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INVITED REVIEW

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Epigenetics in the diagnosis and prognosis of head and neck cancer: A systematic review

Isaac Lim[1] | Jade Tan[1] | Anneka Alam[1] | Majdy Idrees[1] | Peter A. Brenan[2] | Ricardo Della Coletta[3] | Omar Kujan[1]

[1]UWA Dental School, The University of Western Australia, Nedlands, Western Australia, Australia

[2]Department of Oral and Maxillofacial Surgery, Queen Alexandra Hospital, Portsmouth, UK

[3]Department of Oral Diagnosis and Graduate Program in Oral Biology, Piracicaba Dental School, University of Campinas, Piracicaba, São Paulo, Brazil

Correspondence
Omar Kujan, UWA Dental School, The University of Western Australia, 17 Monash Avenue, Nedlands, WA 6009, Australia.
Email: omar.kujan@uwa.edu.au

Abstract

Background: Aberrant epigenetic modifications significantly develop and progress human malignancies including head and neck squamous cell carcinoma (HNSCC). Taking into account issues of late diagnosis and poor prognosis associated with HNSCC, this systematic review is designed to provide an up-to-date insight of epigenetic changes in the management of HNSCC.

Methods: All studies that assessed the diagnostic and prognostic utilities of epigenetic changes (DNA methylation and histone modifications) among patients diagnosed with HNSCC or oral potentially malignant disorders (OPMDs) were considered for inclusion till June 2023. Pre-defined Medical Subject Headings terms were used to search Web of Science, Pubmed, Scopus and Embase Ovid databases.

Results: Twenty-five studies were deemed eligible for inclusion with a total number of 3790 samples (2123 HNSCCs, 334 OPMDs and 1333 as controls). DNA methylation was investigated in 18 studies while the role of histone modifications was assessed in seven studies. The most investigated biomarkers among the studies were H3, DAPK and TIMP3. The diagnostic accuracy of the epigenetic biomarkers in detecting HNSCC was assessed in eight studies where the following biomarkers showed the highest area under the curve values: TIPM3, DCC, DAPK, SEPT9, SHOX9, HOXA9 and TRH. None of the studies assessed the predictability of the epigenetic biomarkers in HNSCC and OPMDs.

Conclusion: Although initial promising results were seen using the epigenetic biomarkers in the early detection of HNSCC, the limited number of patients and the absence of well-designed longitudinal studies limit the clinical applicability of the outcomes.

KEYWORDS

aberrant DNA methylation, epigenetic changes, head and neck cancer, histone modifications, oral potentially malignant disorders

1 | INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is a significant global health issue, accounting for nearly 600 000 new cases annually.[1] Despite the incidence of HNSCC being anticipated to increase by 40% in 2040,[2] HNSCC is known for its unpredictable behaviour and the late diagnosis pattern.[3] Although efforts directed toward early detection of HNSCC to maximise the standards of care,[4,5] its 5-year survival rates have not notably improved over the past two decades.[6]

Like most solid malignancies, HNSCC has a complicated etiology involving genetic and environmental factors.[7] Cumulative evidence shows HNSCC as a consequence of serial molecular and cytological changes rather than a singular event.[7] In most cases, these changes appear phenotypically as oral potentially malignant disorders (OPMDs).[7] Several phenotypic-based criteria were proposed to predict the prognosis of oral lesions. However, neither the HNSCC outcomes nor the malignant transformation potential of OPMDs can be objectively determined.[5] This is usually seen in clinical settings where different outcomes have been reported among patients with HNSCCs or OPMDs regardless of having similar prognostic profiles, indicating various underlying patterns of epigenetic and genetic changes at cellular levels.[8] While hundreds of molecular biomarkers - proposedly implicated in head and neck tumorigenesis - have been extensively investigated in the literature,[4,9] conclusive outcomes have yet to be determined.

During the past few years, scientific evidence has highlighted a strong association between epigenetic mechanisms and the hallmarks of cancer, where tumorigenesis is seen as a consequence of multiple epigenetic events.[8] Contrasting to the irreversible nature of genetic changes, epigenetics are genomic mechanisms that reversibly regulate gene expression and are considered a link between genotype and phenotype.[8] They provide an opportunity to investigate underlying mechanisms implicated in cancer phenotypes and potential therapies.[10]

DNA methylation is the principal epigenetic factor and the critical regulator of several cellular activities like cell growth and differentiation.[8] The fundamental enzymes that control DNA methylation and mediate the transfer of the methyl group from S-adenosylmethionine to the cytosine are called DNA methyltransferases (DNMTs).[10] In cancer cells, aberrant DNA methylation patterns are associated with chromosomal instability and a decrease in the expression of tumour-silencing genes.[8] Loss of DNA methylation, also known as global DNA hypomethylation, usually arises earlier and is linked with genomic instability and mutagenesis.[10] It has also been reported that global hypomethylation of specific sequences (LINE-1, Alu and Sat-α) can negatively impacts tumour prognosis.[11] On the contrary, DNA hypermethylation is associated with carcinogenesis by silencing tumour suppressor genes and impairing the DNA repair genes by adding additional methyl groups to CpG islands located at the promoter regions of these genes.[10] In oral cancer, literatures have reported more than 40 tumour suppressor genes silenced by DNA hypermethylation in a process that impacts several cellular functions like cellular cycles, apoptosis and cell-to-cell adhesion.[12,13]

On the other hand, common histone modifications observed in HNSCCs and OPMDs include histone acetylation, methylation, phosphorylation, parylation and ubiquitination.[14] Unlike DNA methylation, histone modifications can promote DNA transcription rather than only silence the expression of specific genes.[14] Analysis of cancer cells revealed that lower levels of histone modifications are indicators of more aggressive malignancies.[15] Changes in H3K4 histone methylation have also been reported at early events of HNSCCs, highlighting their potential role in head and neck tumorigenesis.[16,17]

Liquid biopsy, as a minimally invasive alternative to conventional surgical biopsies, has gained significant attention in recent years, especially for its cost-effectiveness and low-technique sensitivity.[3] This includes collecting and analysing body fluids like saliva, urine and blood.[18] A previous comparative study revealed a high concordance of the levels of specific nucleic acid biomarkers between liquid and tissue biopsies.[19] In the head and neck medicine field, fluid biopsies showed promising potential for detecting various genetic and epigenetic changes for both diagnostic and prognostic purposes.[3,18]

The primary aim of this systematic review was to summarise the results of published studies that assessed the potential diagnostic and prognostic role of epigenetic biomarkers in head and neck malignancies. In addition, we highlighted the associated limitations and provided recommendations for future studies.

2 | MATERIALS AND METHODS

2.1 | Protocol and focused questions

This systematic review was conducted per the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA).[20] The review was designed to answer the following questions: (a) What are the diagnostic and prognostic values of epigenetic biomarkers in OPMDs and HNSCC? and (b) What are the potential limitations associated with the current studies?

2.2 | Eligibility criteria

Studies published in the English language from 1947 to June 2023 and evaluated the role of any epigenetic biomarker in human malignancies were included according to the following inclusion criteria (a) involved cases with confirmed HNSCC and OPMDs through clinical and histopathological assessments, (b) collected samples utilising saliva, oral rinse, blood, or tissue biopsies, (c) provided a cohort of comparable controls in terms of the exposure and demographic details and (d) provided details about the employed molecular analysis assays.

Systematic reviews and meta-analyses, letters to the editors and case reports were excluded. Data derived from cell lines or animal models were also excluded.

2.3 | Search strategy and data extraction

MEDLINE by PubMed, Scopus, Embase Ovid and Web of Science databases were searched to identify potentially eligible studies using sensitive Medical Subject Headings (MeSH) (Table S1). No restrictions were involved on the date of publication. All results were exported to EndNote X9 (Clarivate, PA, USA). De-duplication was carried out by the automated procedure in EndNote (I.L.) and manually reviewed by two reviewers (J.T. and A.A.) who performed the manual deduplication.

Three independent authors (I.L., J.T. and A.A.) blindly evaluated the retrieved studies by reading their abstracts. Studies that were considered potentially eligible for inclusion were then assessed by full-text reading. Only studies that matched the pre-defined inclusion criteria were included. The authors (I.L., J.T., A.A. and O.K.) completed a calibration exercise before commencing the title and abstract's screening. The inter-rater agreement using Cohen's Kappa coefficient showed substantial agreement between investigators ($\kappa = 0.71$). Discrepancies in the assessment among the authors were resolved by discussion with a senior author (O.K.).

The assessors (I.L., J.T. and A.A.) independently utilised a standardised data collection form to extrapolate the following data from the included studies: (a) authors, publication year and country of publication; (b) patient age and gender; (c) lesion types and numbers (HNSCC or OPMDs), (d) number of samples, (e) study design and molecular assays, (f) epigenetic biomarkers and (g) outcomes. A senior author (M.I.) verified all data extracted by the three assessors (I.L., J.T. and A.A.).

2.4 | Risk of bias assessment

Two independent reviewers (I.L., J.T. and A.A.) assessed the quality of the included studies using the Newcastle–Ottawa quality assessment scale with some modifications due to the design of the studies by removing an item related to the response rate.[21] Accordingly,

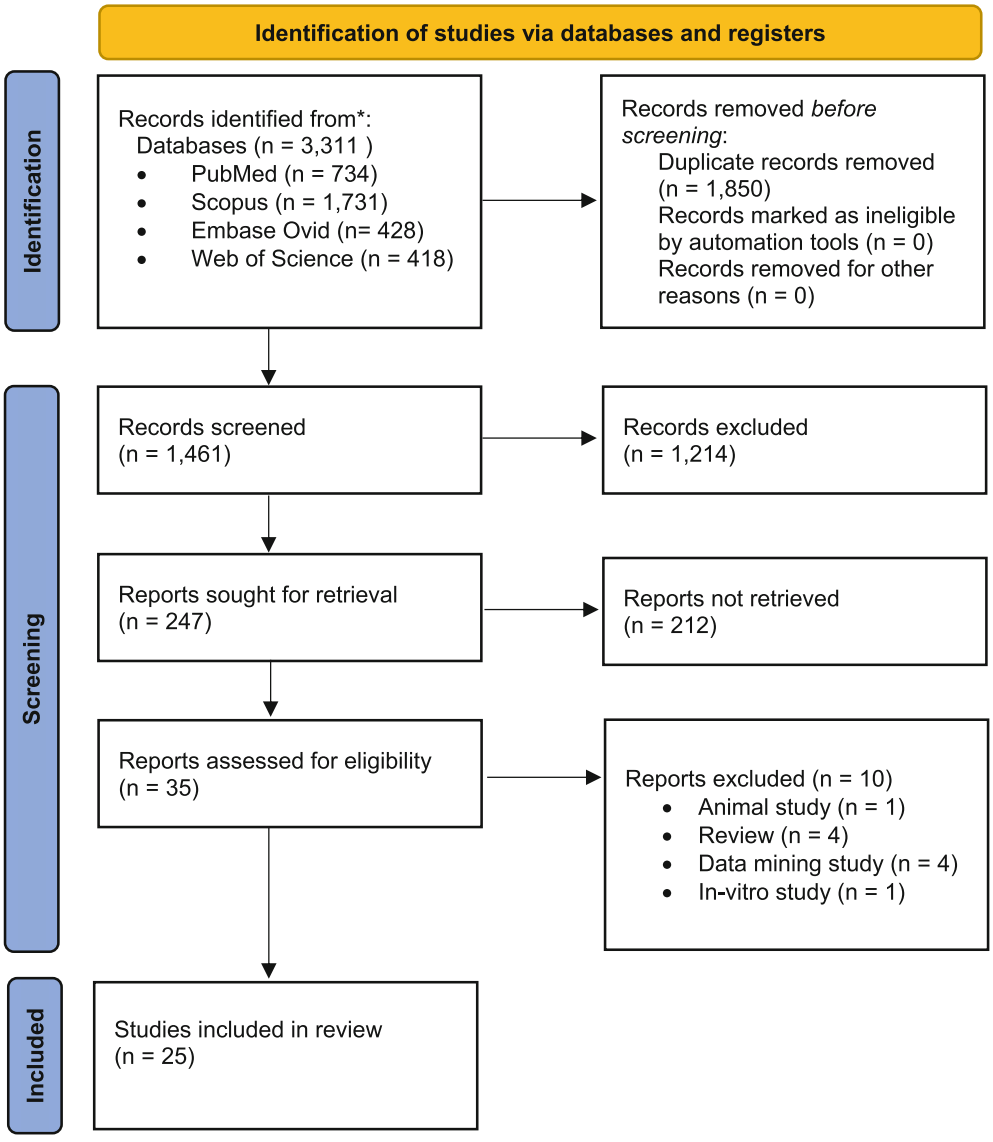

FIGURE 1 PRISMA flow chart of the screened, included and excluded studies.

TABLE 1 General characteristics of the included studies with detailed information about the subjects and sampling methods.

Authors (year)	Inclusion & exclusion criteria	Specimen type	The presence of controls	Total sample size	Subject breakdown	Age (years)	Gender (N) or M:Fe ratio	Follow-up	Anatomical subsite
Arantes et al. (2015)[12]	Inclusion only	Tissue (FFPE) + Saliva	Yes	80	OSCC: 40, Control saliva: 40	Experimental group: R (41–78) Control group: R (37–80)	90% M in both groups	No	Tongue, FoM, Other oral cavity sites
Biron et al. (2012)[23]	No	Tissue (F)	Yes (HPV vs. non-HPV)	44	OPSCC advanced stage: 44	p16 positive: Mean (57) p16 negative: Mean (53)	p16 positive: (M:Fe; 17:1), p16 negative: (M:Fe; 22:4)	No	Base of the Tongue, Tonsil
Chang et al. (2004)[24]	No	Fluid Rinse + Tissue (FFPE)	Yes	121	HNSCC: 31 Control (Nonsmoker/Non-drinker): 37 Control (Smokers/Drinkers): 22	Healthy Nonsmokers/ Non-drinkers: R (24–84) Healthy Smokers/ Drinkers: R (16–81) HNSCC: R (27–77)	Healthy Nonsmokers/ Non-drinkers: (M:Fe; 1:1.47) Healthy Smokers/ Drinkers: (M:Fe; 1:0.1) HNSCC: (M:Fe; 1:0.35)	No	Oral cavity, Oropharynx, Hypopharynx, Larynx
Chang et al. (2012)[25]	No	Tissue (F)	Yes	75	OSCC case: 25 Inverted papilloma: 25 Control: 25	SSC: R (31–79) Inverted papilloma: R (34–75) Normal mucosa: R (19–65)	OSCC: (M:Fe; 1:0.39) Inverted papilloma: (M: Fe; 1: 0.79) Normal Mucosa: (M:Fe; 1:0.47)	No	Sino-nasal region
Chen et al. (2013)[17]	No	Tissue (F)	No	215	OSCC case: 215 (Stage I: 33; Stage II/III/IV: 182)	63 < 50, 152 > 50	M (215), No Fe	Yes (8 years follow-up study)	NR
De Vos et al. (2017)[37]	No	Blood (Plasma)	Yes	598	HNSCC: 137 Control with benign disease: 170 Additional control: 48 Validation of the results, HNSCC 141, Control 102	NR	NR	NR	Tongue, Oral Cavity, Tonsil, Oropharynx, Larynx, Nasopharynx, Pharynx
Dillenburg et al. (2015)[16]	Yes	Tissue (F)	No	42	Atrophic/erosive OLP: 42 (21 had complete recovery; 13 partial clinical recovery; 8 nonresponsive to treatment)	>21	NR	Yes (8 weeks)	Buccal mucosa, tongue, Lips
Foy et al. (2015)[13]	No	Tissue (F)	Yes	148	Baseline: OSCC: 38; Control: 110 Discovery: non-OSCC: 12, OSCC: 12	NR	NR	Yes (OSCC: 2.15 years; non-OSCC: 7.64 years)	Oral Cavity
Franzmann et al. (2007)[40]	Yes	Saliva	Yes	171	HNSCC: 102 (40 Stage I-II, 62 Stage III-IV); Control: 69	NR	NR	Yes (18–>48 months)	Oral Cavity, Pharynx, Larynx, Upper aerodigestive tact

(Continues)

TABLE 1 (Continued)

Authors (year)	Inclusion & exclusion criteria	Specimen type	The presence of controls	Total sample size	Subject breakdown	Age (years)	Gender (*N*) or M:Fe ratio	Follow-up	Anatomical subsite
Gissi et al. (2020)[26]	Yes	Oral brushing specimen	Yes	129	OL case: 31; OLP case: 18 Previously treated OSCC: 26 Control: 54	Healthy subjects: Median (46.33) OL: Median (63.35) OLP: Median (61.36) rOSCC: Median (65.77)	Healthy Control (M:Fe): 1:1 OL (M:Fe): 1:1.2 OLP (M:Fe): 1:2 rOSCC (M:Fe): 1:1	Yes (every 3–6 months)	Oral Cavity
Guerrero-Preston et al. (2011)[41]	No	Tissue (FFPE) + Saliva	Yes	179	OSCC: 4; OL: 4; Control: 4	NR	NR	No	Oral Cavity, Pharynx, Larynx, Upper aerodigestive tact
Laytragoon-Lewin et al. (2010)[27]	No	Tissue (F)	Yes	41	HNSCC: 41; Control: 18	Median: 62	M (30), Fe (11)	No	Tongue, Tonsil, Pharynx.
Liu et al. (2012)[28]	No	Tissue (F) + Blood + Saliva	No	109	OP: 77; OSCC: 32	OP: Mean (56.1) OSCC: Mean (55)	OP: M (54.5%), Fe (45.5%) OSCC: M (59.4%), Fe (40.6%)	No	NR
Moreira et al. (2009)[29]	No	Tissue (F + FFPE)	Yes	26	OKC: 10; Control: 16	OKC: R (11–39), Dental follicle: R (16–28), Normal: R (17–26)	OKC: M (6), Fe (4) Dental follicle: M (5), Fe (5) Normal: M (3), Fe (3)	No	Dental follicle, Oral Mucosa.
Piscopo et al. (2006)[43]	Inc only	Cytobrush sample	Yes	43	OSCC: 23; OL: 20	OSCC Pts: 65.2 years mean/72.8 years OL Pts: 51.3/54.3 years mean	OSCC: M (14), Fe (9); OL: M (12), Fe (8)	No	Oral Cavity
Puttipanyalears et al. (2018)[30]	Inc only	Tissue (F) + oral rinse + oral swab	Yes	194	OSCC: 98; Control: 96	Oral rinse-healthy controls: Control group 1: Median (22), R (19–24), Control group 2: Median (22), R (19–32) Oral swab-OSCC: Median: (67), R (45–90) Oral rinse-OSCC:: OSCC group 1: Median (67), R (45–90), OSCC group 2: Median (57), R (22–92), OPC: Median (57), R (29–70)	OSCC: M (5), Fe (4) Oral rinse- healthy controls: Control group 1: M (17), Fe (16), Control group 2: M (25), Fe (29) Oral swab-OSCC group M (15), Fe (8) Oral rinse-OSCC: group 1: M (15), F (8), OSCC group 2: M (19), Fe (23), OPC: M (0), Fe (24)	No	Tongue, Lips, Gingiva.

TABLE 1 (Continued)

Authors (year)	Inclusion & exclusion criteria	Specimen type	The presence of controls	Total sample size	Subject breakdown	Age (years)	Gender (N) or M:Fe ratio	Follow-up	Anatomical subsite
Puttipanyalears et al. (2013)[31]	No	Tissue (F) + Blood (PBMC) + Oral rinse	Yes	249	OSCC: 88; Control: 161	Group 1: Normal: Mean (47.59), OSCC: Mean (64.33) Group 2: Normal Mean (48.28), OSCC: Mean (63.03) Group 3: Non-smoker: Mean (48.37), OSCC Mean (60.40), OSCC Low stage (I + II): Mean (63.79), OSCC High stage (III + IV): Mean (58.76)	Group 1: Normal M (6), FE (16); OSCC: M (5), Fe (4) Group 2: Normal M (14), Fe (17), OSCC: M (16), Fe (20) Group 3: Non-smoker M (12), (31), | OSC: M (21), Fe (22); OSCC Low stage (I + II): M (5), Fe (9) OSCC High stage (III + IV): M (16), Fe (13)	No	Oral Cavity
Righini et al. (2007)[32]	Yes	Tissue (FFPE) + Saliva	Yes	90	OSCC: 90 (68 newly diagnosed primary tumours; 22 second primary HNSCC)	Median: 57 R (33–74)	Control: M (20), Fe (10) HNSCC: M (77), Fe (13)	Yes (Median 564 days)	NR
Schröck et al. (2017)[38]	Yes	Blood (Plasma)	Yes	649	HNSCC: Training cohort (284), Testing cohort (141) Paired Controls: Training cohort (122), Testing cohort (102)	HNSCC Training cohort: Median 61, R (32–89) Control: Median 60, R (32–87) HNSCC Testing cohort: Median 63, R (37–93) Control: Median 62, R (36–86)	Training HNSCC: M (231), Fe (53) Control: M (95), Fe (27) Testing HNSCC: M (115), Fe (26) Control: M (71), Fe (31)	Yes (duration NR)	NR
Shen et al. (2019)[39]	NR	Blood (PBMC)	Yes	33	EOLP: 12 NEOLP: 11 Control: 10	EOLP: Mean (43.7), NEOLP: Mean (46.4), Control: Mean (43.7)	EOLP: M (3), Fe (9); NEOLP: M (3), Fe (8); Control: M (2), Fe (8)	No	NR
Sun et al. (2012)[42]	No	Saliva + Oral brush sample	Yes	197	HNSCC: 197 (saliva sample from exfoliating brush prospectively collected); 57 paired salivary rinses with/or exfoliating brush	Mean (56.5) R (29–87)	M (44) Fe (13)	No	Oral Cavity, Larynx, Pharynx.
Tawfik et al. (2011)[33]	No	Tissue (F)	Yes	49	HNSCC: 49 (Clinical Stage I: 8; Stage 2: 7; Stage 3: 9; Stage 4: 24)	Mean (57.85), R: 40–79)	M (45), Fe (4)	No	Oral Cavity, Larynx, Pharynx.

(Continues)

TABLE 1 (Continued)

Authors (year)	Inclusion & exclusion criteria	Specimen type	The presence of controls	Total sample size	Subject breakdown	Age (years)	Gender (N) or M:Fe ratio	Follow-up	Anatomical subsite
Temam et al. (2005)[34]	Yes	Tissue (FFPE), blood, oral brush	Yes	38	HNSCC single, untreated, early-stage (T1 or T2): 33 Control: 5	NR	NR	No	Oropharynx, Supraglottic Larynx, Hypopharynx.
Wagner et al. (2017)[35]	No	Tissue (FFPE)	Yes	84	Benign SGTs: 42, Malignant SGTs: 42 (PA: 33; WA: 9; AdCC: 22; MEC: 15; AcCC: 5)	Benign: Mean (54.9) Malignant: Mean (46.55)	Benign M (21), Fe (21) Malignant M (18), Fe (22)	No	Parotid gland, Submandibular gland, Palate.
Ya-Wei Chen et al. (2013)[36]	Yes	Tissue (F)	No	186	OSCC: 186 (Stage I: 32; Stage II: 75; Stage III: 34; Stage IV: 45)	Median 53.8 R (22–88)	M (158), Fe (28)	Yes (Median 48.4 months)	Buccal Mucosa, Tongue, Gingiva, Lip, FoM, Palate

Abbreviations: AcCC, acinic cell carcinoma; AdCC, adenoid cystic carcinoma; EOLP, erosive oral lichen planus; F, frozen; Fe, female; FFPE, formalin-fixed paraffin-embedded; FoM, floor of mouth; HNSCC, head and neck squamous cell carcinoma; HPV, human papilloma virus; M, male; MEC, mucoepidermoid carcinoma; N, number; NEOLP, non-erosive oral lichen planus; NR, not reported; OKC, oral keratocysts; OL, oral leukoplakia; OLP, oral lichen planus; OP, oral precancer; OPSCC, oral pharyngeal squamous cell carcinoma; OSCC, oral squamous cell carcinoma; PA, pleomorphic adenomas; PBMC, peripheral blood mononuclear cells; R, range; rOSCC, recurrent oral squamous cell carcinoma; SGT, salvary gland tumour; WA, warthin's tumour.

reported data in each study was assessed based on three main domains that include eight sub-domains, each sub-domain worth one point, making the total score for each study out of eight (Table S2). The main domains are (a) selection of the study subjects, (b) comparability of cases and controls in terms of the design and analysis and (c) ascertainment of the prespecified outcomes. Finally, the quality of each study was classified according to its final score into (a) high quality (8 points), (b) medium quality (6–7 points) and (c) low quality (<5 points).[22]

3 | RESULTS

3.1 | Results of database searches

A total of 1461 papers were retrieved for abstract screening out of 3311 results initially identified using the proposed search criteria. Of these, 35 studies were assessed by full-text reading, whereby 10 studies were deemed ineligible for inclusion (Figure 1). A general description of the included studies is shown in Table 1. Ten studies were excluded for the following reasons: review (four studies), animal-based study (one), in vitro based study (one) and four data-mining based studies.

3.2 | Descriptions of the included cases and methods of epigenetic biomarker analysis

The publication years of the included studies ranged from 2004 to 2020. A total of 3790 samples from the 25 studies were analysed, including 1392 OSCC samples, 731 HNSCC but not OSCC samples, 334 OPMD samples and 1333 as controls. Several types of OPMDs were reported in the studies; the most common was oral lichen planus with 83 samples, followed by oral leucoplakia in 55 cases. General characteristics of the included studies are listed in Table 1.

Of the included studies, tissue samples were the primary source to study the epigenetic changes in 17 studies[12,13,16,17,23–36] (Table 1), followed by six studies investigating the epigenetic biomarkers in blood samples.[28,31,34,37–39] Saliva[28,32,40–42] and oral brush cytology[26,30,34,42,43] were employed in five studies each, while two studies employed oral rinse in their design[30,31] (Table 1).

Polymerase chain reaction (PCR) was the most employed approach for epigenetic biomarker analysis in 16 studies,[12,13,24,26–30,32,34,37,38,40–43] followed by two studies that used a combination of immunohistochemistry (IHC) and PCR,[25,33] and two studies that used only immunohistochemistry (IHC) as the method of epigenetic biomarkers assessment.[17,35] Additionally, one study used PCR and phosphorimaging in the analysis[31] (Table 2) whilst a combination of IHC and imaging was used in another study.[23] Likewise, a combination of histology and immunofluorescence (IF),[16] and a combination of histone acetylation detection, PCR and cytokine array were used in one study.[39] Finally, IHC and tissue microarray were employed in one study[36] (Table 2).

TABLE 2 Key epigenetic findings in the included studies and the methods of analysis.

Authors/Year	Discovery phase method	Validation method	House-keeping genes	Considering the progression of OPMD to SCC	Key epigenetic biomarkers studied	Key epigenetic expression findings (head and neck cancer sample vs. normal tissue sample)
Arantes et al. (2015)[12]	Previous studies	Q-MSP: quantitative methylation-specific PCR analyses (Applied Biosystem)	ACTB (Actin beta)	No	Methylation status of CCNA1, DAPK, DCC, CDH1, TIMP3, MGMT, HIC1, AIM1	CCNA1: ↑ methylation DAPK: ↑ methylation CDH1: frequently methylated in OSCC and normal tissue TIMP3: ↑ methylation MGMT: ↑ methylation (not as frequently methylated in HNSCC tissue) AIM1: ↑ methylation (not as frequently methylated in HNSCC tissue) HIC1: frequently methylated in OSCC and normal tissue *p*-value not specified
Biron et al. (2012)[23]	Literature	Immunohistochemistry and Imaging	NR	No	Methylation status of histones—H3K4me3, H3K9me3, H3K27me3, H4K20me1, H4k20me3	H3k4me3: moderate in both p16 positive and negative H3K9me3: low in both p16 positive and negative H3K27me3: higher in p16 positive and low in p16 negative H4K20me1: higher in p16 positive and low in p16 negative H4K20me3: higher in p16 negative and low in p16 negative The study did not compare HNSCC and normal tissue but rather p16 positive HNSCC and p16 negative HNSCC *p*-value not specified
Chang et al. (2004)[24]	Literature	Q-MSP: quantitative methylation-specific PCR analyses (Applied Biosystem)	NR	No	Methylation status of p15	p15: ↑ methylation when HNSCC individuals are compared to healthy individuals who do not smoke or drink but ↓ when compared to healthy individuals who smoke and drink. (*p*-value not specified) ↑ methylation in healthy smokers/drinkers vs. non-smokers and non-drinkers ($p < 0.05$)
Chang et al. (2012)[25]	Literature	QRT-PCR IHC MS-PCR: methylation-specific PCR (Applied Biosystem)	Genomic DNA extracted from nasopharyngeal carcinoma as positive control	No	Methylation status of DLEC1	DLEC1: ↑ methylation leading to repression of the gene ($p < 0.01$)
Chen et al. (2013)[17]	Literature	Tissue microarray-based immunohistochemical staining analysis and antibodies	NR	No	Histone phosphorylation proteins: ARK2 Histone methylation proteins: G9a, EZH2, SUV39H1	ARK2: ↑ expression, especially in cases with worse outcomes, has an association with a 3-year survival rate ($p = 0.005$), staging ($p = 0.006$), and T status ($p = 0.026$). G9a: ↑ expression, associated with worse grading ($p = 0.026$) EZH2: ↑ expression, associated with lymph node metastasis ($p = 0.016$) SUV39H1: ↑ expression, associated with staging ($p = 0.009$)

(Continues)

TABLE 2 (Continued)

Authors/Year	Discovery phase method	Validation method	House-keeping genes	Considering the progression of OPMD to SCC	Key epigenetic biomarkers studied	Key epigenetic expression findings (head and neck cancer sample vs. normal tissue sample)
De Vos et al. (2017)[37]	Training cohort	Triplex quantitative methylation specific PCR: Q-MSP (not specified) Quasi-digital PCR	ACTB (Actin beta)	No	Methylation status of SEPT9, SHOX2	SEPT9: ↑ methylation SHOX2: ↑ methylation, showed a higher methylation pattern in normal tissue (higher background methylation) too thus a higher cut-off is needed to distinguish between healthy and HNSCC samples
Dillenburg et al. (2015)[16]	Previous studies	Histology and Immunofluorescence of OLP tissue	NR	No	Histone modification patterns: H3K9ac, phosphorylation of γH2AX	H3K9ac: ↑ expression especially in patients associated with recurrence or patients who do not respond to treatment ($p < 0.001$). Furthermore, in these same group of patients, higher expression increases the likelihood of double-strand break Phosphorylation of γH2AX: ↑ expression especially in inpatients associated with recurrence or patients who do not respond to treatment
Foy et al. (2015)[13]	Discovery screening cohort	Methylation-specific PCR and pyrosequencing (Biotage/Qiagen)	NR	Yes, compared whether or not increased methylation status led to an increased likelihood of OPL transformation in OSCC	Methylation status of AGTR1, FOX12, HOXA9, PENK, ZIC1, LINE1	AGTR1: ↑ methylation ($p = 0.0004$) FOX12: ↑ methylation ($p = 0.0021$) PENK: ↑ methylation ($p < 0.0001$) LINE1: ↓ methylation ($p < 0.0001$) HOXA9: ↑ methylation ($p > 0.05$) ZIC1: ↑ methylation ($p > 0.05$) In patients who develop OSCC in comparison to those who didn't
Franzmann et al. (2007)[40]	Previous pilot study	MSP: methylation-specific PCR (not specified)	Beta-Actin	No	Methylation status of CD44	CD44: ↑ methylation in HNSCC patients with low solCD44 compared to healthy individual ($p < 0.05$)
Gissi et al. (2020)[26]	Previous studies	Q-MSP: quantitative methylation-specific PCR analyses (not specified)	NR	No	Methylation status of ZAP70, ITGA4, KIF1A, PARP15, EPHX3, NTM, LRRTM1, FLI1, MIR193, LINC00599, MIR296, TERT, GP1BB	ZAP70: ↑ methylation ITGA4: ↑ methylation KIF1A: ↑ methylation PARP15: ↑ methylation EPHX3: ↑ methylation NTM: ↑ methylation LRRTM1: ↑ methylation FLI1: ↑ methylation MIR193: ↑ methylation LINC00599: ↑ methylation MIR296: ↓ methylation TERT: ↓ methylation GP1BB: ↓ methylation *p*-value not specified

TABLE 2 (Continued)

Authors/Year	Discovery phase method	Validation method	House-keeping genes	Considering the progression of OPMD to SCC	Key epigenetic biomarkers studied	Key epigenetic expression findings (head and neck cancer sample vs. normal tissue sample)
Guerrero-Preston et al. (2011)[41]	Discovery screening cohort	Q-MSP: quantitative methylation-specific PCR analyses (Applied Biosystem)	ACTB (Actin beta)	No	Methylation status of EDNRB, HOXA9, GATA4, NID2, MCAM, KIF1A, DCC, CALCA	EDNRB: ↑ methylation HOXA9: ↑ methylation GATA4: ↑ methylation NID2: ↑ methylation MCAM: Promoter methylation did not differ much between tumour tissue and normal mucosa KIF1A: ↑ methylation DCC: ↑ methylation CALCA: Promoter methylation did not differ much between tumour tissue and normal mucosa *p*-value not specified
Laytragoon-Lewin et al. (2010)[27]	NR	Q-MSP: quantitative methylation-specific PCR analyses (Not specified)	Beta-Actin	No	Methylation status of p16, DAPK, RASSF1A	p16: ↑ methylation DAPK: ↑ methylation RASSF1A: ↑ methylation *p*-value not specified Increase methylation is in comparison to normal mucosal tissue, at least 5 cm away from the macroscopic tumour edge, biopsy from the same HNSCC patient.
Liu et al. (2012)[28]	Literature	Q-MSP: quantitative methylation-specific PCR analyses (Roche Applied Science, Mannheim, Germany)	ACTB (Actin beta)	Yes	Methylation status of DAPK	DAPK: ↑ methylation (in tissue sample $p = 0.004$, blood sample $p = 0.007$, salivary sample, $p = 1$) Increase methylation is increased in OSCC patients in comparison to OP patients (the study does not include a comparison to normal tissue)
Moreira et al. (2009)[29]	Literature	Methylation-specific PCR (Eppendorf AG)	NR	No	Methylation status of p16, p21, p27, p53, RB1	p16: Slight ↑ methylation in OCK tissues p21: ↑ Methylation in OCK tissues p27: No methylation in normal or OCK tissues but present in dental follicles RB1: No methylation in normal or OCK tissues but present in dental follicles p53: Methylation was not seen in any of the samples *p*-value not specified
Piscopo et al. (2006)[43]	Literature	RT-PCR (Applied Biosystem)	DEPC-treated water	Yes	Level of H3 and H3.3 histone gene expression	H3: ↑ expression ($p > 0.2$) H3.3 histone: ↑ expression ($p > 0.2$)
Puttipanyalears et al. (2018)[30]	Methylation microarray data deposited in the National Centre for Biotechnology Information (NCBI)	Methylation-specific PCR (Applied Biosystem) and pyrosequencing (Qiagen)	NR	No	Methylation status of site-specific TRH	TRH: ↑ methylation in OSCC tissue sample ($p < 0.001$) ↑ methylation in OSCC oral rinse sample ($p < 0.001$) ↑ methylation in OSCC oral swab sample ($p = 0.0012$)

(Continues)

TABLE 2 (Continued)

Authors/Year	Discovery phase method	Validation method	House-keeping genes	Considering the progression of OPMD to SCC	Key epigenetic biomarkers studied	Key epigenetic expression findings (head and neck cancer sample vs. normal tissue sample)
Puttipanyalears et al. (2013)[31]	Literature	COBRA Alu is performed using PCR and quantified using a phosphor-imager (ImageQuant software)	NR	No	Methylation status of Alu	Alu: ↓ methylation in a tissue sample ($p < 0.0002$), the difference in methylation was not significant in the blood sample, ↓ methylation in oral rinse sample ($p < 0.0001$)
Righini et al. (2007)[32]	NR	Methylation-specific PCR (FastStart DNA polymerase)	MyoD	No	Methylation status of TIMP3, ECAD, p16, MGMT, DAPK, RASSF1	TIMP3: ↑ methylation, $p < 0.001$ ECAD: ↑ methylation, $p < 0.001$ p16: ↑ methylation, $p < 0.001$ MGMT: ↑ methylation, $p < 0.001$ DAPK: ↑ methylation, $p < 0.001$ RASSF1: ↑ methylation, $p < 0.001$
Schröck et al. (2017)[38]	Training cohort	Triplex quantitative methylation-specific PCR: Q-MSP (Life Technologies Corporation)	ACTB (Actin beta)	No	Methylation status of SHOX2, SEPT9	SHOX2: ↑ methylation in HNSCC patients, similarly, HNSCC patients who are positive for this methylation are associated with a higher risk of death ($p < 0.001$), worse tumour grading and tumour nodal category ($p < 0.001$) SEPT9: ↑ methylation in HNSCC patients, similarly, HNSCC patients who are positive for this methylation are associated with a higher risk of death ($p < 0.024$), worse tumour grading and tumour nodal category ($p < 0.001$)
Shen et al. (2019)[39]	Literature	Global histone H3/h4 acetylation detection (Epigentek), Epiquik HDAC Activity Assay Kit (Epigentek), Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), Cytokine Array (RayBiotech)	GAPDH	No	Histone modification patterns: H3 acetylation, H4 acetylation Presence of HDAC6, HDAC7	H3 acetylation: ↓ expression ($p = 0.0116$), negatively correlated with amount IL-4 ($p < 0.005$), IL-16 ($p = 0.042$), TIMP-2 (0.042) H4 acetylation: No difference in acetylation level was found between the different groups ($p > 0.05$) HDAC6: ↑ expression ($p = 0.01$), positively correlated with MCP-1 production ($p = 0.019$) HDAC7: ↑ expression ($p = 0.01$), positively correlated with MIP-1a production ($p = 0.037$)
Sun et al. (2012)[42]	Previous study	Q-MSP: quantitative methylation-specific PCR analyses (Perkin-Elmer Applied Biosystem)	ACTB (Actin beta)	No	Methylation status of p16, CCNA1, DCC, TIMP3, MGMT, DAP, MINT31	p16: ↑ methylation CCNA1: ↑ methylation DCC: ↑ methylation TIMP3: ↑ methylation MGMT: ↑ methylation DAP: ↑ methylation MINT31: ↑ methylation *p*-value not given

TABLE 2 (Continued)

Authors/Year	Discovery phase method	Validation method	House-keeping genes	Considering the progression of OPMD to SCC	Key epigenetic biomarkers studied	Key epigenetic expression findings (head and neck cancer sample vs. normal tissue sample)
						The study was mainly focused on the difference between using or not using an oral brush to collect saliva samples and its impact on the ability to detect hypermethylation (which has been found to have no difference in collecting saliva samples with an exfoliating brush or without) but did mention that hypermethylation of the 7 genes above is higher in HNSCC samples
Tawfik et al. (2011)[33]	Literature	IHC staining (Clone ES05) Methylation Specific PCR (Techne Inc)	NR	No	Methylation status of hMLH1	hMLH1: ↑ methylation in HNSCC tissue as well as surrounding normal tissue adjacent to methylation-positive tumour *p*-value not specified
Temam et al. (2005)[34]	Previous studies	Methylation-specific PCR (Applied Biosystem) GeneScan analysis (Applied Biosystem)	NR	No	Methylation status of p16 and microsatellite instability of UT5085	p16: ↑ methylation UT5085: ↑ MSI *p*-value not specified
Wagner et al. (2017)[35]	Literature	Immunohistory	NR	No	Histone modification patterns: acetyl-H3 (lys9)	acetyl-H3 (lys9): ↓ expression in malignant salivary gland tumour (SGT) ($p = 0.04$) This is in comparison to benign SGT
Ya-Wei Chen et al. (2013)[36]	Literature	Immunohistochemistry and Tissue array	Histone H3	No	Histone modification patterns: H3K4ac, H3K18ac, H3K4me3, H3K9me3, H3K27me3	H3K18ac: ↑ expression, positively correlated with OSCC stage ($p = 0.003$), T-status ($p = 0.003$), PNI ($p < 0.001$) H3K27me3: ↑ expression, positively correlated with OSCC stage ($p = 0.002$), T-status ($p = 0.031$), N-status ($p = 0.01$) and PNI ($p = 0.001$) H3K4ac: ↓ expression, negatively correlated with OSCC stage ($p < 0.001$), T-status ($p < 0.001$), N-status ($p = 0.003$) and PNI ($p < 0.001$) H3K9me3: No statistical correlation was found between histone modification and stage, T-status, N-status and PNI H3K4me3: No statistical correlation was found between histone modification and stage, T-status, N-status and PNI

Abbreviations: NR, not reported; OPL, oral premalignant lesion; OSCC, oral squamous cell carcinoma.

TABLE 3 Studies that assessed the accuracy of using epigenetic biomarkers in HNSCC and OPMDs in diagnosis.

Authors/Year	Epigenetic biomarker investigated	Sensitivity (%)	Specificity (%)	ROC	AUC
Arantes et al. (2015)[12]	AIM1, CCNA1, CDh1, DAPK, DCC, HIC1, MGMT, TIMP3	OSCC vs. Control TIMP3 = 82.5 (CI: 0.68–0.91) DCC = 70 (CI: 0.54–0.81) DAPK = 80 (CI: 0.65–0.89) CCNA1 = 30.0 (CI: 0.18–0.45) A1M1 = 17.5 (CI: 0.08–0.31) MGMT = 30 (CI: 0.18–0.45) CDH1 = 95 (0.83–0.98) H1C1 = 95 (0.83–0.98)	OSCC vs. Control TIMP3 = 100 (CI: 0.91–1.00) DCC = 95 (CI: 0.83–0.98) DAPK = 82.5 (CI: 0.68–0.91) CCNA1 = 100 (CI: 0.91–1.00) A1M1 = 100 (CI: 0.91–1.00) MGMT = 85 (CI: 0.70–0.92) CDH1 = 20 (0.10–0.34) H1C1 = 0 (0.00–0.08)	Yes	TIMP3 = 0.913 DCC = 0.825 DAPK = 0.813 CCNA1 = 0.638 A1M1 = 0.587 MGMT = 0.575 CDH1 = 0.575 H1C1 = 0.475
De Vos et al. (2017)[37]	SEPT9, SHOX2	HNSCC vs. Control Absolute Quantification Training Cohort SEPT9 = 61% SHOX2 = 55% Mean SEPT9/SHOX2 = 64% Testing Cohort SEPT9 = 54% SHOX2 = 43% SEPT9/SHOX2 = 49%	Absolute Quantification Training Cohort SEPT9 = 90% SHOX2 = 90% Mean SEPT9/SHOX2 = 90% Testing Cohort SEPT9 = 89% SHOX2 = 94% SEPT9/SHOX2 = 90%	Yes	Absolute Quantification Training Cohort SEPT9 = 0.80 (0.74–0.85) SHOX2 = 0.80 (0.75–0.86) Mean SEPT9/SHOX2 = 0.83 (0.78–0.88) Testing Cohort SEPT9 = 0.74 (0.68–0.80) SHOX2 = 0.78 (0.72–0.83) SEPT9/SHOX2 = 0.80 (0.74–0.85)
Franzmann et al. (2007)[40]	solCD44, CD44 methylation	HNSCC vs. Control 12 ng/mL solCD44 test = 62% 10.5 ng/mL solCD44 test = 70%	HNSCC vs. Control 12 ng/mL solCD44 test = 88% 10.5 ng/mL solCD44 test = 75%	Yes	NR
Guerrero-Preston et al. (2011)[41]	EDNRB, HOXA9, GATA4, NID2, KIF1A, DCC	Prevalence Screen HNSCC vs. Control HOXA9 = 85% NID2 = 87% Diagnostic Panel OSCC vs. OPSCC vs. Control HOXA9 = 63% NID2 = 72% OSCC vs. Control HOXA9 = 75% NID2 = 87%	Prevalence Screen HNSCC vs. Control HOXA9 = 97% NID2 = 95% Diagnostic Panel OSCC vs. OPSCC vs. Control HOXA9 = 53% NID2 = 21% OSCC vs. Control HOXA9 = 53% NID2 = 21%	Yes	Prevalence Screen HNSCC vs. Control HOXA9 = 0.95 NID2 = 0.91 Diagnostic Panel OSCC vs. OPSCC vs. Control HOXA9 = 0.65 NID2 = 0.57 OSCC vs. Control HOXA9 = 0.75 NID2 = 0.73
Liu et al. (2012)[28]	DAPK	OP vs. OSCC DAPK (tissue) = 46.9% DAPK (blood) = 52.2% DAPK (tissue) (blood) = 60.9%	OP vs. OSCC DAPK (tissue) = 80.5% DAPK (blood) = 86.6% DAPK (tissue) (blood) = 71.6%	Yes	OP vs. OSCC DAPK (tissue) = 0.66 (CI: 0.54–0.78) DAPK (blood) = 0.68 (CI: 0.54–0.81) DAPK (tissue) (blood) = 0.70 (CI: 0.56–0.83)
Puttipanyalears et al. (2018)[30]	cg01009664 of TRH	OSCC vs. Control Oral Rinse TRH: 86.15% (CI: 75.34–93.47)	OSCC vs. Control Oral Rinse TRH: 89.66% (CI: 81.27–95.16)	Yes	OSCC vs. Control Oral Rinse TRH: 0.93 (CI: 0.88–0.97) ($p < 0.001$)

TABLE 3 (Continued)

Authors/Year	Epigenetic biomarker investigated	Sensitivity (%)	Specificity (%)	ROC	AUC
		Oral Swab TRH: 91.3% (CI: 71.96–98.93) OPC vs. Control (OR) TRH: 82.61% (CI: 61.22–95.05)	Oral Swab TRH: 84.85% (CI: 68.10–94.89) OPC vs. Control (OR) TRH: 92.59% (CI: 82.11–97.94)		Oral Swab TRH: 0.97 (CI: 0.93–1.01) ($p = 0.0012$) OPC vs. Control (OR) TRH: 0.88 (CI: 0.77–0.98)
Puttipanyalears et al. (2013)[31]	Alu Methylation	OSCC vs. Control mC: 60% mCmC: 66.7% Combination 86.86%	OSCC vs. Control mC: 78.6% mCmC: 73.8% Combination 56.68%	Yes	OSCC vs. Control mC = 0.736 mCmC = 0.773 ($p < 0.0001$)
Schröck et al. (2017)[38]	SEPT9 and SHOX2 methylation in ccfDNA	OSCC vs. Control SEPT9 = 57% SHOX2 = 50% SHOX2 + SEPT9 = 59%	OSCC vs. Control SEPT9 = 95% SHOX2 = 95% SHOX2 + SEPT9 = 96%	Yes	OSCC vs. Control SEPT9 = 0.79 (CI: 0.74–0.85) SHOX2 = 0.80 (CI: 0.75–0.85) SHOX2 + SEPT9 = 0.83 (0.78–0.88) ($p < 0.001$)

Abbreviations: HNSCC, head and neck squamous cell carcinoma; NR, not reported; OP, oral precancer; OPSCC, oral pharyngeal squamous cell carcinoma; OSCC, oral squamous cell carcinoma.

3.3 | Descriptions of the investigated epigenetic biomarkers

Several epigenetic markers were analysed for their diagnostic and prognostic values for the HNSCC and the OPMDs. Of the included studies, 18 investigated DNA methylation,[12,13,24–34,37,38,40–42] and seven studies considered histone modifications in their design[16,17,23,35,36,39,43] (Table 2).

H3[16,23,35,36,39,43] and p16[23,27,29,32,34,42] were the most common epigenetic biomarkers investigated in six studies each, followed by DAPK[12,27,28,32] in four studies. While TIMP3 was analysed in three reports.[12,32,42] DCC,[41,42] SEPT9,[37,38] SHOX2,[37,38] HoxA9,[13,41] RASSF1A,[27,32] MGMT[32,42] and CCNA1[12,42] were investigated in two studies each. The following groups of epigenetic biomarkers were investigated in one study each; (CDH1, HIC1 and AIM1),[12] (ARK2, G9a, EZH2 and SUV39H),[17] (AGTR1, FOXI2, PENK, IC1 and LINE1),[13] and (HDAC6, HDAC7).[39] Other investigated biomarkers that were investigated in one study each are p15,[24] DLEC1,[25] CD44,[40] TRH,[30] Alu,[31] ECAD,[32] DAP,[42] Mint31,[42] hMLH1[33] and UT5085.[34] The methylation statuses of ZAP70, ITGA4, KIF1A, PARP15, EPHX3, NTM, LRRTM1, FLI1, MIR193, LINC00599, MIR296, TERT and GP1BB were investigated in one study.[26] Likewise, the methylation statuses of (EDNRB, HOXA9, GATA4, NID2, MCAM, KIF1A, DCC and CALCA),[41] and (p21, p27, p53, RB1)[29] were assessed in a single study each (Table 2).

3.4 | Diagnostic biomarkers accuracy

Of the included studies, eight provided details concerning the diagnostic accuracy of epigenetic biomarkers in detecting HNSCCs[12,28,30,31,37,38,40,41] (Table 3). However, the prognostic utility of these biomarkers in HNSCC was not investigated, while only one study included OPMDs in its design but without specifying their diagnosis.[28] Noteworthy, out of 20 biomarkers that were assessed for their diagnostic utilities, only two biomarkers were twice evaluated in two studies (SEPT9 and SHOX2).[37,38] In contrast, other biomarkers were assessed once, which hindered the ability to evaluate the utility of these biomarkers in the studies (Table 3).

The associated area under the curve (AUC) for the differentiation between HNSCCs and healthy tissues ranged between 0.475 and 0.95. Biomarkers associated with high AUC values are TIPM3, DCC, DAPK, SEPT9, SHOX9, HOXA9 and TRH. On the contrary, the following biomarkers showed relatively low AUC values: H1C1, CDH1, MGMT and A1M1 (Table 3).

3.5 | Risk of bias assessment

Based on the adopted Newcastle–Ottawa quality assessment scale, none of the studies was classified as high quality. Nine studies were associated with a moderate level of quality,[12,23,26,28,35,37–40] while 16 were considered low-quality studies.[13,16,24,25,27,29–34,36,41–43] Eight

studies included controls matched in age and other confounders with the diseased individuals.[12,23,26,28,37–40] Only one study provided complete details about the recruitment methods of the controls.[26] A complete list of studies with detailed information about the risk of bias assessment is included in Table S2.

4 | DISCUSSION

Various epigenetic biomarkers, in general, and DNA methylation, in particular, have been extensively published in the literature to determine their predictive and diagnostic values in head and neck tumorigenesis. However, unlike other malignancies such as colorectal cancers where minimally invasive tools based on epigenetic biomarkers are commercially available to help screen high-risk patients,[44] the application relevant to HNSCCs is yet to be adopted. Previous reviews were conducted in this field but only focused on the diagnostic and prognostic values of salivary DNA methylation in HNSCCs.[22] Therefore, our review was designed to assess the utility of epigenetic biomarkers, regardless of their type, in the management of cases with HNSCCs and OPMDs, which, to the best of our knowledge, is the first of its kind in the literature.

The most extensively studied epigenetic modification in the HNSCC is aberrant DNA methylation. We found a gap in assessing the pathogenesis of histone modification patterns in head and neck malignancies. Additionally, most papers demonstrated the robustness of using epigenetic biomarkers in diagnosing and differentiating between HNSCC of different grades. However, none was conducted to depict the association between these biomarkers and the likely course of HNSCC progression. This, in turn highlights an urgent need to reconsider this field by adequately designing and conducting longitudinal studies that follow large cohorts over time.

Aberrant methylations of p16, DAPK and TIMP3 are among the most extensively investigated DNA methylation events in head and neck tumorigenesis. P16 plays a significant role in controlling cell growth, and it has been reported that p16 methylation might be implicated in early events of transformation to head and neck malignancies.[27] In support of that, methylation of p16 has been found in immortalised non-tumour and tumour-derived cell lines. Therefore, it has been proposed that p16 methylation in distant mucosal regions may explain the high recurrence rates of secondary tumours in HNSCC patients.[27] Furthermore, a previous study revealed a significant increase in the rate of progression of oral epithelial dysplasia to oral squamous cell carcinoma among p16 methylation-positive patients in comparison to p16 methylation-negative ones.[45]

Death-associated protein kinase (DAPK), on the other hand is a tumour suppressor gene that mediates cell death of INF-Y-induced apoptosis.[32] Promoter hypermethylation of DAPK, which in turn leads to suppression of the expression of DAPK, is implicated in the pathogenesis of several human cancers, including head and neck malignancies.[32,46] A previous meta-analysis revealed that DAPK promoter methylation is significantly associated with HNSCC with a pooled odds ratio of 3.96.[46] Moreover, it has been reported in the literature that higher DAPK hypermethylation is significantly associated with lymph node metastasis among patients with HNSCC.[47]

The tissue inhibitor of metalloproteinase-3 (TIMP3) is a member of the TIMP family and plays a significant role in promoting apoptosis and inhibiting migration and invasion in various human malignancies.[48] A study has found that aberrant methylation of TIMP3 is associated with poor prognosis among patients with HNSCC by significantly increasing the second primary tumours' development rate.[49] Another paper showed that DAPK and TIMP3 were hypermethylated in almost 90% of clinically T1 and T2 OSCC cases.[12] Interestingly, it has also been reported that hypermethylation of TIMP3 detected utilising salivary rinse is an independent prognostic indicator of local recurrence-free survival rate.[50]

Unlike DNA methylation patterns, the prognostic utility of histone modifications in HNSCC is not well-investigated.[36] Our review found that seven studies included histone modifications in their design, and only two prospectively followed patients. Nonetheless, it has been shown that modifications in core histone H3 may play a significant role in the progression to HNSCC and poor prognosis.[36] The low level of H3K4ac was significantly associated with tumour stage, nodal invasion and perineural invasion.[36] Furthermore, another study found that ARK2 expressions are associated with 3-year survival and tumour stage.[17] In OPMDs, it has been found that the increased expression of H3 acetylation (particularly H3k9a) is correlated with oral lichen planus patients who had a poor response to therapy and a high recurrence rate.[16]

It is also worth noting that it is challenging to compare data among studies due to the highly variable measures employed in these studies. The high risk of bias among studies should be considered, especially where 16 out of 25 were associated with low quality, and none were considered high quality. The significant risk of bias among the included studies was related to the selection of the study subject domain, where only one provided details about the selection of the controls. This was also found by previous reviews that a relatively high risk of bias exist amongst studies concerning DNA methylation in head and neck malignancies due to an unclear subject selection process.[22]

Our systematic review has also demonstrated the capacity to use liquid and minimally invasive brush biopsies to detect and manage OPMDs and HNSCC. Saliva, as a reliable source of molecular biomarkers has been extensively investigated in the literature.[3,18] In the head and neck region, saliva proved superior over other body liquids for being preferentially enriched with tumour DNA from the head and neck region.[18] Moreover, a high concordance rate ($\kappa = 0.833$) was reported between tissue and saliva samples in terms of detecting and quantifying epigenetic biomarkers in head and neck cancers. In contrast, this concordance rate became excellent ($\kappa = 1.0$) for oral cavity tumours.[32] Likewise, strong correlations of gene promoter hypermethylation were demonstrated between saliva samples collected with and without an exfoliative brush, highlighting the great potential of employing saliva to detect epigenetic changes in the head and neck region.[42]

Several limitations are potentially associated with this systematic review. First, including only studies published in English highlights the

possibility of missing reports published in other languages. Second, the conducted risk of bias assessment showed that the included studies were associated with medium and low qualities, which may hinder the reliability of the outcomes of some studies. Finally, comparison between studies and providing conclusive results was not feasible due to the heterogeneity among studies in terms of sampling and analysis methods.

In conclusion, the current literature shows promising diagnostic value for epigenetic biomarkers in head and neck tumorigenesis. Nonetheless, the limited number of patients and the absence of standardised criteria to recruit subjects and report outcomes may hinder the ability to compare between studies. Further multi-centre studies that followed patients prospectively are warranted to provide conclusive results.

AUTHOR CONTRIBUTIONS

Isaac Lim: Data curation; formal analysis; writing – original draft. **Jade Tan:** Data curation; formal analysis; writing – original draft. **Anneka Alam:** Data curation; methodology; writing – original draft. **Majdy Idrees:** Methodology; validation; writing – original draft; writing – review and editing. **Peter A. Brenan:** Methodology; writing – review and editing. **Ricardo Della Coletta:** Methodology; writing – review and editing. **Omar Kujan:** Conceptualization; investigation; project administration; supervision; validation; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data available from the corresponding author upon request.

ORCID

Majdy Idrees https://orcid.org/0000-0001-7206-5389
Omar Kujan https://orcid.org/0000-0002-5951-8280

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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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