields]) and (malign* or premalignant* or "potentially malignant disorder" or "precancer" or "cancer" [All Fields] or "Carcinoma, Squamous Cell" [MeSH] or "squamous cell carcinoma" [All Fields] or "oscc" [All Fields] or "transformation" [All Fields] or "risk" [All Fields] or "progression" [All Fields]).

The aforementioned algorithm was conveniently reformulated for each database, as can be seen in more detail in Table A1. Furthermore, to verify whether every relevant report dealing with the topic under study was retrieved, a complementary manual search with the terms: *Candida*, oral cancer, and malignant transformation in an unstructured form was executed.

2.3. Study Selection

In the first step, titles/abstracts of retrieved reports were screened on the basis of the inclusion/exclusion criteria using the Rayyan QCRI platform. In the second step, all selected studies that were considered proper according to these criteria underwent a full text assessment, and then a final decision was made. Excel spreadsheets were created to gather information regarding this decision-making process, to include/exclude each report. Cohen's kappa coefficient (κ) was applied to quantify the reviewers' intra-agreement at the full text screening process; this coefficient was calculated with an Epidat 4.2 statistical package (available at: https://www.sergas.es/Saude-publica/EPIDAT-4-2, accessed on 9 September 2022).

Study selection was performed by 2 independent operators (A.I.L.P. and M.P.S.) with expertise in oral medicine and pathology. In cases of disagreements among these reviewers, they were solved in joint meetings held with the participation of all authors. A third reviewer, blinded to the study hypothesis, performed an intra-agreement analysis by computing a value for κ (A.P.J.).

2.4. Data Extraction

Data were extracted with a specific form and improved by pilot-testing before its implementation. We collected information on author's first name, publication date, country of origin, study design, population size, malignant transformation rates, recruitment/follow-up periods, method of *Candida* spp. detection, histopathological features, topography of lesions, sex, age, smoking, and alcohol intake among individuals. When data on an item were missing in the primary literature, attempts were made to contact the corresponding authors to incorporate lacking information.

Two reviewers (V.C.A.P. and M.P.S.) were responsible for the data extraction. This process was comprehensively reviewed later by a third participant (A.I.L.P.). In the event of disagreements, they were settled by careful discussion.

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2.5. Critical Appraisal

The risk of bias (RoB) was established using a tool developed for systematic reviews and meta-analysis prevalence developed by the Joanna Briggs Institute (JBI), namely the JBI Prevalence Critical Appraisal Checklist [15]. This checklist assesses RoB studies by means of five domains in the form of closed questions: (i) Was the sample frame appropriate to address the target population? (ii) Were participants sampled appropriately? (iii) Was the sample size adequate for the study objective? (iv) Were participants and setting described properly? (v) Was the data analysis conducted with sufficient coverage? (vi) Were valid methods applied for the identification of the outcome? (vii) Was the outcome measured in a standard, reliable manner? (viii) Was there appropriate statistical analysis? (ix) Was the response rate adequate, or at least was the low response rate managed adequately? The studies were defined as a "high RoB" if 49% of items were reached with a "yes"; as "intermediate RoB" if a range between 50% and 69% of questions scored "yes"; and as "low RoB" if scores $\geq$ 70% received "yes". In order to encounter a more detailed explanation regarding this scale, please see: https://jbi.global/sites/default/files/2019-05/JBI_Critical_Appraisal-Checklist_for_Prevalence_Studies2017_0.pdf, accessed on 9 September 2022.

2.6. Statistical Analysis

Study-specific proportions were computed with the portion of CHC cases reported with malignant transformation acting as numerators and the CHC cohort size as denominators. The 95% confidential intervals (CIs) were then estimated for retrieved studies with a simple asymptotic method with a continuity correction for the avoidance of mathematical aberrations or artifacts and to fuel the closeness of the coverage probability to its nominal value [16]. Later, study-specific log prevalence's were weighted by the inverse of their variance to achieve pooled prevalence values with their corresponding 95% CIs. Meta analyses were performed with the DerSimonian and Laird method, which owed to logic dictated by some degree of between-study heterogeneity. Forest plots were generated to graphically display study-specific proportions as pooled proportions derived from the random-effects model. We also performed sensitivity analyses, evaluating the individual influence exerted by every study on the estimation of pooled rates; this approach was applied in order to check the reliability of combined results and to detect studies with aberrant results, bearing in mind the mathematical construct [17]. We also developed a mixed-effect meta-regression to check within-study variation and between-study variation to confront a specific covariate (year of publication) with the main outcome under study (i.e., MT rate).

Cochran's Q test was implemented to assess between-study heterogeneity; $p < 0.1$ was considered a significant figure to assume heterogeneity. The I^2 index was also used to quantify the percentage of heterogeneity. The thresholds established to define heterogeneity intensity were as follows: less than 25% no apparent, between 25% and 49% low, between 50% and 74% moderate, and 75% or greater high heterogeneity [18]. We later planned to explore the origin of heterogeneity across strata by restricting the analysis to subgroups defined by study-specific features, namely study origin and RoB assessment (high versus moderate and low RoB).

Publication bias or "small study effects" were evaluated visually using Begger's funnel plot but also formally through the test described by Egger et al. [19]. The data analysis was performed using free R software (v.3.4.4; https://www.r-project.org, accessed on 9 September 2022, particularly using the Metafor package. A *p*-value less than 5% was considered statistically significant.

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3. Results

3.1. Study Selection

Figure 1 displays the flow diagram of the electronic search, which resulted in 338 studies. After the screening of titles and abstracts, duplicates were removed, and 318 articles were excluded. After the screening of full texts, another 11 studies were excluded on the following basis. Eight articles were excluded because of their cross-sectional nature without any patient follow-up data. Two articles were excluded on the basis of being case reports, whilst one last study was discarded due to its representation of in vitro research results (i.e., off-topic). Nine articles were finally selected to enter the systematic review [6,7,20–26]. The reviewers' intra-agreement was considered appropriate (κ = 0.83 [95% CI: 0.75–0.89].

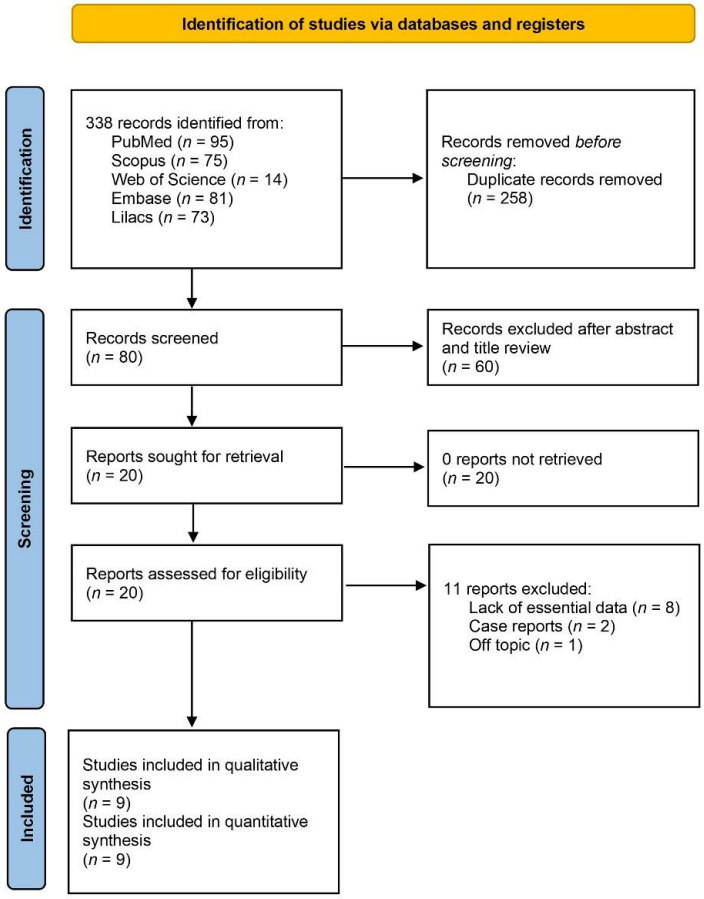


Figure 1. Prisma flow diagram of the searching processes.

3.2. Study Characteristics

Study characteristics in the qualitative synthetizes are shown in Tables 1 and 2. The studies reported a total of 274 patients with CHC. The nine reports included in the present review were conducted in different countries across three continents: Europe [7,20–24], Asia [6,25], and North America [26]. Whilst these reports were published between 1966 and 2021, most studies were case series or retrospective cohort studies, and the target population was always clinic-based.

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Table 1. Main clinicodemographic data of patients with CHC included in the study (F: female; M: male; *n*: number).

Author and Year	Country	Study Type	Patients				Follow-Up (Years)	Method of (Assessment)	Malignant (Development (%)
			n	Sex		Mean Age (Years)			
				F	M				
Cawson, 1966 [7]	UK	Case series	15	4	11	50.5	10	Biopsy (P.A.S)	13.3
Williamson, 1969 [20]	UK	Case series	2	0	2	59.5	-	Biopsy (P.A.S), culture	50
Eyre & Nally, 1971 [23]	UK	Case report	3	0	3	55.7	-	Scraping and culture	66.6
Bánóczy, 1977 [21]	Hungary	Cohort	70	-	-	-	9.8	Biopsy (P.A.S)	28.7
Silverman et al., 1984 [26]	USA	Cohort	80	-	-	54	7.2	Biopsy (P.A.S), culture	4.4
Abdulrahim et al., 2013 [22]	Ireland	Case-control	31	14	17	57.8	2	Biopsy (P.A.S)	6.5
Chiang et al., 2020 [25]	Taiwan	Cohort	22	-	-	-	5	Biopsy (P.A.S),	13.6
McParland & Warnakulasuriya, 2021 [24]	UK	Descriptive	3	-	-	-	16	Biopsy (P.A.S),	33.3
Zhang et al., 2021 [6]	China	Cohort	48	13	35	54.9	6	Biopsy (P.A.S),	4.2
Total			274	31	68	55.4	8	-	12.4

Table 2. Location of CHC lesions at the time of diagnosis (FOM: floor of the mouth; ED: epithelial dysplasia; CHC: chronic hyperplastic candidiasis; SCC: squamous cell carcinoma).

Author and Year	Location							Histopathology			
	FOM	Retromolar	Gingiva	Palate	Tongue	Buccal	Lip	SCC	High-ED	Low-ED	No ED
Cawson, 1966 [7]	0	0	0	4	4	6	1	-	-	-	-
Williamson, 1969 [20]	-	-	-	-	-	-	-	1	-	-	1
Eyre & Nally, 1971 [23]	-	-	-	-	-	2	3	2	-	-	-
Bánóczy, 1977 [21]	-	-	-	-	-	-	-	-	-	-	-
Silverman et al., 1984 [26]	-	-	-	-	-	-	-	2	-	-	-
Abdulrahim et al., 2013 [22]	0	0	2	3	4	22	-	2	8	18	3
Chiang et al., 2020 [25]	-	-	-	-	-	-		3	-	-	-
McParland & Warnakulasuriya, 2021 [24]	-	-	-	-	-	-	-	-	-	-	-
Zhang et al., 2021 [6]	-	-	-	-	-	-	-	0	1	9	38
Total	-	-	2	7	8	30	4	10	9	27	42

The main clinical and histopathological features of the 274 patients are displayed in Tables 1 and 2. A total of seven studies stated the follow-up period of patients [6,7,21,22,24–26]. This time, they ranged between 2 and 16 years [22,24], with a mean of 8 years. All the retrieved studies reported cases of CHC with MT; this rate fluctuated broadly between 4.2% and 66.6% [6,23]. Among all the patients from the included studies, a total of 34 (12.4% of patients) progressed with a MT during the follow-up period.

Regarding the sex of CHC-affected patients, 68.7% were males, whilst 31.3% were females. Most of the patients were quinquagenarians and sexagenarians, with a mean age of 55.4. Lamentably, none of the primary literature reported relevant cofounders that related to MT properly, such as tobacco, alcohol, or betel consumption. The most frequent locations of CHC were buccal mucosa (58.9%), the tongue (15.7%), palate (13.7%), lip (7.8%) and gingiva (3.9%) (Table 2). Regarding ED assessment, different grading systems were applied owing to the studies. In this vein, we divided ED into mild, moderate, and severe, following the WHO proposal [27]. Only two of the included studies reported an ED grade or its absence [6,22], as exhaustively detailed in Table 2.

CHC ascertainment varied across the studies, although the gold standard was the periodic acid–Schiff stain (PAS) [6,7,21,22,24,25]. Moreover, three studies performed a culture to isolate *Candida* clinical species in blood agar, potato dextrose, and Sabouraud dextrose agar [20,23,26]. A single study was based on scraping coupled to the culture and further species identification [23].

3.3. Quality Scores and Data Synthesis

In summary, not all studies used for pooled analysis were conducted with the same vigor. One (11.1%) was classified as low [6], five (55.6%) as moderate [7,21,22,24,26] and three (33.3%) as a high RoB [20,23,25]. The domains showing a higher RoB potential were the condition diagnosis/measure (i.e., domain eight) and data analysis conducted with insufficient coverage of the primary data (i.e., domain five). However, hitherto studies also displayed a sub-optimal quality in some of the remaining domains, as extensively displayed in Figure 2.

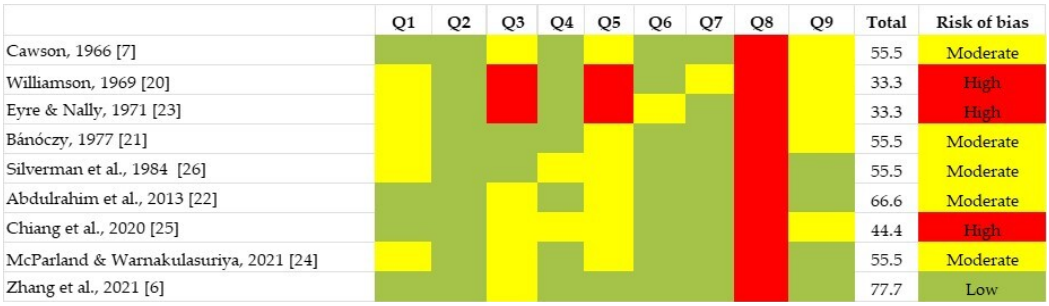


Figure 2. Quality plot graphically depicting the risk of bias among individual studies, assessed using Joanna Briggs Institute Critical Appraisal Checklist designed for systematic reviews addressing prevalence, cumulative incidence questions, and/or for proportion meta-analyses [6,7,20–26].

Overall, the cumulative pooled prevalence of MT in CHC was 12.1% (95% CI, 4.1–19.8%; I^2 = 78.27%, Q test *p* value = 0.002) (Figure 3A). A remarkable asymmetry could be seen in the forest plot, indicating a skewness to the right (Figure 3B). Egger's regression test confirmed the existence of a publication bias (pEgger = 0.001). We stratified the subgroup analysis by either Asian or non-Asian, and the heterogeneity subsided for Asian studies (I^2 = 31.03%), while the pooled estimate was also modified meaningfully [pooled proportion: 6.5% (95% CI, −1.5–14.5)]. On the other hand, in the non-Asian subgroup, heterogeneity was extremely large (I^2 = 82.77). Stratification by a RoB assessment provided

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further insight; high-quality studies showed a pooled proportion of 9.8% (95 % CI: 2.0–17.8), while low-quality studies harbored a higher, albeit non-significant, proportion [pooled proportion = 34.6% (95% CI: −0.2–71.5) (Table 3).

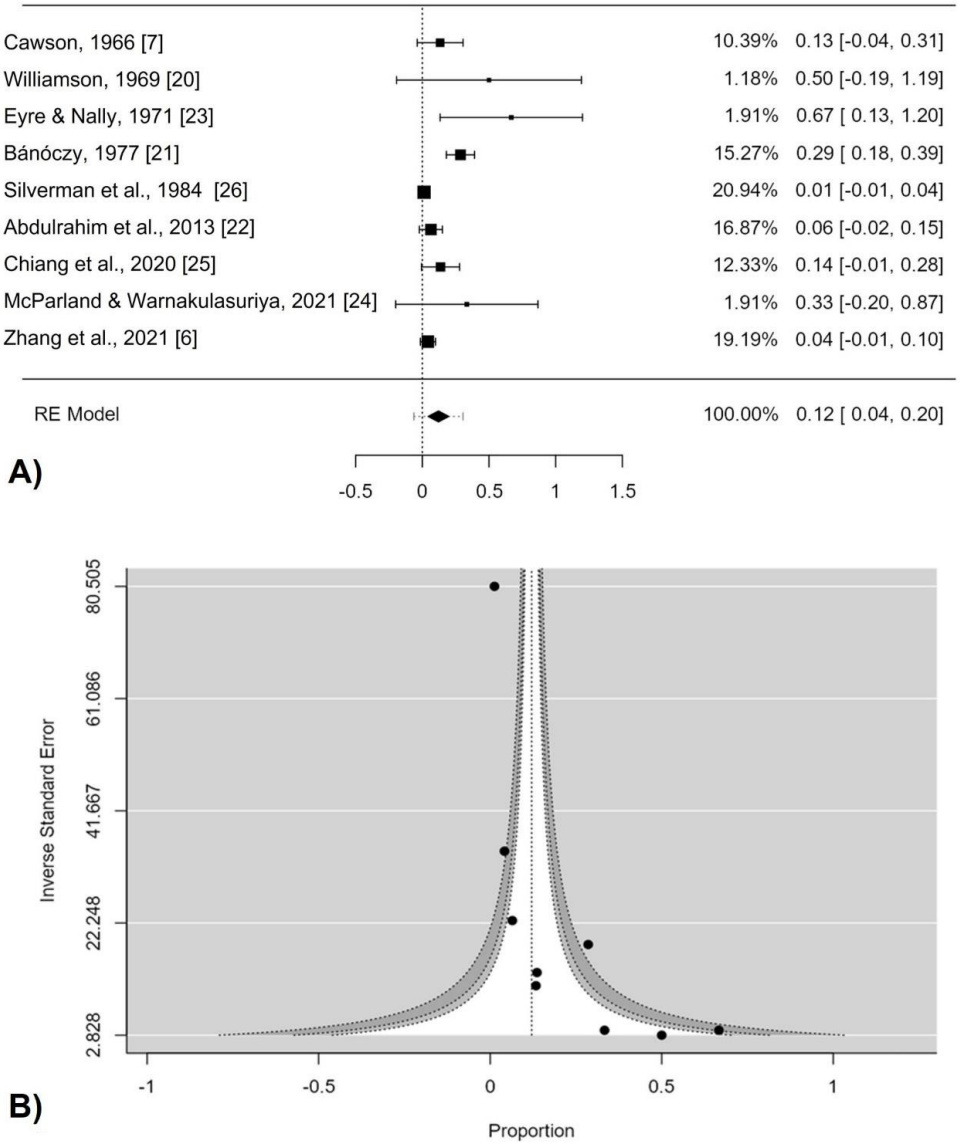


Figure 3. (**A**) Forest plot. Overall malignant transformation of chronic hyperplastic candidiasis. RE (random effects) and weight of each study; (**B**) Funnel plot assessing the publication bias [6,7,20–26].

In our sensitive analysis, the general results did not appreciably fluctuate after the sequential repetition of the pooled analysis with the omission of each individual study and this factor indicates that our estimations are not meaningfully influenced by a particular individual study. A single exception was noted as when Bánóczy et al. study [21] was omitted the pooled prevalence dropped to 6.6% (95% CI 1.1–12.1), which may explain part

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of the heterogeneity found (Table 4). The meta-regression, when executed, displayed a meaningful negative correlation between malignant transformation rates and the year of publication of the included reports (p-value = 0.001). A regression plot shows this feature in more detail [see Figure A1].

Table 3. Pooled prevalence, malignant transformation rate, and subgroup analysis of the initial histopathological diagnosis of CHC (CI: confidence intervals; CHC: chronic hyperplastic candidiasis; PP: pooled proportion).

	Sample Size (n)		Pooled Data		Heterogeneity	
	Studies	**Patients**	**PP (95% CI)**	**p-Value**	**p_{het}**	**I^2 (%)**
Malignant development						
	9	274	PP = 12.1% (4.3–19.8)	0.002	0.001	78.27
Subgroup analysis						
Low and moderate risk of bias	6	247	PP = 9.8% (2.0–17.8)	0.014	0.001	81.98
High risk of bias	3	27	PP = 34.6% (−0.2–71.5)	0.06	0.113	54.21
Asian	2	70	PP = 6.5% (−1.5–14.5)	0.111	0.229	31.03
Non-Asian	7	204	PP = 16.3% (3.9–28.9)	0.010	0.001	82.77

Table 4. Leave-one-out pooled prevalence analysis.

Studies	**Pooled Proportion**	**95% CI**
Overall	12.1	4.3–19.8
Omitting Cawson [7]	12.0	3.7–20.4
Omitting Williamson [20]	11.6	3.9–19.3
Omitting Eyre & Nally [23]	10.8	3.4–18.1
Omitting Bánóczy [21]	6.6	1.1–12.1
Omitting Silverman et al. [26]	15.6	5.9–25.3
Omitting Abdulrahim et al. [22]	13.9	4.6–23.1
Omitting Chiang et al. [25]	12.0	3.6–20.4
Omitting McParland & Warnakulasuriya [24]	11.7	3.9–19.5
Omitting Zhang et al. [6]	15.6	4.7–26.4

4. Discussion

Globally, the results of our systematic review displayed that one over ten patients diagnosed with chronic hyperplastic candidiasis will eventually undergo a malignant transformation. In this vein, the present study unraveled that CHC potential for malignant transformation is underestimated in the current scientific literature, with some authors considering CHC as a low-risk disorder [28,29]. Thus, it is key to perform an early diagnosis and control, as well as to monitor clinicopathological features that might be implicated in its malignancy [8].

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CHC is a prototypical oral lesion caused by chronic *Candida* infection [30]. We consider that this infection is undervalued as a risk factor for squamous cell carcinoma, essentially because it is not registered in most epidemiological studies addressing the MT rate of apparently related conditions, such as canonical OL [31]. In the multifactorial context of oral carcinogenesis, assigning a causal role to *Candida* infection would dismiss the risk of the interaction between this risk factor with other well-known ones such as tobacco or alcohol [32]. In addition, the complex differential diagnosis and variety of nomenclatures for CHC implemented in the scientific literature may be responsible for a misdiagnosis of this disorder and, consequently, a misestimation of its malignant potential. However, we found a noticeable difference between the CHC and OL MT rates [31]. According to previous studies based on pooled analysis, the MT rate of OL ranges from 9.5% to 9.8% [33,34], whilst here we reported a rate of 12.1%. CHC may then accomplish the classical definition of a precancerous condition as "a morphologically altered tissue in which cancer is more likely to occur than in apparently normal counterpart" [35]. However, *Candida* presence in OL lesions was considered as CHC in some of the retrieved scientific literature, and these conditions are considered histologically distinct, although they may frequently display a clinical overlap. This event entails a certain complexity for establishing a definite diagnostic threshold [4]. Overall, the present study demonstrates that our biologically plausible assumption seems to correlate epidemiologically by merging *Candida* infection as a remarkable risk factor for carcinogenesis. Indeed, nowadays, different theories exist on *Candida* infection mechanisms linked to oral carcinogenesis. *Candida* can produce nitrosamines such as N-nitroso-benzyl-methylamine and acetaldehyde, which are cancerogenic compounds [36,37]. Moreover, during infection, there is an increase in proinflammatory cytokines (interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-18, tumor necrosis factor (TNF)-α, IFN-γ, leading to a disbalance in immune surveillance mechanisms and changes in the tissue environment [38–40]. Candidalysin is a cytolytic toxin and was shown to induce NF-kB and MAPK pathways, which result most of the time as being dysregulated in cancer [41].

Sex and age distribution across our strata were similar to those figures reported in previous narrative reviews (i.e., predominantly men in their 50s and 60s) [42]. The buccal mucosa has been the most frequent location of CHC as classically reported, but other regions, such as the tongue or palate, may also be affected [1,3,4]. We could not report a preferential location in which squamous carcinoma may arise due to poor reporting information retrieved from the included studies. In terms of histopathology, and particularly regarding ED, only two studies reported its grade (Tables 1 and 2) [6,22]. None of these studies confirmed that the presence of ED represented a risk factor for MT. On the other hand, several authors consider that *Candida* infection correlates with more pronounced grades of ED and thus indirectly implies a higher risk of malignancy [43]. Odell et al. considered how in CHC histopathology, it is important to identify epithelial changes as reactive or pre-neoplastic. This group also pointed out that re-biopsy should be delayed by 6 weeks after antifungal treatment bearing in mind the turnover time of the oral mucosal epithelium [11]. Following systemic antifungal treatment, CHC resolution has been reported with a reduction in ED [44].

Some potential limitations to our pooled analysis should be highlighted. Meaningful statistical between-study heterogeneity was found as expected, and priori planned random-effects estimations were used to be more conservative. This situation is extremely frequent in prevalent meta-analyses [45]. In this vein, heterogeneity presence should be considered more as the rule rather than the exception [46]. Subgroup analyses also showed that non-Asian and poor-quality studies tended to overestimate the MT rate of CHC. Subgroup analysis, according to RoB assessment, showed that results lacked robustness and probably underpowered our data synthesis in a moderate manner. Furthermore, it should be emphasized that only data from 271 individuals were included in this meta-analysis. In this vein, no conclusive results can be derived from these analyses of the context characteristics of CHC cancerization to avoid generalizations.

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Secondly, publication bias (i.e., the tendency to publish only studies with positive results) may, in part, explain our results. Our methods lack statistical power due to the fact that the number of primary studies does not reach ten [47]. It is also worth mentioning that simulation analyses point out high type 1 error artifacts when assessing publication bias in prevalence meta-analyses and evaluating infrequent outcomes [48].

Thirdly, information bias should be considered. This concept refers to errors that are introduced during the exposure, event, or other co-variables assessment on each specific-study population [49]. The lack of consensus regarding CHC diagnostic criteria is likely to introduce measurement error and, therefore, misdiagnosis. In addition, in terms of the exposure to co-variables that may exert an effect on MT, heterogeneous reports were found regarding risk factors, including histopathology, which hindered more detailed analyses. This study may have a protopathic bias, which is detected when an exposure is influenced by early (subclinical) stages of a pathology [50]. Despite the explained mechanisms that could explain the carcinogenic potential of CHC, other explanations may be plausible such as the presence of a reverse causation. This is due to the lack of any threshold to stablish a differential diagnosis between CHC and a canonical OL without suffering from a fungal infection during its course. The lack of a clear differential diagnosis creates a two-way causal relationship implying a relevant feedback loop.

Finally, large heterogeneity can be caused by errors unrelated to the sample population. The range of sample types and techniques each has their limitations and may have contributed to this large heterogeneity. Each technique has a threshold of detection. Especially microscopy is more prone to overlook part of the sample as it is not entirely analyzed. It is well known that fungi can be well identified using microscopy without making a distinction on identity. Moreover, cultivation techniques may intentionally or unintentionally be targeted to a specific species, jeopardizing the possibility of identifying all fungi. To conclude, no medium contains enough nutrients to cultivate all fungi in a laboratory environment owing to the specific nutritional requirements and metabolic states of some species [51].

Despite the aforementioned limitations, the robust nature of the current pooled analysis is indicated by the forest plot, which demonstrates a consistent statistical malignant transformation rate for chronic hyperplastic candidiasis as its robustness is confirmed by sensitivity analysis.

5. Conclusions

The current available evidence on the malignant transformation rate of CHC remains limited. Nonetheless, the present systematic ascertained a pooled malignant transformation rate of 12.1% derived from a reduced number of longitudinal studies, and so avoiding the draw of broad inferences from these particular observations. CHC is broadly considered to be clinically important but a relatively neglected condition for the scientific community. Based on the current study's estimates of malignant development, CHC-affected patients should be placed on exhaustive surveillance programs. In the author's opinion, *Candida* infection should be seen as a relevant risk factor for OPMDs, particularly in OL. Nonetheless, it remains complex to affirm that CHC is an individual OPMD according to the evidence gathered in the present study. Prospective cohort studies to define the natural history of CHC and a consensus statement to clarify a proper set of diagnostic criteria are strongly needed.

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Author Contributions: Conceptualization, data curation, formal analysis, methodology, resources, software, validation, visualization, and writing-original draft: A.I.L.-P. Conceptualization, investigation, methodology, writing-original draft, writing-review, and editing: A.P.-J. Conceptualization, data curation, formal analysis, methodology, project administration, resources, supervision, validation, writing—review, and editing: V.C.A.C. Data curation, investigation, methodology, writing-original draft: F.S. and C.M.C.-P. Data curation, investigation, methodology, writing-original draft: Ó.Á.-C.-I. Data curation, investigation, methodology, supervision, writing—original draft: P.G.-V. Conceptualization, data curation, formal analysis, investigation, project administration, resources, supervision, validation, writing-review, and editing: L.L.M. and M.P.-S. All authors have read and agreed to the published version of the manuscript.

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Appendix A

Table A1. Database search strategy (June 2022).

Database	Search
PubMed	("Candidiasis, Chronic Mucocutaneous" [Mesh] or "Chronic Hyperplastic Candidiasis" [All Fields] or "candidal leukoplakia" [All Fields] or "Candida leukoplakia" [All Fields]) and (malign* or premalign* or "potentially malignant disorder" or "precancer" or "cancer" [All Fields] or "Carcinoma, Squamous Cell" [Mesh] or "squamous cell carcinoma" [All Fields] or "oscc" [All Fields] or "transformation" [All Fields] or "risk" [All Fields] or "progression" [All Fields]).
Scopus	**TITLE-ABS ("Chronic Hyperplastic Candidiasis" OR "Chronic Mucocutaneous Candidiasis" OR "candidal leukoplakia" OR "candida leukoplakia") AND TITLE-ABS ("malignant" OR "malignant" OR "evolution" OR "evolves" OR "evolve" OR "progress" OR "progresses" OR "progression" OR "transformation" OR "prognosis" OR "prognostic" OR "prognoses")**
Web of Science	TS = ("Chronic hyperplastic candidiasis" OR "CHC") **AND** TS = **("malignant" OR "malignant" OR "evolution" OR "evolves" OR "evolve" OR "progress" OR "progresses" OR "progression" OR "transformation" OR "prognosis" OR "prognostic" OR "prognoses") AND article type: articles**
LILACS	tw:("Chronic hyperplastic candidiasis" OR "candidiasis hiperplasica cronica" OR "candidiase hiperplasica") **AND tw:**("malignant" OR "malignent" OR "maligna" OR "malignas" OR "maligno" OR "malignos" OR "evolution" OR "evolução" OR "evolución" OR "progression" OR "progressão" OR "progresión" OR "avance" OR "avanço" OR "transformation" OR "transformação" OR "transformación" OR "prognosis" OR "prognostic" OR "prognoses" OR "prognóstico" OR "pronóstico" OR "prognósticos" OR "pronósticos")
EMBASE	('Chronic hyperplastic candidiasis') AND ('malignant' OR 'malignent' OR 'evolution' OR 'evolves' OR 'evolve' OR 'progress' OR 'progresses' OR 'progression' OR 'transformation' OR 'prognosis' OR 'prognostic' OR 'prognoses')

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Appendix B

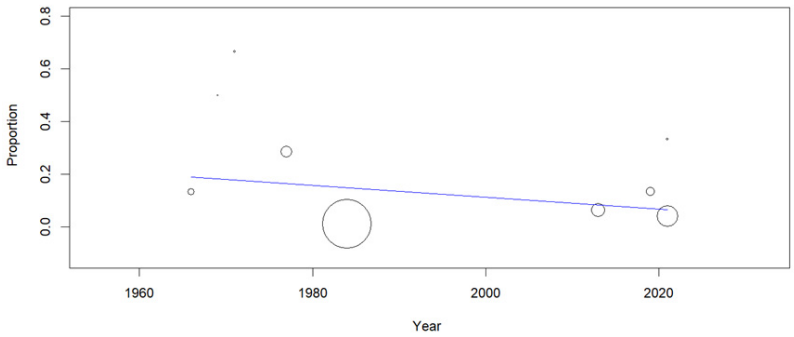


Figure A1. Bubble plot of meta-regression on the chronic hyperplastic candidiasis malignant transformation rate plotted against the year of study.

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ARTÍCULO 7.

Título: DNA Methylation by Bisulfite Next-Generation Sequencing for MLH1 and MGMT in Oral Squamous Cell Carcinomas and Potentially Malignant Disorders: An Integrative Analysis towards Field Cancerization

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DNA Methylation by Bisulfite Next-Generation Sequencing for MLH1 and MGMT in Oral Squamous Cell Carcinomas and Potentially Malignant Disorders: An Integrative Analysis towards Field Cancerization

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Abstract: *Background and Objectives*: MGMT methylation is a well-described biomarker in several solid tumors and MLH1 seems to occur in the initial stages of oral carcinogenesis. The aims of this study were to evaluate MHL1 and MGMT methylation levels in oral squamous cell carcinoma (OSCC) and oral potentially malignant disorders (OPMDs), and to integrate this information with The Cancer Genome Atlas (TCGA) database. *Materials and Methods*: To determine the percentage of gene methylation in MLH1 and MGMT, pyrosequencing analysis was conducted. Samples were divided as follows: (1) patients diagnosed with OSCC (N = 16); (2) patients with OPDM who developed OSCC in the same location (N = 47); and (3) patients with OPDM who developed OSCC in a different location (N = 22). As a validation cohort in this study, data from The Cancer Genomic Atlas (TCGA) database, particularly regarding Head and Neck Squamous Cell Carcinoma, was used. *Results*: Overall MLH1 methylation levels of 8.6 ± 11.5% and 8.1 ± 9.2% for MGMT were obtained. With regard to MHL1, the OSCC presented the highest degree of methylation with 9.3 ± 7.3% (95%CI 5.1–13.6), and with regards to MGMT, the simultaneous malignancy group presented the highest degree of methylation with 10 ± 13.5% (95%CI 6–10), although no significant differences were found between the groups (p = 0.934 and p = 0.515, respectively). The estimated survival was higher for MGMT methylated cases (19.1 months, 95%CI 19.1–19.1) than for unmethylated cases (9.4 months, 95%CI 6–12.8), but not statistically significant. *Conclusions*: Our results did not show a correlation between MGMT and MLH1 methylation and any clinicopathological feature or survival in our institutional cohort. MLH1 methylation was present mainly in OSCC, whilst MGMT in OPMD represented a modest contribution to field cancerization, with an overall consistency with the TCGA database.

Keywords: DNA methylation; oral squamous cell carcinoma; next-generation sequencing; potentially malignant disorders; MLH1; MGMT

1. Introduction

Oral squamous cell carcinoma (OSCC) represents 95% of all cancers that can be found within the oral cavity [1]. It is considered neoplasia with a poor survival rate of around 50–60% [2,3]. OSCC etiology is highly debatable, although a certain genetic disposition coupled with other risk factors such as smoking and alcohol consumption, viral infections,

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actinic radiation and the malignant transformation of oral potentially malignant disorders (OPMDs) such as leukoplakia, erythroplakia, lichen planus, actinic cheilitis andr oral submucous fibrosis, are considered its main cornerstones [4–8].

MMR (mismatch repair) is one of the main DNA repair systems that relates to the homologous MutLS bacterial system (human MutS and MutL proteins) [9]. MLH1 (mutL homolog 1) is a human gene that plays a key role in the DNA duplication error reparation process, and likewise, it also plays a pivotal role in preserving genomic stability. On the other hand, methylguanine-DNA methyltransferase (MGMT) is a specific DNA damage repair protein which plays a key role in maintaining normal cell physiology and genomic stability [10]. Methylation of this promoter is a key predictor of whether alkylating agents can effectively control tumor cell progression [11].

The accumulation of genetic alterations may trigger the development of potentially malignant lesions and subsequently, carcinomas [12]. On the one hand, genetic alterations are considered irreversible changes to the DNA sequence, which result in oncogene activation or the deactivation of the tumor suppressor genes [13]. On the other hand, epigenetic alterations result in reversible and heritable changes. Nonetheless, these do not generate any DNA sequence alteration [10]. DNA methylation is an epigenetic mechanism in which a methyl group is transferred to the cytosine C5 position to form 5-methylcytosine [9]. This phenomenon regulates gene expression by recruiting the proteins that participate in gene repression, or by inhibiting the union of DNA transcription factors. DNA methylation usually takes place in the cytosines that precede a guanine, or alternatively, it can take place in CpG sites. This epigenetic modification is essential to silence the retroviral element and regulate the gene expression for specific tissues, and it is also essential for genomic imprinting and X chromosome inactivation [11].

MHL1 is one of the DNA MMR genes commonly found in Lynch syndrome and associated with germline mutations. Patients with MLH1 gene mutations usually develop colorectal cancers at an earlier age [14]. Different studies have shown that in most cases, MLH1 methylation occurs in the initial stages of oral carcinogenesis, although it can also appear, to a lesser degree, at more advanced stages. Therefore, it is worth noting that MHL1 methylation is an early process that continues throughout the tumor progression [15,16]. On the other hand, it has been proven that maintaining normal MLH1 expression levels is beneficial for maintaining hPMS1 (human post-meiotic segregation 1) and hPMS2 (human post-meiotic segregation 2) levels; that is to say, in the absence of MLH1, these proteins are unstable. Furthermore, the possible influence of smoking on MLH1 methylation has been suggested and it is understood that this could be a reversible process [17]. MGMT methylation is a well-described biomarker in several solid tumors, although on a clinical basis it is simply used in glioblastoma. MGMT is considered a good prognosis biomarker given that its own expression can be silenced, which results in cell death, particularly inducing cancer cells apoptosis [18].

The aims of this study were twofold: (i) to evaluate MHL1 and MGMT methylation levels in OSCC and OPMD to ascertain their prognostic and clinicopathological significance in our institutional database and (ii) to integrate this information with The Cancer Genome Atlas (TCGA) database.

2. Materials and Methods

2.1. Patients and Samples

For this study, eighty-five samples were selected from patients diagnosed with OSCC. The samples were divided as follows: (1) patients diagnosed with OSCC (OSCC group comprised of sixteen samples), (2) patients with OPDM who developed OSCC in the same location (transformation group comprised of 47 samples) and (3) patients with OPDM who developed OSCC in a different location (simultaneous group comprised of 22 samples). The samples were obtained from the Department of Oral Medicine of the University of Santiago de Compostela.

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Patients included in this study were required to sign an informed consent form, and, subsequently, all of the samples were processed at the Pathological Anatomy Service of the University Hospital Complex of Santiago de Compostela (CHUS, by its acronym in Spanish). This project was approved by the Galician Clinical Research Ethics Committee (Register Code: 2019/271). For this study, the principles of the Declaration of Helsinki and its subsequent amendments were followed. The STROBE guidelines were used as the reference for data reporting [19].

2.2. Study of Methylation by MLH1 and MGMT Pyrosequencing

In order to determine the percentage of methylation of the samples studied in the MLH1 and MGMT genes, pyrosequencing analysis was conducted. Samples were initially embedded in paraffin. The total DNA was extracted and treated with bisulphite, using the Epitect Fast DNA Bisulfite Kit (Qiagen®, Hilden, Germany), and following the protocol recommended by the manufacturer: one 5 µm cut of paraffin for MHL1 and two 5 µm cuts for MGMT. The concentration of the samples was measured using the NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA), and all of the samples had 260/280 values of between 1.7–2. Once transformed, the DNA was extracted, and the Therascreen MGMT Pyro kit (Qiagen®) was then used to perform PCR and pyrosequencing for methylated MGMT detection. With regards to MLH1, the PCR was performed using the Pyromark PCR kit 200 (Qiagen®) with a concentration of no greater than 100 ng/microliter. The pyrosequencing was conducted using Pyromark Q24 CPG MLH1 (4 × 24) (Qiagen®). All PCRs were performed in the Agilent Technologies Surecycler 8800, and the pyrosequencing was performed by the Pyromark Q24 and Pyromark Q24 workstation (Qiagen®), using the Pyromark Q24 2.0.7 to compute and analyze the results.

2.3. Methylation Quantification

For each sample, the average of the four and five CpG islands were calculated respectively, and the result was considered positive (meaning methylated) if the value was between 11 and 15% (as indicated by Qiagen®). In any cases in which the result was uncertain, the corresponding CpG island and Pyrogram were checked separately.

2.4. Variables and Collected Data

The qualitative variables determined were as follows: study group (OSCC, transformation and simultaneous), gender (male or female), area (gum, tongue, buccal mucosa, hard palate, floor of the mouth, retromolar trigone, labial mucosa, soft palate or tonsil), initial disorder and subsequent progression (leukoplakia without dysplasia, low degree leukoplakia, high degree leukoplakia, oral lichen planus, papilloma, verrucous carcinoma, non-specific ulcer or OSCC), exitus and qualitative MLH1 and MGMT methylation (yes > 11%, no < 11% or poorly 11–15%).

2.5. TCGA Cohort Sample Selection and Data Downloading

As a validation cohort in this study, data from The Cancer Genomic Atlas (TCGA) database, particularly regarding Head and Neck Squamous Cell Carcinoma (HNCS) was used [20]. The query was restricted to primary OSCCs. Data of the samples were retrieved from GDAC Broad Institute (https://gdac.broadinstitute.org/, accessed on 15 March 2022). After this collection, data was catalogued and manually checked based on the primary site of tumour onset in order to exclude non-OSCC patients, as aforementioned. After the filtration, 342 cases were left and downloaded directly from the server and entered into the cBioPortal for Cancer Genomics (http://www.cbioportal.org, accessed on 17 March 2022). Data for TCGA methylation derived from the Human Methylation-450 Bead Chip assay of MGMT and MLH1 was then gathered. As described elsewhere, for genes with multiple CpG-Islands, as in the case of MGMT, only methylation data from the probe with the strongest negative correlation between the methylation signal and the gene expression were chosen for further analysis. In order to identify the dichotomized methylation status

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(i.e., methylated or not) for the TCGA cohort, k-means clustering was performed, using beta values for selected CpG sites as initially reported by the TCGA program [20].

2.6. Statistical Analysis

The categorical variables were reported as frequencies, while the percentages and quantitative variables were displayed using means and standard deviation. In order to study the relationship between the categorical variables, the chi-square test was used and contingency tables were built. Similarly, to describe the differences in the means, the Student's T-test or one-way ANOVA analysis was used, depending on the data distributions. Survival plots were created based on Kaplan–Meier curves and their related log ranks. SPSS v.24.0 (IBM, Statistics, NY, USA) software for Windows was used to perform this analysis.

All the raw data obtained from the TCGA database was entered in R software v.4.1.0 and its JMV package to perform statistical analyses of correlation with overall survival (in months) for both MGMT and MLH1 methylation among the cohort. Also, correlation analysis was performed to check the mutual exclusivity of the methylation of both genes. For the sake of exhaustivity, both types of correlation estimates were presented (i.e., Spearman and Pearson correlation test), although the most appropriate figure was considered Spearman's coefficient based on database constructors. Multiple linear regression models were conducted to more formally assess the results. Lastly, bearing in mind the post hoc analysis of our institutional cohort, we opted to perform a bioinformatic analysis to ascertain the pathways of the neoplasm involved in the methylation of MGMT and MLH1. Figure plotting was performed using the in-app service implemented in cBioPortal. The level of significance established for the results was $p \leq 0.05$.

3. Results

3.1. Sample Description

The sample was comprised of 16 (18.8%) OSCCs, 47 (55.3%) previous OPDMs transformed lesions and 22 (25.9%) lesions in patients with simultaneous neoplasia with OPDMs in another location in the oral topography. In addition, 48 (56.5%) of the samples were from women and the tongue was the most common location (31.8%), followed by the buccal mucosa (28.2%) and the gingiva (15.3%). With regards to age, the average age was 70 $\pm$ 10.8 years old. The full descriptive data is shown in Table 1.

3.2. Methylation State

Overall methylation levels of 8.6 $\pm$ 11.5% for MHL1 and 8.1 $\pm$ 9.2% for MGMT were obtained. If assessed in a dichotomous way, 25.3% of the samples were methylated for MHL1 (1.2% poorly methylated) and 26.7% were methylated for MGMT (2.4% poorly methylated). Table 2 shows the methylation levels in the different study groups. With regards to MHL1, the OSCC presented the highest degree of methylation with 9.3 $\pm$ 7.3% (95%CI 5.1–13.6), and with regards to MGMT, the simultaneous malignancy group presented the highest degree of methylation with 10 $\pm$ 13.5% (95%CI 6–10). No significant differences were found between the groups (p = 0.934 and p = 0.515, respectively) (Table 3). No differences were found in terms of gender (p = 0.120 for MLH1 and p = 0.444 for MGMT), area (p = 0.780 for MLH1 and p = 0.506 for MGMT), recurrence (p = 0.358 for MLH1 and p = 0.561 for MGMT) or survival in the follow-up period (p = 0.680 for MLH1 and p = 0.413 for MGMT).

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Table 1. Characteristics of the institutional cohort and levels of methylation.

Qualitative Variables		N	%
Study group	OSCC	16	18.8
	Transformation	47	55.3
	Simultaneous	22	25.9
Gender	Woman	48	56.5
	Man	37	43.5
Location	Gingiva	13	15.3
	Tongue	27	31.8
	Buccal mucosa	24	28.2
	Hard palate	2	2.4
	Floor mouth	2	2.4
	Retromolar trigone	8	9.4
	Lip	3	3.5
	Soft palate	3	3,5
	Tonsil	3	3.5
Exitus	No	68	80.0
	Yes	17	20.0
Relapse	No	54	63.5
	yes	31	36.5
Treatment	Only surgery	56	65.9
	Radiotherapy	22	25.9
	Radiotherapy and chemotherapy	7	8.2

Quantitative variables	N	Minimum	Maximum	Average	SD
Percentage MLH1	75	0.4	63.8	8.6	11.5
Percentage MGMT	82	1.0	41.0	8.0	9.2
Time until malignancy	58	0.4	116.6	33.4	39.4

Table 2. Methylation levels stratified according to subgroups of our institutional database.

Lesion		Study Group	MLH1 Methylation		*p*-Value	MGMT Methylation		*p*-Value
			No	Yes		No	Yes	
Initial lesion	Leukoplakia without dysplasia	Transformation	10 (55.6%)	3 (75.0%)	0.474	11 (64.7%)	3 (60.0%)	0.848
		Simultaneous	8 (44.4%)	1 (25.0%)		6 (35.3%)	2 (40.0%)	
	Oral lichen planus	Transformation	2 (40.0%)	0 (0.0%)	0.439	2 (50.0%)	0 (0.0%)	0.221
		Simultaneous	3 (60.0%)	1 (100.0%)		2 (50.0%)	2 (100.0%)	
	Papilloma	Transformation	2 (100.0%)			2 (100.0%)		
		Simultaneous						
	Verrucous carcinoma	Transformation		1 (100.0%)		1 (100.0%)		
		Simultaneous						
	Erythroplasia	Transformation				1 (100.0%)		
		Simultaneous						
	Nonspecific ulcer	Transformation	2 (100.0%)	2 (100.0%)		2 (100.0%)	2 (100.0%)	
		Simultaneous						
Final lesion	Leukoplakia without dysplasia	Transformation	2 (50.0%)	1 (100.0%)	0.361	3 (60.0%)	1 (100.0%)	0.439
		Simultaneous	2 (50.0%)	0 (0.0%)		2 (40.0%)	0 (0.0%)	
	Leukoplakia low grade	Transformation	6 (85.7%)	1 (50.0%)	0.284	6 (60.0%)	1 (100.0%)	0.428
		Simultaneous	1 (14.3%)	1 (50.0%)		4 (40.0%)	0 (0.0%)	
	Leukoplakia high grade	Transformation	2 (100.0%)	1 (100.0%)		5 (100.00%)		
		Simultaneous						
	Oral lichen planus	Transformation	1 (33.3%)	0 (0.0%)	0.505	1 (50.0%)		
		Simultaneous	2 (66.7%)	1 (100.0%)		1 (50.0%)		
	Papilloma	Transformation	2 (100.0%)			1 (100.0%)	1 (100.0%)	
		Simultaneous						
	Verrucous carcinoma	Transformation	3 (100.0%)			3 (100.0%)		
		Simultaneous						
	Nonspecific ulcer	Transformation	2 (100.0%)	1 (100.0%)		2 (100.0%)	2 (100.0%)	
		Simultaneous						
	OSCC	OSCC	8 (25.0%)	6 (42.9%)		12 (33.3%)	4 (30.8%)	0.825
		Transformation	13 (40.6%)	6 (42.9%)		16 (44.4%)	5 (38.5%)	
		Simultaneous	11 (34.4%)	2 (14.3%)		8 (22.2%)	4 (30.8%)	

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Table 3. Clinicopathological features relationship with MLH1 and MGMT methylation.

Methylation			Average	Standard Deviation	Confidential Interval 95%		*p*-Value
					Lower Limit	Upper Limit	
MLH1 methylation	Study group	OSCC	9.3	7.3	5.1	13.6	0.934
		Transformation	8.7	13.8	4.3	13.0	
		Simultaneous	7.9	8.8	3.8	11.9	
	Sex	Woman	6.8	9.3	3.9	9.7	0.120
		Man	11.0	13.8	5.9	16.1	
	Location	Gingiva	10.6	18.0	−1.5	22.7	0.780
		Tongue	11.5	13.4	5.9	17.0	
		Buccal mucosa	6.9	8.2	3.0	10.7	
		Hard palate	2.4	–	–	–	
		Floor mouth	3.2	0	3.2	3.2	
		Retromolar trigone	4.8	3.9	1.2	8.4	
		Lip	3.5	1.3	0.3	6.7	
		Soft palate	11.3	10.0	−13.6	36.3	
		Tonsil	4.8	4.7	−6.8	16.4	
MGMT methylation	Study group	OSCC	6.6	6.4	3.2	10.0	0.515
		Transformation	7.7	7.7	5.4	10.0	
		Simultaneous	10.0	13.5	3.5	16.5	
	Sex	Woman	7.3	9.1	4.6	10.0	0.444
		Man	8.9	9.4	5.7	12.1	
	Location	Gum	9.4	11.4	2.1	16.6	0.506
		Tongue	5.8	5.9	3.5	8.2	
		Buccal mucosa	10.9	12.0	5.6	16.2	
		Hard palate	8.5	6.4	-48.7	65.7	
		Floor mouth	2.1	0.5	−2.6	6.9	
		Retromolar trigone	5.1	5.1	0.8	9.4	
		Lip	4.3	1.9	−0.3	8.9	
		Soft palate	12	10.3	−13.5	37.5	
		Tonsil	12.8	13.9	−21.9	47.4	

3.3. Follow-Up

The mean follow-up time was 60.5 ± 43.7 months, with a timelapse until malignant transformation of 33.4 ± 39.6 months, in which time 17 patients (20%) passed away, and 31 (36.5%) experienced recurrence. In terms of overall survival, it was 9.2 ± 6.5 months, whilst disease-free survival was 29 ± 26.7 months. The estimated survival in accordance with the Kaplan–Meier curves was higher for MGMT methylated cases (19.1 months, 95%CI 19.1–19.1) than for unmethylated cases (9.4 months, 95%CI 6–12.8) (Table 4). However, no statistically significant differences were observed (log rank, p = 0.134). With regards to the estimated time until recurrence, this was longer in MHL1 methylated cases (42.4 months, CI 95% 9.9–74.9) than in unmethylated cases (19.7 months, 95%CI 12–27.3), and no significant differences were found (log rank, p = 0.137).

Table 4. Association of MLH1 and MGMT methylation with survival and recurrences among the cohort.

Kaplan Meier			Months (Mean)	Confidential Interval 95%		Log Rank Test *p* Value
				Lower Limit	Upper Limit	
Survival	MLH1 methylation	No	9.1	5.2	13.1	0.827
		Yes	9.3	1.3	17.3	
	MGMT methylation	No	9.4	6.0	12.8	0.134
		Yes	19.1	19.1	19.1	

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Table 4. *Cont.*

Kaplan Meier			Months (Mean)	Confidential Interval 95%		Log Rank Test *p* Value
				Lower Limit	Upper Limit	
Recurrence	MLH1 methylation	No	19.7	12.0	27.3	0.137
		Yes	42.4	9.9	74.9	
	MGMT methylation	No	30.9	19.2	42.6	0.267
		Yes	18.2	9.4	26.9	

3.4. Validation in External Dataset

Although it was not the intention, restrictions in order to exclusively include OSCC cases were discarded due to the poor levels of methylation in targeted genes within the cohort. In this vein, all head and neck squamous cell carcinomas (HNSC) that provided data on the matter under study were included (n = 504). The k- means clustering analysis clearly separated four groups of samples. According to the analysis of available TCGA data, 495 tumour samples were hypomethylated, while nine tumour samples were hypermethylated for MGMT. In the case of MLH1, the figures were quite similar with only nine cases considered as hypermethylated according to the Methylation-450 Bead Chip assay. Assuming that the correlations of MGMT and MLH1 methylation are A and B, respectively, we performed a pooled analysis of Log2 Odds Ratio to study mutual exclusivity. Log2 Odds Ratio was <−3 showing a q-value of 0.849 derived from the Benjamin–Hochberg false discovery ratio correction procedure (i.e., no patient shows a double methylation for both genes). This asseveration is represented by the non-significant levels of correlation and regression analysis as displayed in Figure 1A.

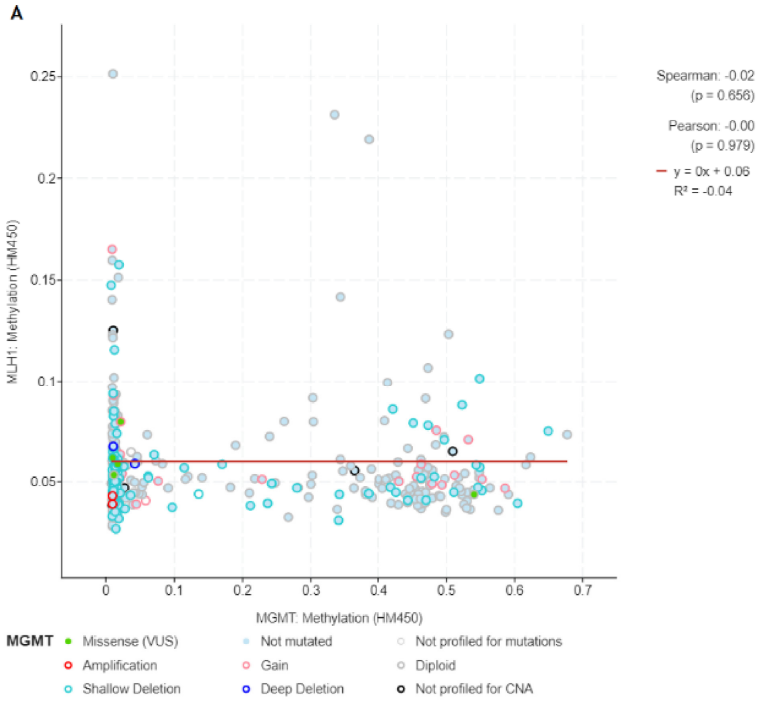


Figure 1. *Cont.*

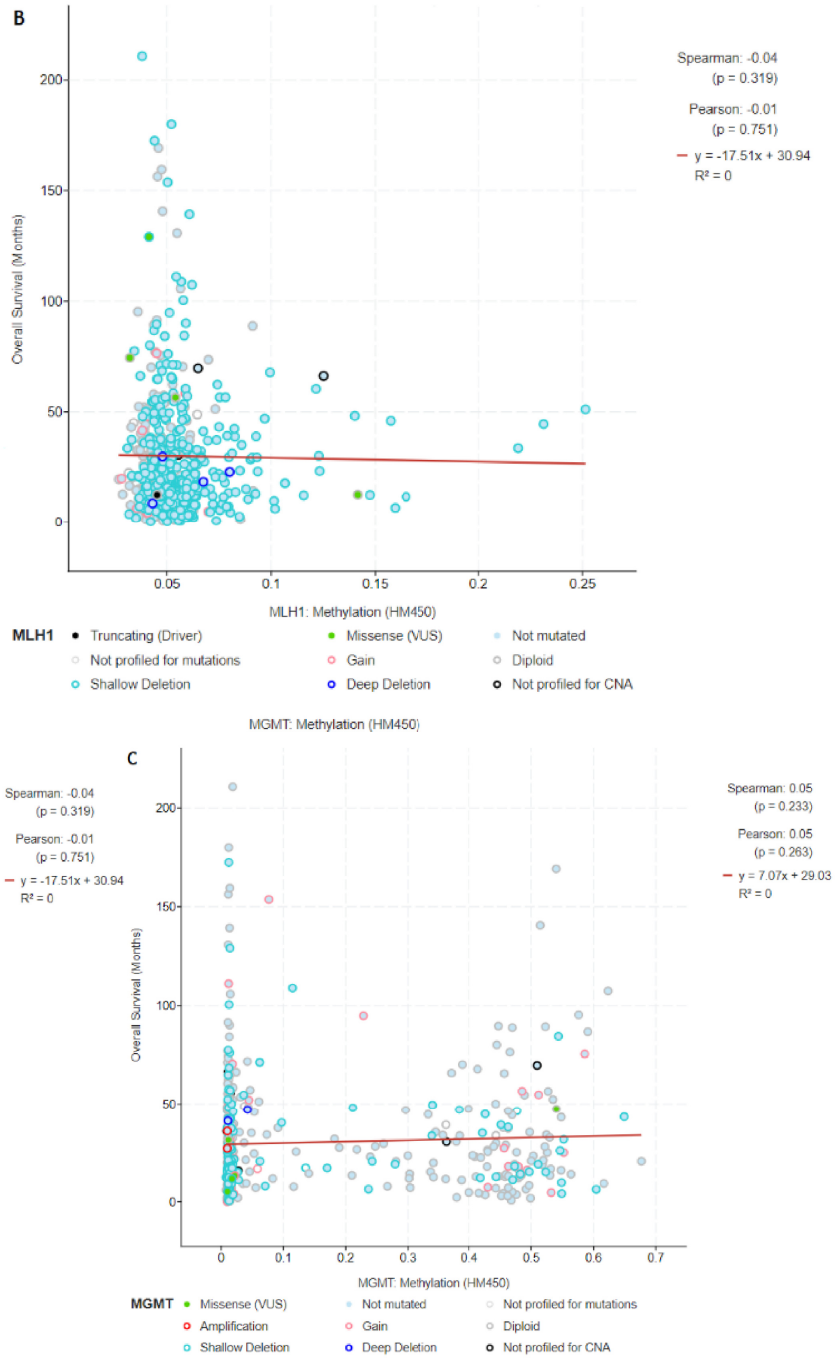


Figure 1. Integrative analysis with the TCGA database. (**A**) Correlation and linear regression model of MGMT and MLH1 methylation. Correlation and linear of MLH1 (**B**) and MGMT methylation (**C**) with overall survival in months.

Owing to the poor levels of hypermethylation, the intention to perform a Kaplan–Meier analysis was discarded and a correlation analysis of MGMT and MLH-1 versus overall survival analysis (in months) was computerised. The analysis is displayed in Figure 1C in the case of MGMT and Figure 1B for MLH1. MGMT hypermethylation was not correlated with overall survival (Spearman's correlation coefficient = −0.05, $p = 0.233$). Similarly, hypermethylation of MLH-1 was also not correlated with overall survival (Spearman's correlation coefficient = −0.04, $p = 0.319$). Figure 1 displays the mathematical analysis by means of regression models.

Lastly, taking the methylation of both genes together we indagated the pathways involved in carcinogenesis in the portion of the TCGA database under study. The most affected pathway seemed to be WNT. These genes can act upon WNT ligands, affecting subsystems such as those involving genes which play pivotal roles in the WNT dual receptor complex, SFRP, DKK and AXIN. At the same time, this orchestrated state can induce cell proliferation through a molecular machinery involving the Groucho/TLE/Grg family of transcriptional co-repressors and TCF/LEF Transcription Factors (Figure 2).

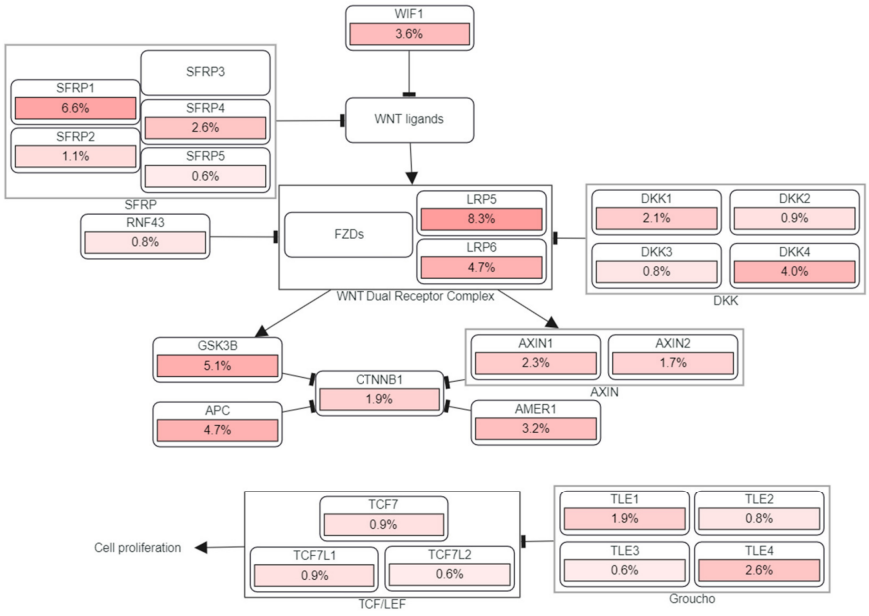


Figure 2. Pathways involved in head and neck carcinogenesis related to MLH1 and MGMT methylation according to the TCGA database.

4. Discussion

Despite epigenetic alterations found in OSCC and OPMD methylation levels and their correlations with clinical outcomes and survival in the present study, our results are inconsistent with the previous literature [21]. These differences reflect the heterogeneity of these diseases in their histology and clinical behavior, with different aetiologias and risk factors, and known tissue and tumor-type specificity of methylation patterns [22]. The prevalence of MGMT and MLH1 methylation detected in this study are also consistent with previously summarized data for HNSC in the TCGA database [23,24].

The determination of MGMT promoter methylation is of great interest to clinicians and patients, given that in recent in vitro studies with cell lines, an association between MGMT promoter methylation and OSCC diagnosis has been observed compared to normal cell lines. Furthermore, this repression was reversed following treatment with a demethylating agent (5-Aza-2′-deoxycytidine) [25], which was corroborated by an increase in gene

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expression [23]. However, the methylation percentage obtained in our samples was not significant for any clinicopathological or survival feature.

The percentage of methylated OSCC cases with MGMT was 9.2%, which was less than the percentage obtained in other studies with a similar sample size. In those studies, a methylation percentage of 27% was obtained, and in their control group, a methylation percentage of 7% was obtained [26]. This team has also observed that combined methylation of p16 and MGMT promoters was only found in the OSCC group. In this study, combined methylation between MGMT and MHL1 genes was analyzed and no association with specific clinical parameters, such as survival or recurrence, was found. Recent meta-analyses have also suggested that MGMT methylation may be related to OSCCs which metastasise; however, these results did not reveal a worse prognosis in methylated samples [27]. A study conducted by Taioli et al. observed the association between MGMT methylation and a poorer prognosis for OSCC [28]. However, our analyses found a higher survival rate in MGMT-methylated cases (19.1 months, 95%CI 19.1–19.1) than in unmethylated cases (9.4 months, 95%CI 6–12.8) without any statistically significant difference. On the other hand, in the present study, MGMT methylation was more marked in the simultaneous malignization group, obtaining $10 \pm 13.5\%$. Based on the field cancerization theory, the DNA methylation results may reflect that the entire epithelial histology adjacent to tumors and premalignant lesions have prominent levels of methylation of this gene, suggesting that MGMT methylation is an early event in oral carcinogenesis [10]. Nonetheless, the methylation of other genes in mouth neoplasm such as APC, DAPK, ECAD, RASSF1, TIMP3 and p16, have retrieved more promising diagnostic and prognostic values [21].

Fewer studies have considered the methylation of the MLH1 gene in oral cancer compared with the MGMT [29,30]. A study found that 100% of mouth neoplasm is affected by this gene methylation, which suggests that this occurs in patients with an advanced stage of the disease [16]. This does not coincide at all with the results obtained in this study, nor with those obtained by Ramirez et al., who suggested that it is an early marker for malignancy in oral lesions [15]. However, according to our results, both OPDMs groups in our institutional cohort did not verify these results, nor did our integration with the TCGA database.

The strength of our study is the well-characterized population of the groups OPMD and OSCC, which has detailed baseline epidemiological data on risk factors and also a comprehensive follow-up. An obvious limitation of the present study is its cross-sectional design and the small sample size. On the other hand, one of the main aims of this study was to assess early methylation in both genes in oral lesions prior to malignancy and particularly its implication in field cancerization. However, methylation in the OPDM groups was not significant, nor was it associated with the correspondent OSCC samples with methylation patterns maintained over time. In this sense, the present results preclude the generalization of results.

5. Conclusions

In conclusion, our results did not show a correlation between MGMT and MLH1 gene methylation and any clinicopathological feature or survival in our institutional cohort. Moreover, our results showed that MLH1 methylation was present mainly in OSCC, whilst MGMT in OPMD represented a modest contribution to field cancerization. There was an overall consistency with the TCGA database, although it should be borne in mind that individually the methylation of these genes has not proven clinical significance. Based on the results of this study, we encourage the development of further studies analyzing the methylation of more genes prospectively in bigger cohorts to unravel a panel able to reach clinical translation.

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Data Availability Statement: Not applicable.

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ARTÍCULO 8.

Título: Predictive value of CDKN2A/p16^{INK4a} expression in the malignant transformation of oral potentially malignant disorders: Systematic review and meta-analysis

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Predictive value of CDKN2A/p16^{INK4a} expression in the malignant transformation of oral potentially malignant disorders: Systematic review and meta-analysis

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ABSTRACT

Background: Management of oral potentially malignant disorders (OPMDs) is still challenging. Despite the diagnostic ascertainment by bioptic examination, this method is poorly informative of the prognosis and subsequent malignant transformation. Prognosis is based on histological findings by grading of dysplasia. Immunohistochemical expression of p16^{INK4a} has been investigated in different studies, with controversial results. In this scenario, we systematically revised the current evidence about p16^{INK4a} immunohistochemical expression and the risk of malignization of OPMDs.

Material and methods: After a proper set of keywords combination, 5 databases were accessed and screened to select eligible studies. The protocol was previously registered on PROSPERO (Protocol ID: CRD42022355931). Data were obtained directly from the primary studies as a measure to determine the relationship between CDKN2A/P16^{INK4a} expression and the malignant transformation of OPMDs. Heterogeneity and publication bias were investigated by different tools, such as Cochran's Q test, Galbraith plot and Egger and Begg Mazumdar's rank tests.

Results: Meta-analysis revealed a twofold increased risk to malignant development (RR = 2.01, 95% CI = 1.36–2.96 - $I^2 = 0\%$). Subgroup analysis did not highlight any relevant heterogeneity. Galbraith plot showed that no individual study could be considered as an important outlier.

Conclusion: Pooled analysis showed that p16^{INK4a} assessment may arise adjunct tool to dysplasia grading, leading to an optimized determination of the potential progression to cancer of OPMDs. The p16^{INK4a} overexpression analysis by immunohistochemistry techniques has a multitude of virtues that may facilitate its incorporation in the day-to-day prognostic study of OPMDs.

1. Introduction

Oral squamous cell carcinoma (OSCC) remains as the most common and deadly neoplasia within head and neck region [1]. It affects more than 750,000 people worldwide per annum and, despite the efforts in terms of research as advances in therapies, during the last three decades the specific survival has marginally improved [2]. OSCC onset is often preceded by a group of pathologies, namely oral potentially malignant disorders (OPMDs) [3]. This group is made up of a plethora of pathologies, oral leukoplakia and lichen planus are the most ubiquitous conditions globally [4]. Worldwide prevalence of this group of disorders has been reported ranging from 2.6% to 4.1% with remarkable geographic variations [5]. Several reports have estimated a median malignant transformation rate from 7.9% to 12.1% with a great variability among

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OPMDs subtypes [6,7]. Clinically, management of patients diagnosed of OPMD is difficult since clinicians are still lacking means to accurately predict this risk of transformation.

Nowadays, OPMDs oncogenesis risk is mainly assessed by the keen evaluation of the presence and grading of oral epithelial dysplasia in a bioptic sample [8]. However, this intervention may present some drawbacks. Some studies highlighted inter- and intra-examiner variability [9,10]; moreover, it is well known that not all dysplasias will become malignancies, and on the other hand non-dysplastic lesions may eventually progress to carcinomas [11]. Due to advances in molecular biology, there is burgeoning interest in defining and characterizing the molecular factors that drive the malignant transition of these disorders [12]. This scenario calls for new biomarkers, aiming to guide therapies from a biological and molecular standpoint. In addition, these approaches may be able to predict from which tissue may arise a tumor [13].

Consistent with the multistep theory of field cancerization, the natural history of OSCC appears to evolve gradually through transitional precursors leading a progressive switch to carcinogenic phenotypes [14,15]. This process is due to the accumulation of genetic and epigenetic alterations [15–17]; indeed, DNA ploidy, and dysregulation in proteins, such as p53, podoplanin were found in patients with OPMDs, but with inconsistent patterns and heterogeneity, leading to limited clinical applications [12].

Cell cycle is the biological phenomena in which a cell replicates its contents and results in two twin cells from a genetic perspective. This molecular machinery in eukaryote cells is basically controlled by three groups of interferers: cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors [18]. In molecular oncology, these entities are classified as tumor suppression genes owed to their ability to knockdown cell proliferation. There are mainly two CDK inhibitors families, namely the CIP/KIP family and the INK4 family [19]. $P16^{INK4a}$ is a part of the INK4 family and exerts a pivotal role as a negative regulator of cell cycle progression by regulating the functions of the protein retinoblastoma. $P16^{INK4a}$ also down regulates the formation of cyclin D/CDK4,6 complexes by CDK4 and CDK6 interactions, which in turn controls pRb phosphorylation. Inactivation of $P16^{INK4a}$ has been classically described as an early event in OSCC [8,20]. Some authors have hypothesized that $p16^{INK4a}$ assessment may turn to be helpful as a predictive marker to evaluate the malignant potential of OPMDs [12]. However, many studies have suffered from significant drawbacks such as cross-sectional design or small sample sizes. In this sense, the ability of CDKN2A/-$p16^{INK4a}$ alterations in predicting malignant progression of OPMDs need to be clearly validated. Therefore, the objective of this systematic review was to evaluate published evidence on the $p16^{INK4a}$ expression as a predictive biomarker for the malignant transformation of OPMDs.

2. Material and methods

2.1. Data sources and search strategy

This systematic review and meta-analysis were performed following the PRISMA-P guidelines [21], as was registered in the PROSPERO database (ID: CRD42022355931). Two of the authors (AILP and MPS) independently performed searches on PubMed via Medline, Scopus, EMBASE, Web of Science and LILACS since their inception until December 2022 without language restriction.

A combination of thesaurus terms specific to each database and free text words were pooled through Boolean operators to build our search strategy. This next syntax was applied in PubMed: ("Tumor Suppressor Protein p16"[MeSH Terms] OR " Genes, p16"[MeSH Terms] OR "p16"[All Fields] OR "CDKN2A"[All Fields]) AND ("mouth"[MeSH Terms] OR "mouth"[All Fields] OR "oral"[All Fields] OR oropharyn*[All Fields]) AND ("carcinoma, squamous cell"[MeSH Terms] OR ("carcinoma"[All Fields] AND "squamous"[All Fields] AND "cell"[All Fields]) OR "squamous cell carcinoma"[All Fields] OR “dysplasia"[All Fields] OR "potentially malignant disorders"[All Fields] OR premalign* [All Fields] OR precancer* [All Fields] OR “leukoplakia"[All Fields] OR “erythroplakia"[All Fields] OR “lichen planus"[All Fields] OR “submucous fibrosis"[All Fields]) AND (“transformation"[All Fields] OR “progression"[All Fields] OR “risk"[All Fields]). Syntax was conveniently modified and optimized for each database (Appendix S1). To maximize sensitivity, references from related reviews and those of included studies were comprehensively checked to supplement manually database search.

The questions addressed was: What is the significance of CDKN2A/$p16^{INK4a}$ expression in the prediction of the malignant transformation risk of patients with oral potentially malignant disorders?

The question was addressed using the PICO methodology:

i. P (population): Patients diagnosed with oral potential malignant disorders (with or without epithelial dysplasia).
ii. I (intervention): CDKN2A/$p16^{INK4a}$ assessment using objective and quantitative methods, in biopsy specimens from the study population.
iii. C (comparison): Control group will be represented by the group of patients from the study population with low CDKN2A/$p16^{INK4a}$ expression.
iv. Differences in the expression of CDKN2A/$p16^{INK4a}$ will be categorized as overexpression for the exposition group, and low expression for the control group, based on the cut-off value chosen by the authors.
v. O (outcome): progression to OSCC.

2.2. Eligibility criteria

To be eligible for inclusions, studies had to: (i) evaluate CDKN2A/$P16^{INK4a}$ alterations in protein expression, assessed by immunohistochemistry in biopsies of OPMDs, (ii) and to correlate with their malignant transformation, including data on malignant development in studies with a longitudinal design, and (iii) providing Hazard ratio (ORs) or Risks Ratio (RRs) as their corresponding 95% confidence intervals (CIs) as a measurement of association or at least sufficient data for their calculation. In the case of results derived from an overlapping study population, the report providing more data was prioritized.

Exclusion criteria were: (i) studies including heterogeneous sampling with potentially malignant disorders not belonging to the mouth, (ii) withdrawn studies, review articles, case reports, commentaries, abstracts from scientific meetings, letters, or book chapters, (iii) studies based on animals or in vitro models, (iv) evaluation of CDKN2A gene alterations, and (v) cross-sectional studies.

In a two steps process, observers (AILP & APJ) independently assessed retrieved reports. First, titles/abstracts selected in our initial search were checked. Then, those apparently meeting set criteria were comprehensively read to reach a decision on their final inclusion or exclusion. Interrater reliability was evaluated by means of the κ statistic. Finally, $\kappa = 0.92$ was obtained. Minor disagreements among reviewers were carefully solved by discussion or with the inclusion of a third participant (MPS). All data management was performed with Mendeley Desktop v1.19.8 (Elsevier, London, UK).

2.3. Data extraction

Three authors (VCAC, AILP & OACI) separately extracted data from every record admitted in the final selection using a pilot-tested sheet, with the software Excel v.2021 (Microsoft. Redmond, WA, USA). From each included report, the following data was extracted: first author, year of publication, study country and continent, sample size, system and grading of dysplasia, type of OPMD, recruitment period, follow-up time, alteration evaluated CDKN2A/$P16^{INK4a}$, HPV presence/absence and method of assessment, methodology applied, alteration frequency, measure of effect and its adjustment. For immunohistochemistry

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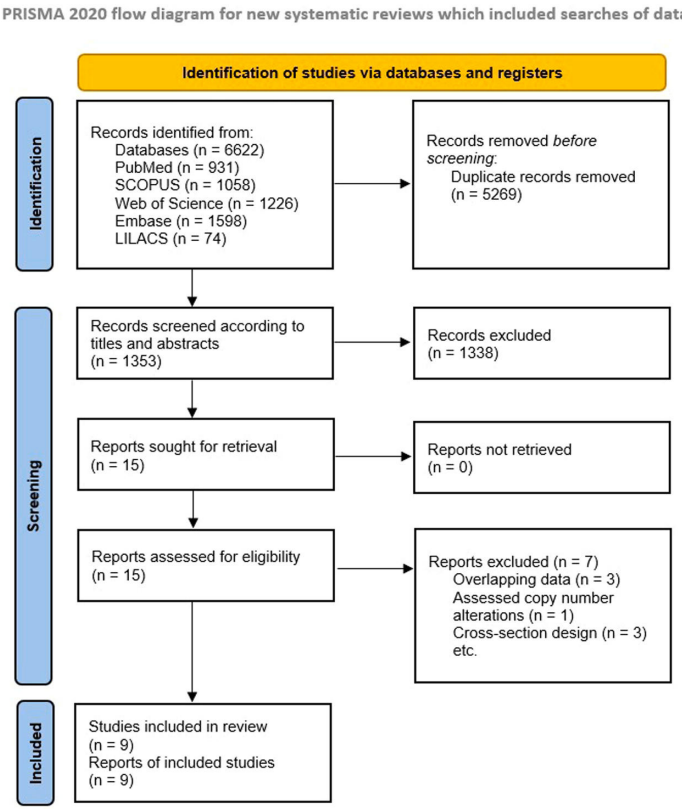


Fig. 1. : Prisma flow diagram of the searching processes.

reports, cut-off point for positivity, anti-P16^{INK4a} antibody, and cellular immunohistochemistry pattern used were also collected. Differences in the expression levels of CDKN2A/p16^{INK4a} were categorized as positive or negative based on the predefined cut-off value of each primary document. A RR value of one study was inquired obtaining a response that allowed inclusion [22].

2.4. Quality appraisal

The "QUality In Prognosis Studies" (QUIPS) tool was implemented to assess the risk of bias (RoB) of the included studies [23]. This tool consists of six domains:

i. Samples: a) Cohort (retrospective or prospective) study with a well-defined study population; b) Medical treatment applied to the patients was explained. Authors have explained if all patients have received the same treatment or not.
ii. Clinical data of the cohort: The basic clinical data such as age, gender, clinical stage, and histopathological grade was provided.
iii. Immunohistochemistry: Well-described staining protocol or referred to original paper.
iv. vi) Prognosis: The analyzed survival endpoints were well defined (e.g., OS and DFS).
v. Statistics: a) Cut-off point, which is used to divide the cases into risk groups was well described; b) Estimated effect describing the relationship between the evaluated biomarker and the outcome was provided; c) Adequate statistical analysis (e.g., Cox regression modelling) was performed to adjust the estimation of the effect of the biomarker for known prognostic factors.
vi. Classical prognostic factor: The prognostic value of other classical prognostic factors and its relationship with the studied factor was reported.

The RoB for each item was allocated as low or high. RoB assessment was performed independently by six authors, with each domain graded by a minimum of two authors. Two participants (AILP & VCAC) carried out RoB, and disagreements were resolved by consensus. An overall rating will be also assigned to individual studies for statistical purposes (i.e., to explore the potential influence of quality/risk of bias on pooled estimates).

2.5. Statistical analysis

Risk ratios and their related 95% confidential intervals were obtained directly from the primary studies as a measure to evaluate the relationship between CDKN2A/P16^{INK4a} overexpression and the malignant transformation of OPMDs. Both multivariate and univariate RRs were retrieved but, when available, the multivariate ones were chosen for further analysis. When RRs data were not reported, HRs were taken as an approximation of this measure [24]. In our output, study-specific RRs were weighted by the inverse of their variance to compute pooled RRs with their 95% CIs. Pooled analyses were obtained using Mantel–Haenszel method and DerSimonian and Laird method. In our computation, high heterogeneity warranted the use of inverse-variance models. On the other hand, fixed-effect models were applied in scenarios of non-significant heterogeneity. Forest plots were produced to graphically display our outputs.

Heterogeneity was evaluated with Cochran's Q test, using $p < 0.05$ as

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Table 1
Extracted data from included reports. Abbreviations: F: female; M: male; MT: malignant transformation; N/R: non reported; NoMT: no malignant transformation; Buccal: buccal mucosa, FOM: Floor of the mouth.* Uses binary grading system for epithelial dysplasia instead of WHO grading system.

Study, year	Country	Sample size	Recruitment period	Follow-up (months)	Sex	Age	Tobacco	Alcohol	Type of OPMDs (n)	Anatomical subsite (n)	Grading of epithelial dysplasia	Progressed (MT)	Not progressed (NoMT)	p16^{INK4a} antibody for IHC	IHC pattern	Cut-off (%)
Shah et al. 2007	India	60	2000–2003	N/R	6 F, 54 M	18–75	53	N/R	Leukoplakias (45)	Buccal mucosa (60)	16 Mild, 5 Moderate and 4 Severe grade transformed	25	35	Clone SC-166 (Monoclonal)	Nuclear	11
Kaur et al. 2013	Canada	110	2000–2010	43	MT: 21 F, 18 M NoMT: 30 F, 41 M	59 (30–88)	MT: 15, NoMT: 31 Yes, 28 No	N/R	Unspecified	Tongue:(26 MT, 53 NoMT) Others (13 MT, 18 NoMT)	Mild (12 MT, 46 NoMT) Moderate (18 MT;21 NoMT), Severe (9 MT, 4 NoMT)	39	71	Clone SC-166 (Monoclonal)	Nuclear	N/R
Nankivell et al. 2014	United Kingdom	148	1996–2008	42	72 F, 76 M	62 ± 14	78	81	Unspecified	Tongue (69), Buccal (38), FOM (20), Other (21)	N/R	39	148	E6H4T (monoclonal)	Nuclear/ cytoplasmic	75
Bazarsad et al. 2017	Sri Lanka	36	N/R	78	MT: 3 F, 2 M NoMT: 9 F, 22 M	N/R	N/R	N/R	Unspecified	Tongue (1 NoMT), Buccal (4 MT, 28 NoMT), Other (2 MT, 1 NoMT)	9/12 dysplastic 22/24 non-dysplastic did not transform	5	31	N/R	Nuclear/ cytoplasmic	5
Zhang et al. 2017	South Korea	160	1994–2009	135	MT: 8 F, 2 M NoMT: 6 F, 4 M	52 (13–89)	N/R	N/R	Leukoplakias: 22 MT 138 No MT	Tongue (10 MT y 34 NoMT) Buccal (6 MT, 38 NoMT) Gingiva (6 MT, 66 NoMT)	13/24 with high grade 45/54 with low grade 80/82 without dysplasia did not transform*	22	138	N/R	Nuclear/ cytoplasmic	N/R
Upadhyaya et al. 2018	USA	20	1994–2016	92	MT: 8 F, 2 M NoMT: 6 F, 4 M	62 (34–87)	MT: 4 Yes, 4 No NoMT: 7 Yes,1 No	N/R	Proliferative verrucous leukoplakia	Multiple affected regions	N/R	10	10	N/R	Nuclear/ cytoplasmic	75
Wu et al. 2019	China	76	2000–2015	72	MT: 27 F, 14 M NoMT: 24 F, 11 M	60	18	10	Leukoplakias: 41 MT 35 no MT	Tongue (30 MT, 29 NoMT), Buccal (9 MT, 5 NoMT), Gingiva (2 MT, 1 NoMT)	Mild (15 MT) Moderate (17 MT) Severe (9 MT, 35 NoMT)	41	35	N/R	Nuclear/ cytoplasmic	> 70
Monteiro et al. 2022	Portugal	52	1995–2006	32	46 M 18 F	58.1 ± 16.8	17	15	Leukoplakias: 6 MT 46 no MT	Tongue (27), Others (11), FOM (2), Buccal (13), Gingiva (11)	41 low grade and 11 high grade *	6	46	OA315 (Monoclonal)	Nuclear/ cytoplasmic	N/R

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Table 2
Quality appraisal using the Quality in Prognosis Studies tool.

Author	Year	1. Study Participation	2. Study Attrition	3. Prognostic Factor Measurement	4. Outcome Measurement	5. Study Confounding	6. Statistical Analysis and Reporting	Overall
Shah et al.	2007	Low	High	High	Moderate	High	Low	High
Kaur et al.	2013	Low	Low	Moderate	Low	Low	Low	Low
Nankivell et al.	2014	Low	Low	Low	Low	Moderate	Low	Low
Bazarsad et al.	2017	Moderate	Low	Low	Low	Moderate	High	High
Zhang et al.	2017	Low	Low	Moderate	Low	Low	Low	Low
Upadhyaya et al.	2018	Low	Low	Low	Low	Low	High	High
Wu et al.	2019	Low	Low	Low	Low	Moderate	Low	Low
Monteiro et al.	2022	Low	Low	Moderate	Low	Low	Low	Low

significant threshold. We also used Higgins I^2 index. We considered the values of 25%, 50%, and 75% to inform about low, moderate, and high heterogeneity [25]. Later, the origin of heterogeneity was studied by subgroup analysis bearing in mind relevant predefined study characteristics (i.e., OPMD type, continent, anti-$P16^{INK4a}$ antibody, cut-off point, adjustment, inmunohistochemical pattern, quality appraisal). Ad hoc Galbraith plot was built to assess the relative contribution of each individual study to global heterogeneity more formally. We also used this method to check for the potential presence of statistical outliers [26].

Publication bias was also assessed using funnel plots. Statistically, we used the regression test proposed by Egger as Begg & Mazumdar's rank correlation test. The trim-and-fill method proposed by Duval et al. [27] was also used. Reviewer Manager 5.4 (Cochrane Collaboration, Copenhagen, Denmark) and Stata 16.1 (Stata Corp, College Station, TX, USA) were employed for data management. A value of $p < 5\%$ was considered significant.

3. Results

3.1. Study selection

The step wise search protocol is depicted in Fig. 1. Globally, 6622 records were initially retrieved. Each database retrieved the following number of documents: 931 from Medline via PubMed, 1058 from Scopus, 1598 from Embase, 1226 from WOS, , 74 from LILACS and 1735 from hand-searching and grey literature. No additional studies were retrieved based on the complementary manual handsearching. Elimination of duplicates resulted in 1353 studies. After, 1338 records were screened according to title/abstract. Among 15 potentially eligible papers selected for full-text assessment, 7 did not meet predefined criteria. Finally, 8 manuscripts fulfilled the inclusion criteria and were yielded for both qualitative and quantitative synthesis [22,28–34].

3.2. Study features and quality appraisal within studies

The 8 included publications encompassed only retrospective cohort studies. Studies were performed across three continents, particularly 4 in Asia [28,31,32,34]; 2 in America [29,33] and 2 in Europe [22,30]. All documents were published between 2007 and 2022 [22,28]. Studies comprised 496 patients with OPMDs; ranging from 20 to 160 patients [32,33]. Out of 8 studies, 3 included mixed OPMDs [28–30], 3 oral leukoplakias [22,32,34], 1 oral submucous fibrosis [31] and 1 included proliferative verrucous leukoplakias [33]. All studies ascertained 16^{INK4a} expression by immunohistochemistry techniques on formalin-fixed paraffin-embedded tissues with different cut-off points to establish positivity as a variety of anti-$p16^{INK4a}$ antibodies (Table 1).

The RoB evaluation ended in 3 studies (37.5%) with a high score, whilst it was low in 5 (62.5%). Most potential biases emerged due to failure in not considering relevant cofounders and due to the use of non-meticulous statistic approaches. Results of the RoB assessment are reported in Table 2.

3.3. Quantitative analysis

Pooled analysis of eight studies evaluating the overexpression of $P16^{INK4a}$ in OPMD revealed a twofold risk to progress into OSCC (RR = 2.01, 95% CI = 1.36–2.96). The rate of between-study heterogeneity was negligible ($I^2 = 0\%$), and for such reason, a fixed-effects model was computed. Individual and pooled RRs are depicted in Fig. 2A. Subgroup analysis pointed out that association was preserved in most of the strata: Asian studies (RR = 1.81, 95%CI 1.06–3.10), studies that used other antibody instead of SC-166 (RR = 1.57, 95%CI 0.76–3.22), studies involving a heterogeneous group of OPMDs (RR = 1.96, 95% CI 1.09–3.50) and those evaluating joint nuclear and cytoplasmatic staining patterns (RR = 2.22, 95%CI 1.40–3.52). No relevant heterogeneity subsided according to our subgroup analysis Complementary, Galbraith plot showed that no individual study could be considered as an important outlier. In this sense, overall heterogeneity was not disproportionately affected by any primary-level study in our pooled analysis (Fig. 2B).

The funnel plot is moderately skewed to the right (Fig. 3). However, the existence of publication bias was not confirmed by Eggers's regression (p = 0.977), nor by the Begg & Mazumdar's correlation test (p = 0.229). However, implementation of the trim-and-fill methodology ended up in the addition of a new "missing" RR. The inclusion not substantively altered our pooled analysis, yielding a close estimate (RR = 1.83, 95% CI = 1.42–2.24). Another simulation was performed to evaluate the robustness of our quantitative synthesis. We calculated how many unpublished negative reports (i.e., RR = 1) with as many cases and controls as the average number of the ones included in our primary studies were necessary to reverse our estimate. Our simulation showed the need of 329 negative studies to obtain a pooled RR equal to the unit and non-significant.

4. Discussion

The progression from normal oral epithelium to neoplasia usually happens in a stepwise process [4]. Accumulation of cellular and histological alterations preceding its development eventually manifest as clinically visible lesions on the topography of the mouth. These OPMDs have potential for reversion as well as for malignant development depending on factors such as cellular stress and tumor microenvironment [11]. Oral carcinogenesis is a complex multistep process requiring both genetic and epigenetic variations. Several related hallmarks represent a cornerstone to properly understand its biology, such as cell cycle. Inside the molecular machinery of the cell cycle, tumor suppression genes such as $P16^{INK4a}$ play pivotal roles in oncogenesis as in the transition of precursor lesions [20].

Nonetheless, no attempt has been made to systematically address the current evidence on $p16^{INK4a}$ as a predictive marker of OPMDs. The current study houses the largest pooled sample analyzed to date (8

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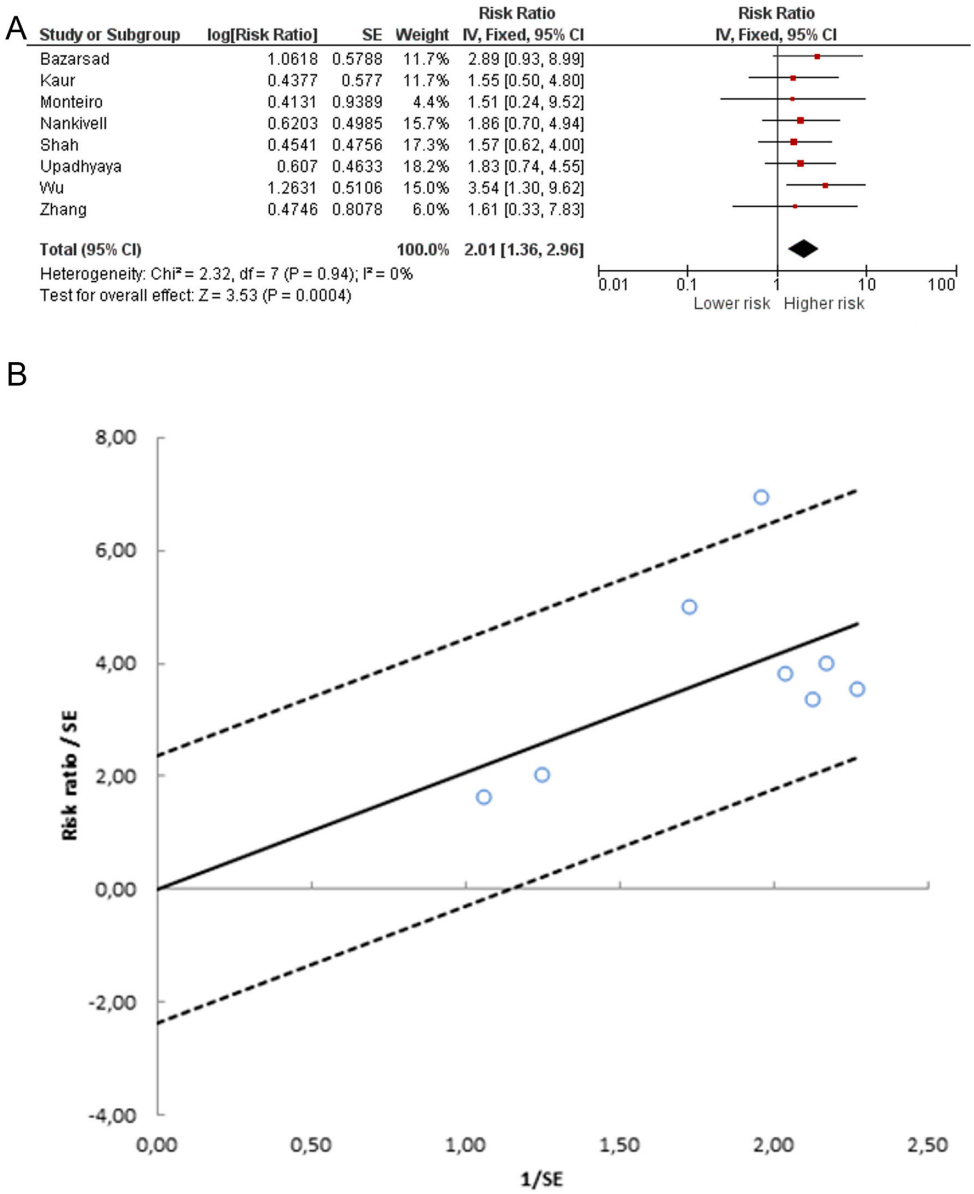


Fig. 2. : A) Forest plot displaying risk of malignant progression in oral potentially malignant disorders positive for P16INK4a overexpression. B) Galbraith plot depicting the individual contribution of studies to the overall heterogeneity. X axis represents individual risk ratios standardized according to standard errors following the next formula, y = RR/SE(RR), whilst the y axis displays precision (x = 1/SE[RR]). The regression diagonal line is projected from the origin of the coordinate system (i.e., x = 0, y = 0). Approximate 95% confidence intervals run between the two parallel lines at ± 2 units around diagonal line.

studies/496 patients), representing the first pooled analysis dealing with prognostic ability of $p16^{INK4a}$ alterations to predict the malignant transformation. Overall, this meta-analysis suggests that $p16^{INK4a}$ overexpression in OPMDs is significantly associated with a greater risk of malignant transformation to OSCC (RR = 2.01, 95% CI = 1.36–2.96) with a negligible heterogeneity among the studies as evidenced by the value of the I^2 index, 0% (Fig. 2, Table 3). In addition to the predictive value attributable to $p16^{INK4a}$ overexpression, a significant fraction of its value as a predictor for malignant transformation of OPMDs, may be caused by distortion derived from residual confounding. It was evidenced that some of the primary studies included in our data synthesis did not provide adjusted RRs estimates for relevant cofounding factors such as for oral epithelial dysplasia or human papilloma virus infection. Nonetheless, overexpression of $p16^{INK4a}$ is known to be indicative of HPV infection [35]. To this regard, only 3 included studies investigated HPV infection in OPMDs [29,33,34], with inconclusive results. Indeed, there is discrepancy in the prevalence of HPV in the mouth, and its role in the oral carcinogenetic process is still under-explored [36,37].

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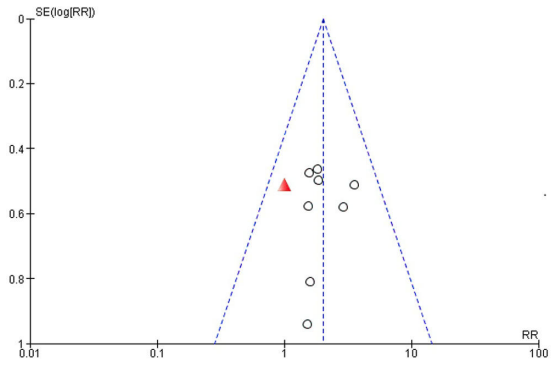


Fig. 3. : Funnel plot. The log RRs of malignant transformation is represented on the x axis, whereas its standard error (SE) values are represented on the y axis. The blue bubbles represent the 8 studies from primary literature and the triangle represents the "new study" added by the trim-and-fill methodology.

Moreover, a growing body of literature points out that the p16 overexpression does not unequivocally represent the real presence or more importantly the active transcription of HPV in these disorders since other mechanisms related to cell cycle and other molecular pathways may fuel the positive staining for $p16^{INK4a}$ [38,39].

We acknowledge some limitations of this systematic review beginning by the plausible presence of publication bias. Small study effects is an unlikely explanation for our outputs due to the asymmetry tests used as the clear interpretation of our funnel plot. We also emphasized that the trim-and-fill method detected one apparently "missing" study (Fig. 3). Its inclusion in our pooled estimate did not appreciably alter our final output (RR = 1.83, 95% CI = 1.42–2.24). Moreover, our quantitative synthesis achieved robust results with almost null heterogeneity, as evidenced by our subgroup analysis and the Galbraith plot interpretation. (Fig. 2, Table 3). The apparent absence of these statistical artefacts is of paramount importance due to in oncological literature on biological markers with prognostic or predictive value there is consistently a relevant tendency to find exclusively positive results [40].

In our subgroup analyses, we were unable to identify a specific rationale that accounted for study heterogeneity due its negligible degree across our global computation. Specifically, we could not trace relevant factors related to immunohistochemistry techniques such as cut-off point, $p16^{INK4a}$ antibodies or histological patters that could enhance the predictive value of this biomarker. Moreover, a specifical OPMD subtype in which $p16^{INK4a}$ assessment could have a stronger predictive value for carcinogenesis was not identified, this assumption may be derived from residual confounding given that several reports included pooled data about unspecified groups of OPMDs (Table 3).

While this study provides valuable insights into the role of $p16^{INK4a}$ immunohistochemical expression in predicting malignant transformation of OPMDs, several drawbacks should be considered. Firstly, the analysis was based on the available published literature, which might be subject to publication bias. Despite efforts to investigate publication bias and assess statistical power, the potential presence of unpublished or inaccessible studies cannot be completely ruled out. Secondly, the number of primary studies included in the meta-analysis was relatively limited, which might affect the statistical power and generalizability of the findings. Additionally, the heterogeneity among the included studies was not significant, but variations in study design, patient characteristics, and methodologies might have influenced the overall results. Additionally, a generalized lack of patients' treatment information as follow-up evaluation and outcome evaluation hindered the possibility to run a more detailed analysis. Moreover, although the use of immunohistochemical expression of $p16^{INK4a}$ shows promise as a prognostic tool, its practical implementation in routine clinical practice may require further validation and standardization across different laboratories and institutions. Lastly, it is important to note that this study focused specifically on $p16^{INK4a}$ and its association with malignant transformation, and other biomarkers or factors that might contribute to the prognosis of OPMDs were not extensively explored.

By and large, the findings of this study have practical implications of paramount importance for the management of OPMDs. The use of $p16^{INK4a}$ immunohistochemical expression as an adjunct tool to dysplasia grading can significantly improve the assessment of the potential progression of OPMDs to cancer. The twofold increased risk of malignant development identified in the meta-analysis underscores the significance of considering $p16^{INK4a}$ expression in the clinical evaluation of patients with OPMDs. Incorporating $p16^{INK4a}$ immunohistochemistry into the day-to-day prognostic study of OPMDs can provide clinicians with valuable information for risk stratification and treatment planning. By enhancing the accuracy of prognostic assessments, this approach may help identify individuals at higher risk of malignant transformation, allowing for early intervention and appropriate management strategies. Furthermore, the multitude of advantages associated with $p16^{INK4a}$ overexpression analysis, such as its availability, simplicity, and cost-effectiveness, make it a feasible and valuable addition to routine clinical practice in the evaluation of OPMDs.

In conclusion, our pooled analysis showed that $p16^{INK4a}$ may be a remarkable adjunct tool to dysplasia assessment leading to a better biological characterization of the potential progression to cancer of OPMDs complementing it from a molecular perspective. Future research

Table 3
Subgroup analysis.

				Pooled data		Heterogeneity		
Subgroups		**No. of studies**	**Stat. model**	**RR (95% CI)**	**P**	**Q**	**P_{het}**	**I^2 (%)**
All		8	F	2.01 (1.36–2.96)	< 0.001	0.941	0.93	0
Continent	Asia	4	F	1.81 (1.06–3.10)	0.03	0.837	0.84	0
	America	2	F	1.72 (0.84–3.48)	0.135	0.050	0.82	0
	Europe	2	F	1.78 (0.75–4.21)	0.192	0.038	0.84	0
Cutoff point	75%	2	F	1.84 (0.94–3.58	0.071	0.001	0.98	0
	Other	6	F	1.74 (1.09–2.78)	0.020	0.923	0.96	0
Antibody	SC-166	2	F	1.57 (0.76–3.22)	0.22	0.001	0.98	0
	Other	6		2.22 (1.40–3.52)	0.001	1.669	0.89	0
Type of OPMDs	Oral leukoplakia	3	F	1.57 (3.29–3.29)	0.23	0.003	0.99	0
	Mixed OPMDs	3	F	1.96 (1.09–3.50)	0.023	0.666	0.72	0
	OSMF	1	F	2.89 (0.93–8.99)	0.001			
	PVL	1	F	1.83 (0.74–4.55)	0.658			
Immunohistochemical pattern	Nuclear/ cytoplasmic	7	F	2.22 (1.40–3.52)	< 0.001	1.669	0.89	0
	Nuclear	2	F	1.57 (0.76–3.22)	0.22	0.001	0.98	0

Abbreviations: CI, confidence intervals; F, Fixed-effect model; OPMDs, oral potentially malignant disorders; RR, risk ratio; OSMF, oral submucous fibrosis; PVL, proliferative verrucous leukoplakia.

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should aim to address identified gaps of knowledge and provide a more comprehensive understanding of the prognostic value of $p16^{INK4a}$ and other related markers in the context of OPMDs.

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CRediT authorship contribution statement

Alejandro I. Lorenzo-Pouso: Data curation; Formal analysis; Investigation; Visualization; Writing-original draft. **Vito Carlo Alberto Caponio:** Investigation; Writing-review & editing. **Fábio França-Vieira-E-Silva:** Formal analysis; Software. **Alba Pérez-Jardón:** Methodology; Visualization. **Óscar Álvarez-Calderón-Iglesias:** Methodology; Writing-review & editing. **Pilar Gándara-Vila:** Supervision. **Giuseppe Pannone:** Conceptualization. **Mario Pérez-Sayáns:** Project administration; Supervision; Writing-review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prp.2023.154656.

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10.ANEXOS

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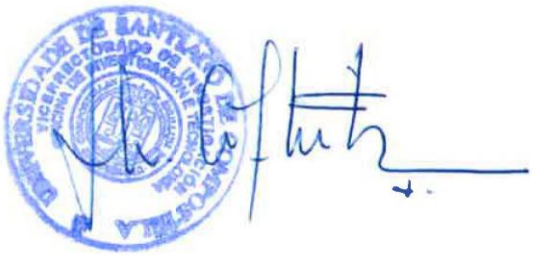
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INFORMA:

Que o estudo de investigación: **"Consumo de alcohol: comprensión de los objetivos de salud entre los estudiantes de Odontología y Medicina",** do que é investigador principal D. **Mario Pérez Sayäns García,** ten sido examinado por o Comité de Bioética desta Universidade, cumprindo o seu protocolo experimental os requisitos éticos esixidos.

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INFORMA:

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DITAME DO COMITÉ DE ÉTICA DA INVESTIGACIÓN DE SANTIAGO-LUGO

Juan Manuel Vázquez Lago, Secretario do Comité de Ética da Investigación de Santiago-Lugo

CERTIFICA:

Que este Comité avaliou na súa reunión do día 19/07/2016 o estudo**:**

Título: Estudio clínico patológico de pacientes con leucoplasia verrugosa proliferativa
Promotor: Pilar Gándara Vila
Tipo de estudo: Outros
Código de Rexistro: 2016/337

E, tomando en consideración as seguintes cuestións:

- A pertinencia do estudo, tendo en conta o coñecemento dispoñible, así coma os requisitos legais aplicables, e en particular a Lei 14/2007, de investigación biomédica, o Real Decreto 1716/2011, de 18 de novembro, polo que se establecen os requisitos básicos de autorización e funcionamento dos biobancos con fins de investigación biomédica e do tratamento das mostras biolóxicas de orixe humana, e se regula o funcionamento e organización do Rexistro Nacional de Biobancos para investigación biomédica, a ORDE SAS/3470/2009, de 16 de decembro, pola que se publican as Directrices sobre estudos Posautorización de Tipo Observacional para medicamentos de uso humano, e o RD 1090/2015, de 4 de decembro, polo que se regulan os ensaios clínicos con medicamentos, os Comités de Ética da Investigación con medicamentos e Rexistro Español de Estudos Clínicos
- A idoneidade do protocolo en relación cos obxectivos do estudo, xustificación dos riscos e molestias previsibles para o suxeito, así coma os beneficios esperados.
- Os principios éticos da Declaración de Helsinki vixente.
- Os Procedementos Normalizados de Traballo do Comité.

Emite un **INFORME FAVORABLE** para a realización do estudo **polo/a investigador/a do centro**:

Centros	Investigadores Principais
Facultad de Medicina e Odontoloxía. Universidad de Santiago de Compostela	Pilar Gándara Vila

En Santiago de Compostela, a 19 de xullo de 2016
O secretario

NOMBRE VÁZQUEZ LAGO JUAN MANUEL - NIF 46829259M

Juan M. Vázquez Lago

XUNTA DE GALICIA
CONSELLERÍA DE SANIDADE
Secretaría Xeral Técnica

Comité de Ética de la Investigación
Santiago Lugo

galicia

DICTAMEN DEL COMITÉ DE ÉTICA DE LA INVESTIGACIÓN DE SANTIAGO-LUGO

Lorenzo Armenteros del Olmo, Secretario suplente del Comité de Ética de la Investigación de Santiago-Lugo,

CERTIFICA:

Que este Comité evaluó en su reunión del día 22 de octubre de 2019 el estudio:

Título: Análisis clínico y molecular de la leucoplasia oral
Versión: 3.0
Promotor/a: Pilar Gándara Vila
Investigador/a: Pilar Gándara Vila
Código de Registro: 2019/271

Y que este Comité, tomando en consideración la pertinencia del estudio, el conocimiento disponible, los requisitos legales aplicables y los Procedimientos Normalizados de Trabajo del Comité, emite un dictamen **FAVORABLE** para la realización del citado estudio.

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Valencia, 26 Marzo del 2024

Como director de la revista Medicina Oral Patología Oral y Cirugía Bucal,

CERTIFICO y AUTORIZO QUE:

Los articulos publicados en nuestra revista que figuran a continuación pueden ser utilizados para presentar una tesis por compendio de publicaciones, por parte de Alba Pérez Jardón.

-Título: Counselling toward reducing alcohol use, knowledge about its morbidity and personal consumption among students of medical and dental courses in north-western Spain
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Drug use, risk perceptions and attitudes towards drug use amongst medical and dentistry students

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Xerostomia, the perception of general and oral health and health risk behaviours in people over 65 years of age

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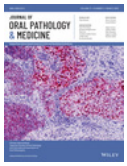
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Critical update, systematic review, and meta-analysis of oral erythroplakia as an oral potentially malignant disorder

Author: José Manuel Aguirre-Urízar, Andrés Blanco-Carrión, Cintia M Chamorro-Petronacci, et al
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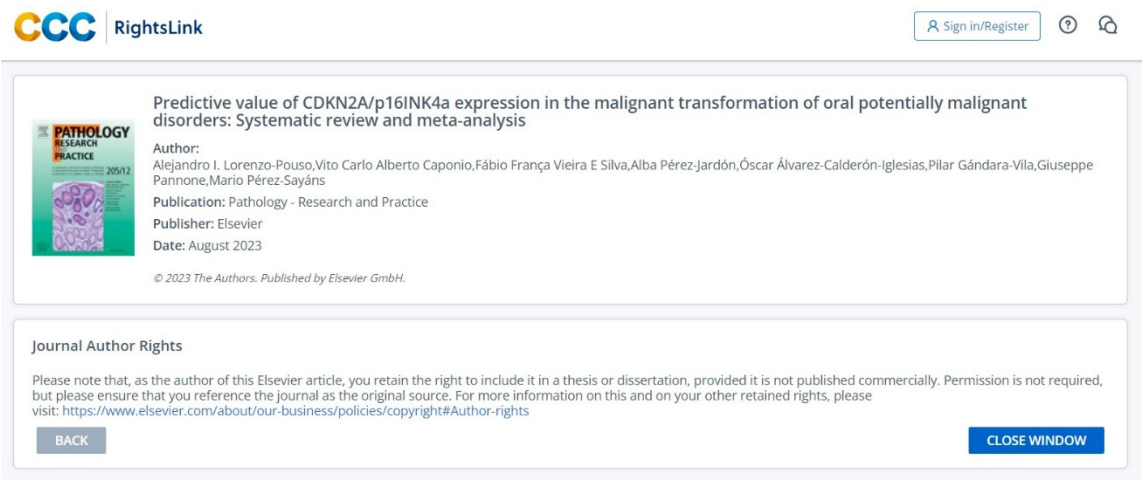
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Predictive value of CDKN2A/p16INK4a expression in the malignant transformation of oral potentially malignant disorders: Systematic review and meta-analysis

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ANEXO 3. TABLA A1

Tabla A1. Datos descriptivos del artículo 4.

		N	%	Xerostomia Inventory					p
				No	Leve	Moderada	Grave	Xeros total	
Sección I. Datos sociodemográficos									
Género	Varón	308	38,5	234 (76,0)	67 (21,8)	7 (2,3)	0 (0)	74 (24,0)	0,003
	Mujer	492	61,5	321 (65,2)	138 (28)	31 (6,3)	2 (0,4)	171 (34,8)	
Edad	De 65 a 74 años	460	575	321 (69,8)	115 (25,0)	23 (5,0)	1 (0,2)	139 (30,2)	0,944
	Más de 74 años	340	42,5	234 (68,8)	90 (26,5)	15 (4,4)	1 (0,3)	106 (31,2)	
Región	Noreste	158	19,8	109 (69,0)	41 (25,9)	8 (5,1)	0 (0)	49 (31,0)	0,649
	Este	117	14,6	82 (70,1)	30 (25,6)	5 (4,3)	0 (0)	35 (29,9)	
	Sur	120	15,0	84 (70,0)	28 (23,3)	8 (6,7)	0 (0)	36 (30,0)	
	Centro	177	22,1	122 (68,9)	48 (27,1)	6 (3,4)	1 (0,6)	55 (31,1)	
	Noroeste	79	9,9	55 (69,6)	24 (30,4)	0 (0)	0 (0)	24 (30,4)	
	Norte Centro	75	9,4	53 (70,7)	16 (21,3)	6 (8,0)	0 (0)	22 (29,3)	
	Islas y ciudades autónomas	74	9,3	50 (67,6)	18 (24,3)	5 (6,8)	1 (1,4)	24 (32,4)	
Estudios	Sin estudios	43	5,4	30 (69,8)	12 (27,9)	1 (2,3)	0 (0)	13 (30,2)	<0,001
	Primarios	287	35,9	183 (63,8)	89 (31,0)	14 (4,9)	1 (0,3)	104 (36,2)	
	Superiores	227	28,4	170 (74,9)	46 (20,3)	11 (4,8)	0 (0)	57 (25,1)	
	FP1	80	10,0	51 (63,7)	24 (30,0)	5 (6,3)	0 (0)	29 (36,3)	
	FP2	150	18,8	114 (76,0)	30 (20,0)	6 (4,0)	0 (0)	36 (24,0)	
	Ns/nc	13	1,6	7 (53,8)	4 (30,8)	1 (7,7)	1 (7,7)	6 (46,2)	
Profesión	Por cuenta ajena	623	77,9	439 (70,5)	152 (24,4)	31 (5,0)	1 (0,2)	184 (29,5)	0,065
	Cuenta propia	129	16,1	88 (68,2)	35 (27,1)	6 (4,7)	0 (0)	41 (31,8)	
	Otro	48	6,0	28 (58,3)	18 (37,5)	1 (2,1)	1 (2,1)	20 (41,7)	
Jubilado	No	84	10,5	57 (67,9)	20 (23,8)	7 (8,3)	0 (0)	27 (32,1)	<0,001
	Sí	710	88,8	495 (69,7)	183 (25,8)	31 (4,4)	1 (0,1)	215 (30,3)	
	Ns/nc	6	0,8	3 (50,0)	2 (33,3)	0 (0)	1 (16,7)	3 (50,0)	
Vive solo/a o acompañado/a?	Solo/a	219	27,4	137 (62,6)	72 (32,9)	9 (4,1)	1 (0,5)	82 (37,4)	<0,001
	Acompañado/a	572	71,5	412 (72,0)	131 (22,9)	29 (5,1)	0 (0)	160 (28,0)	
	Ns/nc	9	1,1	6 (66,7)	2 (22,2)	0 (0)	1 (11,1)	3 (33,3)	
Actividades hogar	Necesita ayuda	91	11,4	49 (53,8)	34 (37,4)	8 (8,8)	0 (0)	42 (46,2)	<0,001
	Autosuficiente	701	87,6	502 (71,6)	168 (24,0)	30 (4,3)	1 (0,1)	199 (28,4)	
	Ns/nc	8	1,0	4 (50,0)	3 (37,5)	0 (0)	1 (12,5)	4 (50,0)	

		N	%	Xerostomia Inventory					p
				No	Leve	Moderada	Grave	Xeros total	
Sección II: Salud general y oral									
Preocupa salud general	Nada	144	18,0	124 (86,1)	18 (12,5)	2 (1,4)	0 (0)	20 (13,9)	0,001
	Poco	128	16,0	85 (66,4)	34 (26,6)	9 (7,0)	0 (0)	43 (33,6)	
	Algo	148	18,5	108 (73,0)	36 (24,3)	3 (2,0)	1 (0,7)	40 (27,0)	
	Bastante	224	28,0	140 (62,5)	69 (30,8)	14 (6,3)	1 (0,4)	84 (37,5)	
	Mucho	156	19,5	98 (62,8)	48 (30,8)	10 (6,4)	0 (0)	58 (37,2)	
Preocupa salud bucodental	Nada	227	28,4	184 (81,1)	40 (17,6)	3 (1,3)	0 (0)	43 (18,9)	<0,001
	Poco	117	14,6	81 (69,2)	28 (23,9)	8 (6,8)	0 (0)	36 (30,8)	
	Algo	103	12,9	67 (65,0)	32 (31,1)	4 (3,9)	0 (0)	36 (35,0)	
	Bastante	216	27,0	149 (69,0)	54 (25,0)	12 (5,6)	1 (0,5)	67 (31,0)	
	Mucho	137	17,1	74 (54,0)	51 (37,2)	11 (8,0)	1 (0,7)	63 (46,0)	
Estado salud general 2019	Muy malo	22	2,8	13 (59,1)	7 (31,8)	2 (9,1)	0 (0)	9 (40,9)	<0,001
	Malo	81	10,1	37 (45,7)	31 (38,3)	12 (14,8)	1 (1,2)	44 (54,3)	
	Normal	302	37,8	200 (66,2)	89 (29,5)	13 (4,3)	0 (0)	102 (33,8)	
	Bueno	293	36,6	222 (75,8)	62 (21,2)	8 (2,7)	1 (0,3)	71 (24,2)	
	Muy bueno	99	12,4	82 (82,8)	14 (14,1)	3 (3,0)	0 (0)	17 (17,2)	
	Ns/nc	3	0,4	1 (33,3)	2 (66,7)	0 (0)	0 (0)	2 (66,7)	
Estado salud bucodental 2019	Muy malo	9	1,1	5 (55,6)	0 (0)	4 (44,4)	0 (0)	4 (44,4)	<0,001
	Malo	35	4,4	16 (45,7)	16 (45,7)	3 (8,6)	0 (0)	19 (54,3)	
	Normal	346	43,3	225 (65,0)	103 (29,8)	17 (4,9)	1 (0,3)	121 (35,0)	
	Bueno	331	41,4	246 (74,3)	71 (21,5)	13 (3,9)	1 (0,3)	85 (25,7)	
	Muy bueno	78	9,8	62 (79,5)	15 (19,2)	1 (1,3)	0 (0)	16 (20,5)	
	Ns/nc	1	0,1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
Sección III: Problemas orales durante 2019									
Frecuencia en 2019 dificultades para comer	Nunca	594	74,3	446 (75,1)	133 (22,4)	13 (2,2)	2 (0,3)	148 (24,9)	<0,001
	Casi nunca	87	10,9	48 (55,2)	31 (35,6)	8 (9,2)	0 (0)	39 (44,8)	
	Ocasionalmente	97	12,1	55 (56,7)	32 (33,0)	10 (10,3)	0 (0)	42 (43,3)	
	Bastante a menudo	11	1,4	4 (36,4)	4 (36,4)	3 (27,3)	0 (0)	7 (63,6)	
	Muy a menudo	9	1,1	1 (11,1)	4 (44,4)	4 (44,4)	0 (0)	8 (88,9)	
	Ns/nc	2	0,3	1 (50)	1 (50)	0 (0)	0 (0)	1 (50)	
Dolor de dientes o encías	Nunca	607	75,9	459 (75,6)	132 (21,7)	15 (2,5)	1 (0,2)	148 (24,4)	<0,001
	Casi nunca	79	9,9	42 (53,2)	31 (39,2)	6 (7,6)	0 (0)	37 (46,8)	
	Ocasionalmente	92	11,5	47 (51,1)	34 (37,0)	10 (10,9)	1 (1,1)	45 (48,9)	
	Bastante a menudo	13	1,6	3 (23,1)	7 (53,8)	3 (23,1)	0 (0)	10 (76,9)	
	Muy a menudo	6	0,8	2 (33,3)	0 (0)	4 (66,7)	0 (0)	4 (66,7)	
	Ns/nc	3	0,4	2 (66,7)	1 (33,3)	0 (0)	0 (0)	1 (33,3)	

		N	%	Xerostomia Inventory					p
				No	Leve	Moderada	Grave	Xeros total	
Problemas en dientes, boca o dentadura	Nunca	601	75,1	446 (74,2)	132 (22,0)	21 (3,5)	2 (0,3)	155 (25,8)	<0,001
	Casi nunca	57	7,1	39 (68,4)	16 (28,1)	2 (3,5)	0 (0)	18 (31,6)	
	Ocasionalmente	101	12,6	52 (51,5)	43 (42,6)	6 (5,9)	0 (0)	49 (48,5)	
	Bastante a menudo	14	1,8	5 (35,7)	5 (35,7)	4 (28,6)	0 (0)	9 (64,3)	
	Muy a menudo	12	1,5	3 (25,0)	4 (33,3)	5 (41,7)	0 (0)	9 (75,0)	
	Ns/nc	15	1,9	10 (66,7)	5 (33,3)	0 (0)	0 (0)	5 (33,3)	
Ha evitado sonreír o hablar por la apariencia de sus dientes o dentadura	Nunca	732	91,5	522 (71,3)	181 (24,7)	28 (3,8)	1 (0,1)	210 (28,7)	<0,001
	Casi nunca	12	1,5	8 (66,7)	2 (16,7)	2 (16,7)	0 (0)	4 (33,3)	
	Ocasionalmente	37	4,6	20 (54,1)	14 (37,8)	3 (8,1)	0 (0)	17 (45,9)	
	Bastante a menudo	12	1,5	5 (41,7)	6 (50)	1 (8,3)	0 (0)	7 (58,3)	
	Muy a menudo	7	0,9	0 (0)	2 (28,6)	4 (57,1)	1 (14,3)	7 (100)	
	Ns/nc	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Sección IV: Consumo de tabaco y alcohol									
Consumo de tabaco	No he fumado nunca	433	54,1	285 (65,8)	123 (28,4)	23 (5,3)	2 (0,5)	148 (34,2)	0,222
	Soy fumador	71	8,9	57 (80,3)	12 (16,9)	2 (2,8)	0 (0)	14 (19,7)	
	Soy exfumador	295	36,9	213 (72,2)	69 (23,4)	13 (4,4)	0 (0)	82 (27,8)	
	Ns/nc	1	0,1	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	
Cigarrillos al día	No fuma	729	91,1	498 (68,3)	193 (26,5)	36 (4,9)	2 (0,3)	231 (31,7)	0,605
	Fumador ocasional, no todos los días	4	0,5	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
	Entre 1 y 5	17	2,1	15 (88,2)	1 (5,9)	1 (5,9)	0 (0)	2 (11,8)	
	Entre 6 y 10	20	2,5	18 (90,0)	2 (10,0)	0 (0)	0 (0)	2 (10,0)	
	Más de 10	29	3,6	20 (69,0)	8 (27,6)	1 (3,4)	0 (0)	9 (31,0)	
	Ns/nc	1	0,1	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	
Años dejó de fumar	Ninguno	505	63,1	342 (67,7)	136 (26,9)	25 (5,0)	2 (0,4)	163 (32,3)	0,973
	Menos de 5 años	26	3,3	17 (65,4)	7 (26,9)	2 (7,7)	0 (0)	9 (34,6)	
	De 5 a 10 años	38	4,8	26 (68,4)	11 (28,9)	1 (2,6)	0 (0)	12 (31,6)	
	De 11 a 15 años	29	3,6	19 (65,5)	9 (31,0)	1 (3,4)	0 (0)	10 (34,5)	
	Más de 15 años	199	24,9	149 (74,9)	41 (20,6)	9 (4,5)	0 (0)	50 (25,1)	
	Ns/nc	3	0,4	2 (66,7)	1 (33,3)	0 (0)	0 (0)	1 (33,3)	
Bebidas alcohólicas	Nunca	281	35,1	180 (64,1)	83 (29,5)	17 (6,0)	1 (0,4)	101 (35,9)	0,120
	Ocasionalmente	375	46,9	273 (72,8)	81 (21,6)	20 (5,3)	1 (0,3)	102 (27,2)	
	Diariamente	140	17,5	100 (71,4)	39 (27,9)	1 (0,7)	0 (0)	40 (28,6)	
	Ns/nc	4	0,5	2 (50)	2 (50)	0 (0)	0 (0)	2 (50)	

		N	%	Xerostomia Inventory					p
				No	Leve	Moderada	Grave	Xeros total	
Cerveza	0	754	94,3	522 (69,2)	193 (25,6)	37 (4,9)	2 (0,3)	232 (30,8)	0,967
	1	30	3,8	18 (60,0)	11 (36,7)	1 (3,3)	0 (0)	12 (40,0)	
	2	13	1,6	12 (92,3)	1 (7,7)	0 (0)	0 (0)	1 (7,7)	
	3	1	0,1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
	4	1	0,1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
	>5	1	0,1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
Vino	0	686	85,8	478 (69,7)	169 (24,6)	37 (5,4)	2 (0,3)	208 (30,3)	0,611
	1	62	7,8	35 (56,5)	26 (41,9)	1 (1,6)	0 (0)	27 (43,5)	
	2	30	3,8	23 (76,7)	7 (23,3)	0 (0)	0 (0)	7 (23,3)	
	3	14	1,8	12 (85,7)	2 (14,3)	0 (0)	0 (0)	2 (14,3)	
	4	5	0,6	4 (80,0)	1 (20,0)	0 (0)	0 (0)	1 (20,0)	
	>5	3	0,4	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
Licor	0	784	98,0	543 (69,3)	201 (25,6)	38 (4,8)	2 (0,3)	241 (30,7)	0,976
	1	12	1,5	8 (66,7)	4 (33,3)	0 (0)	0 (0)	4 (33,3)	
	2	2	0,3	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
	3	2	0,3	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
Sección V: Hábitos dietéticos									
¿Cree que una buena alimentación influye en la salud bucodental?	Nada	30	3,8	23 (76,7)	6 (20,0)	1 (3,3)	0 (0)	7 (23,3)	0,717
	Poco	24	3,0	18 (75,0)	5 (20,8)	1 (4,2)	0 (0)	6 (25,0)	
	Algo	69	8,6	55 (79,7)	11 (15,9)	3 (4,3)	0 (0)	14 (20,3)	
	Bastante	318	39,8	224 (70,4)	78 (24,5)	15 (4,7)	1 (0,3)	94 (29,6)	
	Mucho	323	40,4	212 (65,6)	96 (29,7)	14 (4,3)	1 (0,3)	111 (34,4)	
	Ns/nc	36	4,5	23 (63,9)	9 (25,0)	4 (11,1)	0 (0)	13 (36,1)	
Carne blanca	Nunca	19	2,4	15 (78,9)	3 (15,8)	1 (5,3)	0 (0)	4 (21,1)	0,500
	Esporádicamente	114	14,2	86 (75,4)	25 (21,9)	3 (2,6)	0 (0)	28 (24,6)	
	Cada cuatro o cinco días	226	28,2	149 (65,9)	63 (27,9)	14 (6,2)	0 (0)	77 (34,1)	
	Cada dos o tres días	413	51,6	284 (68,8)	110 (26,6)	17 (4,1)	2 (0,5)	129 (31,2)	
	Todos los días	20	2,5	15 (75,0)	2 (10,0)	3 (15,0)	0 (0)	5 (25,0)	
	Ns/nc	8	1,0	6 (75,0)	2 (25,0)	0 (0)	0 (0)	2 (25,0)	
Carne roja	Nunca	84	10,5	58 (69,0)	21 (25,0)	5 (6,0)	0 (0)	26 (31,0)	<0,001
	Esporádicamente	264	33,0	176 (66,7)	70 (26,5)	18 (6,8)	0 (0)	88 (33,3)	
	Cada cuatro o cinco días	261	32,6	178 (68,2)	72 (27,6)	11 (4,2)	0 (0)	83 (31,8)	
	Cada dos o tres días	175	21,9	131 (74,9)	39 (22,3)	4 (2,3)	1 (0,6)	44 (25,1)	
	Todos los días	5	0,6	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
	Ns/nc	11	1,4	7 (63,6)	3 (27,3)	0 (0)	1 (9,1)	4 (36,4)	

		N	%	Xerostomia Inventory					p
				No	Leve	Moderada	Grave	Xeros total	
Verdura	Nunca	7	0,9	4 (57,1)	3 (42,9)	0 (0)	0 (0)	3 (42,9)	<0,001
	Esporádicamente	28	3,5	20 (71,4)	5 (17,9)	3 (10,7)	0 (0)	8 (28,6)	
	Cada cuatro o cinco días	51	6,4	33 (64,7)	17 (33,3)	1 (2,0)	0 (0)	18 (35,3)	
	Cada dos o tres días	257	32,1	175 (68,1)	69 (26,8)	13 (5,1)	0 (0)	82 (31,9)	
	Todos los días	450	56,3	320 (71,1)	108 (24,0)	21 (4,7)	1 (0,2)	130 (28,9)	
	Ns/nc	7	0,9	3 (42,9)	3 (42,9)	0 (0)	1 (14,3)	4 (57,1)	
Fruta	Nunca	5	0,6	4 (80,0)	1 (20,0)	0 (0)	0 (0)	1 (20,0)	<0,001
	Esporádicamente	26	3,3	19 (73,1)	6 (23,1)	1 (3,8)	0 (0)	7 (26,9)	
	Cada cuatro o cinco días	8	1,0	7 (87,5)	1 (12,5)	0 (0)	0 (0)	1 (12,5)	
	Cada dos o tres días	43	5,4	31 (72,1)	8 (18,6)	4 (9,3)	0 (0)	12 (27,9)	
	Todos los días	712	89,0	491 (69,0)	187 (26,3)	33 (4,6)	1 (0,1)	221 (31,0)	
	Ns/nc	6	0,8	3 (50,0)	2 (33,3)	0 (0)	1 (16,7)	3 (50,0)	
Legumbres	Nunca	9	1,1	4 (44,4)	4 (44,4)	1 (11,1)	0 (0)	5 (55,6)	<0,001
	Esporádicamente	90	11,3	65 (72,2)	19 (21,1)	6 (6,7)	0 (0)	25 (27,8)	
	Cada cuatro o cinco días	217	27,1	147 (67,7)	61 (28,1)	9 (4,1)	0 (0)	70 (32,3)	
	Cada dos o tres días	418	52,3	296 (70,8)	101 (24,2)	20 (4,8)	1 (0,2)	122 (29,2)	
	Todos los días	57	7,1	38 (66,7)	17 (29,8)	2 (3,5)	0 (0)	19 (33,3)	
	Ns/nc	9	1,1	5 (55,6)	3 (33,3)	0 (0)	1 (50,0)	4 (44,4)	
Agua o líquidos al día	Menos de medio litro	26	3,3	19 (73,1)	6 (23,1)	1 (3,8)	0 (0)	7 (26,9)	<0,001
	Medio litro	104	13,0	69 (66,3)	29 (27,9)	6 (5,8)	0 (0)	35 (33,7)	
	Un litro	262	32,8	184 (70,2)	65 (24,8)	12 (4,6)	1 (0,4)	78 (29,8)	
	Un litro y medio	241	30,1	171 (71,0)	62 (25,7)	8 (3,3)	0 (0)	70 (29,0)	
	Dos litros	110	13,8	75 (68,2)	28 (25,5)	7 (6,4)	0 (0)	35 (31,8)	
	Más de dos litros	49	6,1	32 (65,3)	13 (26,5)	4 (8,2)	0 (0)	17 (34,7)	
	Ns/nc	8	1,0	5 (62,5)	2 (25,0)	0 (0)	1 (12,5)	3 (37,5)	

ANEXO 4. TABLA A2

Tabla A2. Datos extraídos de informes incluidos en el artículo 8. Abreviaturas: F: femenino; M: masculino; N/R: no reportado; NoTM: sin transformación maligna; Bucal: mucosa yugal.* Utiliza un sistema de clasificación binario para la DE en lugar del sistema de clasificación de la OMS.

Estudio, año	País	Tamaño de muestra	Reclutamiento	Seguimiento (meses)	Sexo	Edad	Tabaco	Alcohol	Tipo de OPMD	Subsitio anatómico	Clasificación DE	TM	No TM	Anticuerpo $p16^{INK4a}$ para IHQ	Patrón IHQ	Corte
Shah et al. 2007	India	60	2000-2003	N/R	6 F, 54 M	18-75	53	N/R	LO (45)	Bucal (60)	16 leve, 5 moderdo y 4 severa grade se transformaron	25	35	Clon SC-166 (Monoclonal)	Nuclear	11
Kaur et al. 2013	Canadá	110	2000-2010	43	TM: 21 F, 18 M NoTM: 30F, 41M	59 (30-88)	TM: 15, NoTM: 31 Yes, 28 No	N/R	No especificado	Lengua:(26 TM, 53 No TM) Otros (13 TM, 18 No TM)	Leve (12 TM, 46 No TM) Moderado (18 TM,21 No TM), Severa (9 TM, 4 No TM)	39	71	Clon SC-166 (Monoclonal)	Nuclear	N/R
Nankivell et al. 2014	Reino Unido	148	1996-2008	42	72 F, 76 M	62±14	78	81	No especificado	Lengua (69), Bucal (38), suelo de boca (20), otros (21)	N/R	39	148	E6H4T (monoclonal)	Nuclear/ citoplasmático	75
Bazarsad et al. 2017	Sri Lanka	36	N/R	78	TM: 3F, 2M No TM: 9F, 22M	N/R	N/R	N/R	No especificado	Lengua (1 No TM), Bucal (4 TM, 28 No TM), Otros (2 TM, 1 No TM)	9/12 con DE y 22/24 sin DE no se transformaron	5	31	N/R	Nuclear/ citoplasmático	5
Zhang et al. 2017	Corea del Sur	160	1994-2009	135	TM: 8F, 2M No TM: 6F, 4M	52 (13-89)	N/R	N/R	LO: 22 TM 138 No TM	Lengua (10 TM y 34 No TM) Bucal (6 TM, 38 No TM) Encía (6 TM, 66 No TM)	13/24 alto grado 45/54 bajo grado 80/82 sin DE no se transformaron	22	138	N/R	Nuclear/ citoplasmático	N/R

Estudio, año	País	Tamaño de muestra	Reclutamiento	Seguimiento (meses)	Sexo	Edad	Tabaco	Alcohol	Tipo de OPMD	Subsitio anatómico	Clasificación DE	TM	No TM	Anticuerpo $p16^{INK4a}$ para IHQ	Patrón IHQ	Corte
Upadhyaya et al. 2018	EE. UU.	20	1994-2016	92	TM: 8F, 2M NoTM: 6F, 4M	62 (34-87)	TM: 4 Sí, 4 No No TM: 7 Sí ,1 No	N/R	Leucoplasia verrugosa proliferativa	Múltiples regiones afectadas	N/R	10	10	N/R	Nuclear/ citoplasmático	75
Wu et al. 2019	China	76	2000-2015	72	TM: 27 F, 14M NoTM: 24F, 11M	60	18	10	LO: 41 TM 35 no TM	Lengua (30 TM, 29 No TM), Bucal (9 TM, 5 No TM), Encía (2 TM, 1 No TM)	Leve (15 TM) Moderada (17 TM) Severa (9 TM, 35 No TM)	41	35	N/R	Nuclear/ citoplasmático	> 70
Monteiro et al. 2022	Portugal	52	1995-2006	32	46 M 18 F	58.1±16.8	17	15	LO: 6 TM 46 no TM	Lengua (27), Otros (11), suelo de boca (2), Bucal (13), Encía (11)	41 bajo grado y 11 alto grado *	6	46	OA315 (Monoclonal)	Nuclear/ citoplasmático	N/R

Esta tesis aborda la salud oral desde diversas perspectivas, explorando el consumo de sustancias en estudiantes de odontología y medicina, la xerostomía en adultos y el potencial maligno de desórdenes orales. Revela una preocupante prevalencia de consumo de alcohol y otras drogas, destacando diferencias de género y ciclo académico. Se resalta la asociación de la xerostomía con la salud sistémica y oral en adultos mayores. El análisis de la transformación maligna de desórdenes orales potencialmente malignos subraya la necesidad de criterios diagnósticos claros. En el ámbito molecular, se investigan biomarcadores, resaltando la importancia en la sobreexpresión de p16INK4a. Se destaca la importancia de enfoques multidisciplinarios y se ofrecen nuevas perspectivas para la prevención y manejo de la patología oral.