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REVIEW ARTICLE

Periodontal disease metabolomics signatures from different biofluids: a systematic review

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Abstract

Background Periodontitis is resulted from a complex interaction between genetics and epigenetics, microbial factors, and the host response. Metabolomics analyses reflect both the steady-state physiological equilibrium of cells or organisms as well as their dynamic metabolic responses to environmental stimuli.

Aim of review This systematic review of the literature aimed to assess which low molecular weight metabolites are more often found in biological fluids of individuals with periodontitis compared to individuals with gingivitis or periodontal health.

Key scientific concepts of review All the included studies employed untargeted analysis. One or more biological fluids were analyzed, including saliva (n = 14), gingival crevicular fluid (n = 6), mouthwash (n = 1), serum (n = 3) and plasma (n = 1). Fifty-six main metabolites related to periodontitis have been identified in at least two independent studies by NMR spectroscopy or MS-based metabolomics. Saliva was the main biological fluid sampled. It is noteworthy that 14 metabolites of the 56 detected were identified as main metabolites in all studies that sampled the saliva. The majority of metabolites found consistently among studies were amino acids, organic acids and derivates: acetate, alanine, butyrate, formate, GABA, lactate, propionate, phenylalanine and valine. They were either up- or down-regulated in the studies or this information was not mentioned. The main metabolic pathway was related to phenylalanine, tyrosine and tryptophan biosynthesis. Metabolites more frequently found in individuals with periodontitis were related to both the host and to microorganism responses. Future studies are needed, and they should follow some methodological standards to facilitate their comparison.

Keywords Saliva · Metabolomics · Nuclear magnetic resonance · Mass spectroscopy · Systematic review

1 Introduction

Periodontal diseases are a chronic inflammatory condition and involve multiple causal components that play a role simultaneously, and interact with each other, in an unpredictable way (Loos & Van Dyke, 2020). This condition is the result of a complex interaction between genetics and epigenetics, microbial factors and the host response (Loos & Van Dyke, 2020) and can be divided into gingivitis and periodontitis. Gingivitis is considered an incipient dysbiosis because in non-susceptible individuals it does not progress beyond inflammatory signs in the gingival tissue. In contrast, periodontitis is a frank dysbiosis that perpetuates chronic non-resolving and destructive inflammation due to an aberrant host response (Meyle & Chapple, 2015). Periodontitis involves the destruction of the periodontal ligament, bone, and gingival tissues, and can lead to tooth loss. Periodontitis behaves in a nonlinear fashion. It has been established that the disease can involve bursts of activity followed by periods of quiescence or stability, which are not easily measured clinically (Loos & Van Dyke, 2020). For oral health-care clinicians, there can be a level of uncertainty in predicting successful periodontal site-specific treatment outcomes and stability (Korte & Kinney, 2016). Thus, the need exists for supplemental diagnostic tools (Korte & Kinney, 2016).

Personalized medicine is a medical model that uses genetic, genomic, environmental and clinical diagnostic

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testing to individualize patient care (Nguyen et al., 2020). This approach utilizes clinical assessment and subclinical profiles to develop highly individualized diagnosis, prognosis and treatment algorithms (Korte & Kinney, 2016). Personalized medicine for periodontal diseases may involve utilization of biological fluids such as gingival crevicular fluid (GCF) and saliva to develop subclinical profiles, identifying and measuring specific genotypes, phenotypes, putative pathogens, inflammatory markers and collagen-degradation biomarkers to make informed clinical decisions about disease susceptibility, site-specific risk and treatment interventions (Korte & Kinney, 2016). When considering the periodontal pathogenic processes, periodontitis can generally be divided into three phases: inflammation; connective tissue degradation; and bone turnover. During each phase of the disease, specific host-derived biomarkers have been identified and therefore might provide a general sense of what stage of pathologic breakdown the patient is currently experiencing (Korte & Kinney, 2016).

Metabolomics is emerging as an important tool to characterize patient phenotypes in parallel with other -omics platforms. Metabolomics analyses reflect both the steady-state physiological equilibrium of cells or organisms as well as their dynamic metabolic responses to environmental stimuli. In comparison to other human biological measurements, the metabolome is uniquely suited to depict the phenotype and measure the impact of environmental factors on the end products of metabolism (Tolstikov et al., 2020). Changes in metabolite composition are important in understanding the microbial–host response in periodontal disease (Nguyen et al., 2020). Identification of metabolites as potential biomarkers of periodontal disease status or pathogenesis is of interest to understand the metabolic mechanisms underlying periodontal disease and to target patient treatment. Changes in metabolite composition associated with states of disease may allow the identification of metabolic biomarkers that can be used in a variety of applications, including early disease detection, evaluation of current disease status, and examination of pathways triggered or altered in the diseased state (Almeida et al., 2017; de Oliveira et al., 2016; Fidalgo et al., 2013, 2015; Freitas-Fernandes et al., 2020; Nguyen et al., 2020).

The challenges associated with metabolomic studies in periodontal disease include the high variability that exists in the chemical structure and properties of the metabolites (Nguyen et al., 2020). Metabolic biomarkers of periodontal disease have been identified via the analysis of saliva, serum, plaque, and GCF (Nguyen et al., 2020). Such metabolic phenotyping studies are able to provide new insights into disease pathophysiology (Beger et al., 2016). The objective of the present systematic review was to summarize the existing information about human biofluids metabolomic findings in periodontal disease.

2 Material and methods

2.1 Protocol and registration

This review adhered to the PRISMA statement (Preferred Reporting Items for Systematic Review and Meta-Analysis) guidelines (Moola et al., 2020). This systematic review protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO), registration number: CRD42021253486.

2.2 Search strategy

The search procedures were performed independently by two examiners (F.B. and H.F.). The electronic databases PubMed/Medline, SCOPUS, EMBASE, ISI Web of Knowledge and Google-Scholar were searched for studies published until November 2020, without language or year restrictions. The electronic search strategy was developed using the most cited descriptors in previous publications on this theme, combining Medical Subject Heading terms (MeSH) and free terms as title/abstract (tiab). Initially the search strategy was developed for PubMed and then adapted to the other databases. The following MeSH and tiab terms were used to search PubMed: ("Metabolome", "Metabolomics"), "periodontitis", periodontal disease, "humans". The boolean operators "AND" and "OR" were applied to combine the terms and create the search strategy. The search strategies defined for each database are detailes in the Open Science Framework repository (https://doi.org/10.17605/OSF.IO/ZK5HE). No filters or limits were applied in the searches. Disagreements related to the inclusion of studies were resolved via discussion and consultation with the third author (T.K.S.F.). A complementary screening of the references in the selected studies was performed and a hand search in the *Journal of Periodontology, Metabolomics*, *Journal Clinical Periodontology* and *Periodontology 2000* was performed to find any additional studies that did not appear in the primary database search. Articles from different sources were imported to the EndNote Web reference manager (EndNote™), to catalog the references and automatically remove duplicate records. Duplicate studies in the database search were considered only once.

2.3 Eligibility criteria

Human studies that evaluated the low molecular weight metabolites of subjects with and without periodontal disease were included without age restriction. The criteria

adopted for the eligibility of the studies to be included in this research were defined based on the elements of the PECOS strategy, as follows:

P (Population)—Biological fluids

E (Exposure)—Periodontitis

C (Comparison)—Gingivitis or periodontal health

O (Outcome)—Low molecular weight metabolites analyzed through metabolomic techniques

S (Study design)—Cross-sectional, prospective, parallel, and crossover designs

2.4 Exclusion criteria

Articles, or at least the abstract, must have been written in English. Conference abstracts, reviews, meta-analyses, case reports, ecological studies, and letters to the editor were excluded.

Patients with periodontal disease and other systemic diseases other than diabetes mellitus were excluded from the study. Diabetes was not excluded since is an important modifying factor of periodontitis, and according to the New Classification of Periodontal Diseases (Caton et al., 2018), diabetes should be included in a clinical diagnosis of periodontitis as a descriptor since there are no characteristic phenotypic features that are unique to periodontitis in patients with diabetes mellitus (Jepsen et al., 2018).

2.5 Data extraction and metabolic pathway

Two authors (F.B. and H.F.) independently collected the data from the included studies. Eventual disagreements were resolved consensually, and if the disagreement remained, a third experienced author made a decision (T.K.S.F.). Information regarding publication (author and publication year), study design, methods, statistical analysis, source of biological sample, number of subjects in the study, oral condition, number of metabolites detected, main metabolites that presented statistical difference, and perturbed pathways was identified. In the case of missing data, up to three attempts to contact the respective authors were made by email.

Based on the list of the most frequently found metabolites identified in the manuscripts, a pathway analysis was performed using Metaboanalyst 4.0. The following metabolites that presented statistical difference in saliva between groups were included in the analysis: butyrate, choline, ethanol, isopropanol and methanol, acetate, alanine, butyrate, formate, GABA, lactate, propionate, phenylalanine and valine. The pathway analysis was based on saliva since it is easily collected by untrained individuals, it is painless, and especially because the main metabolites found in saliva were not discrepant among the studies. The pathway analysis was obtained using the input type "compound name", the visualization method was scatter plot, the enrichment method was hypergeometric test, the topology analysis was relative-betweenness centrality, the reference metabolome was all compounds in the selected pathway library, and the pathway library was Homo sapiens (KEGG).

2.6 Quality assessment

The methodological quality of the studies was assessed by two independent evaluators (T.K.S.F. and F.B.) using the Joanna Briggs Institute (JBI) Critical Appraisal Tool for cross-sectional studies (Moola et al., 2020). The JBI tool consists of eight questions that address the following parameters: inclusion criteria, description of the population included, risk of bias, outcomes and statistical analysis. The following variables were considered:

- Were the criteria for inclusion in the sample clearly defined?
  Whether the inclusion/exclusion criteria were addressed in the studies.
- Were the study subjects and the setting described in detail?
  Whether the sample of participants was described with information such as age and gender.
- Was the exposure measured in a valid and reliable way?
  Whether the study clearly described which reference was followed to determine periodontal status.
- Were objective, standard criteria used for measurement of the condition?
  Whether the study mentioned how the exam was done and if there was training and calibration.
- Were confounding factors identified?
  Whether there were confounders such as the inclusion of diseases, patients who used antibiotics, pregnant women, smokers and even saliva collection at a standardized time due to the variation in the production of metabolites related to the circadian cycle of saliva.
- Were strategies to deal with confounding factors stated?
  Whether strategies were used to deal with the confounders, such as multivariate analysis or separate analysis of data.
- Were the outcomes measured in a valid and reliable way?
  Whether the metabolomic evaluation methods were correct, that is, validated in the literature.
- Was appropriate statistical analysis used?
  Whether an adequate statistical analysis was performed.

The classification system was determined by the authors, judging each answer as a possible concern, with "No" being assigned for low risk and "Yes" for high risk. When at least one "high risk" response was attributed to studies, the study was classified as high risk of bias.

2.7 Strength of evidence

The Grading of Recommendations, Assessment, Development and Evaluations (GRADE) tool was used to evaluate the strength of the evidence of the included studies (Guyatt et al., 2011). This tool assesses information about the overall included studies, such as the number of participants, the risk of bias, inconsistency, indirectness, imprecision, and publication bias. It allows the classification of the quality of the evidence as very low, low, moderate or high strength of evidence, based on the confounders present, in addition to the summary of the findings.

3 Results

Figure 1 shows the flow diagram of the search strategy. Initially, the search resulted in 353 studies published in the relevant databases and 6 studies were found in other sources. Of these, 114 were excluded due to duplication, 212 were excluded because they were not associated with the topic, and 13 were excluded because they did not satisfy the inclusion criteria. The analysis of titles and abstracts culminated in the selection of 21 suitable published studies (Tables 1, 2, 3).

3.1 Data extraction and metabolic pathway analysis

All the included studies employed untargeted analysis. Three studies did the assessment pre and post biofilm debridement (Kuboniwa et al., 2016; Romano et al., 2019; Sakanaka et al., 2017). According to the results reported by the authors, we divided the studies into three main categories. The first main category included studies in which metabolites were reported to be related to microorganisms (Table 1), the second main category included studies in which metabolites were reported to be related to the host response (Table 2) and the third main category included studies in which metabolites were reported to be related to both microorganisms and to the host response (Table 3).

Identified metabolites that were considered as the main metabolite in at least two independent studies and their expression in periodontal disease (up or downregulated) are shown in Table 4. Figure 2 shows each metabolite's super class (HMDB, 2022), the biological medium from which it was sampled and the type of response to which it was reported to be associated. Figure 3 shows the impacted pathway, the main metabolic pathway was related to phenylalanine, tyrosine and tryptophan biosynthesis. The following 6 metabolic pathways were related (Table 5): Aminoacyl-tRNA biosynthesis, Butanoate metabolism, Pyruvate metabolism, Glycolysis/Gluconeogenesis, Glyoxylate and dicarboxylate metabolism, and Phenylalanine, tyrosine and tryptophan biosynthesis.

3.2 Assessment of the risk of bias

The 17 included studies presented a cross-sectional design (Tables 1, 2 and 3). Citterio et al., (2020) performed a treatment but only their baseline data was included in this systematic review. Similarly, Liebsch et al. (2019) data was part of a bigger cohort study; however, the authors collected the data without follow-up. The quality assessment and risk of bias demonstrated that six studies (Chen et al., 2018; Citterio et al., 2020; Kuboniwa et al., 2016; Pei et al., 2020; Sakanaka et al., 2017; Shi et al., 2020) presented a low risk of bias and 10 high risk (Aimetti et al., 2012; Barnes et al., 2009, 2011, 2014; Huang et al., 2014; Liebsch et al., 2019; Ozeki et al., 2016; Romano et al., 2018; Rzeznik et al., 2017; Singh et al., 2017) (Table 6).

The major concerns were the absence of a standard method, especially the absence of inter-individual assessment of the clinical measurements, and confounding, with the inclusion of patients with systemic diseases of smokers without controlling for these variables in the statistical analysis. Ozeki et al. (2016), Sakanaka et al. (2017) and Singh et al. (2017) did not mention their inclusion criteria, which was considered a concern. Ozeki et al. (2016) and Barnes et al. (2009) did not mention the reference used for classification of the periodontal condition. Romano et al. (2018) and Rzeznik et al. (2017) included smokers, but controlled for this in the statistical analysis. Liebsch et al. (2019) did not mention whether they excluded patients with comorbidities, smokers, those taking antibiotics etc. Barnes et al. (2014) included patients with diabetes, but considered this condition in the statistical analysis; they did not report the cigarette smoking data. Aimetti et al. (2012) included systemic diseases in the analysis such as hypertension, heart vessel alterations, Parkinson's disease, chronic bronchitis, diabetes, and osteoporosis. Barnes et al. (2009) did not mention any exclusions related to smokers.

3.3 Strength of evidence

The strength of evidence was rated very low (Table 7). Regarding the "risk of bias" domain, there was a downgrade due to the presence of confounders, such as the inclusion of patients with systemic diseases and smokers. The "inconsistency" domain was not downgraded, since the results found were consistent among the different studies. The "indirect" domain was not downgraded since the studied population was compatible with the groups. The "imprecision" domain was downgraded because the studies had samples smaller than 300 individuals.

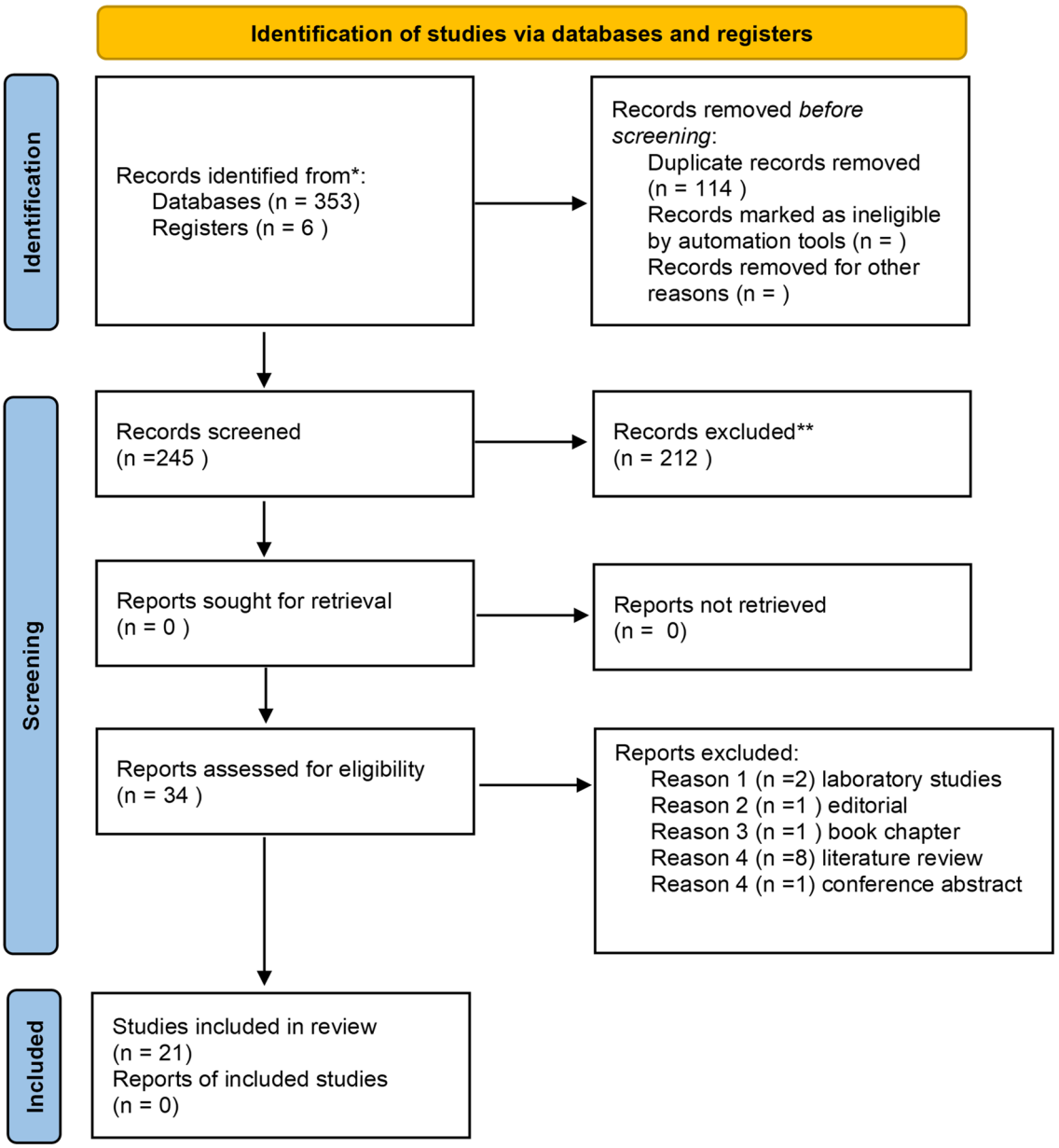

Fig. 1 Selection of studies

4 Discussion

The aim of the current review was to conduct an in-depth analysis of metabolomic approaches to determine the metabolomic profile of periodontitis. It revealed 56 main metabolites related to periodontitis that have been identified in at least two independent studies by HNM or MS-based metabolomics, using untargeted approaches. Due to the large number of metabolites detected, herein we discuss the most commonly detected metabolites, focusing on the biological medium in which they were sampled.

The studies varied considerably in terms of total number of participants and even more in the number of individuals regarding their periodontal diagnosis. The main focus of the studies was to compare periodontitis patients with healthy controls, and some assessed whether the severity of periodontitis could, in any way, influence metabolites (Kuboniwa et al., 2016; Sakanaka et al., 2017; Singh et al., 2017). Four studies compared the two previous extinctic forms of periodontitis, aggressive periodontitis and chronic periodontitis (Aimetti et al., 2012; Romano et al., 2018; Rzeznik et al., 2017; Shi et al., 2020), and the results showed that the metabolic profiles were similar. Three studies assessed

Table 1 Studies in which the main metabolites detected were reported to be related to microorganisms

References	Study type	Method	Statistical analysis	Source of biological sample	Number of subjects in the study	Oral condition	Number of metabolites detected	Main metabolites	Pertubed pathways identified
Shi et al. (2020)	cross-sectional/ case–control	GC–MS	PCA/OPLS-DA	GCF/Subgingival plaque	34	AgP(n = 24)/ HC(n = 10)	103	Glucose, uridine, alanine, isoleucine, maltotriose, putrescine, 5-aminovaleric acid, valine, oxoproline, leucine	Aminosynthesis, pyrimidine metabolism and galactose metabolism
Gawron et al. (2019)	Cross-sectional/ case–control	H-NMR	OPLS-DA	MW/TS	45	CP(n = 30)/ HC(n = 15)	40	Unknown 1, isopropanol, glycerol, acetone, methanol, lactate, unknown 2 and taurine	NI
Rzeznik et al., (2017)	Cross-sectional/case–control	H-NMR	PCA; OPLS	Stimulated saliva	51	GAgP(n = 8), GCP(n = 18)/ HC(n = 25)	10	Butyric acid	NI
Sakanaka et al. (2017)	Cross-sectional/ case–control	GC/MS	OPLS	Stimulated saliva	50	SevP(n = 12), ModP(n = 28)/ HC(n = 10)	69	Cadaverine and hydrocinnamate	Yes/polyamine metabolism, arginine and proline metabolism, butyric acid metabolism, and lysine degradation
Singh et al. (2017)	RCT	H-NMR	PCA; PLS-DA	Unstimulated saliva	114	CP(n = 62)/ HC(n = 52)	100	Propionate, propylene Glycol, butyrate, acetoin, valine, lactate, desaminotyrosine, tyrosine, formate, ethanol, ethane sulfonate, glutamate, NAG, glycine, alanine, phenol, histidine, phenylalanine, phenylacetate, 3-methylhistidine, 5- aminopentanoate	Yes/purine degradation pathway, protein degradation and oxidative stress

Table 1 (continued)

References	Study type	Method	Statistical analysis	Source of biological sample	Number of subjects in the study	Oral condition	Number of metabolites detected	Main metabolites	Pertubed pathways identified
Kuboniwa et al. (2016)	Cross-sectional	GC/MS	OPLS	Unstimulated saliva	19	SevP/ModP/MiP	63	Cadaverine, ornitine, spermidine, 5-oxoproline, valine, proline, hydrocinnamate, histidine	NI

NMR nuclear magnetic resonance spectroscopy, *GC* gas chromatography, *MS* mass spectrometry, *H-NMR* proton nuclear magnetic resonance, *HPLC* high-performance liquid chromatography, *ICP–MS* inductively coupled plasma mass spectrometry, *LC* liquid chromatography, *HPLC–ESI–MS-MS* high performance liquid chromatography-tandem electrospray ionization mass spectrometry, *UHPLC/MS/MS* ultrahigh performance liquid chromatography/tandem mass spectrometry, *PCA* principal component analysis, *PLS* projection to latent structure, *OPLS* orthogonal projection to latent-structure, *OPLS-DA* orthogonal partial least-squares discriminant analysis, *PLS-DA* partial least squares discriminant analysis, *RCT* randomized controlled trial, *GCF* gingival crevicular fluid, *GCP* generalized chronic periodontitis, *CP* chronic periodontitis, *AP* aggressive periodontitis, *HC/I* healthy controls/individuals, *DS* diabetes subjects, *PS* periodontal subjects, *GS* gingivitis subjects, *LCP* localized chronic periodontitis, *LAP* localized aggressive periodontitis, *MiP* mild periodontitis, *ModP* moderate periodontitis, *SevP* severe periodontitis, *SP* subgingival plaque, *Ging* gingivitis; *MW* mouth washout, *TS* tongue swab

only periodontitis patients without a control group (Barnes et al., 2009, 2010; Kuboniwa et al., 2016). In general, this heterogeneity prevented us comparing the results regarding periodontal disease diagnosis.

Among the six studies that reported metabolites that were related to microorganisms, three studies used the NMR spectroscopy by ^{1}H-NMR method (Gawron et al., 2019; Rzeznik et al., 2017; Singh et al., 2017) and three used the GC–MS method (Kuboniwa et al., 2016; Sakanaka et al., 2017; Shi et al., 2020). Among the 11 studies that reported metabolites that were related to the host response, four studies used the NMR spectroscopy by ^{1}H-NMR method (Aimetti et al., 2012; Citterio et al., 2020; Romano et al., 2018, 2019) and seven used different MS methods (Barnes et al., 2009, 2010, 2011, 2014; Elabdeen et al., 2013; Huang et al., 2014; Ozeki et al., 2016). All four studies that reported metabolites that were related to both microorganisms and the host response used different methods of MS (Chen et al., 2018; Liebsch et al., 2019; Marchesan et al., 2015; Pei et al., 2020). Indeed, these two platforms are the most common analytical platforms used in metabolomics (Klupczyńska et al., 2015). It has been observed that the biomarkers indicated by NMR spectroscopy and MS techniques differ, which suggests the complementary nature of these two analytical platforms (Klupczyńska et al., 2015). It seems that the results of this review corroborate this statement. The studies that the heat map matched most were Aimetti et al. (2012), Citterio et al. (2020), Gawron et al. (2019), Romano et al. (), Rzenik et al. (2017) and Singh et al. (2017). All these studies used analytical NMR spectroscopy, mostly ^{1}H-NMR.

Metabolite profiling was the metabolomic approach most employed in the studies included in this review. Metabolite profiling concentrates on the analysis of a wide range of metabolites (for example, amino acids, sugars, lipids, bile acids) and compounds of potential interest that are not known a priori. This approach aims to identify as many compounds as possible, as well as to quantify them (Klupczyńska et al., 2015). This strategy enables the detection of changes in unexpected parts of the metabolome and such changes can be attributed to specific metabolic pathways. Thus, metabolite profiling often leads to the formulation of new scientific hypotheses and the identification of novel metabolic biomarkers (Klupczyńska et al., 2015), which is the main aim in regard to metabolomics in periodontology. Half of the studies in this review identified perturbed pathways (Barnes et al., 2009, 2011, 2014; Chen et al., 2018; Elabdeen et al., 2013; Huang et al., 2014; Liebsch et al., 2019; Pei et al., 2020; Romano et al., 2018; Shi et al., 2020). The pathways identified were mostly related to oxidative stress, purine and the metabolism of pyrimidine and arachidonic acid.

Another common finding among the studies that the heat map matched most (Aimetti et al., 2012; Gawron et al., 2019; Romano et al., 2018, 2019; Rzeznik et al.,

Table 2 Studies in which main metabolites detected were reported to be related to host response

References	Study type	Method	Statistical analysis	Source of biological sample	Number of subjects in the study	Oral condition	Number of metabolites detected	Main metabolites	Pertubed pathways identified
Citterio et al. (2020)	Cross-sectional/ case–control	NMR	OPLS-DA	Unstimulated saliva	23	GCP(n = 12)/ HC(n = 11)	31	Etanol, acetate, valine, acetoin,isoleucine, uracil, hypoxanthine, formate, phenylalanine, lactate,methylamine	NI
Romano et al. (2019)	Cross-sectional/ case–control	NMR	PCA	Unstimulated saliva	51	GCP(n = 19)/ HC(n = 32)	NI	Valine, lactate, proline, pyruvate, tyrosine, and formate	NI
Romano et al. (2018)	Cross-sectional/ case–control	NMR	PCA	Unstimulated saliva	100	GAgP(n = 28)/ GCP(n = 33)/ HC(n = 39)	NI	pyruvate, N-acetyl groups, lactate, proline, phenylalanine, isoleucine, valine, tyrosine, sarcosine, formate	Phenylalanine metabolism; pyruvate metabolism
Ozeki et al. (2016)	Cross-sectional/ case–control	GC/MS	PCA	GCF	30	CP(n = 16)/ HC(n = 14)	19	Galactose, lysine, phosphate,putrescine, phenylalanine, namely, ribose, taurine, 5-aminovaleric acid, galactose, lactic acid, benzoic acid, glycine, malic acid	NI
Huang et al. (2014)	Cross-sectional/ case–control	MS; HPLC; ICP-MS	NI	Unstimulated saliva and serum	100	CP(n = 50)/ HC(n = 50)	40	fatty acids, F2-isoprostanes	Oxidative stress, metabolism of arachidonic acid
Barnes et al. (2014)	Cross-sectional/ case–control	LC/MS; GC/MS	NI	Unstimulated saliva and plasma	161	PS(n = 55)/ GS(n = 53)/ HC(n = 53)	1247	Carnitine, 3-dehydrocarnitine,, arachidonate, 12-HETE, linoleate, linolenate, docosapentaenoate, palmitoyl sphingomyelin	Purine degradation, dipeptides, amino acid metabolites, carbohydrates, energy metabolites, uridine, allantoin, ω-6-fatty acids, fatty acids, acetylcarnitine, carnitine, 3-dehydrocarnitine

Table 2 (continued)

References	Study type	Method	Statistical analysis	Source of biological sample	Number of subjects in the study	Oral condition	Number of metabolites detected	Main metabolites	Pertubed pathways identified
Hager et al. (2013)	Cross-sectional/ case–control	HPLC–ESI–MS-MS	NI	GCF; unstimulated saliva and serum	38	GAgP(n = 19)/ HC(n = 19)	60	n3-PUFAs; n6-PUFAs; PGE2, LXA4; LA; AA; EPA;DHA; LTB4; 20-OH-LTB4; 5-HETE; 5-oxoETE; 15-HETE; 15-oxoETE; 17-HDHA; 5-HETE, 8-HETE, 12-HETE, 15-HETE, HXA3, 9-HODE, 13-HODE, 13-oxoODE, 12-HEPE, 8-HEPE, 4-HDHA, 10-HDHA, 14-HDHA; d15d12PGD2; 20-COOH-AA; 20-HDHA; 11-HETE	12-LOX pathway; 15-LOX pathways; 5-LOX pathways
Aimetti et al. (2012)	Cross-sectional/ case–control	NMR	PCA; PLS	Unstimulated saliva	54	GAgP(n = 2)/ GCP(n = 21)/ LCP(n = 4)/ LAP(n = 2)/ GS(n = 3)/ HC(n = 22)	NI	Pyruvate, N-acetyl groups, acetate, c-aminobutyrate, n-butyrate, succinate, trimethylamine, propionate, valine, phenylalanine	NI
Barnes et al. (2011)	Cross-sectional/ case–control	UHPLC/MS; GC/MS	NI	Unstimulated saliva	68	PS(n = 34)/ HC(n = 34)	390	NI	Peptides, Amino acids, Carbohydrates, Lipids, Nucleotides, Cofactor, Unnamed
Barnes et al. (2010)	RCT	GC/ MS;UHPLC/ MS	NI	GCF	39	CP (n = 39)	10	Inosine, lysine, putrescine, and xanthine	NI
Barnes et al. (2009)	Cross-sectional	LC/MS; GC/MS	NI	GCF	22	CP(n = 22)	228	Inosine, hypoxanthine, xanthine, guanosine, guanine, putrescine, cadaverine, cholie, glycerol-3-phosphate, N-acetylneuraminic acid, ascorbic acid and uric acid	Purine degradation and xanthine oxidase

Table 2 (continued)

NMR nuclear magnetic resonance spectroscopy, *GC* gas chromatography, *MS* mass spectrometry, *H-NMR* proton nuclear magnetic resonance, *HPLC* high-performance liquid chromatography, *HR-NMR* high-resolution nuclear magnetic resonance spectroscopy, *ICP–MS* inductively coupled plasma mass spectrometry, *LC* liquid chromatography, *HPLC–ESI–MS-MS* high performance liquid chromatography-tandem electrospray ionization mass spectrometry, *UHPLC/MS/MS* ultrahigh performance liquid chromatography/tandem mass spectrometry, *PCA* principal component analysis, *PLS* projection to latent structure, *OPLS* orthogonal projection to latent-structure, *OPLS-DA* orthogonal partial least-squares discriminant analysis, *PLS-DA* partial least squares discriminant analysis, *RCT* randomized controlled trial, *GCF* gingival crevicular fluid, *GCP* generalized chronic periodontitis, *CP* chronic periodontitis, *AP* aggressive periodontitis, *HC/I* healthy controls/individuals, *DS* diabetes subjects, *PS* periodontal subjects, *GS* gingivitis subjects, *LCP* localized chronic periodontitis, *LAP* localized aggressive periodontitis, *MiP* mild periodontitis, *ModP* moderate periodontitis, *SevP* severe periodontitis, *SP* subgingival plaque, *Ging* gingivitis, *MW* mouth washout, *TS* tongue swab

2017; Singh et al., 2019) was that saliva was the biological medium sampled (in six out of seven studies). In only one study the saliva was stimulated (Rzeznik et al., 2017) while in the other five studies unstimulated saliva was sampled (Aimetti et al., 2012; Citterio et al., 2020; Romano et al., 2018, 2019; Singh et al., 2019). Gawron et al. (2019) obtained the sample from mouth washout and tongue swab. Jo et al. (2019) demonstrated a very high correlation with the microbiome composition from mouth rinse water, stimulated and unstimulated saliva and suggested that mouth rinse water or mouth washout is a suitable collection method instead of saliva for oral microbiome analysis. To date, no comparison has been made for the oral metabolome but the findings of the above studies suggest the same tendency, as the metabolites from mouth washout were similar to those obtained from the saliva. Interestingly, four of these studies reported that their results were more related to the host response (Aimetti et al., 2012; Citterio et al., 2020; Romano et al., 2018, 2019) while the three other studies reported that their results were more related to the microorganisms' response (Gawron et al., 2019; Rzeznik et al., 2017; Singh et al., 2019), although the main metabolites were coincident. Except for butyrate, ethanol, and methanol, all the following metabolites were coincident among the studies: acetate, alanine, butyrate, formate, lactate, propionate and phenylalanine (Aimetti et al., 2012; Gawron et al., 2019; Romano et al., 2018, 2019; Rzeznik et al., 2017; Singh et al., 2019). They were either up- or down-regulated in these studies, in some of them this information was not mentioned. In general, these metabolites are related to oxidative stress.

Phenylalanine was highlighted as the main metabolite in ten studies in different biological mediums: unstimulated saliva (Aimetti et al., 2012; Barnes et al., 2011; Citterio et al., 2020; Romano et al., 2018, 2019; Singh et al., 2019), stimulated saliva (Rzeznik et al., 2017; Sakanaka et al., 2017) blood and GCF (Chen et al., 2018) and GCF (Barnes et al., 2010; Ozeki et al., 2016). In five of these studies this metabolite was increased (Aimetti et al., 2012; Barnes et al., 2010, 2011; Ozeki et al., 2016; Romano et al., 2018). Phenylalanine is an essential amino acid that is highly concentrated in the human brain and plasma (Blau et al., 2010; Pilotto et al., 2021; Waisbren et al., 2007).

The second most reported main metabolite in seven and six studies respectively was valine and succinate. Valine is an essential branched-chain amino acid (BCAA) that is critical to human life and is particularly involved in stress, energy and muscle metabolism. Succinic acid is also a microbial metabolite that is produced by *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterobacter*, *Acinetobacter*, *Proteus mirabilis*, *Citrobacter frundii*, and *Enterococcus faecalis*. Succinic acid is also found in *Actinobacillus*, *Anaerobiospirillum*, *Mannheimia*,

Table 3 Studies in which main metabolites detected were reported to be related to both biofilm and to the host response

References	Study type	Method	Statistical analysis	Source of biological sample	Number of subjects in the study	Oral condition	Number of metabolites detected	Main metabolites	Pertubed pathways identified
Pei et al. (2020)	Cross-sectional/ case–control	GC/MS	PLS-DA; PCA; OPLS-DA	GCF	58	SevP-CP or ModP-CP(n = 30)/ HC(n = 28)	147	citramalic acid and N-carbamylglutamate 2	Pyrimidine metabolism
Liebsch et al. (2019)	Cross-sectional	UHPLC-MS/MS	NI	Stimulated saliva	909		284	Dihomolinolenate; 5-Oxoproline; 5-aminovalerate	Amino acid metabolism
Chen et al. (2018)	Cross-sectional/ case–control	GC/MS	PCA; OPLS-DA	GCF and serum (venous blood)	40	GAgP(n = 20)/ HC(n = 20)	549	Glutathione, Urea, 2-deoxyguanosine, 2,5-dihydroxybenzaldehyde, Adipic acid, Allo-inositol, Alpha-tocopherol, dehydroascorbic acid, Xanthine, Beta-alanine, Thymidine, Uridine, Fructose-6-phosphate, Galactose, Glucose-1-phosphate, Ribose-5-phosphate, Ribulose-5 phosphate, Ribose, Noradrenaline, Glycine-d5, Lysine, Methionine, Sarcosine, 2-ketobutyric acid, Phosphoglycolic acid	Oxidative stress, purine, degradation, the metabolism of tyrosine and pyrimidine
Marchesan et al. (2015)	RCT	LC/MS; GC/MS	PCA	SP and unstimulated saliva	168	MiP-P/Ging/HC	7	cyclo (-leupro); cyclo (-phe-pro); glycerol-3-phosphate	NI

NMR nuclear magnetic resonance spectroscopy, *GC/MS* gas chromatography/mass spectrometry, *H-NMR* proton nuclear magnetic resonance, *MS* mass spectrometry, *HR-NMR* high resolution nuclear magnetic resonance spectroscopy, *HPLC* high-performance liquid chromatography, *ICP–MS* inductively coupled plasma mass spectrometry, *LC* liquid chromatography, *HPLC–ESI–MS-MS* high performance liquid chromatography-tandem electrospray ionization mass spectrometry, *UHPLC/MS/MS* ultrahigh performance liquid chromatography/tandem mass spectrometry, *PCA* principal component analysis, *PLS* projection to latent structure, *OPLS-DA* orthogonal partial least-squares discriminant analysis, *PLS-DA* partial least squares discriminant analysis, *OPLS* orthogonal projection to latent-structure, *GCF* gingival crevicular fluid, *GCP* generalized chronic periodontitis, *CP* chronic periodontitis, *AP* aggressive periodontitis, *HC/I* healthy controls/individuals, *DS* diabetes subjects, *PS* periodontal subjects, *GS* gingivitis subjects, *LCP* localized chronic periodontitis, *LAP* localized aggressive periodontitis, *MiP* mild periodontitis, *ModP* moderate periodontitis, *SevP* severe periodontitis, *SP* subgingival plaque, *Ging* gingivitis, *MW* mouth washout, *TS* tongue swab, *RCT* randomized controlled trial

Table 4 Main metabolites and their expression in periodontal disease (up or downregulated)

Metabolite	Author	Levels	Metabolite	Author	Levels
12-HETE	Barnes et al. (2014) and Hager et al. (2013)	↑	Isopropanol	Citterio et al. (2020), Romano et al. () and Gawron et al. (2019)	↑
		↑			NI
3-Dehydrocarnitine	Barnes et al., (2011, 2014)	↑			↓
		↑			↓
			Lactate	Citterio etal (2020), Gawron et al. (2019), Romano et al. (2018, 2019), Liebsch et al.(2019) and Rzeznik et al. (2017)	↑
					↑
					NI
5-HETE	Huang et al. (2014) and Hager et al. (2013)	↑			NI
		↑			↓
					↓
			Leucine	Shi et al. (2020), Citterio et al. (2020), Liebsch et al.(2019); Sakanaka et al. (2017) and Barnes et al. (2010)	↑
5-oxoproline	Shi et al. (2020), Sakanaka et al. (2017), Liebsch et al. (2019) and Kuboniwa et al. (2016)	↑			↓
		NI			NI
		NI			NI
		NI			↑
			Lysine	Liebsch et al. (2019), Chen et al. (2018), Ozeki et al. (2016) and Barnes et al. (2010)	NI
5-aminovaleric acid	Shi et al. (2020), Sakanaka et al. (2017) and Ozeki et al. (2016)	↑			↑
		NI			↑
		↑			↑
Acetate	Romano et al., (2018, 2019), Gawron et al. (2019), Rzeznik et al. (2017) and Aimetti et al. (2012)	NI	Methanol	Citterio et al. (2020), Gawron et al. (2019), Romano et al. (2018, 2019) and Rzeznik et al. (2017)	↑
		↓			↑
		↑			NI
		↓			↑
		↑			↓
Acetoin	Citterio et al. (2020), Gawron et al. (2019) and Singh et al. (2017)	↑	Methylamine	Citterio et al.(2020) and Romano et al. (2018, 2019)	↓
		↑			NI
		↑			↑
Acetylcarnitine	Barnes et al., (2011, 2014)	↑			↓
		↑	N-acetyl groups	Romano et al. (2018, 2019)	NI
					↓
Alanine	Citterio et al. (2020), Romano et al. (2018, 2019), Gawron et al. (2019), Singh et al. (2017) and Ozeki et al. (2016)	↓			
		NI	Ornithine	Sakanaka et al. (2017), Kuboniwa et al. (2016)	NI
		↓			NI
		↑	P-cresol sulfate	Barnes et al., (2011, 2014)	↑
		NI			↑

Table 4 (continued)

Metabolite	Author	Levels	Metabolite	Author	Levels
		↑	Phenylalanine	Citterio et al. (2020), Liebsch et al.(2019), Romano et al. (2018, 2019), Sakanaka et al. (2017), Singh et al. (2017), Ozeki et al. (2016), Aimetti et al. (2012) and Barnes et al., (2010, 2011)	↓
					NI
					NI
Aspartate	Citterio et al. (2020) and Liebsch et al. (2019)	↓			↑
		NI			NI
Butyrate	Romano et al. (2018, 2019), Rzeznik et al. (2017), Singh et al. (2017) and Aimetti et al. (2012)	NI			↓
		↑			↑
		↓			↑
		↑			↑
		↑			↑
		↑			
Cadaverine	Sakanaka et al. (2017), Kuboniwa et al. (2016), Barnes et al., (2009, 2010)	NI	Pipecolate	Liebsch et al. (2019) and Marchesan et al. (2015)	NI
		↓			NI
		↑	Proline	Citterio et al. (2020), Romano et al. (2018, 2019), Liebsch et al. (2019)	↓
		↑			NI
Carnitine	Barnes et al., (2011, 2014)	↑			NI
		↑			↑
Choline	Citterio et al.(2020), Gawron et al. (2019), Romano et al. (2018, 2019), Barnes et al., (2009, 2010)	↑	Propionate	Citterio et al.(2020), Romano et al. (2019), Gawron et al. (2019), Gawron et al. (2019), Romano et al. (2018, 2019), Singh et al. (2017) and Aimetti et al. (2012)	↑
		↑			NI
		NI			↓
		↓			↑
		↑			↑
		↑			↑
		↑			↑
Creatine	Citterio et al. (2020), Liebsch et al. (2019) and Gawron et al. (2019)	↓	Propylene Glycol	Gawron et al. (2019) and Singh et al. (2017)	↑
		NI			↑
		↓			
Ethanol	Romano et al. (2018, 2019), Gawron et al. (2019), and Singh et al. (2017)	NI	Putrescine	Shi et al. (2020), Sakanaka et al. (2017), Ozeki et al. (2016), Barnes et al., (2009, 2010)	↑
		↑			NI
		↓			↑
		↓			↑
Formate	Citterio et al. (2020), Gawron et al. (2019), Romano et al. (2018, 2019) and Singh et al. (2017)	↑			↑
		↑	Pyruvate	Romano et al. (2018, 2019), Gawron et al. (2019) and Rzeznik et al. (2017)	NI
		NI			↓
		↑			↓
		↑			↓

Table 4 (continued)

Metabolite	Author	Levels	Metabolite	Author	Levels
GABA	Romano et al. (2018a, 2018b, 2019) and Rzeznik et al. (2017)	NI	Sarcosine	Citterio et al. (2020), Romano et al. (2018, 2019) and Chen et al. (2018)	↓
		↑			NI
		↓			↓
Galactose	Citterio et al. (2020), Chen et al. (2018) and Ozeki et al. (2016)	↑			↑
		↑	Succinate	Gawron et al. (2019), Romano et al. (2018, 2019), Sakanaka et al. (2017), Ozeki et al. (2016) and Aimetti et al. (2012)	↑
		↑			NI
					↑
Glutamine	Sakanaka et al. (2017) and Singh et al. (2017)	NI			NI
		NI			NI
					↑
			Taurine	Citterio et al. (2020), Gawron et al. (2019) and Ozeki et al. (2016)	↓
					↓
Glutathione	Barnes et al., (2009, 2014)	↑			↑
		↓	Thymidine	Liebsch et al. (2019) and Chen et al. (2018)	NI
Glucose	Shi et al. (2020); Citterio et al. (2020); Gawron et al. (2019); Barnes et al. (2009)	↑			↓
		↓	Tryptophan	Sakanaka et al. (2017) and Singh et al. (2017)	NI
		↓			NI
		↑	Tyrosine	Citterio et al. (2020), Pei et al. (2020), Romano et al. (2018, 2019), Liebsch et al. (2019) and Barnes et al. (2011)	↓
Glycine	Romano et al. (2018, 2019) and Ozeki et al. (2016)	NI			NI
		↑			NI
		↑			NI
Glycine-D5	Pei et al. (2020) and Chen et al. (2018)	↑			↑
		↓			↑
Hypoxanthine	Citterio et al. (2020), Liebsch et al.(2019) and Barnes et al. (2010)	↓			
		NI	Uracil	Citterio et al. (2020), Pei et al. (2020) and Liebsch et al. (2019);	↓
		↑			↑
Histidine	Singh et al. (2017) and Kuboniwa et al. (2016)	↓			NI
		NI	Urea	Liebsch et al. (2019) and Chen et al. (2018)	NI
Hydrocinnamate	Sakanaka et al. (2017) and Kuboniwa et al. (2016)	NI			↑
		NI			

Table 4 (continued)

Metabolite	Author	Levels	Metabolite	Author	Levels
Inosine	Liebsch et al.(2019), Barnes et al., (2010, 2014)	NI	Uridine	Shi et al.(2020), Chen et al.(2018) and Barnes et al.(2014)	↑
		↑			↑
		↑			↑
Isoleucine	Shi et al. (2020), Citterio et al. (2020), Romano et al. (2018, 2019), Liebsch et al. (2019), Sakanaka et al. (2017) and Barnes et al. (2010)	↑	Valine	Shi et al. (2020); Gawron et al. (2019), Romano et al. (2018, 2019), Singh et al. (2017), Liebsch et al. (2019), Kuboniwa et al. (2016) and Aimetti et al. (2012)	↑
		→			→
		NI			NI
		NI			↑
		↑			↑
		NI			NI
		↑			NI
			Xanthine	Barnes et al., (2009, 2010, 2014)	↑
					↑
					↑

Corynebacterium and *Basfia*. Succinate was increased in three studies (Aimetti et al., 2012; Gawron et al., 2019; Romano et al., 2018) whereas valine was increased in four studies (Aimetti et al., 2012; Romano et al., 2018; Shi et al., 2020; Singh et al., 2019) and decreased in one (Gawron et al., 2019).

Propionate was reported in six studies as one of the main metabolites (Table 4) whereas acetate and butyrate were reported in five studies as one of the main metabolites (Table 4). Propionic acid (PA) is an end-product of the microbial digestion of carbohydrates. It is a metabolite of *Bacteroides*, *Clostridium*, *Dialister*, *Megasphaera*, *Phascolarctobacterium*, *Propionibacterium*, *Propionigenum*, *Salmonella*, *Selenomonas* and *Veillonella*. Butyrate is produced as an end-product of a fermentation process solely performed by obligate anaerobic bacteria (Rivière et al., 2016). Acetate is an ionic form from acetic acid, one of the simplest carboxylic acids. The acetyl group, derived from acetic acid, is fundamental to the biochemistry of virtually all forms of life. When bound to coenzyme A it is central to the metabolism of carbohydrates and fats. Acetic acid is produced and excreted by certain bacteria, notably the *Acetobacter* genus and *Clostridium acetobutylicum*. Urinary acetic acid is produced by *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterobacter*, *Acinetobacter*, *Proteus mirabilis*, *Citrobacter frundii*, *Enterococcus faecalis*, *Streptococcus* group B, and *Staphylococcus saprophyticus*. Acetic acid is also found in *Akkermansia*, *Bacteroidetes*, *Bifidobacterium*, *Prevotella* and *Ruminococcus*. Butyrate has diverse and apparently paradoxical effects on cellular proliferation, apoptosis and differentiation. It is produced as an end-product of a fermentation process solely performed by obligate anaerobic bacteria. It is a metabolite of *Anaerostipes*, *Coprococcus*, *Eubacterium*, *Faecalibacterium* and *Roseburia* (Duncan et al., 2002).

When analyzing studies in which the biological medium sampled was the GCF (Barnes et al., 2009, 2010; Ozeki et al., 2016; Pei et al., 2020; Shi et al., 2020), the most consistently reported metabolites were lysine and putrescine. Lysine is an essential amino acid. Lysine reduction can also affect immune system since there is a cellular proliferation role and ability to induce humoral and cell mediated immune responses (Datta et al., 2001). Putrescine is a polyamine. Putrescine is related to cadaverine,another polyamine. Both are produced by the breakdown of amino acids in living and dead organisms, and both are toxic in large doses. Putrescine can be found in *Citrobacter*, *Corynebacterium*, *Cronobacter* and *Enterobacter* (Wendisch, 2017). The GCF results reflect inflammatory processes at individual sites with the disease (Barros et al., 2016; Jaedicke et al., 2016; Nazar Majeed et al., 2016; Öngöz Dede et al., 2017; Wassall & Preshaw, 2016). Saliva results, on the other hand, reflect the inflammatory state of the total gingival fluid in the periodontal

Fig. 2 Heat map of the main metabolites in each study, their super class, the biological medium from which they were sampled and the type of response with which the metabolites were associated. **A** Fist 31 metabolites; **B** Other 30 metabolites

pockets of the entire mouth (Taylor & Preshaw, 2016). The results of this review demonstrate that the metabolites from the GCF and the metabolites from the saliva differ. More studies assessing both saliva and GCF simultaneously are necessary for metabolite profiling in periodontal disease, especially if the aim is to analyze the severity of the disease.

Studies in which the biological medium sampled was plasma or serum associated with other biological mediums compared serum and GCF (Chen et al., 2018), serum and unstimulated saliva (Huang et al., 2014), serum and unstimulated saliva and GCF (Elabdeen et al., 2013) and plasma and unstimulated saliva (Barnes et al., 2014). The only metabolites that were common to all studies were 12-HETE

Table 5 Pathways, match status, and p-value

Pathway	Match status	p-value
Aminoacyl-tRNA biosynthesis	3/48	0.006
Butanoate metabolism	2/15	0.006
Pyruvate metabolism	2/22	0.013
Glycolysis/Gluconeogenesis	3/26	0.001
Glyoxylate and dicarboxylate metabolism	2/32	0.027
Phenylalanine, tyrosine and tryptophan biosynthesis	1/4	0.033

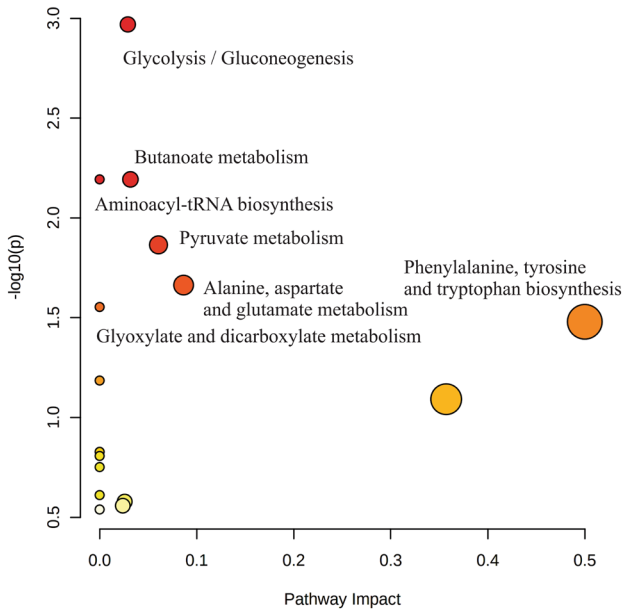

Fig. 3 Overview of salivary biofluid pathway analysis. The metabolic pathway analysis highlighted the main pathways. Aminoacyl-tRNA biosynthesis, Butanoate metabolism, Pyruvate metabolism, Glycolysis/Gluconeogenesis, Alanine, aspartate and glutamate metabolism, Glyoxylate and dicarboxylate metabolism, Phenylalanine, tyrosine and tryptophan biosynthesis

and 5-HETE. 12-hydroxyeicosatetraenoic acid, also known as 12-HETE, is an eicosanoid, a 5-lipoxygenase metabolite of arachidonic acid. 12-HETE is one of the six monohydroxy fatty acids produced by the non-enzymatic oxidation of arachidonic acid. 12-HETE is a neuromodulator that is synthesized during ischemia. 5-hydroxyeicosatetraenoic acid (5-HETE) is an endogenous eicosanoid. 5-HETE is an intermediate in arachidonic acid metabolism. It is also involved in the pathway of leukotriene synthesis (Fruteau de Laclos et al., 1984; Rodriguez-Lagunas et al., 2013). It is important to highlight that the main metabolites in GCF, serum and plasma did not correspond to metabolites in the saliva. This is an issue that should be better investigated in future studies.

As periodontitis is a complex chronic inflammatory disease, a question that arises is whether some metabolite biomarkers of other chronic inflammatory diseases can be detected in individuals with periodontitis. It was demonstrated that in diabetes, for example, phenylalanine, tyrosine, valine and isoleucine were upregulated and are considered metabolite biomarkers of diabetes (Barnes et al., 2014). Interestingly, these metabolites were reported as main metabolites in some studies included in this review. Ulven et al. (2019) identified metabolomic signatures in obese individuals and obesity-related metabolic alterations such as inflammation or oxidative stress that demonstrated the value of an integrative approach to the microbiome and metabolomics. In periodontology, based on the findings of the selected studies, this integrative approach might also help researchers to clarify and understand the interactions of the microbial metabolites with the host organism and to avoid misinterpretation.

It was noticed that the PLS-DA was used in studies with a small number of case–control individuals. Some caveats associated with PLS-DA that need to be considered when using this model are difficulties in the identification of small numbers of variables that are responsible for the separation between two or more groups (classes) and therefore a larger number of variables are required to achieve a good prediction accuracy, scores plot may present an overoptimistic view of the separation between the classes and there is a tendency to overfitting (Gromski et al., 2015). Mendez et al., (2019) suggested that for robust predictive models the most important consideration is statistical power (Mendez et al., 2019). The authors point out there is no magic formula for calculating the number of samples needed for robust metabolomics multivariate machine learning, where estimates are dependent on many factors, including: the dimensionality of the data, the strength of effect, the degree of covariance (strength of latent structure), the heterogeneity of the sample population, the repeatability of the measurement instrument, and the complexity of the model. As the number of individuals are not large in studies about metabolites in periodontal disease and many of it used PLS-DA analysis, we need to be careful to avoid over optimistic reporting of results. Thus, through this critical analysis of the literature, we have carefully to consider if the complete data set is a real representative sample of the biological question about periodontal disease. Studies with larger numbers of participating individuals (tests and controls) should be performed so that we have more consistent data on periodontal disease metabolites.

This systematic review identified 56 metabolites that were detected in at least two independent studies. It is noteworthy that 14 metabolites of the 56 detected were identified as main metabolites in all six studies that sampled the saliva and in the study that sampled the mouthwash and the tongue swab. However, these 14 metabolites were related to both the host and microorganism responses. This finding supports the use of saliva as a potential biofluid for monitoring this disease state. Finally, further long term longitudinal studies considering the analysis before and after periodontal treatment would benefit this knowledge area in order to confirm this findings.

Table 6 Joanna Briggs Institute tool for quality assessment of cross-sectional studies

Concerns identified									
Authors	Inclusion criteria[a]	Subjects description[b]	Validation measure[c]	Standard criteria[d]	Confoundings[e]	Strategies to deal with confoundings[f]	Outcomes[g]	Statistical analysis[h]	Risk of bias
Pei et al. (2020)	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Low
Citterio et al. (2020)	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Low
Shi et al. (2020)	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Low
Liebsch et al. (2019)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	High
Romano et al. (2018)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	High
Chen et al. (2018)	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Low
Rzeznik et al. (2017)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	High
Sakanaka et al. (2017)	No	Yes	Yes	No	Yes	NA	Yes	Yes	Low
Singh et al. (2017)	No	Yes	Yes	No	Yes	NA	Yes	Yes	High
Kuboniwa et al. (2016)	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	Low
Ozeki et al. (2016)	No	Yes	No	No	Yes	NA	Yes	Yes	High
Huang et al. (2014)	Yes	Yes	Yes	No	Yes	NA	Yes	Yes	High
Barnes et al. (2014)	Yes	Yes	Yes	No	No	Yes	Yes	Yes	High
Hager et al. (2013)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	High
Aimetti et al. (2012)	Yes	Yes	Yes	No	No	No	Yes	Yes	High
Barnes et al. (2011)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	High
Barnes et al. (2009)	Yes	Yes	No	No	No	No	Yes	Yes	High

NA not applicable

[a]Were the criteria for inclusion in the sample clearly defined?

[b]Were the study subjects and the setting described in detail?

[c]Was the exposure measured in a valid and reliable way?

[d]Were objective, standard criteria used for measurement of the condition?

[e]Were confounding factors identified?

[f]Were strategies to deal with confounding factors stated?

[g]Were the outcomes measured in a valid and reliable way?

[h]Was appropriate statistical analysis used?

Table 7 Certainty of evidence of included studies

No. of studies	Risk of bias	Inconsistency	Indirectness	Imprecision	Other consideration	Overall certainty of evidence
Cross-Sect. 20 studies	Serious[a]	Not serious	Not serious	Serious[b]	All plausible residual confounding would suggest spurious effect, while no effect was observed	⨁◯◯◯ Very low

[a]Absence of exclusions such as systemic disease and smokers

[b]Less than 300 subjects

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