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Metabolomics- A New Era in the Field of Periodontal Diagnosis

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Abstract:- Periodontology has seen remarkable advancements in terms of both therapeutic applications and research. Metabolomics allows for a thorough examination of small-molecule metabolites in living things. Research in the areas of food, plants, microbes, and medicine frequently makes use of metabolomics.

Keywords: *Periodontitis, Metabolomics, Mass Spectrometry, Liquid Chromatography, Gas Chromatography.*

I. INTRODUCTION

Periodontal disease, along with dental caries, is one of the two major dental diseases and the leading cause of tooth loss. Periodontal disease has recently been linked to systemic diseases such as diabetes and aspiration pneumonia, and is now recognised as a lifestyle-related disease. Metabolome analysis has recently made rapid progress. It is a method of searching for metabolites in cells and biological samples in order to understand the interaction of biomacromolecules and metabolites, novel metabolic pathways, unknown metabolic regulatory mechanisms, and unknown gene and protein functions. To stop tooth loss and enhance the patient's quality of life, periodontal disease must be properly diagnosed and treated. A technology called metabolomics, commonly referred to as metabolomic analysis, is used to thoroughly examine small-molecule metabolites in living organisms. We should be familiar with the fundamentals of metabolomics before getting into the specifics of how metabolomics research is used to periodontal disease.

Oliver coined the term "metabolome" in 1998 to refer to an organism's collection of tiny chemicals. Horning coined the phrase "metabolic profiling" to describe a gas phase analytical technique for metabolite analysis from human urine sample.

➢ *METABOLITES:*

These are substrates intermediates and products of metabolism within the context of metabolomics, a metabolite is defined as any molecule less than 1.5kDa in size. However, there are exceptions to this depending on the sample and detection method.

➢ *METABOLOME:*

These refers to the complete set of small molecules (≤1.5kDa) metabolites (such as metabolic inter mediates, hormones, and other signalling molecules and secondary metabolites) to be found in a biological sample such as a single organism.

Metabolomics is thus, a scientific study of chemical process involving metabolites, the small molecular substrates, intermediates and products of cell metabolism.

II. METABOLOMICS STRATEGIES

Metabolomics strategies cover to primary analysis platforms including "untargeted discovery global "and" targeted-validation tandem" based on the objective of the study.

Untargeted metabolomics	Targeted metabolomics
Discovery Hypothesis generating Relative quantification Qualitative identification and no chemical commercial standard required More than thousand metabolites measured	Validation Hypothesis driven Absolute quantification of specific features Validation identification features (requires commercially available chemical standard for validation) Nearly twenty metabolites have been measured

Table 1:- Metabolomics Strategies

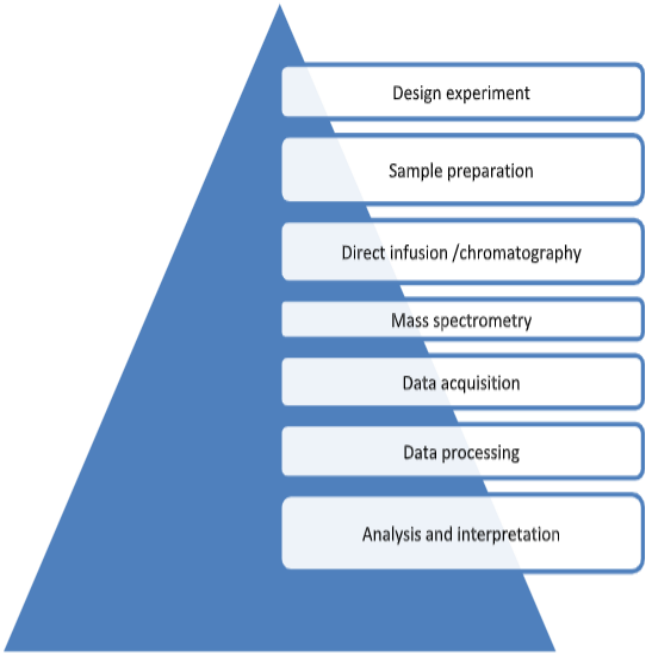

Fig 1:- Metabolomics Workflow

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➢ *Sample Acquisition*

Metabolome analysis can be performed using various biological sampling such as tissue, suspension-cultured mammalian cells, biofluids like serum, plasma, saliva CSF, urine, sweat, feces, bile, seminal plasma.

➢ *Sample Preparation &Extraction*

During sample preparation different extraction solvents are used for recover of both polar and non-polar compounds based on the targeted a non-targeted approach on different biological samples. Basically, applied approaches include optimized -water-chloroform combinations to extract both hydrophilic and hydrophobic compounds. During sample preparation after the centrifugation process the first one of the two phase extraction involves the (upper)aqueous phase/solvent (eg; methanol water) followed by extraction with (lower)non polar solvent (eg; chloroform) of the centrifugal pellet. further the higher recovery of both hydrophobic and hydrophilic compounds, separation extraction applications give better results.

➢ *Chromatographic Separation Techniques*

1. Liquid chromatography (LC)
2. Gas chromatography (GC)
3. High performance liquid chromatography (HPLC)

Liquid chromatography device is a column packed with the porous medium made of a granular solid material (i.e. stationary phase), such as polymerase and silica, where the sample is injected and the solvent (i.e., mobile phase) passes to transport the sample. The components with more affinity towards the stationary phase are the last to separate. This is because high affinity corresponds to more time to travel to the end of the column. Gas chromatography either uses the solid or liquid as stationary phase while using gas as mobile phase. As the sample solution makes contact with the second solid or liquid phase, these solutes will start interacting with other phase due to differences in adsorption rate, ion exchanges, partitioning or sizes. These differences will make the sample mixture pass at different rates through the column, and the compound can be separated. High performance liquid chromatography works by using the basic column chromatography. Compounds are determined based on their retention time in the column using a graph called “chromatogram “and then identified and quantified by spectrometry. Retention time is usually representing the X-axis of the graph; however, the Y-axis depends on the method used for the detection. which is usually a UV detector and measures the intensity of the absorbance. Other detectors are also used like mass spectrometry when higher sensitivity than UV is required. All the three separation techniques mentioned previously are then combined with or followed by Mass spectrometry (MS) Nuclear magnetic resonance (NMR).

A measurement method for tiny molecules like metabolites is mass spectrometry. The MS may receive the smaller molecules directly or via a coupled chromatographic system. the mass-to-charge ratio (m/z) and temporal intensity triplets, which specify the strength of the ion beam and the time at which each detected ion is detected by the spectrometer for each mass, respectively, for the results. Since MS has a higher sensitivity and can detect 300–1000 metabolites, it is preferable to NMR for targeted analysis. To quantify organic and some inorganic substances inside of biological samples, nuclear magnetic resonance is employed (as solid tissue or extracted metabolite). The nuclei in a sample absorb and then re-emit electromagnetic radiation when it is exposed to magnetic field and radio frequency (rf) pulse. The energy that is emitted has a resonating frequency. In metabolomics, proton atom from small molecules are usually investigated.

The two most prevalent statistical methods used in metabolomics are called univariate and multivariate. Results from a univariate study, which only considers one variable, are weighted differently. When a variety of factors are analysed, traits based on relationships between all of the variables are highlighted. The objective of statistical analysis is the classification and prediction of sample properties using successive iterations of models that encapsulate the data matrices' information.

III. SIGNIFICANCE OF METABOLOMICS

The non-invasive nature of metabolomics and its close link to the phenotype adds primarily to its significance. Its importance is ≥95% of all clinical assays test for small molecules, 89% of all known drugs are small molecules in which 50% of all drugs are derived from pre-existing metabolites. 30% of identified genetic disorders occupy diseases of small molecule metabolism. Thus, metabolites are the canaries of the genome and it is more sensitive than other “omics”.

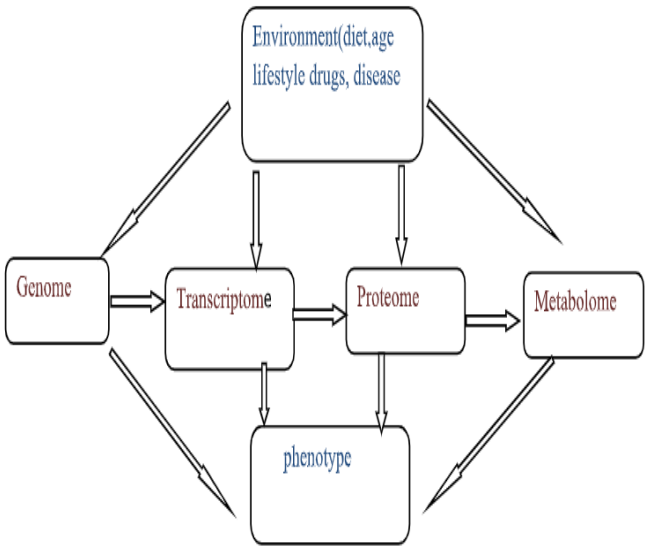

Fig 2:- Significance of Metabolomics

It is essential to try and construct a chain of causality between the inflammation that is clinically evident and the makeup of the periodontal microbiota in order to acquire a thorough grasp of periodontal pathobiology. The relationship between the cause (microbial population) and the effect (inflammation) is reciprocal, and thus contributes to the etiopathogenesis of periodontitis. Van Dyke et al. recently proposed a new model called inflammation mediated

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polymicrobial emergence and dysbiotic exacerbation. This distinctive dysbiosis is influenced not only by changes in the microbial population, but also by changes in the individual's metabolomics. Sakanaka et al. were successful in establishing a clear relationship between the degree of inflammation in periodontitis and the presence of specific metabolites in 2017. Following enrichment analysis of the metabolic compounds, it was possible to create a distinct metabolomic "blueprint" or signature of periodontitis. This study also sought to determine whether a decrease or increase in these levels had any bearing on the severity of the disease as well as the level of inflammation present, in addition to examining the role of specific metabolites and their levels in influencing the pathogenesis of the periodontal disease. The Periodontal Inflamed Surface Area (PISA), an index developed by Nesse et al. in 2008, was used to gauge this level or status of inflammatory load. Following the construction of a metabolomic "profile" of samples obtained both before and after the removal of plaque and calculus and subjecting them to metabolomic analysis in accordance with protocol, the study claims that many incriminating metabolic chemicals were identified. The analysis' findings revealed that, among all the metabolites examined, a few were found to be higher or lower in concentrations specific to inflammation even after plaque had been removed. The scientists suggested that people with a more severe type of periodontitis, i.e., those with a higher PISA score, have elevated metabolic pathways involving each of these substances. With strong evidence that metabolomic analysis plays a part in the dysbiosis and consequent damage associated with periodontitis, the paradigm for treating periodontal disorders switches from a generalised to a tailored approach. This strategy is founded on the central idea of a treatment model that enables the periodontist to design and give a catered or tailored approach toward therapy that differs for each patient, as explained by Bartold in 2018.

The ability to examine different metabolites and detect metabolites at lower concentrations has been made possible by advancements in metabolite analysis techniques using GC-MS, LC-MS, and capillary electrophoresis-MS. Many studies in the realms of food, plants, microbes, and medicine have made use of metabolomics. Quantifying in vivo metabolites can provide a more thorough understanding of cellular functions because they are placed downstream of the core dogma that drives biological activity. In vivo metabolites are the targets of metabolomics. MS is the most sensitive technology and can extract more data from a smaller sample volume. It's crucial to examine as many metabolites as you can at once in order to conduct a thorough examination of them. Combining different MS analysis techniques allows analyse a variety of metabolites. Kuboniwa et al. examined the relationship between salivary metabolites to reflect periodontal inflammation severity using a recently proposed parameter (PISA). The combination of cadaverine, 5-oxoproline, histidine and pyroglutamic acid yielded a satisfactory accuracy for periodontitis diagnosis. Liebsch et al. used a large set (284) of salivary metabolites obtained by LC-MS/MS from a subsample of 909 nondiabetic participants in the Study of Health in Pomerania to describe the clinical attachment level, periodontal probing depth, supragingival plaque, supragingival calculus, number of missing teeth, and removable denture as oral parameters. Periodontal disease metabolites have been linked to tissue destruction, host defence mechanisms, and bacterial metabolism, with the bacterial metabolite phenyl acetate being significantly associated with periodontal disease variables. Thus, bacterial metabolites like phenylacetate are expected to play a significant role in periodontal disease.

In order to unbiasedly identify periodontitis at the molecular level, Ozaki et al. looked into the utility of GC-MS, which may be employed for the on-site detection of metabolites in gingival crevicular fluid (GCF). The proximal fluid, or GCF, which is nearest to the lesion site and best represents the state of the periodontal tissue. GCF is thought to be an important marker of periodontal tissue metabolism, and contains several enzymes and proteins relevant to it. Even though there are only a few proteins and enzymes in GCF, MS can be employed to examine these little amounts. Candidate periodontal disease markers that might be evaluated by metabolomics analysis are anticipated to be present in GCF. As a result, metabolomics is regarded as a key method for comprehending GCF. The gingival metabolome of mice with and without periodontitis and obesity brought on by a high-fat diet (HFD) was studied by Chen et al. By using nontargeted/targeted LC-MS, the gingival metabolome and arginine metabolism were examined. They came to the conclusion that the obese population, fed an excessive HFD, showed a metabolic susceptibility to worsened periodontal deterioration and an amplified metabolic response to periodontitis. This study suggested that periodontal disease research and future applications could benefit from the use of gingival metabolome based on MS technology, both nontargeted and targeted LC-MS. A prospective direction for metabolomics research is the development of a panel of endogenous metabolites produced by the hosts, the periodontal pathogens, or both, and quantification of these metabolites using microfluidics. In addition to causing fundamental alterations in the primary and secondary metabolic pathways of the concerned organism, Derewacz et al., Goodwin and their colleagues found that mutations on the metabolic scale in an organism can also predispose them to demonstrate resistance to antibiotic treatments. On the other hand, it can bring about a degree of co-morbidity for systemic disorders that share the inflammatory backdrop of periodontitis, making it challenging to offer definitive treatment for either. Overmyer et al. conducted 16S rDNA sequencing as well as metabolomics, lipidomics, and proteomics analyses on supragingival dental plaque. Phosphatidylcholines, ceramides containing non-OH fatty acids, and proteins related to actin filament rearrangement were elevated in plaques from PD(periodontal disease samples) samples. New treatments for periodontal disease could be developed by studying the function of proteins that serve as diagnostic markers for the inflammation of periodontal tissues. Studying periodontal metabolite biomarkers involves several challenges, and it is essential to examine how specimens should be collected and analysed. The oral microbiome is the focus of increasing attention in periodontal research. An analysis comparing the oral microbiomes at multiple sites (saliva and plaque) in a

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sample of 1000 people from the Japanese population was recently published. In the future, MS-based oral microbiome and metabolome analyses may be used to elucidate the pathogenesis of periodontal diseases. There is a significant elevation of reactive oxygen species as well as anti-oxidants that are usually present in bodily fluids viz., crevicular fluid, saliva, and other bodily fluids. The presence of these metabolic products such as lactate, acetone, propane-1,2,3-triol, and others supports the assumption that they can contribute elements to chronic inflammatory disease. The researchers report that they will make the results of their microbiome analysis widely available on the Internet. In the future, MS-based oral microbiome and metabolome analyses may be used to elucidate the pathogenesis of periodontal diseases. The economic efficiency of metabolomics techniques is quite high, which makes it a difficult challenge to conduct a conclusive study. A complete examination of each piece of information acquired may be hampered due to the enormous amount of data that is generated at one time of study via metabolomics. Acquisition of experimental data serves as the basis for all metabolomics research. The accuracy of measuring the subject's entire metabolic biome with just one approach is the most crucial challenge in this regard. When metabolites change or degrade while being transported with collected samples for processing, valuable time may be lost. Facilities providing basic health care services might not have the resources and arsenal necessary to prevent this from happening.

IV. APPLICATION AND FUTURE PROSPECTS

- *Application*

1. Toxicology testing
2. Clinical trial testing
3. Drug phenotyping
4. Genetic disease testing

- *Future prospects*

1. Metabolomics informs decision making in the field of biomarker discovery.
2. In personalised medicine, Metabolomics is necessary for rapid disease diagnosis in order to provide the highest level of healthcare customisation.
3. By examining the biochemistry of genetically modified plants at various stages of plant development, metabolomics helps to improve them and quantify associated risk.

Kuboniwa, M.; Sakanaka, A.; Hashino, E.; Bamba, T.; Fukusaki, E.; Amano, A. Prediction of Periodontal Inflammation via Metabolic Profiling of Saliva. J. Dent. Res. 2016	Sixty-three metabolites were identified in whole saliva samples from 19 subjects. Post debridement saliva provided a more refined model for prediction of PISA (periodontal inflamed surface area) than did pre debridement samples. Debridement may improve detection of metabolites eluted from subgingival areas in saliva, thus more accurately reflecting the pathophysiology of periodontitis.
Sakanaka A, Kuboniwa M, Hashino E, Bamba T, Fukusaki E, Amano A. Distinct signatures of dental plaque metabolic byproducts dictated by periodontal inflammatory status. Sci Rep 2017; 7:42818.	The research produced a distinctive metabolomic "blueprint" or signature that can definitely be used to predict or identify the occurrence of periodontal disease. These substances comprised degraded lysine, proline, butanoic acid, and arginine. In addition to these compounds, cadaverine and hydro cinnamate were two other metabolites that were strongly associated with altering the severity of periodontal disease activity in the patients, as well as the level of inflammation.
Bartold PM. Lifestyle and periodontitis: The emergence of personalized periodontics. Periodontol 2000 2018;78:7-11.	Their suggestion offered a highly systematised approach based on a model for periodontal treatment, where the patient population is split and subdivided into many groups/strata, and a profile consisting of personalised clinical decision-making, practise, and treatment alternatives is formed. In addition to genetic and epidemiologic information, this model would also benefit from input from the patient's sociologic, physiologic, molecular, and cellular investigations. A comprehensive data collection and a treatment plan tailored to the individual and their disease severity will be produced in the end as a result of the cumulative effect of all these approaches.
Liebsch, C.; Pitchika, V.; Pink, C.; Samietz, S.; Kastenmüller, G.; Artati, A.; Suhre, K.; Adamski, J.; Nauck, M.; Völzke, H.; et al. The Saliva Metabolome in Association to Oral Health Status. J. Dent. Res. 2019, 98, 642–651.	Periodontal disease has been linked to tissue destruction, host defence mechanisms, and bacterial metabolism. Bacterial metabolites like phenylacetate are expected to play a significant role in periodontal diseases. This study used a large set of salivary metabolites obtained from 909 nondiabetic participants in the Study of Health in Pomerania.
Van Dyke TE, Bartold PM, Reynolds EC. The nexus between periodontal inflammation and dysbiosis. Front Immunol	The massive amount of data that is obtained via metabolomic analysis may be subjected to stratification of various case

2020; 11:511.	phenotypes using machine learning or artificial intelligence, to eliminate personnel error. Based on the results thereof, blueprints can be created for diagnosis, treatment planning as well as determining the prognostic risk of periodontal disease in an individual
Overmyer, K.A.; Rhoads, T.W.; Merrill, A.E.; Ye, Z.; Westphall, M.S.; Acharya, A.; Shukla, S.K.; Coon, J.J. Proteomics, Lipidomics, Metabolomics, and 16S DNA Sequencing of Dental Plaque from Patients with Diabetes and Periodontal Disease. Mol. Cell. Proteom. 2021, 20, 100126	16S rDNA sequencing as well as metabolomics, lipidomics, and proteomics analyses on supragingival dental plaque. Phosphatidylcholines, ceramides containing non-OH fatty acids, and proteins related to actin filament rearrangement were elevated in plaques from PD (periodontal disease samples) samples. New treatments for periodontal disease could be developed by studying the function of proteins that serve as diagnostic markers for the inflammation of periodontal tissues

Table 2 Summary of recent literature supporting the use of metabolomics to advance the field of personalized medicine including periodontics

V. CONCLUSION

In the area of diagnostic medicine, translational metabolomics has already demonstrated astonishing promise as a marker and a predictor of disease activity. However, it is still a cutting-edge method that has plenty of potential dangers. With the ongoing evolution of disease causation and progression as well, addressing these gaps and doing active research to enable their adoption into clinical periodontal practise can be an invaluable resource and ought to be investigated. Periodontal disease biomarkers associated with inflammation, immune response, and tissue destruction have been identified by MS researchers. The analysis of many protein metabolites is hoped to clarify the links between diseases, drugs, and other protein metabolites, as well as elucidate the mechanisms of periodontal lesions.

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