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Metabolomics as Neo Contrivance in Diagnosis of Periodontitis

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ABSTRACT:
Periodontal disease is an infectious inflammatory disease related to the destruction of supporting tissues of the teeth, leading to a functional loss of the teeth. Inflammatory molecules present in the exudate are catalyzed and form different metabolites that can be identified and quantified. With recent advances in mass spectrometry technology, metabolomics research is now widely conducted in various research fields. Metabolomics, which is also termed metabolomic analysis, is a technology that enables the comprehensive analysisof small-molecule metabolites in living organisms. With the development of metabolite analysis, methods using gas chromatography–mass spectrometry, liquid chromatography–mass spectrometry, capillary electrophoresis–mass spectrometry, etc. have progressed, making it possible to analyse a wider range of metabolites and to detect metabolites at lower concentrations. Metabolomics is widely used for research in the food, plant, microbial, and medical fields.

KEYWORDS: Metabolomics, salivary markers, GCF markers, Periodontitis.

I. INTRODUCTION

Molecules present in fluids in the oral cavity may indicate a relationship between processes linked to health and disease, as well as repair processes. Periodontitis is an infectious inflammatory disease that causes destruction of the tissues supporting the teeth. It significantly affects oral health and is the most common cause of tooth loss. With recent advances in mass spectrometry technology, metabolomics research is now widely conducted in various research fields. Metabolomics, which is also termed metabolomics analysis, is a technology that enables the comprehensive analysis of small-molecule metabolites in living organisms. [1,2]

WHAT IS METABOLOMICS?

Metabolomics is the scientific study of chemical processes involving metabolites, the small molecule substrates, intermediates, and products of cell metabolism. Specifically, metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind", the study of their small-molecule metabolite profiles.[3]

HISTORICAL PERSPECTIVE:

The metabolome is a close analogue to the genome, the transcriptome and the proteome. These four ‘omes’ together forms the building blocks of systems biology. The History is old as genomics and proteomics. The first paper was entitled “quantitative analysis of urine vapour and breath by gas liquid partition chromatography”, by Robinson and Pauling in 1971. The name metabolomics was originated in the late 1990’s. Human metabolome project is the first draft of human metabolome. Metabolomics is expanding and trying to catch up with other multiparallel analytical techniques but remains far less developed and accessible. [4]

BEQUEST OF METABOLOMICS:

Recent advances in metabolic studies have enabled the identification and quantification of different metabolites in normal individuals and those with certain diseases using metabolomics. In disease diagnosis, several efforts have been made to identify metabolites in the saliva of individuals with diseases that would provide new biological markers to aid the diagnosis of periodontal disease. Given the heterogeneous characteristics of the diverse immune responses to infection, efforts have been made to identify markers in the fluids in the oral cavity, such as saliva and crevicular fluid, to predict the presence of periodontal disease and its stage.

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Mass Spectrometry Of Metabolomics Analysis:

Developments in metabolite analysis methods by using Gas Chromatography-Mass Spectrometry (GC MS), Liquid Chromatography - Mass Spectrometry (LC MS), and capillary Electrophoresis-Mass Spectrometry have made possible to analyse the various metabolites and to detect them at lower concentrations.[5] Among the various techniques, Mass Spectrometry is currently the mainstay of metabolomics analysis, and it is the most sensitive method and can obtain more information from a smaller sample volume. GC-MS is the most common form of MS analysis. Recently, there has been increasing interest in using this two-dimensional GC-MS to perform a more sensitive analysis. In addition to GC-MS, LC-MS, which is a combination of high-performance liquid chromatography and Mass spectrometry is widely used in metabolomics.**[6,7]**

Applications Of Metabolomics Research In Periodontal Disease Using Ms

The metabolites correlated with periodontal disease were suggested to be related to tissue destruction, host defense mechanisms, and bacterial metabolism. Bacterial metabolite, phenyl acetate, being significantly associated with periodontal disease variables are expected to be deeply involved in periodontal disease**.[8]**

SALIVA AS A DIAGNOSTIC MARKER:

Metabolic profiling of saliva can provide an extensive view of the changes associated with periodontal diseases. From the pool of several markers, it is suggested that the dipeptides leucyl isoleucine, phenyl phenol and serylisoleucine and the fatty acids arachidonate, arachidate and dihomolinolate are attractive candidate markers. Ionomics found decreased salivary levels of Mn, Cu, and Zn in patients suffering from periodontal disease. Superoxide dismutase levels were decreased in saliva and serum in periodontal disease..[9]Increased levels of cyclooxygenase (COX) products (PGE2, PGD2, and PGF2α and TXB2) in periodontal saliva indicate elevated inflammatory response. There is an increased level of lipoxygenase (LOX) products 5-Hydroxyeicosatetraenoic acid (5-HETE). The oxidative stress marker F2-isoprostane was increased in the saliva of periodontal disease.[10] Cyclooxygenase is a functional molecule that influences the inflammatory responses. Lipoxygenases (LOD) are of interest in inflammatory diseases, like atherosclerosis. It is now known that molecules altered by oxidative stress get deposited in various organs causing diseases.[11,12] For example, in diabetes mellitus, oxidized sugars tend to bind with proteins, increasing abnormal glycated proteins. Study on COX products, and LOX products' oxidative stress, and their involvement in periodontal disease is expected in gaining attention.**[13,14]**

SMOKING AND METABOLOMICS:

Smoking adversely can affect the salivary expression of lactate, pyruvate, and sucrose (Takeda et al. 2009), hexanoic acid, cadaverine and G3P[15]. This explains the conflicting increase of pyruvate in periodontitis patients. The concentration of Short chain fatty acids, amines, phenylalanine, glycine, and succinate is strongly correlated with the bacterial load in saliva. Valine was found increased in oral, breast and prostate cancers[16,17], and in Alzheimer's disease[18] and dementia[19], while butyrate levels were interfered by the presence of dental caries[20,21] Furthermore, isoleucine, leucine, valine, and tyrosine were remarkably increased in patients with type 2 diabetes[22]. Due to the multifactorial character of periodontitis, diagnostic power can be increased by evaluating a panel of metabolites (Ramseier et al. 2009; Lee et al).[23,24]

GCF AS MARKER:

Gingival crevicular fluid has been utilized to monitor periodontal diseases. It is an inflammatory exudate, produced by epithelium of the gingival crevice, which contains immunoglobulins and plasma proteins and has antimicrobial properties. Recent biochemical studies have demonstrated its applicability in identifying and monitoring active periodontal diseases (coupled with bone loss). same as saliva, gingival crevicular fluid also collected non-invasively and site-specifically. It is considered as an ideal substrate for the study of periodontal diseases.[25] It is proposed that markers such as inosine, lysine, putrescine and xanthine could serve as a sensitive indicator.[26] A recent report from Elabdeen et al. proposed that by using gingival crevicular fluid from aggressive periodontitis subjects suggests that the ratio of proresolution/proinflammatory lipid mediators can provide specific information about disease status.[27] They analyzed eicosanoid and docosanoid molecules using High Performance Liquid Chromatography–electrospray ionization combined with tandem Mass Spectrometry and enzyme linked immunosorbent assay in serum, saliva and gingival crevicular fluid collected from patients with aggressive periodontitis. They also reported that increased concentration of prostaglandin E2 molecules in gingival crevicular fluid of diseased subjects. It is proposed that gingival crevicular fluid

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is a more suitable biofluid for the diagnosis of periodontal diseases with respect to serum and saliva.[27] Gas Chromatography-Mass Spectrometry, which could be used for the onsite analysis of metabolites in (GCF), to objectively diagnose periodontitis at the molecular level. GCF is the proximal fluid closest to the lesion site, and best reflects the condition of periodontal tissue. GCF contains enzymes and proteins related to periodontal tissue metabolism and is considered a global indicator of disease progression.

METABOLITES SEEN IN DEEP POCKETS:

Using GCF and GC-MS, the peak areas of putrescine, lysine, and phenylalanine were markedly higher in the group of deep-pocket sites than in healthy sites and moderate sites. Further, ribose, taurine, 5-aminovaleric acid, and galactose were significantly higher in the group of deep-pocket sites when compared to healthy and moderate-pocket sites. [28]

DIABETES AND METABOLOMICS

Phosphatidylcholines, plasmenyl phosphatidylcholines, ceramides containing non-alcohol fatty acids, and host proteins related to actin filament rearrangement were increased in plaques from Periodontal disease (PD) versus non-PD samples.[29[The association between Lautropia and monomethyl phosphatidylethanolamine (PE-NMe) is very striking, because PE-NMe synthesis is not common in oral bacteria. [30]Using metabolomics and 16S rDNA sequencing, ametabolic pathway and significant associations of host-derived proteins with PD were observed. It is well known that people with diabetes have a high incidence of periodontal disease, and diabetes has been viewed as a significant risk factor for periodontal disease. [31]

Untargeted Analysis: Metabolites Associated With Chronic Periodontitis:

The most significantly enriched metabolites in Chronic periodontitis group are uracil, N-carbamylglutamate 2, N- acetylβ-D-mannosamine 1, fructose 1, citramalic acid, 5-dihydrocor3, and 4-hydroxyphenylacetic acid are positively correlated with severe clinical parameters, while the opposite trends were observed for thymidine 3 and O-phosphoserine.

Metabolites Associated With Generalized Aggressive Periodontitis:

Noradrenaline, uridine, α-tocopherol, dehydroascorbic acid, xanthine, galactose, glucose-1-phosphate, and ribulose-5-phosphate levels are increased metabolites in patients with GAgP, while thymidine, glutathione, and ribose-5-phosphate levels were decreased.[32] Putrescine, lysine, phenylalanine, ribose, taurine, 5-aminovaleric acid, and galactose displayed a trend toward increasing in deep pocket sites; whereas lactic acid, benzoic acid, glycine, malic acid, and phosphate are decreased from severe periodontitis sites to healthy sites. Ratios of omega 3 to omega 6-polyunsatured fatty acids (PUFAs) and of the direct precursors of the pro-resolution lipid mediators were significantly decreased in the GCF of Aggressive Periodontitis patients than in the healthy controls. [33,34]

Metabolites - Reactive Oxygen Species:

Targeted Gcf Metabolite:

Oxidative stress-related metabolites are the most studied in targeted GCF analyses. Tendency is based on the rationale is that oxidative stress is key pathophysiological mechanisms in periodontitis at the molecular level. Malondialdehyde derived from the peroxidation of PUFA, and a reliable indicator of reactive oxygen species (ROS). Higher GCF level of MDA indicates an increased inflammatory mediated oxidative stress within the periodontal microenvironment. ROS released from neutrophils after interactions with pathogenic antigens directly destroy cell membrane and start the pathway of lipid peroxidation, which in turn leads to more inflammation and tissue degradation. 8-HOdG is a stable marker of oxidative DNA damage, and it is a steady indicator for the harmful effects of ROS .[35,36]4-HNE is one of the major aldehydic end product also derived from lipid peroxidation processes, that possesses strong cytotoxic activities and signaling activities. LPAs are a group of phospholipid mediators that plays a key role in inflammation and their contribution in periodontitis pathogenesis is supported by several lines of evidence. Because of the variations in the responses produced it is important to detect individual LPA species rather than total LPAs. Decreased glutathione (GSH) is an important intracellular antioxidant for Reactive oxygen species detoxification and may serve as an indicator of the antioxidant defense mechanism. GSH levels in the GCF have been found significantly decreased in chronic periodontitis.

UNTARGETED GCF METABOLOMICS:

Metabolites are considered the most proximal reporters of physiopathological states at the histological level, more than proteomic or transcriptomic variations. The metabolic changes encountered in periodontal disease progression and in macromolecular degradation, are D-glutamine and D-glutamate metabolism, histidine metabolism,

and tyrosine metabolism. The overexpression of glycosidase, lipase, and protease activities is associated with periodontal inflammation and provides a more favorable energetic environment for pathogenic bacteria, exacerbating the disease state.[37]

RESEARCH IN METABOLOMICS:

Improved diagnostic methods and new biological markers are needed for periodontal disease diagnosis; the application and interaction of the -omic approaches will help us to broaden our perspectives on the molecular mechanisms involved in periodontal disease progression and enable the optimization of bold diagnosis and prognosis. Mass spectrometry with chromatography is very useful for predicting periodontal diseases in different stages of disease progression. Identification and quantification of metabolites are highly sensitive. older adult patients with chronic periodontal disease, the following metabolites were more prominent**: 5-aminovaleric acid, serine, 1-monopalmitine, aspartic acid, D-mannitol, putrescine, 1-benzoyl-2-t-butyl-5-ethyl-3- methyl-5-vinyl-imidazolidin-4-one, palmitoleate, maltose, lactic acid, oxalic acid, edetic acid and D-glucose-6-phosphate**, of which the first two are remarkably increased always. The relationship between periodontitis and increased aminovaleric acid, in addition to lactic acid, certain sugars, and putrescine, a compound associated with tissue decay, has been found. The observed differences in metabolite profiles are related to the different characteristics of the development of chronic periodontal disease, as well as to the host response to pathogens.[33]

Metabolites from different metabolic pathways assure diagnostic and prognostic specificity and sensitivity. Metabolites from the inflamed site in the periodontium and inflammation-related metabolites from microorganisms make marked dysbiosis in periodontitis; therefore, these metabolites can be a biomarker, pointing at a potential strategy for the prediction, diagnosis, prognosis of periodontal therapy.

Metabolites found in crevicular fluid can be influenced by old age because of increased susceptibility to infections and inflammations.[37] In a metabolomic evaluation of crevicular fluid from individuals with a middle age found the association of two components, citramalic acid and N-carbamylglutamate, as markers of chronic periodontitis. Metabolomic differences were identified between healthy and periodontitis subjects and changes in the concentrations of compounds associated with the biosynthesis of amino acids, galactose, and pyrimidine were observed, and correlation between the metabolic profile and microbial community were found.[38]

FUTURE DIRECTION:

The oral microbiome at multiple sites revealed "differences in community structure between the microbiomes" of the saliva and dental plaque and showed that diversity of microbes correlated with the severity of periodontal diseases. Moreover, numerous study Molecules on periodontal diseases have combined the microbiome and metabolome to explain the pathogenesis of periodontal diseases, such as the functional diversity of the microbial community in healthy adults and patients with periodontitis-based carbon source utilization by Zhang et al. The researchers concluded that results of their microbiome analysis widely available on the Internet without personal identifiers, and detailed results will be distributed for use by researchers nationwide. In the future, MS-based oral microbiome and metabolome analyses may be used to make clear the pathogenesis of periodontal diseases.[39]

II. CONCLUSIONS

It is expected that new treatments and drugs that control the function of proteins and metabolites were found. Biomolecules that are associated with inflammation, immune response, and tissue destruction in periodontal disease are expected to be a valuable biomarker for assessing periodontal disease activity and the response to diagnosis and treatment plan. crevicular fluid collected from the gingival sulcus and periodontal pockets contains the biomarkers that shows the inflammation, immune response, and tissue destruction at the site of periodontal lesions, making metabolomic analysis using Mass Spectrometry an essential tool for evaluation and diagnosis. It is hoped that the through analysis of many protein metabolites may make clear the functional links between metabolites whose expression fluctuates in relation to diseases, drugs and other protein metabolites and elucidate the mechanisms of periodontal disease development and progression.

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