Chapter

Salivary Diagnostics in Oral Diseases

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Abstract

Common oral diseases like dental caries, periodontal diseases and oral cancer have major impact on quality of life. For prevention, treatment and prognosis, it is essential to measure the disease objectively and accurately in a quantitative manner. Quantification of biochemical or molecular specific products of cancers in serum or localized body juices can be one of the current methods of measuring oral diseases objectively. Salivary diagnostics has influenced several researchers and has been verified as an important tool in the diagnosis of many systemic conditions and prognosis of the disease. Developments in the field of molecular biology, salivary genomics and proteomics have directed to the detection of novel molecular markers for oral disease diagnosis, therapeutics and prognosis.

Keywords: oral malignant disorders, dental caries, periodontal disease

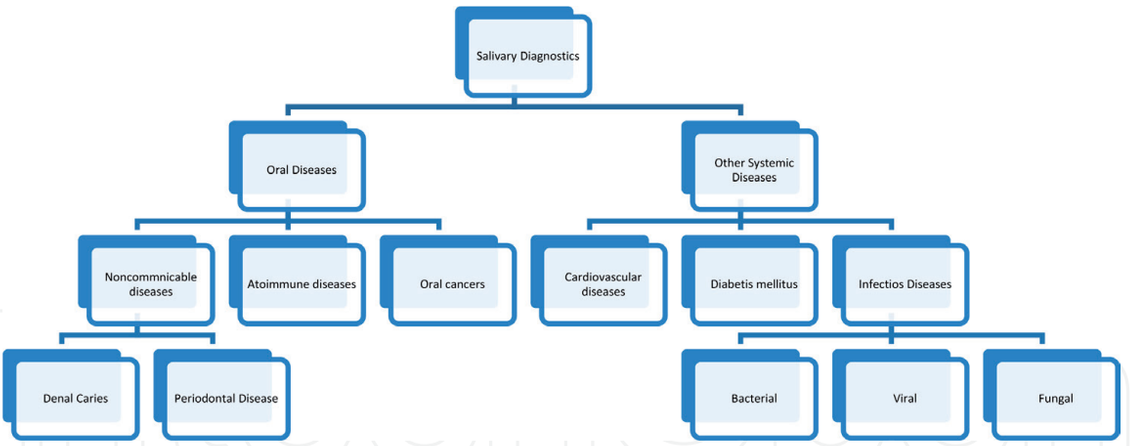
1. Introduction

Early diagnosis of the disease, quantification of the disease and prognosis of the treatment are the vital steps in controlling and preventing the diseases that would damage the person's quality of life. Diagnosing these diseased conditions has become challenging and thus necessitates complementing clinical evaluation with laboratory testing [1]. It is essential to have a thorough knowledge in order to control or prevent a disease. When we know the particular clinical, radiological, and histological and laboratory characteristics of the disease, it is easy to prevent the disease condition.

Chronic non-communicable diseases are the major public health problems faced by many of the developed and developing countries in the world. Unlike most of the communicable diseases, chronic non-communicable diseases are initiated by multiple risk-factors. Identifying such risk factors is vital to control the disease burden. Identifying unique compound in the diseased body, which is sensitive and specific to that particular disease can help in identifying and measuring the disease status.

2. Why saliva is used for diagnosis?

Saliva was used as a screening tool for cystic fibrosis in the early 1960s [2, 3]. Saliva is the exudate of serum; hence saliva also contains all the biological compounds like hormones, growth factors, antibodies, enzymes, microbes and their products [2, 4, 5]. Salivary diagnostics has become popular these days as collecting saliva is non-invasive, inexpensive, less technique sensitive and easy to perform as compared to serum.



3. Oral diseases

3.1 Dental caries and saliva

Dental caries is a multifactorial oral disease resulting in demineralization of mineralised tissues and denaturation of organic tissues of the teeth initiated by acid production by cariogenic bacteria. These are the major reasons for the loss of teeth among the population. Saliva has protective action in maintaining oral health by its buffering action, antibacterial action and cleansing effect. The awareness of functional properties of saliva as well as those of its distinct components may permit a better valuation of dental caries susceptibility [6].

4. Caries susceptible tests

It refers to intrinsic propensity of the host tissue, the tooth, to be affected by the carious process. It uses saliva as a diagnostic tool in detection and progression of dental caries. Saliva used for caries susceptibility measurement can be explained in four parts:

1. Bacterial count measurement (salivary microbiomics)
2. Colorimetric methods
3. Enzymatic biomarkers
4. Physical property of saliva

5. Bacterial count measurement

5.1 Sampling oral microbial community through saliva (salivary microbiomics)

When considering the hard tissue diseases in oral cavity (dental caries), sampling from the acquired pellicle (AP) provides more sensitive and precise protein profile compared to that of saliva [7–10]. Here saliva plays like carrier of the disease biomarkers.

Only few fractions of known proteins found in human saliva (130/2290 proteins), which are originated from acquired pellicle on dental enamel. The exact

biological functions of 51% of known proteins are unknown [8]. This lack of knowledge will provide a new vista to further research.

The particular species of microbial community grow on the AP which is specific to individual oral disease. The quality and quantification of these species can provide a clearer picture on diagnosis and find out the severity of the oral disease.

For example, patients exhibiting dental caries demonstrates dominated acidogenic and acid-tolerant Gram-positive bacteria (i.e., *Streptococcus* and *Lactobacilli* sp) [11].

Some of the salivary determinant tests are as follows

1. *Lactobacillus colony count test*: assessment of the number of acidogenic bacteria can be done by calculating the number of colonies appearing on Tomato peptone agar plates (pH 5.0) after inoculation with a sample of saliva.

2. *Streptococcus mutans level in saliva*: this measures the number of *S. mutans* colony forming units per unit volume of saliva.

3. *Saliva/tongue blade method*: estimation of the number of *S. mutans* in Mutans Salivarius Bacitracin (MSB) agar inoculated by paraffin-stimulated saliva/Saliva-contaminated wooden spatulas [12].

5.2 Colorimetric methods

1. Snyder's test [7, 13]: the caries susceptibility is correlated with production of acid that is assumed to result due to the fermentation of specific amount of glucose by cariogenic Lactobacillus species by inoculating saliva into agar containing bromocresol green indicator.

2. Albans test: this is a simpler version of Snyder's test in which the patient expectorates directly into tubes that contain the medium. In this test, somewhat softer medium is used that permits the transmission of saliva and acids without the requirement of melting the medium [7].

3. Swab test: the principle of this test is same as that of Snyder's test i.e., capacity of salivary microorganism to form organic acids from a specific carbohydrate medium. The medium encompasses a colour changing indicator dye, bromocresol, which changes its colour when the environment changes its Ph.

5.3 Enzymatic biomarkers

1. *Salivary reductase test*: this test quantifies the activity of the reductase enzyme present in salivary bacteria.

2. *Peroxidase*: peroxidase is a salivary enzyme which neutralises the toxic compound (hydrogen peroxide) produced by oral microorganisms and reduces production of acid in the dental plaque. This severely reduces the plaque accumulation thereby reduces the plaque related diseases like dental caries and periodontal diseases [14].

3. *Collagenase*: it represents in extracellular fluids like serum or saliva represents tissue destruction or cell death. In saliva it indicates destruction of pulp (severe dental caries) or destruction of periodontal tissue [15].

6. Physical properties of saliva

1. Salivary buffer capacity test:

   a. This test measures the amount of millilitres of acid involved in lowering the Ph of saliva via an arbitrary Ph interval, such as, from Ph 7.0 to 6.0 or the quantity of acid or base essential to bring colour indicators to their end point [16].

   b. Population level researches salivary flow rate and buffer effect show a contrary correlation with caries susceptibility [17].

2. Flow rate:

   Many researches showed that, higher the flow rate showed quicker is the salivary clearance [17–19] and greater is the buffer capacity [18] Reduced salivary flow rate and the associated reduction of oral defence systems may cause severe caries and mucosal inflammations [16, 17, 20].

7. Salivary ions

Calcium ion is the most extensively researched salivary ion for dental caries and periodontal disease. Demineralisation of teeth or bone leads to leach out excess of calcium ion into saliva. Increased levels of salivary calcium ion indicate the presence and severity of oral diseases. Similarly increased selenium content in saliva and food is the indicative of dental caries in subjects [21].

8. Periodontal disease and saliva

Diagnosing active phases of periodontal diseases, and identifying those at risk for active disease has been challenging for both clinicians and investigators. Since saliva can be easily collected which contains the locally derived and systemically derived markers of periodontal disease, it can provide specific diagnostic test for periodontitis.

Enzymes present in saliva are contributed by the cells of the salivary glands, oral microorganisms, PMNs, epithelial cells and GCF entering the oral cavity. Studies have shown a reliable relationship between enzyme activity and periodontal status and its response to the periodontal treatment.

Saliva can be categorised in to two types, whole saliva and saliva from specific glands [22–24]. Differences in the amount of fluid and constituents of each gland can be determined in gland specific saliva.

Whole saliva which consists of oral fluids, secretions from the major and minor salivary glands, non-salivary constituents, GCF, bronchial secretions, serum, blood cells, food debris and microorganisms along with their products can also be used as a diagnostic tool.

It has been found that, proposed markers for diseases such as proteins of host origin (i.e., enzymes, Ig), phenotypic markers such as epithelial keratins, host cells, hormones, microorganisms, volatile compounds and ions are found in saliva [25].

Some of the major salivary biomarkers are discussed below:

1. Salivary proteins:

Salivary proteins are formed from combined Direction of DNA and RNAs. Any disparity in DNA or RNA stands can lead to altered protein formation which leads

to disease condition and the altered protein becomes the marker of that disease. A patient's salivary protein mapping can provide details on entire body's health because saliva is an exudate of blood and contains juices from gingival crevicular fluid along with major and minor salivary glands, and is much less invasive and more acceptable to the patients compared to blood sampling [10, 26–28] Salivary proteins play a major role in adhesion of microbes on tooth surface through AP formation by stereo-specific mechanism [10, 29]. Salivary proteins can regulate adherence of microorganism by using the carboxylterminal of histatin and of acidic proline-rich proteins (PRPs) by promoting or reducing the attachment to the protein [30–32].

Some of the proteins involved are

A. Enzymes:

   a. Lysozyme

   b. Peroxidase

   c. Collagenase

   d. Acidic and alkaline phosphatase

B. Glycoproteins and proline rich proteins

   a. Dextran

   b. Acquired pellicle forming proteins

   c. Lactic acid and pyruvic acid

   d. Fibronectin

C. Hormones

D. Histatin

E. Matrix metallo proteins

F. Other proteins

   a. Cystatin

   b. Amino acids

   c. Growth factors and vascular endothelial growth factors

A. Salivary enzymes:

   a. *Lysozyme*: lysozyme is an antimicrobial enzyme secreted in human saliva with the ability to hydrolase the 1,4-beta-linkages between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine existing in peptidoglycan, which is the major component of Gram-positive cell wall. Patients with low levels of lysozyme in saliva are more vulnerable to accumulation of dental plaque, which is considered a risk factor for oral disease and increased salivary lysozyme activity indicates recent infection in oral cavity [33].

b. *Peroxidase*: it inhibits the hydrogen peroxide formation by microbes and there by prevents accumulation of plaque formation. This directly stops the plaque related oral diseases like periodontitis and dental caries. Quantification of this enzyme directly proportional to the severity of the disease status [14].

c. *Collagenase*: collagenase is also known to as MMP-13, is collagenolytic MMP with remarkably extensive substrate specificity. Presence of this in extracellular fluid like serum and saliva indicate tissue destruction or cell death. In saliva this may indicate destruction of periodontal tissue, pulp tissue, infective necrosis or carcinogenic destructions [15].

d. *Acid and alkaline phosphatase*: the enzyme alkaline phosphatase (ALP) and acid phosphatase (ACP) are the twin counterpart enzymes which occur in many organisms ranging from bacteria to man, basically functions by catalysing or blocking the hydrolysis of monoesters of phosphoric acid and also catalyse or block a trans-phosphorylation reaction in the presence of large concentrations of phosphate acceptors [34]. Some researches conveyed amplified activity of acid phosphatase and alkaline phosphatase in the acute stage of periodontal disease, and also observed recovery of enzyme level to the normal range after periodontal therapy [35, 36].

B. Glycoproteins and proline rich proteins:

a. *Dextran*: dextran is a complex, branched glucan (polysaccharide made of many glucose molecules) composed of chains of varying lengths which helps the plaque to attach to the host tissue. Measuring the level of salivary dextran level can helps in calculating the plaque attachment status. There by calculating the plaque related diseases quantitatively [37].

b. *Acquired pellicle forming proteins*: salivary proteins play a chief role in bonding of microbes on tooth surface through acquired pellicle formation by stereospecific mechanism [29]. These salivary proteins decide the type of microbes to grow. Hence identifying these proteins which are specific to grow selective bacteria will provide knowledge about the type of disease.

c. *Fibronectin*: fibronectin is a glycoprotein that mediates adhesion between cells and encourages selective adhesion and colonisation of certain favourable bacterial species. It also involved in inflammation, chemotaxis, and wound healing and tissue repair [23, 38]. Quantification of fibronectin in saliva provides a clear picture on periodontal disease status.

C. Hormones:

Cortisol: cortisol is a hormone highly sensitive to emotional changes and is stress related. It provides anti-inflammatory and immunosuppressive effect. This has direct effect on oral infection and dental plaque related illness [38, 39]. Even though lots of researches indicate the relation of cortisol and oral diseases, more specific and confirmatory researches are needed.

D. Histatin:

Histatin is a salivary protein secreted from parotid and submandibular glands with definite antimicrobial properties. It interacts with endotoxic

lipopolysaccharides situated in the membrane of Gram-negative bacteria and neutralises it. It also has antihistaminic action, hence influences oral inflammation [38, 40, 41].

E. Matrix metallo proteins:

They are host proteinases accountable for both tissue degradation and remodelling. The presence of these metallo proteins indicates tissue destruction. MMP 8 is the most predominant MMP found in ill periodontal tissue and GCF. Recent researches reviled that the level of MMP-8 was highly raised in saliva of patients with periodontal disease [38, 42].

F. Other proteins:

a. *Cystatin:* this is a proteolytic enzyme produced by pathogenic bacteria, inflammatory cells, fibroblasts and osteoclasts which have collagenolytic property. It helps in spreading of oral disease to different planes also progress the disease status [23, 38]. Measuring the level in saliva can explain the severity of the oral diseases like periodontal disease and potentially malignant disease.

b. *Amino acids:* some of the amino acids like Proline shows increased levels in saliva of periodontally ill subjects compared to the healthy individuals. This may be due to degradation of salivary proteins by bacterial activities [38, 43, 44]. Presence of proline in saliva directly proportional to the level of bacteria in dental plaque. Hence it quantifies the plaque related oral diseases.

c. *Growth factors and vascular endothelial growth factors:* these are angiogenic cytokines associated with inflammation and healing tissue. Higher level of these proteins in saliva observed in the inflammatory conditions (periodontal diseases) or growth of tumour (Malignant tumours) [23, 38].

G. Human salivary immune system:

a. *Immunoglobulins*: immunoglobulins are specific first-line defence mechanism of saliva. The chief immunoglobulin in saliva is secretory IgA (sIgA), which is produced by the plasma cells in the salivary glands. IgA has 2 subclasses, IgA1 which is predominated in serum and IgA2, which is predominantly present in secretions like saliva, milk and sweats [38, 45]. Many researches show the positive correlation between severity of inflammation (Periodontal disease) and IgA concentration [14, 46].

b. *Salivary neutrophil count*: neutrophils play a major role in the innate immune response. Most of the oral diseases are associated with infection and inflammations, which are explained in the form of neutrophil count in saliva. Very meagre research directed to correlate the neutrophils in plaque, saliva, and gingival crevicular fluid (GCF) to periodontally healthy and diseased subjects. These researches reviled that there is positive correlation between oral diseases with PMN counts [47–49].

H. Salivary ions:

Calcium ion in saliva indicates demineralization of teeth or alveolar bone which leaches out into saliva indicating severity of dental and periodontal diseases [21].

I. Oxidative stress assessment:

Redox (reduction and oxidation) reactions are common in all cells. But imbalance between reduction and oxidation process can lead to oxidative stress within the cell which destroys the cell. Oxidative stress was involved in the progression of periodontal diseases [50–52]. In chronic periodontitis, there was lower serum total antioxidant level when equated to the control individuals [52, 53] Biomarkers of lipid peroxidation (one of the oxidative stress-mediated pathways) such as 8-isoprostane and malondialdehyde (MDA) were elevated in patients with chronic periodontitis [52, 54–56].

J. Salivary Microbiomics:

The particular species of microbial community grow on the AP which is specific to individual oral disease. The quality and quantification of these species can provide a clearer picture on diagnosis and find out the severity of the oral disease. Patients with periodontal disease Express increased percentage of obligately anaerobic bacteria (i.e., Gram-negative species) [10, 57].

Some of the literatures on salivary biomarkers to detect periodontal diseases are as follows:

Author (year)	Biomarkers	Inference
Nakamura and Slots (1983) [35]	Salivary enzymes	Significant difference between diseases and healthy periodontium. No significant difference between LJP and AP
Zambon et al. (1985) [58]	levels of caprylate esterase lipase, leucine, valine and cysteine aminopeptidases, trypsin, B-galactosidase, B-glucuronidase and B-glucosidase, sub gingival black pigmented bacteroides and motile organisms	A decrease was seen in the levels of all the biomarkers after the treatment in AP
Gregory et al. (1992) [59]	Immunoglobulins	Igs in LPJ patients was higher than healthy individuals of same age, race and gender
Gibbons et al. (1986) [30]	Fibronectin-degrading enzymes in saliva	This biomarker was higher in concentration in diseased patients
Nieminem et al. (1993) [60]	Protease levels	Decrease in the levels of protease markers after the treatment in advanced periodontitis
Ingman et al. 1993 [61]	Collagenase, elastase like and trypsin like activity	AP patients' saliva demonstrated a higher level of protease, collagenase and elastase lie activity when compared to LJP and healthy patients
Uitto et al. (1990) [62]	Collagenase	Higher in periodontitis patients
Orner 1976 [63] Halinen et al. 1996 [64]	Collagenase, activated matrix metalloproteinase	Higher in children with Down's syndrome who are more prone to periodontal diseases
Hayakawa et al.1994 [65]	TIMP-1 and total collagenase activity	TIMP-1 was lower in patients with periodontal disease and total collagenase activity was higher in diseased patients

9. Oral cancer and saliva

Oral cancer is 6th most common human malignancies with approximately 50% mortality rate in 5 years. Oral cancer is a potentially lethal disease and the result of

the treatment and prognosis largely determined by primary diagnosis. For prevention, treatment and for prognosis, it is essential to measure the disease objectively and accurately in quantitative manner. Quantification of biochemical or molecular specific products of cancers in serum or localised body juices can be one of the current methods of measuring oral cancer objectively. Salivary diagnostics has influenced several researchers and has been verified as an important tool in the diagnosis of many systemic conditions and prognosis of the disease. Developments in the ground of molecular biology, salivary genomics and proteomics have directed to the detection of novel molecular markers for oral cancer diagnosis, therapeutics and prognosis.

Several research groups have found that salivary levels of specific proteins are increased in whole saliva of patients with oral squamous cell carcinoma. For example, CD44 (a cell surface glycoprotein involved in cell-to-cell interaction), 44 Cyfra 21-1 (a fragment of cytokeratin 19), tissue polypeptide antigen (TPS), and cancer antigen 125 (CA-125) have been suggested as oral cancer bio markers [2, 66].

Abundant biomarkers have been studied for finding of oral potentially malignant diseases and cancer, until now no further research has been done. Whole saliva was used to conduct most of the research projects. Whole saliva comprises of juices from all major and minor salivary glands, as well as liquids from mucosal and periodontal tissues, which are influenced by oral and systemic environments and by host immune responses and carry the essence of disease product with them. Measuring the levels of disease product will provide a specific diagnostic and prognostic value. Additionally, least requirement of money, material and manpower makes it more economical, less time consumption, and no special training make this methodology easily accepted.

10. Usage of stimulated or un-stimulated saliva for collection

Unstimulated saliva is considered as an ideal sample because it has no effect on flow rate and salivary composition of salivary glands. But some researches shows that stimulated saliva also can provide equally or more precise detection of cancer biomarkers [67, 68]. Limited researches were performed in these directions on the effect of stimulated and unstimulated saliva on salivary biomarkers of cancer. Hence, it needs to be studied to further standardise the salivary biomarkers.

11. Storage and transportation of saliva

Saliva is very sensitive sample, which is influenced by systemic, physiological, microbial, environmental (food products) and biochemical changes in oral cavity. It also fluctuates with time of collection, Ph of the surrounding environment, temperature type of saliva collection method, and storage methods [69, 70–73] Some study reported that storage at −80°C provides less biochemical and microbial changes in saliva hence gives better results as compared to −20°C [72] . To avoid altered results, there should be least time gap between sample collection and analysis.

12. Classification of salivary biomarkers for oral cancer

Biomarkers have been classified based on biomolecules and disease states [74, 75].
Based on biological molecules:

1. DNA biomarkers

2. RNA biomarkers

3. Protein biomarkers

   a. Enzymes

   b. Hormones

   c. Glycoproteins

4. Salivary ions

5. Oxidative stress assessment

   i. Based on disease state

   1. Diagnostic biomarkers

   2. Prognostic biomarkers

More than 100 potential salivary biomarkers have been reported till date, they can be explained with following headings:

1. Peptides

2. Proteins

3. DNA

4. Salivary mRNA

1. *Peptides*: some of the researches explain that polypeptides like defensin which possess cytotoxic and antimicrobial properties, which exhibits their presence in azurophil granules of polymorphonuclear leukocytes is one of the potent biomarker of oral cancer. OSCC can be distinguished even in their earlier stages by the raised levels of salivary defensin-1 matched with healthy controls [67, 76].

2. *Proteins*: major portion of salivary biomarkers are protein in nature. Most of the proteins share their presence with other diseases or environmental factors (food), this exhibits higher levels of sensitivity with low specificity. Some of the protein biomarkers showed significant elevated level in saliva such as interleukins (8, 6, 1b), matrix metalloproteinase (MMP 2, 9), transforming growth factor (TGF-1), Ki67, cyclic D1, transferrin, amylase, tumour necrosis factor (TNF-a) and catalase among saliva of oral squamous cell carcinoma by various studies [77]. Protein CD44 showed elevated levels in saliva (oral rinse) of oral squamous cell carcinoma patients (n = 102) matched to controls (n = 69) [78]. some of the researches confirmed that IL-8 and IL-6 are informative biomarkers for OSCC, where IL-8 and IL6 showed elevated levels of concentration in saliva and serum respectively [79].

A. Enzymes:

   1. *Lysozyme*: lysozyme is an antimicrobial enzyme secreted in human saliva with the capability to hydrolase peptidoglycan, which is the major constituent

of Gram-positive cell wall. Lysozyme shows antitumor properties by direct activation of immune cells or it can raise tumour cell immunogenicity. And also, lysozyme can release elements from bacteria (peptidoglycans and/or polyribopyrimidinic acids) responsible for immune-potentiation and therefore antitumor activity [80].

2. *Pyruvate kinase M2 isoenzymes*: cancer cell uses metabolic alteration (Warberg's effect) for their survival and growth. They chose anaerobic respiration for their energy needs even though abundant availability of oxygen. Pyruvate kinase muscle iso-enzyme M2 (PKM2) is a glycolytic enzyme and a vital enzyme in tumour cell metabolism and growth. Increased level in serum and saliva indicate presence of malignancy [81].

3. *Collagenase*: collagenase is the major intracellular enzyme explains the destruction of the tissue if found in extracellular fluids like saliva. In saliva this may indicate destruction of periodontal tissue, pulp tissue, infective necrosis or carcinogenic destructions [15].

4. *Acid and alkaline phosphatase*: a study clarifies that the salivary ALP enzyme displays significantly higher levels in subjects with diabetes mellitus, smokers and subjects with potentially malignant disorders without any periodontitis associated to systemically healthy persons [82]

B. Glycoproteins:

Lactic acid and pyruvic acid: pyruvic acid and lactic acids were produced as the end product in the physiologic process of glycolysis [83, 84]. This energy creation cascade carry on by using the end product (pyruvate) of glycolysis as a fuel to Krebs cycle in mitochondria by oxidative phosphorylation. But this process gets interrupted in malignant cells where cell undergoes fermentation of sugar molecule (anaerobic respiration) even though enough presence of oxygen. This process is known as Warberg's effect. This leads to accumulation of the excess PA and LA. Hence quantification can give a clearer picture on the stages of cancer [83].

C. *Salivary ions*: most of the researches explain excess and deficient levels of copper or zinc were significant correlation with oral cancer risk [85]

D. *Oxidative stress assessment*: oxidative stress is the product of a discrepancy between oxidant factors and protective antioxidant systems; it may occur due to an excess of free radicals, or by the shrinking of the antioxidant systems.

Oxidative stress was also associated with oral cancer, as amplified lipid peroxidation and reduced antioxidants was reported in patients suffering from stage II, III, and IV oral cancer [52, 86].

3. *DNA*: DNA is highly specific type of biomarker used for detection of cancer. It requires high end operating machineries and also requires special training to operate it. This makes it more expensive biomarker to detect. Boyle et al. observed that OSCC exhibits p53 mutations in 71% of saliva samples by using plaque hybridization technique [87]. Aberrant methylation of p16, MGMT and DAP-K in OSCC patients was recognised by Rosas et al.

4. *Salivary mRNAs*: oral carcinogenesis can be detected by measuring the elevated six mRNA molecules such as DUSP1, H3F3A, IL 1B, IL 8, SAT and S100 [88].

   a. *DUSP1* (dual specificity phosphatase 1): DUSP mRNA involved in protein modification, oxidative stress, and signal transduction and participates in MAPK (Mitogen Activated Protein Kinase) pathway. Molecular studies showed that the carcinogenesis is associated with hypermethylation of DUSP1 gene [89].

   b. *H3F3A*: these proteins are nuclear proteins, located in chromosome 1, responsible for the structural integrity of chromosomal nucleosome and acts as a one of the proliferative marker for oral cancer.

   c. *IL IB*: Interleukin 1 beta is a chemical mediator of cell proliferation, differentiation, and apoptosis and also inflammation. Though it represents its presence in other pathological and physiological aspects, it shows Elevated serum levels in patients with oral squamous cell carcinoma.

   d. *IL 8*: Interleukin 8 (neutrophil chemotactic factor) is a pro-inflammatory cytokine which plays a key role in tumour angiogenesis, cell adhesion, and cell cycle arrest. By observing various studies on salivary biomarkers, it concluded that IL 8 in saliva is the best biomarker for squamous cell carcinoma [79].

   e. *SAT*: Spermidine/spermine N1-acetyltransferase 1 a protein that participates in the catabolism of polyamines. Researches show elevated levels of SAT in the saliva of oral cancer patients compared to the healthy controls.

   f. *S100 P*: it is a calcium binding protein P, which is located in the cytoplasm or in the nucleus. Its levels in saliva get deviated among oral cancer subjects.

5. *Salivary microRNA*: MicroRNAs (miRNAs) are short parts of RNA transcripts. They are associated with most of the cellular functions like cell growth, apoptosis, differentiation, motility, and immunity. miRNAs are more specific and potent compared to mRNAs. They exhibit high sensitivity which effectively detect and differentiate poorly differentiated carcinomas. Some of these include miR-125a, miR-200a and miR-31 [88].

13. Conclusion

Saliva contains numerous substances which are potential biomarker of many oral diseases. Saliva could be a key cause of biochemical records capable of identifying some diseases. Hence would be useful for ideal methods to diagnosis, prognosis, and monitoring and management of patients with oral diseases. Even though saliva provides some evidence in early detection of oral diseases with advantages like easy, inexpensive, safe, less time and technique sensitive and non-invasive approach over serum, it showed resistance in the diagnostic and clinical usage. Salivary diagnostics faces lots of challenges for the use of saliva-based oral fluid diagnostics for future application. Salivary diagnostic procedures required more sensitive technological support and quality researches to generalise and implement the procedures.

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