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Omic Paradigms Enhance Interface between Periodontitis Pathogenesis and Human Health

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Introduction

Within public health there are oral health related challenges that pose the great concern to society's wellbeing in terms of microflora and disease progression. Consider the fields of environmental and occupational health where the natural verses built environment and the workplace settings can actually present factors that are deleterious to one's health. Environmental health is concerned with how the environment influences human health and disease. The natural environment is inclusive of air, water, food, and soil, as well as all the physical, chemical, biological and social features of our surroundings. The physical structures of our surroundings where people live and work and the community systems such as roads or other means of transport, land use and waste management practices are considered aspects of the built environment. There are also elements of the social environment such as lifestyle factors and socioeconomic status which can have an indirect effect on a person's health. All of these environmental components interact in unique ways with the quality of life and can inevitably end up compromising one's health status. One such issue that has had a continual impact on worldwide health is periodontal disease. The World Health Organization (2015) indicates that "diseases of the mouth, such as tooth decay or cavities (dental caries) and gum disease (periodontal disease) are among the most common non-communicable diseases in the world and, traditionally, some of the most neglected" [1].

The oral cavity is a unique entity reflecting the condition of the rest of the human body system. It serves to provide the means of mechanical and chemical ways of utilizing nutrition and it also provides the first line of defense from the complex interactions of the environmental and biological variables. It provides an ideal niche for colonies of bacterial plaque that dwells and proliferates under the optimal conditions such as moisture, optimal temperature, nutrition and ideal pH conditioning both aerobic and anaerobic environment within the oral cavity. However, this is balanced with a highly vascularized alveolar mucosa and a strong supporting periodontium structures.

Periodontal disease is a chronic inflammation of the peridontium involving the gingival tissue, the supporting collagen fibers and the compact alveolar bone of the mandible and the maxilla. An estimated 64.7 million or 47.2 percent American suffer from mild, moderate or severe form of periodontal disease during 2009-2010 [2]. The etiology of periodontal disease is a collective interaction of disease causing bacterial plaque with predisposing gene factors of the host resulting in a destructive response of the supporting structures such as the alveolar bone and the collagen fibers. This can lead to poor prognosis of tooth loss. Periodontal disease can be acute or chronic, mild, moderate or severe with bone loss which can begin at any age. Periodontal disease is as prevalent in our nation as cardiovascular disease is which affects half the population in the US. There have been numerous studies on the established association between periodontal disease and the increased risk of systemic conditions such as neutrophil dysfunction, atherosclerosis and cardiovascular disease in children and young adults [3-5]. Type II diabetes and preterm weight births are also linked with the chronic inflammation associated with periodontal disease [6,7].

The oral flora is quite complex as it constitutes several colonies of bacteria. These bacterial colonies live in a sheltered environment thriving on nutrients, harboring within the calculus accretions formed on the teeth surfaces. The bacterial biofilms somehow trigger inflammation that does not stay contained within the gingival connective tissue, but spreads causing tissue degradation and bone loss hindering support to the teeth structure. Based on previous research, the paradigm of slowing the progression of periodontitis is to control the inflammation and the toxins produced during the degradation process. Once bacterial plaque calcifies it is difficult to remove such accretions. Dental professionals are able to minimize the progression by removing the calculus and root planning of teeth surfaces thus disrupting the colonies and the toxins that trigger the immune response. The current scope of practice by dental practitioners only involve the management of the disease, but further research needs to be conducted to understand the pathogenesis of periodontal disease to mediate its prevention. In the early onset of periodontal pathogenesis, Larjava et al. [8] has reported that kindlin-1 mutation and integrin (i.e., alpha 11, beta6 and beta2) are regulators involved in controlling cellular response of neutrophil recruitment thereby promoting inflammation and related cytokine gene expression [8]. Thus, it is indicating the need to detect scaffold like adaptor molecules that trigger sequential inflammatory events at the cellular level using different methodologies in the transgenic mouse model. In addition, there are reported characterization on host defensive factors using family member samples such as mother to son to determine genetic correlation and immunological factors in the host. This is accomplished through analysis of human leukocyte antigen (HLA) phenotypes such as HLA class II antigens, serum immunoglobulin G (IgG) antibody titers against periodontopathic bacteria and microfloral shift from healthy to progressive periodontitis includes distinct bacterial flora appearance likely *Actinobacillus actinomycetemcomitans* in all periodontal pockets [9]. Representative profiles of dental plaque microflora feature the following: Propionibacterium, Actinomyces, Streptococcus, Lactobacillus and

Bifidobacterium. Located on root caries lesions and in periodontal pockets the microflora include: Actinomyces, Prevotella, Actinobaculum, Streptococcus, Olsenella and Eubacterium. Most importantly, in pericoronitis the predominant microflora includes the following: *Prevotella intermedia*, *Peptostreptococcus micros*, *Veillonella species*, *Fusobacterium nucleatum* and *Streptococcus mitis* [10,11]. Geographical study has also shown that *Treponema denticola* is considered a distinct microflora in oral subgingival using Korean and German periodontal patients [12].

Current challenges of environmental health are within the realm of many on going public health issues that include polluted air and water, poor waste management systems, as well as hazardous materials/toxic substances management, and food safety. In previous studies, it was indicated that genetic, environmental, and nutritional factors could be attributed to periodontal disease or other oral disease due to dental plaque deposition associated with biofilm development from microflora colonization in combination with nutritional factors, growth factors, and enhanced abiotrophia species [13-16]. Unfortunately the risks include potential to lead to more serious effects such as varying types of chronic diseases. Inclusive of these diseases are some non-communicable health conditions such as the heart and respiratory diseases, cancer, and diabetes to name a few. Herein, dental health proves to be yet another public health burden. Biological and chemical toxins, contaminants, and food ingredients contribute to the potential health risks. In order to ensure the health of the population the only resolution for public health practitioners is to ascertain ways to prevent the onset of periodontal disease through early detection of predominant microflora. For these reasons, biofilm formation from microflora colonization due to nutritional, genetic, and environmental factors can be better detected and monitored through efforts of integrated molecular methods. OMICS applications as molecular detection tools utilize genomics, proteomics, metabolomics, epigenomics, toxicogenomics, pharmacogenomics, and the microbiome [17-19]. For large data analysis, new OMIC paradigms involve improved systems biology along with scientific techniques such as recombinant biotechnology with gene editing, immune host response, protein array and DNA sequencing such as Next Generation Sequencing (NGS). It is necessary to understand the biological processes of oral pathogens by profiling the genome determine phylogenetic analysis and monitor microflora colonization in clinical samples. In this regard, personalized dental medicine can be realized through advanced molecular assessment, risk prediction of resistance and toxicity in the physiological process of chronic periodontal disease [20-22].

Significance

In the National Health and Nutrition Examination Survey (NHANES) and National Health Examination Follow-Up Study (NHEFS) (epidemiology) of 1982-1984, led by Eklund and Burt [23], correlations were shown between higher periodontal disease scores and variable factors such as perceived poor dental health, perceived need for extractions, history of smoking, and low ascorbic acid intake. In addition, a behavioral prevention program reduced the burden of periodontal disease progression by the addition of semi-annual prophylaxis compared to the control group after five preventive modules such as behavior modification, added weekly chlorhexidine gluconate rinse and semi-annual fluoride varnish [24].

Despite targeted efforts towards prevention in the public, periodontal disease remains prevalent worldwide primarily because early onset of this disease is unknown. However, research has recognized the genetic predisposition behind aggressive forms of the disease which is classified by certain contributing factors as described in the following: behavioral and life style factors such as smoking and medication; systemic diseases such as coronary heart disease, cerebral infarction, diabetes, preterm labor, and hormonal changes; genetic causes that include genetic susceptibility, gene alteration or mutation; and environmental modifying factors such as bone loss [25-28].

In the cellular and molecular level, it was suggested the impact of oxidative stress on microflora of the oral cavity by which the outcome in the cellular properties of mouse gingival fibroblasts (MGFs) attributed to the development of periodontal diseases [29]. The significance of the disease burden of periodontal disease lies in the premature destruction of oxidative mitochondrial DNA seen in patients. An overproduction of reactive oxygen species (ROS)/reactive nitrogen species (RNS) was recorded in 30 patients suffering from periodontal disease using polymerase chain reaction (PCR). It was detected using oxidative marker, 8-hydroxydeoxyguanosine in saliva [30]. The increased burden of oxidative stress in the gingival tissue led to damage and deletion of 5 kbp mtDNA found through blood samples. This is thought to be the resulting activation of polymorph nuclear leukocytes from chronic periodontal inflammation. Neiva et al. [31] suggests that a prophylactic nutrient supplementation such as calcium, ascorbic acid and vitamin B-complex may be considered for the prevention of the onset and treatment of progression of periodontal disease as well as wound healing. It could indicate that oxidative stress plays a critical role accelerating microflora growth in periodontal disease by interaction with host cell physiology.

Overall periodontal disease poses a significant health burden throughout the nation. Recent data from the Centers for Disease Control and Prevention (CDC) have estimated that 47.2% of the population, approximately 65 million people has a form of periodontal disease. The trend tends to increase with age as it is evidenced in 70.1% of people age 65 years and older. It presents more commonly in men than in women with a reported 56.4% *vs.* 38.4%, respectively. There is also a predominant association of disease occurrence in those individuals living below the poverty level as well as in those having lower levels of education. Reported numbers show that 65.4% of the population that is below poverty level and 66.7% of people with less than a high school education has periodontal disease [32,33]. Documented research has showed that periodontal disease contributes to increased mortality in diabetic patients. It was realized to be a strong predictor of death, at 3.2 increased risks from ischemic heart disease and diabetic nephropathy [34]. Data extracted and analyzed from the National Health and Nutrition Examination Survey (NHANES) has also demonstrated that individuals with both periodontitis and chronic kidney disease exhibited a mortality rate of 41% in comparison to a mortality rate of 32% among those individuals who only had chronic kidney disease [35]. Furthermore, the trends in food of a Western diet may also have a contributory role in periodontal disease. Nutritional content is a key in the disease pathogenesis. A healthy diet supplies a healthy body as well as supports a strong immune system and further supplies a healthy oral environment. This maintains a balance between good and bad oral bacteria. Simple infections can be fought before they become severe if an individual is in good health. In this regard, food additives, artificial ingredients, sugary and processed foods can promote periodontal disease [36]. The information presented should raise great concern for public health practitioners as well as the national population. Periodontal disease can be a serious health condition fraught with major complications. It is in the best interest of

population health to utilize necessary interventions and any applicable strategies in order to maximize prevention and curb this ongoing health issue.

Strategy of OMICS Platforms as Advanced Risk Assessment Tool

Gradually, the more traditional tests of culture-based, biochemical, and immunological assays for clinical diagnostics are being replaced by more advanced or complementary nucleic acid based diagnostic tools [37]. As it relates to oral health, it has been reported that advanced molecular diagnostics allow for the detection of nucleic acid molecules to determine microflora using clinical samples [38]. The application of molecular biology techniques and knowledge of disease mechanisms for diagnosis, prognosis and treatment of diseases allows for more advanced analysis. Essentially, it is utilizing molecular materials of DNA, RNA, and proteins to test and determine the state of health or disease for a specific sample. Based on OMICS concepts, new biomarkers for diagnostics can be explored and uncover roles of disease pathogenesis within cells and organisms [19]. In general, OMICS consist of 4 major categories which are described as the following: Genomics, the study of the genome; Transcriptomics, the study of mRNA; Proteomics, the study of functional proteins; and Metabolomics, the study of cellular metabolism. More advanced OMICS include the disciplines of epigenomics, pharmacogenimcs, toxicogenomics, marine metabolomics, neurogenetic, nutrigenomics, and microbiome. The use of OMICS strategies have allowed biologists to analyze the effects of various environmental factors at molecular and cellular levels against microflora and host interaction to understand underlying pathological processes.

Molecular diagnostics has many applications that span many medical disciplines such as infectious and chronic diseases, oncology, cytopathology, genetic disease screening, and even pharmocogenomics. The analytic procedure of an assay is central to molecular diagnostics. For periodontal disease, the most basic of steps to performing a molecular assay include extraction and purification of the crevicular fluid (CF) proteins from gingivitis and periodontitis, and the detection of the amplified target using analysis by 2-dimensional gel electrophoresis (2-D PAGE) or gelatinolytic activity from teeth with apical lesions using gelatinases such as MMP-2 or MMP-9 in gingival crevicular fluid (GCF) [39-41]. It is imperative to understand that molecular testing provides aid is identification of many disease agents whether environmental or biological in origin. We can determine the mechanisms of infection, toxicity, or how agents cause morbidity and mortality. More importantly, it provides a platform for long term study of harmful effects from established health risks.

On the other hand, use of the OMICS approach lends too much, improved analyses. The scope of biomedical research has made great scientific advances through the field of omics methods and applications, especially with bioinformatics. Omics refers to study or investigation of the roles and relationships of molecules within cells and organisms by incorporating the aforementioned scientific platforms of genomics, transcriptomics, proteomics, and metabolomics. Molecules of DNA, RNA, proteins, peptides, lipids and metabolites are studied not only individually but in such a way that their interactions are intricately evaluated. This merging of OMICS technologies provides more comprehensive insight into the biological behavior of cellular systems including microRNA or epigenetic modification. There is a more informative understanding of biological processes and the interplay of systems (so called systems biology). Thus, an OMICS approach allows for enhanced measures of disease detection and diagnosis which can also open up pathways for discovery of more effective means of treatment.

In addition, Comparative analysis of disease detection would reveal significant differences in methodologies between the conventional and the more innovative techniques of the OMICS approach. Some of these more modern techniques include cellomics, nutrigenomics, metabolomics, immunomics and immuno-informatics. These disciplines can mitigate periodontal disease as a health burden as well as explore therapeutic value in the process of disease complications by nutrition or food ingredients.

Periodontal disease so far has been controlled and managed through dental treatment methods to minimize bacterial colonies and toxins that produce and initiate the immune response of the host. This immune response results in self destruction of the supporting periodontium. Therefore, there is a need for advanced detection and risk assessment via molecular basis of the inflammatory process through epigenetics intervention. Study of the alteration of histones, DNA methylation and micro-RNA role in translational repression can help develop medication such as deacetylase inhibitors and demethylation agents. In previous studies, different types of microRNAs (i.e., let-7a, let-7c, miR-130a, miR301a, miR-520d, miR-548a and miR-146a) were involved in the regulation of inflammation due to infection of periodontitis gingivae in chronic patients utilized ApoE (-/-) mice as animal model [42,43].

Epigenetics is the study of the changes in the gene function that is not the expected result of the DNA sequence. Environmental factors of any form can induce epigenetic changes that lead to increased susceptibility to disease. The three gene expressions are the DNA methylation, micro-RNA and modification of histones. Patients that are given similar clinical treatment respond differently thus raising the question of understanding the underlying mechanism involved in periodontal disease. This can be achieved by the utilizing the advanced methodology of epigenetics. For example, Zhang et al. [44] reported that change in the promoter methylation status of Tumor necrosis factor-α (TNF-α) regulated periodontal disease and its pathogenesis in human chronic gingival biopsies and cell lines (THP.1 cells and RAW294.7 cells) [44]. This is indicative that inflammation of periodontitis is correlated to methylation of the Toll-like receptor (TLR) 2 gene and is an innate immune response marker in periodontopathic bacteria using the Methyl Profiler DNA Methylation qPCR assay [45].

The mouth provides an ideal microbial flora to various microbes. The collective characteristics of a moisture, darkness, consistent nutrients and the hard enamel altogether helps microbes thrive in a biofilm that is well attached to the teeth surfaces. The immune response to these thriving microbes is associated with different cytokine regulation such as IL-8 mRNA induction signaling from bacteria. Other responses include that of *P. gingivalis* triggering human gingival epithelial cells (HGECs) through activation of MAPK kinase/extracellular signal-regulated kinase (MEK/ERK) pathways, not mitogen-activated protein kinase (MAPK) p38 pathways. It has also been suggested that gingival epithelial cells (GECs) using *P. gingivalis* affects DNA methyltransferase and histone deacetylase by stimulating gingival cytokine secretions of IL-6, CXCL1, and beta-defnsin-2 [46,47].

Another interesting study concerning epigenetics has documented that hyper methylation of the tumor suppressor gene E-Cadherin and

COX-2 has been observed in biopsies of breast cancer and in chronic periodontitis. Therefore the rise in cytokines affects the DNA methyltransferase which ultimately affects the T-cells thus triggering an adaptive immune response in periodontitis [48].

Increasing body of evidences support that different micro-RNA species regulate gingival mediated inflammation process in periodontitis as compared to healthy gingival tissue studied using quantitative real-time PCR (QPCR) and micro-array techniques. Experimental studies also support the premise that engagement of microRNA between lipid metabolism and inflammation due to epigenetic changes and immune dysfunction result in various pathological events such as glucose intolerance, hypertriglyceridemia, central obesity, rheumatoid arthritis and coronary heart disease [49]. Furthermore, other study for a role of micro-RNA on stem cell driven differentiation indicate that risk factor such as cigarette smoking attributes to delay on regenerative healing process by stimulation of two nicotine specific miR species (i.e., hsa-miR-1305 and hsa-miR-18b) in human periodontal ligament-derived stem cells (PDLSC) [50]. Of note, there is evidence of a connection between the effects of changes by maternal periodontal infection as the oral micro flora with alterations in the placenta and/or the fetal environment result in preterm low birth weight through a case-control study using 124 pregnant or postpartum mothers led by Offenbacher et al. [51].

To confirm the correlation between epigenetic changes and immune alterations in human populations due to exposure from periodontal infection, a clinical study using genomic tools led by Lavu et al. [52] has shown that chronic periodontitis susceptibility is associated with single nucleotide polymorphisms (SNPs) in the IL1B gene and variable number of tandem repeat (VNTRs) polymorphisms in the IL1RN gene after examining blood samples and genotyping in healthy gingiva and chronic periodontitis patients. Furthermore, other research using meta-analysis has also reported that interleukin (IL)-1β C-511T polymorphism is involved in Chinese chronic periodontitis [53]. Therefore, these results emphasize that epigenetic modifications by periodontopathic bacteria is a pivotal genomics signature highlighting the health complications of immune regulation dysfunction as well as exacerbated inflammatory signaling circuits.

Recently, system approach could tackle periodontal bacteria growth along with better understands how to build up biofilm in oral atmosphere. It suggests new concept likely connector which it could pave a new road to open new avenue of therapeutic or diagnostic on periodontal pathogenesis following by exploring therapeutic molecule using various OMICS technologies such as stable isotope labeling by/with amino acids in cell culture (SILAC) and RNA sequencing (RNAseq) [54,55].

Implications

Ultimately both the use of conventional molecular methods and innovative/advanced OMICS strategies within clinical diagnostic practice contribute greatly to disease diagnosis, prognosis and the determination for future risk of disease. They allow clinicians to take advantage of discovering and determining molecular mechanisms that contribute to specific disease development such as cancer, metabolic disease, heart failure, and neuronal disorders. Additionally, both approaches serve valuable purposes for the development and maximization of treatment as well as for the health assessment and management of disease.

Early diagnosis using advanced molecular assessment tools such as OMICS paradigms (i.e., nutrigenomics, metabolomics, immunomics and immunoinformatics) along with radiographic assessment can provide early diagnostic insight and prevention of periodontal disease in both children and adults. To further reduce the public health burden, aggressive research on the potential role of nutrition is necessary and should be considered. Analysis of the ingredients at the sub-molecular level via mapping and epigenetic susceptibility while simultaneously making the connection to periodontal disease can be a great therapeutic asset. This would take into account immune adaptation, oxidative stress response as well as anti-inflammatory response under the integration of nutrigenomics and thus proving beneficial in understanding the host response to exposure of pathogenic microflora.

Conclusion

A new era of personal oral medicine is possible by targeting microflora at the molecular level to mitigate periodontal disease and thereby improving oral health. The integrated molecular OMICS disciplines of genomics, transcriptomics, proteomics, and metabolomics allow for in depth microflora investigation, biomarker exploration, and therapeutic resolution. Use of High Throughput Screening (HTS) module provides more informative understanding of the mechanisms of microflora colonization, environmental factors, biofilm formation, and host cell immunity in periodontal and other oral diseases. OMICS has enabled us to understand that periodontal disease does not only have severe ramifications in the mouth but also effects systemic gene expression and has associations with causing oxidative stress, mutation, DNA-methylation and alteration in the gene expression regulation of micro-RNA. It also has adverse effects on the T-cells which play an important role in the immune response which is not limited to clinical intervention or limitation of dental treatment since the immune response varies in different individuals. It is because of using the advanced methodology of epigenetics that we are able to understand this difference in immune response and why certain patients suffering from periodontitis are not able to respond to the current dental treatments in place. Hence, this can become a turning point in dentistry by providing dental health professionals with the alternative tools to utilize advanced risk assessment before the disease progresses and leads to accompanying health complications.

Conflict of Interest

The authors do not declare any financial interest.

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