Review

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Predictive salivary biomarkers for early diagnosis of periodontal diseases – current and future developments

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Abstract: Periodontal diseases are chronic diseases of oral cavity comprising of inflammatory conditions which effect the supporting structures of dentition. It is a multifactorial disease which is also known to be affected by genetic and environmental factors. However, some of the clinical parameters such as probing depth, attachment level, plaque index, bleeding on probing and radiographic assessment of alveolar bone are known to assess the severity of disease, although the disease activity is not measured. In the current scenario the salivary diagnostic markers for diagnosis of periodontal diseases have included the salivary enzymes, immunoglobulins, bacterial components or products, phenotypic markers such as epithelial markers. Also, saliva is a mirror of oral and systemic health and a valuable source to find out the physiological aspects of periodontal diseases. The present review thus highlights various salivary biomarkers which are quick, easy and reliable method for assessing and monitoring periodontal disease that improves and speeds treatment decisions and moves the field closer to individualized point-of-care diagnostics.

Keywords: biomarkers; diagnostic fluid; inflammation; periodontia; periodontitis; saliva

Introduction

The periodontal disease is a chronic inflammatory disease of the periodontium mediated by anaerobic bacterial infection leading to destruction of the supporting structures of the teeth and alveolar bone [1]. The periodontal diseases have been evaluated by probing the periodontal pocket depth (PD), clinical attachment level, and radiographic assessment of alveolar bone [2]. However, these techniques lack specificity and are error prone, time consuming and present a picture of past incidence and current status of the periodontium. In the modern evidence-based practice, where adequate prevention is more emphasized, with the available tools at the clinician's disposal it is not possible to predict the individuals who are susceptible to the disease [3]. The diagnosis of periodontitis is usually made at the later stage due to the chronic nature of the disease resulting in a painless destruction of the attachment apparatus. The patient seeks dental care after a significant portion of the periodontium has been compromised.

Among the bodily and oral fluids used for clinical assessment, saliva is handy and easily attainable and can be derived from the mucosal surfaces and the gingival crevices, as well from the tooth surfaces of oral cavity. The days of yore, the Chinese physicians employed saliva to diagnose the physical wellbeing of humans over 2000 years [4]. Many biologically active compounds enter into saliva from the serum through intracellular pathways via passive transfer, diffusion and ultrafiltration unchanged [5]. The salivary metabolites, inflammatory markers such as cytokines, signal molecules, hormones have been detected

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in saliva, and this renders it to be accessible biological fluid providing necessary information pertaining to oral health in the mouth. In the recent times, due to advanced technology, salivary biomarkers could be extremely sensitive and easily measured and could be used as an effective modality for the early diagnosis, and for monitoring post therapy status in chronic periodontitis. Saliva also reflects as a mirror of systemic health. In this review, we highlight the importance of saliva as a diagnostic tool in periodontitis. Besides the identical immunological, genetic and environmental factors which includes the diet factors and modified life style which are implicated to be the contributory factors in periodontitis. The novel identified factors such as diabetes mellitus, autoimmune disorders, inflammatory bowel syndrome, Alzheimer's disease and oral cancers also have been reciprocated to cause periodontitis. However our present review highlights the importance of salivary biomarkers in detecting periodontitis which is not restricted to specific etiology. We particularly highlighted the salivary biomarkers and its implications in the diagnosis of periodontitis. Our review does not focus on diagnosis of systemic diseases, malignancies and other diseases. Certainly lots of efforts are needed to collect the literature on the sensitivity and specificity of each salivary biomarker in the diagnosis of general diseases and employ them as routine detection method.

Saliva

Saliva is a complex, slightly acidic biological fluid secreted by major and minor salivary glands of the oral cavity; which include parotid, submandibular and sublingual glands classified under major salivary glands as well the buccal, lingual and palatal glands which represent the minor salivary glands. On an average, individual salivation ranges from to 0.7 mL of saliva per minute, a total of 1–1.5 liters of serous and mucinous saliva [6]. It is composed of minerals, electrolytes, buffers, enzymes, enzyme inhibitors, growth factors, cytokines, immunoglobulins (e.g., secretory immunoglobulin A [IgA], mucins, glycoproteins, hormones,antibodies, growth factors, enzymes, microbes and their products [7]. When compared to serum, saliva can be collected easily without any professional help as well it is inexpensive. Moreover, the storage and transport as well the capacious sampling, makes saliva the best advanced investigative biofluid compared to serum or urine.

Saliva collection

The factors such as salivary composition and salivary flow rate are very important and essential for diagnostic, experimental and clinical protocols. Saliva can be collected as expectorated whole saliva or stimulated conditions by gustatory and masticatory stimuli to increase the salivary flow rate. The unstimulated saliva refers to saliva which can be collected passively by drooling into a sample container. However, the individual secretions refer to saliva collected from a specific salivary gland (parotid or submandibular), whereas whole saliva contains normal metabolites along with other non-salivary compounds such as gingival crevicular fluid (GCF),desquamated epithelial cells etc., since salivary flow rate varies among individuals. The procedure of salivary collection should be standardized [8]. Some of the commonly used methods for collection of unstimulated saliva includes the draining, spitting, suction and absorbent methods, whereas stimulated saliva can be collected by chewing paraffin wax or 2 % citric acid before collection [9]. The parotid saliva is usually collected in plastic single cups such as Carlson and Crittenden cups. However disposable containers should be used more nowadays as it is uncomplicated.

"Saliva: a reliable predictor for diagnosis – an hour of need"

The current clinical diagnostic tests can only detect sites of active periodontal inflammation. This infact is a great challenge in periodontitis as it is insidious, chronic, with asymptomatic progression and patients report to the dentist at an advanced stage of the disease. This makes it imperative to discover innovative and reliable predictors that would diagnose the current periodontal status and help in identifying the futures sites of periodontium which are at risk. As periodontitis progresses, inflammation of gingiva, destruction of soft tissues and supporting structures occur consecutively and release proteins and metabolites into the saliva, in addition to these numerous biological markers associated with periodontium are also contained in it. Biomarker is defined as a characteristic parameter measured as an indicator of normal biological, pathogenic processes or response to an exposure or intervention [10]. Salivary biomarkers representing periodontal health and diseased state can originate from both bacteria and the host. Over the last

decades, several biomarkers have been focused for diagnosing periodontal diseases. However, most of them seemed to end up in terms of reliability and validity. Some of the periodontal bacteria such as *Porphyromonas gingivalis*, *Tanerella forsythia*, *Fusobacterium nucleatum* and *A. Actinomycetans* which exist in periodontium, due to their complex interaction cell mediated inflammatory response can be seen leading to tissue destruction [11, 12].

The biomarkers can be classified based on their source such as Enzymatic markers, inflammatory markers, Specific and Non-Specific markers, Osteogenic markers, Systematic markers, Salivary transcriptomes, and proteomes.

Enzymatic markers

There are various enzymes in the GCF which are released from stromal, epithelial, bacterial and inflammatory cells. Enzymes such as AST (asparatateaminotransferase) ALT (alanine amino transferase), peroxidase, GGT (gamma glutamyl transferase), lysozyme, ALP (alkaline phosphatase) and ACP (acidic phosphatase) have been correlated with severity of periodontal diseases. The mechanism of action of each enzyme is given in Tables 1–4.

Osteogenic markers

The osteogenic markers help in bone remodeling and this could accelerate the diagnosis and management of periodontal diseases. Alveolar bone loss is one of the characteristic traits of periodontal inflammation. During bone resorption, various markers are expressed into the bloodstream which passively diffuses into the saliva and can be readily detected [31]. Some of the regulators of bone cell activity such as osteocalcin, RANKL and OPG which help in mediating bone loss the characteristic of periodontitis has been more in focus. Many different biomarkers are associated with bone formation, resorption, and turnover such as alkaline phosphatase, osteocalcin, osteonectin and collagen telopeptides [32–37].

Table 1: The mechanism of action of immunoglobulins.

Marker	Mechanism of action	References
IgG	Influence the oral microbiota by interfering with the bacterial adherence or by inhibiting bacterial metabolism.	[13]
IgA		[14]

Table 2: The mechanism of action of enzymatic markers.

Marker	Mechanism of action	References
AST (asparatate amino transferase), peroxidase lysozyme	These enzymes reflect the metabolic changes in gingiva and high cellular loss indicating the inflammatory changes in gingiva and destruction of periodontal tissues by catalyzing the transamination of glutamic acid to oxaloacetic and aspartic acids.	[15]
Beta-glucuronidase	Lysosomal acid hydrolase contributes to non-collagenous matrix degradation in periodontal diseases	[16, 17]
Lactoferrin	Sequesters iron from the environment, thus depriving bacteria of this essential element and decreased expression in periodontitis	[18]
Soluble neuropilin-1 (sNRP-1)	These are transmembrane glycoproteins having immunoregulatory and angiogenic mechanisms. Many studies have found a positive correlation between the SNRP's and the periodontal parameters	[19]
Matrix metalloproteinases-8 and 9	MMP8,9 is responsible for tissue destruction and immune responses in saliva and as MMP increases, the tissue inhibitors in saliva are decreased. The MMP,S have been related to periodontal inflammation as collagen diseases induce inflammation.	[20, 21]

Dabra investigated the correlation between levels of enzymatic markers in saliva in patients with periodontitis. They found a significant increase in the enzymatic concentration and concluded that they can be considered for the early diagnosis and prognosis of periodontitis [38]. Lysozyme is an antimicrobial enzyme which has capacity to break the bonds in the bacterial cell wall. It has capacity to interfere with proteases in saliva. It has been implicated that low levels of lysozyme in saliva have more susceptibility for plaque accumulation and thus carries high risk for destruction of periodontal tissue [39]. The metalloproteinases are proteolytic enzymes which are involved in degradation of hard and soft tissues. The class of MMP includes MMP1, 8, 13 grouped as collagenases and MMP3, 10,

Table 3: The mechanism of action of inflammatory mediators.

Marker	Mechanism of action	References
Macrophage inflammatory protein-1 alpha (MIP-1α)	They are chemotactic chemokines as well chemoattractants which play a significant role in chronic inflammatory reaction and periodontal diseases.	[22]
Interleukin-1beta (IL-1β)	The proinflammatory cytokines like IL-1β, IL-6 have been contributed to severe inflammation and tissue breakdown in periodontitis. These predictors can be used as effective tool to intercept and diagnose early stages of periodontitis.	[23]
Interleukin-6		[24]
Tumour necrosis factor TNFα	Stimulates the proliferation and differentiation of osteoclasts precursors and also acts on mature osteoclasts, activating them and presence of TNF factor indicates presence of chronic generalized periodontitis.	[25]
Prostaglandin E2 (PGE2)	It induces vasodilation as well the capillary permeability which, inturn inhibits collagenase and clinical signs such as it induces vasodilation as well the capillary permeability which inturn inhibits collagenase and clinical signs such as oedema, bone resorption and erythematous swollen gums	[26]
C-reactive protein (CRP)	CRP has been important indicator of inflammatory activity. The high levels of CRP has been an indicator for diagnosing cardiovascular disorders. The CRP levels increase with severity of periodontal diseases.	[27]
Neopterin	Its levels increase during periodontal inflammation and it is produced by macrophages which is responsible for nitric oxide radical formation and phagocytosis.	[28, 29]
8-Hydroxy-2′-deoxyguanosine (8- OHdG)	It is formed by oxidation of guanine from damaged DNA and its level increases in saliva during periodontisis.	[30]

Table 4: The mechanism of action of osteogenic markers.

Marker	Mechanism of action	References
Osteocalcin, osteonectin	Ostecalcin plays an important role in bone remodeling and is generally considered as bone formation and prognostic marker for assessment of periodontal disease. Osteonectin is a predictive bone marker a bone regulating protein which maintains hemostasis of PDL during the bone remodeling phase.	[31–33]
Alkaline phosphatase (ALP)	ALP stimulates the calcification process and helps in remodeling of bone. It has vital role in cementogenesis and in maintaining the haemostasis of bone. It is an important bone turn over marker indicating the progression of periodontal diseases.	[34]
Osteopontin,	It is a glycoprotein which plays dual role in bone resorption and mineralization. The levels of osteopontin decreases as the severity of periodontal destruction decreases	[35]
Receptor activaton of the nuclear factor-Kb (RANK)	RANKL acts as ligand and gene regulator which controls activation, proliferation of osteoclasts which can result in resorption of alveolar bone. The RANKL/OPG ratio plays key role in determining the periodontal inflammation.	[36]
Osteoprotegerin (OPG)	OPG is an alveolar bone turn over marker that plays a key role in osteogenesis. It has capacity to suppress its activity through osteoclast formation.	[37]

11 named as stroma lysins and MMP2,9 as gelatinases. Many studies have stated increased levels of MMP8 in periodontal inflammation. The MMP 8,9 are major set of proinflammatory mediators found in saliva and are said to be profound markers for monitoring the periodontal disease activity. The increases in MMP 9 in periodontal diseases have been responsible for tissue destruction. The classes of MMP'S aid in degradation of extracellular matrix and basement membrane constituents. MMP8, 9, 13 are major types involved in periodontal destruction. MMP 8 is responsible for the collagenolytic activity. It is majorly found in polymorphonuclear neutrophils, fibroblasts, plasma cells and macrophages. MMP 9 is responsible for bone resorption, periodontal inflammation, and tissue breakdown [40, 41]. Similarly, Beta-Glucuronidase is an important enzyme a component of acid hydrolase which contributes to

degradation of periodontal tissues. Many researchers have found positive correlation of periodontal compromised individuals and rise in salivary β-glucuronidase levels and probing pocket depth [17].

Hormones

Cortisol hormone has been correlated with emotional stress levels which is a risk for periodontal disease [42]. The increased plaque levels and stress factors are positively correlated and exhibit strong inhibitory effect on inflammatory process and destruction of periodontal tissues. Botelho conducted meta-analysis on influence of cortisol levels in periodontitis and revealed that high cortisol levels were associated with aggressive and chronic periodontitis and can have a negative impact on the periodontium, contributing to worsening of aggressive periodontal disease [43].

Specific markers

The Immunoglobulins (Ig) are specific defense factors seen in saliva. Among IgA, IgM, IgG the IgA type is predominantly found in saliva. They interfere with bacterial adherence and influence the oral microbiota. Several studies have found a positive correlation between the levels of immunoglobulins and severity of periodontal inflammation and salivary levels of IgA [14, 44].

Inflammatory markers

Some of the pro-inflammatory cytokines such as interleukin (IL)-1α, IL-1β, IL-6, Tumor Necrosis Factor-α (TNF-α), Prostaglandin E2 (PGE2), Transforming Growth Factor-β (TGF-β), and Macrophageinflammatory protein-1 alpha (MIP-1α/CCL3) have been used in diagnosing periodontal diseases as they represent cascade of inflammatorycells such as leukocytes, lyzozymes which are responsible for degradation of tissue components [45]. IL-1β is released by activated macrophages, lymphocytes and fibroblasts which secrete PGE2, leading to bone destruction. They also secrete MMP's, determining connective tissue destruction. IL-1β has been a highly reliable marker for assessing the periodontal status with 90 % sensitivity and 76 % specificity [46]. Ebersole reported that IL-1β, IL-6 and MMP-8has a high capacity to distinguish periodontitis patients from healthy individuals [41]. Macrophage inflammatory protein-1 alpha (MIP-1α) has been recently discovered to be secreted in abundance at the sites of periodontal inflammation and bone resorption [47]. The cytokines are produced by phagocytes, fibroblasts in acute as well during the chronic inflammation. It is responsible for the chemotactic effect and proinflammatory response affecting the T Lymphocytes, monocytes, platelets, and NK cells. The cytokines also help in detecting the subclinical inflammation in periodontally compromised individuals. Al-Sabbagh, had demonstrated that MIP-1α has a high sensitivity and specificity of 95 % and 97 %, respectively [48].

Oxidative stress markers

The process of inflammation and tumor proliferation is called as oxidative stress which occurs due to excess of free radicals. The process occurs either by lack of antioxidant mechanism or by increased risk of oxygen species. Sequence of inactivation of enzyme, stimulation of proinflammatory cytokinesand lipid peroxidationare responsible for the destruction of periodontal tissues.8- Hydroxydeoxyguanosine (8-OHdG) an oxidized nucleoside formed by the oxidation of guanine from damaged DNA. Its levels increase in saliva during inflammation of periodontium and decreases with successful intake of anti-inflammatory drugs [49]. It has been demonstrated that the 8-OHdG act as a biomarker of oxidative stress in body fluids. Ascorbate, albumin and melatoninare the main antioxidants, and their effect in salivais correlatedwithextent and intensity of periodontal inflammation. The elevated levels of lipid peroxidation and decreased levels of antioxidants found in saliva are responsible for periodontal tissue destruction [50].

Systemic markers

C-reactive protein is produced by the liver and stimulated by circulating cytokines, such as TNF α andinterleukin-1, which are derived from local and systemic inflammation unlike periodontal inflammation [51]. The C-reactive protein reaches saliva via GCF or the salivary glands. High levels of C-reactive protein have been associated with chronic and aggressive periodontal diseases and with other inflammatory biomarkers. C-reactive protein can be measured in saliva using a lab-on-a-chip method in periodontal subjects. Other protein biomarkers which are useful in periodontitis include fibronectin, cystatin; Neopterin [29]. The level of Neopterin usually increases during inflammation of periodontium. It plays a key role in nitric oxide radical formation and phagocytosis.

Microbial markers

There are almost 600 bacterial species which can be seen in subgingival plaque and they are duplicated to cause periodontal disease. Some of the periodontal pathogens such as *Treponema denticola, Porphyromonas gingivalis, Tanerella forsythesis* has been the main organisms for the cause of periodontal diseases. The "red complex" is mainly indicated in periodontal disease. Species such as *Actinobacillus actinomycetemcomitans* has been associated with chronic, early onset and aggressive periodontitis. The other pathogens such as *C. ochracea, E. corrodens, C.recta, F. nucleatum* has also been associated with chronic periodontitis [52].

Nonspecific biomarkers [53–60]

It is classified as 1) Proteins 2) Growth factors 3) Hormones 4) Inflammatory cells 5) Volatiles 6) Bacteria 7) Epithelial keratins.

Mucins

These are glycoproteins produced by sublingual and submandibular glands. They aid in lubrication, protection against dehydration. They mainly interfere with aggregation of bacteria like AA and levels of mucins are usually decreased with increased concentration of *Actinomycetes comitans*.

Lactoferrin

It is an iron binding glycoprotein, which is increased during inflammation of gingival and higher levels, can be detected in saliva of periodontally compromised individuals.

Histatin

It is a protein, having high antimicrobial properties mainly secreted by submandibular and parotid glands. It is chiefly involved in inhibition of histamine released by mast cells which are responsible for gingival inflammation and destruction of periodontium.

Growth factors

It includes EGF and VEGF. Vascular endothelial growth factor: It plays a key role in wound healing and inflammation. It is classified as multifunctional angiogenic cytokine, which can be detected in whole saliva of patients diagnosed with periodontitis.

Epithelial keratins

These keratins which are phenotypic markers have been used as indicators for periodontal diseases. However no study evidence has been associated with keratins of saliva and progression of periodontitis. It has been majorly detected in odontogenic cysts, tumors and oral cancers.

Amino acids

They are released from bacteria, or by degradation of salivary proteins rich in proline. However, many of the studies have found no association or diagnostic significance for decreased or increased level of amino acids and cause for periodontal disease.

Hormones

Emotional stress and increased cortisol levels have been contributed to be the risk factors for periodontitis. The increased cortisol levels along with emotional stress exert strong inflammatory process and immune response. Although in future further studies may be required to evaluate salivary cortisol levels and association of periodontitis.

Inflammatory cells

Leukocytes in saliva enter the oral cavity via gingival crevice and the orogranulocyte migratory rate reflects the inflammation of oral cavity. This method of screening can be considered as laboratory test to diagnose periodontitis.

Volatiles

Salivary volatile compounds such as methyl mercaptan, hydrogen sulphide have been classified as possible diagnostic marker in periodontal disease.

Bacteria

The microorganisms in dental plaque utilize the components of saliva and act as substrate. The species such as *actinomyces viscous, Streptococcus* serve as growth medium in saliva. Other species such as *A. Actinomycetemcomitans, P. gingivalis* and *prevotella intermedia* are seen in whole saliva of periodontal patients. The species of bacteria mentioned have been found mainly in subgingival environment.

Advanced markers

The translational research on saliva has emerged with toolbox of salivary proteome and the word called **"OMICS"** has been employed more using salivary transcriptome, Proteome and metabolome for diagnosis of periodontal diseases. The salivary proteome and the proteomics, where the portion of genome is expressed. This has paved a way for oral health. The human salivary proteins also have been emerging with "Shot gun proteomics" approach through mass spectrophotometry and gel electrophoresis. A total of 1,159 proteomes have been identified. Jeon analyzed [61] specific RNA expression profiles in saliva samples and gingival tissue in periodontal patients and healthy individuals, to determine their potential as monitoring tool in periodontitis. A vast array of salivary proteomes has been researched into a database called as the Saliva Proteome Knowledge Base and is available to the public (http://www.skb.ucla.edu/) for the identification of combinations of biomarker panels providing reliable approach for saliva-based diagnostic tests [62].

MicroRNAs

They are single stranded RNA, which are implicated in pathological process and chronic inflammatory diseases as well cancers. The dysregulation of these markers has been induced by several bacterial components and oral plaque. It has been markedly a diagnostic and prognostic marker in periodontal diseases [63].

Point of care testing (POC)

The development of diagnostic tests should always be carried out such that active disease and future progression can be determined. The point of care testing is usually performed when the patient needs time care at a certain point. The field of medicine has revolutionized with the development of oral; fluid based POC which can be employed as chair side test for diagnosis of oral diseases. It can be performed when the time care to the patient is required. Development of microfluidic approaches and detection of biomarker molecules in the oral cavity with advanced techniques like PCR for RNA and DNA, ELISA for proteins, makes the POC based oral method a diagnostic reality. Some of the newly introduced POC devices include Integrated Microfluidic Platform for Oral Diagnostics (IMPOD) which is used to detect salivary proteins with a low sample volume requirement (10 μL) and considerable sensitivity. Using this device, rapid (<10 min) measurements of MMP-8, TNF-α, IL-6, and CRP in saliva were performed [64]. Lab-on-a-chip (LOC) system that integrates microfluidics and a fluorescence-based optical system in which sandwich immunoassays are performed on chemically sensitized beads emerged as a great hope for managing fluids like saliva and also in determining the risk of periodontal diseases. It allows multiple measurements of C-reactive protein, MMP-8, and IL-1β, which are related to the clinical expression of periodontitis. However, trials are still undergoing to confirm the validity of the studies [65].

Another unique test kit used in Japan is the Salivary Occult Blood Test (SOBT), the origin of this test started in Japan. It is basically a monoclonal antibody against hemoglobin of humans in determining the periodontal health. The SOBT test kit contains paper strip containing gold labeled anti-human hemoglobin monoclonal antibody which can be easily dipped into the saliva sample. A magentaline is indicated as positive test which is formed by an immune complex with hemoglobin, according to the manufacturer's reference concentration of ≥2 μg/mL human hemoglobin [66]. With a view of maintaining good oral health despite of many drawbacks, this tool has been used effectively in screening regular oral self-care and for maintaining periodontal tissues. However, it has a low specificity and sensitivity rate despite this drawback, it has been used as an effective tool to create oral health awareness among the public.

Limitations

The limitations associated with salivary biomarkers include the flow rate of saliva which varies among individuals based on age, sex, medical condition and the circadian rhythm. The factors undeniably question the accuracy and reproducibility of diagnosis using salivary biomarkers in diagnosing early stages of periodontitis. The biomarker test needs to be real-time, where the patient's periodontal status can be immediately monitored in the dental office. Lastly, biomarkers should not only help in diagnosing the early stages of periodontal diseases but also should help in predicting the future risk by simple and affordable means.

Discussion

The balance between the microbiota and the host response is very important for the periodontal health. The conventional techniques such as bleeding on probing, Pocket depth and radiographic assessment has been employed, although there is always search for the reliable source and saliva has been a potential tool for early diagnosis, also number of salivary biomarkers have been used in diagnosing various oral diseases. The collection of saliva is easy and multiple samples can be taken as it is noninvasive and more acceptable by the patients. The salivary based immunological, microbial and molecular level diagnostics can be easily diagnosed in healthy and diseased individuals. The growing technology and knowledge on molecular, clinical studies and individual biomarker aimed at characterizing the efficacy of individual biomarkers have been useful in monitoring periodontitis. The systematic reviews considering PROBE guidelines need to be put forward with predictivity and reliability of the novel salivary biomarker and whether combination of biomarker signatures would help in better diagnosis of periodontal diseases. The combined microbial, molecular level biomarkers can be employed for successful progression of periodontal diseases. Ebersole tested this hypothesis by evaluating the expression of IL-6 and MMP-8 [67]. Gursoy determined that triple combination of IL-6, MMP8, and IL-1β had predictive value of 100 % and could be reliable in diagnosing periodontitis [65].

Future directions

The concept of personalized medicine with genetic, environmental and genomic as well clinical directions may help in individualized patient care. These set of biomarkers may provide effective reproducibility sensitivity and specificity which directs us to establish a patient diagnostic criteria especially in periodontology. The role of salivary biomarkers in oral cancers have been identified through microarray technology, moreover 3,000 various types of mRNA have been identified in unstimulated saliva, this novel technology can thus help in identifying the periodontal health caused due to oval and systemic effects. The advances in micro fluids technology in periodontics has revolutionized the procedures pertaining to enzymatic, DNA analysis and proteomics specialty through lap on chip technology which helps in detecting the periodontal specialty. Through the lab or chip technology which helps in detecting the periodontal biomarkers during immune response. Moreover combined analysis of biomarkers through novel techniques of salivaomics such as Interatomics, Metabolomics, Peptidomes, Proteomics etc.; might help in breaking the analysis of single biomarker, analysis. The precise combination analysis will thus help in early diagnosis of periodontitis [68, 69].

Clinical implications

The conventional method of periodontal screening was through evaluation of clinical and radiographic parameters. However, in the recent times the evaluation of salivary biomarkers has been used as prospective screening tool and has been correlated between periodontitis and systemic diseases. Large scale studies are however needed with specific sensitivity and specificity for utilization in routine dental practice. It has proposed as complementary method of weighing the risk, benefit ratio.

Conclusions

Salivary biomarkers are promising and a reliable source for diagnosis of oral diseases in future. The expression of these markers would help the clinician with a valued non-invasive procedure apart from the clinical findings, and further research with appropriate study design, clinical implications and prolonged follow up periods are warranted to close the gap between reliability of these markers to the current diseased state.

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