Review Article

Recognizing the salivary panomics for the clinical application in oral potentially malignant disorders

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Abstract

Oral cancer arises as a result of multistep carcinogenic progress from precursor lesion to oral squamous cell carcinoma through collective mutational process occur in the stem cells of mucosal epithelium. The detection of such oral potentially malignant disorders (OPMDs)/cancer in subclinical level will greatly improve the prognosis of a patient. The highly specific and sensitive salivary biomarkers have functioned in detection, prediction, surveillance and therapeutic monitoring of the diseases of interest. The aim of the review is to appraise various salivary biomarkers for the clinical utility in OPMDs. An electronic web-supported search was performed via PubMed, ScienceDirect and Google Scholar search engine since the year 2015–2019. A total of 28 research articles were selected for the review after screening and assessment. The various genomic, transcriptomic, proteomic, metabolomic and miscellaneous markers were analyzed and their characteristics and clinical application in OPMD patients were discussed. miR-21, miR-31, miR-84, H3F3A mRNA + IL-8P, matrix metalloproteinase-9, chemerin, tumor necrosis factor-alpha, cytokeratin-10, ornithine + O-hydroxybenzoate + R5F, 8-hydroxy-2-deoxyguanosine, malondialdehyde, Vitamin E and Vitamin C are identified as potential markers for OPMD patients. Scientifically validated, reliable and economical clinical biomarkers in OPMDs would serve as evidence-based treatment from patient point of view. Further longitudinal studies are needed to verify the accuracy and validate the applicability of these diagnostic/prognostic markers. Saliva has been reported as a valuable noninvasive valuable tool in biomarker identification. Recent advancements in salivary biomarker identification techniques lead to various potential biomarkers with precise outcome. The utilization of these biomarkers for the clinical application in OPMDs depends on the feasibility and personal choice of the clinician.

Keywords: Oral potentially malignant disorder biomarkers, salivary biomarkers, salivary panomics

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INTRODUCTION

Oral potentially malignant disorder (OPMD) has an increased risk for malignant transformation (MT) which could be an epithelial lesion or a disorder. They are considered as the precursor lesion for oral squamous cell carcinoma (OSCC). The commonly encountered OPMD

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lesions are leukoplakia, lichen planus, oral submucous fibrosis, erythroplakia and erythroleukoplakia.[1] The global prevalence of OPMD is 4.5% approximately, and a study revealed that the MT in OPMD is 4.32%, with a range of 6–67-month follow-up in a Taiwanese cohort.[2] The predictors of OPMD turning into malignancy would include clinical parameters, histopathological examination and molecular diagnostic methods. According to clinical parameters, patients with history of alcohol, betel-quid chewing and family history of oral cancer are having increased risk for malignancy. The appearance of verrucous hyperplastic leukoplakia, erythroplakia, multiple sites of occurrence and large size has increased risk than other lesions.[3] Histopathology grading of dysplasia of the OPMD also predicts the cancer risk. Severe dysplastic lesions are considerably having high-risk cancer transformation. Right now, this is the validated diagnostic procedure for the detection of MT in OPMDs.[4]

The molecular-level biomarkers have been extensively studied using whole blood, serum, plasma, tissues, cell lines and saliva. Biomarkers being product of malignant cells, they may also serve as a target for intervention of therapy to prevent disease progression. These include genetic and epigenetic markers which would be helpful in early prediction of cancer in high-risk groups as well as screening of such lesions over a period of time. Serum and salivary biomarkers are also beneficial for convenient disease monitoring, and quantifying them on a scale makes it easier to compare levels during follow-up.[5]

The oral biofluid/saliva as an easily available noninvasive sample makes it striking option for diagnosing, monitoring and prognosis of various human ailments. The reported advantage of salivary sample is being feasible application in the pediatric groups, disabled persons and in frequent follow-up procedures. It would be an excellent alternative when biopsy specimen is insufficient for further processing.

Unlike other areas, oral cavity provides the visibility for the follow-up and predicts the cancer in high-risk OPMD patients. Regardless of the new invention of early diagnostic and advanced therapeutic techniques for OSCC, the 5-year survival rate remains low (50%–60%).[6] Typically, the symptom presentation of OPMD patients to the clinic and the confirmatory diagnosis proceed long time. In India, 80% of OSCCs are reported to have OPMD and majority of the patients are having habit-associated etiology, i.e., tobacco, smoking and alcohol.[7] The early detection of OSCC in high-risk group of OPMD would greatly improve the prognosis of the patient. The treatment decisions can also be quickly made according to the diagnosis and risk for MT. A reliable, cost-effective, precise and noninvasive biomarker would also be useful for cancer screening/preventive programs.

MATERIALS AND METHODS

- Study design: Systematic review
- Objective of the study: Salivary biomarkers for diagnosing OPMDs/predicting early oral cancer in OPMDs
- Materials of the study: Scientific articles
- Units of analysis:

The natural history and types of OPMD, different types of salivary biomarkers and their characteristics, the molecular method of identification of salivary biomarkers in OPMD, parameters such as P values, specificity, sensitivity, receiver operating curve (ROC) and area under curve (AUC) report of the biomarkers.

Study criteria

Inclusion criteria

- 2015–2019 English language studies
- Studies using human saliva for biomarker study in OPMDs
- Descriptive/observational studies
- Analytical/observational studies
- Diagnostic tests.

Exclusion criteria

- Books/book chapters
- Other language studies
- Studies with nonhuman samples
- Studies using oral rinses
- Metagenomic/metaproteomic studies of oral microbiota.

Search strategy

An electronic web-supported search was performed via PubMed, Google Scholar and ScienceDirect search engine from the year 2015–2019. The search words such as oral potentially malignant disorder or salivary biomarkers, leukoplakia and salivary biomarkers, erythroplakia and salivary biomarkers, submucous fibrosis or salivary biomarkers, lichen planus and salivary biomarkers, OSCC and salivary biomarkers and human saliva were used. The supplementary data were collected from reference list of articles and other relevant articles. By using filters, articles were sourced from the year 2015–2019. The relevant article has been chosen by reading the title and abstract of the article. After removing the duplication

of articles and repeated studies, the systematic review was done with the abstracts of all sourced articles and full text of available ones. The article selection process is explained in Figure 1.

RESULTS

The results are tabulated in Tables 1 and 2.[8-34]
It describes the patient/study demographics.
Types of OPMD, comparison groups and cohort size.
Types of biomarkers (genomics/transcriptomics/proteomics/metabalomics).
Sample collection and diagnostic techniques.
Clinical inference of the study
The role of biomarkers in carcinogenesis
Figures 2 and 3 depict the types of OPMD and the various biomarker studies in order.

DISCUSSION

OPMDs being a sign for foreseen malignancy, particularly in high-risk groups, the early detection of MT helps the clinician to start more aggressive therapy and intensive follow-up to give better prognosis for the patient. The latest study reported that the MT in OPMD varies between 1.4% and 36%.[35] Various elements play a role in progression of OPMD into malignancy such as population, gender, habits and grade of dysplasia. A significant number of lesions are reported to be malignant even before the histologic changes of dysplasia.

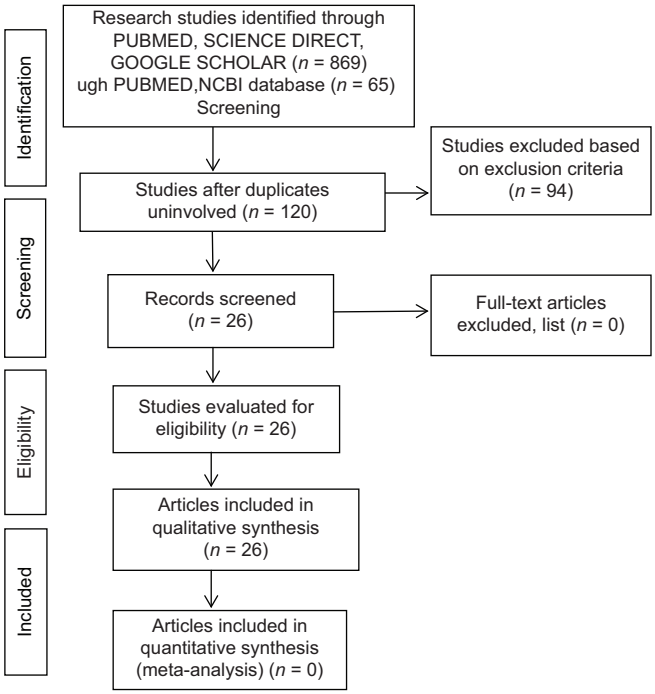

Figure 1: Flowchart

[1] In addition, patients with family history of OSCC with high-risk OPMDs and patients with possibility for second primary can also be benefited with early diagnosis. It will greatly improve the morbidity and the economic burden of a patient.

Biomarkers being products of malignant cells, they may also serve as a target for intervention of therapy to prevent disease progression.[36] Several standard methods with optimum protocol are available for the collection of whole-mouth saliva in a passive unstimulated manner, and various types of salivary collection devices are also available in the market. The collected saliva can be placed in ice or instant frozen in liquid nitrogen and centrifugation done at + 4°C to remove insoluble materials/debris, and the supernatant saliva can be stored at − 80°C till it gets analyzed.[37] Table 3 shows the various study methods to detect and quantify the salivary biomarkers.[38]

The data from the available literature from the year 2015–2019 have been reviewed for the identification of potential salivary biomarkers for screening/diagnosing PMDs. Most of the studies have included oral lichen planus, leukoplakia and oral submucous fibrosis as the study sample.

The comparison group of the study also varies from healthy controls and OSCC patients. Few studies have also included high-risk group (smokers/drinkers) and disease controls such as aphthous stomatitis and persistent suspicious oral lesions as the study sample.

The biomarker of interest in each study depends on the demographical factors such as ethnic group, age, gender and individual habits. Among OPMDs, the etiopathogenesis and prevalence of the particular disease and the clinical course of the disease determine the selection of biomarker in each study.

To minimize the bias in salivary bio marker study the following factors like the methods of collection of sample, sample processing, time of collection, blinding of samples while measurement, the biomarker identification methods, sample attrition, other confounding factors, study follow-up, validation and the methods of statistical analysis has to be considered carefully.

This paper includes studies of individual and combined OPMDs. Biomarkers in OPMD can be used as diagnostic, prognostic or disease-monitoring purposes. In general, the control subjects were normal subjects or OSCC patients. Studies among the various histologic grades

Table 1: Summary of salivary genomic biomarkers in oral potentially malignant disorder

Type of OPMD/ comparison groups (habits, follow-up)	Country	Bio-markers	Sample collection/ Techniques	Cohort size	Age/gender	Clinical inference	Role in carcinogenesis	Reference
Oral lichen planus OSCC age and gender matched controls (smokers, severe alcoholics were excluded)	Iran	microRNA 320a, CRP and IL-6	Whole unstimulated saliva, RT-qPCR, ELISA, clinical chemistry analyzer	Oral lichen planus 32, (22 dysplastic), 15 OSCC, 15 age and gender matched controls	NS	A significant decrease in salivary microRNA-320a in dysplastic OLP and OSCC not in OLP without dysplasia was found which is confirmed by VEGFR-2 expression in tissues. IL-6 level is significantly increased in OLP, dysplastic OLP and OSCC whereas CRP level was significantly increased in OSCC and OLP with dysplasia. A positive co-relation among IL-6 and CRP levels was observed	microRNA-320a might be a regulator for vascular endothelial cell function via targeting Neuropilin (NRP1). I IL-6 is a proinflammatory indicators	Shahidi M *et al.*, 2017 [8]
Potentially malignant oral disorders, OSCC, controls	Spain	Epstein-Barr virus (EBV) DNA	Whole unstimulated saliva/qualitative real-time PCR (qPCR)	12 OSCC patients, 12 PMD patients, 47 healthy control	NS	OSCC group have revealed the highest percentage of positive salivary EBV DNA pursued by the PMD and the controls. The difference among the groups was not statistically significant	Oncogenic human herpes virus affecting epithelial cells and B lymphocytes	Bagan L *et al.*, 2016 [9]
OPMD with healthy control mean follow-up of 820 days	Taiwan	MicroRNA-21, MicroRNA-31	QT-PCR, in situ hybridization	20 saliva samples/24 healthy, 46 tissue samples	Mean of 53.3±3.7 yrs with male predominance.	Significantly increased expression of salivary miRNA 21, miRNA-31 was observed in OPMD than in controls. Further increased levels of miRNA-31 expression are observed in recurrent and lesions with malignant transformation.	MiR- 21 could have a role in invasion and metastasis via some target molecules and it may associate in survival.	Hung KF *et al.*, 2016 [10]
OLP/healthy controls (NS)	Korea	Salivary exosomal miRNA-4484 miRNA-1246 miRNA-1290	NS/MiRNA Microarray, qRT-PCR	20 (14/6)	57.25±11.08/54.8±9.18 years (mean±SD)/NS	miRNA 4484 is significantly unregulated in salivary exosomes of OLP patients than in controls.	Immune response to pathological stimuli.	Byun *et al.*, 2015 [11]
OLP/healthy controls/human whole saliva	Sweden	miR-203	qRT-PCR	21 (7/14)	>18 years/NS	NS	This is epithelium specific and represses ΔNp63 inducing terminal differentiation of skin stem cells and also regulates ΔNp63 upon genotoxic damage in SCCHN cells.	Lundegard M *et al.*, 2015 [12]

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Table 1: Contd...

Type of OPMD/ comparison groups (habits, follow-up)	Country	Bio-markers	Sample collection/ Techniques	Cohort size	Age/gender	Clinical inference	Role in carcinogenesis	Reference
OPMD/OSCC/ RAS/healthy controls with (smokers included with three years follow-up)	Saudi Arabia	miRNA-21 miRNA-184 miRNA-145	WUS/qRT-PCR	100 (40/20/20/20)	Mean±SD54.2±9.7/58±9.2/ 28±7.3/51.1±9.3 years With female predominance	Significantly increased miRNA-21, miRNA-184 OPMD vs normal, OSCC vs normal. OPMD vs OSCC significantly decreased in non dysplasic opmd. miRNA-145 is significantly decreased in OPMD vs normal and in OSCC vs normal.	miRNA-21 has a role in invasion and metastasis miRNA-184 had important effect over anti-apoptotic and proliferative processes in OSCC. miRNA-145 is a tumor suppressor miRNA and plays a role in regulating apoptosis.	Zahran *et al*., 2015 [13]
Oral submucous fibrosis/ leukoplakia/ controls with tobacco related habits	India	E-cadherin-160C/A (CDH1-160 polymorphism)	DNA isolation with PCR amplification	90 (30,30,30)	41.41 yrs in sub mucous fibrosis, leukoplakia, 47.30 yrs in control group with male predominance	14 patients from OSMF demonstrated C/A and 3 revealed A/A polymorphism. 9 patients demonstrated C/A and 4 revealed A/A polymorphism in Leukoplakia and 13 (12,1) of controls with chewing habits also shows polymorphism	E-cadherin is the main adhesive protein of the epithelia and takes part both in tumor invasion and suppression	Hiremath SV *et al*., 2017 [14]
OSCC, OPMDs with dysplasia, healthy control (smokers and drinkers included)	Taiwan, USA	IL8, IL-1β, OAZ1, SAT1, DUSP1, S100p and H3F3A mRNA and IL8 and IL1 β proteins	WUS/qPCR, ELISA	180 patients (60 OSCC, 60 OPMDs with dysplasia, 60 healthy control)	39 to ≥70 predominantly men	The IL-8p Is significantly higher in OSCC and has the highest AUC value between OSCC and PMOD patients. The H3F3A mRNA together with IL-8p offered greatest AUC value for discrimination among OSCC and PMOD patients.	OAZ and SAT are involved in the intracellular polyamine synthesis and involved in cell homeostasis and proliferation IL-1Band IL-8 are cytokines and known as important mediators of carcinogenesis. DUSP1involves in negative regulation of cellular proliferation. S100P regulates cell cycle progression and differentiation. H3F3A plays role in telomere organization and cell growth regulation.	Gieber-Netto FO *et al*., 2016 [15]
Oral Leukoplakia/ Oral Sub Mucous Fibrosis/ OSCC/healthy individual (smokeless, smoked tobacco)	India	Endothelin-1	WUS/ELISA	15/15/15/15	20-80 years with male predominance	Significantly higher expression of salivary endothelin OSCC Followed by OSMF and OL groups. The mean levels were higher in advanced histopathological grading in SMF and OL with positive correlation	ET-1 is involved in tumor growth progression, inducing proliferation of cell, survival, angiogenesis and metastasis through ETAR activation.	Ankita *et al*., 2019 [16]

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Type of OPMD/ comparison groups (habits, follow-up)	Country	Bio-markers	Sample collection/ Techniques	Cohort size	Age/gender	Clinical inference	Role in carcinogenesis	Reference
Oral leukoplakia/ OSCC/ normal control (smokers, tobacco chewers)	India	Tumor necrosis factor-α	WUS/ELISA	90 (30,30,30)	20-74 years with male predominance	Highly significant difference between three groups with increase expression from controls to leukoplakia to OSCC	TNF-α is a pro inflammatory cytokine has both pro and anti-tumorigenic effect.	Deepthi G *et al*., 2019 [17]
Oral lichen planus/Healthy Control/ Recurrent apthous stomatitis (non smokers, non substance abusers)	China	IL-2, IL-4, IL-5, IL-6, IL-10, INF-α, TNF-α, TGF-α, INF-α	WUS, BD CBA Human Enhanced Sensitivity Flex Sets	69 (41, 14, 14)	Mean 56.27±13.03/51.21 ±5.19/50.00±4.22 years with female predominance	IL-6, IL-10, IFN-α, IFN-α/IL-4 were significantly increased in OLP patients compared to healthy controls. IL-10 and IL-6 are correlated with OLP severity	Cellular and humoral immune response	Wei W *et al*., 2018 [18]
First set five oral lichen planus/ five healthy control groups, second set 24 OLP/with age matched healthy controls, third set three pairs of age and sex matched OLP (non smokers)	Thailand	Putative protein biomarkers	WUS/ Two-dimensional gel electrophoresis, mass spectrometry for the first place of patients, ELISA for second place of patients and for the third place Immunoblotting was used.	5+24+3 (5, 24, 3)	30-76 OLP/33-70 years healthy control with female predominance	20 unique proteins were identified among which putative proteins like fibrinogen fragment 3, complement component C3c were exhibited increased expression and decreased expression of cystatin in eliminating the pathogens from an organism. Cystatin SA could reduce human cathepsin L and may be engaged in proteolytic episodes in vivo. SA which were confirmed and validated with ELISA and immunoblotting.	In vitro, fibrinogen has been shown to generate cytokine and chemokine secretion in various cell types. The complement system is a key part of innate immune system, that helps antibodies and phagocytic cells	Talungchit *et al*., 2018 [19]
OLP/OSCC/ healthy control (nonsmokers and non-alcoholics)	Iran	IL-6, C-reactive protein	WUS/ELISA	62 (32/15/15)	NS	Significantly increased levels of CRP and IL-6 in dysplastic OLP, OSCC compared to normal control	IL-6 signaling pathway plays a role in malignant transformation and CRP gene can promote angiogenesis and tumor development.	Shahidi M *et al*., 2017 [8]
Oral premalignant lesions/OSCC/ healthy control (smokers excluded)	Egypt	Chemerin and MMP-9	WUS/ELISA	45 (15,15,15)	42.33±10.99, 47.66±14.07, 43.26±11.82 with female predominance	Salivary Chemerin and MMP-9 were significantly higher than OPMLs and control group	Chemerin is a adipokine that regulates angiogenesis, cell proliferation and inflammation. MMMP-9 has a role in tumor progression, invasion and angioge2nesis.	Ghallab NA *et al*., 2017 [20]

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Type of OPMD/ comparison groups (habits, follow-up)	Country	Bio-markers	Sample collection/ Techniques	Cohort size	Age/gender	Clinical inference	Role in carcinogenesis	Reference
Leukoplakia/ healthy control (smokers, drinkers included) 4months to 6 years with a mean of 2.73 years follow-up	Brazil	Protein identification	MS analysis	20 (10,10)	Mean 73.8/32.3 years with female predominance	Ig alpha-2 chain C region, apolipoprotein A1, mature metal chelatase catalytic antibody with hapten, chain A protein were shown increased folds whereas Cystatin SN precursor (Cystatin -1) and serum albumin were shown decreased folds in leukoplakia compared to normal control. The CK10 fragment was found in the saliva of all OL group and missing in the control group.	Ck-10 is involved in keratinocyte differentiation and keratinization process	Camisasca DR *et al.*, 2017 [21]
Oral Lichen Planus/Healthy control (smoking and alcohol consumption were excluded)	Spain	IgA, Adiponectin and cortisol	WUS, IgA and Adiponectin by ELISA. Cortisol by solid -phase competitive chemiluminescent enzyme immunoassay.	65 (33,32)	Mean 57±15.8 53±12 with female predominance	IgA and Cortisol levels were significantly higher in OLP than in control	Cortisol is an indicator of psychological stress. IgA has major role in mucosal pathogenesis. Adiponectin has effects on immune and inflammatory components.	Lopez J *et al.*, 2016 [22]
Leukoplakia/ healthy control (smoking, alcohol)	Brazil	Epidermal growth factor	WUS/ELISA	64 (32,32)	Above/equal and below 60 years male predominance	Among the patients and the controls there were no significant difference in the salivary EGF levels. Dysplastic lesions demonstrated a tendency toward presenting increased salivary EGF levels.	EGF can play a role both in the maintenance of epithelial integrity and in carcinogenesis.	Jaeger F *et al.*, 2015 [23]
Oral lichen planus/healthy volunteer (non-smokers)	Iran	Interferon-ɑ Interleukin-4	WUS/ELISA	Sixty three (30 reticular, 33 erythematous and ulcerative)/63	Mean 41.5±0.4/37±0.6 yrs with female predominance	Significant higher levels of IFN-ϒ and IL-4 in reticular OLP subjects compared to controls. Increase of salivary IFN-ϒ/IL-4 ratio explained Th1 might have leading role in the pathogenesis of OLP.	INF-ϒ is involved in keratinocyte apoptosis and disease chronicity of OLP and IL-4 is involved in humoral immune response.	Malekzadeh H *et al.*, 2015 [24]
OLP/OSCC/ control group (not mentioned)	Iran	P53 (wild type)	Unstimulated whole saliva/ELISA	34 OLP (17 erosive, 17 reticular forms), 24 OSCC, 41 controls	28-74/31-81/23-67 years with women predominance	Salivary P53 concentration is significantly higher in OSSC than in healthy and OLP patients	P53protein coordinates various responses like arrest of cell growth, apoptosis and DNA repair.	Agha-Hosseini F *et al.*, 2015 [25]

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Type of OPMD/ comparison groups (habits, follow-up)	Country	Bio-markers	Sample collection/ Techniques	Cohort size	Age/gender	Clinical inference	Role in carcinogenesis	Reference
OLP, healthy volunteers (not mentioned)	China	IL-17, IL-23 and oral microbe	Unstimulated whole saliva/ELISA/ PCR-DGGE	30 OLP (reticular, erosive), 15 healthy volunteer	26-61/27-54 years with female predominance.	Salivary IL-17 level in erosive lichen planus is significantly increased than reticular OLP patients and healthy controls. There were significantly fewer bacterial diversity and complexity in saliva of OLP patients than in healthy volunteers.	Important role in inflammatory response against microbial pathogens	Wang K *et al*., 2015 [26]
OSCC/Oral Epithelial Dysplasia/ Persistent suspicious oral mucosal lesions	Japan	Ornithine, Carnitine, Arginine, O-Hydroxybenzene, N-Acetylglucosamine 1-phosphate, Ribose 5 -phosphate	WUS (capillary electrophoresis mass spectrometry)	48 (6,10,32)	21-86 years/male predominance	The OSCC/OED group revealed significantly decreased levels of all six metabolites than the PSOML group with best AUC values.	Intermediate metobolites in various metabolic pathways.	Ishikawa Shigeo *et al*., 2019 [27]
Leukoplakia/ healthy control/ tobacco usage	India	Lipid peroxidation (TBARS) Glutathione S transferase, nitrite, uric acid	WUS/ bio-chemical assay (ultraviolet visible spectrophotometer)	80 (40,40)	Not specified	Significantly decreased levels of uric acid and GST and significantly increased levels of TBRS and nitrites compared to controls. Similar results were seen along with clinical stages and histopathological grades of LP.	The reactive oxygen species and anti-oxidant balance plays key role in inflammation-mediated carcinogenesis.	Srivatsava, 2019 [28]
Oral submucous fibrosis/healthy control/history of tobacco use	India	Lactate dehydrogenase	WUS-LDH (P-L) kit.	40 (20,20)	18-60 years/male patients	Statistically significant increased levels of salivary LDH level in OSMFvs Controls.	Lactate dehydrogenase (LDH) is a hydrogen transfer enzyme and plays a role in the final step in the metabolic chain of anaerobic glycolysis	Mishra S *et al*., 2018 [29]
OSCC, PMD's, smokers or drinkers without lesion group (risk group), control group	India	Cortisol	Unstimulated saliva, serum (ELISA)	100 subjects (25 in each groups)	35-60 years Only male patients	Significantly increased salivary cortisol levels were observed in patients in OSCC groups compared to other study groups.	The stress harmone like cortisol induces the cancer cells to produce free radicals which further damages the DNA .	Sharma P *et al*., 2018 [30]
Lichen planus (reticular/ non reticular)/ control (smokers and drinkers excluded)		Aldehyde dehydrogenase 1	Whole saliva/ unstimulated, ELISA	30 (9, 21), 30 healthy volunteers	27-66 years with majority female patients.	Significant higher levels of ALDH1 in the non-reticular vs reticular group. OLP vs control does not show any significant differences.	The salivary ALDH plays a role as a primary defensive enzyme against toxic aldehydes.	Mansourian A *et al*., 2017 [31]

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Table 1: Contd...

Type of OPMD/ comparison groups (habits, follow-up)	Country	Bio-markers	Sample collection/ Techniques	Cohort size	Age/gender	Clinical inference	Role in carcinogenesis	Reference
Oral lichenplanus, oral leukoplakia, Oral sub mucous fibrosis, oral squamous cell carcinoma and controls with history of smoking and alcohol	Belgium	8 -hydroxy -2-deoxyguanosine (8-OHdG), malondialdehyde (MDA), Vitamin C and Vitamin E	Unstimulated whole mouth saliva, biochemical, ELISA, HPLC.	Total 200 patients in each category (40+40+40+40+40)	Age ranges from 41-64 years 20 males and 20 females in each sample category	The salivary levels of 8-OHdG, MDA were significantly higher in OSCC while vitamin c and Vitamin E levels were decreased pursued by advanced stages of precancer patients relative to the earlystage patients. The levels of 8-0HdG, MDA were significantly higher with lower levels of Vitamin c and Vitamin E in lichenplanus, leukoplakia, submucous fibrosis patients compared with healthy controls. The combination of markers have high specificity and sensitivity when compared to individual biomarker approach.	The reactive oxygen species and anti-oxidant balance plays key role in inflammation -mediated carcinogenesis.	Kaur J *et al*., 2016 [32]
Lichen planus, healthy controls (non-smokers)	Romania	8-OHdG, MDA, Uric acid, TAC, GPx, CTX, MMP-8	whole mouth saliva (biosystem kit, spectrophotometric, ELISA), serum.	30 OLP, 30 controls	18-68 years (OLP 15 males and 15 females, in control 20 males and 10 females)	Significantly increase in salivary MDA, MMP-8, CTX 1, 8-OHdG levels in the OLP patients than in controls and significantly decreased TAC, GPxand uric acid level in the saliva compared to controls. There was a negative correlations between TAC and GPx and between uric acid and 8-OHdG in salivary samples of OLP patients.	The reactive oxygen species and antioxidant balance plays key role in inflammation -mediated carcinogenesis. The collagen degradation markers such as mmp-8 and CTx may represent the inflammation intensity in OLP.	Totan A *et al*., 2015 [33]
PMD, healthy control	India	Pyruvic acid	Unstimulated saliva, blood	50 subjects (25 in each group)	13 males and 12 females in healthy group with 53.8 mean age and 16 males, 9 females in PMD with mean age 52.6	Significant increase in salivary Pyruvic acid levels in PMD group than controls.	Pyruvic acid is an intermediary in carbohydrate, fat and protein metabolism. Increased glycolysis was detected in cancer cells and this metabolic pathway is very essential for the production of ATP to face their energy requirement	Bhat A *et al*., 2015 [34]

LDH: Lactate dehydrogenase, 8-OHdG: 8-hydroxy-2-deoxyguanosine, MDA: Malondialdehyde, TBARS: Thiobarbituric acid reactive substances, GST: Glutathione S-transferase, OSMF: Oral submucous fibrosis, OSCC: Oral squamous cell carcinoma, PMD: Potentially malignant disorder, OLP: Oral lichen planus, TAC: Thrombin affinity column, HPLC: High-performance liquid chromatography, MMP: Matrix metalloproteinase, OPMD: Oral potentially malignant disorder, OED: Oral epithelial dysplasia, AUC: Area under the curve, PSOML: Persistent suspicious oral mucosal lesion, OPML: Oral potentially malignant lesion, MS: Mass spectrometry, IL-6: Interleukin, DGGE: Denaturing gradient gel electrophoresis, PCR: Polymerase chain reaction, EDF: Epidermal growth factor, TNF-α: Tumor necrosis factor-α, CRP: C-reactive protein, SMF: Submucous fibrosis, CBA: Cytometric bead array, NRP: Neuropilin, EBV: Epstein-Barr virus, RT: Real-time, SD: Standard deviation, SCCHN: Squamous cell carcinoma of the head and neck, VEGFR: Vascular endothelial growth factor receptor, NS: Nonstimulated cell, miRNA: MicroRNA, OAZ: ornithine decarboxylase antizyme, SAT: spermidine/spermine N1-acetyltransferase

Table 2: Summary of Sensitivity, Specificity and AUC values of bio markers in OPMDs

Author and year	Biomarker identified	Type of biomarker	Result	Comparison	Sensitivity (%)	Specificity (%)	AUC	Inference
Hung *et al*., 2016[10]	miR-21 and miR-31	Genomic marker	Increased expression	OPMD versus normal	100	Not mentioned	0.74 and 0.76	Diagnostic value for OPMD screening
Zahran *et al*., 2015[13]	miRNA-184	Genomic marker	Increased expression	OSCC versus OPMD with dysplasia	80	75	0.86	Precise biomarker for oral malignant transformation
Gleber-Netto *et al*., 2016[15]	IL-8p+H3F3A mRNA	Transcriptomic and proteomic marker	Higher level of IL-8p and H3F3A mRNA	OSCC versus OPMDs with dysplasia	0.9	0.45 (maximum spec)	0.752	Useful for the discrimination between OSCC and PMOD
Deepthi *et al*., 2019[17]	TNF-α	Proteomic marker	Increase in expression	OPMD with healthy control	90	95	0.968	This can be used as a biomarker to diagnose OPMD and as an indicator for neoplastic progression of OPMDs
Ghallab and Shaker, 2017[20]	Chemerin and MMP-9	Proteomic marker	Elevated levels	OSCC versus OPML	93, 100	80 93.3	0.880 0.991	Both are considered as diagnostic biomarkers for OPMLs and for detection of early malignancy in OPMLs
Ishikawa *et al*., 2019[27]	Ornithine+O-hydroxybenzoate+R5F	Metabolomic marker		OSCC/OED with PSOML	Not mentioned	Not mentioned	0.871	Screening to discriminate OSCC/OED from PSOML
Kaur *et al*., 2018[32]	8-OHdG, MDA, Vitamin C and Vitamin E	Miscellaneous oxidative marker	8-OHdG, MDA and lower Vitamin C and Vitamin E	OSCC versus advanced precancerous lesions	80	80	NS	Useful marker for diagnosing oral precancerous lesions

Sensitivity, Specificity and AUC values of biomarkers in OPMDs. OPMD: Oral potentially malignant disorder, OSCC: Oral squamous cell carcinoma, PMOD: Potentially malignant oral disorder, OPML: Oral potentially malignant lesion, OED: Oral epithelial dysplasia, PSOML: Persistent suspicious oral mucosal lesion, 8-OHdG: 8-hydroxy-2-deoxyguanosine, MDA: Malondialdehyde, MMP: Matrix metalloproteinase, AUC: Area under the curve, TNF-α: Tumor necrosis factor-α

Table 3: Study methods to detect and quantify a salivory bio markers

Type of biomarker	Methods of study	Inference
Salivary metabolomics	GC-MS, LC-MS, UPLC-MS, CE-MS, 1H-NMR-spectroscopy, RPLC, HILIC, biochemical methods	Upregulated or downregulated expression could be measured
Salivary proteomics	MS, microarray, two-dimensional DIGE, gel-LC-MSMS, iTRAQ, ELISA	Expression can be observed as mass peaks, spots and quantification of proteins
Salivary transcriptomics	qPCR and microarrays followed by qPCR ELISA	Transcription expression analysis
Salivary genomics	NGS	Quantification of gene expression level (mean fold change) by delta threshold cycle calculation

MS: Mass spectrometry, GC-MS: Gas chromatography-MS, LC-MS: Liquid chromatography-MS, UPLC: Ultra-performance liquid chromatography, CE-MS: Capillary electrophoresis-MS, 1H-NMR: Proton nuclear magnetic resonance, RPLC: Reversed-phase liquid chromatography, HILIC: Hydrophilic interaction liquid chromatography, DIGE: Difference in gel electrophoresis, iTRAQ: Isobaric tag for relative and absolute quantification, qPCR: Quantitative polymerase chain reaction, NGS: Next-generation sequencing

of OPMD would be useful for disease-monitoring purposes.

Since there is lot of heterogeneity among the selected studies, the results were analyzed and concluded according to their statistically significant results, follow-up periods, validation methods and specificity, sensitivity and AUC analysis as they highlight the study accuracy and important appraise of biomarker performance in distinguishing OPMDs from controls.

In general, salivary genomic studies reveal the genetic expression such as miRNA changes, DNA hypermethylation/hypomethylation, gene

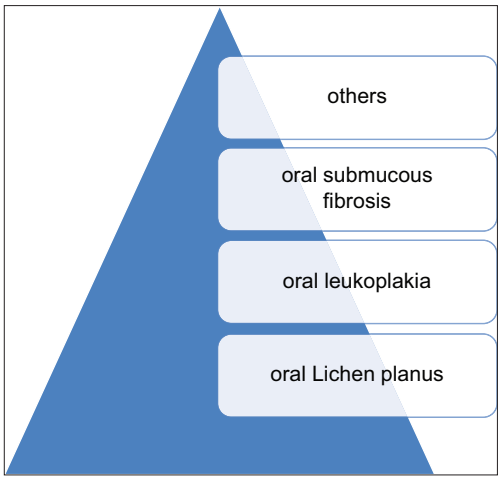

Figure 2: Types of oral potentially malignant disorders included in the review

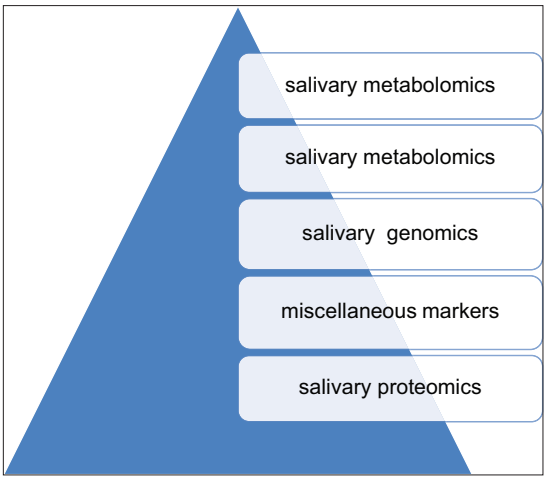

Figure 3: Types of salivary panomics assessed in this review

polymorphism, histone acetylation/deacetylation, loss of imprinting and chromosome inactivation. Promoter hypermethylation of DNA and miRNA studies in OPMDs provided promising results for their diagnostic and predictive values. The long noncoding RNA (lncRNA), salivary exosomal studies, studies on salivary extracellular vesicle, circulating cell-free DNA and circulating tumor DNA expression studies are the emerging areas in salivary genomic studies.

The miRNA studies revealed the upregulation or downregulation of various miRNA expressions in the particular disease of interest. Only two studies have come up with AUC value analysis. Hung *et al.* recommended that miR-21 and miR-31 were significantly higher in OPMD patients than in controls. This study also compared these miR expressions in tissue sample with a follow-up period of 820 days. It only mentions the sensitivity of the markers.[10] Zahran *et al.* in their studies suggested that miRNA-184 is significantly increased in OPMD with dysplasia patients when compared to normal and OSCC patients with maximum sensitivity (80%) and specificity (75%) and a high AUC value (0.86). This study also includes recurrent aphthous stomatitis as one of the disease control groups and found no differences from healthy control group. This study has a follow-up period of 3 years.[13] These three miRNA studies gave promising results and can be clinically utilized as potential markers in OPMD patients.

Salivary transcriptomic analysis takes accounts of RNA biomarker analysis of particular transcripts of genes. Various mRNA biomarkers and their predictive value have been studied individually and as a panel of markers. The significantly higher expression of mRNA and protein panel of H3F3A + IL-8 biomarkers could have a great AUC value of 0.752 in differentiation between OSCC and PMOD patients.[15]

Salivary proteomic studies include the study of either individual proteins of interest with total protein analysis or panel of proteins and peptides, and the particular proteins could be further validated.

Tumor necrosis factor-alpha (TNF-α) receptors are expressed on both epithelial and stromal cells. TNF-α is a pro-inflammatory cytokine that has both pro- and antitumorigenic effects. The cytotoxic effect is through necrosis which inhibits tumor progression. It can also stimulate angiogenesis, proliferation, migration and survival of tumor cells in cancer. Deepthi *et al.* found that there were extermely significant differences across three groups with elevated TNF-α expression from controls to leukoplakia to OSCC. The sensitivity is 90% and specificity 95%, with the 0.968 AUC value between leukoplakia and healthy control.[17]

Within the proteomic markers discussed, chemerin and matrix metalloproteinase (MMP)-9 also showed a significantly higher level, satisfactory AUC with high sensitivity and specificity in distinguishing OSCC from oral potentially malignant lesions. Chemerin is commonly witnessed in adipose tissue, fibroblast, endothelium and keratinocytes. Various studies stated that chemerin is a multifunctional adipokine which participates in regulating angiogenesis, inflammation and cell proliferation. MMP-9 is the largest member among the 26 members of MMP gene family. By degrading type IV collagen, fibronectin and elastin and also through regulation of angiogenesis, MMP-9 plays a major role in the pathogenesis of tumor.[20]

Camisasca *et al*. in their study suggested the presence of another interesting protein cytokeratin-10 (CK-10) fragment in all leukoplakia samples with decreased folds of cystatin-A when compared to control with a mean of 2.73-year follow-up. Further studies are needed to validate the use of CK-10 as a potential biomarker in predicting the progress of malignancy in leukoplakia. The tissue immunohistochemistry confirmed the presence of CK-10 in the superficial layers of the Oral Leukoplakia (OL) patients, which verifies the readily available of this protein in saliva. This was absent in the control group. However lower level of cystation SN indicates that cysteine protease may be involved in this cleavage.[21]

Salivary metabolomics is the study of metabolites that are small molecules released during metabolism that can provide the information regarding the early changes associated with the OPMDs.

This review includes only one study of salivary metabolomics. The study results revealed significantly decreased arginine, carnitine, ornithine, o-hydroxybenzoate, N-acetylglucosamine-1-phosphate and ribose-5-phosphate levels in the OSCC/oral epithelial dysplasia group than in persistent suspicious oral mucosal lesions (PSOMLs). The decrease in the R5P, one of the intermediate metabolites in the pentose phosphate pathway, specified a Warburg effect. The precursors of polyamines such as arginine and ornithine are intermediate metabolites in the urea cycle and are considered as a biomarker in various cancers. Since the increased polyamines are the indicators of the reduced ornithine and arginine, the study results were reasonable. The ROC analysis of the combined ornithine + O-hydroxybenzoate + R5F metabolites has shown that the AUC was sufficient to discriminate OSCC/Oral Epithelial Dysplasia from PSOML groups.[27]

The miscellaneous marker includes inflammatory/oxidative biomarkers and markers associated with anaerobic glycolysis. The reactive oxygen and nitrogen species give rise to oxidative damage to DNA and could be crucial in carcinogenesis and mutagenic. The UV light exposure, radiation and reactive oxygen and nitrogen species lead to the creation of 8-hydroxy-2-deoxyguanosine (8-OHdG). The membrane phospholipids are injured by the reactive oxygen and nitrogen species and identified as lipid peroxidation with malondialdehyde (MDA), a well-known biomarker of cell exposed to oxidative stress. In addition, the cytotoxic nature of MDA is reported to be responsible for tumor promotion and carcinogenesis. In contrast, Vitamin C and Vitamin E are accounted for defensive role against oxidative harm to DNA.

The salivary levels of 8-OHdG and MDA were significantly higher in OSCC, while Vitamin C and Vitamin E levels were decreased pursued by advanced phases of precancer patients compared to the early phase patients. The levels of 8-OHdG and MDA were significantly higher with lower levels of Vitamin C and Vitamin E in lichen planus, leukoplakia and submucous fibrosis patients compared with healthy controls. The combination of markers has high specificity (80%) and sensitivity (80%) when compared to individual biomarker approach.[32]

The age, gender, ethnic background, geographic location, dietary factors and medications taken can also influence the outcome of biomarker research. Other important confounding factors in biomarker studies are other associated mucosal inflammatory conditions, nonneoplastic systemic diseases and/or systemic cancers that can influence the outcome of study variables. In order to circumvent these factors, a proper study design, consistent research method should be planned and implemented accordingly.[39]

The majority of the selected papers showed statistically significant results within the study limits. Few studies have come up with sensitivity and specificity with AUC characteristics in OPMD patients as diagnostic/prognostic applications. Studies among different grades/different clinical types of OPMD were less.

Biomarkers with high sensitivity, specificity and optimum AUC values are considered as potential markers as a diagnostic tool. The physician's choice of biomarker selection to differentiate OPMDs from controls depends on these remarks and the practicality. Regarding the validation of biomarkers, different study methods can be applied for the same marker to arrive at reliable results. The studies can be repeated on different ethnic cohorts with various geographic conditions. Large-scale studies would also be helpful to validate the biomarkers of interest. Regarding OPMD, more longitudinal studies with uniform study methodology are needed for further validation.

The biomarker identity in OPMD depends on the individual lesion etiology, molecular biology and genetic behavior of the disorder. Hence, the biomarker of interest also varies in each disorder. The main purpose of a reliable biomarker in OPMD is early detection of the disease progress and cancer prediction. A reliable, precise and valid panel of

biomarkers for uniform application in various high-risk OPMDs in detecting MT is the need of hour.

The painless, quick and easily accessible panel of salivary biomarkers with the scientific credential for clinical/individual application in early diagnosis of oral cancer is the need of an hour. If a panel of biomarkers would be applicable for early detection of cancer in OPMD, it would also serve as an evidence-based treatment for the patients as well as it greatly reduces the psychological/economic burden of the patient.

The ultimate aim of any biomarker study is the development of point of care-monitoring system to deliver feasible patient care either clinical or personal monitoring. The integrative panoramic approach extends its anticipation to the invention of novel biomarkers and targeted therapeutics which leads to the new precision medicine era with enhanced patient care in the health-care system.

CONCLUSION

The early intervention is possible, if the genetic or epigenetic level markers are identified in early stages of OPMDs before the advanced clinical manifestation would emerge. The diagnostic ability of the biomarkers in OPMD is clearly evident from various studies, and these potential biomarkers can definitely delineate the OPMD patients from either normal or OSCC patients. The success of prediction/prognostic biomarker depends on the identification and validation of early MT in OPMD patients. For the disease-monitoring purposes, the expression of particular marker over a period of time would be an indicator for patient counseling and follow-up. The painless, quick and easily accessible panel of salivary biomarkers with the scientific credential for clinical/individual application in early diagnosis of oral cancer is the need of hour. The salivary biomarkers would also serve to monitor tumor heterogeneity over a period of time on a scale which is difficult to achieve with biopsy alone. The application of oral biofluid as a biomarker in detecting early oral cancer diagnosis would be more valuable in OPMD cases rather than Stage III and Stage IV oral cancer since the major side effect of postradiation therapy patients is xerostomia. Genomic salivary marker studies to detect long noncoding RNA and promoter hypermethylation of DNA and other genomic studies such as salivary exosomal study, extracellular vesicle study, circulating tumor cells and cell-free DNA studies were relatively less in OPMD patients. This could be the future areas of interest for the researchers. It is advisable to perform more longitudinal studies involving all the types of OPMDs for the homogeneous application in early cancer diagnosis.

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Conflicts of interest
There are no conflicts of interest.

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