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# MATRIX METALOPROTEINASE AS A POTENTIAL SALIVARY BIOMARKER FOR ORAL SQUAMOUS CELL CARCINOMA

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**KEYWORDS**

biomarker, MMP, oral cancer, OSCC, saliva

**ABSTRACT**

***Introduction****: Oral squamous cell carcinoma (OSCC) is the sixth most common cancer worldwide and the most prevalent form of oral cancer. The five-year survival rate for OSCC varies considerably depending on the stage of the disease, ranging from 40% to 60%. The majority of cases are diagnosed at an advanced stage, resulting in a significantly reduced life expectancy. Therefore, there is a clear need for effective strategies to detect cancer at an early stage.* ***Aim:*** *The objective of this article is to identify the potential of matrix metalloproteinase (MMP) as a salivary biomarker for the early detection of oral squamous cell carcinoma (OSCC).* ***Review:*** *Saliva is considered a potential source of biomarkers for oral cancer due to its continuous contact with cancerous lesions in the oral cavity and the various enzymes, hormones, antibodies, antimicrobial constituents, and cytokines it contains. MMP is an extracellular endopeptidase enzyme present in saliva and associated with the carcinogenesis process. It has been identified as a salivary biomarker for the early detection of OSCC. The levels of several MMP proteins in the saliva of OSCC patients have been found to be elevated, including MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-11, MMP-12, and MMP-13.* ***Conclusion:*** *The increased levels of salivary MMP, which were most specifically found in OSCC patients, included MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, MMP-12, and MMP-13. This suggests that MMP may be a potential salivary biomarker for the early detection of OSCC.*

## INTRODUCTION

Oral squamous cell carcinoma (OSCC) represents the sixth most common malignancy worldwide.[1,2] Based on data from Basic Health Research of the Republic of Indonesia (Riset Kesehatan Dasar or RISKESDAS) in 2013, the prevalence of oral cancer in Indonesia was 1.4%, this number increased by 4.2% in five years to 5.6% in 2018,[3] . Therefore, it is evident that efforts must be made to reduce the incidence of oral cancer in Indonesia. The survival rate for patients with OSCC is poor, with 5-year survival rates ranging from 40 to 50%.[4] This is due to delays in establishing a diagnosis or if a diagnosis is established at an advanced stage.[5,6] Efforts are needed to establish an early diagnosis and to obtain additional examination tools to reduce morbidity and mortality due to cancer,[6] since general practitioners who are not oral

surgeons discover the majority of oral cancer cases.[7]

The diagnosis of OSCC can be made through clinical and histological examination; however, this method is considered less effective in the early stages of cancer development.[8] The biopsy has long been regarded as the gold standard for diagnostic purposes. However, it is a relatively time-consuming procedure and is site-specific.[9] Another issue associated with incisional biopsy is the elevated risk of metastasis resulting from the disruption to the tissue barrier.[2] The establishing of a diagnosis of OSCC at an early stage can prevent the necessity for more extensive treatment procedures by the utilisation of diagnostic tools, including biomarkers. Salivary biomarkers, which consist of DNA, RNA, and protein markers, are one of the diagnostic modalities that have a significant relevance in the field of molecular biology.[5]

Some studies have indicated that saliva is a valuable supporting medium for examination due to its continuous interaction with the cancerous lesion in the oral cavity. Saliva contains a variety of analytes that can be utilized as biological markers (biomarkers) for the early detection of cancer. One such biomarker is an enzyme known to be involved in the cancer invasion process, namely matrix metalloproteinases (MMPs). MMPs play a pivotal role in the breakdown of the extracellular matrix (ECM), which is essential for the invasion and metastasis of cancer.[8,9]

The objective of this literature review is to identify the potential and role of using matrix metalloproteinase (MMP) enzyme protein biomarkers in saliva as an examination medium for the early diagnosis of oral cancer. This approach has the potential to reduce morbidity and mortality due to cancer, particularly oral squamous cell carcinoma (OSCC).

## REVIEW

### Oral Squamous Cell Carcinoma

Cancer is defined as uncontrolled cell proliferation caused by irreversible genetic alterations related to a variety of external environmental stimuli. Even after the discontinuation of exposure to these stimuli, the growth will continue if induced. Cancer is essentially genetic in nature, yet it is not always inherited.[10] One of the hallmarks of cancer is invasion and metastasis, which are the primary causes of morbidity and mortality. The metastatic cascade is divided into several phases, namely (1) extracellular matrix (ECM) invasion and (2) vascular dissemination, tissue homing, and colonization. The stages of ECM invasion comprise a number of active processes, including (1) "loosening up" the interactions between tumor cells, (2) ECM degradation, (3) attachment to the "remodelled" ECM components, and (4) migration and invasion of tumor cells.[11] The degradation of the basal membrane and interstitial connective tissue is a part of the invasion and metastasis process.

Tumor cells can invade by secreting proteolytic enzymes or by inducing stromal cells (e.g., fibroblasts and inflammatory cells). A range of proteases, including matrix metalloproteinases (MMPs), chaptesin D, and plasminogen activator urokinase, are overexpressed in tumors and play a role in tumor cell invasion.[11]

OSCC is the most prevalent form of cancerous lesions in the head and neck region. Approximately 90% of oral cancers have a histologic origin in squamous cells, a condition that is clinically defined as oral squamous cell carcinoma.[12] OSCC is an invasive epithelial neoplasia, which histologically exhibits varying degrees of differentiation. Based on the International Classification of Diseases (ICD version 9, a category: 140- 146), there are many different sub-areas within the oral cavity, including the buccal mucosa, alveolus, retromolar triangle, maxillary and mandibular gingiva, tongue, floor of the mouth, palate. The location of intraoral cancer in Asian populations is more prevalent in the buccal mucosa, whereas in Western populations, it is more on the lower portion of the tongue and the floor of the mouth.[13]

Carcinogenesis is a molecular process that alters molecular functioning, cell structure, and, most importantly, cellular characteristics. It is not only restricted to the epithelium but also includes dynamic interactions between connective tissue and immune function. The primary genes consist of oncogenes and tumor suppressor genes (TSGs), and the role of regulatory genetic molecules is implicated in OSCC. Genetic alterations describe allele loss or additions to the chromosomal areas related to proto-oncogenes and TSGs, or epigenetic alterations such as deoxyribonucleic acid (DNA) methylation or histone deacetylation. Viruses and carcinogens, as well as extracellular enzymes, cell surface molecules, and immune function, are important factors in the development and spread of oral malignancy.[14]

The ability of oral squamous cell carcinoma to metastasize lies in its ability to degrade the basal membrane, penetrate surrounding tissues and vascular structures, as well as forming new vasculature for tumor expansion.[15] Squamous cell carcinoma spreads predominantly through direct, local, and regional extension, particularly via the lymphatic pathway. The regional extension of the oral mucosa can occur through direct and submucosal extension, resulting in the vastness of the area involved. The production of type-I collagenase, heparinase, prostaglandin E2, interleukin-1 (IL-1), and connective tissue growth factor (CTGF) affects the ECM and the motility of epithelial cells leading to invasion. Alterations to the basal membrane, such as damage to collagen and laminin, occur in the presence of invasion. MMPs and tissue-metalloproteinase inhibitors have a significant role in the development and progression of cancer and have prognostic significance. Understanding the biological

invasion of cancerous cells leads to other approaches to diagnosis and management.[14]

**OSCC Examination and Early Detection**

Over the years, the improvement in survival rates has been minimal. This is because most of OSCC cases go undiagnosed until they are at an advanced stage.[12,16,17] This condition occurs due to a lack of knowledge from both the patient and the physician; therefore, the physician is not precise in diagnosing. Delays in diagnosis can drastically reduce the survival rate, even though many treatment methods are available.[12] Contrary to popular belief, early detection and diagnosis can increase survival rates and are essential to the success of clinical treatment.[18] Early detection of oral cancer is paramount because the best prognosis and even the chance of cure are offered by treatment at the pre-invasive stage.[16]

Early diagnosis of OSCC is considered difficult because there is generally no pain in the initial stages of the disease's development and no burning sensation in the mouth until the lesion gets into an advanced stage.[18] Generally, oral cancer is found during conventional extraoral examination (COE) or conventional visual and tactile examination (CVTE), which are the most common oral cancer screening methods, followed by a needle biopsy and followed by a histopathological evaluation to obtain a definitive diagnosis. Conventional extraoral examinations are routinely carried out by dental health care providers or dental clinicians under normal lighting conditions; therefore, they are inadequate for cancer risk assessment.[19,20]

Histological examination is the "gold standard", but under certain conditions, it is considered less practical due to the nature of the tumor (benign or malignant) and the tumor's response to the therapy provided.[16,17] This histological examination takes more time,[21] and is an area-specific procedure.[22] Saliva, cerebrospinal fluid, blood serum, and urine are examples of bodily fluids where gene alterations can be detected. These bodily fluids provide information on changes to proteins and nucleic acids, making them useful biomarkers for the early detection of OSCC at the initial stage.[23] The most typical source of biomarkers is blood (plasma and serum). However, saliva is in direct interaction with lesions in the oral cavity, making it a possible source of oral cancer biomarkers.[20,24] Saliva is also generally available in large volumes, does not clot, and can be collected as frequently as necessary, making it far easier to collect, store, transport, and use as a component of clinical tests than blood and tissue.[20,25]

**Matrix Metalloproteinases (MMPs)**

MMPs or matrixin[26] are a group of extremely homologous extracellular endopeptidases that are zinc- and calcium-dependent. MMPs combine with their specific inhibitors, such as tissue metalloproteinase inhibitors (TIMPs).

MMP has enzymatic activity, is involved in maintaining homeostasis of ECM components, processing inflammation, carcinogenesis, and migration.[27,28] MMP is currently recognized as a biomarker in various fields including diagnosis, monitoring, and treatment efficacy, where overexpression of MMP is found to be specific and elevated in a disease.[29] The fundamental biological activity of MMPs are ECM proteins and glycoproteins, membrane receptors, growth factors, and cytokine degradation. MMPs contribute to a wide range of biological processes, including tissue regeneration and repair, cellular differentiation, embryogenesis, morphogenesis, cell mobility, angiogenesis, cell proliferation and migration, wound healing, apoptosis, as well as substantial reproductive events such as ovulation and endometrial proliferation. MMP activity deregulation leads to the development of several pathological processes, which can be classified into (1) tissue destruction, (2) fibrosis, and (3) matrix degeneration. MMP overexpression is known to be involved in several diseases.[30] There are 23 MMP paralogues, of which 14 are expressed in the vascular endothelium. These paralogues are made up of a duplicated MMP-23 gene that encodes two similiar forms of MMP-23. The MMP family is classified into six subfamilies: collagenase, gelatinase, stromelysin, matrilysin, membrane-type MMP (MT)-MMP, and other MMPs (Table 1).[31]

**Table 1.** Specificity of matrix metalloproteinase substrate.[31]

| Group | MMP | ECM Mayor Substrate |
|---|---|---|
| Collagenases | MMP-1, MMP-8, MMP-13, and MMP-18 | Type I, II, III, VII, VIII, and X fibrillar collagen, gelatin, aggrecan |
| Gelatinases | MMP-2 and MMP-9 | Gelatin, elastin, galectin 3, aggrecan, and fibrinoectin; collagen type IV, V, VII, X, and XIV |
| Stromelysins | MMP-3, MMP-10, MMP-11, MMP-12 | Collagen type III, IV, IX, and X, casein, gelatin, proteoglycans, fibrinoectin, and laminin |
| Matrilysins | MMP-7, and MMP-26 | Type IV collagen, fibrinoectin, laminin, proteoglycans, and VE-cadherin |
| Membrane-type | MMP-14, MMP-15, MMP-16, and MMP-25 | Tenascin-C large, fibrinoectin, laminin |

**MMP in Carcinogenesis**

During carcinogenesis, MMPs with their inhibitors are associated with the invasion regulation and metastasis of oral cancer. An imbalance interaction between MMPs and their specific inhibitors, such as tissue inhibitor metalloproteinase (TIMPs), contributes to cancer initiation and progression.[32] MMP overexpression in tumors and stromal cells in various cancers was discovered in the early 1990s and was reported to be related to the invasion and

progression of tumorigenesis. Moreover, MMPs generated from distant organs, as well as growth factors against tumor cells, are known to have a part in the metastasis initiation.[27]

Cancer metastasis is the major cause of mortality. The metastatic process is divided into various stages involving the stroma, blood vessels, and other related factors (eg, epithelial-mesenchymal transition [EMT])[33]. These processes include (1) local infiltration of tumor cells in the surrounding tissue; (2) intravasation or trans-endothelial migration of cancer cells into blood vessels; (3) tumor defense in the circulatory system; (4) extravasation; and (5) proliferation of organs leading to colonization.[34] The effectiveness of tumor metastasis depends on many factors, including cell invasion, migration, angiogenesis, host immune escape, and extravasation.[33] Metastasis not only depends on the capacity of cancer cells to migrate from the primary tumor but also requires the formation of a receptive environment, known as the metastatic niche, which is suitable for tumor attachment to distant organs. MMPs are involved in the formation of metastatic niches. MMP-9 is an important substance in the formation of metastatic niches that can release VEGF (vascular endothelial growth factor) and induce angiogenesis.[35]

Angiogenesis is the growth and formation of new capillaries and blood vessels originating from previous blood vessels that function for nutrient diffusion and provide tissue oxygenation or cell metabolism related to the wound healing process, myeloid cells, and stromal cells. Angiogenesis is a physiological developmental process and is a part of the healing process, but it can also characterize pathological conditions such as cancer. The development of new blood vessels may facilitate the development, maintenance, and spread of cancer. MMP affects the regulation of angiogenesis and the immune function in the initiation and spread of cancer. The pro- and anti-angiogenic properties of MMP significantly influence the ECM or several substrates-degradation. A group of proteins called collagenases, which includes MMP-1, MMP-8, and MMP-13, are related to angiogenesis. Furthermore, MMP-2 and MMP-9 modulate the remodeling of ECM by changing aggrecan, collagen, elastin, fibronectin, laminin, and glycosaminoglycans which affect the process of angiogenesis.[26]

Invasion is a local process that takes place between the host and the tumor surface. Tumor and stromal cells exchange biological molecules (such as enzymes and cytokines), thereby modulating the local ECM and stimulating cell migration. MMPs regulate the ability of growth factors and receptors of the cell surface during the invasion process while supporting the formation of specialized structures known as invadopodia. Invadopodia use a variety of secreted and activated MMPs to break down ECM macromolecules, modulate the release of membrane-anchored ligands (like epidermal

growth factor receptor [EGFR]), regulate integrin-proliferative effects, change antiapoptotic signaling by cleaving Fas ligands, and control tumor vasculature. MMP can also decrease chemotactic and inflammatory responses by inactivating MCP3 (monocyte chemotactic protein 3). MMPs not only contribute to cell invasion but can frequently also prevent it, depending on the equilibrium of the expressed components.[33]

**Saliva and Salivary Biomarker**

The National Cancer Institute defines a biomarker as a biological molecule that can be detected in blood, other bodily fluids, or tissues and functions as a marker for a condition, disease, or the sign of a normal or abnormal process. It is also known as molecular markers that are widely used to establish the diagnosis, the prognosis of a health condition or disease, and monitor treatment (Figure 1).[36] Biomarkers or biological markers are substances that are generally used as indicators of the biological status of oral diagnosis. The most significant issues in the use of biomarkers are the identification of biomarkers for screening, prognosis, and disease activity evaluation, as well as treatment efficacy (diagnostic testing). There are two types of biomarkers used in cancer detection, including diagnostic and prognostic biomarkers. Diagnostic biomarkers assist in the detection or confirmation of the disease or condition to be assessed, while prognostic biomarkers assist in determining the progression, course and recurrence of the disease.[37]

Laboratory evaluation is an important method and has high accuracy to establish the diagnosis and to determine the prognosis of the disease. Among all laboratory tests, liquid biopsy is a non-invasive procedure as it does not require tissue removal. [25] Over the past 20 years, various oncology fields have regarded liquid biopsy as an essential test based on the detection of circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating tumor RNA (ctRNA), proteins, and exosomes.[38] Samples of liquid biopsies include blood, urine, saliva, and other body fluids such as plasma, seminal fluid, pleural effusion, cerebrospinal fluid, sputum, and stool samples.[39] Over the past few decades, saliva has been evaluated as an important bio-fluid or biological fluid for detecting various diseases. Saliva is mostly made of water (>99%), protein (1%), electrolytes, and other low molecular weight substances.[19,40] It has a complex component originating from the salivary glands (major and minor), blood, oropharynx, gastrointestinal reflux, and gingival crevicular fluid (GCF). Because changes in components present in saliva can indicate the physiological as well as pathological states of the body's health, analysis of salivary components is regarded as a useful procedure for monitoring health status.[19,41]

Saliva represents the whole picture of the body and is known as a "mirror of health" or "window of health".[18] Saliva samples can be used to diagnose a variety of diseases such as cancer, cardiovascular, neurological, and metabolic disease.[19] Oral cancer lesions that are in constant interaction with saliva make the tumor marker examination in saliva an alternative for serum and tissue examination.[18]

Numerous analytes found in saliva can be utilized as biomarkers in research and therapeutic applications. It comprises an array of cytokines, hormones, antibodies, and antibacterial components. Numerous blood constituents are identified in saliva. The majority of these constituents enter the saliva through the blood by passing through the cells via intracellular, transcellular, and paracellular diffusion through extracellular ultrainfiltration within the salivary glands or through the gingival sulcus. Saliva is seen as an essential sign of both systemic and oral health.[42] In general, there are five categories of salivary biomarkers for OSCC: metabolic, proteomic, transcriptomic, genomic, and epigenomic (Figure 2).[43]

Carcinogenesis is a dynamic process involving a wide range of genotypic and phenotypic alterations at molecular and tissue levels. The extensive application of genomic and proteomic methods has resulted in the finding of numerous biological substances that are essential for the formation of distinct tissue malignancies. Identification of biomarkers facilitates the process of examining, diagnosing, and monitoring the development of cancer.[20] Saliva is classified as a specimen that can be used to make in diagnosis because it is easy to collect and non-invasive. Saliva surrounds OSCC cells, making it practicable to conduct an evaluation using saliva. More than 100 molecules contained in saliva have been reported as OSCC potential biomarkers, including proteins, nucleotides (DNA, mRNA, and microRNA), and metabolites.[44] Saliva as an examination medium is safer to use than blood, allows the use of larger volumes for testing and sampling, can be carried out repeatedly for monitoring from time to time because it does not clot, is easy to collect with minimal training requirements and can be done by the patients themselves, is easy to transfer and store, remove the requirement for direct interaction with medical professionals and researchers, hence lowering nosocomial infections and reducing the need for personal protective equipment, as well as can be used for mass examinations in a large populations. Due to its simplicity of sample, low cost, and non-invasive nature, saliva is being used more frequently as a diagnostic fluid. This is its main advantage. [42,45]

Based on Zaidi *et al.*, saliva was chosen to identify lesions suspected of being OSCC in areas that are difficult to reach by the biopsy, as an alternative to incisional biopsy procedures that may trigger metastases, as well as to prevent complications in medically

compromised individuals who are at high risk after surgical intervention.[2] In addition to having several advantages, the salivary examination also has disadvantages, including that the concentration of most analytes in the saliva is considered insufficient (100–1000 times) compared to concentrations in blood, but this is not a restriction for salivary sampling in oral cancer because biomarkers are usually produced spontaneously and locally to the tumor area. Moreover, the currently available technology is considered very sensitive for examination using salivary media.[42]

The most probable diagnosis in the use of saliva as an examination tool must be based on a combination of standardized biomarker panels, so that it can be used as an effective examination tool to increase the accuracy of early detection and diagnosis. In addition, the combination of biomarkers with conventional technology is considered to provide additional and strong diagnostic value for the early detection of precancerous lesions and cancer in the oral cavity.[21,45]

The use of this salivary biomarker is not without obstacles. Finding new diagnostic indicators in saliva with high sensitivity and specificity is now a major difficulty for researchers. Therefore, it is crucial to develop standardized techniques and develop efficient tools for the estimation of these potential biomarkers. Comprehensive research is required on various scientific platforms for the biomarker discovery, their estimation procedures, and how to make those biomarker assays acceptable for routine use to empower saliva as a diagnostic tool in health surveillance.[46]

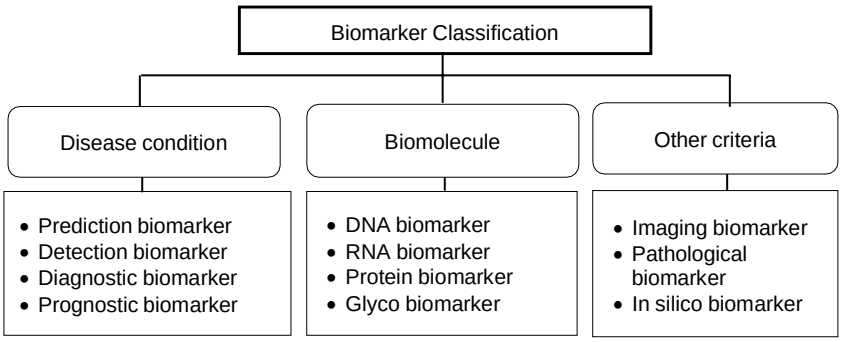


**Figure 1.** Biomarker classification. Adapted from a paper by Mishra and Verma, which in turn was adapted from Nguyen et al.[36]

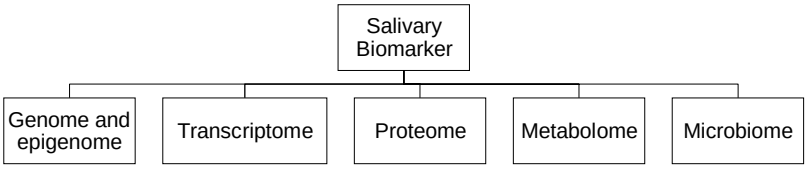


**Figure 2.** Saliva contains a diverse range of biomarkers with a high utility index in the initial detection of oral malignancy.[43]

## DISCUSSION

The purpose of this literature review was to identify the potential and function of MMP as salivary biomarkers for the early identification of OSCC (

Table ***2***). Identifying biomarkers can provide three advantages, including detecting tumors at an early stage, acting as a prognostic marker, and therapeutic targets.[15] MMP is considered as a potential biomarker that theoretically fulfills all these purposes. It is well-known that MMP and ECM are known to be important components in the etiology of oral cancer, contributing to the invasion and metastasis.[37] The estimation of MMP levels in various tissues serves as an easy-to-handle, does not require a lot of time, and non-invasive tool for the diagnosis of disease along with monitoring of the prognosis of the disease.[15,47] An increased number of both one and several MMPs is to be found in most cancers.[48,49] The most frequently overexpressed MMPs in head and neck malignancies are MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-13, and MMP-14.[33] When compared to normal mucosa, tumors had significantly greater levels of MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-11, and MMP-13. MMP-9 is attributed to a poor prognosis for OSCC patients without cervical node metastases, and overexpression of MMP-2 and MMP-9 are both implicated in the invasion process of OSCC. In addition, MMP-9 is considered useful for determining the potential for malignancy in head and neck squamous cell carcinoma (HNSCC).[48] The use of a validated combination of salivary biomarkers will accelerate a highly sensitive screening tool for detecting early-stage OSCC.[50]

According to several studies, MMP levels (including MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, and MMP-13) are increasing in the saliva of OSCC patients, making MMP potentially useful as a diagnostic marker for OSCC.[16,18,25,51,52] Based on a study conducted by Zaidi et al., MMP-1 was not proven to be an accurate biomarker for OSCC. This is because the sensitivity (50%) of MMP-1 levels is considered very low as an examination tool to detect OSCC.[2] On the other hand, according to Chang et al., MMP-1 is a beneficial biomarker for early-stage OSCC screening in susceptible groups, monitoring OSCC progression or disease recurrence, and evaluating the cervical lymph node's metastatic status in OSCC.[51] Dalirsani et al. found an increase in MMP-2 and MMP-9 levels in the saliva of HNSCC patients compared to normal patients.[53] MMP-2 protein, also known as gelatinase, is involved in the degradation of bound fibrillar collagen, elastin, endothelin, fibroblast growth factor, MMP-9, MMP-13, plasminogen, and transforming growth factor-β (TGF-β). MMP-2 is known to have an essential function in the degradation of the ECM, which is a vital

component for primary tumor cells to invade and migrate.[47]

MMP-2 expression is also known to have an association with the epithelial-mesenchymal transition (EMT) process through the breakdown of type-IV collagen, which is the most common element in the basal membrane.[47] Epithelial-mesenchymal transition or epithelial-mesenchymal transformation (EMT) is a biological process of changing epithelial cells into mesenchymal cells which can be temporary or permanent. This mechanism, specifically invasion and metastasis, is thought to have a significant function in the progression of cancer. During the epithelial-mesenchymal transition, epithelial cells lose intercellular attachment, basal- apical polarity, epithelial markers, and acquire the ability of cell motility, have spindle cell shapes, and appear to have mesenchymal markers.[54] In addition to affecting cell signaling and polarity, the basal membrane is an important component that maintains the arrangement of tissues and forms the supporting structures of cells. Several studies have also demonstrated that basal membrane damage is an important stage in the initiation of the invasive and metastasizing nature of most types of cancer.[47]

MMP-7 has been associated with several different cancers, including lung, breast, esophageal, gastric, colorectal, bladder, cervical, astrocytoma, pediatric leukemia, and renal cell carcinoma; however, oral cancer is a rare case of this association. However, it is also known that MMP-7 is only expressed in squamous cell carcinoma and not in stromal cells and that the importance of resistance is indicated by high MMP-7 expression, which can also be used as a prognostic indicator.[27] Abnormal MMP-7 expression was strongly correlated to the biological characteristics of OSCC, and MMP-7 may be facilitated by COX-2, which contributes to OSCC invasion and metastasis.[48]

MMP-9 significantly contributes to the induction of the initial angiogenic stage during carcinogenesis.[16] Vilen et al. stated that MMP-9 plays a dual role, changing from a pro-angiogenic molecule to an anti-angiogenic molecule in cancer angiogenesis.[55] MMP-9 also contributes to the aggressive behavior of OSCC by degrading type IV collagen, which is a major aspect of the basal membrane. It is suggested that SNAIL (a transcription factor) upregulates MMP-9, which triggers the epithelial-mesenchymal transition, thereby allowing carcinoma cells to migrate by changing structure and lowering cell adhesion molecules. MMP-9 is thought to have a dual role in the control of angiogenesis by both inhibiting angiogenesis by releasing antiangiogenic substances from the precursors and enhancing angiogenesis by releasing and activating VEGF from extracellular proteoglycans.[56]

According to Peisker et al., MMP-9 levels in saliva in OSCC patients compared to healthy control patients showed significant changes (increased by 19.2%), therefore it can be

concluded that diagnosing OSCC using saliva is considered very promising due to direct contact between cancerous oral lesions and saliva. MMP-9 has an important role in OSCC pathogenesis so it can be used as an early detection tool for OSCC.[18] According to Deraz et al., HNSCC can invade more easily and spread more quickly when MMP-10 is overexpressed. Invasions affected by MMP-10 may be related to p38 MAPK inhibition.[57] Metastatic tumors were found to have higher MMP-7, MMP-10, and MMP-13 levels than non-metastatic tumors.[48]

MMP-12 expression in saliva was higher in OSCC patients than in healthy individuals.[15,58,59] Prior research has also demonstrated a correlation between the range of salivary protease and the state of oral health, with an increase in salivary protease levels in OSCC patients compared to individuals with other oral disorders.[28] MMP-12 and other MMPs, including MMP-1, MMP-2, MMP-3, MMP-10, and MMP-13, are only detected in the saliva of OSCC patients. Moreover, MMP-1, MMP-2, MMP-10, and MMP-12 levels were discovered to be significantly higher in OSCC patients compared to healthy individuals, individuals with benign oral masses, and individuals with moderate chronic periodontitis. According to Saleem et al., OSCC patients had a higher concentration of MMP-12 in their saliva (1300 pg ml-1) than healthy individuals (700 pg ml-1), individuals with benign oral masses (900 pg ml-1), and individuals with chronic periodontal disease (900 pg ml-1).[25]

**Table 2.** Summary of literature search results.

| Author(s) | Years | Type of MMP | Results |
|---|---|---|---|
| Gkouveris et al. | 2017 | MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-13, and MMP-14 | The overexpression of MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-13, and MMP-14 are the most frequently found in head and neck malignancies |
| Ren et al. | 2020 | MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-11, and MMP-13. | Tumors of oral mucosa had significantly greater levels of MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-11, and MMP-13. MMP-2 and MMP-9 are implicated in OSCC invasion. |
| Feng et al.<br>Peisker et al.<br>Ghallab et al.<br>Chang et al. | 2019<br>2017<br>2017<br>2020 | MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, and MMP-13 | MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, and MMP-13 are increasing in the saliva of OSCC patients |
| Zaidi et al. | 2021 | MMP-1 | MMP-1 was not accurate for OSCC salivary biomarker |
| Chang et al. | 2020 | MMP-1 | MMP-1 is a beneficial biomarker for early-stage OSCC |
| Dalirsani et al. | 2019 | MMP-2 and MMP-9 | Increasing levels of MMP-2 and MMP-9 in the saliva of HNSCC patients. |
| Shih et al. | 2018 | MMP-7 | Overexpression of MMP-7 can be used as prognostic factor in OSCC |
| Peisker et al. | 2017 | MMP-9 | Increasing level of salivary MMP-9 in OSCC patients. |
| Deraz et al. | 2011 | MMP-10 | Overexpression of MMP-10 in oral cancer. |

| | | | |
|---|---|---|---|
| Choudhry et al.<br>Saleem et al.<br>Holmström et al. | 2019<br>2021<br>2019 | MMP-12 | MMP-12 expression in saliva was higher in OSCC patients. |
| Feng et al. | 2019 | MMP-1, MMP-2, MMP-3, MMP-10, MMP-12, MMP-13 | MMP-1, MMP-2, MMP-3, MMP-10, MMP-12, and MMP-13 are only detected in the saliva of OSCC patients |

Abbreviations: MMP, matrix metalloproteinase; OSCC, oral squamous cell carcinoma.

## CONCLUSION

The most frequently elevated salivary MMPs identified in OSCC patients include MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, MMP-12, and MMP-13. MMP can be an effective and potential biomarker for identifying OSCC at an early stage.

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