Inflammatory and metabolic profiles of patients with end-stage renal disease: potential strategy for predictive, preventive, and personalized medicine

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Abstract

Objectives Few studies reported the periodontal disease-related metabolic profile of end-stage renal disease (ESRD) patients. The present study aimed to compare the inflammatory and metabolic differences between patients with ESRD and healthy controls, and to identify potential useful biomarkers for predictive, preventive, and personalized medicine (PPPM) in GCP and serum of ESRD patients.

Methods Patients with ESRD (ESRD group; n = 52) and healthy controls (HC group; n = 44) were recruited. Clinical periodontal parameters were recorded. The differential metabolites in the GCF and serum were identified by liquid chromatography/mass spectrometry. Inflammatory markers including Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Interleukin-8 (IL-8) and C-reactive protein (CRP) were also assessed.

Results In ESRD group, IL-8 and CRP were significantly higher in GCF, whereas IL-6 and CRP were significantly higher in serum, compared with HC group (all $P < 0.05$). In the case of GCF, taurine levels were positively correlated with IL-8 levels in both groups (all $P < 0.05$). In the case of serum, L-phenylalanine and p-hydroxyphenylacetic acid levels were positively correlated with CRP levels in both groups (all $P < 0.05$). Significant positive correlation was observed between pseudouridine and IL-6 levels only in ESRD group.

Conclusions IL-8 and CRP were potential inflammatory makers. Metabolites of taurine in GCF as well as L-phenylalanine and p-hydroxyphenylacetic acid in serum were possible biomarkers that correlated with inflammatory cytokine. All these biomarkers may consider as a potential strategy for the prediction, diagnosis, prognosis, and management of personalized periodontal therapy in the population with ESRD.

1 Introduction

End-stage renal disease (ESRD) refers to the end stage of chronic kidney disease (CKD) that requires renal replacement therapy, including peritoneal dialysis, hemodialysis, or kidney transplantation [1]. ESRD patients with hemodialysis therapy have a relatively lower life expectancy than the general population, whose 5-year survival rate is estimated to be 76% [2]. Evidence from observational studies suggests that a larger proportion of patients undergoing dialysis treatment suffer from periodontal diseases [3]. And it is reported that 58.9% of hemodialysis patients have moderate to severe periodontitis [4]. Currently, a number of studies have shown the association between periodontal health and ESRD [4–7].

According to a recent systematic review, there is a mild evidence that CKD has an impact on periodontal status [7]. Immune response to the etiological factors of periodontal diseases is the determinant of disease susceptibility. ESRD patients are more susceptible to periodontal disease due to general debilitation and depression of the immunological system. CKD, especially ESRD, may affect periodontal condition through systemic immune disorders, decreased saliva secretion, and accumulation of waste products in the blood [8–11].

Studies based on gingival crevicular fluid (GCF) analysis exhibit the examination of the levels of inflammatory chemokines such as IL-8 can be used to assess periodontal health status [12–14]. IL-8 is an important chemokine and can be produced by many cell types in gingival epithelial tissues such as endothelial cells and neutrophils [15, 16]. Higher expression of IL-8 can be detected when the gingival tissue is in an inflammatory state in comparison to healthy gingiva [17, 18]. In addition to inflammatory chemokines as the biological markers to the periodontal inflammation, the dynamics of the metabolic composition in GCF also hints development of periodontal disease. Previous studies have reported that significant differences in metabolites related to inflammation, oxidative stress, tissue degradation, and bacterial metabolism could be observed in GCF between the population with and without periodontitis [19, 20]. Metabolites can not only reflect metabolic activities but affect clinical phenotypes, as they are the end products of biological processes and indicate the expression levels of many functional genes and proteins [21]. Metabolite changes in GCF could serve as a useful tool to reflect the periodontal inflammation status.

Considering these previous observations, the levels of certain metabolites may be associated with inflammatory levels in both GCF and serum. Therefore, the aim of present study was to compare the inflammatory markers and metabolite levels in serum and GCF between ESRD group and healthy controls (HC) group using metabonomics, and to explore the association between inflammatory markers and metabolites. Integration of inflammatory and metabolomic data with clinical data will provide more valuable information for PPPM in GCP and serum of the individuals especially ESRD patients.

2 Materials And Methods

2.1 Participants

Prior to the implementation of the study, a full-mouth periodontal examination was performed on all participants by an examiner using a sterile periodontal probe (PCPUNC 15, Hu-Friedy, Chicago, IL, USA). Periodontal pocket depth (PPD), gingival Bleeding Index (GBI) and calculus Index (CI) were recorded to identify the periodontal health status. Structured questionnaire was used to collect the information on sociodemographic background (age and sex), smoking habit, oral health-related behaviors (toothbrushing frequency and use of dental floss), and medical history (presence of hypertension or diabetes) of each participant.

Ethical approval was obtained by the Research Ethics Committee of Shanghai Ninth People's Hospital. Each participant was informed about the purpose and procedure of the study. Each participant signed a written informed consent prior to the implementation of the study.

2.2 Collection of biological samples

2.2.1 Serum

A total of 3 mL peripheral blood was collected in vacutainer tubes in the morning from all participants. The blood samples were incubated at room temperature for not more than 2 h and centrifuged for 10 min at 2,000 × g at 4°C. Serum was collected in the empty tubes and then stored at -80°C.

2.2.2 GCF

All participants were instructed not to eat or drink within 2 h prior to the collection of GCF. PerioPaper Strips® (Oraflow Inc, New York, USA) were used to collect GCF samples from the disto-buccal and mesio-buccal sites of all the first molars. The targeted area was separated with cotton rolls, PerioPaper Strips® were placed in the gingival sulcus for 30 s to collect GCF, and all periopapers strips from different sites were pooled in the same tube. The samples were then stored in an empty tube at -80°C.

2.3 Estimation of inflammatory markers in GCF and serum

Accordingtothekitinstructions, the concentrations of Interleukin-1β (IL-1β), Interleukin-6 (IL-6), and Interleukin-8 (IL-8) in GCF and serum as well as C-reactive protein (CRP) in GCF were measured using Human High Sensitivity T Cell Magnetic Bead Panel (Luminex Corporation, Austin, TX, USA*).* Further, the concentration of CRP in serum was measured using an CRP ELISA kit (R&D Systems, Minneapolis, MN, USA*).*

2.4 Metabolite extraction

For metabolite extraction from GCF, 200 µL extraction solution (methanol: acetonitrile: water = 2:2:1, with isotopically labelled internal standard mixture) was added to periopaper strips. The mixture was vortexed for 30 s and grinded at 35 Hz for 4 min. Thereafter, the samples were sonicated for 15 min in ice-water bath. For the extraction of serum metabolites, 50 µL of sample was mixed with 200 µL of extraction solution (acetonitrile: methanol = 1: 1, containing isotopically labelled internal standard mixture). The mixture was vortexed for 30 s and sonicated for 10 min in ice-water bath. Both mixtures were incubated for 1 h at -40^{o}C and centrifuged at 12,000 rpm for 15 min at 4^{o}C. The supernatant was separated and mixed with quality control (QC) samples for machine testing.

2.5 Metabonomics analysis

Liquid chromatography/mass spectrometry (LC/MS) was used for metabonomics analysis. It was performed using a UHPLC system (Vanquish, Thermo Fisher Scientific) containing a UPLC BEH Amide column (2.1 mm × 100 mm, 1.7 µm) with Q Exactive HFX mass spectrometer (Orbitrap MS, Thermo). HPLC contained aqueous phase (25 mmol/L ammonium acetate and 25 mmol/L ammonia) and acetonitrile. The analysis was processed with elution gradient. Other LC parameters were as follows: mobile phase flow rate: 0.5 mL/min, column temperature: 30^{o}C, sample pan temperature: 4^{o}C, injection volume: 4 µL. The QE HFX mass spectrometer was used to collect primary and secondary mass spectrometry data under the control of the acquisition software (Xcalibur, Thermo).

2.6 Data preprocessing and annotation

The raw data were transformed into the mzXML format, using R software to process peak detection, extraction, alignment, as well as integration. Further, an in-house MS2 database (BiotreeDB) was used for metabolite annotation with the cutoff for annotation set at 0.3.

2.7 Sample size calculation

A pilot study (n=15 in each group) was performed to obtain information about inflammatory markers. The levels of IL-8 were significantly different between two groups, which we chose as the primary outcome. We assumed two-sided hypothesis testing under the 5% type I error and 90 % statistical power to detect a difference of 0.815 ng/ml with estimated group standard deviations of 1.331 ng/ml and 0.603 ng/ml. A total of 36 participants were required in each group, which was calculated using *PASS11.0*(NCSS, LLC*. Kaysville,Utah,USA)*.

2.8 Statistical data analysis

The Kolmogorov–Smirnov test was performed to test the normality of data distribution. Numerical variables were presented as mean (SD) when parameters were normally distributed or as median (interquartile range) when the distribution was skewed. Student's t-test or Mann–Whitney U test was used to compare the differences between ESRD and HC groups depending on whether variances were homogenous and normal. Chi-square test or Fisher's exact test was used to compare the differences of sociodemographic background, lifestyle, and medical history between the two groups. Based on SIMCA software V16.0.2 (Sartorius Stedim Data Analytics AB, Umea, Sweden), principal component analysis (PCA) was used to show the sample distribution within the two groups. Orthogonal partial least-squares discriminant analysis (OPLS-DA) was also used to assess total differences of metabolites between the two groups. Multivariate models were performed to determine various metabolites with variable importance (VIP) values >1.0 and P <0.05 as the key metabolites for a further analysis. Pearson correlation coefficient was used to calculate the correlations between metabolites and inflammatory markers. Multiple factor ANOVA analysis was used to assess the association between system diseases and the levels of IL-8 in the CGF. P <0.05 was considered to be statistically significant.

3 Results

3.1 Participant characteristics

A total of 96 participants were enrolled in the study. Among these, 52 were ESRD patients receiving hemodialysis treatment (30 males and 22 females; group designated as ESRD), and 44 were healthy controls (25 males and 19 females; group designated as HC). Characteristics of all patients were summarized in Table 1. There were no significant differences regarding age, smoking history and oral health related behaviors between the two groups (all P > 0.05). The proportion of participants with diabetes and hypertension was significantly higher in the ESRD group than in the HC group (all P < 0.05). There were significant differences in periodontal parameters including PPD, GBI and CI between two groups (all P < 0.05).

Table 1
Participant characteristics of the ESRD and HC groups

		ESRD group	HC group	P value
Total		52	44	
Age groups; n (%)				0.574
	≤ 40	4 (7.7%)	5 (11.4%)	
	41–50	10 (19.2%)	11 (25.0%)	
	51–60	17 (32.7%)	16 (36.4%)	
	≥ 61	21 (40.4%)	12 (27.2%)	
Sex; n (%)				0.548
	male	30 (57.7%)	25 (56.8%)	
	female	22(42.3%)	19 (43.2%)	
Smoking history; n (%)				0.462
	Yes	15 (28.8%)	14 (31.8%)	
	No	37 (71.2%)	30 (68.2%)	
Toothbrushing frequency				0.064
	≤ 1 per day	25 (48.1%)	13 (29.5%)	
	≥ 2 per day	27 (51.9%)	31 (70.5%)	
Use of dental floss				0.222
	Yes	5 (9.6%)	8 (18.2%)	
	No	47 (90.4%)	36 (81.8%)	
Diabetes history*; n (%)				0.019
	Yes	13 (25.0%)	3 (7.0%)	
	No	39 (75.0%)	40 (93.0%)	
Hypertension history*; n (%)				< 0.001
	Yes	40 (76.9%)	10 (23.3%)	
	No	12 (23.1%)	33 (76.7%)	
GBI (%), median (IQR)		20.60 (17.86–23.68)	14.29 (12.96–17.86)	0.008

	ESRD group	HC group	P value
CI (%), median (IQR)	10.79 (7.41-12.00)	7.28 (3.70-10.71)	0.013
PPD (mm), median (IQR)	3.46 (3.33–3.62)	3.32 (3.19–3.44)	0.020
*: One missing value; ESRD: End-stage renal disease; HC: Healthy controls; PPD: Periodontal pocket depth; GBI: Gingival bleeding index; CI: Calculus index			

3.2 Comparison of inflammatory markers in serum and GCF

The levels of IL-8 and CRP of GCF were significantly higher in the ESRD group than in the HC group (P = 0.032 and P < 0.001, respectively). In addition, the levels of IL-6 and CRP of serum were higher in the ESRD group (P = 0.008 and P < 0.001, respectively). However, there were no significant differences on the IL-1β and IL-6 levels of GCF. Moreover, IL-1β and IL-8 levels in serum did not show significant differences between the two groups (Table 2).

Table 2
Comparison of inflammatory markers between the ESRD and HC groups

Inflammatory markers	ESRD group Median (IQR)	HC group Median (IQR)	P value
Serum			
IL-1β (pg/ml)	0.75 (0.60–1.01)	0.88 (0.60–1.25)	0.225
IL-6 (pg/ml)	5.76 (4.89–7.68)	4.46 (3.36–5.03)	0.008
IL-8 (pg/ml)	46.55 (38.98–82.49)	38.86 (32.29–47.41)	0.073
CRP (ng/ml)	2.20 (1.60–4.09)	0.74 (0.50–1.26)	< 0.001
GCF			
IL-1β (ng/ml)	0.26 (0.20–0.37)	0.34 (0.20–0.53)	0.186
IL-6 (pg/ml)	2.75 (2.14–3.23)	2.46 (2.19–3.24)	0.622
IL-8 (ng/ml)	1.44 (0.81–2.26)	0.88 (0.55–1.23)	0.032
CRP (ng/ml)	0.48 (0.29–0.78)	0.16 (0.11–0.23)	< 0.001
ESRD: end-stage renal disease; HC: healthy controls; IL-1β: Interleukin-1β; IL-6: Interleukin-6; IL-8: Interleukin-8; CRP: C-reactive protein; IQR, interquartile range			

3.3 Results of multiple factor ANOVA of the level of IL-8 in the GCF

Since the distribution of diabetes and hypertension history between ESRD and control groups were significantly different, multiple factor AVOVA were performed to explore the association between groups

(ESRD and HC group), diabetes, hypertension history and level of IL-8 in the GCF. As shown in the Table 3, only the group significantly associated with the IL-8 levels of GCF (β = 1.17; P = 0.001), while diabetes and hypertension history did not associated with IL-8 level (β = 0.27, P = 0.486 and β = -0.61, P = 0.071).

Table 3
Results of multiple factor ANOVA analysis of the level of IL-8 in the GCF

Variables		Beta	95% CI	P value
Group				0.001
	ESRD	1.17	0.49–1.84	
	HC [a]			
Diabetes				0.486
	Yes	0.27	-0.5–1.04	
	No [a]			
Hypertension				0.071
	Yes	-0.61	-1.28–0.05	
	No [a]			

GCF: Gingival crevicular fluid; IL-8: Interleukin-8; ESRD: End-stage renal disease; HC: Healthy controls; CI: Confidence interval; R^2 = 0.130; [a] reference

3.4 Metabonomics analysis of serum samples

A total of 261 differential metabolites with VIP > 1 and P < 0.05 were identified in the serum samples for the hierarchical cluster analysis (Fig. 1a). In the plot of PCA scores, the obvious divergence of metabolites in serum samples could be observed between ESRD group and HC group (Fig. 1b). The OPLS-DA model (Fig. 1c) was constructed many times to obtain the R^2Y and Q^2 values of the random model (R^2Y = 0.983 and Q^2 = 0.973), demonstrating that the original model had a good robustness and there was no overfitting (Fig. 1d). The four main metabolic pathways that differed dramatically in serum included arginine and proline metabolism, phenylalanine metabolism, glycine/serine/threonine metabolism, and pyrimidine metabolism (Fig. 1e). Among these various pathways, 19 key metabolites were selected for further analysis, and fold changes (FCs) of key metabolites (P < 0.05) were calculated. The metabolites 4-hydroxybenzoic acid, 4-acetamidobutanoic acid, and alpha-N-phenylacetyl-L-glutamine (log_2 (FC): 7.29, 7.34 and 7.54, respectively) showed the highest FCs in the ESRD group in comparison with HC group (Fig. 1e). To assess the discriminating accuracy of the metabolites in differentiating two populations, the area under curve (AUC) was employed to identify 19 metabolites of serum (Fig. 1f). And the high AUCs of metabolites were observed, most of which were up to 1.00.

3.5 Metabonomics analysis of GCF samples

A total of 88 differential metabolites with VIP > 1 and P < 0.05 were identified for the hierarchical cluster analysis (Fig. 2a). The PCA score showed a good separation of metabolites between the two groups (Fig. 2b). The OPLS-DA model (Fig. 2c) was constructed many times to obtain the R^2Y and Q^2 values of the random model ($R^2Y = 0.682$ and $Q^2 = 0.616$, Fig. 2d). The seven main metabolic pathways that showed the significant differences in GCF samples were galactose metabolism, nicotinate and nicotinamide metabolism, phenylalanine metabolism, purine metabolism, starch and sucrose metabolism, urine metabolism, and pyrimidine metabolism based on the KEGG pathway enrichment analysis (Fig. 2e, P < 0.05). As shown in Fig. 2e, the metabolites significantly upregulated in ESRD group in comparison to HC group were raffinose, alpha-D-glucose, alpha-D-glucose, D-maltose, dUMP, and N1-methyl-2-pyridone-5-carboxamide. Other compounds, including taurine, 3-sulfinoalanine, xanthine, inosine, hypoxanthine, m-coumaric acid, hydrocinnamic acid, and niacinamide were downregulated in ESRD group. Metabolites with the greatest FCs (P < 0.05) that were increased in ESRD group relative to HC group were dUMP, alpha-D-glucose, and raffinose (log_2 (FC): 3.04, 3.07 and 4.92, respectively). Two metabolites–niacinamide and hydrocinnamic acid–with the greatest FCs were decreased (P < 0.05) in ESRD group compared to HC group (log_2 (FC): -1.49 and − 1.72, respectively). The AUCs of 15 metabolites in the GCF were shown in the Fig. 2f, of which the highest AUC was 0.84.

3.6 Correlation between inflammatory markers and key metabolites in serum and GCF

The correlations between key metabolites found in serum and GCF and four inflammatory markers including IL-1β, IL-6, IL-8, and CRP were calculated. Only statistically significant positive or negative correlations (P < 0.05) between the two groups are shown in Table 4. In serum samples, the levels of L-phenylalanine and p-hydroxyphenylacetic acid positively correlated with the levels of CRP in both groups but positively correlated with the levels of IL-6 only in ESRD group. Significant positive correlation was observed between pseudouridine and IL-6 only in ESRD group. In the case of GCF samples, the levels of taurine were positively correlated with IL-8 levels in ESRD group and HC group, whereas 3-sulfinoalanine levels were positively correlated with IL-8 levels only in ESRD group.

Table 4
Correlation between various inflammatory markers and key metabolites in serum and GCF between the two groups

Sample		ESRD group	HC group
Serum	p-Hydroxyphenylacetic acid and IL-6	0.519**	-0.081
	L-Phenylalanine and IL-6	0.492**	-0.146
	Pseudouridine and IL-6	0.377**	-0.132
	p-Hydroxyphenylacetic acid and CRP	0.324*	0.309*
	L-Phenylalanine and CRP	0.309*	0.507**
GCF	Taurine and IL-8	0.570**	0.416**
	3-Sulfinoalanine and IL-8	0.469**	0.016

*: P < 0.05, **: P < 0.01. ESRD: End-stage renal disease; HC: Healthy controls; GCF: Gingival crevicular fluid; IL-6: Interleukin-6; IL-8: Interleukin-8; CRP: C-reactive protein

4 Discussion

The significantly associations between periodontal health status and ESRD have been assessed among a number of studies [22]. It is evident that that patients with kidney diseases have poor periodontal health in comparison to healthy individuals [23, 24]. However, no study could be traced about comparing GCF metabolites between ESRD patients and the healthy as well as the association between metabolites and periodontal inflammation status, thus the present study was conducted to compare inflammation markers, the significantly altered metabolites and their potential associations between two groups. In the present study, we found that IL-8 and CRP levels of GCF in ESRD group were significantly higher than that in HC group, in which IL-8 positively correlated with taurine and 3-sulfinoalanine. Meanwhile, we observed the periodontal parameters (PPD, GBI and CI) of ESRD patients was worse than that of healthy participants.

According to the 2018 American Academy of Periodontology and European Federation of Periodontology, periodontal health would be defined as a clinically non-inflammatory status, which means that absence of inflammation in periodontal tissue is a prerequisite for defining periodontal health [25]. And the success of treatment for periodontitis is to minimize inflammation and improve periodontal parameters [25]. We utilized inflammatory markers, especially IL-8, to reflect the periodontal inflammation status between the proups with and without ESRD. IL-8 can help neutrophils migrate from gingival tissue to the gingival crevice, thus facilitates the inflammatory cells infiltration and release of granule enzymes to efficiently degrade periodontal connective tissue [16, 26]. Previous studies proved that IL-8 was mainly detected in deeper layers of the pocket epithelium and indicated that IL-8 was involved in the induction

and development of periodontal disease [17, 27, 28]. Dag A et.al reported that IL-8 levels in GCF were found higher in hemodialysis patients compared with the healthy individuals [29]. The finding was consistent with our study. IL-8 may play an important role in the periodontal condition of ESRD patients.

When periodontal tissue is in an inflammatory state, inflammatory factors such as cytokines, bacterial antigens, various cells, metabolites and other degradation products are released in the GCF [30]. Interestingly, IL-8 showed a significant positive correlation with taurine and 3-sulfinoalanine in our study. Taurine, a type of free amino acid with antimicrobial and antioxidant activities, has a great impact on important biological processes related to inflammation [31, 32]. It was reported that taurine could inhibit oxidation of hypochloric acid associated with myeloperoxidase-hydrogen peroxide-halide system of neutrophils [31]. 3-sulfinoalanine, a product of cysteine dioxygenase, is involved in several processes, such as pyruvate production and taurine/hypotaurine synthesis [33]. This finding suggests that abnormally elevated inflammatory markers may induce the production of taurine and 3-sulfinoalanine to inhibit the inflammation. We also find other significant metabolites of GCF were more abundant in hemodialysis patients than in the healthy. However, the mechanism is not clear due to a shortage of the relevant researches.

Furthermore, though the compositions of GCF mainly origninated from serum,(30) there were many discrepancies between serum and GCF with regard to metablites and inflammatory markers. IL-6 levels were significantly different in the serum between two groups rather than IL-8 levels. IL-6-associated and CRP-associated metabolites including pseudouridine, L-phenylalanine, and p-hydroxyphenylacetic acid were reported to be associated with renal disease [34–36], which were seen obviously abundant in the serum instead of GCF in the ESRD patients. The discrepancy between serum and GCF existed due to the interaction of host defense, oral microbial species, and external factors [30]. Evidence supports ESRD could make periodontal microbial community different from that of those without ESRD [37]. The interplay between microbial agent and the adjacent periodontal tissues often increases gingival inflammation and releases biologically active substances [38].

GCF, a kind of non-invasive tissue fluid and easily processed, contains tremendous metabolites from the host as well as bacteria [39, 40]. GCF is likely to reflect the periodontal health status [40]. ESRD patients are susceptible to infection due to the impairment of immune system, leading to an increase of proinflammatory cytokines [41]. Our study indicated that there were some correlations between key metabolites and inflammation. Therefore, the inflammatory markers and metabolites in GCF may be valuable biomarkers for patient stratification, disease surveillance, predictive diagnosis and targeted prevention. Through the omics analysis of GCF, we can determine unique metabolite information and then analyze the related molecular pathway in global terms, which can predict whether there is any disorder in periodontal tissue or system health [42]. Specifically, based on the results in this study, the individuals may be in a state of periodontal inflammation and at a risk of periodontal disease when the most direct biomarkers such as taurine in GCF decreased greatly compared with the previous levels or were at a low level. The prediction can trigger targeted preventions, such as increased attention to oral hygiene (the frequency of tooth brushing) and regular oral care (supragingival scaling) [43]. Meanwhile,

the metabolites with anti-inflammatory and antioxidant properties found in this study can aid in understanding the pathogenesis of periodontal disease of ESRD patients and help in the development of therapeutics.

Some limitations of this study need to be addressed. Selection bias may exist as the proportions of participants with the history of diabetes and hypertension were uneven in the two groups; The bias is difficult to avoid as diabetes and hypertension are the leading causes for ESRD, account for 61% of dialysis cases [44–46]. However, multiple factor analysis were performed to analyze the association between inflammatory level and these two variables. And the result showed hypertension and diabetes did not significantly associated with the IL-8 levels of GCF. Secondly, clinical attachment loss (CAL) was no included in periodontal parameters of the study as it was challenging and time-consuming to measure gingival recession and cemento-enamel junction in the chairside operation [47].

Much work has already been done to focus on the interaction between ESRD and periodontal health. However, the relationship between periodontal and kidney diseases was not explored deeply so far. Our results provide some clues regarding their relationships based on significant differences of inflammatory markers and metabolites. To our best knowledge, it is the first study that reported the metabolites and its association with inflammation in the GCF of ESRD patients, the extent to discuss these findings are limited for us. The role of the hemodialysis may lead to the poor periodontal health status.(6) However, further evidence is certainly needed to draw causal conclusions in the future.

5 Conclusions

The periodontal inflammation of ESRD patients appeared to be severer than that of healthy individuals. And IL-8 levels correlated with some metabolites with anti-inflammatory and antioxidant properties. Our findings can provide insights regarding the pathogenesis of periodontal health status of ESRD patients. The differential inflammatory markers and metabolites in GCF between ESRD patients and healthy individuals are possibly biomarkers, pointing to a potential strategy for the prediction, diagnosis, prognosis, and management of personalized periodontal therapy in the population with ESRD.

Declarations

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Conflicts of interest

The authors declare no conflict of interests.

Availability of data and material

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Code availability

All software applications used are included in this article.

Authors' contributions

Xiaoxin Ma contributed to the study conception and design, data analysis, and drafted the manuscript. Yongli Wang contributed to the study conception and design, and drafted the manuscript. Hongyu Wu, Fei Li, Yingxin Xie, Danshu xie and Wenji Wang contributed to the sample collection. Xiping Feng and Edward. Chin. Man. Lo contributed to the study conception and design, and critically revised the manuscript. Haixia Lu contributed to the literature review, study conception and design, data analysis, and drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Ethics Committee of Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine (Ethical approval number: SH9H-2018-T32-3), and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent to participate

The protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Institutional Ethics Committee of Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. Written informed consent was obtained from individual or guardian participants.

Consent for publication

Not applicable

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Figures

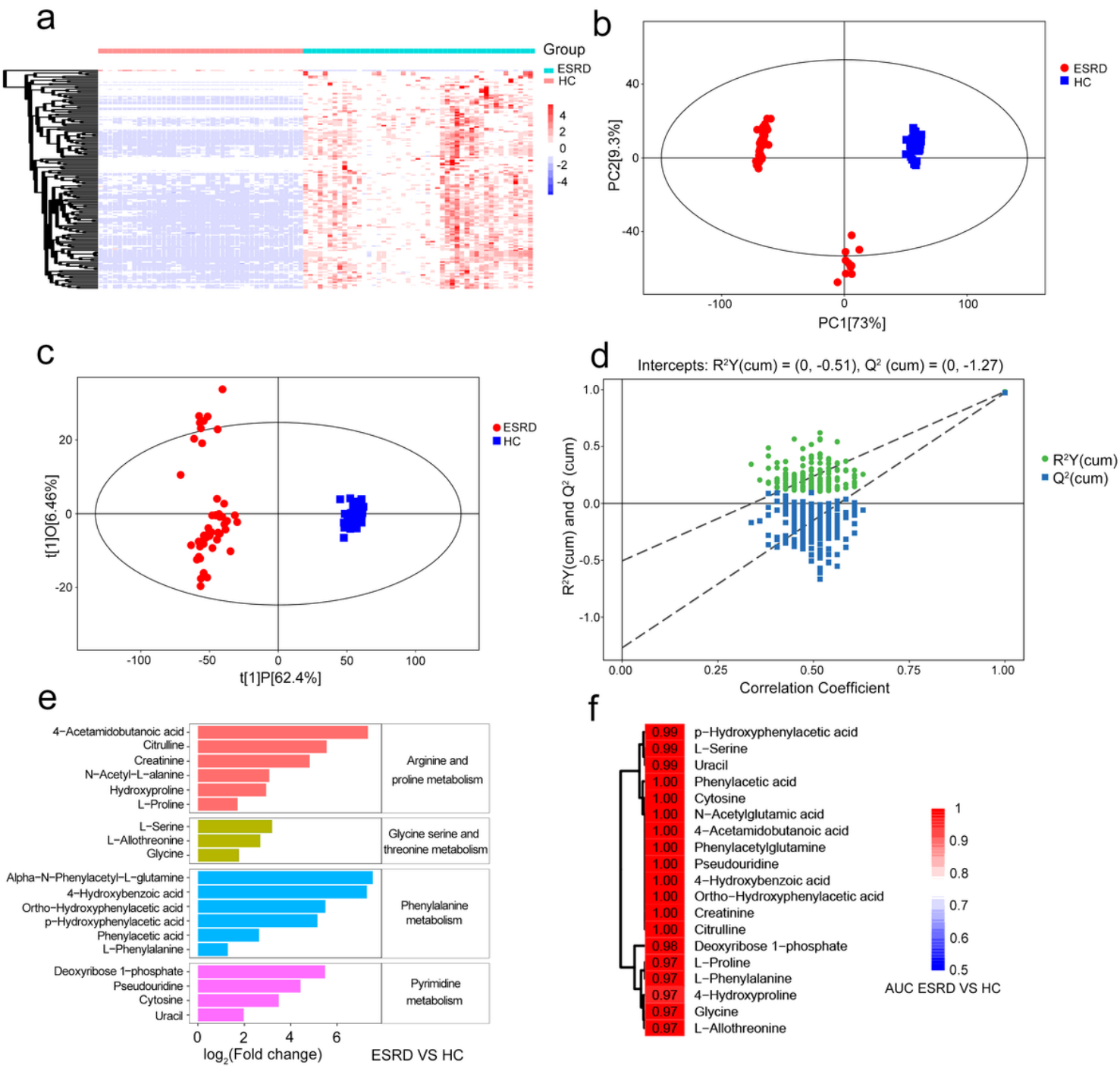

Figure 1

<p>Metabolic profile of serum based on LC-MS. (a) Heatmap of metabolites between the ESRD group and the HC group; (b) PCA score plot model of serum samples; (c) OPLS-DA score plot of serum samples; (d)

OPLS-DA permutation plot of serum samples; (e) Fold change (FC) of the key metabolites and the related pathways: ESRD vs HC. When the metabolite of serum in ESRD group was higher than that in HC group, the value of log2 (FC) was positive, otherwise it was negative; (f) Area under the receiver-operating-characteristic curve (AUC) to assess the discriminating accuracy of significant metabolites of serum in differentiating ESRD group and HC group: The redder the AUC value was, the higher discriminating accuracy of the metabolite was; LC-MS: Liquid chromatography/mass spectrometry; ESRD: End-stage renal disease; HC: Healthy controls; OPLS-DA: Orthogonal partial least-squares discriminant analysis; PCA: Principal component analysis</p>

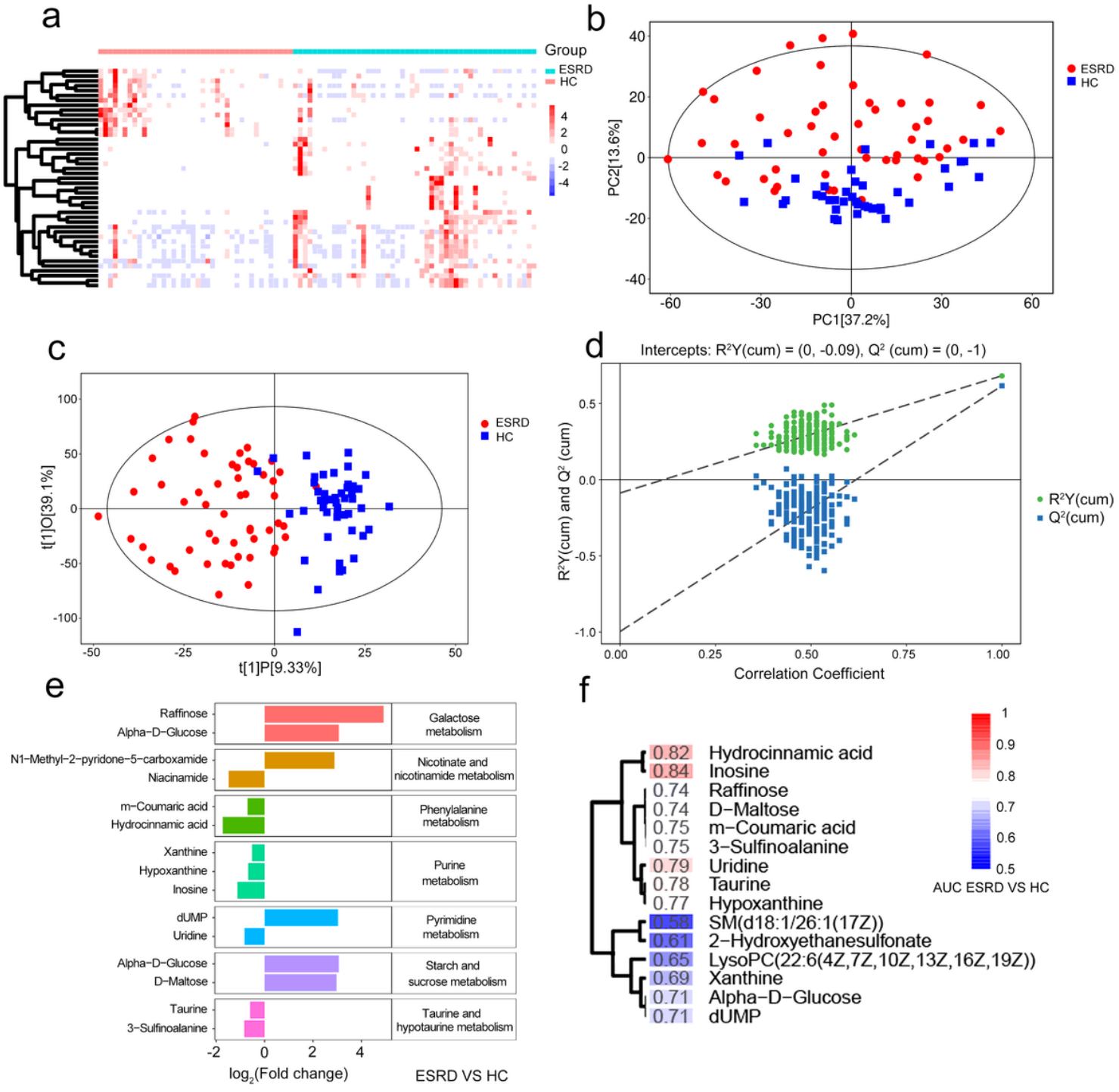

Figure 2

Metabolic profile of GCF based on LC-MS. (a) Heatmap of metabolites between ESRD group and HC group; (b) PCA score plot model of GCF samples; (c) OPLS-DA score plot of GCF samples; (d) OPLS-DA permutation plot of GCF samples; (e) Fold change (FC) of the key metabolites and the related pathways: ESRD vs. HC. When the metabolite of GCF in ESRD group was higher than that in HC group, the value of log2 (FC) was positive, otherwise it was negative; (f) Area under the receiver-operating-characteristic curve (AUC) to assess the discriminating accuracy of significant metabolites of GCF in differentiating ESRD group and HC group: The redder the AUC value was, the higher discriminating accuracy of the metabolite was; LC-MS: Liquid chromatography/mass spectrometry; ESRD: End-stage renal disease; HC: Healthy controls; OPLS-DA: Orthogonal partial least-squares discriminant analysis; PCA: Principal component analysis