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Metabolomic Status of The Oral Cavity in Chronic Periodontitis

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Abstract. *Chronic periodontitis is an inflammatory disease of tooth-supporting tissues associated with Porphyromonas gingivalis. Expansion and invasion of this bacterium into the periodontium is associated with changes in the metabolome of the oral cavity. Materials and Methods: Metabolomics analysis of mouth washout and tongue swab samples based on proton nuclear magnetic resonance (^{1}H-NMR) method was employed to determine metabolic status of the oral cavity in chronic periodontal disease. Results: Mouth washout extracts contained a total of 23 metabolites and tongue swab extracts contained 17. Identified metabolites partially overlap with the content of saliva and gingival crevicular fluid. The colonization of the oral cavity of patients with periodontitis by bacteria was manifested in the change in levels of eight metabolites. Conclusion: NMR-based metabolomics analysis is a potentially useful methodological approach for monitoring the pathological processes observed in the oral cavity in the course of periodontitis.*

Chronic periodontal disease (CPD) is a bacterial-driven inflammatory malady affecting tissues supporting the teeth (1, 2). Disease severity ranges from mild and reversible inflammation of soft tissue of the gingiva to chronic destruction of periodontal tissues, including periodontal ligaments and alveolar bone, which leads to formation of periodontal pockets with eventual exfoliation of teeth (3-5). CPD in adults is associated with colonization and proliferation of the gram-negative anaerobic bacterium *Porphyromonas gingivalis* in periodontal gingival pockets. The presence of *P. gingivalis*, and several other gram-negative anaerobic bacteria, *i.e.* *Agregatibacter actinomycetemcomitans, Prevotella intermedia, Tannerella forsythia, Fusobacterium nucleatum, Campylobacter rectus, Peptostreptococcus micros, Streptococcus intermedius, Treponema denticola, Eikenella corrodens* acting possibly in concert with the absence of beneficial species and the presence of certain immunological deficiencies in the host, appears to play an essential role in disease severity and progression (6, 7). The primary site of oral cavity colonization by *P. gingivalis* includes the supragingival tooth surface and subgingival crevice (the space between the tooth root and the gingiva). *P. gingivalis* represents a late colonizer of the oral cavity, requiring antecedent organisms to create the necessary environmental conditions. The antecedent bacterial species may facilitate colonization by *P. gingivalis via* provision of attachment sites for interspecies adherence, supply of nutrients, and reduction of oxygen tension to the low levels required for growth and survival of this obligate anaerobe (7-9).

P. gingivalis does not produce a single potent exotoxin, but is equipped with a set of enzymes, *e.g.* proteinases, hemolysins, peptidylarginine deiminase and cellular constituents, such as fimbriae and lipopolysaccharides, which along with toxic metabolites, have the potential to impinge upon host tissue integrity thus causing destruction of periodontal supporting tissues (5, 7, 10-12). The repertoire of enzymes and metabolites that can be detrimental to the host also includes phospholipase A, prostaglandins, alkaline

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and acid phosphatases, *etc*. (13-17) and volatile sulfur compounds such as hydrogen sulfide, methylmercaptan, and dimethyl sulfide, which are cytotoxic and can be responsible for protein synthesis inhibition (18, 19).

Beyond the primary site of colonization, the oral cavity provides a variety of surfaces to which *P. gingivalis* can efficiently adhere or invade (13, 16). Previous studies have reported detection of periodontopathic bacteria in tongue coating in close association with those in dental plaque (20-22) and periodontal conditions (23-25), indicating that periodontopathic microorganisms in the tongue, equally to dental plaque, are an important factor in the etiopathogenesis of periodontal disease. Therefore, similarly to gingival crevicular fluid (GCF), other oral fluids and surfaces may also constitute a potential reservoir of a variety of inflammatory mediators and tissue-destructive molecules associated with increased host–bacterial interactions compared to individuals with healthy periodontium (25-28).

Metabolomics is a method of choice for diagnosis of different pathological conditions. It allows for comparison of the signature of low molecular weight compounds (MW<1,000-1,500 Da) in biological samples from healthy and disease-affected individuals, mainly by employing nuclear magnetic resonance (NMR) and mass spectrometry (MS) combined with various separation techniques (29-34). Salivary metabolomics studies have been conducted for the detection of many diseases including oral cavity (35). The total composition shown by analysis of salivary gland products should reflect two metabolomes, *i.e.* first that of the investigated individual, within which all metabolites are associated with the health status, and second, that which represents the oral microbiome. Therefore, the output of different studies may differ due to the contribution of different microbial metabolomes. Among many diseases, metabolomics analysis of oral cavity samples allows distinction between patients with different periodontal diseases and healthy individuals (36-38).

The aim of our study was to demonstrate the feasibility of NMR analysis in determining the metabolic status of the oral cavity in CPD, in easily accessible samples of mouth washout and tongue swab employing an untargeted metabolomics profiling method.

Materials and Methods

Study participants. The study was carried out in accordance with the Declaration of Helsinki and was approved by the Bioethics Committee of the Jagiellonian University, Medical College in Krakow, Poland (KBET/310/B/2012). Adult males and females from Krakow, Malopolska area (Poland) were enrolled in this study. All participants read and signed a written informed consent form prior to inclusion in the study. The study group consisted of 30 donors diagnosed with advanced CPD with a minimum of 20 natural teeth present (aged 37-68 years; 11 males, 19 females). The control group comprised 15 donors in good general health (aged 23-62 years; four males and 11 females). Exclusion criteria included: diagnosis of a medical condition which required pre-medication/pre-treatment prior to dental visits and procedures; five or more decayed untreated teeth at screening (cavities); diagnosis of other diseases of the hard or soft oral tissues; use of antibiotics or antimicrobial drugs within 30 days prior to the first study visit; a history of systemic disease *e.g.* rheumatoid arthritis, aspiration pneumonia, diabetes mellitus, atherosclerosis or other uncharacterized systemic disease; pregnant and lactating women; tobacco smokers; immune-compromised individuals *e.g.* diagnosed with HIV, AIDS, taking immuno-suppressive drug therapy, radio- and chemotherapy.

Clinical and periodontal examination. Each participant's medical, dental, and medication history was recorded by the clinician. The diagnosis of CPD was confirmed by radiographs and peripheral blood samples collected for routine laboratory tests. The following parameters were examined to assess the periodontal status: aproximal periodontal index (API), sulcus bleeding index (SBI), probing pocket depth (PPD) and clinical attachment level (CAL). API was classified as optimal at <25%, moderately good at 26-39%, average at 40-70%, and poor at 71-100% and expressed as the average value per whole oral cavity. SBI was classified as: normal/healthy periodontium at <12%; mild inflammation (slight change in color, little change in texture) at 13-20%; moderate inflammation (redness, edema and hypertrophy) at 21-50%; chronic inflammation (marked redness and edema/hypertrophy, spontaneous bleeding or ulceration) at 51-100% and also expressed as the average value per whole periodontium. PPD and CAL were assessed for five teeth including at least one central incisor and one first molar in the group with periodontitis group and for five random teeth in control group, using the following classification: PPD>7 mm and CAL>5 mm advanced periodontitis; PPD 5-7 mm and CAL 3-4 mm moderate periodontitis; PPD 3-5 mm and CAL 1-2 mm mild periodontitis. All in individuals with periodontal disease included in the study group were classified as having chronic (advanced) periodontitis. In the control group, all assessed parameters were within the reference range.

Sample collection. Participants were asked to refrain from eating or drinking, excluding water, from 11:00 pm of the night preceding sample collection and to brush their teeth and entire mouth in the evening before the visit, but not on the morning of collection. Tongue swabs were obtained before mouth washouts collection. A sterile cotton-tipped swab was used to collect the specimen from the external surface of the tongue, placed in a 1.5 ml sterile polypropylene tube with phosphate-buffered saline (sterile PBS, pH 7.2) and kept for 15 min at 4˚C to dissolve the swab content in the buffer. Next, the samples were frozen in a dry-ice bath and stored at −80˚C until shipping to Wroclaw University of Technology (Poland) for metabolomics analysis. To obtain mouth washout, 0.5 ml of sterile saline (0.9% solution of NaCl) was given to participants to rinse the oral cavity for 20-30 seconds, then the sample was collected in a 2 ml sterile polypropylene tube and immediately frozen in a dry-ice bath until shipping for metabolomics analysis. No preservatives were added to the samples.

Sample preparation for ^{1}H-nuclear magnetic resonance (NMR) measurements. Biological samples were thawed at room temperature and vortexed. Each sample of 120 μl of mouth washout and 120 μl of tongue swab in PBS were transferred to new Eppendorf tubes. The biological samples were then centrifuged for 10 min at 4˚C at 13,845

× *g*. After centrifugation, 100 µl of supernatant was transferred to a new Eppendorf tube and mixed with 400 µl deuterium oxide (D_2O) and 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt (TSP) (0.3 mM), and then transferred to an NMR cuvette (5 mm, Type 5SP; ARMAR GmbH, Leipzig, Saxony, Germany).

Proton NMR measurements. The NMR spectra of mouth washout and tongue swab samples were recorded at 300 K using an Avance II spectrometer (Bruker, GmBH Hamburg, Germany) operating at proton frequency of 600.58 MHz. The CPMG pulse sequence with water presaturation (*cpmgpr1d* in Bruker notation) was used for both types of biological material. Spectra for each sample were collected by 128 subsequent scans with relaxation delay of 3.5 seconds; acquisition time of 2.73 seconds; time domain (TD) of 64k; spectral width (SW) of 20.01 ppm. The ^{1}H-NMR spectra were processed with line broadening of 0.3 Hz and manually phased, baseline-corrected using Mestrelab Research v 11.0, (MestReNova, Santiago de Compostela, Spain). Chemical shift was adjusted to reference signal of TSP at δ=0.00 ppm. The resonance signals were aligned with the use of the correlation optimized warping algorithm (COW) and the *icoshift* algorithm implemented in Matlab 2014a (Mathworks Inc., Natick, MA, USA) (39,40). Probabilistic quotient normalization (PQN) method was used for spectra normalization (41).

Processing for data analysis. Calculation of the relative integral of any NMR-measured metabolite was obtained as a sum of data points of the non-overlapping resonances (or a cluster of partly overlapping resonances). The metabolite resonances were identified according to assignments published in the literature, the v 8.2 Chenomx software (Chenomx Inc. Edmonton, Alberta, Canada) and on-line databases (Biological Magnetic Resonance Data Bank (www.bmrb.wisc.edu) and Human Metabolome Data Base (www.hmdb.ca). Only those metabolites values that could be calculated based on assumptions of non-overlapping resonances were used for further analysis. A total of 21 mouth washout and 18 tongue swab metabolites were assigned. Data matrix was scaled by unit variance scaling (UV, Autoscaling).

Multivariate data analysis. The Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) with a 7-fold cross-validation procedure was performed to determine variation between studied groups. Sample order in the data matrix was randomized. The reliability of OPLS-DA models was assessed by cross validation analysis of variance at a significance level α=0.05. The importance of the variables explaining the part of X related to Y were presented by VIPpred plot (Predictive Variable Importance for the Projection). Multivariate analysis calculations were carried out in SIMCA 15 software (Sartorius Stedim Biotech, Göttingen, Lower Saxony, Germany).

Statistical data analysis. Calculated relative integral of metabolites assigned for mouth washouts and tongue swabs were used for univariate statistics. The Shapiro–Wilk test was calculated for each variable at a significance level α=0.05. Depending on the results of normality test, a parametric (Student's *t*-test) or a nonparametric (Mann–Whitney–Wilcoxon test) test was used, both at a significance level α=0.05. The changes between relative integrals of metabolites of studied groups were verified by average percentage difference (APD) and median percentage difference (MPD). APD and MPD were calculated in relation to values for the control group, whereby a positive value refers to the value being higher in the patient group and negative one being lower in the patient group than in the control group. The boxplot data visualization and receiver operating characteristics (ROC) curve calculation (classical univariate ROC curve analyses) were prepared with MetaboAnalyst 4.0 platform (42). The cutoff value for area under the ROC curve (AUC-ROC) of 0.750 was implemented for selection of the metabolites most predictive of CPD.

Results

The ^{1}H-NMR measurements allowed assignment of 21 recognized and four unknown metabolites from tongue swabs and mouth washouts as listed in Table I.

The analysis of the tongue swabs by ^{1}H-NMR method detected 17 metabolites: acetate, acetone, creatine, ethanol, glycerol, glycine, isopropanol, lactate, methanol, O-phosphocholine, propionate, pyruvate, succinate, and four unknown compounds. Among all these metabolites, only three were of statistical significance: Unknown 1, isopropanol, and glycerol (Table II). The first two metabolites were found at a higher level and the third was reduced in the CPD group compared to the control group.

Analysis of the mouth washouts revealed the presence of 23 assigned metabolites, namely: acetate, acetoin, acetone, alanine, choline, ethanol, formate, glucose, glycerol, glycine, isopropanol, lactate, methanol, propionate, propylene glycol, pyruvate, succinate, taurine, valine and unknown compounds 1-4 (Table I). Differences in levels of four metabolites were statistically significant between healthy and study groups: acetone, methanol and unknown 2 were lower, while that of lactate was higher in CPD than in controls (Table III).

The OPLS-DA analysis showed separation between patient and healthy control groups with satisfactory parameters (Figures 1 and 2; Table IV), where both models passed the validation test at the level of p=0.05.

The identified metabolites were verified by the ROC curve by their discrimination potential. The value of 0.75 of AUC-ROC was utilized as the cutoff point for selection of metabolites. According to this limit, metabolites such as unknown 1, isopropanol, and glycerol were important based on tongue swab samples (Table II). Moreover, for mouth washout, acetone, methanol, lactate, unknown 2 and taurine passed the determined critical value for the ROC curve (Table III).

Discussion

To date, metabolomics analysis pertinent to periodontitis has focused only on the metabolome of saliva and GCF (43-47). In this study, we investigated the metabolic status of the oral cavity in CPD using easily accessible, simple to collect and non-invasive sampling of mouth washouts and tongue swabs. The changes in the metabolite levels presented in Tables II and III seem to be related to two phenomena. First,

Table I. *Proton nuclear magnetic resonance (^{1}H-NMR) resonance signals with their chemical shift δ, multiplicity and identification, which were used in data analysis for tongue swabs and mouth washouts.*

No.	Resonance signal assignment	^{1}H δ=(ppm)		HMDB ID
		Tongue swabs	Mouth washout	
1	Acetate	1.91 (s)	1.92 (s)	HMDB00042
2	Acetoin	-	1.38 (d)	HMDB03243
3	Acetone	2.22 (s)	2.21 (s)	HMDB01659
4	Alanine	-	1.48 (d)	HMDB00161
5	Creatine	3.02 (s)	-	HMDB00064
6	Choline	-	3.20 (s)	HMDB01565
7	Ethanol	1.17 (t)	1.18 (t)	HMDB00108
8	Formate	-	8.48 (s)	HMDB00142
9	Glucose	-	5.23 (d)	HMDB00122
10	Glycerol	3.56 (m)	3.58 (m)	HMDB00131
15	Glycine	3.55 (s)	3.56 (s)	HMDB00123
11	Isopropanol	1.16 (s)	1.17 (d)	HMDB00863
12	Lactate	1.31 (d)	1.33 (d)	HMDB00190
13	Methanol	3.34 (s)	3.36 (s)	HMDB01875
14	O-Phosphocholine	3.21 (s)	-	HMDB01565
16	Propionate	1.04 (t)	1.06 (t)	HMDB00237
17	Propylene glycol	-	1.15 (d)	HMDB01881
18	Pyruvate	2.36 (s)	2.37 (s)	HMDB00243
19	Succinate	2.39 (s)	2.41 (s)	HMDB00254
20	Taurine	-	3.41 (t)	HMDB00251
21	Valine	-	1.05 (d)	HMDB00883
22	Unknown 1	0.89 (t)	0.89 (t)	No ID
23	Unknown 2	1.25 (s)	1.25 (s)	No ID
24	Unknown 3	1.29 (m)	1.56 (q)	No ID
25	Unknown 4	3.71 (m)	3.02 (m)	No ID

Table II. *Analysis of tongue swab samples showing average percentage difference (APD), median percentage difference (MPD) and relative standard deviation (RSD) of relative integrals for metabolites for the study groups.*

			RSD	
Tongue swabs	APD (%)	MPD (%)	Controls (%)	Patients (%)
Unknown 1[a#*]	79.54	97.03	76.98	72.1
Propionate	−0.34	−3.73	30.19	44.46
Isopropanol[a#*]	35.71	67.9	74.27	38.79
Ethanol	−2.59	−4.26	18.94	19.24
Unknown 2	34.32	32.15	90.55	69.92
Unknown 3	4.46	4.85	45.29	49.99
Lactate	28.9	16.39	37.94	49.4
Acetate	34.74	70.65	86.45	81.6
Acetone	−1.46	−2.04	9.25	12.17
Pyruvate	8.46	−4.79	50.93	41.57
Succinate	−13.28	−5.15	49.99	51.71
Creatine	4.99	23.49	63.94	62.21
O-Phosphocholine	8.1	15.48	60.41	38.28
Methanol[a]	−8.11	−11.57	14.41	15.33
Glycine	−5.07	−24.13	94.76	97.92
Glycerol[a#*]	−39.39	−51.15	29.67	39.79
Unknown 4[a]	−37.56	14.23	129.64	77.83

[a]Predictive variable importance prediction value above 1.00. *Statistically important metabolites. [#]Metabolites with area under the receiver operating characteristics curve >0.75.

to the activity of the consortium of microorganisms in the oral cavity, which utilize various energy sources during colonization and survival, and second, to the host response to ongoing pathological processes. Eight metabolites altogether can be used as potential markers of periodontitis-associated processes, however, some of them (methanol and isopropanol), which are considered as exogenous compounds, were reported as endogenous metabolites of saliva (43). Moreover, the trends in metabolite level changes in the month washouts and tongue swabs did not always match (Tables II and III). This might have been a result of the methodology of sample collections. Whereas tongue swabs were obtained through physical rubbing on the mucosal surface, the mouth washouts involved gentle rinsing of the oral cavity. A second reason may be associated with the change in solubility or local concentration of the metabolite and gingival response to these stimuli.

Lactic acid was a statistically important metabolite in mouth washout samples. This metabolite is recognized as a side product of oral microbiome activity, *e.g.* a carbohydrate fermentation (48), due to poor hygiene of the oral cavity. On the other hand, the presence of the Lactobacilli species was associated with their protective function against pathogenic strains (49). Interestingly, in the context of the analysis of variation in trends for metabolites in mouth washout samples from patients with CPD, a similar metabolic profile (formate, propionate) to that found in this study was detected in patients with generalized aggressive periodontitis (G-AgP) (50). The analysis showed significantly increased concentrations of lactic acid, while that of formic acid was lower in comparison to the control group (50) and its concentration was associated with *P. gingivalis*-positive sites.

Previous studies exploring the levels of antioxidants and oxidative stress in serum, saliva, and GCF of patients with CPD have shown elevated lipid peroxidation and a disturbed antioxidant status (51-53). Although in this study, we did not detect unambiguously any products of protein/lipid peroxidation in CPD-derived mouth washouts, elevated level of taurine was detected. We hypothesize that mobilization of taurine within the oral cavity may constitute a protective effect mediated by its antioxidative properties and regeneration of inflammatory gingival tissue (54).

Table III. *Analysis of mouth washout samples showing average percentage difference (APD), median percentage difference (MPD) and relative standard deviation (RSD) of relative integrals for metabolites for the study groups.*

Mouth washout	APD (%)	MPD (%)	RSD Controls (%)	RSD Patients (%)
Unknown 1	−7.78	51.49	109.02	68.61
Valine	−7.12	42.26	133.92	122.95
Propionate	10.56	59.98	87.8	41.82
Propylene glycol	20.98	−29.01	135.02	197.17
Isopropanol	3.82	31.49	125.18	106.86
Ethanol	9.84	−6.55	33.24	50.50
Unknown 2[a#*]	−153.25	−134.53	109.44	63.12
Lactate[#*]	101.96	61.82	89.77	123.44
Acetoin	0.08	−9.19	164.21	180.46
Alanine	19.5	26.76	106.73	83.20
Unknown 3	−15.72	1.62	99.33	82.00
Pyruvate	17.81	36.44	129.55	77.07
Acetate[a]	−26.89	10.9	83.76	44.98
Acetone[a#*]	−23.71	−22.96	23.57	17.84
Succinate	8.07	−35.7	47.43	96.88
Unknown 4[a*]	−56.35	−38.07	99	75.50
Choline	−0.54	1.9	124.39	83.45
Methanol[a#*]	−37.52	−39.63	26.12	34.39
Taurine[#]	57.47	77.4	122.29	88.10
Glycine[a]	−26.66	29.13	105.12	58.54
Glycerol	50.24	4.9	37.7	108.95
Glucose	67.86	86.74	126.82	121.67
Formate	−39.29	−53.64	84.46	132.82

[a]Predictive variable importance prediction value above 1.00. *Statistically important metabolites. [#]Metabolites with area under the receiver operating characteristics curve >0.75.

Table IV. *Summary of Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) model parameters with CV-ANOVA p-value.*

Comparison	N	Apred	Aorth	R^2X	R^2Y	Q^2	*p*-Value
Tongue swabs	34	1	2	0.363	0.757	0.391	0.026
Mouth washout	35	1	1	0.455	0.522	0.322	0.017

Apred: Number of predictive components; Aorth: number of orthogonal components; R^2X: cumulative fraction of the variation of X variables explained by the model; R^2Y: cumulative fraction of the variation of Y variables explained by the model; Q^2: cumulative fraction of the variation of Y variables predicted by the model.

Acetone originates from breakdown of acetoacetate and α-hydroxybutyrate, accompanying fatty acid degradation, glycolysis, and pyruvate metabolism (55-57). Acetone mouth odor is mainly associated with diabetes and halitosis, however, its higher level in body fluids may be used in clinical diagnostics (56-58). The metabolism of acetone is closely associated with the production of lactic acid, which in this study was increased, therefore can be the product, at least in part of acetone biotransformation (57). Increased level of isopropanol in samples from CPD-affected patients is rather surprising, considering that it is used as a common disinfectant and antiseptic agent. However, the positive correlation between isopropanol and acetone was found, where acetone can also be the product of isopropanol breakdown (56).

In general, glycerol is a metabolite that can originate from glucose, proteins, pyruvate, triacylglycerols, and other metabolic pathways (59). A part of glycerol of biological origin can also be derived from its use as an ingredient of toothpaste. Surprisingly, its level was found to be significantly decreased in CPD, and this phenomenon can be explained by utilization of glycerol as a carbon source or an osmoregulatory agent by bacteria and fungi (59) colonizing the oral cavity during periodontitis. The literature data showed that glycerol-3-phosphate, which is the product of glycerol phosphorylation by glycerol kinase, was found to be elevated in periodontal disease (25, 60).

The presence of methanol in the oral cavity remains in conflict with its toxic properties. In general, methanol is considered a toxic agent in mammals. However, it is commonly found as an endogenous metabolite, especially in exhaled breath (61,62). Very recently it was suggested that methanol might regulate mammalian gene activity (63). The role of methanol and its sources in humans are not well known. However, anaerobic fermentation by bacteria in the human gut associated with a pectin-rich plant diet is considered the source of methanol (64). Another process is the transformation of S-adenosyl methionine to methanol (65). Therefore, the presence of methanol in the oral cavity is not surprising and its lowered level in those with CPD than in healthy individuals may be related to its use as a carbon or energy source for bacteria associated with CPD.

All these metabolites might contribute to CPD and determining the pattern of their changes could be helpful to highlight specific biochemical disturbances associated with the transition from oral health to dysbiosis observation in CPD.

Conclusion

The current study was performed to recognize the feasibility of using metabolomic analysis in tracing pathological changes in the oral cavity during the transition from health to CPD. Based on the multivariate models, ROC, and statistical analysis, it may be possible to find correlation between specific patterns of metabolite variation and the activity of the microbial consortium in the oral cavity, which utilize various energy sources during colonization.

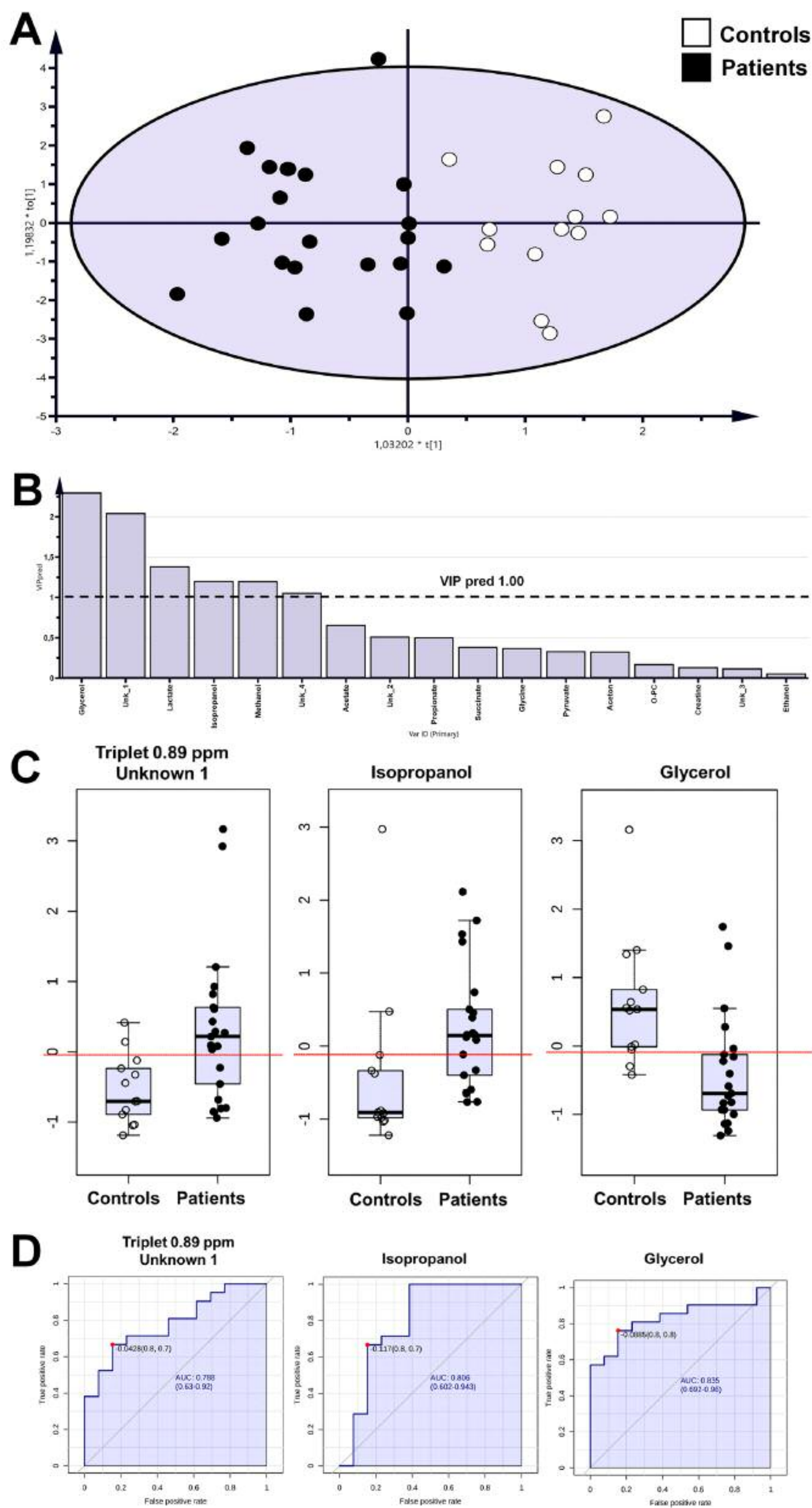

Figure 1. *Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) models for tongue swab samples (A), predictive variable importance for the projection plot (B), boxplot for statistically important metabolites and these with area under the receiver operating characteristics curve (AUCROC)>0.75 (C), univariate ROC curves for selected metabolites (D).*

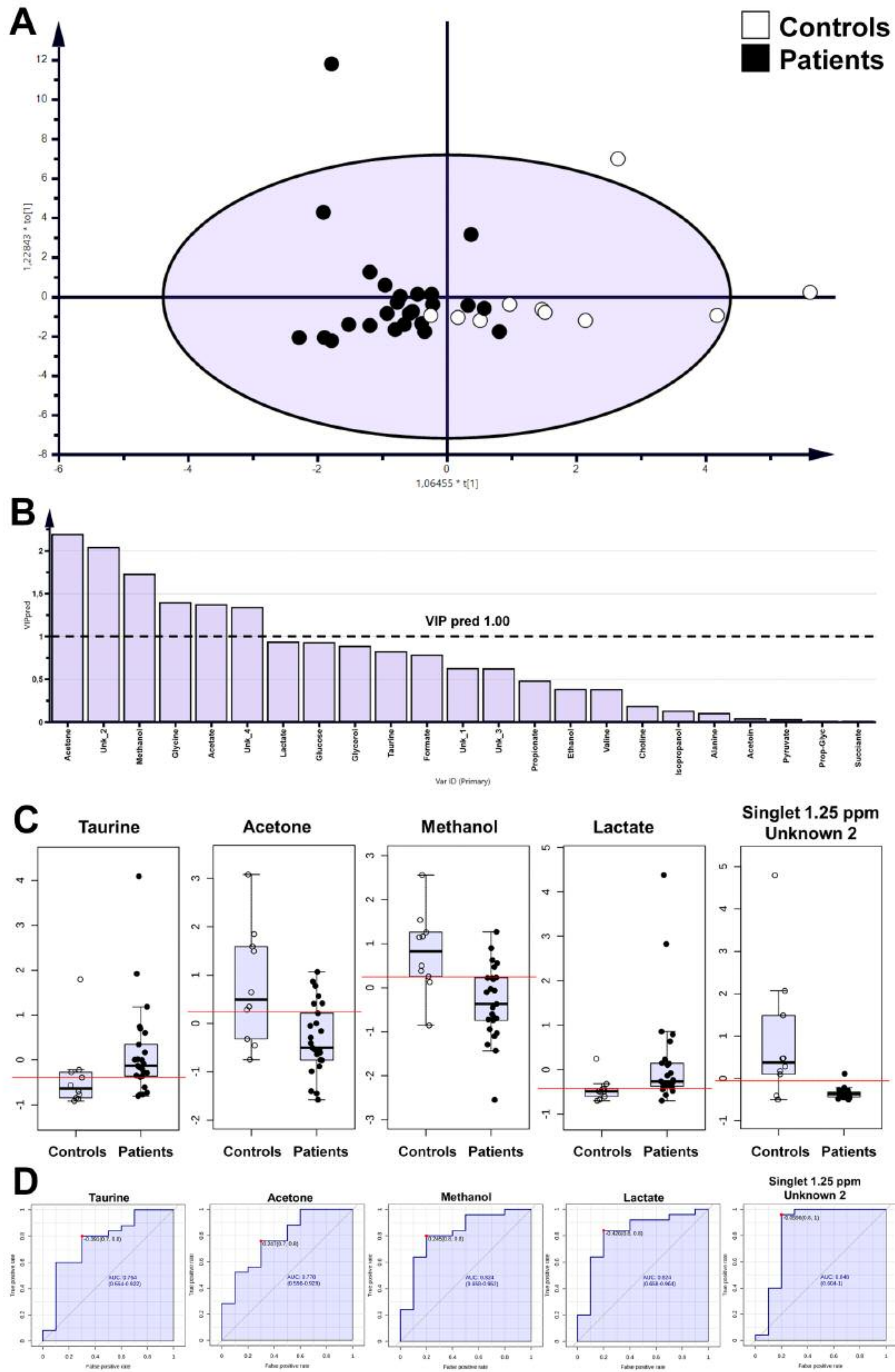

Figure 2. *Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) models for mouth washout samples (A), predictive variable importance for the projection plot (B), boxplot for statistically important metabolites and/or these with area under the receiver operating characteristics (AUCROC)>0.75 (C), univariate receiver operating characteristics (ROC) curves for selected metabolites (D).*

We conclude that CPD-associated bacterial have a higher metabolic activity as revealed by changes of lactate, acetone, isopropanol, glycerol and methanol levels in comparison to commensal microbiota in a healthy mouth. We suggest that these metabolites are potential candidate biomarkers of CPD and propose NMR-based metabolomics analysis as a potentially useful methodological approach for monitoring the pathological processes observed in the oral cavity in the course of this disease. Further insight is needed to confirm this preliminary observation through a prospective study on a larger cohort of patients with CPD matched by sex and age with healthy individuals.

Ethics Approval and Consent to Participate

The study was carried out in accordance with the Declaration of Helsinki. The Bioethical Committee at the Jagiellonian University (KBET/310/B/2012), Medical College in Krakow approved the protocol, including the clinical/periodontal examination, collection of tongue swabs and mouth washouts.

Consent for Publication

All participants read and signed a written informed consent prior to enrollment in the study.

Availability of Data and Material

The datasets generated during and/or analyzed during the current study are available from the corresponding Author upon a reasonable request.

Conflicts of Interest

The Authors declare that they have no conflict of interests in regard to this study.

Authors' Contributions

KG: Experiment idea; clinical sample collection; clinical part of the study conduction; manuscript preparation; substantive discussion. WW: Performed the metabolomics experiment; data calculation, article preparation. BQ: Manuscript edition, data preparation. AL: Data preparation. KŁ-B: Clinical part of the study conduction; substantive discussion. PM: Substantive discussion. MC-G: Substantive discussion; JP: Substantive discussion. PM: Experiment idea; manuscript preparation; substantive discussion.

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