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METABOLOMICS IN PERIODONTICS: CHEMICAL DIVERSITY

Periodontology

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ABSTRACT

Various recent advances in cellular biology and technology have led to the development of newer approaches in diagnostic research at a molecular level. Metabolomics facilitates the comprehensive evaluation of small-molecule metabolites in cells that interact between biomacromolecules and metabolites, novel metabolic pathways, unknown metabolic regulatory mechanisms, and unknown gene and protein functions by spectroscopic techniques. It has potential for use in diagnosis and treatment of periodontal diseases as identifying metabolites from oral fluids such as saliva and gingival crevicular fluid helps us understand the complex pathogenesis of periodontitis.

KEYWORDS

Metabolomics, metabolites, spectral analysis.

INTRODUCTION:

Recent advances in cellular biology and technology have led to the development of newer approaches in diagnostic research at a molecular level.

Metabolomics, also termed as metabolic profiling, is an analytical method which facilitates the comprehensive evaluation of small-molecule metabolites in cells that interact between biomacromolecules and metabolites, novel metabolic pathways, unknown metabolic regulatory mechanisms, and unknown gene and protein functions by spectroscopic techniques.(1,2)

Metabolomics, is being widely used in various research fields as an essential tool for the diagnosis of and estimation of severity of several diseases, ranging from autoimmune disorders to chronic diseases. One such application is in exploring the pathogenesis of periodontal diseases. It has potential for use in diagnosis and treatment of periodontal diseases as identifying metabolites from oral fluids such as saliva and gingival crevicular fluid helps us understand the complex pathogenesis of periodontitis.

The molecules present in fluids of oral cavity may imply association between various processes linked to health and disease, as well as repair processes.

METABOLITES

Metabolites are transitional end products released by altered cellular metabolism of host and microbes. They are chemically diverse in nature which can be classified into ionic species, alcohols, hydrophilic carbohydrates, volatile ketones, lipids, and organic acids.(3)

Many metabolites related with inflammation, oxidative stress, tissue degradation, and bacterial metabolism are found to be significantly induced by the disease. The metabolic integration of oral fluids such as saliva and GCF offers a wide range of biochemical basis for considering the pathogenesis of periodontitis.

Sample collection and preparation

Intrinsic

Age
Gender
Glands & secretion
Physical activity
Systemic health

Extrinsic

Diet & nutrition
Oral hygiene
Oral diseases
Oral microbiota
Smoking & alcohol

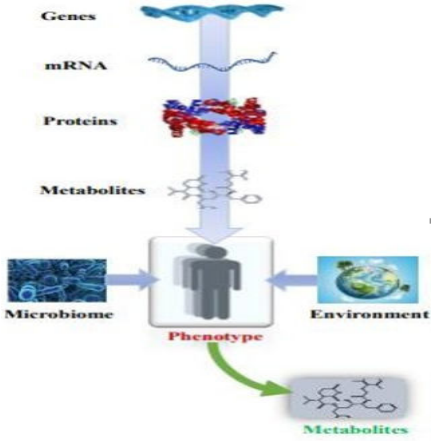

Figure 1: Diagrammatic representation of linking omics

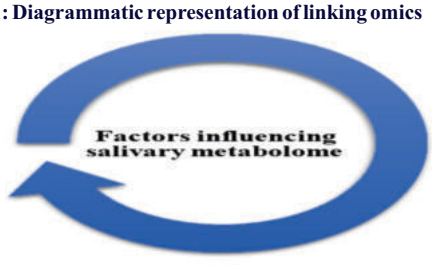

Figure 2: Factors influencing salivary metabolome

METABOLOMICS IN PERIODONTICS:

Periodontitis is as an inflammatory disease of supporting tissues of teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with periodontal pocket formation, gingival recession or both.(4)

Resulting in production of various inflammatory markers and other biomarkers produced due to immune response and tissue destruction. Metabolomics hence can facilitate the identification and quantification of various metabolites in normal individuals and those with certain disease including periodontitis(5,6,7) and hence can be a beneficial tool for detection of periodontal inflammation.(8) Salivary metabolites may originate from the microbiome on buccal mucosal membranes or plaque. GCF also has rich source of metabolites and associated with host-bacterial interactions.(9)

Metabolomics Methodology & Workflow: (10, 11)

I. Pre-analytical work (Sample preparation)

1. Sample collection: Saliva, GCF, serum, etc
2. Sample pre-processing:

a) Metabolic quenching or extraction:

i) Protein precipitation- 2.5 ml Methanol (MeOH), Acetonitrile (ACN)

ii) at -20°C

iii) Liquid- liquid extraction (LLE)- MeOH, 1.25 ml Chloroform ($CHCl_3$)

iv) Dilution: H_2O

b) Centrifugation: for 10 min then collect supernatant

c) Filtration

d) Sample storage: at -80 ºC- 20ºC and freeze-thaw cycles avoided

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II. Analytical work

1. Sample processing- diluted with buffered solution
2. Pre-acquisition normalization- Chemical: Internal standardization
3. Data acquisition (NMR, GC-MS, LC-MS)

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III. Data processing

1. Data pre-processing: filtering, alignment
2. Statistical analysis
3. Metabolite identification
4. Metabolite quantification: univariate, multivariate

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IV. Data analysis

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V. Interpretation

Figure 3: Flowchart of Metabolomics workflow

Spectral analysis tools for metabolomics

Numerous (ten to hundreds) of metabolites are rapidly measured using non-chemical, non- colorimetric methods such as gas chromatography- mass spectrometry (GC–MS), liquid chromatography—mass spectrometry (LC–MS), capillary electrophoresis (CE), Matrix- Assisted Laser Desorption/Ionization-Time-of -Flight type Mass Spectrometer (MALDI-TOF MS), Fourier transform mass spectrometry (FT-MS) and Proton Nuclear Magnetic Resonance spectroscopy (NMR). It is possible even to detect metabolites at lower concentrations.

GC-MS has high sensitivity and mature database, which is currently a suitable way for non- targeted metabolome detection, for chromatographic and mass separation. LC-MS combines high-performance liquid chromatography (HPLC) and triple quadrupole mass spectrometry which separates the organic compounds in a liquid and analyzes them by mass. MALDI-TOF MS combines MALDI as the ionization method and TOF as the analyzer. NMR based metabolomics analysis is a potentially useful method for examining the pathological processes observed in the oral cavity during course of periodontitis.

There are two distinct ways for collecting, processing and interpreting metabolomic data. (19) In chemometric or non-targeted 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. (20, 21) In targeted profiling, the compounds are formally identified and quantified. The resulting list of compounds and concentrations i.e., metabolic profile is then used to diagnose, identify phenotypes or draw conclusions. (19, 22)

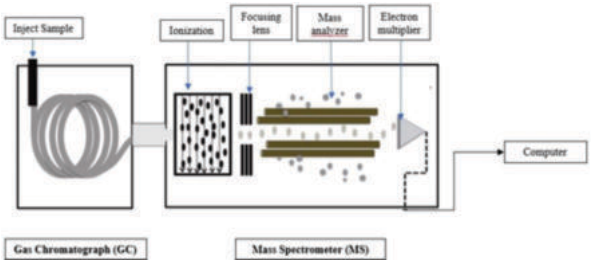

Figure 4: Schematic diagram of a gas chromatography mass spectrometer (GC-MS).

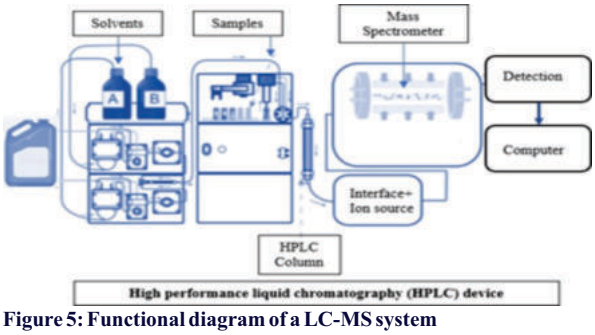

Figure 5: Functional diagram of a LC-MS system

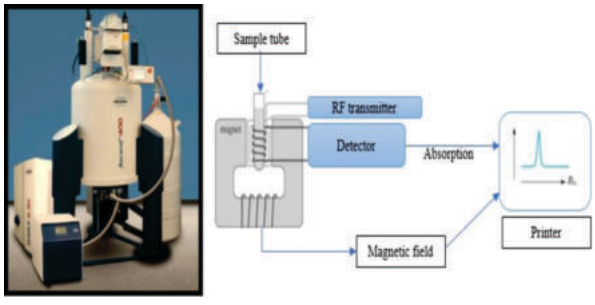

Figure 6: NMR Spectrometer

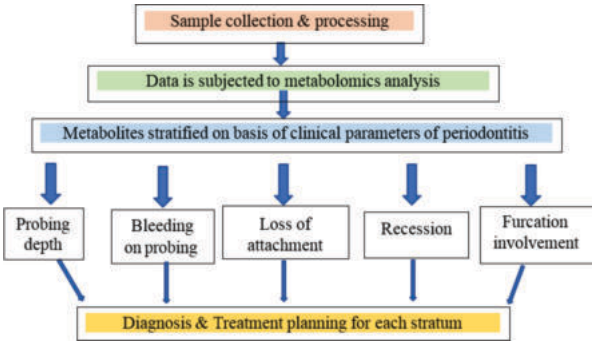

Figure 8: Flowchart of metabolomics in periodontics

Table 1: Various studies conjoining periodontal diseases and metabolome are as follows:

Author and Year	Study
V.M. Barnes et al (2011)	Carried out a study to evaluate the metabolic role of saliva in the diseased state and found increase in lipase, protease, and glycosidase activities to be associated with periodontitis that created a more favourable environment for oral bacteria, potentially exacerbating the disease state. (9)
Zhang et al, (2014)	Compared the metabolic functional diversity of the microbial community in healthy subjects and periodontitis patients to assess the sole carbon source utilization and found significant difference in microbial functional diversity between healthy subjects and periodontitis patients. (12)

Kuboniwa et al, (2016)	Conducted a study to detect the complex biochemical processes and host-bacteria interactions in periodontitis patients and healthy individuals and stated that metabolites, which are released due to bacterial metabolism or host-induced inflammatory processes into oral fluids may help offer potential biomarkers reflecting the severity of periodontitis. Cadaverine, 5-oxoproline, histidine seen to be higher in periodontitis than in healthy individuals. (6)
Sakanaka et al, (2017)	Performed a study to identify robust biomarkers for periodontal inflammation severity using saliva metabolomic data and stated that cadaverine and hydrocinnamate were two metabolites that were majorly implicated in altering not only the degree of inflammation but also the severity of periodontal disease activity in the patients.(13)
Gawron et al, (2019)	Conducted a study to determine metabolic status of the oral cavity in chronic periodontal disease and stated that NMR-based metabolomics analysis is potentially useful methodological approach for monitoring the pathological processes observed in the oral cavity during the course of periodontitis. (14)

Advantages

1. Non-invasive diagnostic technique.
2. Large numbers (tens to hundreds) of metabolites are rapidly measured, in a matter of minutes.
3. Offers the ability to gain a snapshot of different individual and intersecting metabolic pathways.
4. Minimizes the requirement of onsite instrumentation.
5. Offers the possibility of distant diagnosis.

Limitations

1. More diverse, containing many different biological molecules, making it physically and chemically more complex than the other "omics" methods. Limiting the number of metabolites can lead to overlooking of some significant by-products, which might be a bias for targeted method.
2. Relatively small size of the number of endogenous molecules to analyse as compared to the number of genes, mRNAs or proteins.
3. Greater sensitivity and responsiveness of metabolomics approaches suggest its susceptibility in complicating experimental design and interpretation.
4. High set-up costs and requirement for complex informatics.
5. The standard diagnostic approach of periodontal diseases involves clinical and radiological investigations.
6. As these techniques rely on visual and morphological changes associated with disease, they have limitations in early prediction.

Future Perspectives:

Metabolomics can help in developing a prospective disease-specific biomarker based on the molecules. Integrating the microbiological and metabolomic data helps in identifying the physiological effects of microorganisms on host physiology by producing, modifying, or degrading bioactive metabolites as the oral microbiome is emphasized in periodontal research.

It is expected that the analysis of many protein metabolites will simplify the functional links between metabolites whose expression alters in relation to diseases, drugs and other protein metabolites and explicate the mechanisms of periodontal disease development. Newer treatments and drugs, regulating the function of proteins and metabolites is predicted to be established.

Improved diagnostic methods and new biomarkers are needed for diagnosis of periodontal disease to enable the optimization of bold diagnosis and monitoring prognosis. In single omics studies, since other parameters are not available, subgrouping is not commonly performed. Multi-omics approach provides rich and complementary perception in the analysis of periodontitis compared to single-omics studies.(16)

Thus, integrating metabolomics with other omics (e.g., genomics, transcriptomics and proteomics) will enhance the data on the biological stratification of periodontitis and help assembling the best discriminative diagnostic standard and allow us to widen our perspectives on the molecular mechanisms involved in periodontal diseases.(18) Metabolomics, an important challenge can be established to these platforms for routine clinical use.

CONCLUSION:

Saliva and GCF have been broadly inspected in attempts to assess the oral disease status. Contemporary periodontal screening methods are deficient, there is an urgent need for novel approaches in periodontal screening procedures. At present, there are no valid screening tests that detect periodontitis among affected subjects and predict prospective periodontal tissue destruction. Prediction of risk and accurate diagnosis of current disease activity may facilitate effective prevention and treatment of disease. Several different approaches have confirmed that identifying a single marker is unlikely; rather, a combination of biomarkers would be an effective clinical test.

Therefore, there is an essential need for an easy, non-invasive method to detect periodontitis in early stages to allow simple intervention prior to advanced periodontal destruction. Hence, mass spectrometry based oral microbiome and metabolomic analysis has potential for use to expound the pathogenesis of periodontal diseases, thus, contributing towards refining research outcomes and the enhance treatment prediction in the periodontal disease with several advantages as compared with other –omics.

Diagnosis is not the end, but the beginning of practice.

-Martin H Fischer

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