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Genomic Biomarkers: Revolutionizing Diagnosis and Resolution of Periodontal Disease

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Abstract

Periodontal disease encompasses an array of conditions affecting the health of the periodontium. It is one of the most common diseases affecting 10-15% of world's population. Traditional methods of assessing the periodontal status have been proved to be insufficient in recognizing the prognosis of the disease. Therefore, there is a need for an advanced innovative diagnostic test. Current genomics and biotechnology promise the development of biomarkers to identify individual at higher risk of developing periodontal disease, enable its early detection, and improve diagnostic classification for personalized treatment based on genomic makeup of an individual. It has also been used in the field of pharmacology for monitoring and providing informational feedback for drug discovery and development. This article highlights the types of genomic biomarkers, obstacles to future success and its clinical use towards personalized medicine.

Keywords: Genomic biomarkers; Diagnostic; Periodontitis; Personalized medicine; Pharmacogenomics

Introduction

Periodontal disease is a chronic inflammatory disease of diverse origin that involves degradation of periodontal tissues, including alveolar bone-. It affects 10% to 15% of the total world's population and can eventually result in tooth loss [1]. Development of periodontal disease requires the presence of bacterial plaque which induces pathological changes in the tissues by both direct (through pathological effects of bacteria and their products on the periodontium) and indirect means (initiation of a number of host-mediated destructive processes. After the initiation of gingival inflammation, the disease progresses with the loss of periodontal ligament and its attachment to cementum leading to the formation of a periodontal pocket, tooth mobility, and subsequent tooth loss if left untreated [2,3].

Conventionally, periodontitis has been treated with numerous therapeutic strategies aimed principally at eradication of periodontal pathogens. These treatment strategies integrate both surgical and non-surgical approaches along with anti-microbial therapy for the management of the periodontal disease. However, according to a study by Haffeeje AD et al. (1997), traditional treatments fail 32% of the time within 9 months [4]. This is firstly because of the inability of the traditional diagnostic tool to assess the actual current disease status3 and secondly because most of the drugs are effective for only 25-60 % of patients [5]. Annual adverse drug reactions in the United States alone are seen in more than 2 million cases, with 100,000 involving deaths [6]. The quest for identifying these variances in the disease morphology and treatment outcome dates back to a century when Garrod first introduced the term "chemical individuality" [7]. Subsequently, many studies were conducted and it was concluded that the differences in the genetic makeup of a population form the basis for most of the disease including periodontitis [8,9]. This hypothesis has led to the development of various pioneering diagnostic tests that focus on the identification of genomic biomarkers so as to identify and quantify the periodontal risk and treatment outcome.

"Genomic Biomarkers - The chronicle of life"

Genomic Biomarkers are the evidence of past or present life as they reflect the entire spectrum of health as well as disease from its earliest manifestations to the terminal stages. For a biomarker to be used as a diagnostic tool, it should have high sensitivity, specificity along with high predictive value [10]. It should be safe and easy to measure accompanied by a verified treatment to modify the biomarker. The follow-up tests should be cost effective as well as consistent across genders and ethnic groups.

Genomic biomarkers comprise tools and technologies that aid in understanding the aetiology, diagnosis, progression, regression, or outcome of treatment of disease.

At present the genomic biomarker arena can be divided into two broad subsets.

1. Genetic and epigenetic biomarkers (Disease related biomarkers)
2. Drug related biomarkers

1. Genetic and epigenetic biomarkers (Disease related biomarkers): Genetic alterations include the disruption of normal DNA sequence that causes disease or is associated with the increased susceptibility to disease (Table 1), whilst epigenetic changes result in changes in gene expression without alterations to the DNA sequence. Epigenetic variations include DNA methylation, histone modifications etc.; deregulating the mechanisms such as transcriptional control leading to the inappropriate silencing or activation of periodontitis-associated genes. Measurement of these

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Table 1: Genetic biomarkers.

Genetic Biomarker	Remarks	References
Cathepsin C gene	1. Mutation in the gene causes Papillion Lefevre syndrome.	[11]
	2. Contribute to increased susceptibility in generalized aggressive periodontitis.	[12]
Collagen Gene	1. COL1A1 gene mutation - Osteogenesis imperfect. 2. COL1A2 gene mutation- Ehlers–Danlos syndrome.	[13]
IL-1 Polymorphism	1. Individuals carrying the positive genotype have significantly greater risk for developing chronic periodontitis.	[14]
	2. Positive association between AgP and the presence of the IL-1B polymorphism.	[15]
	3. A correlation exists between IL-1 polymorphism and peri-implantitis	[16,17]
IL-6 Polymorphism	1. Interleukin 6 polymorphism increases the risk of aggressive and chronic periodontitis	[18]
IL-10 Polymorphism	1. May confer a relative increase in the risk for chronic periodontitis.	[19]
Tumour Necrosis factor polymorphisms	1. Associated with severe chronic periodontitis	[20]

Table 2: Epigenetic biomarkers.

Gene	Epigenetic Alterations	References
TNFA	Hypermethylation at promoter and decreased expression	[21]
IL-6	Hypomethylation and increased expression Another study , No altered DNA methylation & increased expression	[22,23]
IL-8	Hypomethylation and increased expression	[24]
E-Cadherin, COX-2	Hypermethylation at promoter	[25]
IFNG	Hypomethylation at promoter and increased expression	[26]
PTGS2	Hypermethylation at promoter and lower level of PTGS2 transcription	[27]

changes in tissues or peripheral fluids like blood, plasma serum, urine and stool samples, can be considered as an essential marker of disease detection, its advancement and response to therapy (Table 2).

2. Drug-Related Biomarkers: The second extensive area where biomarkers are used are the pharmaceutical and biotechnology industries; which have adopted them as a wide-ranging tool aimed at monitoring and providing informational feedback for drug discovery and development. The rapid development of techniques in the area of genome analysis has facilitated identification of new pharmacogenomics biomarkers. These biomarkers mainly originate from genes encoding enzymes responsible for metabolizing and transporting drug, drug targets and human leukocyte antigens [28]. Classic examples are the cytochrome P450 enzymes, CYP2C9 and 2C19, which are responsible for phenytoin conversion to its hydroxylated form in the liver. Polymorphisms in CYP2C9 were reported and particularly the 2C9*3 haplotype that includes the 2C9 polymorphism has been suggested to exert great influence on the metabolism of phenytoin [29], leading to increased serum concentration which ultimately leads to adverse drug reactions such as gingival overgrowth. Therefore, it has been hypothesized to be a candidate gene for prevention and early diagnosis of the gingival overgrowth severity [29]. Thus, the identification of these pharmacologic biomarkers has the potential to facilitate development of safer and more effective drugs in terms of their benefit/risk profiles. Hassell in 1981 [30] discussed the phenotypic differences between gingival fibroblasts and later it was suggested that gingival overgrowth was due to direct or indirect stimulation of "responsive" fibroblasts which further justifies the individual susceptibility to those drugs [31].

The mechanisms by which these biomarkers are elevated in biological fluid include gene overexpression, increased protein secretion and shedding, angiogenesis, invasion and destruction of tissue architecture [32].

Biomarker discovery and omics technology

The medical term "biomarker discovery" describes the process by which biomarkers are identified. Regularly used sources for identifying potential periodontitis biomarkers includes blood, saliva and gingival crevicular fluid.

The recent interest in biomarker discovery is driven by new molecular diagnostic techniques, which have the potential to find appropriate markers rapidly, without detailed insight into the mechanisms of a disease. Genomics, proteomics, transcriptomics, and metabolomics are some technologies used in this process. Secretomics, along with other OMICS tool, also help in high-throughput searches for biomarkers, thus emerging as an important technology. The main advantages of proteomics and other technologies are that low levels of a specific biomarker can be detected, and that this can be achieved easily and noninvasively by using saliva or gingival crevicular fluid [33]. Investigators are designing "lab-on-a-chip" prototypes. This handheld, automated, easy to use integrated system enables simultaneous and rapid detection of multiple salivary protein and nucleic acid targets by using small samples.

Challenges faced in biomarker discovery

Experimental design: It is probably one of the most ignored and least appreciated components of biomarker discovery. Appropriate number of samples should be analysed to achieve statistically significant data outcomes. Adequate controls are a very necessary unit in the design of such studies and can influence, for example, whether a global or targeted analysis is appropriate, and whether tissue or body fluid should be analysed [34].

Sample quality: The quality of samples analysed will ultimately

determine the quality of biomarkers produced. A number of factors must be taken into consideration. For example, a clear lineage and adequate care for animals is necessary, whereas in the case of human samples, history, outcomes and storage conditions are all very important. In particular, one must also consider whether to pool samples or analyze individual samples. Though pooling sample makes the experiment very cost effective [35], many researchers believe that pooling is not appropriate as the individual samples provide more important information as they contain intrinsic biological variability [34].

Technology platforms: An old proverb "Garbage in Garbage Out" is popular computing slang for "if the input data is wrong, the results will also be wrong" and a modern modification of this saying "Garbage in = Gospel Out" refers to the blind acceptance of the answer obtained from computers. This can result in faulty decision making and therefore, it is necessary to check and re-check the data and coding to ensure that the results are valid.

a. There has been a tremendous development in -omics platform capability over the past decade. However there still remain a number of concerns. Precision and reproducibility of this approach are still a question mark. One of the major limitations of current technologies, predicated on chromatography and mass spectrometry, is the limited measurable dynamic range i.e. typically 10^4 but in biological tissue and fluids the dynamic range can vary from 10^6 to 10^{10} [36]. This creates significant problems in terms of extent of coverage and limited sensitivity [36].

b. Also, the ability to integrate data from different platforms (Genomics, Proteomics, Transcriptomics, and Metabolomics) is very difficult because of the limited number of commercially available tools. Also there exists different types of customized approaching methods for biomarker analysis such as signal to noise ratio, t-tests, and Ecombo, (a whole genome comparative browser) [37] but there are no unifying standards.

Economics: Many authors have focused on the important -omics platforms used to undertake biomarker discovery. However, a number of authors have pointed out that "while there is genomics, transcriptomics, proteomics, metabolomics, pharmacogenomics and secretomics, the only really important -omics is ECON-omics"! [37,38]. The cost of developing these technologies for the biomarker discovery still remains very high and therefore it cannot yet be used as a routine diagnostic measure [39].

Errors in the assessment of a biomarker: One mistake often made when assessing the significance of biomarkers is to assume a strong association between a biomarker and disease by misinterpreting the statistical data and thus using the results inappropriately. Another potential misuse centers on the generalizability of biomarkers. There are at least five different "modifiers" that should be considered before using a biomarker for a specific patient: sex, co-morbidities, race, age and pathology [40]. An example of biomarker misuse would be to generalize results from a study of patients with cardiovascular disease to predict clinical outcomes in patients with no systemic disease.

Clinical use of biomarkers: Towards personalized medicine

The traditional treatment strategies used a "one size fits all" approach but as our knowledge of the underlying molecular causes of periodontitis continues to grow and along with the completion of human genome project, it is possible to develop personalized therapeutics that targets an individual patient's needs based on the genotype. The concept of personalized medicine differs from that of evidence based medicine as the latter derives treatment decisions mainly from mean responses in studies designed either to minimize variability in response by inclusion and exclusion criteria or to tolerate the variability by increased size. But personalized medicine over turns this model to identify and exploit genetic differences among individuals within populations. In clinical practice, it means classifying individuals according to different biological pathways that may produce similar clinical signs and symptoms but produce different responses to disease initiators or treatments [41]. Accompanying the development of targeted therapeutics, there has been an increase in development of tests called companion diagnostics, or theranostics, that identify the disease or drug related biomarkers. Interest in their development has recently boosted, promoting the promise of personalized medicine. Examples of the companion diagnostics identifying disease related biomarkers include the Periodontal susceptibility test (PST®) which analyses two interleukins (IL-1α and IL-1β) genes for variations. Since IL-1 polymorphism is associated with increased of developing aggressive periodontitis [42] and peri-implantitis, this test can be used to identify high risk patients and modify treatment decisions accordingly. Another companion diagnostic kit available is the Periodontitis ++ (Autoimmu Diagnostika Aid, GMBH) which not only identifies five common bacterial pathogens (*Actinobacillus actinomycetemcomitan, Bacteroides forsythus, Prevotella intermedia, Treponema denticola,Porphyromonas gingivalis*) but also HLA DR4 antigen known to be commonly associated with aggressive periodontitis [43].

The AmpliChip CYP450, F. Hoffmann-La Roche Ltd, Switzerland test is the first FDA approved pharmacogenetic test to determine the genotype of the patient in terms of two cytochrome P450 enzymes: 2D6 and 2C19. They are responsible for the majority of the inter-individual variability in the ability to metabolize drugs. If a drug, for example, phenytoin is given to the patient as a medication, and if the patient has reduced CYP2D6 or CYP2C19 activity, the patient will have elevated drug concentration in their body, and therefore severe side effects such as gingival overgrowth may occur. On the other hand, for the patient with increased activity, the drug concentration might be too low to have a therapeutic effect. So testing the phenotype of the patient is important to help determine the optimum dosage of the drug. The test analyses the DNA of a patient to determine the genotype, with the sample obtained from patients' blood, buccal swab or saliva [44].

Conclusion

The use of biomarkers is growing, with new products being constantly brought in the market via diagnostics. Some of these biomarkers assist in identification, while others are channel either towards monitoring disease progression or evaluating the effectiveness of therapeutic options. These "novel biomarkers" have become the basis for preventive medicine, as it helps in promptly recognizing the disease or the risk of disease; and takes appropriate measures to prevent its progress. Furthermore, they offer another means for time-bound, rational drug design and development

besides accelerating translational drug progress from animal to man. Biomarkers are now seen as the key to personalized medicine that helps in providing customized treatments to the individuals for highly efficient intervention in disease processes. However, in many cases, the evidence which supports the use of these new methods as opposed to traditional biochemical tests has not yet been demonstrated. Therefore, the ability to recognize, evaluate and understand the uses of existing and emerging biomarkers is an essential skill required of all biomedical health care professionals. Dentistry is at a crossroads at which we can either continue to use our traditional tools and traditional biomarkers to define oral disease or embrace what is emerging as the future tools of precision medicine.

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