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Review Article

Metabolomics as a diagnostic tool for periodontal diseases- An overview

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ABSTRACT

Periodontal disease results in gingivitis and periodontitis, is the most prevalent chronic inflammatory condition affecting the teeth and its supporting tissues. High prevalence of periodontal disease has an adverse effect on systemic health, mastication, and esthetics. There are many local and systemic factors that contribute to clinical manifestations of periodontal disease. The diagnosis is still based on conventional clinical examinations despite the high prevalence and greater understanding of the pathogenesis of periodontal disease. The term 'metabolome' was first coined by Steven Oliver and colleagues in the late 1990s which are active participants in metabolic reactions that are essential for normal physiological functions. GCF and saliva have been used for metabolomics based periodontal diagnosis: Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are the two main methods used to collect data on metabolomics. In a way that genomics, transcriptomics, and proteomics could not fully utilize, metabolomics offers a special chance to affect discovery-driven science. The field of salivary research is one that is emerging, and it is hoped that the analysis of numerous protein metabolites will enlighten the mechanisms of periodontal disease development and focus attention on the functional relationships between metabolites whose expression varies over time in relation to diseases, medications, or other protein metabolites.

1. Introduction

Periodontal disease, which frequently results in gingivitis and periodontitis, is the most prevalent chronic inflammatory condition affecting the teeth and its supporting tissues. High prevalence of periodontal disease has an adverse effect on systemic health, mastication, and esthetics.[1] There are many local and systemic factors that contribute to clinical manifestations of periodontal disease. The primary etiological factor contributing to periodontal pathogenesis is bacterial plaque or dental biofilms. The duration and severity of the ensuing illness are determined by how the bacteria interact with the host. These responses are mediated by variety of factors that can be divided into innate and adaptive immunity.[2]

The diagnosis is still based on conventional clinical examinations despite the high prevalence and greater understanding of the pathogenesis of periodontal disease. The implementation of an accurate prognostic system is another crucial element of the therapeutic strategy. Moreover, there are currently no periodontitis prognostic indicators supported by evidence.[3]

2. What is Metabolome?

The term 'metabolome' was first coined by Steven Oliver and colleagues in the late 1990s.[4] The entire collection of small molecule metabolites found in an organism

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or cell is known as the metabolome such as glucose, cholesterol, adenosine triphosphate (ATP), lipids, amine neurotransmitters, amino acids, organic acids, and steroids that are synthesized during metabolism, which are active participants in metabolic reactions that are essential for normal physiological functions.[5]

The metabolome can essentially be categorized into four groups:

1. Intracellular metabolome or endometabolome
2. Extracellular metabolome or exometabolome
3. Microbial metabolome
4. Xenometabolome

2.1. Omics: Its growth and evolution

According to the central dogma of molecular biology, deoxyribonucleic acid (DNA) is transcribed into ribonucleic acid (RNA), which is then translated into proteins, the activities of which result in the formation of myriad metabolites. The closest molecules of the biochemical activity that takes place in an organism in response to physiological and pathological stimuli are metabolites because they are the pinnacle of all regulatory complexity existing in the cells and are also the most accurate phenotypic predictor.[6]

The omics cascade revolutionised from genomics, which is the study of DNA, to transcriptomics which is the study of complete set of RNA, to proteomics which is the study of proteins and finally led to metabolomics which is the study of metabolites. (Figure 1)

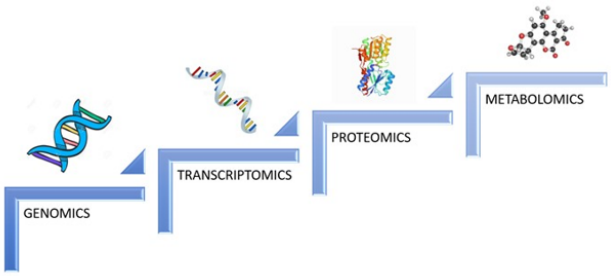

Figure 1: Omics cascade

This concept has led to the development of a more informed diagnostic and management process and there has been a paradigm shift in diagnosis, as molecular diagnostics has now begun to overweigh clinical diagnostics in periodontal practice.

3. What is Metabolomics ?

Periodontal diagnosis has always been at the forefront of a comprehensive rather than simplified approach. Accurately predicting the likelihood of dysbiosis in a patient has become essential given the progress of diagnostic science's practical implementation.

Metabolomics is the study of small molecules in biological materials at specific states through qualitative and quantitative measurement and characterization. Small molecules or metabolites could be found in the tissues and cells of the body or could be freely moving through bioorganic fluids. These, which are essentially chemical substances, can either be made by the body itself or obtained from outside sources. Assessing the pattern of these metabolites in a person's body could possibly help to reveal semi-quantitative data about their entire metabolome. The technique monitors and assesses carbohydrates, amino acids, lipids, and their related by-products through intracellular processes. Any value that differs significantly from the standard suggests an associated condition, not all of which are limited to periodontal disease.[7]

4. How Does Metabolomics Work ?

The primary research is the key factor that determines the samples used in metabolomic studies. Typically, complex matrices used in metabolic investigations include tissues, biofluids, and cell culture materials. Biofluids are researched to seek new biomarkers, while cells and tissues are typically used to investigate the mechanisms of action linked to inflammatory pathways. Since these sample preparation stages might be sources of variance, how samples are collected and prepared can have a big impact on the metabolomics data and findings of a study. A poor process may result in significant variability, instrument interference, metabolite loss, or even the production of breakdown metabolites. Depending on the analytical technique, different methods are used, but generally, peak or spectrum alignment, baseline correction, deconvolution, peak detection, normalization, and scaling are considered standard steps in pre-processing.[8]

Although univariate methods are also employed to extract the statistical significance of the differences detected according to a crucial threshold, multivariate analysis is the major data analysis technique used in metabolomics. The presence of interactions of multiple metabolic features and the impact of potential confounding variables, which can increase the likelihood of obtaining false positive or false negative results, are not considered by this statistical analysis, even though it is simple to use and interpret. To maximise the extraction of important details from metabolomic datasets, it is strongly advised to combine the use of multivariate and univariate data analysis.[9]

One of the major obstacles in the untargeted metabolomics technique is the translation of parameters into metabolite identities. However, this is a crucial requirement to combine data from various investigations and carry out a suitable biological interpretation. (Figure 2)

Figure 2: Work flow in metabolomics

5. GCF and Saliva for Metabolomics Based Periodontal Diagnosis

GCF is regarded as the finest biofluid to study the pathogenesis of periodontitis, whereas metabolites are the closest mediators of pathophysiological conditions. GCF has been used to identify several inflammatory mediators, including cytokines, proteinases, and proteins. A matrix metalloproteinase-8 (MMP-8) test can discriminate between healthy, gingivitis and periodontitis sites. Interleukin-1β (IL-1β) and receptor-activated nuclear factor-kappa B ligand (RANKL) are two additional often mentioned biomarkers linked to periodontitis in the GCF. It might help in the early diagnosis of periodontitis progression in addition to serving as a future diagnostic tool. Increased levels of active collagenase-2, MMP-8, IL-1β, and RANKL in the GCF have been proven to be predictors of alveolar bone resorption and were linked to the advancement of periodontitis.[10]

Thus, it may be possible to predict a patient's susceptibility to subsequent attachment loss by analysing biomarkers in GCF. Findings from metabolomics should also offer fresh perspectives on the biomarkers for the advancement of periodontal disease. The purine degradation pathway, which is essential to the generation of reactive oxygen species (ROS), has been discovered to be remarkably prevalent in disease states. This may demonstrate that the oxidative stress and inflammation in periodontitis sites may be mediated by this mechanism stating that periodontal disease could be predicted as well as diagnosed using GCF's metabolomic analysis.[11]

Salivary metabolic profiling can give a broad overview of the changes caused by periodontal diseases. Various possible biomarkers, including host or bacterial enzymes and prostaglandin E2, have been suggested as diagnostic candidates for periodontitis, which is already thought to be caused by salivary indicators in the form of enzymes including alkaline phosphatase, esterase, glucuronidase, aminopeptidase, immunoglobulins, and steroid hormones. The by-product of oxidative DNA damage known as 8-hydroxy-deoxyguanosine has also been proposed as a new salivary diagnostic for periodontitis.[3]

6. Methods of Analysis of Metabolomics

Metabolomics has developed at the ideal time from a technological standpoint. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are the two main methods used to collect data on metabolomics.[12] When doing metabolomic research, MS and NMR spectroscopy each offer unique advantages and limitations. Modern mass spectrometers can frequently detect analytes in the femtomolar to attomolar range, making sensitivity the primary benefit of MS. A single sample can have hundreds of different species measured by combining MS with liquid chromatography (LC) or gas chromatography (GC). Identification of these metabolites is becoming more frequent due to the mass precision. Quantification is one of the primary concerns with MS in metabolomics. Any compound's MS signal strength is influenced by the sample preparation method and molecular surroundings.[13] The primary advantages of NMR spectroscopy outweigh the primary drawbacks of MS. Quantitative measurements of compounds in a complex combination can be done with extreme precision since the peak area of a molecule in the NMR spectrum is closely associated to the concentration of certain nuclei. The resonance positions of a metabolite's nuclei in the NMR spectrum or the use of different pulse sequences like total correlation spectroscopy, heteronuclear single quantum coherence, and heteronuclear multiple bond correlation can be used to identify a metabolite that has been detected as being more abundant in a particular sample. The ability to analyze metabolites in intact tissues like tumors or in vivo as well as in liquid states like serum and urine is another unappreciated feature of NMR spectroscopy.[14]

Although cryogenically cooled probe technology, higher field-strength superconducting magnets and miniaturized radiofrequency coils have increased sensitivity, NMR spectroscopy is still less sensitive than MS. Despite being less developed than genomics and proteomics, metabolomics is already having a significant impact in a wide range of scientific fields.

The simplest form of MS is direct infusion-mass spectrometry (DI-MS), which is based on the direct injection of samples into the spectrometer without prior chromatographic or electrophoretic separation. This considerably reduces the analysis time, avoids sample dilution, and improves the repeatability and accuracy, but also results in ion suppression/ion enhancement and low ionization efficiency and does not allow quantification. To reduce ion-suppression problems, MS can be coupled with several separation techniques, such as capillary electrophoresis, liquid chromatography, or gas chromatography.[15]

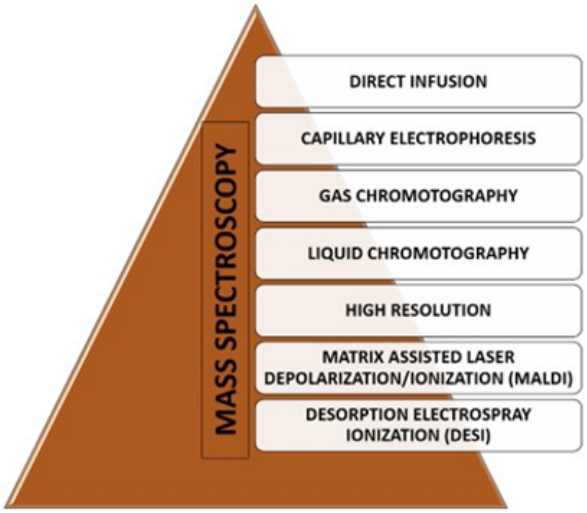

Figure 3: Variants in mass spectrometry

Capillary electrophoresis-mass spectrometry (CE-MS) has the following strengths such as small volume of samples, short analysis time, minimal sample preparation, wide variety of molecules can be analysed. It also possesses the following weaknesses such as poor reproducibility, low sensitivity, affected by salts in the sample and it being less stable.[8]

Liquid chromatography-mass spectrometry (LC-MS) with the advantages of having very high sensitivity, robust, enables analysis of thermolabile metabolites, simple sample preparation, suitable for the study of lipids, and other macromolecules, also has certain disadvantages such as ion suppression or enhancement which makes novel compounds identification difficult.[8]

Gas chromatography-mass spectrometry (GC-MS) with very high sensitivity, enables simultaneous analysis of different classes of metabolites but it requires extensive sample preparation which can be destructive and is limited to volatile compounds.[16]

Another variation that is being utilized more often to create metabolomics data is high-resolution mass spectrometry (HRMS). HRMS significantly enhances the quality of metabolomic data due to its high mass resolution and mass measurement accuracy and is particularly useful for metabolite identification in complicated biological mixtures.[17]

Other intriguing and cutting-edge MS-based metabolomics technologies have now been developed, including matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging and desorption electrospray ionization (DESI) mass spectrometry imaging. These approaches preserve the morphological integrity of the examined tissues or cells while simultaneously providing in situ spatial information for several metabolites.[18] (Figure 3)

7. Limitations

1. Misapprehension: Metabolomics receives substantially less exposure than other omics-based technologies by a significant margin due to the enormous amount of data that is created at a single instance of analysis using metabolomics.
2. Investigational costs: Metabolomics approaches have a great economic efficiency, which makes it a challenging task requiring to invest in a setup for a lab capable of carrying out such an experiment.
3. Attainment of trial facts: Any metabolomics inquiry must start here. When metabolites undergo transformation or degradation, valuable time may be lost, necessitating the presence of resources and armamentarium that might not be present at facilities offering basic health services.
4. Identification and mapping of metabolites: For the time being, not all the metabolites that occur naturally in organisms can be measured or evaluated commercially, and kits for such an evaluation still need to be developed and tested.
5. 5. Quantification of results: At best semi-quantitative data makes it difficult to successfully combine data from metabolomic analysis with data from other-omics methods, or a "multi-omics interaction."[19,20]

A definitive quantification of the levels of metabolites is currently the focus in consideration of these problems. Metabolites can be used in this way and may present a good possibility for periodontal diagnostics in the future because they are easier and more frequently measured than other biological units.

8. Future Trends & Prospects

In a way that genomics, transcriptomics, and proteomics could not fully utilize, metabolomics offers a special chance to affect discovery-driven science. We now have a larger potential to integrate metabolomics data with those collected for the other biomolecules since metabolomics is maturing at a time when the other three omic technologies are much more advanced. Years of study have shown that approaching difficult biological problems in technical storage facilities is ineffective, particularly in the domains of transcriptomics and proteomics.[20]Public health will be greatly impacted by the ability to convert discoveries into assays that can be used often in the clinic. Since healthcare practitioners have been told that omic technologies would address this requirement, the desire for disease-specific biomarkers continues to be heard. The issue of biomarker finding will finally be resolved by the field of metabolomics. However, the evidence required to confidently identify those indicators that are valuable enough to warrant the resources

required for validation will be provided by integrating data from genomic and proteomic research that support metabolomics findings.[21]

9. Closing Thoughts

For assessing and identifying the activity of periodontal disease and the response to diagnostic treatment, biomarkers linked to disease activity, can be aided by advancements in technology and data analysis methodologies in the many "omics" disciplines. Metabolomic analysis is a crucial tool for evaluation and diagnosis since GCFs from the gingival sulcus and periodontal pockets contain biomarkers that signify inflammation, immunological response, and tissue degradation at the site of periodontal diseases. The field of salivary research is one that is emerging. Additionally, it is hoped that the analysis of numerous protein metabolites will enlighten the mechanisms of periodontal disease development and focus attention on the functional relationships between metabolites whose expression varies over time in relation to diseases, medications, or other protein metabolites. The field of diagnostic medicine may benefit from metabolomics, either as a predictor of disease activity or as an indicator of it. However, it is still a relatively new approach, and it has its share of drawbacks. Since the pathogenesis and course of the disease are always changing, it is more important than ever to overcome these barriers and conduct ongoing research to make it possible for incorporation into clinical periodontal therapy.

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11. Conflict of Interest

None.

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