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Original Research Paper

Periodontics

METABOLOMICS: A PERIODONTAL DIAGNOSTIC PARADIGM SHIFT

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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. Periodontology has made significant advances in both therapeutic applications and research. Metabolomics, also termed as metabolomic analysis, is a technology that enables the comprehensive analysis of 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 analyze a wider range of metabolites and to detect metabolites at lower concentrations. This review attempts to better understand the arising need for metabolites for better diagnosis and treatment of periodontitis.

KEYWORDS : Periodontitis, Metabolomics, Mass Spectrometry, GCF markers, Saliva markers

INTRODUCTION

The WHO world global status report (2022) estimated that periodontal disease affects more than 1 billion worldwide with the overall prevalence of severe periodontal disease in the Indian population being 19.6% - 27.3%. Periodontitis is a multifactorial disease resulting in inflammation of the periodontium. It has a major impact on oral health and is a reason for tooth loss. According to Ozeki M et al., (2016) a clinical test that detects regions of high disease activity and predicts periodontal tissue destruction is crucial to perform effective therapy; yet, it is still difficult to predict periodontal tissue breakdown using conventional methods. One of the diagnostic tools that have lately advanced quickly is metabolome analysis. Dr. Anju K.S & Dr. Nandini N Krishnamurthy (2022) outlined metabolomics as a method of searching for metabolites in cells and biological samples to understand the interaction of biomacromolecules and metabolites, novel metabolic pathways, unknown metabolic regulatory mechanisms, and unknown gene and protein functions.

Periodontal disease metabolites can be seen in serum, saliva, GCF, and plaque, which are released as a result of tissue degradation, inflammation, bacterial metabolism, and oxidative stress. Rodrigues WF et al., (2021) found metabolites such as 2,3-Dihydroxypropyl icosanoate, glycerol, serine, 5-amino valeric acid, putrescine, lactulose, oxalic acid, 1-benzoyl-2-t-butyl-5-ethyl-3-methyl-5-vinyl-imidazolidin-4-one, and maltose are found to be two-fold greater in the periodontitis. The purpose of this review is to better understand the implications of metabolomics in periodontal disease.

Conventional Diagnostic Methods

Traditional clinical periodontal assessment methods, such as pocket probing depth (PPD), bleeding on probing (BOP), clinical attachment level (CAL), and radiological assessment of the alveolar bone volume, are widely used. However, these traditional periodontal parameters fail to provide noteworthy information on current disease activity, severity, extent of breakdown, future progression, and therapy response. More importantly, Ramenzoni et al., (2021) suggested that the clinical assessment methods do not adequately reflect the patient's biological phenotype, and the host response to periodontal bacteria and the consequent inflammatory burden, i.e., the influence of biological phenotype, may largely determine periodontitis progression.

Other diagnostic methods are as follows

- Perio temp probe
- Ultrasonographic probe
- Computed tomography and cone-beam computed tomography
- Enzyme-linked immunosorbent assay (ELISA)
- Computer assisted densitometric image analysis (CADIA)
- Polymerase Chain Reaction (PCR)

METABOLOMICS

The metabolome is a close analog to the genome, the transcriptome, and the proteome. Dettmer K et al., (2007) in their review stated "The genome can tell what could happen, the transcriptome can tell what appears to be happening, the proteome can tell what makes it happen and the metabolome can tell what has happened and what is happening". The study of metabolites in a biological sample that comprises heterogeneous small molecules is known as metabolomics.

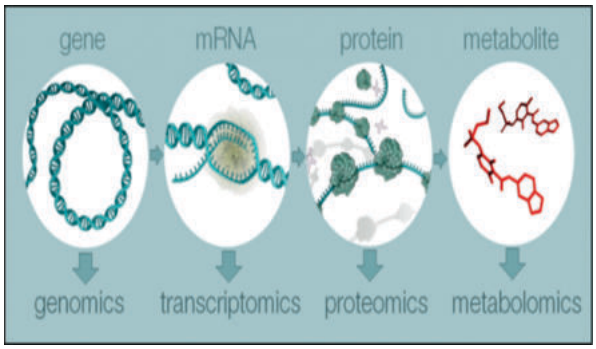

Figure 1: Overview Of The Four Major "omics" Fields, From Genomics To Metabolomics.

In 1998, the word metabolomics was coined by Oliver SG. The term "metabolic profiling" was given by Horning to describe a gas phase analytical technique for metabolite analysis from a human urine sample. The first paper was entitled "Quantitative analysis of urine vapor and breath by gas-liquid partition Chromatography", by Robinson and Pauling in 1971. In line with Siddiqui A et al., (2021) the metabolites can be exogenous or endogenous. Endogenous metabolites are of 2 types i.e., primary and secondary. Primary metabolites are those which are involved in the primary process of life such as glycolytic intermediates. However, secondary metabolites are those which have species-specific functions such as hormones, and biologic-specific functions such as alkaloids.

Metabolites In Chronic Periodontitis

A decrease in the level of gamma amino butyric acid, butyrate, and lactate is linked to an increased risk for periodontal disease development. On the other hand, after a prolonged period of periodontal treatment, the level of isovaleric acid and butyric acid increases significantly due to the recolonization of the microbes. Thus, butyric acid is the marker for periodontal destruction and impairs healing.

Tsuchida S et al., (2022) in their analysis with mass spectrometry concluded that the most significantly enriched metabolites in the chronic periodontitis group are uracil, N-carbamyl glutamate 2, N- acetylβ-D mannosamine 1, fructose 1, citramalic acid, 5- dihydrocor 3, and 4-hydroxyphenyl acetic acid are positively correlated with severe clinical parameters, while the opposite trends were observed for thymidine 3 and O-phosphoserine.

Also, there is an elevation in the levels of lipid peroxidation free radicals and salivary F2, isoprostanes. There is a decrease in the levels of 9- hydroxyeicosatetraenoic acid and 13- hydroxyeicosatetraenoic acid while there is an increase in the level of 5- hydroxyeicosatetraenoic acid.

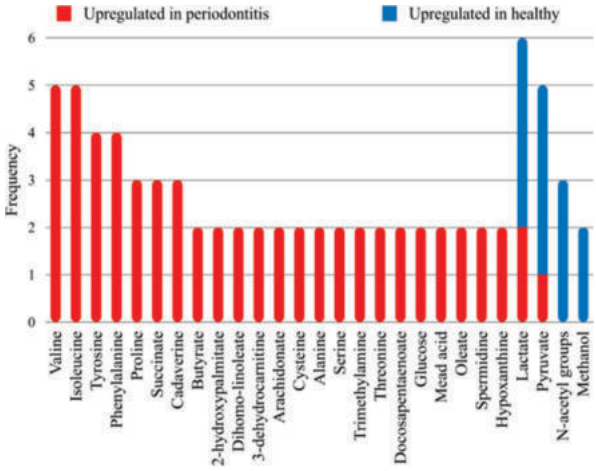

Figure 2: Histogram Depicting The Metabolites Significantly Upregulated Or Downregulated In Periodontitis

Metabolites In Aggressive Periodontitis

Noradrenaline, uridine, α-tocopherol, dehydroascorbic acid, xanthine, alloinositol, urea, galactose, glucose1-phosphate, and ribulose-5-phosphate levels are increased metabolites in patients with generalized aggressive periodontitis, while thymidine, glutathione, acetic acid, 2- deoxyguanosine and ribose-5-phosphate levels were decreased as observed by Tsuchida S et al., (2022).

Siddiqui et al., (2021) impliced that there is a decrease in the level of 2 keto butyric acid, thymidine, glycine-d5, and glutathione, and an increase in xanthine, lysine, ribose, dehydroascorbic acid, and noradrenaline is seen in GCF. These indicate purine degradation, oxidative stress, pyrimidine metabolism, tyrosine metabolism, and biochemistry of bacteria in generalized aggressive periodontitis.

The ratio of omega 3 to omega 6-polyunsaturated fatty acids (PUFAs) and the direct precursors of the pro-resolution lipid mediators were significantly decreased in the GCF of aggressive periodontitis patients than in the healthy controls.

Metabolites In Deep Pockets

Tsuchida et al., (2022) found that the levels 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-amino valeric acid, and galactose were significantly higher in the group of deep-pocket sites when compared to healthy and moderate-pocket sites. Also, a significant increase in the levels of benzoic acid, lactic acid, malic acid, and glycine.

Metabolites In Bone Homeostasis And Bone Loss

Metabolism plays a crucial role in bone homeostasis, and disturbances can cause or are caused by several diseases. Fan J et al., (2021) mentioned, energy metabolisms, including glycolysis and TCA cycle, are pivotal for bone cell differentiation and function. Specifically, arginine and its related metabolism pathways are critical in osteo-immunology. Energy-related metabolites such as lactate and glutamine are altered in bone cancer. During osteoclastogenesis, both glycolysis and oxidative phosphorylation (OxPhos) were found increased, as indicated with a higher glucose consumption and lactate production. This means that glycolysis and mitochondrial processing both play a role in the differentiation and absorption. Hypoxia-inducible factor 1α (HIF-1α) can promote glycolysis and inhibit oxidative phosphorylation (OxPhos). Increased expression of glycolytic genes, including hexokinase, phosphofructokinase, and pyruvate kinase, was found during osteoclastogenesis.

Higher level of creatine, dimethylglycine, hydroxyproline, and glycine seen in cases of bone loss. Increased concentration of hydroxyproline indicates the degradation of collagen type I from the bone matrix. Similarly, other catabolites developed in bone from collagen, such as deoxypyridinoline (collagen stabilizer) and pyridinoline (cross-linking compound of collagen fibers), can also serve as the markers of osteoporosis.

As of Surrati A et al., (2021), hydantoin-5-propionate, a metabolite of histidine, significantly increased in the extracellular space during osteogenic treatment. 4-Imidazolone-5-propanoate is another histidine metabolite that increased extracellularly during osteogenic differentiation. L-Lysine, L-proline, L-pipecolate, L-2-aminoadipate, L-glutamate 5-semialdehyde and N-acetylputrexcine, L-citrulline, 4-hydoxy-2-oxoglutarate, L-tyrosine, L-histidine, L-methionine, L-methionine S-oxide, N(pi)-methyl-L-histidine, S-adenocyl L-methionine and L-adrenaline also showed a significant increase under osteogenic conditions.

Metabolites In Peri-implantitis

In the longitudinal study of Alassy & Hatem. (2021) cadaverine, lysine, putrescine, alanine, tyramine, and valine were the top metabolites that were strongly linked with peri-implantitis. Alpha-ketoglutarate, methionine, and uracil were the main metabolites that were associated with implant health.

Novel metabolites such as cadaverine, lysine, and putrescine may help with peri-implantitis diagnosis. Cadaverine is a diamine formed when lysine is decarboxylated by bacteria during the putrefaction of tissues. Putrescine can be

synthesized by both bacterial and mammalian metabolic pathways, in contrast to cadaverine, which is entirely of bacterial origin.

Peri-implantitis and alpha-ketoglutarate have a significant inverse relationship. alpha-ketoglutarate (α-KG), is a key molecule in various physiologic processes such as the tricarboxylic acid cycle and is necessary for the production of type I collagen. Proline and 1-3-diamino propane predict future peri-implant bone loss while glucose, biotin, propionate, betaine, and arginine predict implant stability.

Metabolomics Methodology & Workflow

Methodologies used in metabolomics fall into two categories; namely targeted and untargeted metabolomics.

Roberts LD et al., (2012) expressed targeted metabolomics as the measurement of defined groups of chemically characterized and biochemically annotated metabolites, whereas Untargeted metabolomics aims to analyze all quantifiable analytes in a sample, including chemical unknowns.

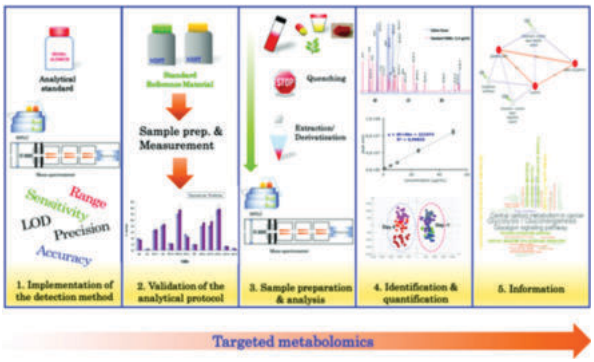

Figure 3: Steps Involved In Metabolomics.

The compounds are formally identified and quantified in targeted profiling. The resulting list of compounds and concentrations i.e., the metabolic profile is then used to diagnose, identify phenotypes or draw conclusions.

Figure 4: Steps Involved In Untargeted.

In a non-targeted or chemometric approach, the compounds are not formally identified, only their spectral patterns and intensities are recorded, compared, and used to diagnose, identify phenotypes or draw conclusions.

Metabolite Detection Methods (Dr. Zeba Samsher Nandrekar et al.,2022)

1. Mass-Spectrometry
2. Liquid chromatography (LC)
3. Gas chromatography (GC)
4. High-performance liquid chromatography (HPLC)
5. Capillary electrophoresis
6. Nuclear magnetic resonance (NMR) spectroscopy
7. Fourier-transform infrared (FTIR) spectroscopy techniques.
8. Matrix-Assisted Laser Desorption/Ionization Time-of - Flight type Mass Spectrometer (MALDI-TOF MS).

Mass-Spectrometry mostly coupled with chromatographic techniques is gaining attention in metabolomics.

Dr. Anju K.S & Dr. Nandini N Krishnamurthy (2022) described that tissue, suspension-cultured mammalian cells, and biofluids such as serum, plasma, saliva, GCF, CSF, urine, sweat, feces, bile, and seminal plasma can all be used for metabolome analysis.

Liquid chromatography device is a column filled with porous media made of granular solid material (i.e., stationary phase), such as polymerase and silica, through which the sample is injected and the solvent (i.e., mobile phase) flows to transport the sample. The components with more affinity towards the stationary phase are the last to separate. This is because strong affinity requires more time to travel to the end of the column.

In gas chromatography, the stationary phase is either a solid or a liquid, while the mobile phase is gas. As the sample solution comes in contact with the second solid or liquid phase, the solutes begin interacting with the other phase due to differences in adsorption rate, ion exchanges, partitioning, or sizes. Because of these differences, the sample mixture will pass through the column at different rates, allowing the compound to be separated.

In High-performance liquid chromatography compounds are determined based on their retention time in the column using a graph called "chromatogram" and then identified and quantified by spectrometry. The X-axis of the graph typically represents retention time; however, the Y-axis depends on the method used for the detection.

MALDI-TOF MS combines MALDI as the ionization method and TOF as the analyzer. All three separation techniques above mentioned are then combined with or followed by Mass spectrometry (MS) and Nuclear magnetic resonance (NMR).

Mass Spectrometry & Nuclear Magnetic Resonance

A measurement method for tiny molecules like metabolites is mass spectrometry. The mass-to-charge ratio (m/z) and temporal intensity triplets specify the strength of the ion beam. For targeted analysis, mass spectrometry is preferred to NMR because it has a higher sensitivity and can detect 300–1000 metabolites.

Nuclear magnetic resonance is employed to quantify organic and some inorganic substances inside biological samples (solid tissue or extracted metabolite). When the sample is exposed to the magnetic field and radio frequency (rf) pulse, nuclei in the sample absorb and then re-emit electromagnetic radiation. The emitted energy has a resonating frequency. Dr. Zeba Samsher Nandrekar et al., (2022) recommended NMR-based metabolomics analysis as a potentially useful method for investigating the pathological processes observed in the oral cavity during the course of periodontitis.

Statistical Analysis

The objective of statistical analysis is to classify and predict sample properties using successive repetitions of models that encapsulate the information in data matrices. The two most prevalent statistical methods used in metabolomics are called univariate and multivariate. The results of the univariate study are weighted differently because only one variable is taken into account. When a variety of factors are analyzed, traits based on relationships between all of the variables are highlighted.

Advantages

The non-invasive nature of metabolomics and its close link to the phenotype primarily increases its significance. Its importance is ≥95% of all clinical assays test for small

molecules, 89% of all known drugs are small molecules, and 50% of all drugs are derived from pre-existing metabolites. 30% of identified genetic disorders occupy diseases of small molecule metabolism. Large numbers (tens to hundreds) of metabolites are rapidly measured, in a matter of minutes, which offers the ability to gain a snapshot of different individual and intersecting metabolic pathways. It minimizes the requirement for onsite instrumentation and offers the possibility of distant diagnosis.

Limitations

Metabolites can be a derivative of host tissue breakdown, supragingival and sub-gingival plaque, saliva, or bacterial communities. One of the major problems with oral metabolites is that the exact origin of a particular metabolite is difficult to determine. It is more diverse and contains many different biological molecules, making it physically and chemically more complex than the other "omics" methods. Limiting the number of metabolites may result in the negligence of some significant by-products, which may be a bias for the targeted method. The number of endogenous molecules to be studied is relatively small in comparison to the number of genes, mRNAs, or proteins. Greater sensitivity and responsiveness of metabolomics approaches suggest its susceptibility to complicating experimental design and interpretation. High set-up costs and requirements for complex informatics. The standard diagnostic approach to periodontal diseases involves clinical and radiological investigations. According to Dr. Zeba Samsher Nandrekar et al., (2022) these techniques rely on visual and morphological changes associated with disease, they have limitations in early prediction.

Future Perspectives

Metabolomics can aid in the development of a prospective disease-specific biomarker based on molecules. In periodontal research, the oral microbiome is emphasized. Therefore, integrating the microbiologic and metabolomic data helps in identifying the physiological effects of microorganisms on host physiology by producing, modifying, or degrading bioactive metabolites. Improved diagnostic methods and new biomarkers are required for diagnosing periodontal disease and monitoring prognosis. Multi-omics approach provides rich and complementary perception in the analysis of periodontitis compared to single-omics studies. The data on the biological stratification of periodontitis will be improved by integrating metabolomics with other omics (such as genomics, transcriptomics, and proteomics), which will also help to assemble the best discriminative diagnostic standard and open up new perspectives on the molecular mechanisms underlying periodontal diseases.

CONCLUSION

Modern periodontal screening techniques, which examine GCF and saliva to determine the state of periodontal disease, are inadequate. Prediction of risk and accurate diagnosis of current disease activity may facilitate effective disease prevention and treatment.

Translational metabolomics has already shown astounding promise as a marker and a predictor of disease activity in the field of diagnostic medicine. With the ongoing evolution of disease causation and progression, addressing these gaps and conducting active research to enable their adoption into clinical periodontal practice can be invaluable resource and should be investigated. Metabolomic analysis has the potential to be used to expound the pathogenesis of periodontal diseases, thereby contributing to improving research outcomes and treatment prediction in periodontal disease.

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