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Significance of Serum, Salivary, Bone Biomarkers in the Progression of Periodontal Diseases

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Abstract

Periodontitis is a multi-factorial poly microbial disease of oral cavity, that is generally caused by the complex interactions between the host immune system and sub-gingival microbiota that leads to chronic gingival inflammation, loss of periodontal ligament attachment, alveolar bone loss and finally resulting into tooth loss. The earliest diagnosis and treatment of progressive periodontitis is very important because of the irreversible nature of this disease. Periodontal diagnosis is the pillar of a successful periodontal strategy, since prevention and treatment plans depend on the perfection and precision of the respective diagnostics. Whatever the clinical measurements are used in the diagnosis of periodontal diseases are frequently of limited usefulness in that they are indications of previous periodontal disease as compared to the present disease progression. Nowadays, a huge number of periodontal biomarkers have been detected in the in the field of oral diagnostics to diagnose or detect the periodontal diseases in patients. Biomarkers are used to objectively evaluate normal biologic processes, pathogenic processes and pharmacologic response to therapy protocols. Biomarkers are the significant molecules that could be used to keep an eye on health status, disease onset, treatment response and outcome. Biomarkers are one of the best early indicators of periodontal disease. The biomarkers like salivary, serum and bone biomarkers

help in determining the inflammatory mediators as they are the good indicators of inflammatory activity. This review article highlights the recent advances as well as the importance of periodontal biomarkers in the field of periodontics.

Keywords: Biomarkers, serum, salivary, bone, peri Oscan, periocheck.

Introduction

Biomarkers of disease in succession play an important role in life sciences and have begun to assume a greater role in diagnosis, monitoring of therapy outcomes, and drug discovery. The challenge for biomarkers is to allow earlier detection of disease evolution and more robust therapy efficacy measurements.[1]

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.[2] Furthermore, periodontitis is time-consuming and expensive to treat and, therefore, to overcome such major challenges in the field of periodontics, biomarkers have come into the evolution to identify as well as to detect the progression of the periodontal diseases.

Pathogenesis and Progression of Periodontitis

The pathogenesis of chronic periodontitis is exaggerated through a microbial imbalance that disrupt the normal symbiotic relationship between the commensal residing microbial species (dental plaque) and the host defensive mechanisms, leading to inflammation and propagation of disease.[3]

Approximately, 800 different species of microbial colonies have been identified from the dental plaque.[4] However, pathogenic microflora alone is not responsible for the pathogenesis of periodontal disease. Host immune responses also play a vital role in progression of periodontitis as shown in (Figure 1).

Bacterial pathogens present in the dental plaque triggers activation of intrinsic host cells which secrete pathogen-associated molecules (PAMPs) such as lipopolysaccharide (LPS) and glycoconjugates. These PAMPs in turn recruit and activate certain pro-inflammatory mediators including neutrophils, cytokines and osteoclasts (MMP-8, MMP-9-, β-glucuronidase, Interleukin-1β and Interleukin-8). Macrophages and lymphocytes (T-and B-lymphocytes) are also activated and mediate release of other secondary inflammatory mediators (tumor necrosis factor alpha, interleukin-12, interleukin-17 and interleukin-18).

The ecological imbalance between microflora and neutrophil concentration is reflected by raised levels of Interleukin (IL)-β and IL-8. Consistent inflammatory response, ultimately leads to loss of soft and hard connective tissues of Periodontium (Figure 2).[5]

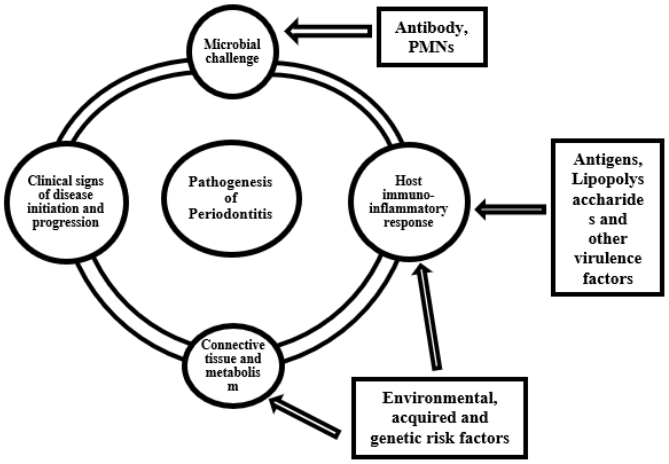

Figure 1: Pathogenesis and progression of periodontitis.

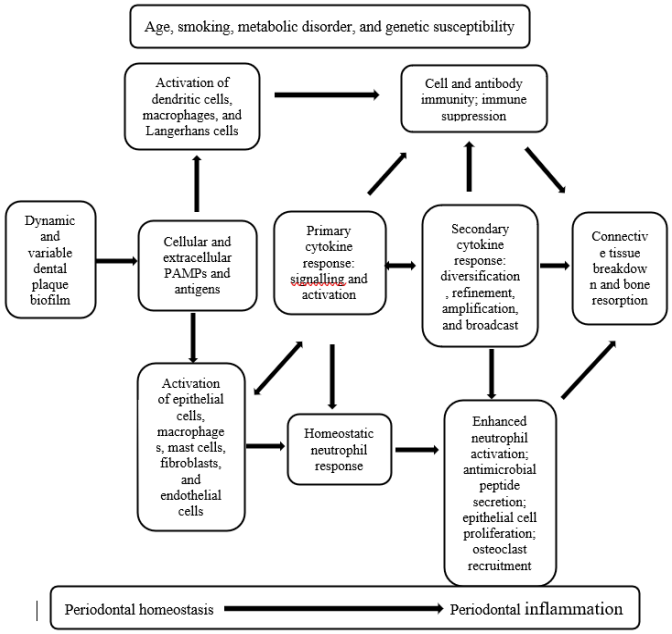

Figure 2: Schematic illustration of the pathogenesis of periodontitis.

A Quick Summary on Biomarkers

General Biomarkers

A biomarker can be 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.[6] Biomarkers have been classified by Perera and Weinstein [7] based on the sequence of events from exposure to disease. They can be classified into five categories based on their application in different disease stages as explained in (Figure 3):[8]

- Antecedent biomarkers to identify the risk of developing an illness,
- Screening biomarkers to screen for sub-clinical disease,
- Diagnostic biomarkers to recognize overt disease,
- Staging biomarkers to categorize disease severity, and
- Prognostic biomarkers to predict future disease course, including recurrence.

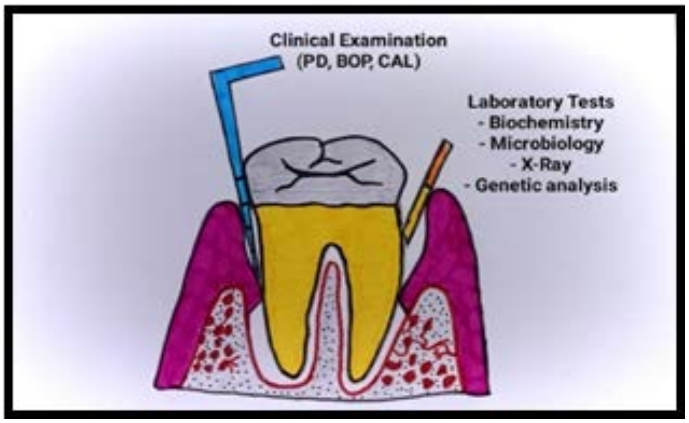

Figure 3: Current and future diagnostic tools

Traditional Biomarkers

Traditional biomarkers in medicine include heart rate, blood pressure, imaging (X-rays), and the Prostate-specific Screening Antigen test (PSA) for prostate cancer. A traditional biomarker for periodontal disease is bleeding on probing (BOP), supposedly the best disease predictor available today (Figure 4). According to researchers there are many false positives associated with it, but the absence of BOP is considered a very precise negative predictor of disease activity,[9] periodontal diagnosis is based upon subjective clinical examination procedures that may be time-consuming and poorly implemented by the operator. Many a time diagnosis is solely based upon periodontal probing measurements due to time constraints, which lead to under diagnosis & inappropriate treatment and low rates of appropriate therapeutic intervention. So, to overcome these difficulties, periodontal biomarkers have come into the way as one of the best diagnostic tools to predict the severity and progression of periodontal diseases for a better treatment plan.

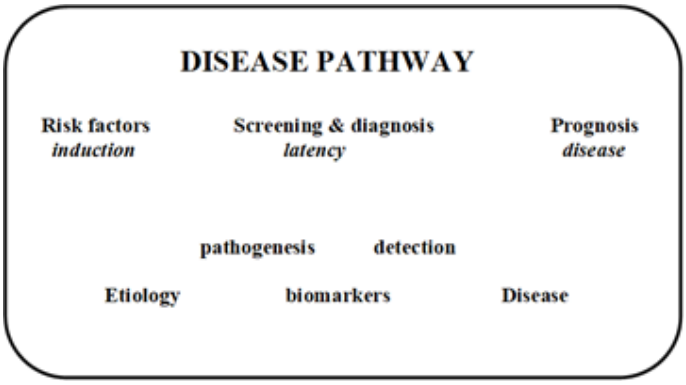

Figure 4: Disease pathway and potential impact of biomarkers

Periodontal Biomarkers

Periodontal biomarkers are the type of biomarkers that are used to monitor health status, disease onset, treatment response and outcome. These biomarkers serve as early surveillance of periodontal disease.

Recent advances in the use of biomarker-based diagnostics for disease activity include mediators that are released into GCF and saliva can be broadly grouped according to their sources are grouped as:[10]

1. Microbial plaque: Endotoxins (lipopolyssachrides), Enzymes, Metabolic end products, DNA probes, Cultures of putative periodontal pathogens.
2. Host derived: - 1L-β, Aspartate, Aminotransferase, Transferase, Matrix proteins, Lactoferrin, Lysozyme etc.
3. Connective tissue breakdown products: Collagen-telopeptides, Osteocalcin, Proteoglycans, Breakdown products, Fibronectin fragments.
4. Inflammatory mediators: Complement, Cytokines, Interleukins, Tumor necrosis factor-α, Interferon-α, Antibacterial Antibodies IgG, IgM, IgA, Substance P, Prostaglandin E2, Acute-phase proteins, transferrin, C-reactive protein.

Also, specific periodontal biomarkers for periodontal disease, which are considered to be the mirror of the body are classified as below (Table 1).[9]

Table 1: Classification of Periodontal Biomarkers.

Proteoic biomarkers	Genetic biomarkers	Microbial biomarkers	Other biomarkers
Cystatins, αglucosidase, Acid phosphatase, Alkaline phosphatase, Aminopeptidase, Lactoferrin, Translactoferin, IgM, MMP13, MMP-8, MMP-9, Cathepsin B, Osteonectin, Osteocalcin, Osteopontin, Elastase Platelet-activating factor, Epidermal growth factor, Platelet-derived growth factor, Esterase, Pyridinoline crosslinked carboxy –terminal telopeptide, Fibronectin, sIgA (secretory IgA) Gelatinase, IgA, Trypsin, Vascular endothelial growth factor, IgG	Cathepsin Cgene Mutation, Collagen gene mutation, IL-1 polymorphisms, IL-10 polymorphisms, Tumor necrosis factor, Polymorphisms	Aggregatibacter actinomycetemcomitans, Campylobacter rectus, Mycoplasmas, Porphyromonas gingival is, Prevotella intermedia, Pepto streptococcus Micros, Prevotella nigrescens, Treponema denticola, Tannerella forsythia. Treponema socransky.	Calcium, Cortisol, Hydrogen sulphide, Methyl mercaptan, Pyridine.

Different Types of Periodontal Biomarkers Based on Their Mode of Action

Salivary Biomarkers

Studies reveal a promising outlook for saliva as a key diagnostic medium for determining systemic diseases or health statuses of individuals.[11] Because collecting saliva involves noninvasive methods and due to the fact that it is an abundant and easily accessible biofluid, saliva is attractive for diagnostic purposes greatly due to its highly enriched content of disease biomarkers that can be deciphered and analyzed. Biomarkers for the detection of specific diseases, such as Sjogren's syndrome, pancreatic, breast, and oral cancer, or periodontal diseases can be detected in saliva. [12-16]

Different Types of Salivary Biomarkers

There are various types of salivary biomarkers including:[17]

1. Matrix metalloproteinases (MMPs):

- MMPs are divided into five major groups:
- Collagenases (MMP-1, MMP-8, MMP-13),
- Gelatinases (MMP-2, MMP-9),
- Strome lysins (MMP-3, MMP-10, MMP-11),
- Membrane-type MMPs (MMP-14, MMP-15, MMP-16, MMP-17), and
- Others.

2. Salivary alpha amylase

3. Immunoglobulin A2, Immunoglobulin G and Immunoglobulin M: IgA2 and IgG levels are higher in the proteomic profiles of whole unstimulated saliva obtained from generalized aggressive periodontitis patients than in healthy individuals.

4. Alkaline phosphatase (ALP)

5. Neutrophil-derived myeloperoxidase (MPO)

6. Cystatins

7. Lysozyme

8. Lactoferrin

9. Interleukin 1α and Interleukin 1β, Interleukin 4, 6, 17, 23

10. Tissue inhibitor metalloproteinase-1

11. Nod-like receptor family pyrin domain containing protein 3

12. Aspartate aminotransferase (AST)

13. Alanine aminotransferase (ALT)

14. Lactate dehydrogenase (LDH)

Role of Salivary Biomarkers in the Diagnosis and Progression of Periodontitis

A wide variety of etiological factors have been implicated in periodontitis, a severe gingival infection that can lead to the destruction of periodontal ligament and alveolar bone.[18,19] Most cases have a bacterial etiology, generating an anti-inflammatory response mediated by cytokines, chemokines, and other biomolecules.[20-22] IL1α is produced by cells in numerous periodontal tissues and plays an important role in the immune response to plaque bacteria in periodontitis and other oral diseases.

This cytokine frequently acts synergistically with TNF-α and prostaglandin E2 (PGE2) to produce various vascular inflammation-related modifications, and this action is especially important in the migration of neutrophils from the bloodstream to the periodontium. The increased expression of IL1β, TNF-α, and PGE2 in oral cavity fluids and tissues in periodontitis suggests their potential use as biomarkers of its presence and progression. These proteins participate in the activation of osteoclasts, the secretion of infiltrating neutrophils, and the resorption of alveolar bone in chronic periodontitis.[23]

The success and progress of periodontal therapy could be followed by measuring the activity of enzyme markers, such as arginase. It has been shown that a proper and complete periodontal therapy leads to a

decreased arginase activity in saliva of patients with chronic periodontitis.[24] In adults with periodontitis, the activity of salivary arginase has been 2.5 times higher than in healthy controls. After one month treatment, the level of activity decreased in patients and it was 1.5 times higher than in controls.[25]

Serum Biomarkers

Periodontitis is initiated by periodontal pathogens that elicit both an innate and humoral immune response; antibodies to some of these pathogens have been detected both locally at the gingival tissue as well as systemically in the blood stream. In order to determine if the levels of systemic antibodies to Porphyromonas gingival is, the most researched periodontal pathogen, correlate with periodontal status, Trindade et al. evaluated patients with different types of periodontitis compared to healthy controls.[26]

Different Types of Serum Biomarkers

1. Serum albumin
2. Immunoglobulins- IgG, IgA and IgM
3. Serum hsCRP (high-sensitivity C-reactive protein)
4. Salivary alpha amylase
5. Serum cortisol
6. Serum oncostatin M
7. Interleukin-6
8. Serum osteocalcin

Role of Serum Biomarkers in The Diagnosis and Progression of Periodontitis

It has been found in the studies that scaling and root planning resulted in a significant decrease in serum hsCRP and IL-6 up to 3 months post treatment in otherwise healthy patients with periodontitis, while other inflammatory biomarkers were not affected.[27] Based on the existing data, serum hsCRP and IL-6 are one of the best systemic biomarkers for monitoring the response to periodontal treatment in patients who are systemically healthy, non-smokers, non-obese as well as those who do not take anti-inflammatory medications.

Oncostatin M, a member of the IL-6 family of cytokines, is synthesized and secreted by T cells and monocytes in response to bacterial products; it has been shown to have a key role in regulating periodontal bone resorption, by acting on both osteoblast and osteoclast receptor activator of nuclear factor-κ B ligand (RANKL)regulation.[28] Serum oncostatin M was significantly higher in patients with chronic periodontitis compared to healthy and gingivitis controls.[29] Serum IgG levels to P. gingival is may be useful systemic biomarkers for diagnosis of both chronic and aggressive periodontitis and serum cortisol may be a useful systemic biomarker for diagnosis of periodontitis in non-smokers.

Bone Biomarkers

Biomarkers of periodontal disease are mostly represented as molecules that are related to three pathological stages including, inflammation, collagen degradation, and alveolar bone turn over. Bone is constantly undergoing the process of bone remodeling, which is an inescapable process in which the rate of bone deposition is faster as compared to the rate of bone resorption to maintain bone hemostasis. Biomarkers of bone remodeling can be of two types, bone resorption markers and bone formation markers which reflect osteoclastic activity (degradation products of type1 collagen and osteoblastic activity (products of collagen synthesis, matrix proteins or osteoblastic enzyme).

Different types of bone biomarkers are listed below (Table 2).[30]

Table 2: Different Types of Bone Biomarkers.

Bone Formation Markers	Tissue Origin	Analytical Sample
Total Alkaline Phosphatase (ALP)	Bone, liver	Serum
Bone Alkaline Phosphatase (BALP)	Bone	Serum
Osteocalcin	Bone	Serum
C-terminal propeptide of type I procollagen (PICP	Bone, skin, soft tissues	Serum
N-terminal propeptide of type I procollagen (PINP)	Bone, skin	Serum
Bone Resorption Markers	Tissue Origin	Analytical Sample
Hydroxyproline, total and dialyzable	Bone, skin, cartilage, soft tissues	Urine
Pyridinoline	Bone, tendon, cartilage	Urine
Deoxypyrindoline	Bone, dentine	Urine
Bone sialoprotein (BSP)	Bone, dentine, hypertrophic cartilage	Serum
Tartar at-resistant acid phosphatase	Bone, blood	Plasma/ serum
Free gamma carboxyglutaminacid (GLA)	Blood, bone	Serum/ urine

Role of Bone Biomarkers in the Diagnosis and Progression of Periodontitis

Alkaline phosphatase (ALP) is associated with the calcification process and an elevated ALP level commensurate with active bone remodeling. ALP and periodontal disease in an experimental gingivitis model showed a significant correlation between ALP and pocket depth and between ALP and inflammation.[31] As a predictive indicator for future periodontal breakdown, ALP therefore might serve as a marker in periodontal treatment planning and monitoring.

Kunimatsu et al.,[32] reported a positive correlation between GCF osteocalcin, N-terminal peptide levels and clinical parameters in a cross-sectional study of patients with periodontitis and gingivitis.

Osteocalcin is considered as a marker of bone formation, but due to the role of it in recruiting osteoclasts to the site of bone resorption, it is now mostly accepted as a vital bone turnover marker. The salivary levels of osteocalcin were significantly correlated with the clinical attachment level. Similarly, several other observations also revealed high levels of osteocalcin in the saliva of chronic periodontitis patients. Conclusively, it could be stated that osteocalcin not only holds a significant diagnostic

potential but also can be used as a prognostic marker to predict the likely outcome of the disease.[33]

Periodontal Biomarkers for Detecting the Progression of Disease

Salivary Biomarkers and How It Responds To Treatment

In an intervention study evaluating the effect of scaling and root planning on salivary AST, ALT, and LDH levels 1 month after treatment, Yoshie et al. found that treatment resulted in significant decrease in LDH in all patients with chronic periodontitis; AST and ALT were also significantly decreased in patients who were not carriers of the IL-1A allele, whereas no significant change was observed in the AST and ALT levels in IL-1A allele carriers.[34]

Serum Biomarkers and How It Responds to Treatment

Pharmacologic anti-inflammatory therapy alone in patients with periodontitis was shown to result in significant decrease in serum hsCRP, IL-6, and IFN, while other inflammatory biomarkers remained unaffected.[35] In a pilot intervention study, Duarte et al. evaluated the serum concentration of several cytokines in patients with generalized chronic periodontitis, generalized aggressive periodontitis and healthy controls, and the effect of periodontal therapy on these cytokines 6 months after treatment.[36] They found that TNF-alpha and IL-17 concentrations were statistically significantly higher in aggressive periodontitis patients compared to healthy controls or chronic periodontitis, while there was no difference in other cytokines.

Bone Biomarkers and How It Responds to Treatment

Bone biomarkers of disease play an important role in detecting the diagnosis and progression of periodontal diseases. Gibert et al.,[37] analyzed serum levels of alkaline phosphatase (ALP) from patients with chronic periodontal disease and compared with control patients. There was a positive relationship between attachment loss in the periodontal group and a drop-in ALP activity in serum. ALP might serve as a marker in periodontal treatment planning and monitoring.

Osteonectin and N-propeptide alpha I type I collagen were significantly elevated in patients with periodontal disease. Osteonectin is considered to be the more sensitive marker for detection of periodontal disease status, when compared with N-propeptide alpha I type I collagen.

Osteopontin (OPN) holds a dual function in bone maturation and mineralization as well as bone resorption. GCF OPN secretion increased proportionally with the progression of disease and with non-surgical treatment it was significantly reduced.[36]

Advantages and Disadvantages of Biomarkers

Advantages

1. Objective assessment
2. Precision of measurement
3. Reliable; validity can be established
4. Less biased than questionnaires
5. Disease mechanisms often studied
6. Homogeneity of risk or disease

Disadvantages

1. Timing is critical
2. Expensive (costs for analyses)
3. Storage (longevity of samples)
4. Laboratory errors
5. Normal range difficult to establish
6. Ethical responsibility

Available Diagnostic Kits for The Evaluation of Biomarkers (Table 3) [38]

Table 3: Available diagnostic kits for the evaluation of biomarkers.

S. No	Assay	Commercial Diagnostic Kit	Functions
1.	Culture & biochemical Identification (GOLD STANDARD)	Laboral Prognostik	Quantification/identification after bacterial culture of A. a, B. f, C. r, F. n, P. i, P. g, P. M aids in detection of proteinase, elastase
2.	Immunological detection (ELISA	Evaluisite test	Detects bacterial antigens of A.a, P. i, P. g. can be used at chairside.
3.	Bacterial enzymes	Peri Oscan	Detects enzymatic activity of Aa, Bf, Pg detects enzymatic activity of B. f, P. g, T. d

		BANA periodontal test	It utilizes the BANA test for bacterial trypsin like proteases.
4.	Bacterial toxins	TOPAS	Detects toxins derived from anaerobic metabolism and measures GCF protein level.
5.	Host enzymes	Periocheck	Detects enzymatic activity derived from GCF (Matrix metalloproteinases and neutral protease enzymes)
6.	Nucleic. acid technology	Affirm DP BTD test OMTL test, Omni gene Periodontal microbial identification test. ANAWA, DMDx® / Pathotec® Paro gene Explore IAI Pado test 4.5 Micro dent® test Micro Dent test kit	DNA probes for A. a, B. f, P. i, P. g, T. D DNA probes for A.a, C. r, E. c, F. n, P. i, P. g. DNA probes for B. f, P. g DNA probes for A. a, P. i, P. g, E. c, F. n, T. d, C. r, B. f DNA probe for b. f, P. g DNA probes for A.a, P. g, P. i, E. c, B. f, C. r, T. d, F. n DNA probes for A.a, P. g, P. i, E. c, B. f, C. r rRNA quantification for P. g, A.a, B. f, T. d DNA probes for A.a, P. g, B. f, T. d. PCR detection for A.a, P. g, P. i, B. f, T. d Detection of A.a, P. g, P. i, P. d

Available test kits and their evaluation

Sl no.	Assay	Diagnostic kit	Critical Evaluation
	Culture & biochemical Identification (GOLD STANDARD)	Laboral	It is highly technique sensitive & time consuming. But it can be used for testing against resistant pathogens. Pathogens of secondary importance can also reported if found in high percentage.
	Bacterial enzymes & host enzymes	BANA (benzoyl-DL-arginine-β-napthylamide) Periocheck Peri Oscan	Pathogens like T. d, B. f, P. g can be detected. With 90-96% sensitivity, 83-92% specificity. But A. a can't be detected; using BANA. Clinical studies have shown the usefulness of BANA hydrolytic activity, the presence of which is significantly correlated with pocket probing depths and attachment loss greater than 4 mm. Both periocheck & peri Oscan were not very successful & some are no longer distributed.
	Immunological detection	Evaluisite	Polyclonal & monoclonal antibodies are conjugated along with fluroscent reporters to enhance the specificity & sensitivity. Both are relatively low for this test. Therefore, this commercial product is not widely marketed.

A. a: Aggregatibacter actinomycetemcomitans, B. f: Bacteroides forsythus, C. r: Campylobacter rectus, E. c: Eike Nella corrodens, P.m.: Pepto streptococcus micros, P. g: Porphyromonas gingival is, P. i: Prevotella intermedia, P. d: Prevotella denticola, T. d: Treponema denticola.

Future Aspect of Biomarkers

Biomarkers must be the one which is analyzable; interpretable, and can provide clear information such as being able to predict onset, measure activity, and monitor disease progression, for example, from gingivitis to periodontitis. However, there are several scientific, clinical, and technological interrogations in achieving the successful clinical application of salivary diagnostics in the treatment plan of periodontitis.

The fluids obtained from the oral cavity have been used for the whole and sole purpose of HIV diagnosis. A commercially available kit (Ora Sure, Ora Sure Technologies, Bethlehem, Pennsylvania) has an oral specimen collection device to collect HIV-1 antibodies (not the virus) from the tissues of the cheek and gingival. It does not collect saliva but a sample called oral mucosal transudate. For different fluids (oral fluid, finger-stick or venipuncture whole blood or plasma specimens), the alternative test Ora Quick (Ora Sure Technologies) provides accurate results for HIV-1 and HIV-2 in 20 minutes.[39]

Proteomics presents as a novel science and shows immense potential in this field. In contrast to gene expression studies employing oligonucleotide chips ('transcriptomics'), proteomics directly addresses the level of gene products present in a given cell state and can characterize protein activities, interactions and sub cellular distributions. The future of biomarkers in the field of periodontics is although challenging, but is also fruitful in diagnosing the all types of periodontal diseases, which in turn is helpful for a dentist/periodontist to plan the treatments accordingly.

Conclusion

In this challenging generation of oral-fluid based diagnostics, biomarkers are preferred as one of the best diagnostic tools for better monitoring, diagnosis, and clinical management of periodontitis. There are various quantitative and qualitative diagnostic techniques, including genomic profiling, proteomic and microbial analysis, that have helped several researches to best define human physiology and pathology based on thorough screening of these biomarkers. So, it can be easily concluded that these biomarkers aids in the detection of periodontal disease at an earlier stage and reduces the stress or a hectic schedule of a dentist.

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