Study on pulp metabolism in patients with pulpitis via ultra-performance liquid chromatography coupled with Orbitrap mass spectrometry

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Abstract

Background

Pulpitis, a pulp disease caused by several factors, including caries and trauma, has a high clinical incidence. In this study, our aim was to identify possible metabolic biomarkers in individuals with pulpitis and analyze the associated metabolic pathways to the end of providing a theoretical basis for pulpitis diagnosis and prevention.

Methods

Pulp samples from 12 individuals (six patients with pulpitis and six individuals with normal teeth) were analyzed via serum metabolomics based on ultra-high-performance liquid chromatography (UPLC)/Orbitrap mass spectrometry. Thereafter, to identify important biomarkers, we performed multivariate analysis and also established an orthogonal partial least squares discrimination analysis model. Further, we performed correlation analysis as well as biomarker pathway enrichment analysis to determine the associations between the differentially expressed biomarkers as well as their association with different biological pathways.

Results

22 biomarkers (13 upregulated and nine downregulated) we found to be significantly associated with 18 metabolic pathways in pulpitis. Specifically, the major biomarkers included ascorbic acid, inosine, allopurinol riboside, and L-asparagine, and among these, ascorbic acid and inosine were most significantly downregulated and showed the strongest correlation with pulpitis. Additionally, aminoacyl-tRNA biosynthesis and retrograde endocannabinoid signaling showed positive correlation with pulpitis.

Conclusions

These identified pulpitis-associated biomarkers and metabolic pathways may serve as a theoretical basis for further clarifying the pathogenesis of pulpitis and can be applied in the development of preventive drugs.

Background

Pulpitis, the most common oral disease, is an inflammatory disease characterized by pain as its primary symptom. It is caused by bacterial infection that activates the host's immune defense response, and often develops from the untimely and ineffective treatment of caries, trauma, and other oral diseases. The incidence of pulpitis is relatively high. Specifically, its incidence in association with dental caries is up to 88.8%, of which 80% seek medical treatment. Thus, pulpitis is an urgent oral problem requiring an effective solution. Further, pulpitis is an irreversible condition. Even though patients can save the affected tooth via complex treatment, the tooth does not recover and become as strong and durable as healthy teeth. Therefore, the prevention of pulpitis is crucial [1].

Root canal therapy, which reduces inflammation and relieves pain based on pulp and infection removal, is currently the preferred treatment for pulpitis. However, it has several limitations, including (1) pulp removal is required for treatment; this results in the loss of blood supply to the affected tooth, sensory loss, and brittleness; (2) dental tissue damage is inevitable during the treatment process; this reduces flexural strength and increases the risk of tooth splitting; (3) tooth discoloration after treatment affects aesthetics; and (4) due to the complicated nature of the root canal system, even with continuous improvement of materials and equipment, its success rate peaks at approximately 85% [2, 3]. Consequently, the identification of better pulpitis prevention and therapeutic strategies is of great significance. Further, even though revascularization has been used for pulp regeneration in clinical practice, it is primarily applicable only to young permanent teeth, and has a limited range of indications. Therefore, preventing pulpitis as well as inflammation at an early stage, restoring pulp vitality, and shifting from treatment to prevention are of great clinical significance.

Pulpitis prevention has been primarily focused on the prevention and treatment of dental caries via oral hygiene, fluorination of teeth, closure of pits and grooves, and filling of caries cavities. However, no preventive measures for pulpitis have been reported to date. A focus on genes and proteins to the end of preventing the occurrence of pulpitis appears unrealistic. However, small molecules, including small-molecular-weight peptides and metabolites, can easily access cells with no adverse reactions (e.g., polypeptides and metabolites have been used in various fields of medicine). Further, as reported by Ghosh et al. [4], intestinal microbial metabolite generation is strongly associated with intestinal epithelial cells and has important effects on intestinal barrier function and immune response. Additionally, a variety of
peptides derived from BMP-2, such as OP peptide BMP-2 residues 32–48 and 73–92, promote MSC level in Runx2 gene and related alkaline phosphatase proteins, indicating that BMP-2-derived peptides play a role in enhancing osteogenic differentiation [5, 6, 7]. Thus, it is possible to use small molecules to prevent pulpitis, and the discovery of such active small-molecule materials is urgently needed.

In this regard, metabolomics offers the possibility to not only screen disease markers, but also identify metabolites with biological activity. Specifically, metabolomics, which has gradually become a strategy for studying the function of metabolites, is an emerging field that is utilized to qualitatively and quantitatively analyze low-molecular-weight (LMW) metabolites in cells or in an organism under pathological or physiological conditions [8]. It is extensively utilized in the diagnosis of several disorders [9], such as neuropsychiatric diseases [10], liver fibrosis [11], or hepatoma [12], and has been applied in the screening of metabolites to assess their associated disease causing risk. It has also been used to predict the occurrence of cardiovascular diseases (CVDs), such as hyperlipidemia or acute coronary syndrome [13–16]. Furthermore, metabolomics is extensively utilized in stomatology. Specifically, Sakanaka et al. [17] identified a salivary metabolite catalog that possibly reflects cardiac metabolism alterations. By studying these salivary metabolites, they found that periodontal inflammation is related to liver function and lipid metabolism disorders. Garcia-Contreras et al. [18] also reviewed changes in metabolic profiles resulting from the presence of TiO2 nanoparticles within a human model of gingivitis via metabolomics. Additionally, according to Nijakowski et al. [19], saliva contains numerous metabolites that can be used for the diagnosis and staging of oral squamous cell carcinoma at the early stage. Awaad et al. [20] also reported that bio-guided Aspergillus terreus extract fractionation induced separate the formation of the new terpenoid secondary metabolite, which together with its total alcoholic extract, remarkably suppresses oral microbial infections. However, for regular oral diseases, such as pulpitis, studies with a focus on the application of metabolomics are scarce, meanwhile to address issues related to pulpitis prevention, such studies are necessary [21, 22].

Therefore, in this study, we focused on investigating differences in pulp metabolites between pulpitis-affected teeth and normal teeth by screening differentially expressed metabolites (DEMs) to the end of identifying functional metabolites with potential for application in pulpitis prevention. Specifically, to analyze pulp specimens from pulpitis-affected and normal teeth, we performed liquid chromatography-mass spectrometry (LC-MS). Thus, specific metabolites were identified. Thereafter, we performed statistical analysis to identify metabolites with lower or higher expression levels in pulpitis-affected than normal teeth to the end of providing a theoretical foundation for pulpitis prevention.

Materials And Methods

Equipment and materials

We used a table top high-speed refrigerated centrifuge (TGL-16MS, Lu Xiang Yi Centrifuge Instrument Co., Ltd., Shanghai, China), an ultrasonic cleaning machine (F-060SD, Fuyang Technology Group Co., Ltd., Shenzhen, China), a high-performance liquid chromatography (HPLC) equipment (Nexera UPLC, Shimadzu, Japan), and a high-resolution mass spectrometer (QE, Thermo Fisher Technologies, Cleveland, OH, USA) for analysis.

Sample collection

Patients who underwent impacted tooth extraction at the Stomatology Department of Shanghai Tongren Hospital within the January–May 2021 period were enrolled in this study. Specifically, the study population included six patients with normal impacted teeth (healthy dental pulp group) and six patients with pulpitis (the pulpitis group). After extraction, the impacted teeth were immersed in physiological saline and immediately transported to the laboratory. At the laboratory, after the periodontal tissue attached to the root surfaces of the collected specimens were cleaned on an ultra-clean table, the root tips were clipped, and the pulps were removed. Thus, a total of 12 groups of samples were obtained and thereafter, quickly stored in liquid nitrogen until further analysis. Patient data, including genetic, family, and medical histories were collected upon admission.

The inclusion criteria for the pulpitis group were as follows: patients aged between 18 and 60 years; all the affected teeth were diagnosed with acute and chronic pulpitis by dental and pulp specialists according to clinical and imaging examinations; the source of the pulp infection was caries; the pulp could be completely removed; and the patients had not taken any medication within the past 3 months. The exclusion criteria for pulp tissue samples from healthy teeth without caries were as follows: patients with serious brain, heart, kidney, liver, or endocrine system diseases, and an association of the affected tooth with severe periodontitis.

Sample preparation and analysis

After inactivation, all the dental pulp samples were subjected to 30-min sterilization at 56°C. Thereafter, equivalent amounts of samples (100 µL) were placed in 1.5-mL centrifuge tubes, followed by the addition of 0.3 mg/mL L-2-chlorophenylalanine (10 µL) as well as 0.01
mg/mL LysoPC17:0 in methanol as the endogenous standard under 10-s vortexing. Next, a pre-chilled methanol-acetonitrile mixture (300 µL, v:v = 2:1) was added under 1-min vortexing, followed by 10-min ultrasonication at 0°C and 30-min incubation at -20°C. After 15-min centrifugation at 13,000 rpm and 4°C, supernatant samples were collected in novel centrifuge tubes, followed by drying using a freeze-concentration centrifugal dryer. Lyophilization was then performed. The powder samples obtained were then re-dissolved in a methanol-water mixed solution (100 µL, v:v = 1:4), followed by 30-s vortexing and 2-min standing at 4°C. The resultant mixed sample was then subjected to 5-min centrifugation at 13,000 rpm and 4°C to collect supernatants, which were then filtered using 0.22-µm microfilters into LC vials prior to LC-MS analysis. The supernatant samples (200 µL) were added to the glass sampling vials and allowed to dry at 25°C under vacuum conditions, followed by addition into methoxylamine hydrochloride (15 mg/mL) in pyridine (80 µL). The resulting mixed sample was then subjected to 2-min vigorous vortexing and 90-min incubation and 37°C, after which BSTFA containing 1% TMCS (80 µL) and n-hexane (20 µL) was added.

The mixture was then subjected to 2-min vortexing as well as 60-min derivatization at 70°C. This was followed by the incubation of the samples for 30 min at 25°C prior to LC-MS analysis. To prepare the quality control (QC) sample, equal volumes of diverse sample extracts were mixed, with the same QC volume as the sample volume.

**Untargeted metabolomics analysis based on LC-MS**

The Acquity UHPLC system (Waters Corporation, Milford, USA) coupled with an AB SCIEX Triple TOF 5600 System (AB SCIEX, Framingham, MA, USA), functioning in both the ESI positive- and negative-ion modes was used to obtain plasma metabolic profiles. Afterwards, we used the Waters BEH C18 column (1.7 m, 2.1 × 100 mm) (Waters Corporation, Milford, MA, USA), with the mobile phase containing water (supplemented with 0.1% formic acid) and acetonitrile for chromatography. The analysis conditions were as follows: injection volume, 1 µL; column temperature, 45°C; and flow rate, 0.4 mL/min. Further, data were obtained under the full-scan mode at 70–1000 m/z and in the IDA mode, with the collision energy at 30 eV and the m/z range at 25–1000. To evaluate data repeatability, QC samples were loaded at intervals of 10 runs. Moreover, Progenesis QI software (Waters Corporation) was utilized to analyze the raw LC-MS data using the following parameters: fragment tolerance, 10 ppm; precursor tolerance, 5 ppm; noise elimination level, 10.00; and retention time (RT) tolerance, 0.02 min. During the data analysis, we eliminated isotopic peaks, with the minimal intensity set at 15% of the base peak intensity. Additionally, LIPID MAPS (https://lipidmaps.org/), HMDB (https://hmdb.ca/), together with self-built databases, were adopted for metabolite identification based on tandem mass spectrometry (MS/MS) spectra and RT-m/z pairs. Finally, we exported the output data matrix, which included 3-D datasets (m/z, peak RT, and intensity data) for subsequent analyses.

**Statistical analysis**

**Using Thermos Xcalibur 2.2 software**

Peaks were aligned and extracted using Thermo Xcalibur 2.2 software (Thermo Fisher Scientific, San Jose, CA, USA). Thus, we obtained a data table containing RT-m/z values as well as the adj. p-values corresponding to the peak area data.

**Multivariate analysis**

To observe sample distribution and analyze process stability, unsupervised principal component analysis (PCA) was performed for multivariate analysis using Simca-P software version 13.0 (Umetrics, Umea, Sweden). Thereafter, the intergroup heterogeneities of the metabolic profiles and DEMs were analyzed via supervised orthogonal partial least squares analysis (OPLS-DA).

Further, we screened variables with variable importance in the projection (VIP) values above 1.0, and also determined two-tailed p values via Student's t-tests using SPSS software version 18.0.0 (IBM, Armonk, NY, USA). Statistical significance was set at p < 0.05.

**Univariate analysis**

In this study, we performed univariate analysis to describe the centralized and discrete trends of the samples. Specifically, we applied univariate statistics to infer the overall situation based on the obtained sample data. This included a comparison of metabolites based on statistical hypothesis testing and interval estimation via Student's t-test as well as fold change (FC) analysis. Further, both FC and p values, which facilitated the selection of DEMs, were visualized using volcano maps.

**Differential metabolite screening**

In this study, we applied VIP to select DEMs. Specifically, VIP was adopted as it offers the possibility to measure impact intensity as well as the explanatory ability of metabolite expression patterns to the end of classifying and discriminating samples, mining biologically significant DEMs, and verifying their significance using the t-test. Significant DEMs and the top 50 differential metabolites with VIP values above 1.0 showed hierarchical clustering.
Correlation analysis

Correlation analysis based on Pearson's correlation coefficients was performed to determine the correlation between the DEMs and the biological pathways. We also performed correlation analysis to determine the degree of linear correlation between any two DEMs.

Metabolic pathway enrichment

The DEMs were subjected to metabolic pathway enrichment analysis to determine the mechanisms underlying the alterations of metabolic pathways within normal and pulpitis samples. The enriched metabolic pathways were determined based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Results

Metabolite screening

Using Compound Discoverer software (Thermo Fisher Scientific), 22 biomarkers, including 13 and 9 that were upregulated and downregulated, respectively, were found to be associated with 18 major metabolic pathways. Age, sex, body mass index (BMI), and other indicators (Table 1) were not significantly different between two groups (p > 0.05).
<table>
<thead>
<tr>
<th>m/z</th>
<th>Retention time (min)</th>
<th>Ion mode</th>
<th>Metabolites</th>
<th>Mass Error (ppm)</th>
<th>VIP</th>
<th>p-Value</th>
<th>log2(FC)</th>
<th>Compound ID</th>
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<td>175.0242</td>
<td>1.11</td>
<td>neg</td>
<td>Ascorbic acid</td>
<td>-3.39</td>
<td>2.18</td>
<td>3.40E-03</td>
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<td>267.0739</td>
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<td>Inosine</td>
<td>1.60</td>
<td>2.58</td>
<td>5.42E-03</td>
<td>-1.50</td>
<td>HMDB000195</td>
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<tr>
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<td>Allopurinol riboside</td>
<td>2.50</td>
<td>2.95</td>
<td>8.10E-03</td>
<td>-1.27</td>
<td>HMDB000481</td>
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<td>133.0612</td>
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<td>L-Asparagine</td>
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<td>1.11</td>
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<tr>
<td>834.5303</td>
<td>13.37</td>
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<td>PE(18:3(6Z,9Z,12Z)/22:4(7Z,10Z,13Z,16Z))</td>
<td>1.54</td>
<td>3.12</td>
<td>5.33E-04</td>
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<tr>
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<td>neg</td>
<td>5'-Phosphoribosyl-N-formylglycinamide</td>
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<td>1.38</td>
<td>7.72E-03</td>
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<tr>
<td>284.0996</td>
<td>2.25</td>
<td>pos</td>
<td>Guanosine</td>
<td>2.22</td>
<td>1.18</td>
<td>3.36E-02</td>
<td>-0.56</td>
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<tr>
<td>289.0683</td>
<td>1.22</td>
<td>neg</td>
<td>Uridine</td>
<td>0.66</td>
<td>4.08</td>
<td>1.94E-02</td>
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<tr>
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<td>Tryptophan</td>
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<td>5.88</td>
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<td>2.41</td>
<td>2.51E-02</td>
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<td>HMDB000148</td>
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<td>502.2942</td>
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<td>Fexofenadine</td>
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<td>1.94</td>
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<td>1.26E-03</td>
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<td>1.34</td>
<td>2.88E-02</td>
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<td>HMDB0010395</td>
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<td>pos</td>
<td>Asymmetric dimethylarginine</td>
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<td>1.61</td>
<td>1.55E-03</td>
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<td>480.3100</td>
<td>11.11</td>
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<td>Dodecanedioylcarnitine</td>
<td>3.17</td>
<td>3.00</td>
<td>4.20E-02</td>
<td>0.98</td>
<td>HMDB001327</td>
</tr>
<tr>
<td>310.1140</td>
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<td>1.66</td>
<td>1.99E-02</td>
<td>1.02</td>
<td>HMDB000230</td>
</tr>
<tr>
<td>360.1508</td>
<td>0.82</td>
<td>pos</td>
<td>Inulobiose</td>
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<td>8.87</td>
<td>1.08E-05</td>
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<td>4.94E-02</td>
<td>12.90</td>
<td>HMDB0006061</td>
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</table>

**Biomarker selection and detection**

Based on PCA analysis (Fig. 1), an isolation trend was observed within and between the control and pulpitis groups, with the blank controls and pulpitis groups showing better separation trends.
An OPLD-DA model was established for biomarker screening. As shown in Fig. 2, the results corresponding to the pulpitis group were obviously different from those corresponding to the control group, suggesting the stability and reliability of model. Altogether, 22 DEMs satisfying VIP > 1 and p < 0.05 were detected, and among them, ascorbic acid, inosine, allopurinol riboside, asymmetric dimethylarginine, dodecanediolcarnitine, and N-acetyleneuraminic acid showed very significant changes. Further, asymmetric dimethylarginine, dodecanediolcarnitine, and N-acetyleneuraminic acid showed significantly elevated levels in the pulpitis samples, while the levels of ascorbic acid, inosine, and allopurinol riboside were significantly reduced.

Further, Fig. 3 shows a volcano plot, which facilitates the visualization of FC and p-values and is also conducive for selecting DEMs. In this figure, the red and blue origins represent significantly upregulated and downregulated DEMs, respectively, whereas the gray origin represents non-DEMs. Samples from the same group appeared in the same cluster based on clustering, and metabolites within one cluster had similar expression profiles. These observations showed that the blank control and blood stasis model groups could be clustered into two categories, indicating that the screened metabolites were reasonable.

Hierarchical clustering was performed for the significant DEMs. Thus, 50 DEMs with the highest VIP values were used to obtain a heat map. As shown in Fig. 4, the x- and y-axes represent sample name and DEMs, respectively, while the blue-to-red color represents low-to-high abundances of the DEMs, i.e., a more intense red color represents a greater DEM level. Further, after verifying the reasonability and accuracy of the selected DEMs, it was observed that samples from an identical group appeared in the same cluster, and metabolites within one cluster had similar expression profiles. Our experimental analysis also showed that the blank control group and pulpitis group could cluster into two categories, further indicating that the screened metabolites were reasonable.

Furthermore, Pearson's correlation analysis was performed to determine the degree of linear correlation between any two metabolites. The top 50 most significantly changed metabolites with the highest VIP values were selected for visual analysis. From Fig. 5, which displays the correlations between the DEMs, it was evident that ascorbic acid and inosine were positively correlated with allopurinol riboside, asymmetric dimethylarginine, and dodecanediolcarnitine, while N-acetyleneuraminic acid and ascorbic acid showed negative and negative correlations with tryptophan, respectively. Further, inosine was found to show a negative relation with fexofenadine.

### Biomarker pathway analysis

The KEGG database was used for biomarker pathway enrichment analysis (Fig. 6). Thus, we observed that the biomarkers were predominantly associated with aminoacyl-tRNA biosynthesis, retrograde endocannabinoid signaling, glutamate, alanine, and aspartate metabolism, central carbon metabolism within malignancies, and protein digestion and absorption. Further, among these pathways, aminoacyl-tRNA biosynthesis and retrograde endocannabinoid signaling were found to be most significantly correlated with pulpitis.

### Discussion

Pulpitis is caused by plaque biofilm invasion of dental pulp tissue and pulp tissue inflammation. Notably, dental pulp tissue and other damaged tissues present defensive reactions in response to infection. Therefore, tissue metabolism in pulpitis differs significantly from that in normal dental pulp tissue. Additionally, even though irreversible pulp damage caused by pulpitis can be managed considerably via root canal therapy, it is of greater significance to pay attention to preventing pulpitis [23]. In this regard, advances in metabolomics can be applied to develop novel strategies for pulpitis prevention.

Specifically, metabolomics refers to the study of the dynamic metabolic changes in biological systems caused by biological stimuli, pathophysiological disturbances, or changes in genetic information. As the most downstream omics of systems biology, metabolomics represents a key branch of science that offers the possibility to comprehensively study functional alterations within living systems. Metabolomics can be used to account for complex biological systems given that it offers the possibility to use several common biological samples, including biological fluids (urine, serum, plasma, saliva, bile, tears, etc.), animal or human tissue (brain tumor tissue, adipose tissue, etc.), and cells [24, 25] for analysis. Further, the main analytical methods utilized in metabolomics include chromatography-mass spectrometry techniques, such as nuclear magnetic resonance (NMR), CE-MS, and LC-MS. Particularly, UPLC and atmospheric pressure ionization mass spectrometry (AP-MS), show good sensitivity, and owing to their high dynamic range and versatility, they have become the mainstream analysis methods in metabolomics studies. Thus, they can be used to analyze and evaluate target metabolites to the end of clarifying their metabolic pathways and functions, thereby laying a good foundation for the diagnosis and treatment of clinical disorders [26-29].

In this study, we conducted metabolomics to identify metabolites that are closely related to pulpitis to the end of clarifying the mechanism associated with the progression of the disease and developing strategies for its prevention. Specifically, we conducted untargeted...
metabolomics via UPLC-Orbitrap/MS on possible biomarkers within pulp tissues from individuals with pulpitis. Thus, we identified 22 biomarkers (13 and nine that were significantly upregulated and downregulated, respectively) that were associated with 18 major metabolic pathways. These major metabolites included ascorbic acid, inosine, allopurinol riboside, and L-formaldehyde. Further, among the identified pathways, aminoacyl-tRNA biosynthesis and retrograde endocannabinoid signaling were found to be closely associated with pulpitis.

Ascorbic acid, also known as vitamin C, represents one of the essential vitamins for normal physiological function in the human body. It also exhibits antitumor and antioxidation activities. Even though the human body is incapable of vitamin C synthesis, it can be obtained from natural sources, e.g., some food types contain vitamin C. Further, it is added during the development of antitumor drugs as it has a critical effect on suppressing tumor occurrence and progression via different mechanisms, such as reactive oxygen species (ROS) scavenging and the selective ROS generation, which enhance its toxicity to tumor cells [30]. Additionally, it protects the body against free radical oxidation and as an antioxidant, destroys cancer cells. It also participates in numerous physiological activities, such as collagen formation and tissue or fractured bone/tooth/cartilage repair [31], and owing to its antioxidation functions, it can be used to treat CVDs. It can also decrease monocyte adhesion onto endothelium, promote vasodilation, and endothelium-dependent nitric oxide generation, while attenuating vascular smooth muscle cell apoptosis, thereby avoiding unstable plaque formation in atherosclerosis [32]. In oral diseases, it can regulate the inflammatory processes associated with periodontitis, and reportedly, its deficiency impedes dentin generation based on the dentine/pulp complex in pathological/physiological situation [33]. However, there are no clear reports on the use of ascorbic acid in pulpitis. In study, the pulpitis group showed a dramatically lower ascorbic acid expression level compared with the control group. This observation suggested that ascorbic acid deficiency may accelerate pulp tissue necrosis and degeneration, thus accelerating pulpitis progression.

In this study, we also identified inosine as a major metabolite with a significantly lower expression level for the pulpitis group than the normal. Inosine represents the first base modification observed within nucleic acids and in 1965, it was identified as one part of the initially-sequenced transfer RNA (tRNA), tRNA Ala [34]. Further, its presence is indicative of the presence of purine nucleoside generated based on hypoxanthine, which serves as an important intermediate in purine biosynthesis and degradation pathways. It also plays a vital role in neuronal signal transduction. Further, it was first discovered in 1965 in yeast tRNA Ala, and presently, it is considered as an extensively distributed nucleic acid component that has different functions and activities. Among other biological effects, inosine exists within diverse RNAs that regulate transfection accuracy and rate [35]. It also represents the critical mediator of the purine biosynthetic pathway as well as the secondary metabolite generated from purine degradation. Given that its biochemical properties are similar to those of adenosine, molecular inosine has several physiological functions, e.g., in purine, it can act as neural protection during signal transduction involving purine analogs. Furthermore, inosine, located at a swing position in tRNA anticodon, significantly alters codon recognition, whereas within mRNA, it modifies post-translated polypeptide sequences or regulates transcript splicing, stability, and localization. It has also been detected within exogenous and non-coding RNA, and exerts significant effects on the structure and functioning of these RNA. It has also been observed that molecular inosine, which is the critical secondary metabolite obtained from purine metabolism, functions as a molecular messenger in cellular pathways [36]. In this study, the pulpitis group evidently showed decreased inosine levels relative to the control group. Thus, we predicted that the decreased inosine levels associated with pulpitis exacerbate pulp nerve necrosis and contribute to the progression of the disease. However, further studies on other metabolites with significant reductions in expression level in pulpitis are necessary.

Conclusions

In this study, our results indicated that metabolites with significantly altered levels in patients with pulpitis can not only be used as diagnostic markers of pulpitis, but may also be explored as potential drugs for the prevention of the disease. Specifically, based on the PLC-Orbitrap/MS system, we conducted a metabolomics study on pulpitis pulp samples to determine the key metabolites associated with the progression of pulpitis as well as the biological pathways that are associated with pulpitis progression. Thus, we provide a foundation for analyzing the relationship between metabolites and pulpitis incidence. These results could serve as a theoretical basis for pulpitis pathogenesis and preventive drug development.

Abbreviations

ultra-high-performance liquid chromatography (UPLC); low-molecular-weight (LMW); cardiovascular diseases (CVDs); liquid chromatography-mass spectrometry (LC-MS); differentially expressed metabolites (DEMs); quality control (QC); retention time (RT);

Declarations

Ethical Approval and Consent to participate
The study design was approved by the Ethical Committee of Shanghai Tongren Hospital, China. The registration number is 2022-031. Further, all participants provided informed consent prior to sample collection. All experiments were performed in accordance with the declaration of Helsinki.

Consent for publication

Not applicable.

Availability of supporting data

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

None.

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Authors' contributions

The authors made great equal contributions to the present study. KG was responsible for study conception, literature screening, animal experimental implementation, manuscript writing and revision. XDX was in charge of animal experimental implementation. JFG contributed to data extraction. YZ, YGZ, ZFZ and YW were in charge of data analysis. XZC, ZXZ and WJW contributed to study development, literature screening, manuscript writing and reviewing. The above authors read and approved the eventual version.

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Not Applicable

References


Figures

**Figure 1**

Score plot of PCA analysis. ▲, control; ■, pulpitis group

(A) Score plot of the PCA model in the positive mode; (B) Score plot of the PCA model in the negative mode.

**Figure 2**

(A) Score plot of the OPLS-DA model in the positive mode; (B) Score plot of the OPLS-DA model in the negative mode; (C) R² and Q² plots for 200 permutations of 1 component; (D) R² and Q² plots for 200 permutations of 1 component.
Score plot of the OPLS-DA model. ▲, control; ■, pulpitis group.

(A) Score plot of the OPLS-DA model in the positive mode; (B) Score plot of the OPLS-DA model in the negative mode; (C) Permutation test of the OPLS-DA model in the positive mode; (D) Permutation test of the OPLS-DA model in the negative mode.

Figure 3

Volcano plot. (A) Core plot of univariate statistical analysis results in the positive mode; (B) Score plot of univariate statistical analysis results in the negative mode. The red and blue origins represent significantly upregulated and downregulated metabolites in the experimental group, respectively. The gray point represents non-significant metabolites.
Figure 4

Heat map for differentially expressed metabolites: IP1-1-IP1-6, control groups; IP2-1-IP2-6, pulpitis group. The color from blue to red indicates the abundance of the expression of metabolites from low to high; i.e., a higher red color intensity indicates the higher expression level for the differential metabolites.
Figure 5

Diagram of correlation analysis involving differentially expressed metabolites. Red, positive; blue, negative.
Figure 6

Enrichment map of the top-20 metabolic pathways. The red line indicates a p-value of 0.01, while the blue line indicates a p-value of 0.05. When the top of the column is higher than the blue line, it implies that the signal pathway represented by the p-value is significant.