The Abnormal Metabolites in Tongue Coating of Chronic Gastritis Patients Reflect the Changes in Indexes of Gastroscopic Observation and Gastric Mucosal Pathology

Yifeng Xu

Shanghai University of Traditional Chinese Medicine <https://orcid.org/0000-0001-8366-2322>

Renling Zhang

Shanghai University of Traditional Chinese Medicine

Junhong Lu

Shanghai University of Traditional Chinese Medicine

Zhujiang Zhu

Shanghai University of Traditional Chinese Medicine

Yiqin Wang

Shanghai University of Traditional Chinese Medicine

Yiming Hao (✉ hymjj888@163.com)

Shanghai University of Traditional Chinese Medicine

Research

Keywords: chronic gastritis, tongue coating, metabolite, gastroscopy, gastric mucosal pathology

Posted Date: November 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1029371/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Objective

In this study, we analyzed the correlation between different metabolites in the tongue coating of patients with chronic gastritis, gastroscopy and pathological indexes, and discussed the metabolic mechanism at different pathological stages in the development of chronic gastritis.

Methods

We used GC-TOF-MS and UHPLC-QE-MS metabonomics to detect the distribution of metabolites in the tongue coating of patients with chronic gastritis, and analyzed the correlation between different metabolites in the tongue coating of patients with chronic gastritis and gastroscopy and pathological indexes.

Results

Compared with 50 healthy people, 54 metabolites were upregulated and 47 metabolites were downregulated in 350 patients with chronic gastritis. The main differential metabolites were Lipids and lipid-like molecules, which contain 47 metabolites. The best diagnostic model was composed of 5 metabolites, with an accuracy of 95.4%, a specificity of 87.4% and a sensitivity of 88.0%. These 5 metabolites were 1-methyladenosine, Sphinganine 1-phosphate, 3-Hydroxycapric acid, 4-lpomeanol, and Nervonic acid. Compared with healthy people, Sphinganine 1-phosphate, 4-lpomeanol, and Nervonic acid were significantly upregulated in chronic gastritis patients, and 1-methyladenosine and 3-Hydroxycapric acid were significantly downregulated in chronic gastritis patients. After correlation analysis between differential metabolites in tongue coatings and gastroscopic indexes, we found that Trimethylaminoacetone, Sphinganine1-phosphate, alpha-Carboxy-delta-decalactone, and 5,6-Dihydroxyindole were positively correlated with intestinal metaplasia. Conduritol-beta-expoxide, Tetracosanoic acid, Lactosylceramide(d18:1/26:0), Chondrillasterol 3-[glucosyl-(1->4)-glucoside], Azelaic acid, and 1-Methyladenosine were negatively correlated with intestinal metaplasia. Sphinganine1-phosphate, alpha-Carboxy-delta-decalactone, and 5,6-Dihydroxyindole were positively correlated with atrophic. Octadecanol, conduritol-beta-expoxide, Tetracosanoic acid, Smilanippin A, Lactosylceramide(d18:1/26:0), Chondrillasterol 3-[glucosyl-(1->4)-glucoside], and Azelaic acid were negatively correlated with atrophic. 6-deoxyglucitol was negatively correlated with bile reflux, methylmaleic acid, 4-methylcatechol, and 2,4-dichloro-1-(2-chloroethenyl)-benzene were negatively correlated with Hp, 3-benzoyloxy-11-oxo-12-ursen-28-oic acid was negatively correlated with gastric mucosal erosion. From the change trend of different metabolites in different pathological stages, we found that the content of conduritol-beta-expoxide decreased significantly in mild atrophic compared with moderate atrophic and the content of conduritol-beta-expoxide decreased significantly in mild intestinal metaplasia compared with moderate intestinal metaplasia.

Conclusions

We found that Lipids and lipid-like molecules were the main abnormal metabolites in patients with chronic gastritis. Among them, Sphinganine 1-phosphate, which was significantly positively correlated with the aggravation of atrophic and intestinal metaplasia, could be used as one of the diagnostic model markers for chronic gastritis. Additionally, the amount of conduritol-beta-expoxide significantly decreased with the aggravation of atrophic and intestinal metaplasia. We believe that these differential markers in tongue coating may help us to establish a noninvasive and convenient auxiliary detection method for gastritis and gastric precancerous lesion in the future.

1 Introduction

Nowadays, people are living at an increasing pace, the work pressure is increasing, and the diet structure is changing, more and more people are suffering from digestive system diseases. Studies have shown that chronic gastritis, as one of the most common severe epidemic infections worldwide, has now affected more than half of the people in the world [1]. Chronic gastritis can be divided into chronic atrophic gastritis and chronic non-atrophic gastritis, and it is worth noting that gastric mucosal atrophic may relate to the development of gastric cancer [1]. Therefore, early detection and treatment of chronic gastritis play an important role both in preventing further injury to the disease and improving the quality of life. At present, gastroscopy is the main method of diagnosis and curative effect evaluation of chronic gastritis, which is of great significance for the early detection and treatment of chronic gastritis. However, gastroscopy cannot be performed frequently in a short time [2]. Thus, finding a noninvasive and repeatable monitoring method to observe the progress of chronic gastritis is one of the main directions of our research.

Tongue diagnosis is one of the important diagnostic methods of Traditional Chinese Medicine (TCM), which is non-invasive and repeatable. The main way of tongue diagnosis is to observe the changes of tongue body and tongue coating. According to the theory of TCM, tongue coating is a very sensitive index that can reflect the physiological and pathological state of the spleen and stomach.

In recent years, metabolomics has been widely used in medical research and also used to find diagnostic biomarkers of various diseases. In the study of tongue coating metabolites of chronic gastritis, some researchers used ultra-performance liquid chromatography and mass spectrometry (UPLC-MS) technology or liquid chromatography and mass spectrometry (LC-MS) technology to detect and analyze the substances in tongue coating of chronic gastritis patients. They found that there were differences in tongue coating metabolites between healthy people and chronic gastritis patients [3–4]. However, these studies only used LC-MS techniques, and the sample size was less than 100 cases, used only one detection method and under conditions of low sample size, the metabolites in tongue coating could not be fully reflected. Meanwhile, studies on the correlation of tongue coating metabolites and gastroscopic indexes in chronic gastritis patients have not been reported.

Gas chromatography-time-of-flight-mass spectroscopy (GC-TOF-MS) is also a metabonomic method, GC-TOF-MS has a higher separation ability than liquid chromatography-Q exactive orbitrap-mass spectroscopy (UHPLC-QE-MS) in the analysis of polar metabolites. Besides, the GC-TOF-MS system's mass spectra are more repeatable than UHPLC-QE-MS, which makes GC-TOF-MS libraries more comprehensive. Insufficient is that GC-TOF-MS can only detect more than a

hundred metabolites while UHPLC-QE-MS can detect thousands of metabolites that contain semi-polar metabolites [5]. Due to the complex composition of metabolites on tongue coating, the combination of two detection methods can be more comprehensively used to detect the characteristics of tongue coating metabolites.

Therefore, this study combined UHPC-QE-MS and GC-TOF-MS technology to detect and analyze the metabolites in 350 patients with chronic gastritis. The abnormal fluctuation of these metabolites is helpful for us to better understand the changes of the metabolites of tongue coating in patients with chronic gastritis from different aspects.

2 Materials And Methods

2.1 Samples

To explore the composition of tongue coating related metabolites in patients with chronic gastritis, we selected a case-control study approach. We selected 350 chronic gastritis patients in Longhua Hospital (an affiliate of the Shanghai University of traditional Chinese medicine) to perform gastroscopy and pathological examination of gastric mucosa, and selected 50 teachers and students of Shanghai University of traditional Chinese medicine as the healthy control group. People in the selected healthy control group had no history of stomach disease or stomach discomfort at present. The results of their blood cell analysis, liver function, renal function, blood lipid, blood glucose, blood pressure, tumor index, color Doppler ultrasound of neck and abdomen, chest CT and barium metal fluoroscopy were normal. All participants were Chinese (self-reported), they participated in the study from December 2018 to October 2019. Participants underwent gastroscopy and gastric mucosal pathology immediately after being sampled for tongue coating specimens. A summary of their demographic and clinical information was provided in Table 1.

Table 1
Summary of demographics and clinical information of the participants

Demographics and clinical information	Chronic gastritis patient group	Normal control group
Sample number	350	50
Ratio of male to female	1:0.74	1:0.92
Average age (year)	45.71±13.83	34.04±10.34
Number (percentage) of samples diagnosed this time	3 (0.86%)	N/A
Number (percentage) of samples diagnosed for less than 10 years	259 (74.00%)	N/A
Number (percentage) of samples diagnosed for 10-20 years	71 (20.29%)	N/A
Number (percentage) of samples diagnosed for 20-30 years	15 (4.29%)	N/A
Number (percentage) of samples diagnosed for 30-40 years	4 (1.14%)	N/A
Number (percentage) of samples diagnosed over40 years	1 (0.29%)	N/A
Number (percentage) of samples untreated	83 (23.71%)	N/A
Number (percentage) of samples only taking western medicine	174 (49.71%)	N/A
Number (percentage) of samples only taking traditional Chinese medicine	180 (51.43%)	N/A
Number (percentage) of samples taking western medicine and traditional Chinese medicine	84 (24.00%)	N/A

2.2 Ethics Approval

The study was approved by the Ethics Committee of Shanghai University of TCM in China in December 2018 and performed in accordance with the Declaration of Helsinki. All the subjects had signed informed consent agreements before collecting samples.

2.3 Criteria

Diagnostic Criteria

- 1) Endoscopy was performed on patients and biopsies were taken from suspicious lesion sites such as gastric antrum, angle, body and cardia.
- 2) The six gastroscopic and pathological indexes were inflammatory activity, atrophic, intestinal metaplasia, Helicobacter pylori (Hp), bile reflux and gastric mucosal erosion. The inflammatory activity, atrophic and intestinal metaplasia were divided into four grades: none, mild, moderate and severe.
- 3) Patients with Hp infection should confirm that the Hp test of gastric mucosa was consistent with the positive result of the C13 breath test.
- 4) The histopathological assessment was performed by two experienced pathologists in accordance with the clinical guidelines of “the updated Sydney System” [6].

Exclusion Criteria

Patients who had gastroesophageal reflux disease, gastric ulcer, gastric hemorrhage, gastric cancer, duodenal ulcer, or other intestinal diseases were identified by endoscopic examination.

Patients who had diseases of the motor system, nervous system, endocrine system, circulatory system, respiratory system, urinary system, and reproductive system.

Patients who had a mental illness or female subjects were during their pregnancy and lactation period.

Patients who had lesions of the tongue, mouth, nose, or pharynx within one month prior to the collection of samples.

Patients who had received probiotics or antibiotics within one month prior to sample collection.

Patients who had used tobacco or consumed alcohol. The participants had a body mass index (BMI) over 28 [7].

2.4 Tongue coating samples collection

Samples of tongue coating were collected before participants had breakfast. Before collection, the participants rinsed the mouth with a stroke-physiological saline solution 3 times in order to remove the residues in the mouth. The collector used a sterile specimen collection swab (CY-98000, iClean, Huachenyang Technology Co., Ltd, CN) to scrape the thick part of the tongue coating of participants 5 times, then put the head of the tongue coating sample swabs into a sterile centrifuge tube. All tongue coating samples were collected by the same researcher. The tongue coating samples were stored for use at -80°C.

2.5 GC-TOF-MS Metabolomics Processing

The reagents used in the GC-TOF-MS experiment were listed in Supplementary Table S1a. The experimental steps were as follows:

- 1) Transfer the swab head of the tongue coating sample to a 5ml sterile Eppendorf (EP) tube for weighing, and add the pre-cold extraction mixture (Vmethanol:Vchloroform = 3:1) 1500 µL with internal standard solution (adonitol, 0.5 mg/mL stock) 15 µL. The sample underwent ultrasonication in the ice water for 30 min. And then removed the swab head. After centrifugation at 4°C, 10000 rpm for 15 minutes, 500 µL supernatant was transferred to fresh tubes.
- 2) We took 100 µl from each sample and combined it together to prepare a quality control (QC) sample. After evaporation in a vacuum concentrator, add 40 µL methoxyaminated hydrochloride (20 mg/ml pyridine), incubated at 80°C for 30 min, and then incubated with 60 µL bis-(trimethylsilyl)-trifluoroacetamide reagent (containing 1% trimethylchlorosilane, V/V) was derived for 1.5h. After gradually cooled the sample to room temperature, 5 µL fatty acid methyl ester (in chloroform) was added to QC samples.
- 3) GC-TOF-MS detection was performed using an Agilent 7890 gas chromatograph and TOF mass spectrometer. The system employed a db-5ms capillary column. Inject a sample of 1 µl aliquot in a splitless mode. With Helium as the carrier gas, the purge flow at the front inlet was 3 mL/min, and the gas flow rate through the chromatographic column was 1 mL/min. The initial temperature was maintained at 50°C for 1 min, and then raised to 310°C at a rate of 20°C/min, then maintained at 310°C for 6 min. The implantation, the transfer line, and ion source temperatures were 280, 280, and 250°C. The energy was -70eV in electron collision mode. The mass spectrum data were obtained in full-scan mode with the m/z range of 50-500 at a speed of 12.5 spectrum/s and solvent delay of 6.30 min.
- 4) The original data were analyzed by using Chroma TOF (V 4.3x, LECO) software [8], including peak extraction, baseline adjustment, deconvolution, alignment and integration. The mass spectrum and retention index identification of metabolites were performed by using the LECO-Fiehn Rtx5 database.
- 5) The peaks detected in QC samples were less than half of the peaks were removed in QC samples with RSD>30% [9].

2.6 UHPLC-QE-MS Metabolomics Processing

The reagents used in the UHPLC-QE-MS experiment were listed in Supplementary Table S1. The experimental steps were as follows:

- 1) Add 1500 µL of extract containing the isotopically-labeled internal standard mixture (Vacetonitrile:Vmethanol: Vwater = 2:2:1). After being vortexed for 30s, the solution was sonicated on ice for 30 min.
- 2) - 40°C for 1 h and centrifuged at 12000 rpm for 15 minutes at 4°C. The resulting supernatant was transferred to a fresh glass vial for analysis. The supernatants of all the samples were mixed well to make QC samples.
- 3) LC-MS detection was performed on a UHPLC system (Vanquish, Thermo Fisher Scientific) with a UPLC BEH Amide column (2.1 mm×100 mm, 1.7 µm) coupled to Q Exactive HFX mass spectrometer (Orbitrap MS, Thermo). The mobile phases were 25 mmol/L ammonium acetate and 25 mmol/L ammonia hydroxide in water (pH = 9.75) (A) and acetonitrile (B). The elution gradient as follows: 0-0.5 min, 95% B; 0.5-7.0 min, 95%-65% B; 7.0-8.0 min, 65%-40% B; 8.0-9.0 min, 40% B; 9.0-9.1 min, 40%-95% B; 9.1-12.0 min, 95% B. The column temperature was 25°C. The auto-injection temperature was 4°C, and the injection volume was 3 µL.
- 4) The QE HFX mass spectrometer acquired MS/MS spectra in the information-dependent acquisition mode under the control of the acquisition software (Xcalibur, Thermo). In this mode, the acquisition software continuously assessed the full scan mass spectrum. The ESI source conditions were set as following: sheath gas flow 50 Arb, auxiliary gas flow 10 Arb, capillary temperature 320°C, full MS resolution as 60000, MS/MS resolution as 7500, collision energy 10/30/60 in nice mode, spray Voltage 3.5 kV (positive) or -3.2 kV (negative), respectively.

5) The raw data were converted to the mzXML format using ProteoWizard and peak detection, extraction, alignment, and integration were performed using an in-house XCMS based program developed in R.

6) metabolite annotation was adopted from the company’s own MS2 database (BiotreeDB V2.1). The cutoff value for annotation was set to 0.3 [10].

2.7 Statistical analysis

The peak number, sample names, and standardized peak areas were input into Simca-p+13.0 software (Umea, Sweden) for principal components analysis (PCA) and orthogonal projections to latent structures-discriminant analysis (OPLS-DA). To further validate the model, a drive substitution experiment was carried out. The false discovery rate (FDR) of the rank-sum test and the p-value ($P<0.05$), variable importance in the projection (VIP) of the first principal component of the OPLS-DA model ($VIP>1$), similarity value (SV) detected by GC-TOF-MS ($SV>700$) [11], and MS2 score detected by UHPLC-QE-MS detection ($MS2\ score>0.6$)[12] were used to determine the significant difference of metabolites between the two groups. The mean value of the metabolites peak areas was compared between the two groups, and the log fold change (FC) value was calculated.

Spearman algorithm was used for correlation analysis, and correlation coefficient (Corr) matrix and correlation P-value matrix were calculated for subsequent analysis and graphics drawn.

Correlation analysis was performed using the Spearman algorithm, and the Corr matrix, correlation P-values were calculated for subsequent analysis and graphically plotted.

3 Results

3.1 Distribution of patients with chronic gastritis in different indexes of gastroscopic observation and gastric mucosal pathology

Table 2 Distribution of patients with chronic gastritis in different indexes of gastroscopic observation and gastric mucosal pathology

indexes of gastroscopic observation and gastric mucosal pathology	Mild	Moderate	Severe	Proportion of total samples, %
inflammatory activity	48	45	3	96 (27.43%)
atrophic	101	22	6	129 (36.86%)
intestinal metaplasia	87	21	6	114 (32.57%)
Hp	10	20	19	49 (14.00%)
bile reflux	N/A	N/A	N/A	60 (17.14%)
gastric mucosal erosion	N/A	N/A	N/A	171 (48.86%)

3.2 Different peaks findings in tongue coating samples

We performed GC-TOF-MS and UHPLC-QE-MS metabolomic profiling of tongue coating samples from 350 patients with chronic gastritis and 50 healthy controls.

The GC-TOF-MS and UHPLC-QE-MS spectra examples of the same person were shown in Figure 1. As shown in Figure 1a Figure1b and Figure 1c, there were some different mass spectrum peaks between the chronic gastritis patients and healthy control people.

We adopted the criteria of P-value < 0.05 and VIP > 1, there were 32 peaks (20 peaks increased and 12 peaks decreased) in the tongue coating of patients with chronic gastritis by GC-TOF-MS analysis. 98 peaks (62 peaks increased and 36 peaks decreased) in the tongue coating of patients with chronic gastritis by UHPLC-QE-MS positive analysis, and 14 peaks (10 peaks increased and 4 peaks decreased) in the tongue coating of patients with chronic gastritis by UHPLC-QE-MS negative analysis.

We used the criteria of similarity SV > 700 or MS2 score > 0.6, there were 19 peaks (11 peaks increased and 8 peaks decreased) in the tongue coating of patients with chronic gastritis by GC-TOF-MS analysis. 72 peaks (36 peaks increased and 36 peaks decreased) in the tongue coating of patients with chronic gastritis by UHPLC-QE-MS positive analysis. And 10 peaks (7 peaks increased and 3 peaks decreased) in the tongue coating of patients with chronic gastritis by UHPLC-QE-MS negative analysis.

3.3 Different metabolites analysis

As shown in Table 3, 85 metabolites were divided into 11 categories, of which the largest category was Lipids and lipid-like molecules, with 47 metabolites, Organic acids and derivatives with 13 metabolites, Organoheterocyclic compounds with 8 metabolites, Organic nitrogen compounds with 6 metabolites, Organic oxygen compounds with 4 metabolites, and Benzenoids with 2 metabolites, There was one metabolite in Homogeneous non-metal compounds, Nucleosides, nucleotides, and analogues, Phenylpropanoids and polyketides, Organosulfur compounds, and Hydrocarbons.

3.4 Diagnostic model of chronic gastritis

The diagnostic model was constructed by screening the different metabolites of tongue coating in 350 chronic gastritis patients and 50 healthy people. And the best diagnostic model was composed of 5 tongue coating metabolites. They were 1-methyladenosine and Sphinganine 1-phosphate screened by UHPLC-QE-MS positive ion mode, 3-Hydroxycapric acid, 4-Ipomeanol, and Nervonic acid screened by UHPLC-QE-MS negative ion mode. Compared with healthy people,

Sphinganine 1-phosphate, 4-Ipomeanol, and Nervonic acid were significantly upregulated in chronic gastritis patients, 1-methyladenosine and 3-Hydroxycapric acid were significantly downregulated in chronic gastritis patients. This diagnostic model had an accuracy of 95.4%, a specificity of 87.4%, and a sensitivity of 88.0%.

3.5 Correlation analysis between differential metabolites in tongue coating and gastroscopy indexes

As can be seen from Figure 3-1, the differential metabolic markers of tongue coating obtained by GC-TOF-MS analysis between chronic gastritis group and healthy controls, octadecanol, and conduritol-beta-expoide were negatively correlated with atrophic, and conduritol-beta-expoide was negatively correlated with intestinal metaplasia. 6-deoxyglucitol was negatively correlated with bile reflux, and methylmaleic acid, 4-methylcatechol, and 2,4-dichloro-1-(2-chloroethenyl)-benzene were negatively correlated with Hp.

It can be seen from Figure 3-2 that UHPLC-QE-MS analyzes the differential metabolic markers of tongue coating obtained by comparing the chronic gastritis group with the healthy control group, including Trimethylaminoacetone, Sphinganine1-phosphate, alpha-Carboxy-delta-decalactone, and 5,6-Dihydroxyindole were positively correlated with intestinal metaplasia. Tetracosanoic acid, Lactosylceramide(d18:1/26:0), Chondrillasterol 3-[glucosyl-(1->4)-glucoside], Azelaic acid, and 1-Methyladenosine were negatively correlated with intestinal metaplasia. 3-benzoyloxy-11-oxo-12-ursen-28-oic acid was negatively correlated with gastric mucosal erosion. Sphinganine1-phosphate, alpha-Carboxy-delta-decalactone, and 5,6-Dihydroxyindole were positively correlated with atrophic. Tetracosanoic acid, Smilanippin A, Lactosylceramide(d18:1/26:0), Chondrillasterol 3-[glucosyl-(1->4)-glucoside], and Azelaic acid were negatively correlated with atrophic.

Table3 Identification of significant differential metabolites in tongue coating by comparison of chronic gastritis patients and healthy people.

Metabolite name	RT (min)	M/Z	Mean E	Mean N	<i>P</i>	VIP	Log fold change	Detection method
Lipids and lipid-like molecules								
Arachidonic acid	37.582	303	17.019	4.430	<0.01	1.569	1.942	UHPLC-QE-MS negative ion
Prostaglandin D2	46.555	351	1.937	1.098	<0.01	1.657	0.819	UHPLC-QE-MS negative ion
9,10-DHOME	81.276	313	0.133	0.634	<0.01	1.256	-2.256	UHPLC-QE-MS negative ion
Azelaic acid	359.713	187	0.191	0.796	<0.01	1.731	-2.062	UHPLC-QE-MS negative ion
Ethyl oleate	41.892	309	0.692	0.332	<0.01	1.241	1.060	UHPLC-QE-MS negative ion
8,11,14-Eicosatrienoic acid	37.965	305	0.760	0.229	<0.01	1.364	1.727	UHPLC-QE-MS negative ion
Nervonic acid	37.630	365	0.309	0.128	<0.01	1.521	1.275	UHPLC-QE-MS negative ion
Tetracosanoic acid	37.748	367	0.561	0.357	<0.01	1.040	0.651	UHPLC-QE-MS negative ion
Foeniculoside VII	71.454	349	0.517	1.452	<0.01	1.168	-1.490	UHPLC-QE-MS positive ion
Glycerol tripropanoate	61.345	261	1.163	2.718	<0.01	1.078	-1.225	UHPLC-QE-MS positive ion
Solavetivone	115.309	219	0.950	0.134	<0.01	1.408	2.826	UHPLC-QE-MS positive ion
Patchoulenone	61.062	219	11.219	3.010	<0.01	1.438	1.898	UHPLC-QE-MS positive ion
Gibberellin A79	71.856	365	0.064	0.152	<0.01	1.063	-1.247	UHPLC-QE-MS positive ion
Oleic acid	33.290	283	0.263	0.421	<0.01	1.132	-0.678	UHPLC-QE-MS positive ion
Dexamethasone	77.202	393	0.262	1.323	<0.01	1.249	-2.335	UHPLC-QE-MS positive ion
3-O-Methylniveusin A	77.445	409	0.044	0.277	<0.01	1.373	-2.641	UHPLC-QE-MS positive ion
Hoduloside VII	248.651	932	0.926	0.579	<0.01	1.398	0.678	UHPLC-QE-MS

								positive ion
Fucoxanthin	259.139	659	0.077	0.392	<0.01	1.576	-2.344	UHPLC-QE-MS positive ion
N-Cyclopropyl-trans-2-cis-6-nonadienamide	46.068	194	0.174	0.114	<0.01	1.364	0.611	UHPLC-QE-MS positive ion
3-Hydroxyisovalerylcarnitine	57.688	262	0.119	1.688	<0.01	1.457	-3.830	UHPLC-QE-MS positive ion
Lyciumoside III	142.803	649	0.025	2.219	<0.01	1.524	-6.491	UHPLC-QE-MS positive ion
Eicosadienoic acid	33.311	309	0.795	3.662	<0.01	1.584	-2.203	UHPLC-QE-MS positive ion
3beta-Acetoxy-11alpha-methoxy-12-ursen-28-oic acid	193.697	543	0.092	0.044	<0.01	1.400	1.071	UHPLC-QE-MS positive ion
Antibiotic X 14889C	257.759	615	0.082	0.406	<0.01	1.561	-2.308	UHPLC-QE-MS positive ion
2-Ethyl-2-Hydroxybutyric acid	58.656	133	0.727	2.302	<0.01	1.406	-1.663	UHPLC-QE-MS positive ion
Palmitoyl glucuronide	293.193	419	0.408	0.261	<0.01	1.422	0.648	UHPLC-QE-MS positive ion
Physalin O	314.512	529	0.129	0.082	<0.01	1.426	0.663	UHPLC-QE-MS positive ion
Desglucocoroloside	220.265	505	0.030	0.017	<0.01	1.078	0.792	UHPLC-QE-MS positive ion
3-Methylglutaryl carnitine	84.851	290	0.257	0.182	<0.01	1.289	0.502	UHPLC-QE-MS positive ion
Smilanippin A	113.780	725	0.594	0.375	<0.01	1.408	0.662	UHPLC-QE-MS positive ion
PS(16:1(9Z)/18:4(6Z,9Z,12Z,15Z))	207.222	754	0.069	0.573	<0.01	1.592	-3.047	UHPLC-QE-MS positive ion
CPA(18:0/0:0)	278.918	421	0.210	0.131	<0.01	1.368	0.682	UHPLC-QE-MS positive ion
Cucurbitacin I 2-glucoside	205.358	677	1.712	1.092	<0.01	1.092	0.648	UHPLC-QE-MS positive ion
Glycerol tributanoate	125.367	303	0.170	0.563	<0.01	1.081	-1.728	UHPLC-QE-MS positive ion
Fasciculic acid C	116.186	710	0.470	1.367	<0.01	1.406	-1.539	UHPLC-

								QE-MS positive ion
Hebevinoside I	38.382	809	0.097	0.365	<0.01	1.375	-1.917	UHPLC- QE-MS positive ion
2,4,6-Octatriynoic acid	440.442	133	0.296	0.478	<0.01	1.127	-0.692	UHPLC- QE-MS positive ion
Lycoperoside D	154.407	740	0.568	11.651	<0.01	1.352	-4.358	UHPLC- QE-MS positive ion
Chondrillasterol 3-[glucosyl-(1->4)- glucoside]	97.620	737	0.687	1.821	<0.01	1.488	-1.406	UHPLC- QE-MS positive ion
Physapruin B	227.103	603	0.099	0.058	<0.01	1.112	0.778	UHPLC- QE-MS positive ion
Traumatic acid	281.345	229	0.497	0.421	0.047	1.196	0.239	UHPLC- QE-MS positive ion
Lactosylceramide (d18:1/26:0)	31.388	1003	0.003	1.964	<0.01	1.364	-9.313	UHPLC- QE-MS positive ion
Sphinganine 1-phosphate	51.580	382	0.166	0.069	<0.01	1.554	1.256	UHPLC- QE-MS positive ion
3-Benzoyloxy-11-oxo-12-ursen-28- oic acid	34.377	575	2.140	1.439	<0.01	1.278	0.573	UHPLC- QE-MS positive ion
Momordicoside K	69.766	649	0.674	1.394	<0.01	1.508	-1.049	UHPLC- QE-MS positive ion
PI(22:5(4Z,7Z,10Z,13Z,16Z)/16:0)	244.507	886	0.154	0.528	<0.01	1.368	-1.779	UHPLC- QE-MS positive ion
Vinaginsenoside R3	84.853	932	0.466	1.065	<0.01	1.445	-1.192	UHPLC- QE-MS positive ion
Organic acids and derivatives								
glycolic acid	5.611	147	0.001	0.002	0.005	1.229	-0.518	GC-TOF- MS
pantothenic acid	11.358	103	4.356E-04	2.233E-04	<0.01	1.143	0.964	GC-TOF- MS
3-Hydroxycapric acid	83.306	187	0.214	0.441	<0.01	1.383	-1.043	UHPLC- QE-MS negative ion
Bortezomib	285.056	407	0.080	0.811	<0.01	1.026	-3.350	UHPLC- QE-MS positive ion
Ustiloxin D	287.091	495	0.044	0.450	<0.01	1.239	-3.339	UHPLC- QE-MS positive ion
Symmetric dimethylarginine	158.848	203	4.835	6.288	<0.01	1.032	-0.379	UHPLC- QE-MS

								positive ion
Oseltamivir	314.576	313	0.145	0.096	<0.01	1.397	0.593	UHPLC-QE-MS positive ion
Arginyl-Arginine	296.631	331	2.106	1.462	0.045	1.439	0.526	UHPLC-QE-MS positive ion
Arginyl-Histidine	166.723	312	0.423	0.284	0.010	1.342	0.574	UHPLC-QE-MS positive ion
Dihydro-3-(2-octenyl)-2,5-furandione	281.391	211	0.127	0.101	0.012	1.196	0.323	UHPLC-QE-MS positive ion
N-Acetylleucine	265.067	174	0.154	0.099	<0.01	1.231	0.632	UHPLC-QE-MS positive ion
Valyl-Isoleucine	221.874	231	0.092	0.032	<0.01	1.161	1.498	UHPLC-QE-MS positive ion
L-cis-3-Amino-2-pyrrolidinecarboxylic acid	75.782	131	0.032	0.683	<0.01	1.399	-4.437	UHPLC-QE-MS positive ion
Organoheterocyclic compounds								
1-(1-Pyrrolidiny)-2-propanone	53.003	128	1.823	2.480	<0.01	1.031	-0.444	UHPLC-QE-MS positive ion
Pyrimidine	112.505	81	0.097	0.320	<0.01	1.275	-1.728	UHPLC-QE-MS positive ion
5,6-Dihydroxyindole	45.066	150	0.251	0.169	<0.01	1.128	0.574	UHPLC-QE-MS positive ion
N1-Methyl-4-pyridone-3-carboxamide	79.822	153	0.212	0.176	0.032	1.002	0.269	UHPLC-QE-MS positive ion
2-Methylpiperidine	299.990	100	3.360	2.030	<0.01	1.371	0.727	UHPLC-QE-MS positive ion
alpha-Carboxy-delta-decalactone	125.959	215	0.080	0.336	<0.01	1.087	-2.065	UHPLC-QE-MS positive ion
I-Urobilin	174.113	591	0.121	0.476	<0.01	1.214	-1.974	UHPLC-QE-MS positive ion
Garcimangosone C	282.076	413	0.857	0.656	0.048	1.287	0.387	UHPLC-QE-MS positive ion
Organic nitrogen compounds								
1-Butylamine	268.847	74	12.807	7.239	<0.01	1.402	0.823	UHPLC-QE-MS positive ion
Nervonyl carnitine	237.324	102	636.919	429.936	<0.01	1.351	0.567	UHPLC-QE-MS

								positive ion
Isobutylpropylamine	231.650	116	0.444	0.294	<0.01	1.294	0.596	UHPLC-QE-MS positive ion
2-Diethylaminoethanol	300.009	118	196.915	117.700	<0.01	1.441	0.742	UHPLC-QE-MS positive ion
Dipyridamole	47.761	253	0.366	9.287	0.016	1.060	-4.666	UHPLC-QE-MS positive ion
L-Histidinol	62.697	142	0.424	0.160	<0.01	1.289	1.406	UHPLC-QE-MS positive ion
Organic oxygen compounds								
glycerol	7.006	85	1.092E-04	2.011E-04	<0.01	1.884	-0.882	GC-TOF-MS
4-Ipomeanol	72.941	167	0.405	0.072	<0.01	1.626	2.499	UHPLC-QE-MS negative ion
Leonuriside A	372.037	333	0.012	0.235	<0.01	1.365	-4.293	UHPLC-QE-MS positive ion
Trimethylaminoacetone	206.235	116	0.123	0.095	<0.01	1.324	0.380	UHPLC-QE-MS positive ion
Benzenoids								
Dictagymnin	60.998	203	0.237	0.059	<0.01	1.007	2.008	UHPLC-QE-MS positive ion
Salmeterol	57.091	416	0.458	1.005	<0.01	1.466	-1.134	UHPLC-QE-MS positive ion
Homogeneous non-metal compounds								
hydroxylamine	5.968	85	6.208E-04	0.001	<0.01	1.240	-0.834	GC-TOF-MS
Nucleosides, nucleotides, and analogues								
1-Methyladenosine	185.868	282	0.375	0.692	<0.01	1.015	-0.884	UHPLC-QE-MS positive ion
Phenylpropanoids and polyketides								
Sofalcone	285.882	451	0.064	0.639	<0.01	1.259	-3.311	UHPLC-QE-MS positive ion
Organosulfur compounds								
(Â±)-2-Pentanethiol	32.769	105	0.419	0.279	<0.01	1.195	0.590	UHPLC-QE-MS positive ion
Hydrocarbons								
1-Methyl-1,3-cyclohexadiene	32.571	95	0.476	0.315	<0.01	1.290	0.595	UHPLC-QE-MS positive ion
Others								

6-deoxyglucitol	10.173	117	1.979E-04	1.416E-04	<0.01	2.679	0.483	GC-TOF-MS
myristic acid	10.642	117	9.197E-04	0.002	<0.01	1.264	-1.213	GC-TOF-MS
octadecanol	12.158	327	2.58849E-05	3.63256E-05	<0.01	1.036	-0.489	GC-TOF-MS
1,2,4-benzenetriol	9.344	124	0.006	0.003	<0.01	2.238	0.835	GC-TOF-MS
threitol	8.562	191	4.8463E-05	1.651E-04	<0.01	1.286	-1.769	GC-TOF-MS
2,4-dichloro-1-(2-chloroethenyl)-benzene	7.925	170	0.012	0.008	0.011	2.553	0.645	GC-TOF-MS
asparagine dehydrated	8.628	110	2.811E-04	2.228E-04	<0.01	1.493	0.335	GC-TOF-MS
conduiritol-beta-expoxide	11.541	103	0.005	0.003	<0.01	1.674	0.696	GC-TOF-MS
methylmaleic acid	7.923	170	0.012	0.008	0.037	2.782	0.601	GC-TOF-MS
4-methylcatechol	7.928	170	0.012	0.008	0.036	2.120	0.607	GC-TOF-MS
glucose-6-phosphate major	12.806	111	5.8939E-05	4.41292E-05	<0.01	2.808	0.417	GC-TOF-MS
uridine minor	13.354	145	0.002	0.001	0.015	2.825	0.387	GC-TOF-MS
methylmalonic acid	6.698	85	7.14578E-05	1.287E-04	<0.01	1.764	-0.849	GC-TOF-MS
5-hydroxynorvaline	8.88	85	9.138E-04	0.002	<0.01	1.157	-0.977	GC-TOF-MS
sulfuric acid	6.326	117	1.023E-04	7.39354E-05	<0.01	1.921	0.469	GC-TOF-MS
(10S,11R)-Pterosin C 4-glucoside	282.232	397	15.388	11.382	0.024	1.345	0.435	UHPLC-QE-MS positive ion

3.6 Change trends of differential metabolite in different pathological stages

Table 4 The content of metabolites in the tongue coating of chronic gastritis patients at different pathological stages and healthy people.

Metabolites	Mild	Moderate	Severe	Healthy controls
atrophic				
octadecanol	1.534E-05	1.045E-05	1.994E-05	3.63256E-05
conduritol-beta-expoxide	4.46E-03	3.17E-03*	4.66E-03	2.89E-03
Tetracosanoic acid	5.98E-01	5.27E-01	5.57E-01	3.57E-01
Sphinganine 1-phosphate	1.71E-01	1.60E-01	1.17E-01	6.93E-02
Smilanippin A	5.64E-01	5.67E-01	6.73E-01	3.75E-01
Lactosylceramide (d18:1/26:0)	3.17E-03	3.19E-03	2.68E-03	1.96E+00
Chondrillasterol 3-[glucosyl-(1->4)-glucoside]	6.59E-01	6.46E-01	7.64E-01	1.82E+00
Azelaic acid	1.88E-01	1.85E-01	1.70E-01	7.96E-01
alpha-Carboxy-delta-decalactone	7.91E-02	8.74E-02	8.03E-02	3.36E-01
5,6-Dihydroxyindole	2.55E-01	2.55E-01	2.48E-01	1.69E-01
intestinal metaplasia				
conduritol-beta-expoxide	4.62E-03	3.20E-03*	4.66E-03	2.89E-03
trimethylaminoacetone	1.24E-01	1.16E-01	1.12E-01	9.46E-02
tetracosanoic acid	6.08E-01	5.24E-01	5.57E-01	3.57E-01
Sphinganine 1-phosphate	1.64E-01	1.58E-01	1.17E-01	6.93E-02
Lactosylceramide (d18:1/26:0)	3.51E-03	3.34E-03	2.68E-03	1.96E+00
Chondrillasterol 3-[glucosyl-(1->4)-glucoside]	6.75E-01	6.56E-01	7.64E-01	1.82E+00
Azelaic acid	1.84E-01	1.85E-01	1.70E-01	7.96E-01
alpha-Carboxy-delta-decalactone	8.14E-02	8.77E-02	8.03E-02	3.36E-01
5,6-Dihydroxyindole	2.54E-01	2.56E-01	2.48E-01	1.69E-01
1-Methyladenosine	3.60E-01	3.92E-01	4.52E-01	6.92E-01
Hp				
methylmaleic acid	1.17E-02	1.19E-02	1.22E-02	8.13E-03
4-methylcatechol	1.03E-02	1.13E-02	1.22E-02	7.82E-03
2,4-dichloro-1-(2-chloroethenyl)-benzene	1.17E-02	1.13E-02	1.22E-02	7.79E-03

* There were differences between moderate and mild, $P<0.05$.

According to Figure 4-1 to Figure 4-3 and Table 4 we can see that in the tongue coating of atrophic, the contents of octadecanol, Lactosylceramide (d18:1/26:0), Chondrillasterol 3-[glucosyl-(1->4)-glucoside], Azelaic acid, and alpha-Carboxy-delta-decalactone in the three stages of atrophic were lower than those in the healthy control group. The contents of conduritol-beta-expoxide, Tetracosanoic acid, Sphinganine 1-phosphate, Smilanippin A and 5,6-Dihydroxyindole in the three stages of atrophic were higher than those in the healthy control group. The content of conduritol-beta-expoxide decreased significantly in mild atrophic compared with moderate atrophic ($P<0.05$). The changing trend of alpha-Carboxy-delta-decalactone content in the tongue coatings of atrophic patients was first increased and then decreased. The changing trend of octadecanol, conduritol-beta-expoxide, Tetracosanoic acid, and Chondrillasterol 3-[glucosyl-(1->4)-glucoside] contents in the tongue coatings of atrophic patients was first decreased and then increased. The content of Smilanippin A in the tongue coating of atrophic patients showed an upward trend. The contents of Sphinganine 1-phosphate, Lactosylceramide (d18:1/26:0), Azelaic acid, and 5,6-Dihydroxyindole in the tongue coating of atrophic patients showed a downward trend.

In the tongue coating of intestinal metaplasia patients, the contents of Lactosylceramide (d18:1/26:0), Chondrillasterol 3-[glucosyl-(1->4)-glucoside], Azelaic acid, alpha-Carboxy-delta-decalactone, and 1-Methyladenosine in the three stages of intestinal metaplasia were lower than those in the healthy control group. The contents of conduritol-beta-expoxid, trimethylaminoacetone, tetracosanoic acid, Sphinganine 1-phosphate, and 5,6-Dihydroxyindole in the three stages of intestinal metaplasia were higher than those in the healthy control group. The content of conduritol-beta-expoxide decreased significantly in mild intestinal metaplasia compared with moderate intestinal metaplasia ($P<0.05$). The changing trend of Azelaic acid, alpha-Carboxy-delta-decalactone, and 5,6-Dihydroxyindole contents in the tongue coatings of intestinal metaplasia patients was first increased and then decreased. The changing trend of conduritol-beta-expoxide, tetracosanoic acid, and Chondrillasterol 3-[glucosyl-(1->4)-glucoside] contents in the tongue coatings of intestinal metaplasia patients was first decreased and then increased. The content of 1-Methyladenosine in the tongue coating of intestinal metaplasia patients showed an upward trend. The contents of trimethylaminoacetone, Sphinganine 1-phosphate, Lactosylceramide (d18:1/26:0) in the tongue coating of intestinal metaplasia patients showed a downward trend.

In the tongue coating of Hp patients, the contents of methylmaleic acid, 4-methylcatechol, and 2,4-dichloro-1-(2-chloroethenyl)-benzene in the three stages of Hp were higher than those in the healthy control group. The contents of Methylmaleic acid and 4-methylcatechol in the tongue coating of Hp infection patients showed an upward trend. The changing trend of 2,4-dichloro-1-(2-chloroethenyl)-benzene content in the tongue coatings of Hp infection patients was first decreased and then increased.

4 Discussion

In this study, we used UHPC-QE-MS and GC-TOF-MS to detect the metabolites in the tongue coating of 350 patients with chronic gastritis, established the diagnostic model of the screened differential metabolites, and analyzed the correlation between the detected differential metabolites and the results of gastroscopic pathological indexes, and we explored the variation pattern of differential metabolite levels according to the degree of chronic gastritis lesions.

It can be seen from the metabolomic analysis of chronic gastritis tongue coating in Table 3, compared with the healthy control group, the number of differential metabolites of Lipids and lipid-like molecules in the tongue coating of chronic gastritis patients was the largest (there were 47 substances in total, of which 22 substances were upregulated and 25 substances were downregulated), accounting for 55.29% of the total metabolites, suggesting that Lipids and lipid-like molecules were the main metabolic disorder in patients with chronic gastritis. Some researchers have also found that Lipids and lipid-like molecules were the main differential metabolites in the serum of patients with chronic gastritis by using the method of serum metabolomics, and believe that Lipids and lipid-like molecules may promote the development of chronic gastritis to gastric cancer [13].

Among the differential metabolites of Lipids and lipid-like molecules there is a substance called Sphinganine 1-phosphate, which is significantly higher than that of healthy people. Sphinganine 1-phosphate is one of the markers of the diagnostic model of chronic gastritis. It has also been found to be significantly positively correlated with atrophic and intestinal metaplasia. In addition, we also found that although the content of Sphinganine 1-phosphate in patients with gastric mucosal atrophic and intestinal metaplasia was always significantly higher than that in healthy controls, the level of Sphinganine 1-phosphate decreased gradually with the aggravation of the severity of gastric mucosal atrophic and intestinal metaplasia in patients with gastritis. This is a very interesting phenomenon, and its mechanism needs to be further discussed. Other studies have also shown that Sphinganine 1-phosphate was related to the occurrence of cancer, which can promote tumor growth and metastasis [14–15]. Among the differential metabolites screened by the diagnostic model of chronic gastritis, except Sphinganine 1-phosphate, there was another substance belonging to the differential metabolites of Lipids and lipid-like molecules, which was Nervonic acid. Some researchers found Nervonic acid in the mucosa of patients with gastric and colon cancer [16]. It can be seen that nervonic acid exists not only in the upper digestive tract, but also in the lower digestive tract.

In our study on the correlation between differential metabolites in tongue coatings and pathological indexes in patients with chronic gastritis, we found that the number of differential metabolites of Lipids and lipid-like molecules was the largest, they were Sphinganine 1-phosphate, Tetracosanoic acid, Smilanippin A, Lactosylceramide(d18:1/26:0), Chondrillasterol 3-[glucosyl-(1→4)-glucoside], Azelaic acid and 3-Benzoyloxy-11-oxo-12-ursen-28-oic acid.

In Figure 3-[link rid="fig3">1</link>-e](#) and 3-[link rid="fig3">1</link>-f](#), we found that the content of Smilanippin A in the tongue coating of patients with severe atrophic was higher than that of patients with mild and moderate atrophic, and the content of this metabolite in the tongue coating of patients with different degrees of atrophic was higher than that of healthy people. The content of Lactosylceramide (d18:1/26:0) in the tongue coating of patients with severe atrophic was lower than that of patients with mild atrophic and patients with moderate atrophic, and the content of this metabolite in the tongue coating of patients with different degrees of atrophic was lower than that of healthy people. In addition, as shown in figure 3-2-e, the content of Lactosylceramide (d18:1/26:0) gradually decreases with the aggravation of intestinal metaplasia, and the content in the three stages of intestinal metaplasia was lower than that in the tongue coating of healthy people. Studies have found that Lactosylceramide can promote the proliferation of colon cancer cells [17], and researchers have found that this substance exists in the serum of children with inflammatory bowel disease [18]. It can be seen that this substance will aggravate digestive system diseases. In Figure 3-1-g and 3-2-f, the content of Chondrillasterol 3-[glucosyl-(1→4)-glucoside] in the tongue coating of patients with severe atrophic and intestinal metaplasia was higher than that of patients with mild and moderate atrophic and intestinal metaplasia. Moreover, the content of this substance in the three stages of atrophic and intestinal metaplasia was lower than that in the tongue coating of healthy people.

In Figure 3-1-h and 3-2-g, the content of Azelaic acid gradually decreases with the aggravation of atrophic, and its content in the tongue coating of subjects with different degrees of atrophic was lower than that of healthy people. Moreover, the content of Azelaic acid in severe intestinal metaplasia subjects was lower than that in mild and moderate patients, and the content in the three stages of intestinal metaplasia was lower than that in the tongue coating of healthy people.

Organic acids and derivatives were the second largest metabolites in the tongue coating of patients with chronic gastritis. There were 13 metabolites (7 metabolites were upregulated and 6 metabolites were downregulated), among which pantothenic acid was upregulated. Some researchers have found that this substance was a potential biomarker of gastric cancer and was of great significance for the diagnosis of gastric cancer [19]. The differential metabolite 3-Hydroxycapric acid screened by the diagnostic model of chronic gastritis also belongs to Organic acids and derivatives.

Other metabolites include Organoheterocyclic compounds (8 metabolites, 4 substances upregulated and 4 substances downregulated), Organic nitrogen compounds (6 metabolites, 5 substances upregulated and 1 substance downregulated), and Organic oxygen compounds (4 metabolites, 2 substances upregulated and 2 substances downregulated), Benzenoids (there are 2 metabolites, 1 upregulated and 1 downregulated), Homogeneous non-metal compounds, Nucleosides, nucleotides, and analogues, Phenylpropanoids and polyketides, Organosulfur compounds, and Hydrocarbons each have 1 metabolite. In these metabolic species, L-histidinol expression was upregulated. Some studies have shown that histidinol has the effect of reducing gastric acid and reducing gastrointestinal pain. In addition, it can also inhibit the proliferation and migration of lung cancer cells, thus play an anti-tumor role [20–21]. Dictagymnin expression was upregulated, and this substance has anti-inflammatory and tumor growth inhibitory effects [22]. It can be shown that patients with chronic gastritis were probably also undergoing self-repair by the body during their illness. From the correlation between chronic gastritis and

pathological indexes, we found that the differential metabolites belonging to Organoheterocyclic compounds were alpha-Carboxy-delta-decalactone and 5,6-Dihydroxyindole. In figure 3-1-i and 3-2-h, we can see that the content of alpha-Carboxy-delta-decalactone in the tongue coating of patients with moderate atrophic and intestinal metaplasia was higher than that of patients with mild and severe atrophic, and the content of this substance in the tongue coating of patients with different degrees of atrophic and intestinal metaplasia was lower than that of healthy people. In figure 3-1-j and 3-2-i, the content of 5,6-Dihydroxyindole in the tongue coating of patients with severe atrophic and intestinal metaplasia was lower than that of patients with mild and moderate atrophic. Moreover, the content of this substance in the tongue coating of patients with different degrees of atrophic and intestinal metaplasia was higher than that of healthy people.

Among the differential metabolites screened by the diagnostic model of chronic gastritis, 4-Ipomeanol belongs to Organic oxygen compounds, and 1-Methyladenosine belongs to Nucleosides, nucleotides, and analogues. 1-Methyladenosine was also found in the results of correlation of pathological indexes. In the trend figures of chronic gastritis with intestinal transformation, the content of 1-Methyladenosine increased with the increase of intestinal degree, but the content in the three stages of intestinal transformation was lower than that of healthy people.

In addition, we also found three substances in Figure 3-3 of the Hp trend figure: methylmaleic acid, 4-methylcatechol and 2,4-dichloro-1-(2-chloroethenyl)-benzene. The contents of methylmaleic acid and 4-methylcatechol gradually increased with the aggravation of Hp. The content of 2,4-dichloro-1-(2-chloroethenyl)-benzene in the tongue coating of moderate intestinal metaplasia patients was lower than that of mild and severe patients. Experiments on rats showed that 4-methylcatechol was correlated with the occurrence of gastric cancer [23]. While Hp infection was considered a risk factor for gastric cancer [24]. We suggest that 4-methylcatechol might be involved in the transformation from chronic gastritis with Hp infection to gastric cancer. In Table 4, we can also found that the content of conduritol-beta-expoide decreases significantly with the aggravation of atrophic and intestinal metaplasia. However, we have not found the content change of this substance in digestive system diseases.

This study also has the following shortcomings. First, the proportion of sample cases in chronic gastritis group and healthy control group is large. Second, the age difference between chronic gastritis group and healthy control group is large. Although our previous study showed that the significant difference between the number of sample cases and age ratio between groups had a minor effect on the altered results of metabolite content in the tongue coating of patients with gastric precancerous lesions [25], in future studies, we will narrow the difference of sample cases and age between groups as much as possible. Last, this study only conducted a cross-sectional study of 350 patients with chronic gastritis. In the follow-up study, we will conduct a cohort study to further verify our current findings.

5 Conclusions

This study found that Lipids and lipid-like molecules were the main differential metabolites in the tongue coating of patients with chronic gastritis. Among these lipid metabolites, Sphinganine 1-phosphate was one of the metabolites to establish the diagnostic model of chronic gastritis. Its content change was significantly positively correlated with the occurrence of atrophic and intestinal metaplasia, but the level of this metabolite decreases with the aggravation of atrophic and intestinal metaplasia. Therefore, we believe that the change of Sphinganine 1-phosphate content in tongue coating has a certain correlation with the pathological progress of chronic gastritis, but its specific mechanism needs to be further discussed. We also found that conduritol-beta-expoide was negatively correlated with atrophic and intestinal metaplasia, and its content decreased significantly with the aggravation of atrophic and intestinal metaplasia. Other researchers have not reported the above results. In future studies, we will further verify the changes of these tongue coating differential metabolites in patients with gastritis through cohort studies. We believe that in the future, these differential markers in tongue coating may help us to establish a noninvasive and convenient method to detect chronic gastritis and gastric precancerous lesion.

Abbreviations

TCM: Traditional Chinese Medicine

UPLC-MS: Ultra-performance liquid chromatography and mass spectrometry

LC-MS: Liquid chromatography and mass spectrometry

GC-TOF-MS: Gas chromatography-time-of-flight-mass spectroscopy

UHPLC-QE-MS: Liquid chromatography-Q exactive orbitrap-mass spectroscopy

Hp: *Helicobacter pylori*

BMI: Body mass index

EP: Eppendorf

QC: Quality control

PCA: Principal components analysis

OPLS-DA: Orthogonal projections to latent structures-discriminant analysis

FDR: False discovery rate

VIP: Variable importance in the projection

SV: Similarity value

FC: Fold change

CORR: Correlation coefficient

Declarations

Funding

This paper was funded by the National Natural Science Foundation of China (No. 82174279; No. 81703982). This work was also supported by Shanghai Biotree Biotech Co. Ltd.

Conflicts of Interest

The authors declare that the research was conducted in the absence of financial relationships or any commercial.

Acknowledgments

This work was also supported by Shanghai Biotree Biotech Co. Ltd.

Consent to publish

All authors agree to publish this article.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Shanghai University of TCM in China in December 2018 and performed in accordance with the Declaration of Helsinki. All the subjects had signed informed consent agreements before collecting samples.

Authors' contributions

Yiming Hao was responsible for the study. Yifeng Xu wrote the manuscript. Renling Zhang helped collect clinical samples. Junhong Lu helped revise the text and grammar of the manuscript. Yiqin Wang helped with the ideas of the study. Zhuqing Zhu helped with the experimentation. All authors of this study were agreed to be accountable for all aspects of the work.

Availability of data and materials

The Ethics Committee of Shanghai University of Traditional Chinese Medicine limited the measurement data used to support the results of this study in order to protect the privacy of patients. For researchers who meet the criteria for obtaining confidential data, the data of this study can be obtained from Yiming Hao (Email: hymjj888@163.com).

References

- [1] Sipponen P, Maaroos HI. Chronic gastritis. *Scand J Gastroenterol*. 2015, 50(6): 657-667
- [2] Cui J, Cui H, Yang M, et al. Tongue coating microbiome as a potential biomarker for gastritis including precancerous cascade. *Protein Cell*. 2019 Jul;10(7):496-509
- [3] Li FF, Zhao J, Qian P, et al. Metabolite changes in the greasy tongue coating of patients with chronic gastritis. *Zhong Xi Yi Jie He Xue Bao*. 2012,10(7):757-65
- [4] Sun ZM, Zhao J, Qian P, et al. Metabolic markers and microecological characteristics of tongue coating in patients with chronic gastritis. *BMC Complement Altern Med*. 2013,13:227
- [5] Zeki ÖC, Eylem CC, Reçber T, et al. Integration of GC-MS and LC-MS for untargeted metabolomics profiling. *J Pharm Biomed Anal*. 2020 Oct 25; 190:113509
- [6] Dixon MF, Genta RM, Yardley JH, et al. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol*. 1996,20(10):1161-81
- [7] National Health and Family Planning Commission of PRC. Criteria of weight for adults. Vol. 2. Beijing, China: Standards Press of China; 2013
- [8] Kind T, Wohlgemuth G, Lee DY, et al. FiehnLib: mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. *Anal Chem*. 2009,81(24):10038-10048
- [9] Dunn WB, Broadhurst D, Begley P, et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nat Protoc*. 2011,6(7):1060-1083

- [10] Smith CA, Want EJ, O'Maille G, et al. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. *Anal Chem*. 2006;78(3):779-787
- [11] Yang C, Hao R, Du X, et al. GC–TOF/MS-based metabolomics studies on the effect of protein sources in formulated diet for pearl oyster *Pinctada fucata martensii*. *Aquaculture*. 2018;486:139-147
- [12] Wang W, Zhao L, He Z, et al. Metabolomics-based evidence of the hypoglycemic effect of Ge-Gen-JiaoTai-Wan in type 2 diabetic rats via UHPLC-QTOF/MS analysis. *J Ethnopharmacol*. 2018;219:299-318
- [13] Yu L, Lai Q, Feng Q, et al. Serum Metabolic Profiling Analysis of Chronic Gastritis and Gastric Cancer by Untargeted Metabolomics. *Front Oncol*. 2021 Mar 11; 11:636917
- [14] Plano D, Amin S, Sharma AK. Importance of sphingosine kinase (SphK) as a target in developing cancer therapeutics and recent developments in the synthesis of novel SphK inhibitors. *J Med Chem*. 2014 Jul 10;57(13):5509-24
- [15] Schneider G. S1P Signaling in the Tumor Microenvironment. *Adv Exp Med Biol*. 2020; 1223:129-153
- [16] Pacuszk T, Jóźwiak W, Miller-Podraza H, et al. Neutral glycolipid composition in human cancer. *Cancer Biochem Biophys*. 1980;5(1):1-6
- [17] Machala M, Procházková J, Hofmanová J, et al. Colon Cancer and Perturbations of the Sphingolipid Metabolism. *Int J Mol Sci*. 2019 Nov 30;20(23):6051
- [18] Filimoniuk A, Blachnio-Zabielska A, Imierska M, et al. Sphingolipid Analysis Indicate Lactosylceramide as a Potential Biomarker of Inflammatory Bowel Disease in Children. *Biomolecules*. 2020 Jul 21;10(7):1083
- [19] Jiang W, Zhou L, Lin S, et al. Metabolic profiles of gastric cancer cell lines with different degrees of differentiation. *Int J Clin Exp Pathol*. 2018;11(2):869-87
- [20] Zu GX, Sun QQ, Chen J, et al. Urine metabolomics of rats with chronic atrophic gastritis. *PLoS One*. 2020 Nov 11;15(11): e0236203
- [21] Escobar-Reséndiz R, Reyes-Esparza J, Blake IO, et al. Evaluation of antitumoral effect of the combination of L-histidine methyl ester hydrochloride of anfotericin B with antineoplastics on A549 cells. *FASEB J*. 2020;34: 1
- [22] Wang JY, Wang Z, Li MY, et al. Dictamnine promotes apoptosis and inhibits epithelial-mesenchymal transition, migration, invasion and proliferation by downregulating the HIF-1 α and Slug signaling pathways. *Chem Biol Interact*. 2018 Dec 25; 296:134-144
- [23] Asakawa E, Hirose M, Hagiwara A, et al. Carcinogenicity of 4-methoxyphenol and 4-methylcatechol in F344 rats. *Int J Cancer*. 1994 Jan 2;56(1):146-52
- [24] Malfertheiner P, Megraud F, O'Morain CA, et al. Management of *Helicobacter pylori* infection—the Maastricht IV/ Florence Consensus Report. *Gut*. 2012 May;61(5):646-64
- [25] Hao Y, Zhang R, Morris R, et al. Metabolome and microbiome alterations in tongue coating of gastric precancerous lesion patients. *Expert Rev Gastroenterol Hepatol*. 2021 Aug;15(8):949-963

Figures

Figure 1

Mass spectrum peaks of GPL group and healthy control group. a: GC-TOF-MS, b: UHPLC-QE-MS negative ion modes, c: UHPLC-QE-MS positive ion modes. As the examples of the GC-TOF-MS and UHPLC-QE-MS spectra, there were some different mass spectrum peaks between the chronic gastritis patients and healthy control people

Figure 2

Diagnostic model of tongue coating metabolites in patients with chronic gastritis. The accuracy of the diagnostic model is 95.4%, the specificity is 87.4%, and the sensitivity is 88.0%.

Figure 3

Figure 3-1. Correlation figure between metabolic markers of tongue coating and gastroscopic indexes and pathological indexes in patients with chronic gastritis analyzed by GC-TOF-MS. Draw a thermodynamic diagram to show the correlation analysis results, which were represented by red (corr = 1), blue (corr = -1) and white (corr = 0). Data with correlation P values less than 0.05 were marked with "*" and "+" in the figures. The abscissa was the differential metabolic marker and the ordinate was the gastroscopic characteristic indicator. It can be seen that octadecanol, and conduritol-beta-expoxide were negatively correlated with atrophic; 6-deoxyglucitol was negatively correlated with bile reflux; methylmaleic acid, 4-methylcatechol, and 2,4-dichloro-1-(2-chloroethenyl)-benzene were negatively correlated with Hp; conduritol-beta-expoxide was negatively correlated with intestinal metaplasia.

Page 21/24

Figure 5

Figure 4-1. The change figure of tongue coating metabolites in chronic gastritis patients with different degrees of atrophic. The abscissa "1, 2, and 3" represent "light, medium, and severe", and the ordinate represents the content of metabolites. The changing trend of alpha-Carboxy-delta-decalactone content in the tongue coatings of atrophic patients was first increased and then decreased. The changing trend of octadecanol, conduritol-beta-expoxide, Tetracosanoic acid, and Chondrillasterol 3-[glucosyl-(1->4)-glucoside] contents in the tongue coatings of atrophic patients was first decreased and then increased. The content of Smilaniptin A in the tongue coating of atrophic patients showed an upward trend. The contents of Sphinganine 1-phosphate, Lactosylceramide (d18:1/26:0), Azelaic acid, and 5,6-Dihydroxyindole in the tongue coating of atrophic patients showed a downward trend.

Figure 6

Figure 4-2. The change figure of tongue coating metabolites in chronic gastritis patients with different degrees of intestinal metaplasia. The abscissa "1, 2, and 3" represent "light, medium, and severe", and the ordinate represents the content of metabolites. The changing trend of Azelaic acid, alpha-Carboxy-delta-decalactone, and 5,6-Dihydroxyindole contents in the tongue coatings of intestinal metaplasia patients was first increased and then decreased. The changing trend of conduritol-beta-epoxide, tetracosanoic acid, and Chondrillasterol 3-[glucosyl-(1->4)-glucoside] contents in the tongue coatings of intestinal metaplasia patients was first decreased and then increased. The content of 1-Methyladenosine in the tongue coating of intestinal metaplasia patients showed an upward trend. The contents of trimethylaminoacetone, Sphinganine 1-phosphate, Lactosylceramide (d18:1/26:0) in the tongue coating of intestinal metaplasia patients showed a downward trend.

Figure 7

Figure 4-3. The change figure of tongue coating metabolites in chronic gastritis patients with different degrees of Hp infection. The abscissa "1, 2, and 3" represent "light, medium, and severe", and the ordinate represents the content of metabolites. The contents of Methylmaleic acid and 4-methylcatechol in the tongue coating of Hp infection patients showed an upward trend. The changing trend of 2,4-dichloro-1-(2-chloroethenyl)-benzene content in the tongue coatings of Hp infection patients was first decreased and then increased.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [tables1.docx](#)