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Evaluation of salivary biomarkers in patients with oral epithelial dysplasia: A systematic review

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Review Article

Abstract

BACKGROUND AND AIM: Early detection of premalignant oral lesions, especially in high-risk patients, is important to prevent mortality. Dysplastic changes are one of the elements of premalignant lesions which can be perceived in histopathologic examinations. The use of saliva is a promising method for diagnosing epithelial dysplasia, because it is non-invasive and easy to collect. This review evaluated the salivary biomarkers in patients with oral epithelial dysplasia (OED).

METHODS: In this systematic review study, all English articles were searched in the PubMed, Cochrane Library, Web of Science, and Scopus databases until February 2021. The searches were done using the Medical Subject Heading (MeSH) terms and free keywords. Textual data were analyzed manually and significant differences in salivary levels of biomarkers between patients with dysplastic lesions and healthy controls were reported and analyzed.

RESULTS: Originally, 1726 articles were found, of which 17 case-control articles were selected according to the inclusion/exclusion criteria. In 85% of studies, proinflammatory cytokine levels were significantly increased in the groups with epithelial dysplasia compared to the control groups. Tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1α showed an increase in all OED cases, but IL-1β showed no significant difference between epithelial dysplasia and control groups. Salivary levels of 14 types of micro-ribonucleic acid (miRNA) were studied, the most important of which were miRNAs 21 and 31, indicating a significant increase in the epithelial dysplasia groups compared to the control groups.

CONCLUSION: Based on the results of this systematic review, evaluation of salivary cytokines (TNF-α, IL-6, and IL-1α) and miRNAs 21 and 31 may be a non-invasive method in the early detection and prognosis of epithelial dysplasia and may also be useful in developing new prevention and treatment strategies.

KEYWORDS: Precancerous Conditions; Interleukins; Saliva; Biomarkers

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The prevalence of oral cancer has increased over the past decades and is often diagnosed in its late stages. Despite many advances in cancer treatment, early and timely diagnosis plays a major role in successful treatment and increasing patient survival as well as improving their quality of life.[1,2] A premalignant lesion is a disease or syndrome that, without treatment, can lead to cancer. Oral premalignant lesions (OPMLs) such as oral leukoplakia (OL) and oral lichen planus (OLP) occur in about 2.5% of the general population. Leukoplakia and erythroplakia or erythroleukoplakia are most important premalignant lesions and their importance originates from high percentage of cases in which dysplasia or even carcinoma in situ (CIS) was shown in biopsy.[3] While researchers suggest a genetic etiology for

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OPMLs, similar changes can be found in tobacco and alcohol-induced lesions.[4] Early detection of OPMLs, especially in high-risk populations, is very important to prevent disease and mortality, because the rate of cancer transformation within a mean period of 7 years after diagnosis is up to 17%.[3] Dysplastic changes are one of the elements of premalignant lesions which can be detected in histopathologic studies which are obtained from tissue biopsy. Pain and bleeding especially in patient with coagulation problems, inadequate removal of tissue, tissue removal from improper site, and misdiagnosis of the pathologist are complications of conventional biopsy. Hence, clinical and histological features alone cannot properly predict whether such a lesion would remain static, regress, or evolve into malignancy. Such characteristics of premalignant disorders require recognition of molecular markers, which can predict the disease progression.

Although the latest development in medicine recommends conservative methods in diagnostic procedures, further research is required for additional evaluation.[5] The use of saliva is a promising method for diagnosing epithelial dysplasia, because it is non-invasive and easy to collect. Human saliva contains proteins, electrolytes, peptides, and inorganic and organic salts, which are secreted from salivary glands, and mucosal transudates and gingival crevicular fluids (GCFs) also contribute to this mixture.

Biomarkers are the molecular indicators of normal and pathological processes, and pharmacological response to treatment, thus, can be useful in diagnosis and prognosis of the disease. Biomarkers may be used alone or in combination to assess health or disease. An ideal biomarker should have an easy and cost-effective measurement method. Evaluation of a biomarker in cancer and dysplastic lesions helps to develop diagnostic and therapeutic methods that can target the biomarker and reduce the diagnostic and therapeutic costs.[6,7]

In a review study by Cristaldi et al., micro-ribonucleic acids (miRNAs) were introduced as salivary biomarkers for early detection, prevention of cancerous lesions, and improvement in treatment outcomes in these patients.[8] In a review study conducted by Maheswari et al., miRNA-184, miRNA-21, and miRNA-145 were shown as biomarkers with potential in early detection of malignancy.[9] A review study conducted in Romania by Roi et al. showed that abnormal cytokine levels played an important role as biomarkers for oral squamous cell carcinoma (OSCC).[10]

Given the importance of the issue and that more attention is paid to saliva analysis and its use in diagnosing diseases and monitoring public health, the aim of this study was to investigate the role of saliva in the diagnosis of oral mucosal dysplasia as a systematic review.

Methods

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[11] have been used for reporting this systematic review. A focused question was produced according to the patient, intervention, comparison, outcome (PICO) principles. The main question for this study was "Are patients with OPMLs (P) who have increased levels of salivary cytokines/miRNAs (I) compared with healthy controls (C) at increased risk for epithelial dysplasia (O)"?

In this review study, published articles in English were searched by librarian (FS) from PubMed, Web of Science, Cochrane, and Scopus databases until February 2021. Besides, related sources in the selected studies were manually searched. The searches were restricted to human studies. The free and Medical Subject Heading (MeSH) terms were used in various combinations for collecting data. The search keywords included: 'oral' AND 'saliva' AND 'epithelial dysplasia' AND 'oral premalignant lesions' OR 'oral lichen planus' OR 'OLP' OR 'oral leukoplakia' OR 'submucous fibrosis' OR 'tobacco pouch keratosis' AND 'biomarkers' OR 'micro-RNA' OR 'inflammatory cytokine' OR 'interleukins' OR 'interferon' OR 'tumor necrosis factor' OR 'TNF' OR 'IFN' OR 'IL'.

After extracting the articles from the databases, they were screened by two experts in three steps. In the initial stage, titles and abstracts were reviewed by two independent reviewers (PM and KK) based on the inclusion and exclusion criteria. Disputes were resolved with the discussion with the third author (FP). In the next step, the full text of the selected articles was reviewed. The evaluation checklist of Joanna Briggs Institute (JBI) was used to appraise the selected articles; thus, the risk of bias of studies was assessed. The JBI checklist for case-control studies has 10 criteria. Each item was answered as "yes", "no", "unclear", or "not applicable". With 1-3 "yes" scores, the risk of bias was classified as high risk and was excluded from the study, 4-6 "yes" scores were rated as moderate risk, and 7-10 "yes" scores were considered as low risk. Microsoft Excel software was used to organize the extracted data from each study. The extracted information included the first author, year of publication, type of marker(s), sample size, marker evaluation method, and study results (significant relationship between marker and the presence of dysplasia). The target variables were inflammatory cytokines and miRNA.

Cross-sectional and case-control studies that examined salivary biomarkers in oral epithelial dysplasia (OED) were included in the review. Exclusion criteria included review studies, case-report studies, studies that examined salivary biomarkers in cancerous lesions, and studies that examined salivary biomarkers in patients who had inflammatory conditions in addition to epithelial dysplasia that could affect the biomarkers.

Textual data were analyzed manually, and significant differences between these variables and dysplastic and non-dysplastic lesions and healthy tissue were examined and analyzed. The studies involved a high rate of heterogeneity, and many different biomarkers and miRNAs were examined in different articles; thus, no meta-analysis was performed. The study was approved by the Regional Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran (Ethical code: IR.TBZMED.VCR.REC.1399.415).

Results

After a systematic search of sources, 1726 articles were identified. 797 articles were excluded because of duplication, and 879 articles were excluded after reviewing the title and the abstracts. After reviewing the full text of the articles, 17 articles were included in this study. The flow chart for the identified and included articles is shown in Figure 1. The details of the studies included in the study are given in Tables 1 and 2.[12-28]

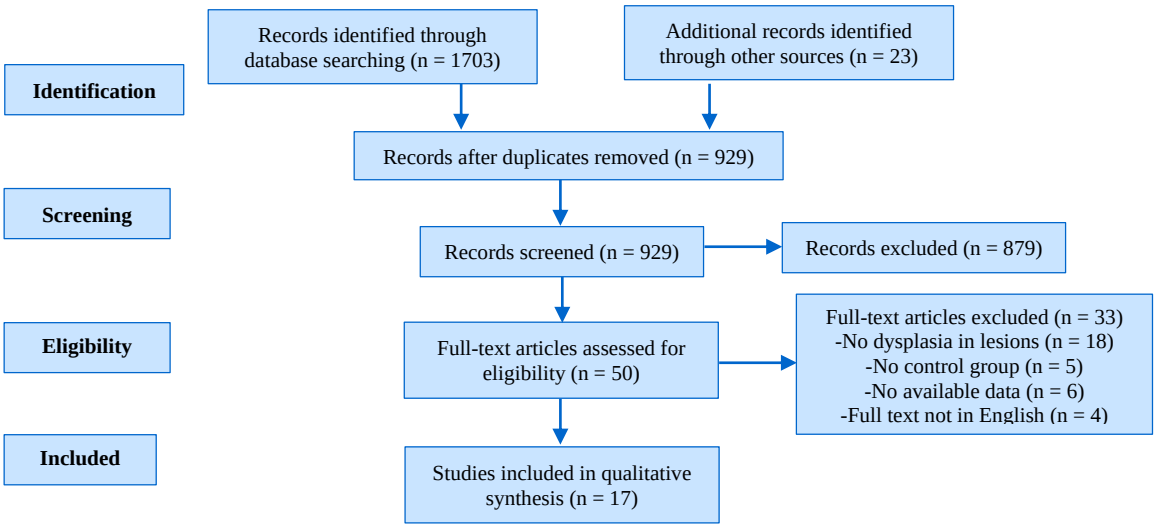

Figure 1. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart of the study selection

Table 1. Levels of salivary micro-ribonucleic acid (miRNA) in oral epithelial dysplasia (OED)

Authors	Country	Sample size (control/case)	Method of miRNA detection	Type of OED	Markers	Results in OED group compared with healthy control group
Uma Maheswari et al.[12]	India	36/36	qRT-PCR	12: OSMF 8: OL 9: OLP 7: OSMF and OL	miRNA-21 miRNA-31	An increase in OPMLs with severe dysplasia An increase in OPMLs with severe dysplasia
Mehdipour et al.[13]	Iran	15/20	qRT-PCR	OLP	miRNA-21 miRNA-31 miRNA-125a miRNA-200a	An increase in OLP with dysplasia An increase in OLP with dysplasia A decrease in OLP with dysplasia No significant differences
Shahidi et al.[14]	Iran	15/22	qRT-PCR	OLP	mi miRNA-320a	A decrease in OLP with dysplasia
Hung et al.[15]	Taiwan	20/24	qRT-PCR	OPMLs	miRNA-21 miRNA-31	An increase in OPMLs with dysplasia An increase in OPMLs with dysplasia
Zahran et al.[16]	Saudi Arabia	20/20	qRT-PCR	OPMLs	miRNA-21 miRNA-184 miRNA-145	An increase in OPMLs with dysplasia An increase in OPMLs with dysplasia A decrease in OPMLs with dysplasia
Yang et al.[17]	China	7/8	qRT-PCR	Progressive OL	miRNA-10b miRNA-145 miRNA-99b miRNA-708 miRNA-181c miRNA-30e miRNA-660 miRNA-197	An increase in OL with dysplasia A decrease in OL with dysplasia A decrease in OL with dysplasia An increase in OL with dysplasia A decrease in OL with dysplasia An increase in OL with dysplasia An increase in OL with dysplasia A decrease in OL with dysplasia

qRT-PCR: Quantitative reverse transcriptase-polymerase chain reaction; OED: Oral epithelial dysplasia; OLP: Oral lichen planus; OPMLs: Oral premalignant lesions; OL: Oral leukoplakia; OSMF: Oral submucose fibrosis; miRNA: Micro-ribonucleic acid

Table 2. Levels of salivary cytokines in oral epithelial dysplasia (OED)

Authors	Country	Sample Size (control/ case)	Method of cytokine detection	Type of OED	Markers	Results in OED group compared with healthy control group
Babiuch et al.[18]	Poland	7/7	ELISA	OPMLs	IL-1α, IL-6, IL-8, TNF-α	An increase in OED
Deepthi et al.[19]	India	30/30	ELISA	OL	TNF-α	An increase in OED (positive significant correlation with grading of dysplasia)
Ameena and Rathy[20]	India	30/30	ELISA	OPMLs	TNF-α	An increase in OED (positive significant correlation with grading of dysplasia)
Shahidi et al.[14]	Iran	15/22	ELISA	OLP	IL-6	An increase in OED
Michailidou et al.[21]	Greece	31/20	RT-PCR	OPMLs	IL-1β, IL-8	No significant differences
Gleber-Netto et al.[22]	Taiwan	60/60	ELISA	OPMLs	IL-1β, IL-8	No significant differences
Dineshkumar et al.[23]	India	100/50	ELISA	oral leukoplakia	IL-6	An increase in OED (positive significant correlation with grading of dysplasia)
Kaur and Jacobs[24]	India	50/141	ELISA	43: OL 48: OLP 50: OSMF	IL-6, IL-8, TNF-α	An increase in OED (positive significant correlation with grading of dysplasia)
Krishnan et al.[25]	India	100/50	ELISA	OL	TNF-α	An increase in OED (positive significant correlation with grading of dysplasia)
Juretic et al.[26]	Croatia	19/19	ELISA	OPMLs	IL-6, TNF-α	An increase in OED
Rhodus et al.[27]	2005/USA	13/13	ELISA	OLP with moderate and sever dysplasia	IL-1α, IL-6, IL-8, TNF-α	An increase in OED
Rhodus et al.[28]	2005/USA	13/13	ELISA	OPMLs	IL-1α, IL-6, IL-8, TNF-α	An increase in OED

ELISA: Enzyme-linked immunosorbent assay; RT-PCR: Reverse transcriptase-polymerase chain reaction; OED: Oral epithelial dysplasia; OLP: Oral lichen planus; OPMLs: Oral premalignant lesions; OL: Oral leukoplakia; OSMF: Oral submucose fibrosis; IL: Interleukin; TNF: Tumor necrosis factor

All of the articles selected for this systematic review were case-control studies. The studied biomarkers were miRNA and inflammatory markers. In 12 studies, proinflammatory cytokine levels were examined.[18-28] In 10 (85%) of these studies, salivary levels of cytokines were significantly increased in the groups with epithelial dysplasia compared to the control groups.[18-20,23-28] Based on the results of this systematic review, tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1α showed an increase in all OED cases.[18-20,23-28] In addition, salivary levels of these cytokines were positively correlated with the degree of dysplasia.[19,20,23-25] IL-1β showed no significant difference between epithelial dysplasia and control groups.[21,22] In the case of IL-8, 4 studies showed an increase in the level of this cytokine in the saliva of patients with epithelial dysplasia,[18,24,27,28] and 2 studies showed no significant difference between patients and control groups; therefore, sufficient evidence to support the role of this cytokine in epithelial dysplasia lesions was not achieved.[21,22]

Salivary levels of 14 types of miRNA were studied in 6 studies. MiRNAs 21 and 31 were the main markers studied in the included articles and they both showed a significant increase in lesions with epithelial dysplasia groups compared to the control groups.[12,13,15,16] There was no significant diference in miRNA-200a levels in one study.[13] MiRNA-145 showed a significant decrease in the epithelial dysplasia in 2 studies.[16,17]

Discussion

The aim of this review study was to evaluate proinflammatory cytokines and salivary miRNAs in patients with epithelial dysplasia. It was observed that cytokines such as TNF-α, IL-6, IL-1α, and miRNAs such as miRNAs 21 and 31 showed a significant increase in the epithelial dysplasia group, which were positively correlated with an increase in the degree of dysplasia.[12-28]

Evidence shows that the clinical appearance and histopathologic changes that indicate OPMLs are caused by specific molecular changes that accumulate over time, ultimately leading to malignant transformation. Dysplasia is an early event, it is followed by some molecular changes essential to the progression to malignancy, and therefore, the use of biomarkers would assist in the prediction of premalignancies' transformation with higher specificity and sensitivity when combined with clinical and histopathological findings.

As saliva contains a wide range of compounds and its sampling is non-invasive and relatively safe with low risk of pathogen transmission, the use of salivary biomarkers is growing. Moreover, saliva can be stored easily and does not coagulate.[29,30]

Due to miRNA stability in the extracellular environment and obtainability in different body fluids circulating, it is the best biomarker for diagnosing and evaluating the progression of the disease.[31] miRNA has a utility in the pathogenesis of cancers and it acts by targeting some tumor suppressor genes or oncogenes.[32,33] Amongst the selected studies, 6 studies examined miRNAs in lesions with dysplasia,[12-17] in 4 of which,[12,13,15,16] miRNA-21, and in 3 of them,[12,13,15] miRNA-31 were evaluated. Mehdipour et al. showed that significant increases in miRNAs 21 and 31 levels were found in samples originated from patients with dysplastic OLP, compared to those of healthy controls and patients with non-dysplastic OLP.[13] In addition, Hung et al. showed that salivary miRNA-21 and miRNA-31 were up-regulated in patients with OED and that further increase in miRNA-31 was associated with dysplasia progress.[15] Uma Maheswari et al. showed that salivary miRNA-21 could be used as a potential auxiliary biomarker to assess early malignant alterations in OPMLs, because its levels were significantly increased in saliva of patients with dysplastic OPMLs, compared to healthy controls.[12] This increase may be associated with the inhibition of tumor suppression or

malignant transformation; moreover, inability of miRNA-21 to detect premalignancies was much higher when compared to salivary miRNA-31.[34,35]

Shahidi et al. also showed a significant decrease in salivary miRNA-320a in dysplastic OLP but not in OLP without dysplasia.[14] Mehdipour et al. reported that miRNA-200a might not be considered as a proper biomarker for detection of dysplastic OPMLs. It was observed that miRNA-125a levels were more significantly reduced in patients with dysplastic OPMLs.[13] Considering the additional decrease or increase of miRNA levels observed in dysplastic OPMLs samples, these miRNAs may serve as a biomarker for detection of malignant transformation in patients with OPMLs.

The transcription factor, nuclear factor-kappa B (NF-κB) is an early response gene, stimulating the expression of a chain of cytokines with pro-angiogenic, pro-inflammatory, and immunoregulatory characteristics, which have a crucial role in carcinogenesis.[36-38] Amongst the selected studies, 12 studies examined NF-κB-dependent cytokines in dysplastic lesions. In 85% of studies, the level of these cytokines in the epithelial dysplasia groups was significantly increased compared to the control group,[18-20,23-28] and in 15% of the studies, no significant difference was observed between the epithelial dysplasia and control groups.[21,22] The contradictory results may be described by the limitations in sample sizes and the various detection methods used in different studies. The levels of salivary TNF-α, IL-1α, IL-6, and IL-8 were statistically and significantly higher in advanced stages of precancerous lesions as compared to early stages.[19,20,23-27]

It has been presented in multiple studies that the chronic inflammation develops a cytokine-based micro environment which can influence cell survival, growth, proliferation, and differentiation, therefore, resulting in cancer initiation and progression.[39] It has not been proven whether this activation is a necessity for angiogenesis and malignant transformation or its result, nonetheless present evidence would probably support both. It does not seem unreasonable to link the inflammation and malignant transformation of OPMLs by activation of NF-κB and NF-κB-dependent cytokines. These investigations highlight the possibility that the inflammatory micro environment mediated by NF-κB and its related cytokines is responsible for beginning or advancing the malignant transformation of OPMLs. The NF-κB-dependent cytokines are increased as a result of localized production from lesional epithelium and activated T lymphocytes in the connective tissue affected with OPMLs.[40]

Considering that some of the biomarkers that have been studied were cytokines, it may be suggested that inflammatory diseases in oral cavity may have a confounding effect in the analysis. Inflammatory diseases, such as periodontitis, are one of the most common pathologies present in oral cavity.[41,42] Therefore, biomarker research should differentiate and validate potential epithelial dysplasia biomarkers in patient with oral inflammatory diseases.

The present study had also some limitations. First, sample size was limited for some groups. Second, the search was limited to articles with English abstract that can be considered as language bias. Since the search and review show few studies on salivary biomarkers in oral precancerous lesions, further studies with larger sample sizes and with long-term follow-ups are recommended. The efficiency of these biomarkers can only be estimated based on the well-designed, prospective multi-institutional trials with larger sample sizes.

Conclusion

Saliva has advantages over other body fluids and is a convenient and simple diagnostic tool. Based on the results of this systematic review, evaluation of salivary cytokines (TNF-α, IL-6, and IL-1α) and miRNAs 21 and 31 may be a non-invasive and cost-effective

method in the early detection and prognosis of epithelial dysplasia and may also be useful in developing new prevention and treatment strategies. However, further studies are necessary for validation of salivary biomarkers for clinical uses. By using newer and more sensitive techniques with standard reference values in the near future, salivary diagnosis will become the method of choice in the early detection of OED and progression of malignancy.

Conflict of Interests

Authors have no conflict of interest.

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