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Bio markers in periodontics

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Abstract

Periodontal disease, one of the prevalent oral diseases, is characterized by gingival inflammation and periodontal tissue destruction. Diagnosing this disease is challenging to the clinicians as the disease process is discontinuous and shows periods of exacerbation and remission. Traditional diagnostic methods basically tells about the past tissue destruction so new diagnostic methods are required which is able to detect the active state of the disease, determine the future progression and also estimates the response to the therapy, thereby helping in the better clinical management of the patient.

Both saliva and Gingival crevicular fluid (GCF) are believed to be reliable medium to detect the biomarkers which plays a pivotal role in measuring the disease activity. Keeping these observations in mind rapid chairside tests are developed to diagnose periodontal disease called as Point of Care (POC) diagnostics which simplifies diagnosis and helps in improving the prognosis. This review article highlights about the biomarkers used in the diagnosis and throws light on the various available points of care diagnostic devices.

Keywords: Periodontal Diseases, Diagnostic, Prognostic, Biomarkers

Introduction

Periodontitis is a group of inflammatory diseases that affect the connective tissue attachment and supporting bone around the teeth whose initiation and progression depends on the presence of virulent microorganisms capable of causing disease.

The course of periodontal disease is marked by discontinuous pattern of disease activity and inactivity showing exacerbation and remission. The traditional clinical assessment methods include attachment level, probing depth, bleeding on probing, radiographic assessment of alveolar bone loss, but they neither provide information on the measures of disease activity nor do they identify the individuals who are susceptible to future disease progression as the biologic phenotypes are not reflected properly in the clinical phenotype[1,2].

Biological phenotypes may then be taken into consideration which will be of help in assessing the burden of microbial and inflammatory load, which further affects the progression of periodontitis. Earlier the disease is diagnosed, more likely it is to be cured

successfully. Periodontitis is considered to be a multifactorial disease with no clear-cut etiology, so its identification and early diagnosis becomes more difficult. The current clinical diagnostic parameters were introduced more than 50 years ago. But all the methods provide disease severity rather than disease activity[3].

A biomarker is a substance used to indicate a biologic state and is an objective measure to evaluate the present and future disease activity. It is defined as – A substance that is measured objectively and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Various biological media like saliva, serum and gingival crevicular fluid are used to determine biomarkers in periodontal health and disease. A single biomarker will not able to predict periodontal disease activity and severity. So combinations of biomarkers are used to predict the disease activity[4].

Thus, the aim of this review is to present recent advances in the development of proteomic, genomics and microbial biomarkers and potential clinical applications.

Need for a bio marker

Since the 1920s, there have been many changes in the classification of periodontal diseases in an attempt to reach the most accurate diagnosis in order to facilitate treatment planning. The latest classification system aimed to address issues associated with the previous classification system and to provide a standard universal platform that can be easily used by periodontists.

A search of the literature reveals the number of studies examining biomarkers in oral fluids as diagnostic tools for periodontal disease has markedly increased in the last decade. It is anticipated that increased understanding of biomarkers in periodontal health and disease will lead to the further development of chair-side technologies enabling dental practitioners to diagnose periodontal diseases and to predict the prognosis and responsiveness to periodontal therapy. Furthermore, biomarkers may be useful in screening as an adjunct in epidemiological studies.

Under diagnosis periodontal therapy leads to failure of periodontal treatment. For that researcher phrased biomarkers that indicated the presence or absence of periodontal disease. The biological media of choice included saliva, serum and gingival crevicular fluid.

Sources of biomarkers of periodontal disease in the oral cavity

Saliva, Gingival Crevicular Fluid (GCF), Peri-Implant Sulcular Fluid (PISF), and mouth rinse remnant constitute reliable sources of biomarkers in the oral cavity that are readily available. These fluids may be collected non-invasively, with a high potential to reflect periodontal health and disease status through examining the biomarkers within them[5].

However, certain limitations affect the quality and quantity of each fluid collected. Several methods have been described for the collection of GCF, such as absorption onto paper strips, microcapillary pipetting, and sulcular washing methods. Despite the fact that GCF provides high levels of different biomarkers, the volume of this fluid is drastically altered in response to health or disease. This fluctuation greatly influences collection time by microcapillary pipetting, which ranges from 10 min for diseased sites up to 40 minutes in healthy sites[6,7].

Saliva is rich in a wide array of biomarkers that can be easily collected and stored in larger amounts than GCF without any potential trauma to the periodontal tissues. Errors associated with interpretation of salivary samples are mostly related to variations in the volume and

composition of saliva. These may be due to differences in pathological and physiological states between individuals, as well as within the same person at different times[8,9].

Potential bio markers of periodontal diseases

A biomarker was defined by the National Institutes of Health Biomarkers Definitions Working Group as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention[10].

Ideally, the biomarker must be valid, safe to use, easily measured, affordable, and able to be collected non-invasively. In addition, it should be highly sensitive to correctly identify those with disease (true-positive) and specific to precisely identify those without disease (true-negative). These criteria increase the accuracy of the biomarker as a predictive and diagnostic tool and for efficiently reflecting the patients' responses to treatment. Furthermore, consistency of results across different ethnic groups, ages, and genders is an important characteristic of an ideal biomarker. This section describes the most promising biomarkers of periodontal and peri-implant diseases[11].

Proteomic biomarkers

The word "proteome" is a blend of "protein" and "genome", and was coined by Marc Wilkins. The proteome is the entire complement of proteins, including the modifications of a particular set of proteins. Proteomics offers a new approach to the understanding the changes occurring as oral micro-organisms adapt to environmental change within their habitats in the mouth[12].

Alkaline phosphatase (alp)

ALP is a catalyzing enzyme that accelerates the removal of phosphate groups in the 5 and 3 positions from a variety of molecules, including nucleotides, proteins, and alkaloids. Although it is present in all tissues, ALP is particularly concentrated in the bone, liver, bile duct, kidney and placenta. The enzyme is likely to be largely derived from the periodontal tissues[13].

The major source of ALP in early inflammation is polymorphonuclear leukocyte. There is a significant correlation of ALP with pocket depth and inflammation. There is a relationship between attachment loss in the periodontitis group to a drop-in ALP activity in serum. Contrary to these results, the measurements of periodontal destruction (probing depth, gingival bleeding, and suppuration) are related to higher levels of ALP in saliva. As a predictive indicator for the future periodontal breakdown, ALP may serve as a marker in periodontal treatment planning and monitoring[9].

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are genetically distinct but structurally related zinc dependent metalloendopeptidases. MMPs are host proteinases responsible for both tissue degradation and remodeling. MMPs degrade extracellular matrix and further potentiate proteolysis and inflammation by processing of bioactive non-matrix substrates, such as cytokines, chemokines and growth factors, and also by activating other MMPs.

The 23 MMPs expressed in humans can be classified into different subgroups based on their primary structures and substrate specificities: Collagenases (MMP-1, -8 and -13), Gelatinases (MMP-2 and -9), Membrane type MMPs (MT-MMPs, MMP-14, -15, -16, -17, -24 and -25) and other MMPs. In the healthy

condition, the periodontal ligament apparatus is protected from matrix metalloproteinases mediated proteolytic attack by tissue inhibitors of metalloproteinases (TIMP)[14].

Gelatinase (MMP-9)

Gelatinase (MMP-9), another member of the collagenase family, is produced by neutrophils and degrades collagen extracellular ground substance. There is a twofold increase in mean MMP-9 levels is reported in patients with recurrent attachment loss.

After giving one dose of systemic metranidazole, the levels of MMP-9 in mouth rinse samples from patients with initial elevated MMP-9 concentrations markedly decreased. Given these results, future use of MMP-9 in oral diagnostics may best serve as a guide in periodontal treatment monitoring[15].

Calprotectin

Calprotectin is released from neutrophils. It is a calcium and zinc binding protein, has both antimicrobial and antifungal activity and play a vital role in inflammation. It inhibits immunoglobulin production and act as a proinflammatory protein. Increased expression of calprotectin at the site of inflammation offer protection against bacterial invasion to epithelial cells especially P. gingival is. Calprotectin appears to improve resistance to P. gingival is by boosting the barrier protection and innate immune functions of the gingival epithelium[16].

Osteopontin (open)

OPN is released by both osteoblasts and osteoclasts. The concentration of OPN is higher at the clear zone where osteoclasts are attached. It helps in bone remodeling. In periodontitis, OPN levels are increased. There is a positive correlation between increased levels of OPN to probing pocket depth. When nonsurgical periodontal treatment is provided GCF OPN levels are significantly reduced[17].

Fibronectin

Fibronectin is a glycoprotein that promotes selective adhesion and colonization of certain bacterial species .it is involved in chemotaxis, migration, inflammation, wound healing and tissue repair. Changes in oral cleanliness may contribute to the rapid fluctuations in salivary proteases and epithelial cell fibronectin[18].

Immunoglobulins

The predominant immunoglobulin in saliva is secretory IgA (sIgA) which is derived from plasma cells in the salivary glands. There are two subclasses of IgA – IgA1 and IgA2. IgA1 is predominates in serum while IgA2 is found in higher concentrations in external secretions. Saliva from treated periodontitis patients has higher IgA and IgG levels than saliva from control subjects.

These higher antibody levels are observed for periodontal pathogens (P. gingival is and Treponema denticola), but also for the normal inhabitant of the oral cavity Strep to cocussali varius. Significantly elevated levels of IgG antibody to A. actinomycetemcomitans are found. High level of salivary IgA is directed against bacteria in dental plaque and may protect against the development of gingivitis[19].

Genetic biomarkers

Interleukin polymorphisms: A study reported that a “composite” IL-1 genotype consisting of at least one copy of the rarer allele at both an IL-lα and IL-1β loci was associated with severe periodontitis[20].

Cathepsin c polymorphisms

The underlying causation of Papillon-Lefevre syndrome has been the subject of considerable debate in the literature. Papillon-Lefevre syndrome is caused by mutation in gene coding cathepsin C. This enzyme is

expressed at high levels in many immune cells including polymorphonuclear leukocytes and macrophages and their precursors. In addition, it has been found that cathepsin C is expressed in areas of epithelium often affected by hyperkeratosis lesions such as palms, soles, knees and oral keratinized gingiva. But hyperkeratosis present only in homozygous trait[21].

TNFα gene polymorphism

The TNFα gene is located on chromosome 6 within the major histocompatibility complex (MHC) gene cluster at the location 6p21.3. It is an important mediator in inflammatory reactions and appears to play a central role in the pathogenesis of severe chronic inflammatory diseases. Differences in the rate of production of TNF have been demonstrated and a familial ability to produce higher or lower cytokine levels seems to exist. The TNF synthesis may be influenced by the presence of certain gene polymorphisms. Some consistent results on association of TNFα gene polymorphisms with diseases are reported for infectious diseases particularly malaria. TNFα gene polymorphisms were also investigated in association with periodontitis[22].

CD14 gene polymorphism

The CD14 gene is on the chromosome 5 at the location 5q31.1. The production of the sCD14depend on C to T transition at position –159 (also called -260). Subjects with the homozygous TT genotype exhibited significantly higher sCD14 levels which influenced the activation of Th2- to Th1 type cells in the response to bacterial challenge. The -260 CD14 gene polymorphism. has been associated with Crohn's disease and also with periodontitis[23].

Other biomarkers

Cortisol: A study evaluated the association of stress, distress, and coping behaviors with periodontal disease and concluded that higher salivary cortisol levels were detected in individuals exhibiting severe periodontitis[24].

Calcium

A study conducted to examine differences in salivary calcium levels in periodontitis patients in comparison to periodontally healthy subjects. The results show that subjects in the high salivary CA group had significantly more intact teeth than their pairs in the low salivary Ca group and concluded that an elevated calcium concentration in saliva was characteristic of patients with periodontitis[24].

Volatiles

Volatile Sulphur compounds, primarily hydrogen sulfide and methyl mercaptan, are associated with oral malodor. Salivary volatiles have been suggested as possible diagnostic markers and contributory factors in periodontal disease. For example, pyridine and Pico lines were found only in subjects with moderate to severe periodontitis. Furthermore, saliva seems to be a useful medium to evaluate oral malodor[25].

Microbial markers

Although there are almost 600 bacterial species present in subgingival plaque, only few of them are causing periodontal disease in a susceptible host. A number of specific periodontal pathogens have been implicated in periodontal diseases, including Tannerella forsythensis, Porphyromonas gingival is, and Treponema denticola[26]. These three organisms are members of the ''red complex'' of bacteria that are highly implicated in the progression of periodontal diseases. Action bacillus actinomycetemcomitans has been linked with early-onset forms of periodontal disease and aggressive

periodontitis, whereas red complex bacteria are associated with Chronic Periodontitis. A study conducted to determine whether the presence of bacterial antigens for Porphyromonas gingival is (Pg), Prevotella intermedia (Pi), and Action bacillus actinomycetemcomitans (A.a) in sub gingival plaque of periodontitis patients after periodontal treatment was associated with progressive alveolar bone loss[27,28].

Progressive alveolar bone loss was determined using digital subtraction radiography with standardized radiographs taken at baseline and 6 months after treatment and concluded the presence of P. gingival is in plaque after treatment was significantly associated with progressive bone loss[29,30].

Periodontal point-of-care test kits

PoC technology aims to evaluate the levels of biomarkers that have shown to be associated with the disease status. These tests have already been used in general medicine for blood coagulation, immunological, and cardiovascular biomarkers. Moreover, some of these tests, such as pregnancy tests and for blood glucose levels, are available for home use. There is potential for developing further PoC tests in medicine, and the WHO has introduced the ASSURED criteria for the characteristics of PoC devices. This stipulates that such devices should be "affordable, sensitive, specific, user friendly, rapid, and robust, with no complex equipment and deliverable to end-users"[31,32,33].

The development of a PoC test for periodontal diseases that meets the above criteria would be of great value and make life easier for researchers, clinicians, and patients. Since the 1990s, many test kits that have been introduced as prototypes or for commercial use have relied upon chemical, immunological, and microbiological techniques for the evaluation of biomarkers. The idea was to develop a test kit with enhanced diagnostic and prognostic capabilities.

This section will review the applicability and usefulness of these kits through the studies that have examined them. In general, the chairside kits can be classified into three groups[34,35,36].

Microbiological test kits

Microbiological test kits have been used to detect period on to pathogenic bacteria that play a role in periodontal diseases, such as A. actinomycetemcomitans, P. gingival is, P. intermedia, T. forsythia, and T. denticola. Evaluation of these bacteria can be used to determine the most common forms of the disease, such as gingivitis and periodontitis (formerly called chronic and aggressive periodontitis). These tests were used to assess the reduction or eradication of periodontal pathogens during periodontal therapy; however, they could not fully satisfy clinical needs. For example, Omnigen diagnostics takes hours to days to perform, Evaluisite has very low sensitivity, and Peri Oscan can only determine the severity of the disease[37].

Biochemical test kits

These kits have mainly been used to determine levels of biomarkers in oral fluids. Molecules, such as enzymes (bacterial and host enzymes), mediators of inflammation, and extracellular matrix components that represent the alteration of periodontal tissues have been investigated. Amongst the molecules, enzymes (MMP8 in particular) have been mainly examined and translated as chair side tests. Generally, these tests are not widely used in the clinic because of complex procedure, low sensitivity and specificity, whereas, the more recently developed PoC test kits, namely Peri Safe® and Implant Safe®, can provide results within 5–7 min, with sensitivity and specificity of 76.5% and 96.7%, respectively[38].

Genetic test kits

Genetic polymorphisms of IL-1α and IL-1β are likely to be related to an individual's genetic susceptibility to periodontitis. Although these genes do not cause or initiate the disease, they might enhance earlier development and severity of the periodontitis. Geno Type PST® and My perio ID tests are used to determine the genetic susceptibility to periodontitis.

The biomarkers that have been examined in relation to PoC test kits have been shown to identify the severity of periodontal diseases. However, apart from PerioSafe® and Implant Safe®, none of these tests have demonstrated the prognostic capabilities of importance to both clinician and patient. Additionally, these tests have been shown not to comply with ASSURED criteria for diagnostic devices. Thus, some of these tests, indeed the majority, are no longer available or rarely used in clinics[39].

Table 1: Summary of diagnostic biomarker test kits for periodontal diseases[40]

Assay	Commercial kit	Sample	Target
Microbial test	Peri Oscan	Subgingival plaque	Utilizes the BANA test for bacterial trypsin-like proteases
	Omni gene	Saliva	Pg, Pi, Aa, Fn, Tf, Td, Ec, and Cr
Bio chemical test	Prognostik	GCF	Serine proteinases and elastase
	Perioguard	GCF	Detects the presence of aspartate aminotransferase
Genetic test	Genotype	Saliva	Interleukin (IL-1α and IL-1β) genes polymorphism
	My perio id	Saliva	Genetic variation/polymorphism within the IL-1 gene

Bio markers of periodontal disease in urine

Among the urine biomarkers, β2-MG, α1-MG, and NGAL were seen to be positively correlated with the clinical periodontal status. Although, urinary albumin was reported to have a relationship with periodontitis, no association with parameters of periodontitis was observed. β2-MG and α1-MG are low-molecular-weight proteins (27 and 11.8 kDa, respectively), with the former being produced by all cells expressing major histocompatibility complex class I antigen and the latter being synthesized mainly by the liver and existing in various body fluids.

Proteins are readily filtered through the glomerulus in a healthy kidney, and approximately 99% is reabsorbed and catabolized by the proximal tubular cells. Therefore, increased β2-MG or α1-MG excretion in urine has been reported to indicate early signs of renal tubular dysfunction. The concentration of urine β2- MG is also known to increase during various inflammatory conditions or viral infections independent of kidney injury.

In the current study, higher inflammatory activity was observed in the PD and BOP (indicating severe periodontitis) of individuals included in the high β2-MG group compared to those in the normal β2-MG group. he mechanism by which periodontitis affects the urinary levels of β2- MG is still unclear, and future studies should also focus on examining the levels of β2-MG in gingival tissues. High concentrations of β2-MG in inflamed periodontal tissues may disseminate into the systemic circulation and be excreted through the urine, thus exhibiting increased levels.

On the contrary, due to the bidirectional relationship between renal function and periodontitis, urine β2-MG possibly increased along with renal dysfunction and associated with periodontitis. However, the participants of the current study were not diagnosed as renal dysfunction. Further studies are necessary to better understand the mechanism underlying increased urinary concentrations of β2-MG in patients with periodontitis[41].

Conclusion

In the field of oral disease diagnosis, there has been a steady growing trend during the last two decades to develop tools to monitor periodontitis. From physical measurements such as periodontal probing to sophisticated genetic susceptibility analysis and molecular assays for the detection of biomarkers on the different stages of the disease, substantial improvements have been made on the understanding of the mediators implicated on the initiation and progression of periodontitis.

At the same time, this evolutionary process has promoted the discovery of new biomarkers and the development of new therapeutic approaches mainly using host modulation. It is clear that no single marker will fulfill all the criteria necessary for assessment of the clinical state of the periodontium, and future research should be directed at the production of "marker packages". The development of a wide spectrum of marker factors will be a primary goal of periodontal research.

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