DOI: 10.7860/JCDR/2023/63670.18525

Review Article

Dentistry Section

Metabolomics: A Pioneering Technology for Periodontal Research and Personalised Medicine

LEYA MATHEWS[1], DEVAPRIYA APPUKUTTAN[2], DHAYANAND JOHN VICTOR[3], PSG PRAKASH[4], SANGEETHA SUBRAMANIAN[5]

ABSTRACT

Metabolomics involves the identification and quantitative analysis of all small metabolites present in cells, tissues, and bodily fluids that are formed as a result of biochemical reactions within the cell. These metabolites form a large pool of substrates and can be modified by serving as a substrate for enzymes involved in other metabolic pathways. Therefore, the metabolome in an organism is so dynamic that there is variation in their quantity and chemical composition over time. Nuclear Magnetic Resonance (NMR), Gas Chromatography Mass Spectrometry (GC-MS), and Liquid Chromatography Mass Spectrometry (LC-MS) are the most commonly used technologies in metabolomics. The metabolites are first isolated based on their polarity, chemical composition, and structural resemblances, after which they undergo specialised processes and are then analysed. Metabolomics, coupled with MS, has advanced rapidly and found widespread use in periodontal research. The presence of distinct metabolic and microbiological profiles in different types of periodontitis, as well as their link to clinical indicators of periodontal inflammation, has demonstrated the usefulness of metabolomics in screening, preventing, and monitoring prognosis. Conventional diagnostics fail to detect periodontitis in its early stages, cannot discriminate between past and present disease activity, and are incapable of analysing the entire repertoire of biomarkers in the biological system. Therefore, metabolomics, in conjunction with other omics technologies, can provide tailored periodontal disease therapy. The present review aimed to explore metabolomics, its applications in periodontics, and the potential for personalised treatment.

Keywords: Mass spectrometry, Microbiome, Nuclear magnetic resonance spectroscopy, Omics, Periodontal disease, Personalised medicine

INTRODUCTION

"The Omics Revolution" Life is the culmination of genomic influence, which can be defined as the "master design of intracellular and extracellular genome-directed functions that occur throughout the entire lifetime." In the last decade, many diseases have been identified in their early stages through molecular diagnostic techniques that are part of a new category of tools known as "omics-based diagnostics." These high-throughput screening technologies utilise cellular nucleic acids in their pre-transcriptional and post-transcriptional states, along with complex data analysis and bioinformatics, to enable precision medicine for managing diseases at their inception, potentially preventing them, and assisting in providing personalised therapy for individuals [1].

Data regarding the organisation of the body's biochemistry can be obtained at different omics levels, namely genomics, transcriptomics, proteomics, and metabolomics. The first discipline introduced during the omics revolution was genomics, which focuses on studying entire genomes and exploring complex phenotypes [2]. Epigenomics emphasises the reversible genomic modifications of DNA or DNA-associated proteins, which also regulate gene transcription and ultimately cellular fate. Transcriptomics focuses on RNA, which acts as a molecular intermediate between DNA and proteins. It examines the entire pool of transcripts, both qualitatively and quantitatively, including non coding RNAs [3]. Metabolomics involves the quantitative analysis of all metabolites present in cells, tissues, and bodily fluids. The metabolites are first isolated based on their polarity, chemical composition, and structural resemblances, and then subjected to specialised processes for analysis [4]. The presence of unique metabolic and microbial profiles in various forms of periodontitis and their relationship to clinical markers of periodontal inflammation have unquestionably demonstrated the value of metabolomics in screening, preventing, and monitoring prognosis. Furthermore, when combined with other omics technologies, personalised treatment for periodontitis can be achieved. Proteomics involves profiling all the proteins produced by an organism (proteome) under specific conditions, including their quantity, localisation, post-translational modifications, isoforms, and molecular interactions [5].

The oral cavity harbours numerous bacteria that contribute to the aetiology of various oral diseases. Furthermore, these bacteria have an impact on overall health and are associated with multiple conditions, including diabetes, arthritis, cardiovascular disease, and more. The field of metabolomics has found applications beyond human medicine in animal sciences, agriculture, drug testing, the food industry, environmental sciences, microbiology, and other fields [6]. In periodontitis, the inflammation mediated by the host in response to bacterial biofilm, along with other predisposing factors, is responsible for tissue breakdown. Dentists and Medical professionals have also observed individual differences in clinical presentation and symptoms, highlighting the need to tailor therapy based on the patient's specific genotype, phenotype, and clinical presentation. Numerous clinical studies and supporting research provide evidence for this [7]. Metabolomics addresses this need and offers an advantage over conventional diagnostics by simultaneously identifying various metabolites that indicate health or disease at a specific time point.

Metabolomics

Metabolomics involves the identification and quantification of pools of small metabolites, which are typically less than 1000 Da and serve as intermediates or end products of metabolism in biofluids or tissues. This is achieved through the use of spectroscopic assay techniques [8]. In 1971, Pauling L et al., suggested, based

on urine and breath analysis, that substances in bodily fluids are characteristic of an individual and can be altered to maintain health and treat disease [9]. The term "metabolic profiling" was later introduced by Horning, and Oliver SG described the "metabolome" as the complete set of small molecules in a biological system [2]. The metabolome in an organism is highly dynamic, with variations in both quantity and chemical composition over time, as well as variations across organisms of the same species. External factors such as stress, physical activity, nutrition, pharmaceutical drugs, etc. can influence metabolic pathways, and metabolomics can identify these effects, giving it an advantage over genomics [10].

The workflow of metabolomics can be categorised into untargeted metabolomics, which provides an overall profile of a large number of metabolites, both known and unknown, in biological samples. On the other hand, targeted metabolomics focuses on the analysis of a specific subset of known metabolites or metabolites associated with specific pathways [11]. MS and NMR are essential techniques for data acquisition in metabolomics. NMR spectroscopy offers high reproducibility and accuracy in identifying and quantifying small molecules. It does not require sample pre-treatment, making it non-disruptive and preserving the chemical structure of the metabolites. NMR can also identify phase changes, conformational alterations, solubility, and diffusion potential, and it is used to determine sample purity. However, it requires a large sample volume and has lower sensitivity compared to MS, making it unable to detect low concentration metabolites. Additionally, NMR spectroscopy is quite expensive [11-14].

MS, in combination with chromatographic separation techniques such as GC-MS, LC-MS, and Capillary Electrophoresis (CE-MS), allows for improved separation, high resolution, and quantification of a large number of molecules, leading to better characterisation and analysis [15]. The MS process involves sample quenching, sample extraction, metabolite separation on a column, ionisation of the metabolites, detection of metabolites, data processing, metabolite identification, data analysis, and documentation [16]. Mass analysers in MS separate molecules based on their mass/charge ratio and consist of an ionisation source that adds charge to the molecules to be analysed, a mass analyser, and a detector. GC-MS is suitable for separating and detecting volatile natural metabolites, as well as metabolites that become volatile after derivatisation [17]. LC-MS employs ionisation techniques such as Electrospray Ionisation (ESI), Atmospheric Pressure Chemical Ionisation (APCI), and Atmospheric Pressure Photoionisation (APPI). These ionisation processes do not modify or fragment biomolecules, making them considered soft ionisation techniques. CE-MS uses CE to achieve high efficiency, selectivity, peak capacity, fast analysis, and small sample volume. It is used to analyse polar and ionic compounds without the need for a derivatisation step [18]. The role of metabolomics in oral diseases is significant. It has found broad application in real-time monitoring of underlying pathology in dental caries, periodontitis, oral cancers, and precancerous lesions such as oral leukoplakia, lichen planus, Recurrent Aphthous Ulcers (RAU), and Sjogren's syndrome [7,11,13,19,20].

Although the composition of the dental caries biofilm has been extensively studied, limited information is available regarding its biochemical characteristics. Metabolomics has contributed to filling this knowledge gap. For example, Heimisdottir LH et al., discovered that 16 supragingival plaque metabolites were significantly correlated with the incidence and severity of Early Childhood Caries (ECC). They found lower levels of catechin and epicatechin (which have anti-caries properties) and higher levels of fucose and N-acetylneuraminate [21]. Similarly, Li K et al., used UHPLC-MS to demonstrate that children with ECC have a distinct salivary metabolic profile and microbiota [22]. Takahashi N and Washio J employed CE-MS to target metabolites of the Embden-Meyerhof-Parnas (EMP) pathway, pentose-phosphate pathway, and Tricarboxylic Acid (TCA) cycle in supragingival plaque. They found a comparable metabolite profile with oral microorganisms Streptococcus and Actinomyces. They also observed distinct expressions of metabolites in oral bacteria and plaque samples after a glucose rinse. Furthermore, they demonstrated the usefulness of metabolome analysis in evaluating the therapeutic agent fluoride based on its enolase inhibition and decreased lactate production [23].

Metabolomics has made significant contributions to cancer research by facilitating early detection and diagnosis of oral cancer, leading to improved therapy and survival rates [24]. It has also provided insights into the unique metabolic pathways and metabolism of cancer cells, enhancing the understanding of the underlying pathophysiology and enabling personalised treatment approaches [25]. Furthermore, metabolomics can be used to assess the effectiveness of anti-cancer medications, their impact on specific pathways, and the emergence of drug resistance, ultimately supporting precision medicine [26]. Saliva, tissues, cells, and blood samples are commonly analysed for metabolomic profiling, and scientific evidence indicates the presence of unique profiles that can distinguish between cancerous and pre-cancerous lesions such as oral squamous cell carcinoma, oral lichen planus, and oral leukoplakia [26-28].

Metabolomic profiling has also found applications in the diagnosis of Sjogren's syndrome and the differentiation of the condition from other ocular diseases. Studies have reported differences in metabolites between healthy controls and individuals with Sjogren's syndrome [20,29]. Similarly, the expression of certain proteins in tears has been found to be more accurate in predicting Sjogren's syndrome compared to conventional ocular tests [30]. Salivary metabolomics has been evaluated for the diagnosis of RAU, and dysregulated metabolites associated with tryptophan metabolism, steroid hormone synthesis, and other metabolic pathways have been identified as potential diagnostic markers for RAU [19]. Metabolomic profiling has also shown promising results in evaluating the efficacy of traditional Chinese medicinal herbs in the management of oral ulcers aggravated by sleep deprivation in a rat model and in diagnosing Behcet's disease [31,32].

Various sources of samples have been used for metabolomic evaluations, including saliva, Gingival Crevicular Fluid (GCF), dental plaque, oral rinse, tongue swab, plasma, and serum [21,23,33-36]. Saliva has been extensively explored for metabolic profiling in periodontitis and oral cancer. It is a simple and non-invasive sample to collect, requiring minimal training. Saliva is a complex fluid that contains components from salivary glands, GCF, bacteria, desquamated epithelial cells, immune cells, and cellular products from both the host and microorganisms [37]. The metabolites released into saliva through bacterial metabolism or host-induced inflammatory processes provide valuable insights into the host-bacteria interaction. Salivary metabolomics shows promise but also presents challenges in fully understanding the physiological and pathological processes in the oral cavity, particularly in the diagnosis and prognosis of periodontal disease [38].

The GCF is another significant biofluid present in the periodontal sulcus/pocket, consisting of microbes, host-derived metabolites, and host-microbe-derived metabolites. It can be collected non-invasively, is cost-effective, and provides site-specific information. Therefore, GCF has the potential to reflect the periodontal status based on biomarkers [39]. Dental plaque biofilm is another sample associated with dental caries. It is known that microorganisms in the oral biofilm metabolise dietary carbohydrates to produce organic acids, which decrease pH and initiate demineralisation of dental hard tissues. Metabolic profiling has facilitated the identification of dysregulated metabolic pathways, the characterisation of the microbiota linked to dental caries, and the understanding of the mode of action of therapeutic drugs effective against dental caries, marking the beginning of a new era in the field [21-23,33]. Additionally, dental plaque fluid, a component of dental plaque, contains various metabolites as byproducts of microbial metabolism, which can

also be evaluated to understand the pathogenesis of various oral conditions [19,28-30,33,40].

Metabolomics in Periodontics

Dysbiotic microbiota and periodontal inflammation are significant factors contributing to the pathophysiology of periodontitis. According to Van Dyke's Inflammation-Mediated Polymicrobial Emergence and Dysbiotic Exacerbation (IMPEDE) model from 2020, disease progression occurs due to a shift from a commensal to a pathogenic microbiota in the oral microbiome, triggered by the host's inflammatory response to the bacterial biofilm. This dysbiotic microbial flora is associated with changes in the individual's metabolomics profile [41]. Unlike other omics technologies, metabolomics has the ability to link biological function in both health and disease. This validates the potential of metabolomic analysis as a diagnostic tool for predicting the likelihood of dysbiosis in the periodontium, ultimately leading to improved therapeutic outcomes in personalised medicine.

Chronic periodontitis is primarily characterised by the colonisation and proliferation of gram-negative obligate bacteria, particularly *P. gingivalis* and other bacteria from the red complex, within the periodontal pocket [42]. Metabolic profiling has revealed that *P. gingivalis*, through exotoxins, enzymes such as proteinases, haemolysins, deminases, and toxic metabolites, induces tissue destruction and compromises the integrity of the periodontal tissue [43-45]. Tonzetich J and McBride BC hypothesised that higher levels of Volatile Sulfur Compounds (VSC) also contribute to periodontitis, as they are positively correlated with increased pocket depth [46]. Similarly, lactic acid, a byproduct of oral microbiome activity due to poor oral hygiene and carbohydrate fermentation, also contributes to periodontitis. Lu R et al., demonstrated significantly higher levels of lactic acid in patients with generalised aggressive periodontitis, and these levels correlated with *P. gingivalis*-positive sites [47-48]. Another important metabolomic byproduct, acetone, which is produced from isopropanol, is elevated in chronic periodontitis. Acetone is closely associated with higher lactic acid production, suggesting its presence in *P. gingivalis*-positive sites [49,50]. Glycerol, a crucial component of many metabolic processes, acts as a marker for the presence of the periodontal pathogenic community. The reduced levels of glycerol seen in periodontitis may be attributed to its use as a carbon source or for osmoregulatory functions during bacterial colonisation [51].

These alterations in metabolites reflect the transition from oral health to dysbiosis in chronic periodontitis. The ability of metabolites associated with oxidative stress to distinguish between periodontal disease and health has been demonstrated in a systematic review and meta-analysis by Baima G et al., Increased levels of Malondialdehyde (MDA), 8-hydroxy-deoxyguanosine (8-OHdG), Lysophosphatidic Acid (LPA), 4-hydroxynonenal, and neopterin were consistently observed in GCF levels [40]. Additionally, reduced levels of glutathione in both oxidised and reduced forms were observed in diseased states. The authors suggested that these metabolites could serve as diagnostic markers and be used to monitor periodontal disease activity [52,53].

Kuboniwa M et al., evaluated salivary metabolites using GC/TOF-MS after removal of supragingival plaque and identified eight metabolites in post-debridement saliva that correlated significantly with Periodontal Inflamed Surface Area (PISA), indicating their potential as markers of periodontal inflammation [33]. Similar increases in salivary levels of these metabolites were previously reported by Barnes VM et al., and Aimetti M et al., [54,55]. Furthermore, cadaverine, 5-oxoproline, and histidine showed better sensitivity and specificity in diagnosing moderate and severe periodontitis [33]. In 2017, the authors extended their study and identified certain metabolites in saliva that correlated with PISA, with higher PISA groups showing overexpression of specific metabolic pathways [56]. Similarly, in a large scale study, Liebsch C et al., identified 284 metabolites in non-diabetic subjects using UHPLC-MS/MS and correlated these metabolites with periodontal clinical parameters. They observed that approximately 107 metabolites were associated with at least one periodontal parameter and were linked to tissue degradation, host defence systems, and microbial metabolism. Phenylacetate positively correlated with probing pocket depth across all subjects, regardless of age [57].

Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), GC-MS, and LC-MS, Huang Y et al., conducted groundbreaking research on the metabolomic analysis of the ionome and lipidome at the systems biology level, revealing their contribution to the pathogenesis of chronic periodontitis. The study highlighted the role of altered arachidonic acid and linolenic acid metabolism in oxidative stress in the periodontal environment, as well as the impact of unbalanced metal ions on the cellular redox status in promoting periodontal inflammation. Metabolomics was shown to aid in early diagnosis and intervention at the onset of the disease [58-60].

Barnes VM et al., performed metabolic profiling of saliva in periodontitis subjects and reported a significantly different metabolic profile between healthy and diseased periodontium [61]. Similarly, in 2014, the authors demonstrated the potential utility of metabolomics in periodontitis associated with systemic diseases, such as diabetes [62]. Ozeki M et al., showed elevated levels of specific metabolites in GCF from deep pockets compared to healthy and moderate pockets, suggesting a change in metabolite profile with disease progression [63]. These studies indicate that a comprehensive analysis of metabolites allows for better assessment of disease progression and monitoring of therapeutic interventions. [Table/Fig-1] presents a review of these studies on "Metabolomics in Periodontics" [7,16,33-36,54,55,57,58,61-63].

Metabolomics and its Role in Precision Medicine in Periodontics

A significant limitation of conventional diagnostic techniques in periodontics, such as periodontal pocket probing and clinical attachment loss, is that they detect tissue breakdown only when it has already caused irreparable damage to the periodontal tissues, leading to tooth loss and reduced quality of life. Radiographic imaging also provides information on past disease activity. Additionally, there are inter-individual variations in the clinical presentation and intensity of inflammation based on predisposing factors and co-morbidities.

Author and year of the study	Source of sample, clinical parameters evaluated	Number of samples/ study groups/study population	Technique used for metabolome analysis	Metabolites identified and the inference
Barnes VM et al., 2009 [54]	GCF, PPD, CAL, BOP	N=22, periodontitis patients, GCF Collected from six healthy, six gingivitis and six chronic periodontitis sites in each subjects. 330 GCF samples. The samples were pooled into the above 3 categories: 66 pooled samples American population	GC-MS and LC-MS untargeted	A total of 103 metabolites were identified out of the 228 detected. Increased levels of inosine hypoxanthine, xanthine, guanosine, and guanine, in periodontally diseased areas, indicating purine degradation pathway. Lower levels of antioxidants such uric acid, glutathione, and ascorbic acid was noticed which correlated with the dysregulated redox balance. Putrescine and cadaverine, end products of amino acid degradation, were increased indicating host-microbiome interactions. The findings demonstrated the potential of metabolomics in periodontal disease diagnosis, monitoring and therapy.

Barnes VM et al., 2011 [61]	Unstimulated saliva samples, modified gingival index, BOP, PPD, CAL	Total N=68, 34 healthy controls 34 periodontitis American population	UHPLC/MS/MS for basic species, UHPLC/MS/MS for acidic species, GC-MS	A total of 390 metabolites were identified, 250 were known metabolites, 72 metabolites were increased in periodontitis. Increased mono and oligo saccharides, 15 dipeptides, lysolipids, monoacyl glycerol, fatty acids and metabolites associated with nucleotide metabolism were demonstrated in periodontitis samples indicating inflammatory environment. p-cresol sulfate, phenol sulfate, carnitine and 3-dehydrocarnitine were elevated indicating dysregulated host-microbe interaction in periodontitis. The findings indicated that metabolites in the inflamed periodontium were favourable for dysbiotic microflora thereby potentiating disease progression.
Aimetti M et al., 2012 [55]	Unstimulated saliva sample, PPD, CAL X-rays	N=54, 22 healthy 32 patients with gingivitis, localised and generalised chronic periodontitis and localised and generalised aggressive periodontitis. Italian population	NMR Targeted metabolomics	Increased levels of acetate, γ-aminobutyrate, η-butyrate, succinate, trimethylamine, propionate, phenylalanine and valine, and decreased levels of pyruvate and N-acetyl groups seen in generalised chronic periodontitis. Metabolomics can be used to discriminate the different types of periodontitis.
Zein Elabdeen HR et al., 2013 [35]	Unstimulated saliva and peripheral blood, PPD, CAL	N=38, 19 aggressive periodontitis 19 healthy controls, sudanese population	LC-MS untargeted metabolomics	The results of this study showed increased levels of eicosanoids and docosanoids and various n6- and n3-PUFAs in the GCF, saliva and serum of AgP patients compared with healthy controls. In addition, there were significantly increased concentrations of PGE2 in GCF of AgP patients compared with healthy controls.
Barnes VM et al., 2014 [62]	Unstimulated Saliva sample and plasma, modified gingival index, plaque index, PPD, CAL, HbA1c >6.5%	Total N=161, 81 systemically healthy 80 diabetic patients American population	GC-MS and LC-MS untargeted metabolomics	A total of 772 metabolites and 475 metabolites were identified in plasma and saliva respectively. 69 metabolites were altered in saliva. increased levels of oxidative stress biomarkers like oxidised glutathione and cysteine-glutathione disulfide, increased purine degradation metabolites like guanosine and inosine and ω-6 fatty acid (linoleate and arachidonate) signatures were observed in periodontitis patients. Subjetcs with diabetes and periodontitis showed increased purine degradation, reduced redox balance, dysregulated lipid profile, and increased aminoacid levels.
Huang Y et al., 2014 [58]	Unstimulated saliva and blood, PPD, CAL, BOP, food frequency questionnaire	Total N=50 25 controls 25 periodontitis Chinese population	ICP-MS, GC-MS, LC-MS targeted metabolomics	Decreased levels of Cu, Mn, and Zn and superoxide dismutase was observed. K, Mg, and Ca levels were also reduced suggesting the role of metal ions in maintaining cellular redox balance. Arachidonic metabolites like PGD2, PGE2, PGF2, thromboxane, and 5-HETE, were increased in periodontitis. The data supported the role of arachidonic acid metabolism contributing to oxidative stress, thus leading to periodontal inflammation. Elevated levels of 5-F2t- and 15-F2t-isoprostane in periodontitis indicated oxidative stress. The study emphasised the significance of nutrition in maintaining periodontal homeostasis.
Kuboniwa M et al., 2016 [33]	Unstimulated whole saliva-pre and post supragingival plaque sample removal. PISA -Periodontal Inflamed Surface Area, PPD, CAL, BOP	N=19 (periodontally healthy, mild, moderate and severe periodontitis) 38 saliva samples both pre and post debridement. Japanese population	GC-MS Targeted metabolomics	A total of 63 metabolites were identified and 8 metabolites i.e., Ornithine, cadaverine, valine, proline, spermidine, histidine, 5-oxoproline, hydrocinnamate were significantly associated with the severity of periodontal inflammation. Cadaverine, 5-oxoproline, and histidine together showed better sensitivity and specificity in diagnosing the severity of periodontal inflammation.
Ozeki M et al., 2016 [63]	GCF, PPD, CAL	Total N=30 16 periodontitis 14 healthy patients Japanese Population	GC-MS Untargeted metabolomics	Out of the 19 metabolites identified, significant higher levels of putrescine, lysine, and phenylalanine were present in group of deep-pocket sites in comparison to the group of healthy sites and moderate-pocket sites. Other metabolites like, ribose, taurine, 5-aminovaleric acid, and galactose were also significantly higher in the group of deep-pocket sites in comparison to the group of healthy sites and moderate-pocket sites.
Sakanaka A et al., 2017 [7]	Unstimulated whole saliva-pre and post supragingival plaque sample removal. PISA- Periodontal Inflamed Surface Area, PESA-Periodontal epithelial surface area, modified plaque index	N=50, Totally 100 samples pre and post debridement. 10 healthy controls 28 moderate periodontitis 12 severe periodontitis Japanese population	GC-MS Targeted metabolomics	A total of 69 metabolites were identified. Nine and six metabolites were specific for high and low PISA group. cadaverine and hydrocinnamate correlated with high PISA, uric acid and ethanolamine corelated with lower PISA. Higher PISA groups had overexpression of polyamine metabolism, arginine and proline metabolism, butyric acid metabolism, and lysine degradation pathways. Specific metabolic profile observed in periodontitis.
Chen HW et al., 2018 [16]	GCF and blood, PPD, CAL	Total N=40, 20 healthy controls 20 aggressive periodontitis Chinese population	GC-MS Untargeted metabolomics	A total of 349 metabolites were detected in GCF samples and 200 in serum samples. Patients with GAgP showed increased levels of serum urea and allo-inositol levels and GCF levels of noradrenaline, ribose, lysine, dehydroascorbic acid and xanthine. Decreased levels of GCF glutathione, 2 ketobutyric acid, glycine -d5 and thymidine and serum glutathione, adipic acid, 2,5 dihydroxybenzaldehyde.
Liebsch C et al., 2019 [57]	Stimulated saliva collection, CAL, PPD, missing teeth, caries, fillings, crowns, calculus and plaque scores	N=909 subjects	UHPLC/MS/MS Targeted metabolomics	A total of 284 metabolites were identified and 107 metabolites associated with at least one clinical parameter. PPD related metabolites were phenylalanine, tyrosine catabolites, N6-acetyllysine, pipecolate, isovalerate, isocaproate and ω-6 fatty acid dihomo-linolenate. In terms of plaque and calculus, positive associations with metabolites like 5-oxoproline and 5-aminovalerate (calculus) as well as inversely with levels of urea and phosphate (plaque) were observed. Phenylacetate correlated significantly with periodontal parameters, indicating its utility as a biomarker. These metabolites were associated with the pathways involved in periodontal disease pathogenesis suggesting the utility of metabolomics in periodontics.
Shi M et al., 2020 [34]	GCF and subgingival plaque, PPD, CAL, BI, PI	N=34 24 aggressive periodontitis 10 periodontally healthy	GC-MS Untargeted metabolomics	A total of 103 GCF metabolites were identified, 27 potential metabolites significantly different in GAgP. levels of glucose, uridine, alanine, isoleucine, maltotriose, putrescine, 5-aminovaleric acid, valine, oxoproline, and leucine were significantly higher in AgP. periodontitis-associated genera were positively correlated with clinical indicators, such as treponema, filifactor, tannerella, and peptostreptococcaceae. glucose, alanine, and aspartic acid had positive relationship with increased clinical indices.

Rodrigues WF et al., 2021 [36]	GCF, PPD, CAL	N=120, 60 healthy controls 60 periodontitis patients, older Brazillian adults	GC-MS	A total of 969 metabolites were identified among those 64 were identified in both the groups .The metabolites 2,3-dihydroxypropyl icosanoate, glycerol, serine, 5-aminovaleric acid, and putrescine levels were elevated by 5 times in periodontitis subjects.lactulose, oxalic acid, 1-benzoyl-2-t-butyl-5-ethyl-3-methyl-5-vinyl-imidazolidin-4-one, and maltose were elevated in periodontally healthy subjects. 5-aminovaleric acid and serine showed significantly increased levels in periodontitis. Specific metabolites can be used as biomarkers for evaluating periodontitis in elderly adults.

[Table/Fig-1]: Review of studies discussed in "Metabolomics in Periodontics" [7,16,33-36,54,55,57,58,61-63].
PPD: Pocket probing depth; CAL: Clinical attachment level; BOP: Bleeding on probing; BI: Bleeding index; PI: Plaque index; GC: Gas chromatography; LC: Liquid chromatography; MS: Mass spectrometry; GCF: Gingival crevicular fluid; PISA: Periodontal inflamed surface area; UHPLC: Ultra high performance level chromatography; ICP-MS: Inductively coupled plasma mass spectrometry; NMR: Nuclear magnetic resonance; PG: Prostaglandin; 5-HETE: 5-Hydroxyeicosatetraenoic acid; GAgP: Generalised aggressive periodontitis

To overcome these challenges and provide early and accurate diagnosis, advanced diagnostic technologies need to be considered. There is now a greater emphasis on the effects of nutrition and lifestyle on periodontal health. Therefore, it is important to integrate nutrigenomics, proteomics, and metabolomics in a synergistic manner to develop personalised therapy approaches. By integrating omics technologies, we can study the various biological interactions within the system and the system's response to the environment, allowing for a more comprehensive characterisation of individuals at the systems level and enabling personalised medicine [64].

The detailed and accurate quantification of metabolites can facilitate the identification of specific metabolic pathways involved in periodontal disease. This information can help in identifying new drug targets and evaluating drug efficacy and toxicity. This approach to treatment aligns with the P4 model of healthcare, which focuses on prediction, prevention, precision, and personalisation.

Drawbacks/Challenges in the Application of Metabolomics

- Metabolite measurement involves multiple platforms, and during this process, metabolites can undergo transformations or degradation. To obtain accurate data, it is crucial to maintain standardisation in metabolomics applications, including proper sample storage and transportation. This may require expensive equipment and highly skilled operators.
- Choosing the appropriate analytical method can be challenging because no single tool can capture the entire metabolome. Additionally, the raw data generated from analysis is complex and voluminous, increasing the likelihood of errors at various steps.
- There are inconsistencies and discrepancies in the detection of metabolites related to periodontitis reported in the literature. These discrepancies can arise from variations in sample processing methods and the use of different data analysis tools.
- Studies have shown that systemic factors, such as inflammatory bowel disease and alterations in the gut microbiota, can influence the oral microbial flora and its metabolites. Similarly, conditions like obesity can lead to pathological changes in the periodontium. Therefore, it is important to consider systemic factors and interpret the results carefully.
- Integrating multiomics data can improve the accuracy of predicting disease conditions. However, as the data becomes semiquantitative,, the accuracy decreases [65].

CONCLUSION(S)

Metabolomics is rapidly emerging as a cutting-edge and expanding technology in periodontal research, supported by a growing body of evidence. Advances in instrumentation and data analysis software are continuously improving the accuracy, reproducibility, specificity, and sensitivity of metabolomics analysis using techniques like NMR and MS. In the field of periodontal research, metabolomics has shown great potential in several areas. It can serve as a tool for early diagnosis, monitoring disease progression, differentiating between different types of periodontitis, evaluating the effectiveness of therapy, assessing drug efficacy, and distinguishing periodontitis associated with systemic diseases. By overcoming the limitations of conventional diagnostic tools, metabolomics opens up the possibility of diagnosing periodontal disease at its early stages. When combined with other "omics" technologies, it enables personalised periodontics and individualised therapy approaches.

REFERENCES

[1] Tebani A, Afonso C, Marret S, Bekri S. Omics-based strategies in precision medicine: Toward a paradigm shift in inborn errors of metabolism investigations. International Journal of Molecular Sciences. 2016;17(9):1555.
[2] Oliver SG. From DNA sequence to biological function. Nature. 1996;379(6566):597-600.
[3] Horgan RP, Kenny LC. 'Omic'technologies: Genomics, transcriptomics, proteomics and metabolomics. The Obstetrician & Gynaecologist. 2011;13(3):189-95.
[4] Goodacre R, Vaidyanathan S, Dunn WB, Harrigan GG, Kell DB. Box 1. Classification of metabolomic approaches. Trends in Biotechnology. 2004;22(5):245-252.
[5] Overmyer KA, Rhoads TW, Merrill AE, Ye Z, Westphall MS, Acharya A, et al. Proteomics, lipidomics, metabolomics, and 16S DNA sequencing of dental plaque from patients with diabetes and periodontal disease. Mol Cell Proteomics. 2021;20:100126.
[6] Kaddurah-Daouk R, Weinshilboum R. Pharmacometabolomics Research Network. Metabolomic signatures for drug response phenotypes: Pharmacometabolomics enables precision medicine. Clinical Pharmacology & Therapeutics. 2015;98(1):71-75.
[7] Sakanaka A, Kuboniwa M, Hashino E, Bamba T, Fukusaki E, Amano A. Distinct signatures of dental plaque metabolic byproducts dictated by periodontal inflammatory status. Scientific Reports. 2017;7(1):1-0.
[8] Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, et al. HMDB 3.0-the human metabolome database in 2013. Nucleic Acids Research. 2012;41(D1):D801-07.
[9] Pauling L, Robinson AB, Teranishi R, Cary P. Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. Proceedings of the National Academy of Sciences (PNAS). 1971;68(10):2374-76.
[10] Jeong H Tombor B, Albert R, Oltvai ZN, Barabasi AL. The large-scale organization of metabolic networks. Nature. 2000;407:651-54.
[11] Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: Beyond biomarkers and towards mechanisms. Nat Rev Mol cell Biol. 2016;17(7):451-59.
[12] Gowda GN, Zhang S, Gu H, Asiago V, Shanaiah N, Raftery D. Metabolomics-based methods for early disease diagnostics. Expert Review of Molecular Diagnostics. 2008;8(5):617-33.
[13] Zia K, Siddiqui T, Ali S, Farooq I, Zafar MS, Khurshid Z. Nuclear magnetic resonance spectroscopy for medical and dental applications: A comprehensive review. European Journal of Dentistry. 2019;13(01):124-28.
[14] Nicholson JK, Lindon JC. Metabonomics. Nature. 2008;455(7216):1054-56.
[15] Luan H, Wang X, Cai Z. Mass spectrometry-based metabolomics: Targeting the crosstalk between gut microbiota and brain in neurodegenerative disorders. Mass Spectrom Rev. 2019;38(1):22-33.
[16] Chen HW, Zhou W, Liao Y, Hu SC, Chen TL, Song ZC. Analysis of metabolic profiles of generalized aggressive periodontitis. Journal of Periodontal Research. 2018;53(5):894-901.
[17] Dettmer K, Aronov PA, Hammock BD Mass spectrometry-based metabolomics. Mass Spectrom Rev. 2007;26(1):51-78.
[18] Gao P, Xu G. Mass-spectrometry-based microbial metabolomics: Recent developments and applications. Anal and Bioanal Chem. 2015;407(3):669-80.
[19] Li Y, Wang D, Zeng C, Liu Y, Huang G, Mei Z. Salivary metabolomics profile of patients with recurrent aphthous ulcer as revealed by liquid chromatography–tandem mass spectrometry. Journal of International Medical Research. 2018;46(3):1052-62.
[20] Kageyama G, Saegusa J, Irino Y, Tanaka S, Tsuda K, Takahashi S, et al. Metabolomics analysis of saliva from patients with primary Sjögren's syndrome. Clinical & Experimental Immunology. 2015;182(2):149-53.
[21] Heimisdóttir LH, Lin BM, Cho H, Orlenko A, Ribeiro AA, Simon-Soro A, et al. Metabolomics insights in early childhood caries. Journal of Dental Research. 2021;100(6):615-22.
[22] Li K, Wang J, Du N, Sun Y, Sun Q, Yin W, et al. Salivary microbiome and metabolome analysis of severe early childhood caries. BMC Oral Health. 2023;23(1):01-08.
[23] Takahashi N, Washio J. Metabolomic effects of xylitol and fluoride on plaque biofilm in vivo. Journal of Dental Research. 2011;90(12):1463-68.
[24] Wang Q, Gao P, Wang X, Duan Y. Investigation and identification of potential biomarkers in human saliva for the early diagnosis of oral squamous cell carcinoma. Clinica Chimica Acta. 2014;427:79-85.

[25] Chen X, Yu D. Metabolomics study of oral cancers. Metabolomics. 2019;15:01-05.
[26] Wang H, Chen J, Feng Y, Zhou W, Zhang J, Yu YU, et al. 1H nuclear magnetic resonance-based extracellular metabolomic analysis of multidrug resistant Tca8113 oral squamous carcinoma cells. Oncology Letters. 2015;9(6):2551-59.
[27] Gupta A, Gupta S, Mahdi AA. 1H NMR-derived serum metabolomics of leukoplakia and squamous cell carcinoma. Clinica Chimica Acta. 2015;441:47-55.
[28] Wei J, Xie G, Zhou Z, Shi P, Qiu Y, Zheng X, et al. Salivary metabolite signatures of oral cancer and leukoplakia. International Journal of Cancer. 2011;129(9):2207-17.
[29] Fernández-Ochoa Á, Borrás-Linares I, Quirantes-Piné R, Alarcón-Riquelme ME, Beretta L, Segura-Carretero A, Precisesads Clinical Consortium. Discovering new metabolite alterations in primary sjögren's syndrome in urinary and plasma samples using an HPLC-ESI-QTOF-MS methodology. Journal of Pharmaceutical and Biomedical Analysis. 2020;179:112999.
[30] Versura P, Giannaccare G, Vukatana G, Mulè R, Malavolta N, Campos EC. Predictive role of tear protein expression in the early diagnosis of Sjögren's syndrome. Ann Clin Biochem. 2018;55(5):561-70. Doi: 10.1177/0004563217750679. Epub 2018 Jan 30. PMID: 29310465.
[31] Chen P, Yao H, Su W, Zheng Y, Fan W, Zhang L, et al. Pharmacodynamic and metabolomics studies on the effect of Kouyanqing granule in the treatment of phenol-induced oral ulcer worsened by sleep deprivation. Frontiers in Pharmacology. 2020;11:824.
[32] Park SJ, Park MJ, Park S, Lee ES, Lee DY. Integrative metabolomics of plasma and PBMCs identifies distinctive metabolic signatures in Behçet's disease. Arthritis Research & Therapy. 2023;25(1):01-03.
[33] Kuboniwa M, Sakanaka A, Hashino E, Bamba T, Fukusaki E, Amano A. Prediction of periodontal inflammation via metabolic profiling of saliva. Journal of Dental Research. 2016;95(12):1381-86.
[34] Shi M, Wei Y, Nie Y, Wang C, Sun F, Jiang W, et al. Alterations and correlations in microbial community and metabolome characteristics in generalized aggressive periodontitis. Frontiers in Microbiology. 2020;11:573196.
[35] Zein Elabdeen HR, Mustafa M, Szklenar M, Rühl R, Ali R, Bolstad AI. Ratio of pro-resolving and pro-inflammatory lipid mediator precursors as potential markers for aggressive periodontitis. PloS one. 2013;8(8):e70838.
[36] Rodrigues WF, Miguel CB, Agostinho F, da Silva GV, Lazo-Chica JE, Naressi Scapin SM, et al. Metabolomic evaluation of chronic periodontal disease in older adults. Mediators of inflammation. 2021;2021:1796204.
[37] Patil PB, Patil BR. Saliva: A diagnostic biomarker of periodontal diseases. J Indian Soc Periodontol. 2011;15(4):310-17.
[38] Hyvärinen E, Savolainen M, Mikkonen JJ, Kullaa AM. Salivary metabolomics for diagnosis and monitoring diseases: Challenges and possibilities. Metabolites. 2021;11(9):587.
[39] Baima G, Corana M, Iaderosa G, Romano F, Citterio F, Meoni G, et al. Metabolomics of gingival crevicular fluid to identify biomarkers for periodontitis: A systematic review with meta-analysis. Journal of Periodontal Research. 2021;56(4):633-45.
[40] Bowen WH, Burne RA, Wu H, Koo H. Oral biofilms: Pathogens, matrix, and polymicrobial interactions in microenvironments. Trends Microbiol. 2018;26(3):229-42.
[41] Van Dyke TE, Bartold PM, Reynolds EC. The nexus between periodontal inflammation and dysbiosis. Frontiers in Immunology. 2020;11:511.
[42] Mysak J, Podzimek S, Sommerova P, Lyuya-Mi Y, Bartova J, Janatova T, et al. Porphyromonas gingivalis: Major periodontopathic pathogen overview. Journal of Immunology Research. 2014;2014:476068.
[43] Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 2000. 1994;5(1):78-111.
[44] Nachnani S, Scuteri A, Newman MG, Avanessian AB, Lomeli SL. E-test: A new technique for antimicrobial susceptibility testing for periodontal microorganisms. Journal of Periodontology. 1992;63(7):576-83.
[45] Lamont RJ, Jenkinson HF. Life below the gum line: Pathogenic mechanisms of Porphyromonas gingivalis. Microbiology and molecular biology reviews. 1998;62(4):1244-63.
[46] Tonzetich J, McBride BC. Characterization of volatile sulphur production by pathogenic and non-pathogenic strains of oral Bacteroides. Arch Oral Biol. 1981;26(12):963-69.
[47] Lu R, Meng H, Gao X, Xu L, Feng X. Effect of non-surgical periodontal treatment on short chain fatty acid levels in gingival crevicular fluid of patients with generalized aggressive periodontitis. Journal of Periodontal Research. 2014;49(5):574-83.
[48] Sookkhee S, Chulasiri M, Prachyabrued W. Lactic acid bacteria from healthy oral cavity of Thai volunteers: Inhibition of oral pathogens. J Appl Microbiol. 2001;90(2):172-79.
[49] Ruzsányi V, Kalapos MP. Breath acetone as a potential marker in clinical practice. Journal of Breath Research. 2017;11(2):024002.
[50] Kalapos MP. On the mammalian acetone metabolism: From chemistry to clinical implications. Biochim Biophys Acta. 2003;1621(2):122-39.
[51] Kato T, Hayashi Y, Inoue K, Yuasa H. Glycerol absorption by Na+-dependent carrier-mediated transport in the closed loop of the rat small intestine. Biological and Pharmaceutical Bulletin. 2005;28(3):553-55.
[52] Tonguç MÖ, Öztürk Ö, Sütçü R, Ceyhan BM, Kılınç G, Sönmez Y. The impact of smoking status on antioxidant enzyme activity and malondialdehyde levels in chronic periodontitis. J Periodontol. 2011;82(9):1320-28.
[53] Bathena SP, Huang J, Nunn ME, Miyamoto T, Parrish LC, Lang MS. Quantitative determination of lysophosphatidic acids (LPAs) in human saliva and gingival crevicular fluid (GCF) by LC–MS/MS. J Pharm Biomed Anal. 2011;56(2):402-07.
[54] Barnes VM, Teles R, Trivedi HM, Devizio W, Xu T, Mitchell MW, et al. Acceleration of purine degradation by periodontal diseases. Journal of Dental Research. 2009;88(9):851-55.
[55] Aimetti M, Cacciatore S, Graziano A, Tenori L. Metabonomic analysis of saliva reveals generalized chronic periodontitis signature. Metabolomics. 2012;8:465-74.
[56] Nesse W, Abbas F, Ploeg I van der, Spijkervet FK, Dijkstra PU, Vissink A. Periodontal inflamed surface area: Quantifying inflammatory burden. J Clin Periodontol. 2008;35(8):668-73.
[57] Liebsch C, Pitchika V, Pink C, Samietz S, Kastenmüller G, Artati A, et al. The saliva metabolome in association to oral health status. Journal of Dental Research. 2019;98(6):642-51.
[58] Huang Y, Zhu M, Li Z, Sa R, Chu Q, Zhang Q, et al. Mass spectrometry-based metabolomic profiling identifies alterations in salivary redox status and fatty acid metabolism in response to inflammation and oxidative stress in periodontal disease. Free Radical Biol Med. 2014;70:223-32.
[59] Williams CS, Mann M, DuBois RN. The role of cyclooxygenases in inflammation, cancer, and development. Oncogene. 1999;18(55):7908-16.
[60] Hartung NM, Ostermann AI, Immenschuh S, Schebb NH. Combined targeted proteomics and oxylipin metabolomics for monitoring of the COX-2 pathway. Proteomics. 2021;21(3-4):1900058.
[61] Barnes VM, Ciancio SG, Shibly O, Xu T, Devizio W, Trivedi HM, et al. Metabolomics reveals elevated macromolecular degradation in periodontal disease. Journal of Dental Research. 2011;90(11):1293-97.
[62] Barnes VM, Kennedy AD, Panagakos F, Devizio W, Trivedi HM, Jönsson T, et al. Global metabolomic analysis of human saliva and plasma from healthy and diabetic subjects, with and without periodontal disease. PloS one. 2014;9(8):e105181.
[63] Ozeki M, Nozaki T, Aoki J, Bamba T, Jensen KR, Murakami S, et al. Metabolomic analysis of gingival crevicular fluid using gas chromatography/mass spectrometry. Mass Spectrometry. 2016;5(1):A0047.
[64] Sengupta A, Uppoor A, Joshi MB. Metabolomics: Paving the path for personalized periodontics-A literature review. J Indian Soc of Periodontol. 2022;26(2):26(2):98-103.
[65] Pei J, Li F, Xie Y, Liu J, Yu T, Feng X. Microbial and metabolomic analysis of gingival crevicular fluid in general chronic periodontitis patients: Lessons for a predictive, preventive, and personalized medical approach. EPMA J. 2020;11(2):197-215.

PARTICULARS OF CONTRIBUTORS:
1. Postgraduate, Department of Periodontics, SRM Dental College, Ramapuram, Chennai, Tamil Nadu, India.
2. Professor, Department of Periodontics, SRM Dental College, Ramapuram, Chennai, Tamil Nadu, India.
3. Professor and Head, Department of Periodontics, SRM Dental College, Ramapuram, Chennai, Tamil Nadu, India.
4. Professor, Department of Periodontics, SRM Dental College, Ramapuram, Chennai, Tamil Nadu, India.
5. Professor, Department of Periodontics, SRM Dental College, Ramapuram, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Devapriya Appukuttan,
Professor, Department of Periodontics, SRM Dental College, Ramapuram
Chennai-600089, Tamil Nadu, India.
E-mail: devapriyamds@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Mar 06, 2023
- Manual Googling: Jun 10, 2023
- iThenticate Software: Aug 19, 2023 (8%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? NA
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Feb 23, 2023**
Date of Peer Review: **May 26, 2023**
Date of Acceptance: **Aug 22, 2023**
Date of Publishing: **Oct 01, 2023**