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Expert Review of Molecular Diagnostics

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iero20

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To cite this article: Nadisha S. Piyarathne, R.M.S.G.K Rasnayake, Randilini Angammana, Pushpika Chandrasekera, Samantha Ramachandra, Manjula Weerasekera, Surangi Yasawardene, Rasha Abu-Eid, J. A. P Jayasinghe & Ekta Gupta (2021) Diagnostic salivary biomarkers in oral cancer and oral potentially malignant disorders and their relationships to risk factors – A systematic review, Expert Review of Molecular Diagnostics, 21:8, 789-807, DOI: 10.1080/14737159.2021.1944106

To link to this article: https://doi.org/10.1080/14737159.2021.1944106

Published online: 12 Jul 2021.

EXPERT REVIEW OF MOLECULAR DIAGNOSTICS
2021, VOL. 21, NO. 8, 789–807
https://doi.org/10.1080/14737159.2021.1944106

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REVIEW

Diagnostic salivary biomarkers in oral cancer and oral potentially malignant disorders and their relationships to risk factors – A systematic review

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ABSTRACT

Introduction: Oral squamous cell carcinoma (OSCC) and oral potentially malignant disorders (OPMD) are a significant health burden globally. Smoking, alcohol, and betel quid are the main risk factors. Lack of screening methods has been highlighted as a significant challenge in management. Salivary biomarkers are proposed as noninvasive diagnostic tools. The aim of this systematic review was to study salivary biomarkers reported in OSCC and OPMD. Specific objectives were to select a salivary biomarker panel suitable for early detection of OSCC and OPMD and to assess relationships between salivary biomarkers and risk factors.

Methods: Electronic literature search was conducted in academic databases (Scopus, Medline, Embase and Web of Science) without any restrictions. Following calibration, two blinded reviewers screened the studies and extracted data. A risk of bias assessment was conducted using Newcastle Ottawa scale. 295 studies were included with descriptive data analysis.

Expert opinion: A salivary biomarker panel including Interleukin (IL) 1β, IL6, and IL8 was selected for OSCC and OPMD. Reported relationships between salivary biomarkers and risk factors are discussed and research gaps are highlighted. Future research should be directed to assess potential salivary biomarkers and their relationships to risk factors in order to understand the biomarker's role in disease initiation.

ARTICLE HISTORY
Received 15 April 2021
Accepted 14 June 2021

KEYWORDS
Oral squamous cell carcinoma; oral potentially malignant disorders; risk factors; salivary biomarkers

1. Introduction

Oral cancer is a significant public health problem worldwide. More than 90% of oral cavity malignancies are oral squamous cell carcinomas (OSCC) [1]. Some patients develop OSCC from clinically distinguishable pre-cancer stage. These conditions are collectively identified as oral potentially malignant disorders (OPMD). OPMD are defined as clinical presentations that carry an increased risk to develop into OSCC [2]. Common OPMD conditions are leukoplakia, erythroleukoplakia, oral lichen planus, and oral submucous fibrosis.

According to the global health statistics, lip and oral cavity cancers reported more than 177,000 deaths and accounted for more than 350,000 new cases in the year 2020 [3]. The worldwide prevalence of OPMD was estimated as 4.47% [4]. More than two-third of OSCC were reported from Asia [5]. In 2012, OSCC was the 12th common cancer type in Asia; in 2018, it had advanced to the 11th position demonstrating an increasing trend with time [6].

Compared to other cancers, OSCC demonstrate low five-year survival rates, the survival rate is about 20% when diagnosed at advance stage and it can improve up to 80% when diagnosed at early stages [7]. The five-year survival rate has not improved with time despite advances in treatment [8,9]. Early detection is important to reduce mortality and morbidity associated with this disease. Lack of effective screening protocols was highlighted as a major barrier for early detection [10]. Identifying which OPMDs will develop into a malignancy remains a challenge, as the malignant transformation of OPMD is not consistent [11]. Hence, the need of biomarkers for screening, diagnosis and prognosis in OSCC and OPMD has been emphasized [12,13].

A biomarker is defined as 'A characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention' [14]. Different DNA, RNA, proteins and metabolites were identified as biomarkers in OSCC [13]. Due to the noninvasiveness and the presence of variety of biomolecules, saliva was proposed as a suitable biological sample to study biomarkers [15]. In OSCC, certain biomarkers that appeared non-significant when analyzed in serum reported significant differences when analyzed in saliva [16,17]. There is no consensus on the most suitable salivary biomarkers for clinical use in head and neck cancer, up to date [18]. In-depth assessment of already identified salivary biomarkers is as important as introduction of novel targets as biomarkers [19].

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Article highlights

- Interleukin 1 beta, 6 and 8 is a biomarker panel suitable for early detection of OSCC and OPMD through salivary analysis
- Salivary biomarkers in OSCC and OPMD have relationships with risk factors
- Relationships between salivary biomarkers and risk factors are important to assess the biomarker's role in disease initiation

Smoking, high alcohol consumption and betel quid are the main risk factors for OSCC and OPMD [20]. Human papillomavirus (HPV) infection, smokeless tobacco use, genetic predisposition, poor oral hygiene, denture wearing, mouthwash use, dietary factors, low socio economic status, co-morbidities, and genetic disorders are other associated risk factors for OSCC and OPMD [21,22].

This systematic review was conducted with the aim of studying salivary biomarkers reported in OSCC and OPMD with two primary objectives. The first objective was to identify suitable salivary biomarkers for early detection and screening of OSCC and OPMD. The second objective was to identify reported relationships between salivary biomarkers and risk factors in OSCC and OPMD.

2. Methodology

2.1. Data Sources

A systematic electronic literature search was conducted to identify relevant published studies using Medline (Ovid), Web of Science, Embase and Scopus databases. The original literature search was conducted in May 2018, and the search was updated in September 2020.

2.2. Search strategy

The keywords (Figure 1) were combined with AND/OR Boolean to generate the search syntax. The search was conducted without time or language restrictions. Search syntax used for Web of Science database was as follows: 'oral cancer*' MeSH terms (mouth cancer, mouth neoplasm, mouth carcinoma, squamous cell carcinoma) OR 'oral premalignant' MeSH terms (pre-cancerous) AND 'saliva* biomarkers*' MeSH terms (saliva, biomarkers).

2.3. Screening and study selection

Abstracts retrieved from the search were exported to Refwork library. Following title and abstract screening 346 articles were selected for the full-text screening. These were screened by two blinded reviewers with four pairs of reviewers (NP+SR, NP+PC, NP+RA, NP+RMSR) assessing approximately 65 papers per pair. Study selection at all stages was conducted using the following eligibility criteria.

2.3.1. Inclusion criteria

- Original research articles containing primary data.
- Studies including patients with head and neck cancer including oral cavity, OSCC or OPMD aged 18 and above.

2.3.2. Exclusion criteria

- Reviews, systematic reviews, meta-analysis, conference proceedings, case reports and case series.
- Full text articles published in languages other than English.
- Studies using non-human subjects.
- Studies that did not analyze biomarkers in saliva or salivary rinse of participants.

Study selection is summarized using PRISMA (preferred reporting systems for systematic reviews and meta-analysis) flow chart (Figure 2).

2.4. Reviewer calibration

All reviewers extracted and analyzed data from five randomly selected papers for training and calibration. Once calibration was achieved two reviewers extracted data from each paper independently and blinded to one another's scores. Four pairs of reviewers (NP+SR, NP+PC, NP+RA, NP+RMSR) conducted the data extraction. Disagreements were resolved through discussion and when necessary with the involvement of a third reviewer (EG).

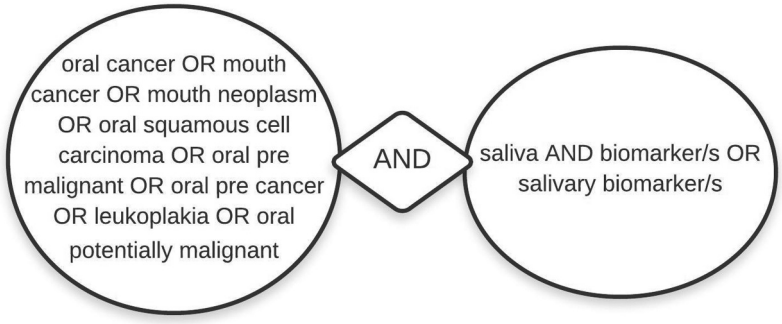

Figure 1. Key words used for electronic literature search.

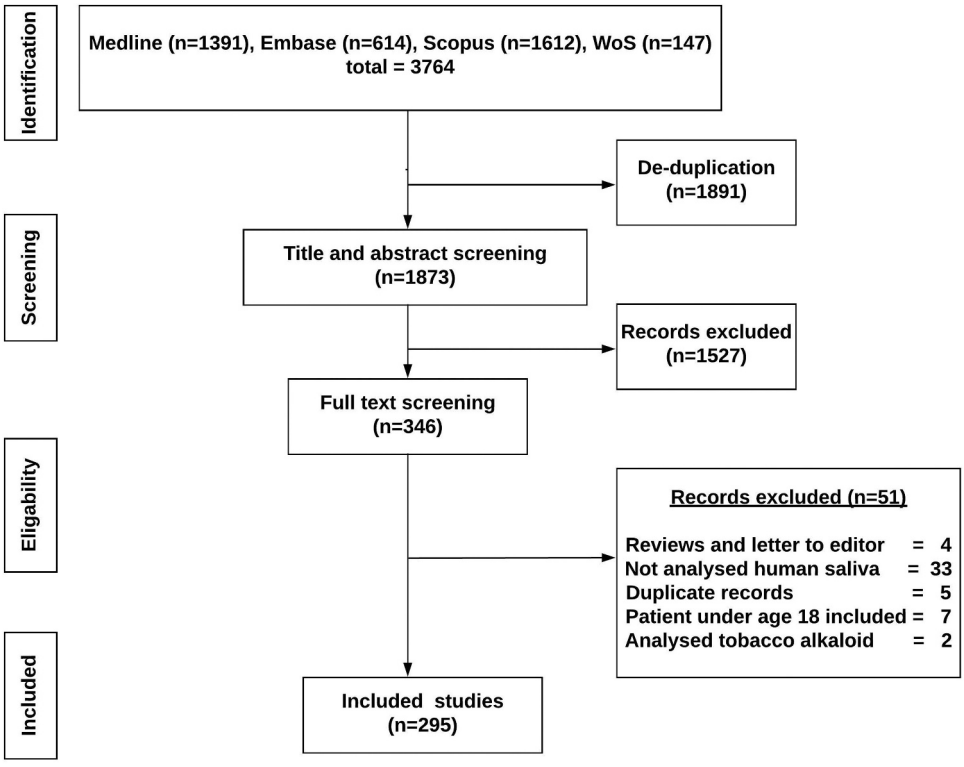

Figure 2. Study selection according to PRISMA format.

2.5. Data extraction

The variables extracted from included articles were: first author, published year, country where the study sample was obtained, study design, age, gender, sample size, biomarkers, method used to analyse the biomarkers, relationships between salivary biomarkers and risk factors and main conclusions. The data were recorded and summarized using bespoke Microsoft Excel spreadsheet and descriptive data analyses were performed.

2.6. Quality assessment

Risk of bias assessment of the studies was conducted using Newcastle Ottawa scale. A star (*) was awarded to the feature of the study that minimized risk of bias in each category. Studies with 6–9 stars were graded with high quality. Studies with 5–4 stars were graded with fair quality. Studies with 3 or less stars were graded with poor quality.

3. Results

From the literature search, total of 3764 abstracts were retrieved. Following de-duplication, 1873 abstracts were subjected to title and abstract screening by application of exclusion criteria. Subsequently, 346 articles were selected for the full-text screening stage. From the screened full-text articles, a total of 295 met the selection criteria and were included in the systematic review. Biomarkers reported in the studies were categorized based on molecular type. 52% of the studies reported protein biomarkers followed by DNA (12%), RNA (8%), metabolites (3%), and microbial (2%) biomarkers.

3.1. Identification of a salivary biomarker panel for OSCC and OPMD

From different biomarker categories, proteins were selected for further study. Among proteins, Interleukins (IL) were selected as suitable biomarkers to assess disease progression in OSSC and OPMD. Studies that reported IL biomarkers in saliva in cases with OSCC and OPMD compared to controls were selected for the objective one (n = 28).

Manuscripts assessed for objective one were published from 2004 to 2020 (Table 1). Majority of the studies were from USA (n = 6), followed by India (n = 5), Croatia (n = 3), Hungary (n = 2), Iran (n = 2), Pakistan (n = 2), Taiwan (n = 2), and one study each from Iraq, Japan, New Zealand, Poland, Serbia and Spain. Sample size ranged from 18 to 300. Enzyme-Linked immuno-sorbent assay (ELISA) and magnetic-bead-based assay were used for biomarker quantification. All researchers used resting whole saliva samples. In the risk of bias assessment, four studies were graded with high quality with low risk of bias, majority of the studies (n = 19) were graded with fair quality with moderate risk of bias and five studies were graded with poor quality with high risk of bias (Table 2).

Nine IL were reported in the work assessed for objective one. These were IL1α, IL1β, IL2, IL4, IL5, IL6, IL8, IL10, and IL13. Statistical significance of the salivary biomarker concentrations were reported using p values and area under the curve (AUC) of the receiver operating characteristics curves. P values less

Table 1. Studies reporting interleukin biomarkers in saliva.

Reference	First author, year	Country	Design	Groups	Sample size	Biomarkers	Method of analysis	Sample	Statistical data/conclusions
[23]	Arellano-Garcia, 2008	USA	Case control	OSCC, periodontitis, controls	40	IL8, IL1β	ELISA, Immune bead based assay	Resting whole saliva	Both biomarkers were statistically higher in OC group than controls, AUC for cancer/controls IL1β = 0.77, IL8 = 0.8, There was no statistically significant difference between IL 8 of periodontitis and controls p value = 0.098, This study report that Luminex xMAP technology is a reliable method to validate and quantitate protein levels in saliva
[53]	Aziz, 2015	Pakistan	Case control	OSCC, controls	66	IL4, IL10, IL13, IL1 receptor antigen	Millipex human cytokine/ chemokine assay kit	Resting whole saliva	P values comparing study groups IL10=0.004, IL13=0.01, this study propose immunosuppressive cytokines as potential salivary biomarkers for OSCC
[24]	Babiuch, 2020	Poland	Case control	OSCC, OED, OLP, controls	45	IL1α, IL6, IL8	ELISA	Resting whole saliva	P values comparing OSCC to controls IL1α=0.017, IL6=0.0012, IL8=0.0001. P value comparing OED to controls IL8=0.04, P value comparing OSCC to OED IL8=0.03. This study identified IL8 in saliva as an important biomarker of malignant process in oral mucosa
[25]	Bagan, 2016	Spain	Case control	OSCC, PVL, controls	60	IL6	ELISA	Resting whole saliva	P value comparing study groups <0.01, patients with OC had the highest salivary biomarker levels followed by PVL and controls, this study suggest salivary IL6 as a potential biomarker to assess disease progression in PVL
[60]	Brailo, 2006	Croatia	Case control	Leukoplakia, controls	64	IL6	ELISA	Resting whole saliva	P value comparing study groups IL6<0.05, the results of this study demonstrate that the patients with oral leukoplakia have an increase in salivary levels of IL6 which might indicate an altered immune response
[65]	Brailo, 2012	Croatia	Case control	OSCC, Leukoplakia, controls	98	IL1β, IL6	ELISA	Resting whole saliva	P values comparing study groups IL1β <0.001, IL6 = 0.012, patients with OC have significantly higher salivary IL1β and IL6 concentrations compared to patients with leukoplakia and healthy controls
[26]	Brinkmanna, 2012	Serbia	Case control	OSCC, controls	86	IL1β, IL8,	ELISA, RT-qPCR	Resting whole saliva	P values comparing protein of IL8, IL1β and mRNA of IL8 p < 0.05, combination of biomarkers from the proteome and transcriptome yielded the highest predictive power for OC
[93]	Csosz, 2017	Hungary	Case control	OSCC, controls	107	IL1α,IL1β, IL6, IL8	Millipex magnetic bead based assay	Resting whole saliva	P values comparing study groups IL1α = 0.96, IL1β = 0.02, IL6 = 0.0002, IL8 = 0.10, OC group had higher biomarker levels, this study demonstrate the potential utility of IL6 as a biomarker in saliva for OSCC in Hungarian population
[27]	Gleber-Netto, 2016	Taiwan	Case control	OSCC, OPMD, controls	180	IL1β, IL8,	ELISA	Resting whole saliva	P values comparing study groups IL1β = 0.0061, IL8 < 0.0001, both biomarkers were higher in OSCC compared to OPMD and controls, this study propose that salivary IL8 protein levels combined with other biomarkers and risk factors have the potential for early detection of OSCC and OPMD
[28]	Hamad, 2011	Iraq	Case control	OSCC, controls	50	IL1α,IL6, IL8	ELISA	Resting whole saliva	P values comparing study groups IL1α<0.001, IL6 = 0.05, IL8 < 0.001, biomarkers were elevated in OC compared to controls, this study propose these biomarkers as diagnostic tools for OSCC
[29]	Hamzavi, 2013	Iran	Case control	Head and neck cancer/ controls	54	IL10	ELISA	Resting whole saliva	P value comparing study groups 0.5, although the salivary levels of IL10 in patients were a little higher than controls, this difference was not statistically significant, this study report that saliva levels of IL10, does not reflect tissue expression
[78]	Marton, 2019	Hungary	Case control	OSCC, controls	175	IL6	ELISA, RT-qPCR	Resting whole saliva	P values comparing study groups for the protein and mRNA <0.001, AUC for IL6 protein 0.69, AUC for IL6 mRNA 0.9, this study propose that salivary IL6 mRNA is suitable biomarkers for OC and suggest clinical validation

(*Continued*)

Table 1. (Continued).

Reference	First author, year	Country	Design	Groups	Sample size	Biomarkers	Method of analysis	Sample	Statistical data/conclusions
[66]	JuretiŰ, 2013	Croatia	Case control	OSCC, OPMD, controls	57	IL6	ELISA	Resting whole saliva	P values comparing study groups IL6 < 0.001, patients have higher salivary concentrations of biomarker compared to controls, this study suggest IL6 as a potential biomarker for OSCC
[81]	Katakura, 2007	Japan	Case control	OSCC, controls	39	IL1β,IL6,IL8	ELISA	Resting whole saliva	P values comparing study groups IL 1β< 0.05, IL 6 < 0.05, IL 8 > 0.05, IL6 was significantly elevated in patients compared to controls, this study report IL6 as a potential biomarker in saliva for OSCC
[30]	Khyani, 2017	Pakistan	Case control	OSCC, OPMD, controls	105	IL6, IL8	ELISA	Resting whole saliva	P values comparing study groups IL8 < 0.05, IL6 no significant difference, IL6 and IL8 are probable biomarker for early detection of OSCC and OPMD in Pakistan population
[31]	Korostoff, 2011	USA	Case control	Tongue cancer, controls	32	IL1α,IL6, IL8	ELISA	Resting whole saliva	P values comparing endophytic tongue cancer/controls IL1α < 0.0001, IL 6 < 0.0001,IL8 < 0.0001, all cytokines were markedly elevated in saliva of endophytic TC patients, this study propose the potential use of these biomarkers for screening, and prognosis of survival in tongue OSCC
[42]	Lee, 2018	Taiwan	Case control	OSCC, controls	65	IL1α,IL1β,IL6, IL8, IL10	Millipex magnetic bead based assay	Resting whole saliva	P values comparing study groups IL1α = 0.625,IL1β = 0.002, IL6 < 0.001, IL8 = 0.001,IL10 = 0.355.AUC for OSCC IL6 = 0.8, IL8 = 0.7, IL1β = 0.7, this study indicate that salivary biomarkers may serve useful as adjuncts for the early detection of OSCC
[32]	Lisa Cheng, 2014	USA	Case control	OSCC, OLP, Periodontitis, controls	101	IL6, IL8	ELISA	Resting whole saliva	P values comparing study groups IL6 < 0.001, IL8 = 0.001, salivary biomarkers were higher in patients with OSCC, compared to OLP, CP and controls, this study propose that IL6 as a useful biomarker in the detection of OC, not influenced by OLP or CP
[33]	Panneer, 2015	India	Case control	OSCC, Leukoplakia, controls	75	IL6	ELISA	Resting whole saliva	P values for OSCC/controls, and OSCC/Leukoplakia <0.0001, biomarker levels were highest in OC, followed by leukoplakia and controls, this biomarker is proposed for further validation to assess its clinical utility
[34]	Punyani, 2013	India	Case control	OSCC, OPMD, controls	75	IL8	ELISA	Resting whole saliva	P values comparing OC/OPMD <0.0001, OC/controls <0.0001, OPMD/ controls = 0.7, IL8 is a potential biomarker for OSCC
[35]	Rajkumar, 2014	India	Case control	OSCC, OPMD, controls	300	IL8	ELISA	Resting whole saliva	P values comparing study groups <0.05, AUC 0.9, IL8 measurement in saliva is a better medium for OC prediction than serum
[36]	Rhodus, 2005	USA	Case control	OSCC, OLP, controls	39	IL1α,IL6, IL8	ELISA	Resting whole saliva	P values comparing study groups IL1α<0.05, IL6 < 0.05, IL8 < 0.05, these biomarkers may be potential targets for disease monitoring in OLP
[37]	Sahebjamee, 2008	Iran	Case control	OSCC, controls	18	IL1α, IL6, IL8	ELISA	Resting whole saliva	P values comparing study groups IL1α>0.05, IL6 < 0.05, IL8 > 0.05, IL6 was elevated in OC compared to controls with statistical significance
[38]	Sharma, 2011	India	Case control	Leukoplakia, periodontitis, controls	60	IL6	ELISA	Resting whole saliva	P value comparing study groups <0.001, all the leukoplakia patients with coexisting periodontitis had higher IL6 levels when compared with patients with periodontitis alone, this study report an increase in IL6 levels with severity of dysplasia grading, IL6 may be a useful biomarker for monitoring of leukoplakia
[39]	Singh, 2020	India	Case control	OSCC, OPMD, controls	159	IL1β, IL8	ELISA	Resting whole saliva	P values comparing study groups IL1β<0.0001, IL8 = 0.0006. AUC for total OSCC were 0.7 for both markers, for stage three and four OSCC, IL1β = 0.9, IL8 = 0.7, these biomarker were significant in Indian population, this study propose the potential use of studied biomarkers for screening and early detection in OSCC

(*Continued*)

Table 1. (Continued).

Reference	First author, year	Country	Design	Groups	Sample size	Biomarkers	Method of analysis	Sample	Statistical data/conclusions
[40]	St. John, 2004	USA	Case control	OSCC, controls	64	IL6, IL8	ELISA RT-qPCR	Resting whole saliva	P values comparing study groups IL8 < 0.01, IL6 > 0.05. AUC for salivary IL8 = 0.9, IL8 at both the mRNA and protein levels were detected in higher concentrations in the saliva of patients with OSCC compared to controls, this study propose salivary IL8 as a potential biomarker for OSCC
[41]	Tan, 2008	USA	Case control	OSCC, controls	40	IL8	ELISA, optical protein sensor	Resting whole saliva	AUC = 0.8, optical sensor and ELISA have similar performances as diagnostic tests, this study report that accurate diagnosis is possible with proposed optical protein sensor platform compared to ELISA for the assessment of salivary IL8
[77]	Vesty, 2018	New Zealand	Case control	Head and neck cancer/ dentally compromised/ controls	30	IL8, IL1β	Cytometric bead array	Resting whole saliva	P values comparing head and neck cancer to controls IL1β = 0.03, IL8 = 0.04, significantly higher concentrations of IL1β and IL8 were detected in HNC and dentally compromised patients, IL1β and IL8 concentrations positively correlated with of C. albicans.

OSCC: oral squamous cell carcinoma, OC: oral cancer, TC: tongue cancer, OPMD: oral potentially malignant disorders, OED: oral epithelial dysplasia, HNC: head and neck cancer, OLP: oral lichen planus, PVL: proliferative verrucous Leukoplakia, CP: chronic periodontitis, IL: interleukin, ELISA: enzyme linked immune sorbent assay, RT-qPCR: reverse transcriptase quantitative polymerase chain reaction, AUC: area under the curve of receiver operating characteristics curve, p < 0.05 and AUC>0.65 was taken as significant

than 0.05 and the AUC values more than 0.65 were taken as statistically significant difference. From the reported data, IL1β, IL6, and IL8 were selected as most suitable salivary biomarkers for early detection of OSCC and OPMD.

3.2. Relationships between salivary biomarkers and risk factors

Studies that reported relationships between biomarkers in saliva (all biomarker types were included) and risk factors in OSCC and OPMD with statistical data were selected for the objective two (n = 33). Findings of these manuscripts are reported in Table 3. Three studies used cohort designs, the rest (n = 30) used case control design. Sample size ranged from 18 to 747. Majority of the data was from USA (n = 8), followed by India (n = 3), Taiwan (n = 3), Brazil (n = 3), Croatia (n = 3), Italy (n = 2), Pakistan (n = 3), and one study each from Australia, Japan, Poland, New Zealand, Sri Lanka, Syria, Hungary, China, and Thailand. Most research analyzed protein biomarkers (n = 22), followed by DNA (n = 8), antioxidant (n = 1), metabolite (n = 1) and both protein and RNA (n = 1). For biomarker analysis, one research has used oral rinse obtained using a mouthwash, four studies used normal saline mouth rinse, three studies used normal saline mouth rinse with exfoliative brush, four studies used stimulated saliva and the rest of the studies (n = 21) used resting whole saliva samples.

In the risk of bias assessment using Newcastle Ottawa scale, four studies were graded with high quality with low risk of bias. Majority of the studies (n = 24) were graded with fair quality with moderate risk of bias and five studies were graded with poor quality with high risk of bias (Table 4).

3.2.1. Salivary biomarkers and smoking

Significant differences between epidermal growth factor (EGF), EDNRB gene, loss of hetero-zygosity (LOH), secretory leukocyte protease inhibitor (SLPI), IL10, hypermethylation of the promoter region of MGMT gene and macrophage migration inhibitory factor (MIF) in patients with smoking habit were reported [47,54,48–52].

Protein biomarker SLPI was studied in saliva of patients with OSCC. SLPI level in current smokers was nearly 1.5-fold higher compared to former smokers, and sixfold higher to never smokers [49]. LOH in 25 gene loci was studied in DNA extracted from salivary cell pellet in patients with OSCC. This study found that LOH in former smokers was in between of current smokers and never-smokers [48]. These researchers identified a pattern where the studied biomarkers were lowest in never-smokers, followed by former smokers with the highest level in current smokers.

Protein biomarkers (IL 4, IL10, IL13 and IL 1 receptor antigen) were assessed for their correlation with smoking, yielding statistically non-significant relationships [53]. Associations between mitochondrial DNA biomarkers (cox 1 and 2 genes), and smoking status were variable; one study identified a significant difference with smoking in the healthy control group but not in the patient cohort [54]. The mean change of mitochondrial DNA biomarker in pre-operative and post-operative saliva samples significantly differed with smoking category in patients with head and neck cancer [55]. On the

Table 2. Risk of bias assessment of studies reporting interleukin biomarkers.

		Selection					Exposure			
Reference	First author, year	Case definition	Representativeness of cases	Control selection	Definition of controls	**Comparability**	Ascertainment of exposure (AOE)	Same AOE for groups	Non response rate	**Quality grade**
[23]	Arellano-Garcia, 2008	Medical records assessed for case selection *	Appropriate sample of cases*	Hospital controls	Controls have no history of outcome*	Matched in design for age*, adjusted in analysis*	Not reported	Not reported	Not reported	Fair
[53]	Aziz, 2015	Medical records assessed for case selection *	Appropriate sample of cases*	Community controls *	Controls were selected from relatives of patients	Matched in design *	Interview not blinded to case control status	Yes *	Not reported	Fair
[24]	Babiuch, 2020	Medical records assessed for case selection *	Appropriate sample of cases*	Not reported	Controls have no history of disease*	Cases and controls not matched in design and not adjusted in analysis. But absence of significant difference of socio demographics reported	Not reported	Not reported	Not reported	Poor
[25]	Bagan, 2016	Diagnosis criteria given*	Appropriate sample of cases*	Community controls*	Controls have no history of disease*	Not matched, but the absence of age difference in groups reported	Not reported	Not reported	Not reported	Fair
[60]	Brailo, 2006	Medical records assessed for case selection *	Appropriate sample of cases*	Community controls*	Controls have no history of disease*	Absence of difference in groups reported	Not reported	Not reported	Not reported	Fair
[65]	Brailo, 2012	Medical records assessed for case selection *	Appropriate sample of cases*	Not reported	Controls have no history of disease*	Cofounders adjusted in the analysis*	Not reported	Not reported	Not reported	Fair
[26]	Brinkmanna, 2012	Medical records assessed for case selection *	Appropriate sample of cases*	Not reported	Controls have no history of disease*	Cofounders adjusted in the analysis*	Not reported	Not reported	Not reported	Fair
[93]	Csosz, 2017	Medical records assessed for case selection *	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design* Adjusted in the analysis*	Questionnaire filled by participant	Yes*	Not reported	High
[27]	Gleber-Netto, 2016	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design* Adjusted in the analysis*	Questionnaire	Yes*	Not reported	High
[28]	Hamad, 2011	Medical records assessed for case selection*	Appropriate sample of cases*	Not reported	Controls have no history of disease*	Patients below the age 18 included in the control group	Not reported	Not reported	Not reported	Poor
[29]	Hamzavi, 2013	Medical records assessed for case selection*	Appropriate sample of cases*	Not reported	Controls have no history of disease*	Matched in design*	Medical records	Not reported	Not reported	Fair
[78]	Marton, 2019	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design* Adjusted in the analysis*	Medical records	Yes*	Not reported	High

(*Continued*)

Table 2. (Continued).

Reference	First author, year	Selection				Comparability	Exposure			Quality grade
		Case definition	Representativeness of cases	Control selection	Definition of controls		Ascertainment of exposure (AOE)	Same AOE for groups	Non response rate	
[66]	Juretić, 2013	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design*	Questionnaire filled by participant	Yes*	Not reported	Fair
[81]	Katakura, 2007	No adequate details on diagnosis	Appropriate sample of cases*	Not reported	Not reported	Only the mean age and female to male ratio reported	Not reported	Not reported	Not reported	Poor
[30]	Khyani, 2017	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Only the age distribution of groups reported	Medical records	Yes*	Not reported	Fair
[31]	Korostoff, 2011	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Adjusted in the analysis*	Not reported	Not reported	Not reported	Fair
[42]	Lee, 2018	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Not reported	Medical records	Yes*	Not reported	Fair
[32]	Lisa Cheng, 2014	Diagnosis criteria given*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design*	Not reported	Not reported	Not reported	Fair
[33]	Panneer, 2015	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design*	Not reported	Not reported	Not reported	Fair
[34]	Punyani, 2013	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design*	Not reported	Not reported	Not reported	Fair
[35]	Rajkumar, 2014	Medical records assessed for case selection*	Appropriate sample of cases*	Community controls*	Controls have no history of disease*	Matched in design*	Not reported	Not reported	Not reported	Fair
[36]	Rhodus,2005	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design* Adjusted in the analysis*	Not reported	Not reported	Not reported	Fair
[37]	Sahebjamee, 2008	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design*	Medical records	Yes*	Not reported	Fair
[38]	Sharma, 2011	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Adjusted in the analysis*	Medical records	Yes*	Not reported	Fair
[39]	Singh, 2020	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design*	Medical records	Yes*	Not reported	Fair

(*Continued*)

Table 2. (Continued).

Reference	First author, year	Selection				Comparability	Exposure			Quality grade
		Case definition	Representativeness of cases	Control selection	Definition of controls		Ascertainment of exposure (AOE)	Same AOE for groups	Non response rate	
[40]	St. John, 2004	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design* Adjusted in the analysis*	Interviews not blinded to case control status	Yes*	Not reported	High
[41]	Tan, 2008	No adequate data is reported	No adequate data is reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Poor
[77]	Vesty, 2018	Medical records assessed for case selection*	Appropriate sample of cases*	Community controls*	No adequate data	Age range and habits of groups reported	Medical records	Not reported	Not reported	Poor

Quality grade: High quality was given to studies with 6–9 stars. Fair quality was given to studies with 5–4 stars. Poor quality was given to studies with 3 or less stars, a star (*) was awarded to the feature of the study that minimized risk of bias in each category.

contrary, a study reported a non-significant difference of mitochondrial DNA biomarker with smoking habit [56].

Protein biomarker MIF was analyzed in patients with OSCC before and after surgical treatment. In this research MIF was decreased in current smokers compared to former-smokers, the difference was statistically significant in the post-operative samples [47]. Significantly low EGF levels in smokers in patients with OSCC were identified [57]. IL10 was significantly higher in smokers in patients with OSCC [50]. EDNRB gene studied in salivary rinse was significantly low in smokers compared to never-smokers in patients with head and neck cancer [58].

Studies reported non-significant differences of IL6, IL1, IL1a, IL8, TIMP3, CCNA1, DCC, DAPK, p16, CD44, malondialdehyde, HP, IGHA2, PRDX-2, ZAG, TNF-α, VGEF, TGF, mitochondrial DNA, choline, betaine, pipecolinic acid, L-carnitine, MMP1, ANXA2, KNG, HSPA and EGFR biomarkers in saliva in smokers compared to nonsmokers [47,50,51,54–56,70,59–68,57,58,69].

3.2.2. Salivary biomarkers and alcohol

The potential associations of the biomarkers EGF, TIMP3, MGMT, MINT31, CCNA1, DCC, DAPK and p16, CD44, HP, IGHA2, PRDX-2, ZAG, Cox 1 and cox 2 genes, SLPI, IL10, TNF-α, VGEF, TGF-β, choline, betaine, pipecolinic acid, L-carnitine and EGFR with alcohol consumption habit were assessed, all these revealed statistically non-significant relations [47,49,50,55,78,61–63,66–70].

3.2.3. Salivary biomarkers and betel quid chewing

Researchers reported non-significant associations of betel quid chewing habit with malonaldehyde, lactate dehydrogenase, ANXA protein, KNG protein, and HSPA protein analyzed in saliva [61,71,72].

Hypermethylation of the promoter region of genes were studied in DNA in saliva of patients with OSCC and oropharyngeal cancer [73]. This study reported a significant difference in the methylation profile of the studied gene panel in patients with and without betel quid chewing habit [73]. Protein biomarker S100A7 was studied in saliva in patients with oral sub-mucous fibrosis, there was a significant negative correlation between biomarker concentration and duration of Arecanut (dried nuts of the plant *Areca catechu*, this is a component of the betel quid and smokeless tobacco products) use [74].

3.2.4. Salivary biomarkers and viral infections

The potential correlations between HPV and Epstein Barr virus (EBV) infections and salivary biomarkers were analyzed. DNA biomarkers (hypermethylation of the promoter region of genes TIMP3, MGMT, MINT31, CCNA1, DCC, DAPK, and p16), reported non-significant differences with HPV infection in patients with head and neck cancer [67]. Lim and colleagues (2016) reported that methylation status of the promoter region of RASSF1α, $p16^{INK4a}$, TIMP3 and PCQAP genes identified in saliva was able to discriminate both HPV status and the presence of head and neck cancer [75]. Possible associations between protein biomarkers IL10, TGFβ, TNFα, VGEF and HPV infection were assessed in patients with OSCC [50]. This study reported significant difference of the IL10 biomarker in

Table 3. Studies reporting relationships between salivary biomarkers and risk factors.

Reference	Country	Design	Groups	N	Biomarker type	Salivary biomarkers	Risk factors	Relationships	Sample
[59]	Italy	Case–control	Oral cancer/ controls	104	Protein	IL6	Smoking	Smokers have higher IL6 $p > 0.05$	Resting whole saliva
[53]	Pakistan	Case–control	Oral cancer/ controls	63	Protein	IL 4, IL10, IL13, IL 1 receptor antigen	Smoking, smokeless tobacco	All cytokines except IL10 positively correlated with the duration of smoked tobacco in months $p > 0.05$	Resting whole saliva
[57]	Brazil	Case–control	Oral cancer/ controls	92	Protein	Epidermal growth factor	Smoking, alcohol	Biomarker difference in smokers $p = 0.03$ and alcoholics $p = 0.08$	Resting whole saliva
[60]	Croatia	Case–control	OPMD/ controls	64	Protein	IL 6, tumor necrosis factor-alpha (TNFα)	Smoking	No significant differences between smokers and nonsmokers. P values for OPMD IL6 = 0.243, TNF = 0.229, control IL6 = 0.051, TNF = 0.845	Resting whole saliva
[65]	Croatia	Case – control	Oral cancer/ OPMD/ controls	88	protein	IL1beta, IL6, Tumor necrosis factor-alpha (TNFα)	Smoking	No significant difference. OC p values for IL1 = 0.232, IL6 = 0.106, TNF = 0.94 Leukoplakia IL1b = 0.619 IL6 = 0.714 TNF = 0.09, controlsIL1b = 0.73,IL6 = 0.24, TNF = 0.92	Resting whole saliva
[67]	USA	Cohort	Head and neck cancer	61	DNA	Promoter hypermethylation of 7 genes	Smoking, alcohol, HPV	There were no statistically significant difference between the methylation status and smoking $p = 1.0$ alcohol $p = 1.0$ and HPV $p = 0.6$	Normal saline oral rinse with exfoliate brush
[47]	Brazil	Cohort	Oral cancer	50	Protein	Macrophage migration inhibitory factor (MIF)	Smoking, alcohol	Difference in smokers pre-operative $p = 0.07$, post-operative $p = 0.04$. In alcohol drinkers, pre-operative $p = 0.58$ and post-operative $p = 0.91$	Resting whole saliva
[58]	USA	Case – control	Head and neck cancer/ controls	132	DNA	Endothelin receptor type B (EDNRB)	Smoking	Current or former smokers had lower EDNRB levels than nonsmokers $p = 0.026$	Normal saline oral rinse with exfoliate brush
[48]	USA	Case – control	Oral cancer/ controls	47	DNA	Microsatellite analysis- loss of heterozygocity (LOH)	Smoking	Quadruple combinations of markers identified in 15 of the 18 smokers $p = 0.0001$. patients who quit smoking showed an LOH frequency that was between that of smokers and nonsmokers	Resting whole saliva
[68]	USA	Case – control	Head and neck cancer/ controls	36	Protein	Soluble CD44	Smoking, alcohol	No significant difference with risk factors of patients $p = 0.34$	Normal saline oral rinse
[70]	USA	Case – control	Head and neck cancer/ controls	186	Protein	Soluble CD44	Smoking, tobacco, alcohol	No statistically significant relationship between smoking status (former, current, never) $p = 0.46$, tobacco use $p = 0.68$ or drinking level (light, moderate, heavy) $p = 0.25$	Normal saline oral rinse
[71]	India	Case–control	Oral cancer/ OPMD/ controls	50	Antioxidant	Lipid peroxidation	Smoking, betel quid chewing	No statistically significant association, smokers in the OPMD $p = 0.13$ oral cancer $p = 0.67$ and chewers in the OPMD $p = 0.77$ and oral cancer groups $p = 0.41$	Resting whole saliva
[69]	Thailand	Case – control	Oral cancer/ controls	25	Protein	Haptoglobin (HP), Ig alpha-2 chain C region (IGHA2), peroxiredoxin-2 (PRDX-2) and zinc-alpha -2-glycoprotein (ZAG)	Smoking, alcohol, betel quid chewing, HPV, EBV	No significant difference to smoking, alcohol or betel quid chewing. Up regulation of HP, PRDX-2 and ZAG and down-regulation of IGHA2 were not influenced by HPV and EBV infection	Resting whole saliva
[43]	Taiwan	Case – control	Oral cancer/ controls	18	Protein	Martrix metalloprotinase 9	Betel quid chewing	Biomarker level rises 3.1 fold after 5 min after areca quid chewing stimulation and decline up to 1.8 fold after 2–4 hours	Stimulated saliva
[54]	USA	Case – control	Head and neck cancer/ controls	747	DNA	Mitochondrial DNA for cytochrome c oxidase (Cox I and Cox II).	Smoking, alcohol	Comparing with never smoker, average Cox I (Pcurrent < 0.0001, Pformer = 0.01) and Cox II (Pcurrent < 0.0001, Pformer = 0.02) content significantly increased with smoking category in the study cohort. Increases by smoking category were limited to control group	Normal saline oral rinse with exfoliative brush

(*Continued*)

Table 3. (Continued).

Reference	Country	Design	Groups	N	Biomarker type	Salivary biomarkers	Risk factors	Relationships	Sample
[78]	Hungary	Case–control	Oral cancer/ controls	175	Protein RNA	IL6	Smoking, alcohol, DMFT, gingival index	P values for protein smoking = 0.95, alcohol = 0.37, DMFT = 0.09, gingival index = 0.1 mRNA level smoking = 0.23, alcohol = 0.07, DMFT = 0.03, gingival index = 0.48	Resting whole saliva
[66]	Croatia	Case–control	Oral cancer/ OPMD/ controls	57	Protein	Tumor necrosis factor alpha (TNFα), IL6	Smoking	No significant differences, p values for TNFα = 0.4 and IL6 = 0.1	Normal saline oral rinse
[44]	India	Case–control	Oral cancer/ OPMD/ controls	300	Protein	Tumor necrosis factor-alpha	Smoking, alcohol, betel quid chewing	No significant difference $p < 0.08$	Resting whole saliva
[75]	Australia	Case–control	Head and neck cancer/ controls	255	DNA	Hyper-methylation of promoter region in a gene panel	HPV	This methylation panel could discriminate HPV-negative and HPV-positive HNC patients from normal healthy control smokers and nonsmokers	Resting whole saliva
[45]	Taiwan	Case–control	Oral cancer/ controls	18	Protein	Martrix metalloprotinase 2	Betel quid chewing	The increase after areca quid chewing stimulation was significant $p < 0.05$	Stimulated saliva
[73]	Sri Lanka	Case–control	Oral cancer/ oro-pharyngeal cancer/ controls	148	DNA	Hyper-methylation of promoter region in a gene panel	Smoking, alcohol, betel chewing, HPV	P vales for p16INK4a all risk factors <0.05, RASSF1A for smoking alcohol betel chewing<0.05 and HPV = 0.06, TIMP3 with smoking = 0.19 alcohol = 0.66 betel quid <0.001, HPV<0.001, PCQAP/MED15 with smoking = 0.24 alcohol = 0.06, betel quid <0.05, HPV<0.001	Resting whole saliva
[49]	USA	Case–control	Head and neck cancer/ controls	240	Protein	Secretory leukocyte protease inhibitor (SLPI)	Smoking, alcohol, education level, BMI, mouthwash	Significantly associated with smoking, current smokers having values nearly 1.5-fold higher than former smokers and six-fold higher than never smokers p = 0.035. Other risk habits were non-significant p values for education = 0.9, BMI = 0.33, mouthwash = 0.5 alcohol = 0.7	Oral rinse using mouthwash
[50]	Poland	Case–control	Oral cancer/ controls	118	Protein DNA	IL10, tumor necrosis factor (TNFα), Transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF)	Smoking, alcohol, HPV, EBV	Significant associations P values for smoking and IL10 = 0.0017, EBV with IL10 = 0.0002 and TNF = 0.0456, HPV with IL10 = 0.03 and TGF = 0.03. Non-significant associations p values for smoking and TNF = 0.15, VEGF = 0.46, TGF = 0.98, alcohol with IL10 = 0.13, TNF = 0.09, VEGF = 0.14, TGF = 0.32, EBV with VEGF = 0.05, TGF = 0.2, HPV with TNF = 0.73, VEGF = 0.9	Resting whole saliva
[74]	Pakistan	Case–control	OSMF/ controls	60	Protein	S100A7	Betel quid chewing, Arecanut use	P values for betel quid chewing duration = 0.03, frequency of betel quid use = 0.3, duration of Arecanut use = 0.009 frequency of Arecanut use = 0.9 in OSMF group	Resting whole saliva
[51]	Brazil	Case–control	Head and neck cancer/ controls	216	DNA	Hypermethylation of the promoter region of the genes	Smoking	No significant association CCNA1 p = 0.70, DAPK p = 0.6, DCC p = 0.83, and TIMP3 p = 1.0. Significant association MGMT p = 0.008	Normal saline oral rinse
[36]	USA	Case – control	Oral cancer/ OPMD/ controls	39	Protein	Tumor necrosis factor-alpha (TNFα), IL1a, IL6, IL8	Smoking	No significant association in the oral cancer group TNF p = 0.64, IL1a p = 0.3, IL6 p = 0.59, IL8 p = 0.5, in the OPMD group TNF p = 0.2, IL1a p = 0.8, IL6 p = 0.31, IL8 p = 0.8	Resting whole saliva
[76]	Japan	Cohort	Oral cancer/ controls	48	Protein	IL6	Smoking, periodontitis	No significant differences smokers p = 0.48, periodontal disease p = 0.37	Stimulated saliva
[56]	Syria	Case – control	Oral cancer/ OPMD/ controls	58	DNA	mitochondrial DNA	Smoking, oral health	No significant differences smoking p = 0.749, oral health p = 0.460	Resting whole saliva
[72]	India	Case – control	OSMF/ controls	60	Protein	lactate dehydrogenase	Betel quid chewing	Biomarker level in patients with habit ≤ 5 years was greater than those with habit > 5 years p = 0.539	Stimulated saliva

(*Continued*)

Table 3. (Continued).

Reference	Country	Design	Groups	N	Biomarker type	Salivary biomarkers	Risk factors	Relationships	Sample
[77]	New Zealand	Case–control	Head and neck cancer/dentally compromised/controls	27	Protein	IL1β, IL8	Microbial species	Positive correlation with C. albicans IL8 p = < 0.001, IL1β p = < 0.001, Eight bacterial genera were significantly negatively correlated with both IL1β and IL8 p < 0.01	Resting whole saliva
[46]	China	Case – control	Oral cancer/controls	60	Metabolites	choline, betaine, pipecolinic acid, L-carnitine	Smoking, alcohol	No significant differences smoking, p values for choline = 0.320, betaine = 0.928, pipecolinic acid = 0.291, L-carnitine = 0.684, alcohol choline = 0.584, betaine = 0.816, pipecolinic acid = 0.200, L-carnitine = 0.301	Resting whole saliva
[61]	Taiwan	Case – control	Oral cancer/controls	227	Protein	Annexin II ANXA2, heat shock 70 kDa protein 5 HSPA5, kininogen KNG1, Matrix metalloproteinase MMP1	Smoking, betel nut chewing	No significant relationship smoking MMP1 p = 0.42, ANXA2 p = 0.13, KNG p = 0.49, HSPA p = 0.77 and betel nut chewing MMP p = 0.17, ANXA p = 0.55, KNG p = 0.37, HSPA p = 0.5	Resting whole saliva
[62]	Italy	Case – control	Oral cancer/controls	100	Protein	Epidermal growth factor receptor	Smoking, alcohol	No significant relationship smoking p = 0.7 and alcohol p = 0.49	Resting whole saliva

N = total sample size included for salivary biomarker analysis, OC: oral cancer, HNC: head and neck cancer, OPMD: oral potentially malignant disorders, OSMF: oral sub mucous fibrosis, HPV: Human papillomavirus, EBV: Epstein Barr virus, BMI: body mass index, DMFT: number of decayed, missing and filled teeth due to caries, p > 0.05 was taken as significant

patients with smoking habit and viral infections. Low methylation profile of the promoter region of the tumor suppressor genes was reported in HPV positive OSCC and oropharyngeal cancer patients [73].

3.2.5. Salivary biomarkers and other risk factors

Assessment of the possible relationships between mitochondrial DNA and oral health status, SLPI protein and mouthwash use, body mass index, education level, IL6 biomarker with periodontitis revealed non-significant relationships [49,56,76]. There were significant associations between salivary IL1β and IL8 levels and microbial species in the oral cavity in head and neck cancer patients [77]. A significant relationship of salivary IL6 mRNA and DMFT (decayed, missing, filled number of teeth due to decay) was reported in OSCC [78].

4. Discussion

More than two-third of OSCC is diagnosed at advance stage of disease [79]. Delayed diagnosis was identified as a major reason for increased mortality, morbidity and low five-year survival rates [7]. Screening and early detection is important to upgrade the management of OSCC [10,11]. Early detection of OPMD lesions will allow the chance to apply secondary preventive measures and thereby reduce the incidence of malignant transformation [2]. Due to noninvasiveness of the sample and presence of variety of biomolecules, saliva is proposed as a suitable specimen to identify biomarkers associated with diseases [80]. As it bathes the oral cavity, salivary biomarkers are proposed as important diagnostic and screening adjuncts for oral diseases, particularly OSCC and OPMD. This systematic review was conducted to identify salivary biomarkers reported in OSCC and OPMD.

The first objective of the present study was to select suitable salivary biomarkers for OSCC and OPMD for the purpose of early detection. Proteins are the working state of molecules and therefore prone to demonstrate acute changes with disease. As proteomic biomarkers, cytokines [81], growth factors [82], angio-genic factors [83], antigens [84], cytokeratin [85], cell surface receptors [86], and enzymes [87,88] were reported in saliva. IL have a diverse role in inflammation and immune reactions in carcinogenesis. IL are involved in the pathogenesis of OSCC and malignant transformation of OPMD [89,90]. Hence, IL were selected as suitable biomarkers to assess disease progression in OSCC and OPMD. Through descriptive data analysis of 28 studies, IL1β, IL6 and IL8 were identified as suitable biomarkers for early detection of OSCC and OPMD. These three biomarkers reported significant differences in the disease group compared to controls. Majority of the studies were graded with fair quality with moderate risk of bias.

Absence of a meta-analysis is a limitation of the present study. Rezaei and colleagues (2019) conducted a meta-analysis on IL6 and IL8 biomarkers in OSCC [91]. This study concluded that these biomarkers have significant predictive power for OSCC.

The second objective was to identify relationships between salivary biomarkers and risk factors. In the phases of biomarker development presented by Pepe and colleagues (2001), one requirement is the assessment of the relationships between

Table 4. Risk of bias assessment of studies reporting relationships between salivary biomarkers and risk factors using the Newcastle Ottawa scale

Reference (case control design)	Selection				Comparability	Exposure			Quality grade
	Case definition	Representativeness of cases	Control selection	Definition of controls		Ascertainment of exposure (AOE)	Same AOE for groups	Non response rate	
53	Medical records assessed for case selection *	Appropriate sample of cases*	Community controls *	Controls were selected from relatives of patients	Matched in design *	Interview not blinded to case control status	Yes *	Not reported	Fair
52	Medical records assessed for case selection*	Appropriate sample of cases*	Not reported	Controls have no history of disease*	Matched in design *	Medical records	Not reported	Not reported	Fair
60	Medical records assessed for case selection *	Appropriate sample of cases*	Community controls*	Controls have no history of disease*	Absence of difference reported	Not reported	Not reported	Not reported	Fair
65	Medical records assessed for case selection *	Appropriate sample of cases*	Not reported	Controls have no history of disease*	Cofounders adjusted in the analysis*	Not reported	Not reported	Not reported	Fair
47	Medical records assessed for case selection*	Appropriate sample of cases*	No control group, comparison before and after treatment	Not applicable	Cofounders adjusted in the analysis*	Medical records	Yes*	Not reported	Fair
58	Medical records assessed for case selection*	Appropriate sample of cases*	Community controls*	Not reported	Cofounders adjusted in the analysis*	Written survey	Yes*	Not reported	Fair
48	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design *	From database	Not reported	Not reported	Fair
68	Medical records assessed for case selection*	Appropriate sample of cases*	Community controls*	Not reported	Cofounders adjusted in the analysis*	Not reported	Not reported	Not reported	Fair
70	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design* Cofounders adjusted in the analysis*	Medical records	Yes*	Not reported	High
71	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Cofounders adjusted in the analysis*	Not reported	Not reported	Not reported	Fair
69	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design* Cofounders adjusted in the analysis*	Not reported	Not reported	Not reported	Fair
43	Medical records assessed for case selection*	No adequate data	No adequate data	No adequate data	No adequate data	No adequate data	Not reported	Not reported	Poor

(*Continued*)

Table 4. (Continued).

Reference (case control design)	Selection				Comparability	Exposure			Quality grade
	Case definition	Representativeness of cases	Control selection	Definition of controls		Ascertainment of exposure (AOE)	Same AOE for groups	Non response rate	
54	Medical records assessed for case selection*	Appropriate sample of cases*	Not reported	Controls have no history of disease*	Cofounders adjusted in the analysis*	Medical records for patients, survey for controls	No	Not reported	Fair
78	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design* Adjusted in the analysis*	Medical records	Yes*	Not reported	High
66	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design*	Questionnaire filled by participant	Yes*	Not reported	Fair
44	Medical records assessed for case selection*	Appropriate sample of cases*	Community controls*	Not reported	Cofounders adjusted in the analysis*	Not reported	Not reported	Not reported	Fair
75	Medical records assessed for case selection*	Appropriate sample of cases*	Not reported	Not reported	Cofounders adjusted in the analysis*	Not reported	Not reported	Not reported	Poor
45	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Absence of habits in the controls reported	Not reported	Not reported	Not reported	Poor
73	Medical records assessed for case selection*	Appropriate sample of cases*	Not reported	Controls have no history of disease*	Matched in design* Cofounders adjusted in the analysis*	Interview for patients	Not reported	Not reported	Fair
49	Medical records assessed for case selection*	Appropriate sample of cases*	Community controls*	Controls have no history of disease*	Matched in design* Cofounders adjusted in the analysis*	Questionnaire filled by participant	Yes*	Not reported	High
50	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Cofounders adjusted in the analysis*	Not reported	Not reported	Not reported	Fair
74	Medical records assessed for case selection*	Appropriate sample of cases*	Not reported	Controls have no history of disease*	Cofounders adjusted in the analysis*	Interviews	Yes *	Not reported	Fair
51	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Not reported	Cofounders adjusted in the analysis*	Medical records	Yes*	Not reported	Fair

(*Continued*)

Table 4. (Continued).

Reference (case control design)	Selection				Comparability	Exposure			Quality grade
	Case definition	Representativeness of cases	Control selection	Definition of controls		Ascertainment of exposure (AOE)	Same AOE for groups	Non response rate	
64	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Matched in design* Cofounders adjusted in the analysis*	Not reported	Not reported	Not reported	Fair
56	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Cofounders adjusted in the analysis*	Medical records	Yes*	Not reported	Fair
72	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Not reported	Matched in design for age*	Not reported	Not reported	Not reported	Poor
77	Medical records assessed for case selection*	Appropriate sample of cases*	Community controls*	No adequate data	Age range and habits reported	Medical records	Not reported	Not reported	Poor
63	Medical records assessed for case selection*	Appropriate sample of cases*	Not reported	Controls have no history of disease*	Matched in design*	Medical records	Not reported	Not reported	Fair
61	Medical records assessed for case selection*	Appropriate sample of cases*	Hospital controls	Controls have no history of disease*	Cofounders adjusted in the analysis*	Medical records	Yes*	Not reported	Fair
62	Medical records assessed for case selection*	Appropriate sample of cases*	Not reported	Controls have no history of disease*	Matched in design*	Medical records	Not reported	Not reported	Fair
Reference (cohort design)		**Selection**			**Comparability**		**Exposure**		**Quality grade**
	Representativeness of exposed	**Selection of non -exposed**	**Ascertainment of exposure**	**Outcome was absent at the start**		**Ascertainment of the outcome**	**Follow up long enough**	**Adequacy of follow up**	
59	Yes *	Yes*	Secure record*	Yes * Outcomes were recurrence and survival	Co-founders adjusted in the analysis*	Record linkage*	Mean 39.4 months, range 12 to 60 months	No loss to follow up	High
67	Yes *	No non-exposed cohort	Secure records*	Yes* outcomes were recurrence and survival	Co-founders adjusted in the analysis*	Record linkage*	Median 2.1 years, range 1 day-9.8 years	Loss to follow up not reported	Fair
76	Yes*	Yes*	Secure records*	Yes* outcome was recurrence	Non exposed were age matched*, Co-founders were reported in the analysis	Record linkage*	48 months	One patient loss to follow up, descriptionprovided*	High

biomarkers and variables such as age, sex, and risk factors [92]. If such factors were found to have strong associations with the studied biomarker, threshold levels must be defined separately for subpopulations. In a study by Csosz and colleagues (2017), researchers highlighted that salivary biomarker expression in OSCC is population tailored as different protein biomarkers were discovered in different population groups with OSCC [93]. This variation reflects the heterogeneity of the pathogenesis of OSCC. This heterogeneity can be due to the difference in risk factors in populations. Risk factors induce molecular and cellular changes in the body. These changes accumulate to give rise to cancer. With different risk factors, the mechanisms of disease initiation can be different; these can be reflected in salivary analysis.

Relationships between biomarkers and risk factors can help to understand the mechanisms involved in disease initiation through risk factors. Loss of heterozygosity (LOH) is a genetic event identified in cancer cells. Significantly high amount of LOH in 25 gene loci was identified in saliva of patients with OSCC. These biomarkers were high in patients who were smokers, followed by ex-smokers and lowest in nonsmokers [48]. These findings suggest that LOH may be an event induced by smoking in the initiation of OSCC.

SLPI is a protein also known as 'antileukoproteinase'. Its main function is inhibition of enzymes secreted by leukocytes. A significant increase in the SLPI biomarker was observed in patients who were smokers compared to never smokers. However, there was no statistically significant elevation of the risk of head and neck cancer with elevated SLPI levels [49]. This result suggests that even though salivary SLPI levels may be induced by smoking, this may not be involved in disease initiation in head and neck cancer.

EGF and EGF receptors are involved in signaling pathways commonly altered in cancer cells. Significantly low levels of salivary EGF in patients with OSCC compared to controls were identified. The same biomarker was significantly lower in smokers compared to never smokers [52]. These findings indicate that decrease in EGF may be involved in the pathogenesis of OSCC by smoking.

Silencing of tumor suppressor genes (TSG) via promoter hypermethylation is an event in the malignant transformation of normal cells into cancer cells. Significantly low methylation status of the TSG was reported in HPV positive head and neck cancer patients compared to HPV negative counterparts [75]. The same biomarkers were assessed in saliva of patients with OSCC and oropharyngeal cancer, the findings of this study support the results reported by Lim and colleagues (2016) as low methylation levels were reported in HPV positive cases [73]. In addition, a higher methylation profile of the same gene panel was reported in patients with betel quid chewing [73]. Results reported in these studies suggest that methylation status of the promoter region of TSG may be a biomarker differently expressed in saliva in relation to risk factors.

Protein biomarkers (IL10, TNFα, TGFβ and VEGF) were studied in patients with head and neck cancer, this study reported higher salivary levels of all biomarkers in cases compared to controls [50]. In the same research, significantly higher IL10 levels were reported in smokers and cases infected with HPV and EBV. From these results, it may be deduced that elevation of anti-inflammatory cytokine IL10 is a mechanism involved in disease initiation in both smoking and viral induced head and neck cancer.

From the results of the present study, following research gaps were identified. From the many salivary biomarkers reported in OSCC and OPMD, only a small proportion was assessed for their relationships to risk factors. Most biomarkers were related to smoking and alcohol habits. Other risk factors such as betel quid, oral health indices and viral infections were less frequently assessed. No records were identified assessing the relationships between biomarkers and risk factors such as genetic predisposition, smokeless tobacco preparations such as snuff, indicators of socioeconomic status, denture wearing, dietary factors, genetic diseases and drug use. These were identified as research needs in the assessment of salivary biomarkers in OSCC and OPMD.

5. Expert opinions

The present systematic review included data extracted from 295 research articles reporting salivary biomarkers in OSCC and OPMD. Identifying biomarkers with high performance in terms of sensitivity and specificity to OSCC help to accelerate future research. Through descriptive data analysis, the present study has identified a proteomic salivary biomarker panel (IL1β, IL6 and IL8) useful for early detection and screening of OSCC and OPMD. This biomarker panel is proposed as suitable for clinical validation as screening and early detection tools for OPMD and OSCC. One disadvantage of the selected biomarkers is that they are inflammatory cytokines; this can be overcome by using a combination of three biomarkers, and using threshold values optimized for the specific patient population.

Relationships between biomarkers reported in saliva and risk factors have been discussed to elucidate mechanisms induced by risk factors in disease initiation. Research gaps in the assessment of salivary biomarkers with respect to their relationships to risk factors have been highlighted. Results of this systematic review indicate that future studies should be directed to assess potential salivary biomarkers for their relationships to risk factors in order to understand the biomarker's role in initiation of disease.

Acknowledgments

N Piyarathne was financially supported by the University of Aberdeen (Elphinstone scholarship), UK and the University grants commission (UGC/VC/DRIC/PG2017(11)/PDN/03) Sri Lanka. Authors would like to thank the library staff at Medical library and Sir Duncan Rice library of the University of Aberdeen for their support in the literature search and recovery of research articles.

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict

with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Conway DI, Purkayastha M, Chestnutt IG. The changing epidemiology of oral cancer : definitions, trends, and risk factors. Nat Publ Gr. 2018;225(9):867–873.
2. Warnakulasuriya S. Oral potentially malignant disorders: a comprehensive review on clinical aspects and management. Oral Oncol. 2020;102: 104550.
3. World Health Organization. GLOBOCAN 2020 [Internet]. Cancer today. 2020. Available from: https://gco.iarc.fr/today/home
4. Mello FW, Miguel AFP, Dutra KL, et al. Prevalence of oral potentially malignant disorders: a systematic review and meta-analysis. J Oral Pathol Med. 2018 Aug;47(7):633–640.
5. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov 1;68 (6):394–424.
6. Sarode G, Maniyar N, Sarode SC, et al. Epidemiologic aspects of oral cancer. Disease-a-Month. 2020;66(12):100988.
7. Jin LJ, Lamster IB, Greenspan JS, et al. Global burden of oral diseases: emerging concepts, management and interplay with systemic health. Oral Dis. 2016 Oct;22(7):609–619.
8. Ghani WMN, Ramanathan A, Prime SS, et al. Survival of oral cancer patients in different ethnicities. Cancer Invest. 2019;37(7):275–287.
9. Capote-Moreno A, Brabyn P, Muñoz-Guerra MF, et al., Oral squamous cell carcinoma: epidemiological study and risk factor assessment based on a 39-year series. Int J Oral Maxillofac Surg. 49(12): 1525–1534. 2020.
• **A study of OSCC over four decades.**
10. Tirelli G, Gatto A, Bonini P, et al. Prognostic indicators of improved survival and quality of life in surgically treated oral cancer. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018 Jan;126(1):31–40.
11. Speight PM, Epstein J, Kujan O, et al. Screening for oral cancer-a perspective from the Global Oral Cancer Forum. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017 Jun;123(6):680–687.
12. Nikitakis NG, Pentenero M, Georgaki M, et al. Molecular markers associated with development and progression of potentially premalignant oral epithelial lesions. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018;125(6):650–669.
13. Rai V, Mukherjee R, Ghosh AK, et al. "Omics" in oral cancer: new approaches for biomarker discovery. Available from Arch Oral Biol. 2018;87February2017:15–34.
14. Califf RM. Biomarker definitions and their applications. Exp Biol Med (Maywood). 2018 Feb;243(3):213–221.
15. Kaczor-Urbanowicz KE, Martin Carreras-Presas C, Aro K, et al. Saliva diagnostics – current views and directions. Exp Biol Med. 2017;242 (5):459–472.
16. Chi LM, Hsiao YC, Chien KY, et al. Assessment of candidate biomarkers in paired saliva and plasma samples from oral cancer patients by targeted mass spectrometry. J Proteomics. 2020;211:103571.
• **This study reported significnt difference of protein biomarkers in saliva of patietns with OSCC, the same panel was non-significant when analysed in serum.**
17. Lee LT, Wong YK, Hsiao HY, et al. Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 2018;47(6):699–707.
18. Amenabar JM, Da Silva BM, Punyadeera C. Salivary protein biomarkers for head and neck cancer. Expert Rev Mol Diagn. 2020;1–9.
19. Adeoye J, Brennan PA, Thomson P. "Search less, verify more"-Reviewing salivary biomarkers in oral cancer detection. J Oral Pathol Med. 2020;49(8): 711–719.
• **A recent review on different biomarker panels identified in saliva for early detection of OSCC.**
20. Warnakulasuriya S. Risk assessment in oral cancer. Springer; 2020. p. 119–132.
21. Al Moustafa AE. Development of oral cancer. 2017.
22. Diajil A, Thomson P Risk factors of oral cancer and potentially malignant disorders (PMDs) – developing a high/low risk profiling system. 2016.
23. Arellano-Garcia ME, Hu S, Wang J, et al. Multiplexed immunobead-based assay for detection of oral cancer protein biomarkers in saliva. Oral Dis. 2008 Nov;14(8):705–712.
24. Babiuch K, Kuśnierz-Cabala B, Kęsek B, et al. Evaluation of Proinflammatory, NF-kappaB Dependent Cytokines: IL-1α, IL-6, IL-8, and TNF-α in Tissue Specimens and Saliva of Patients with Oral Squamous Cell Carcinoma and Oral Potentially Malignant Disorders. J Clin Med. 2020;9(3):867.
25. Bagan L, Sáez GT, Tormos MC, et al. Salivary and serum interleukin-6 levels in proliferative verrucous leukoplakia. Clin Oral Investig. 2016;20(4):737–743.
26. Brinkmanna O, Kastratovicb DA, Milovan V, et al. Oral squamous cell carcinoma detection by salivary biomarkers in a Serbian population. Oral Oncol. 2012;47(1):51–55.
27. Gleber-Netto FO, Yakob M, Li F, et al. Salivary biomarkers for detection of oral squamous cell carcinoma in a Taiwanese population. Clin Cancer Res. 2016;22(13):3340–3347.
28. Hamad AWR, Gaphor SM, Shawagfeh MT, et al. Study of serum and salivary levels of proinflammatory cytokines, potential biomarkers in the diagnosis of oral squamous cell carcinoma. Acad J Cancer Res. 2011;4(2):47–55.
29. Hamzavi M, Tadbir AA, Rezvani G, et al. Tissue expression, serum and salivary levels of IL-10 in patients with head and necksquamous cell carcinoma. Asian Pacific J Cancer Prev. 2013;14 (3):1681–1685.
30. Khyani IAM, Qureshi MA, Mirza T, et al. Detection of interleukins-6 and 8 in saliva as potential biomarkers of oral pre-malignant lesion and oral carcinoma: a breakthrough in salivary diagnostics in Pakistan. Pak J Pharm Sci. 2017 May;30(3):817–823.
31. Korostoff A, Reder L, Masood R, et al. The role of salivary cytokine biomarkers in tongue cancer invasion and mortality. Oral Oncol. 2011;47(4):282–287.
32. Lisa Cheng Y-S, Jordan L, Gorugantula LM, et al. Salivary interleukin-6 and −8 in patients with oral cancer and patients with chronic oral inflammatory diseases. J Periodontol. 2014;85 (7):956–965.
33. Panneer Selvam N, Sadaksharam J. Salivary interleukin-6 in the detection of oral cancer and precancer. Asia Pac J Clin Oncol. 2015;11(3):236–241.
34. Punyani SR, Sathawane RS. Salivary level of interleukin-8 in oral precancer and oral squamous cell carcinoma. Clin Oral Investig. 2013;17(2):517–524.
35. Rajkumar K, Nandhini G, Ramya R, et al. Validation of the diagnostic utility of salivary interleukin 8 in the differentiation of potentially

malignant oral lesions and oral squamous cell carcinoma in a region with high endemicity. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;118(3):309–319.
36. Rhodus NL, Cheng B, Myers S, et al. The feasibility of monitoring NF-κB associated cytokines: TNF-α, IL-1α, IL-6, and IL-8 in whole saliva for the malignant transformation of oral lichen planus. Mol Carcinog. 2005;44(2):77–82.
37. Sahebjamee M, Eslami M, Atarbashimoghadam F, et al. Salivary concentration of TNF? IL1? IL6, and IL8 in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2008;13(5):292–295.
38. Sharma M, Bairy I, Pai K, et al. Salivary IL-6 levels in oral leukoplakia with dysplasia and its clinical relevance to tobacco habits and periodontitis. Clin Oral Investig. 2011;15(5):705–714.
39. Singh P, Verma JK, Singh JK. Validation of salivary markers, IL-1β, IL-8 and Lgals3bp for detection of oral squamous cell carcinoma in an Indian population. Sci Rep. 2020 Apr;10(1):7365.
40. St. John MAR, Li Y, Zhou X, et al. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol - Head Neck Surg. 2004;130 (8):929–935.
41. Tan W, Sabet L, Li Y, et al. Optical protein sensor for detecting cancer markers in saliva. Biosens Bioelectron. 2008;24 (2):266–271.
42. Lee LT, Wong YK, Hsiao HY, et al. Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 2018;47(6):699–707.
43. Hu SY, Lin MH, Yang SC, et al. Areca quid chewing enhances the expression of salivary matrix metalloproteinase-9. J Formos Med Assoc. 2005;2(104):113–119.
44. Krishnan R, Thayalan DK, Padmanaban R, et al. Association of serum and salivary tumor necrosis factor-α with histological grading in oral cancer and its role in differentiating premalignant and malignant oral disease. Asian Pacific J Cancer Prev. 2014;15 (17):7141–7148.
45. Liu SY, Lin MH, Yang SC, et al. Increased expression of matrix metalloproteinase-2 in oral cells after short-term stimulation and long-term usage of areca quid. J Formos Med Assoc. 2005;06 (104):390–397.
46. Wang Q, Gao P, Cheng F, et al. Measurement of salivary metabolite biomarkers for early monitoring of oral cancer with ultra performance liquid chromatography-mass spectrometry. Talanta. 2014;119:299–305.
47. De Souza MB, De Carvalho MB, De Carvalho MB, et al. Serum and salivary macrophage migration inhibitory factor in patients with oral squamous cell carcinoma. Oncol Lett. 2014;8(5):2267–2275.
48. El-Naggar AK, Mao L, Staerkel G, et al., Genetic heterogeneity in saliva from patients with oral squamous carcinomas. J Mol Diagnostics. 3(4): 164–170. 2001.
- **A study reporting significant difference of DNA biomarkers in saliva with smoking habit.**
49. Pierce Campbell CM, Giuliano AR, Torres BN, et al. Salivary secretory leukocyte protease inhibitor (SLPI) and head and neck cancer: the cancer prevention study II nutrition cohort. Oral Oncol. 2016;55:1–5.
50. Polz-Dacewicz M, Strycharz-Dudziak M, Dworzański J, et al. Salivary and serum IL-10, TNF-α, TGF-β, VEGF levels in oropharyngeal squamous cell carcinoma and correlation with HPV and EBV infections. Infect Agent Cancer. 2016;11(1):1–8.
- **A study reporting significant associations between protein biomarker (IL10) in saliva and smoking habit and viral infections.**
51. Rettori MM, De carvalho AC, Bomfim longo AL, et al. Prognostic significance of TIMP3 hypermethylation in post-treatment salivary rinse from head and neck squamous cell carcinoma patients. Carcinogenesis. 2013;34(1):20–27.
52. Bernardes VF, Gleber-Netto FO, Sousa SF, et al. EGF in saliva and tumor samples of oral squamous cell carcinoma. Appl Immunohistochem Mol Morphol. 2011;19(6):528–533.
53. Aziz S, Ahmed SS, Ali A, et al. Salivary immunosuppressive cytokines IL-10 and IL-13 are significantly elevated in oral squamous cell carcinoma patients. Cancer Invest. 2015;33(7):318–328.
54. Jiang WW, Masayesva B, Zahurak M, et al. Increased mitochondrial DNA content in saliva associated with head and neck cancer. Clin Cancer Res. 2005;11(7):2486–2491.
55. Jiang WW, Rosenbaum E, Mambo E, et al. Decreased mitochondrial DNA content in posttreatment salivary rinses from head and neck cancer patients. Clin Cancer Res. 2006;12(5):1564–1569.
56. Sayall L, Mashlah A, Kassem I. Evaluation of mitochondrial DNA content in saliva of oral squamous cell carcinoma and leukoplakia as non-invasive biomarker. Int J PharmTech Res. 2015;7(4):573–579.
57. Bernardes VF, Gleber-Netto FO, Sousa SF, et al. Clinical significance of EGFR, Her-2 and EGF in oral squamous cell carcinoma: a case control study. J Exp Clin Cancer Res. 2010;29(1):1–7.
58. Demokan S, Chang X, Chuang A, et al. KIF1A and EDNRB are differentially methylated in primary HNSCC and salivary rinses. Int J Cancer. 2010;127(10):2351–2359.
59. Arduino PG, Menegatti E, Cappello N, et al. Possible role for interleukins as biomarkers for mortality and recurrence in oral cancer. Int J Biol Markers. 2015;30(2):e262–6.
60. Brailo V, Vučićević-Boras V, Cekić-Arambašin A, et al. The significance of salivary interleukin 6 and tumor necrosis factor alpha in patients with oral leukoplakia. Oral Oncol. 2006;42(4):370–373.
61. Yu J-S, Chen Y-T, Chiang W-F, et al. Saliva protein biomarkers To detect oral squamous cell carcinoma in a high-risk population in Taiwan. Proc Natl Acad Sci U S A. 2016;113(41):11549–11554.
62. Zanotti L, Paderno A, Piazza C, et al. Epidermal growth factor receptor detection in serum and saliva as a diagnostic and prognostic tool in oral cancer. Laryngoscope. 2017;127(11):E408–14.
63. Wang Q, Gao P, Wang X, et al. Investigation and identification of potential biomarkers in human saliva for the early diagnosis of oral squamous cell carcinoma. Clin Chim Acta. 2014;427:79–85.
64. Rhodus NL, Ho V, Miller CS, et al. NF-κB dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. Cancer Detect Prev. 2005;29(1):42–45.
65. Brailo V, Vucicevic-Boras V, Lukac J, et al. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. Med Oral Patol Oral Cir Bucal. 2012;17(1):6–11.
66. Juretić M, Cerović R, Belušić-Gobić M. et al. Salivary levels of TNF-α and IL-6 in patients with oral premalignant and malignant lesions. Folia Biol. 2013;59(2):99–102.
67. Carvalho AL, Henrique R, Jeronimo C, et al. Detection of promoter hypermethylation in salivary rinses as a biomarker for head and neck squamous cell carcinoma surveillance. Clin Cancer Res. 2011;17(14):4782–4789.
68. Franzmann EJ, Reategui EP, Carraway KL, et al. Salivary soluble CD44: a potential molecular marker for head and neck cancer. Cancer Epidemiol Biomarkers Prev. 2005;14(3):735–739.
69. Heawchaiyaphum C, Pientong C, Phusingha P, et al. Peroxiredoxin-2 and zinc-alpha-2-glycoprotein as potentially combined novel salivary biomarkers for early detection of oral squamous cell carcinoma using proteomic approaches. J Proteomics. 2018;173:52–61.
70. Franzmann EJ, Reategui EP, Mateus Pereira LH, et al. Salivary protein and solcd44 levels as a potential screening tool for early detection of head and neck squamous cell carcinoma. Head Neck. 2012;34(5):687–695.
71. Ganesan A, Gautham Kumar N. Assessment of lipid peroxides in multiple biofluids of leukoplakia and oral squamous cell carcinoma patients - A clinico- biochemical study. J Clin Diagn Res. 2014;8 (8):10–13.
72. Sivaramakrishnan M, Sivapathasundharam B, Jananni M. Evaluation of lactate dehydrogenase enzyme activity in saliva and serum of oral submucous fibrosis patients. J Oral Pathol Med. 2015;44 (6):449–452.
73. Liyanage C, Wathupola A, Muraleetharan S, et al. Promoter Hypermethylation of Tumor-Suppressor Genes p16(INK4a), RASSF1A, TIMP3, and PCQAP/MED15 in Salivary DNA as

a Quadruple Biomarker Panel for Early Detection of Oral and Oropharyngeal Cancers. Biomolecules. 2019;9(4):148.

- **A study reporting associations between DNA biomarkers in saliva and betel quid chewing habit in OSCC and oropharyngeal cancer.**

74. Raffat MA, Hadi NI, Hosein M, et al. Differential expression of salivary S100A7 in oral submucous fibrosis. Saudi Dent J. 2019;31 (1):39–44.
75. Lim Y, Wan Y, Vagenas D, et al., Salivary DNA methylation panel to diagnose HPV-positive and HPV-negative head and neck cancers. BMC Cancer. 16(1): 1–12. 2016.

- **A study reporting associations between DNA biomarkers in saliva and HPV infection in head and neck cancer.**

76. Sato J, Ohuchi M, Wada M, et al. Differences in sequential post-treatment salivary IL-6 levels between patients with and patients without locoregional recurrences of oral squamous cell carcinoma: part III of a cohort study. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;120(6):751–760.e2.
77. Vesty A, Gear K, Biswas K, et al. Microbial and inflammatory-based salivary biomarkers of head and neck squamous cell carcinoma. Clin Exp Dent Res. 2018 Nov 28;4(6):255–262.
78. Márton IJ, Horváth J, Lábiscsák P, Márkus B, Dezső B, Szabó A, Tar I, Piffkó J, Jakus P, Barabás J, Barabás P, Olasz L, Kövér Z, Tőzsér J, Sándor J, Csősz É, Scholtz B, Kiss C. Salivary IL-6 mRNA is a Robust Biomarker in Oral Squamous Cell Carcinoma. J Clin Med. 2019 Nov 13;8(11):1958. doi:10.3390/jcm8111958. PMID: 31766212; PMCID: PMC6912409
79. Jayasooriya PR, Pitakotuwage TN, Lombardi T. Descriptive study of 896 Oral squamous cell carcinomas from the only University based Oral Pathology Diagnostic Service in Sri Lanka. BMC Oral Health. 2016 Jan 8;16(1). 10.1186/s12903-015-0139-y.
80. Pfaffe T, Cooper-White J, Beyerlein P, et al. Diagnostic potential of saliva: current state and future applications. Clin Chem. 2011;57 (5):675–687.
81. Katakura A, Kamiyama I, Takano N, et al. Comparison of salivary cytokine levels in oral cancer patients and healthy subjects. Bull Tokyo Dent Coll. 2007;48(4):199–203.
82. Upile T, Jerjes W, Kafas P, et al. Salivary VEGF: a non-invasive angiogenic and lymphangiogenic proxy in head and neck cancer prognostication. Int Arch Med. 2009;2(1):1–4.
83. van der Merwe L, Wan Y, Cheong HJ, et al. A pilot study to profile salivary angiogenic factors to detect head and neck cancers. BMC Cancer. 2018 Jul;18(1):734.
84. He H, Chen G, Zhou L, et al. A joint detection of CEA and CA-50 levels in saliva and serum of patients with tumors in oral region and salivary gland. J Cancer Res Clin Oncol. 2009;135 (10):1315–1321.
85. Malhotra R, Urs AB, Chakravarti A, et al. Correlation of Cyfra 21-1 levels in saliva and serum with CK19 mRNA expression in oral squamous cell carcinoma. Tumor Biol. 2016;37 (7):9263–9271.
86. Ghalwash DM, El GK, Zahran FM, et al. The diagnostic and prognostic value of salivary sCD44 level determination in oral malignant and potentially premalignant lesions. Adv Environ Biol. 2012;6(1):302–310.
87. Lokesh K, Kannabiran J, Rao MD. Salivary lactate dehydrogenase (LDH)- A novel technique in oral cancer detection and diagnosis. J Clin Diagn Res. 2016;10(2):ZC34–7.
88. Totan A, Miricescu D, Parlatescu I, et al. Possible salivary and serum biomarkers for oral lichen planus. Biotech Histochem. 2015;90 (7):552–558.
89. Sahibzada HA, Khurshid Z, Khan RS, et al. Salivary IL-8, IL-6 and TNF-α as potential diagnostic biomarkers for oral cancer. Diagnostics. 2017;7(2):21.
90. Dikova VR, Principe S, Bagan JV. Salivary inflammatory proteins in patients with oral potentially malignant disorders. J Clin Exp Dent. 2019 Jul;11(7):e659–64.
91. Rezaei F, Mozaffari HR, Tavasoli J, et al. Evaluation of serum and salivary interleukin-6 and interleukin-8 levels in oral squamous cell carcinoma patients: systematic review and meta-analysis. J Interferon Cytokine Res. 2019 Dec;39(12):727–739.

•• **A Meta-analysis on IL6 and IL8 biomarkers in OSCC.**

92. Pepe MS, Etzioni R, Feng Z, et al. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst. 2001;93 (14):1054–1061.
93. Csosz É, Lábiscsák P, Kalló G, et al., Proteomics investigation of OSCC-specific salivary biomarkers in a Hungarian population highlights the importance of identification of population-tailored biomarkers. PLoS One. 12(5): 1–22. 2017.

•• **A study highlighting the need for poppulation specific salivary biomarker panels for early detection of OSCC.**