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Dynamic Metabolomics Study of the Bile Acid Pathway During Perioperative Primary Hepatic Carcinoma Following Liver Transplantation

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Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Background: There are many situations of abnormal metabolism influencing liver graft function. This study aims to provide data for the development of liver function recovery after liver transplantation by dynamically analyzing metabolites of bile acids pathway in serum.

Material/Methods: A comprehensive metabolomics profiling of serum of 9 liver transplantation patients before transplantation, on the 1st, 3rd, and 7th days after liver transplantation, and healthy individuals were performed by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Multivariate data and dynamic analysis were used to search for biomarkers between the metabolomics profiles present in perioperative liver transplantation and normal controls.

Results: Thirty-three differential endogenous metabolites were screened by the threshold of variable importance in the projection (VIP) from an orthogonal partial least square discriminant analysis (OPLS-DA) greater than 1.0, q-value <0.05, and fold change (FC) ≤0.8 or ≥1.2 between the preoperative group and the normal controls in negative mode. The metabolite intensities of taurocholic acid, taurochenodeoxycholic acid, chenodeoxycholic acid glycine conjugate, and glycocholic acid pre-transplantation were significantly higher than those of normal controls. The average metabolite intensities of taurocholic acid and taurochenodesoxycholic acid on the first day after liver transplantation were lower than those observed pre-transplantation. The average metabolite intensities on day 3 after liver transplantation showed a sudden increase and then decreased after 7 postoperative days. The average metabolite intensities of glycocholic acid and chenodeoxycholic acid glycine conjugate showed an increasing trend on the 1st, 3rd, and 7th days after liver transplantation.

Conclusions: Use of taurocholic acid and taurochenodeoxycholic acid-related bile secretion, liver regeneration, and *de novo* bile acid synthesis may help clinical evaluation and provide data for the development of liver function recovery after liver transplantation.

MeSH Keywords: **Liver Transplantation • Metabolomics • Perioperative Period**

Abbreviations: **UPLC-MS** – ultra-performance liquid chromatography – mass spectrometry; **VIP** – variable importance in the projection; **OPLS-DA** – orthogonal partial least square discriminant analysis; **FC** – fold change; **PHC** –primary hepatic carcinoma; **Pre** – preoperative patients; **P1** – 1-day postoperative patients; **P3** – 3-day postoperative patients; **P7** – 7-day postoperative patients; **HbsAg** – hepatitis B surface antigen; **NC** – normal controls; **AST** –aspartate aminotransferase; **ALT** – alanine aminotransferase; **UPLC** – ultra-performance liquid chromatography; **MS** – mass spectrometry; **KEGG** – Kyoto Encyclopedia of Genes and Genomes; **LC-MS** – liquid chromatograph mass spectrometer

Full-text PDF: https://www.annalsoftransplantation.com/abstract/index/idArt/921844

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Background

As a new research area built upon successive genomics and proteomics development, metabolomics studies the collection of all metabolites from cells, tissue, or organs [1]. A series of different chemical types of molecules contain peptides, carbohydrates, lipids, nucleic acids, and catabolic products of exogenous compounds [2]. Metabolomics often responds to the genetic, disease, and environmental impacts of the final stages in the body or "downstream" of genes and proteins. Unlike genetics and proteomics, the metabolomics reaction is phenotypic changes, revealing more functional changes. Some researchers have used metabolomics as potential biomarkers to investigate therapeutic responses [3,4].

Primary liver cancer (PHC) is one of the 5 most common cancers in the world, and it is also one of the most common malignant tumors in China [5,6]. Liver transplantation is the most effective treatment for PHC [7]. Preoperative and postoperative liver transplantation has different situations, which are assessed with many indexes [8]. Some studies have shown that bile acids can be used as a tool to assess the quality and function of the donor liver early after transplantation [9]. However, the relationship between the metabolites of the bile acid pathway and perioperative liver transplantation recipients has not been reported. Analysis of the metabolites of the bile acid pathway in perioperative liver transplant recipients will help improve our understanding of liver function recovery in patients after liver transplantation. This study was performed by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) combined with pattern recognition analysis multiplatform next-generation sequencing, which were used to analyze the amount of metabolites produced and the pathways involved [10].

Material and Methods

Patients

Serum samples were collected from 9 patients with primary hepatic carcinoma. The patients were recruited from the Nephrology Department at Guilin No. 181 Hospital (Guilin No. 181 Hospital officially changed its name to Guilin No. 924 Hospital), Guangxi, China. All of the patients had undergone transplantation surgery without recurrence of acute rejection after surgery, and without recurrence of hepatocellular cancer after liver transplantation. Serum samples were collected from patients at various time points: preoperative patients (Pre), 1-day postoperative patients (P1), 3-day postoperative patients (P3), and 7-day postoperative patients (P7). The transplanted livers were obtained from brain-dead donors. The donors did not have a history of liver disease, a medical history of cancer in the last 10 years, hepatitis B surface antigen (HBsAg), hepatitis C virus, or human immunodeficiency virus (HIV) antibodies. Fat infiltration (macrovesicular steatosis >40%), any fibrosis and atherosclerosis of the hepatic artery observed post-transplantation of the liver, and donors after cardiac death were also excluded [11]. Serum samples were also collected from 9 healthy individuals as normal controls (NC) from the Physical Center of the Guilin No. 181 Hospital. The clinical characteristics of patients and healthy individuals were extracted from the hospital database and are summarized in Table 1. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) <50 IU/L were considered normal; therefore, the liver function of patients gradually recovered to normal. All of the peripheral serum samples were obtained after receiving informed consent from the participating subjects. This study was performed according to the Hospital Ethics Committee, which abides by the Helsinki Declaration on ethical principles for medical research involving human subjects.

Serum collection and storage

Whole-blood samples were taken from a peripheral vein between 7: 00 and 9: 00 AM. Serum samples were collected from 9 patients and 9 healthy individuals between 2014 and 2015. The serum samples used for metabolite detection were collected from patients either in the Emergency Department or during physical examination. Every sample was centrifuged at 3500×g for 5 min, and 2 mL of the supernatant was transferred into clean tubes and frozen at -80°C until UPLC-MS/MS analysis. Each 250 µL serum sample was mixed with 750 µL of cold methanol and vortexed for 1 min. After centrifugation at 20 000×g for 20 min, 90 µL of supernatant was transferred into clean tubes and freeze-dried. The dried samples were reconstituted with 90 µL of 10% aqueous methanol.

UPLC-MS/MS analysis

The chromatographic separation was performed on a Waters Acquity™ 2777C ultra-performance liquid chromatography (UPLC) system (Waters, U.K.) and a SYNAPT G2 XS QTOF spectrometer (Waters, U.K.). The sample was loaded onto an Acquity UPLC HSS T3 (1.8 µm) (Waters, U.K.) column held at 30°C. The UPLC mobile phase consisted of water (solution A) and methanol (solution B), with a splitless flow rate of 0.4 mL/min and an injection volume of 10 µL. The following gradient elution program was used: 0–2 min, 100%–100% A; 2–12 min, 100% A-0% A; 12–14 min, 0% A-0% A; and 14–15 min, 0% A-100% A.

Mass spectrometry

A quadrupole time-of-flight (Q-TOF) instrument (Waters Corp., Milford, USA) was used to carry out the mass spectrometry with electrospray ionization (ESI), which had a capillary voltage of 1000 V in positive mode and 2000 V in negative mode and a

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Table 1. Characteristics of the patients and the normal controls.

Characteristic	NC	Patients			
		Pre	P1	P3	P7
Peripheral samples(n)	9	9	9	9	9
Age (years)	43±12	46±15	46±15	46±15	46±15
Sex: Male/Female (n/n)	8/1	8/1	8/1	8/1	8/1
ALT (IU/L)	23.0±8.0	72.0±16.0	286.0±52.0	212.0±43.0	84.0±31.0
AST (IU/L)	16.0±6.8	54.1±21.9	257.0 ±58.0	181.4±46.2	56.3±25.7
TBIL (μmol/L)	12.4±4.2	13.9±3.8	38.9±11.1	35.4±8.6	19.5±9.8

sampling cone voltage of 40 V in both modes. The desolvation temperature was set at 350°C, and the cone gas flow was set at 50 L h^{-1}. The source temperature was set at 120°C. Mass spectrometry (MS) data range was from 100 to 1200 m/z. The instrument performance and data quality were monitored by analyzing all samples with a rigorous quality assurance strategy [12].

Statistical analyses

All the UPLC-MS raw files were converted using MarkerLynx software (Version 4.1 Waters, U.K.) for the compound statistics principal component analysis (PCA), the orthogonal partial least square discriminant analysis (OPLS-DA) [13,14], and the correlation analysis. Variables with a threshold of variable importance in the projection (VIP) from the OPLS-DA greater than 1.0, a q-value <0.05, and fold change (FC) ≤0.8 or ≥1.2 were considered to be differential ions. The Q-value means that the p-value was assessed using a *t* test and corrected using the false discovery rate (FDR). Differential ions were identified using Progenesis QI (version 2.1). Progenesis QI was used to obtain the mass-to-charge ratio, the retention time, and the ion area of metabolites by extracting the peak.

Identification of biomarkers and metabolic pathway analysis

The accurate MS fragments of the metabolites were matched from the Human Metabolome Database (*http://www.hmdb.ca/*) for identifying the metabolites. The Kyoto Encyclopedia of Genes and Genomes (KEGG) (*http://www.genome.jp/kegg/pathway.html*) was used for pathway analysis, which visualizes the metabolomics.

Results

Multivariate statistical analysis of the metabolite profiles

The PCA score map was used for multivariate data analysis to observe the clustering between perioperative liver transplant patients and the control groups (Figure 1A, 1B), which suggested a clear separation between the perioperative liver transplantation patients and healthy individuals, with a few overlaps. The OPLS-DA score plot of preoperative patients and healthy controls showed remarkable group separation, with no sample overlapping for negative and positive patients (Figure 2A, 2B). When the R^2 and Q^2 values of the model parameters are higher, the OPLS-DA model is more reliable. A more rigorous model validation procedure was applied by class permutation testing, and the results strongly confirmed model consistency and reliability. The model parameters were permutation tested 1000 times. The p-value of Pre *vs.* NC was 0.012 for the model of positive ions and 0.003 for the model of negative ions; $p<0.01$ indicates the difference between the 2 groups was extremely significantly. Therefore, we chose negative ions of Pre *vs.* NC to select differential makers. Metabolite identification was subsequently performed to yield 33 annotated endogenous metabolites; detailed information is depicted in Table 2. The 4 ions belong to the bile acid pathway among the metabolites.

Changes in the metabolites of the bile acid pathway

Four differential metabolites from the Pre patient group were distinguished from those of the controls (VIP from the OPLS-DA greater than 1.0, q-value <0.05 and fold change ≤0.8 or ≥1.2). Overall, these metabolite intensities displayed dynamic changes between control and perioperative liver transplantation samples, including taurocholic acid (Figure 3A), taurochenodeoxycholic acid (Figure 3B), chenodeoxycholic acid glycine conjugate (Figure 3C), and glycocholic acid (Figure 3D). The 4 types of metabolites had varying degrees of up- and down-regulation before and after transplantation, which indicates that the metabolites of the bile acid pathway play an important role in perioperative liver transplantation. The results showed that the metabolite intensities of taurocholic acid (VIP=3.7255, FC=121.63, q=0.0250), taurochenodeoxycholic acid (VIP=2.9581, FC=33.078, q=0.0442), chenodeoxycholic acid glycine conjugate (VIP=2.5999, FC=6.2715, q=0.0106), and glycocholic acid (VIP=2.9258, FC=9.1140 q=0.0151) were significantly higher in pre-transplantation patients than in

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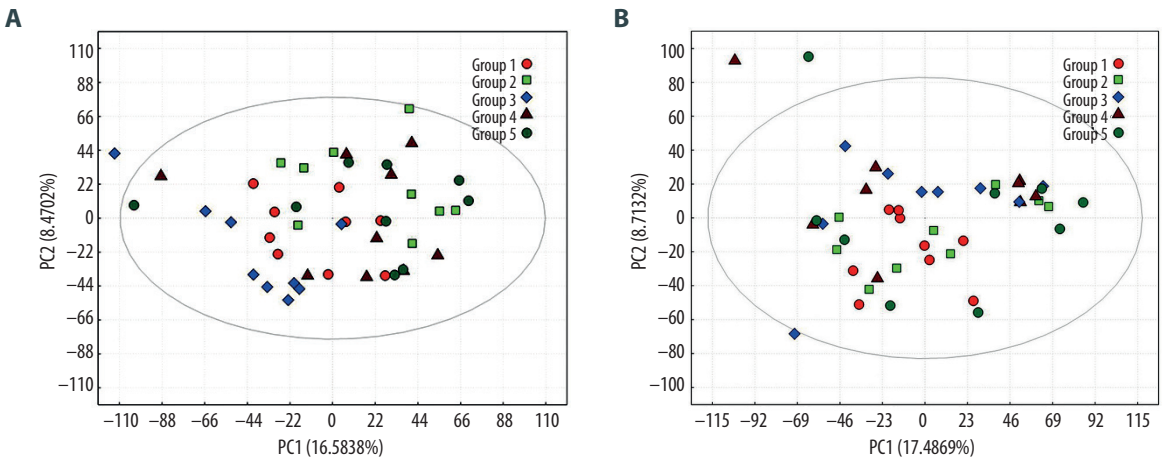

Figure 1. PCA scores for metabolic pattern to visualize group clustering between perioperative liver transplantation and NC samples. PCA score plots of serum samples collected from NC (group 1), Pre (group 2), P1 (group 3), P3 (group 4), and P7 (group 5) groups in negative ion mode (**A**) and positive ion mode (**B**). The sample clusters of negative ion mode were tighter than those of positive ion mode, and no extreme outliers were observed.

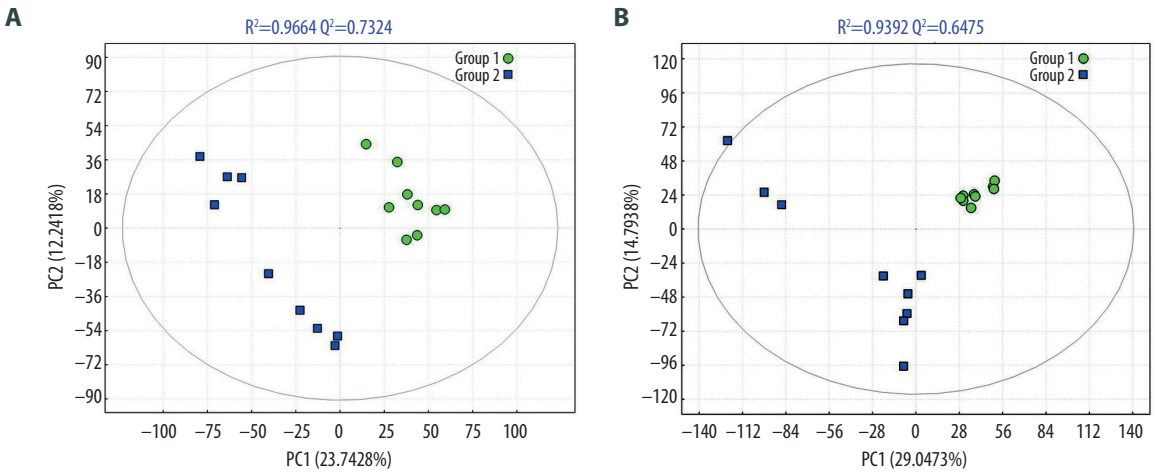

Figure 2. The PLS-DA models of NC (group 1) and Pre (group 2) in negative ion mode (**A**) and positive ion mode (**B**). The PLS-DA score plot for the negative ion mode has a clearer separation than that for the positive ion mode between Pre and NC samples.

normal controls. The average metabolite intensities of taurocholic acid, taurochenodeoxycholic acid, and chenodeoxycholic acid glycine conjugate at the first day after liver transplantation were lower than those detected pre-transplantation. The average metabolite intensities of taurocholic acid and taurochenodeoxycholic acid on day 3 after liver transplantation had a sudden increase and then decreased after 7 postoperative days. The average metabolite intensity on day 7 after liver transplantation tended to approach NC. The average metabolite intensities of chenodeoxycholic acid glycine conjugate and glycocholic acid showed an increasing trend on the 1st, 3rd, and 7th days after liver transplantation. The average ionic intensities of glycocholic acid on postoperative day 7 were significantly higher than those of NC (VIP=2.9072, FC=27.001, q=0.0384). The results of the differential comparison between groups are listed in Table 3.

Metabolic network visualization for bile acid pathway

Figure 4A shows the significantly enriched metabolites of pathways for primary and secondary bile acid biosynthesis in the perioperative liver transplantation samples. Among these metabolites, taurocholic acid, taurochenodeoxycholic acid, chenodeoxycholic acid glycine conjugate, and glycocholic acid were involved in both primary and secondary bile acid biosynthesis. Primary and secondary bile acid biosynthesis were

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Table 2. Potential metabolite makers of negative ions from Pre *vs.* NC.

Metabolite name	m/z	Change	VIP	Fold change	q-value	Related pathway
Threonic acid	135.0296	↑	2.920519	6.722945	0.002249	Ascorbate and aldarate metabolism
Threonic acid	135.0299	↑	3.101811	8.680666	0.002504	Ascorbate and aldarate metabolism
Phosphoribosyl formamidocarboxamide	365.0538	↑	1.429341	1.667861	0.015919	Purine metabolism; Metabolic pathways; Biosynthesis of secondary metabolites
8(R)-Hydroperoxylinoleic acid	311.2199	↑	1.985069	3.354787	0.049389	Linoleic acid metabolism
13-L-Hydroperoxylinoleic acid	311.2199	↑	1.985069	3.354787	0.049389	Linoleic acid metabolism; Metabolic pathways
9(S)-HPODE	311.2199	↑	1.985069	3.354787	0.049389	Linoleic acid metabolism; Metabolic pathways
Taurocholic acid	514.2828	↑	3.725514	121.6349	0.025012	Primary bile acid biosynthesis; Secondary bile acid biosynthesis; Taurine and hypotaurine metabolism; Metabolic pathways; Bile secretion
8-HETE	319.2261	↓	3.539139	0.104499	0.007557	Arachidonic acid metabolism; Metabolic pathways; PPAR signaling pathway
8,9-Epoxyeicosatrienoic acid	319.2261	↓	3.539139	0.104499	0.007557	Arachidonic acid metabolism; Metabolic pathways; Vascular smooth muscle contraction
19(S)-HETE	319.2261	↓	3.539139	0.104499	0.007557	Arachidonic acid metabolism; Metabolic pathways
11(R)-HETE	319.2261	↓	3.539139	0.104499	0.007557	Arachidonic acid metabolism
15(S)-HETE	319.2261	↓	3.539139	0.104499	0.007557	Arachidonic acid metabolism
20-Hydroxyeicosatetraenoic acid	319.2261	↓	3.539139	0.104499	0.007557	Arachidonic acid metabolism; Metabolic pathways; Vascular smooth muscle contraction
5,6-Epoxy-8,11,14-eicosatrienoic acid	319.2261	↓	3.539139	0.104499	0.007557	Arachidonic acid metabolism; Metabolic pathways; Vascular smooth muscle contraction
16(R)-HETE	319.2261	↓	3.539139	0.104499	0.007557	Arachidonic acid metabolism
14,15-Epoxy-5,8,11-eicosatrienoic acid	319.2261	↓	3.539139	0.104499	0.007557	Arachidonic acid metabolism; Metabolic pathways; Vascular smooth muscle contraction
11,12-Epoxyeicosatrienoic acid	319.2261	↓	3.539139	0.104499	0.007557	Arachidonic acid metabolism; Metabolic pathways; Vascular smooth muscle contraction
5-HETE	319.2261	↓	3.539139	0.104499	0.007557	Arachidonic acid metabolism
Docosapentaenoic acid	329.2469	↓	1.976434	0.413162	0.008974	Biosynthesis of unsaturated fatty acids
Arachidonic acid	303.2320	↓	2.263972	0.331697	0.000831	Arachidonic acid metabolism; Linoleic acid metabolism; Biosynthesis of unsaturated fatty acids; Metabolic pathways; Vascular smooth muscle contraction; Fc epsilon RI signaling pathway; Fc gamma R-mediated phagocytosis; Long-term depression; Phototransduction – fly; GnRH signaling pathway; Leishmaniasis; Amoebiasis
Taurochenodeoxycholic acid	498.2879	↑	2.958113	33.07833	0.044239	Primary bile acid biosynthesis; Secondary bile acid biosynthesis; Bile secretion
9,10-Epoxyoctadecenoic acid	295.2257	↓	1.591986	0.55364	0.02713	Linoleic acid metabolism; Metabolic pathways
Alpha-dimorphecolic acid	295.2257	↓	1.591986	0.55364	0.02713	Linoleic acid metabolism; PPAR signaling pathway

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Table 2 continued. Potential metabolite makers of negative ions from Pre *vs.* NC.

Metabolite name	m/z	Change	VIP	Fold change	q-value	Related pathway
12,13-EpOME	295.2257	↓	1.591986	0.55364	0.02713	Linoleic acid metabolism; Metabolic pathways
13S-hydroxyoctadecadienoic acid	295.2257	↓	1.591986	0.55364	0.02713	Linoleic acid metabolism; PPAR signaling pathway
Chenodeoxycholic acid glycine conjugate	448.3056	↑	2.59988	6.271501	0.010639	Primary bile acid biosynthesis; Secondary bile acid biosynthesis; Bile secretion
Glycocholic acid	464.3005	↑	2.925803	9.113965	0.015133	Primary bile acid biosynthesis; Secondary bile acid biosynthesis; Metabolic pathways; Bile secretion
9,12,13-TriHOME	329.2304	↑	2.3531	3.288932	0.006036	Linoleic acid metabolism
9,10,13-TriHOME	329.2304	↑	2.3531	3.288932	0.006036	Linoleic acid metabolism
Adipate semialdehyde	129.0544	↓	1.13189	0.720488	0.025688	Caprolactam degradation; Metabolic pathways; Microbial metabolism in diverse environments
Ketoleucine	129.0544	↓	1.13189	0.720488	0.025688	Valine, leucine and isoleucine degradation; Valine, leucine and isoleucine biosynthesis; Glucosinolate biosynthesis; Metabolic pathways; Biosynthesis of secondary metabolites
Phosphoribosyl formamidocarboxamide	401.0251	↑	1.649845	1.751775	0.003328	Purine metabolism; Metabolic pathways; Biosynthesis of secondary metabolites
Trans-Cinnamic acid	147.0438	↑	1.2355	1.388167	0.001396	Phenylalanine metabolism; Phenylpropanoid biosynthesis; Metabolic pathways; Biosynthesis of secondary metabolites; Microbial metabolism in diverse environments

The markers were chosen on the basis of VIP from the OPLS-DA greater than 1.0, q-value <0.05, and fold change ≤0.8 or ≥1.2. "↑" and "↓" represent the compound is up- and down-regulated, respectively, in preoperative patients compared with the normal control.

Table 3. The differential comparison for ionic strength of metabolites.

Groups	Taurocholic acid			Taurochenodeoxycholic acid			Chenodeoxycholic acid glycine conjugate			Glycocholic acid		
	VIP	Fold change	q-value	VIP	fold change	q-value	VIP	fold change	q-value	VIP	fold change	q-value
Pre *vs.* NC	3.7255*	121.63*	0.0250*	2.9581*	33.078*	0.0442*	2.5999*	6.2715*	0.0106*	2.9258*	9.1140*	0.0151*
P1 *vs.* Pre	0.1995	0.8706	0.58367	0.9535	0.6365	0.3168	1.4928	0.6484	0.1004	0.9049	1.1897	0.3272
P3 *vs.* P1	1.0553	2.1777	0.5727	0.3641	1.8009	0.7085	0.5288	1.1475	0.6667	1.7125	1.8460	0.3533
P7 *vs.* P3	0.6284	0.3481	0.9997	0.1090	0.32701	0.9997	0.7702	1.1803	0.9997	0.0039	1.3490	0.9999
P7 *vs.* NC	3.7079*	80.275*	0.0243*	1.9267	12.399	0.1930	1.2458	5.5076	0.2866	2.9072*	27.001*	0.0384*

* VIP from the OPLS-DA greater than 1.0, q-value <0.05 and fold change ≤0.8 or ≥1.2 indicates the difference between the 2 groups was significantly.

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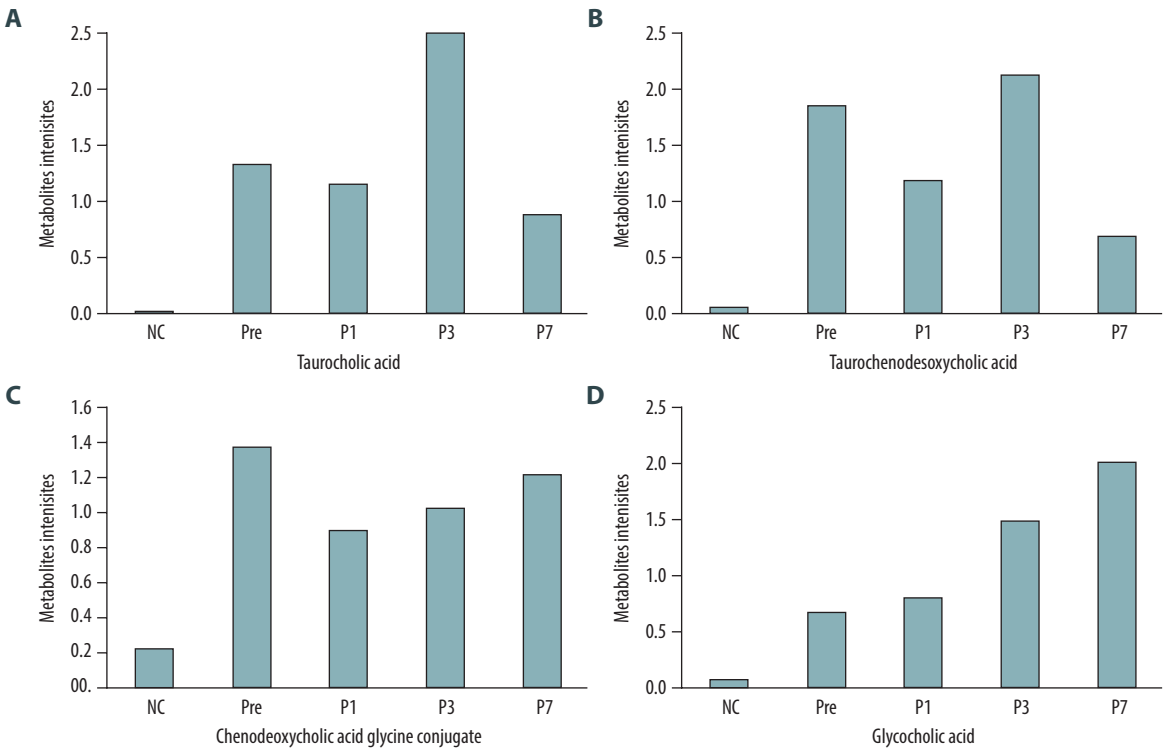

Figure 3. (**A–D**) Intensities of the metabolites of the bile acid pathway in the control and perioperative liver transplantation groups.

involved in bile secretion (Figure 4B). Finally, bile acids were produced through synthetic circulation. Taurocholic acid, taurochenodeoxycholic acid, chenodeoxycholic acid glycine conjugate, and glycocholic acid are metabolites of circulation. The results showed that these metabolites circulate through bile acid pathways by active transport in the liver, and recovered bile acids are secreted through bile [15,16].

Discussion

Endogenous metabolites are involved in human metabolite pathways and are associated with the development of some diseases [17]. It is important that some methods detect the metabolites of these pathways to find diagnostic biomarkers. Although many studies have reported metabolite biomarkers for liver disease and liver transplantation [10,18–22], which also included bile acids [23], the dynamic analysis value of the metabolites of the bile acid pathway for monitoring perioperative liver transplantation remains largely unknown.

Liquid chromatograph mass spectrometer (LC-MS)-based metabolomics, which process large datasets and classifies sample groups as well as the nature of these metabolites, could be an advanced tool to find metabolites [10,24,25]. In this study, we used UPLC-MS/MS to conduct differential metabolite profiling between Pre and NC samples. The PCA revealed a significant separation between the perioperative liver transplantation and control samples. According to the PLS-DA model based on the R^2 and Q^2 values, we chose negative ions of Pre *vs.* NC to select 33 differentially expressed endogenous metabolites, which were chosen according to VIP from OPLS-DA greater than 1.0, q-value <0.05, and fold change ≤0.8 or ≥1.2. Patients who usually undergo liver transplantation for primary liver cancer will be observed for whether they have transplant rejection after surgery, whether hepatocarcinoma recurs, and whether liver function is restored. In this study, we found that patients had no transplant rejection and recurrence of hepatocellular carcinoma after surgery; therefore, we chose to discuss the liver function with dynamic changes after surgery. The bile acids in transplant recipients provide information on the pattern of recovery of bile secretion immediately after graft reperfusion and may be early indicators of the development of primary graft dysfunction [18]. We assume that metabolites of the bile acid pathway could help to further potentiate the role of bile acid analysis as a tool to assess graft function during perioperative liver transplantation. Therefore, we chose 4 out of 33 metabolites in the bile acid pathway to dynamically analyze the metabolic level between NC and perioperative liver transplantation. The KEGG analysis for visualizing the metabolic network of the bile acid pathway showed that UPLC-MS/MS is reliable for detecting metabolites of the bile acid pathway.

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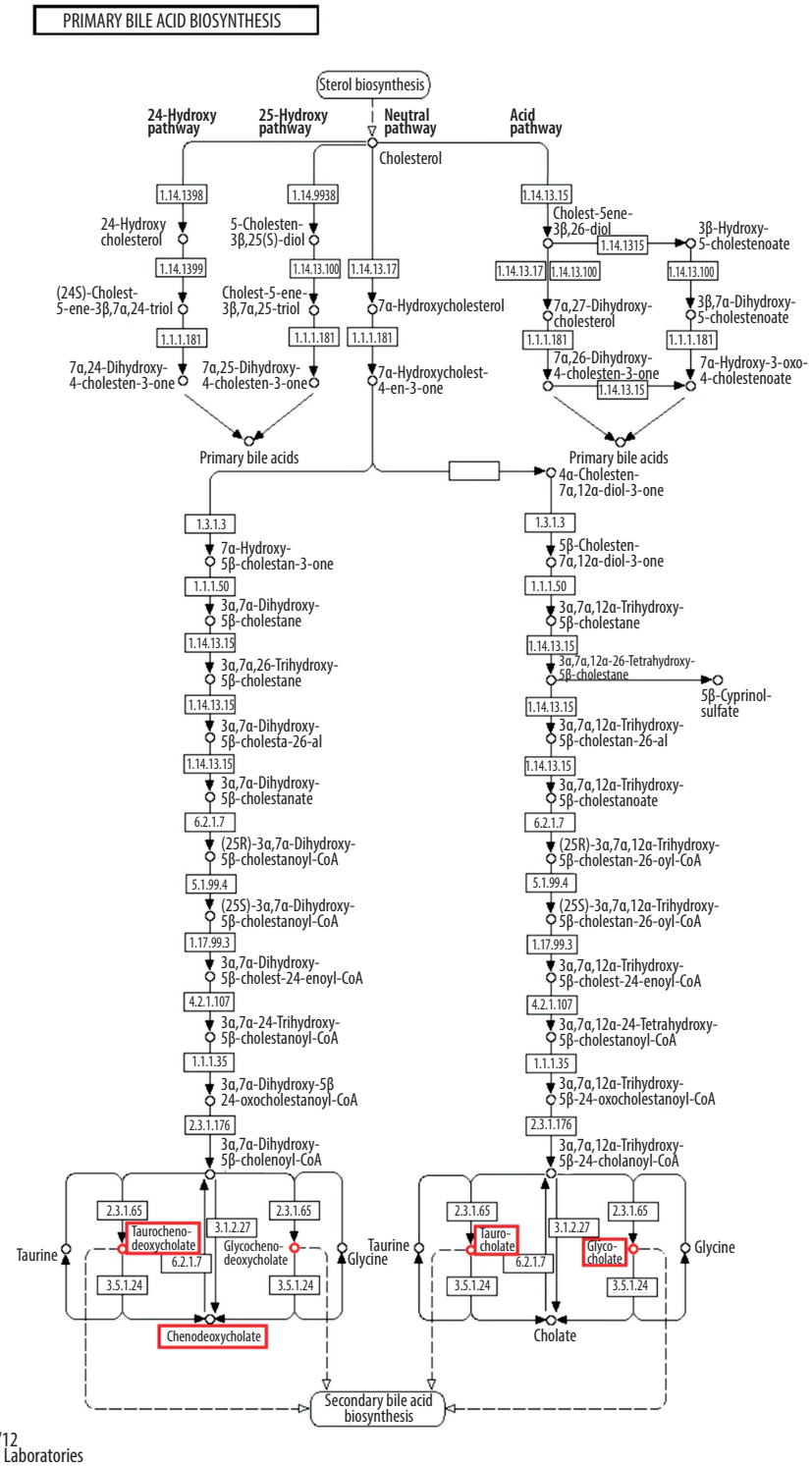
A


PRIMARY BILE ACID BIOSYNTHESIS
Sterol biosynthesis
24-Hydroxy pathway
25-Hydroxy pathway
Neutral pathway
Acid pathway
Cholesterol
1.14.1398
1.14.9938
1.14.13.15
24-Hydroxy cholesterol
5-Cholesten-3β,25(S)-diol
Cholest-5ene-3β,26-diol
1.14.1315
3β-Hydroxy-5-cholestenoate
1.14.1399
1.14.13.100
1.14.13.17
1.14.13.17
1.14.13.100
1.14.13.100
(24S)-Cholest-5-ene-3β,7α,24-triol
Cholest-5-ene-3β,7α,25-triol
7α-Hydroxycholesterol
7α,27-Dihydroxy-cholesterol
3β,7α-Dihydroxy-5-cholestenoate
1.1.1.181
1.1.1.181
1.1.1.181
1.1.1.181
1.1.1.181
7α,24-Dihydroxy-4-cholesten-3-one
7α,25-Dihydroxy-4-cholesten-3-one
7α-Hydroxycholest-4-en-3-one
7α,26-Dihydroxy-4-cholesten-3-one
1.14.13.15
7α-Hydroxy-3-oxo-4-cholestenoate
Primary bile acids
Primary bile acids
4α-Cholesten-7α,12α-diol-3-one
1.3.1.3
1.3.1.3
7α-Hydroxy-5β-cholestan-3-one
5β-Cholesten-7α,12α-diol-3-one
1.1.1.50
1.1.1.50
3α,7α-Dihydroxy-5β-cholestane
3α,7α,12α-Trihydroxy-5β-cholestane
1.14.13.15
1.14.13.15
3α,7α,26-Trihydroxy-5β-cholestane
3α,7α,12α-26-Tetrahydroxy-5β-cholestane
5β-Cyprinol-sulfate
1.14.13.15
1.14.13.15
3α,7α-Dihydroxy-5β-cholesta-26-al
3α,7α,12α-Trihydroxy-5β-cholestan-26-al
1.14.13.15
1.14.13.15
3α,7α-Dihydroxy-5β-cholestanate
3α,7α,12α-Trihydroxy-5β-cholestanoate
6.2.1.7
6.2.1.7
(25R)-3α,7α-Dihydroxy-5β-cholestanoyl-CoA
(25R)-3α,7α,12α-Trihydroxy-5β-cholestan-26-oyl-CoA
5.1.99.4
5.1.99.4
(25S)-3α,7α-Dihydroxy-5β-cholestanoyl-CoA
(25S)-3α,7α,12α-Trihydroxy-5β-cholestan-26-oyl-CoA
1.17.99.3
1.17.99.3
3α,7α-Dihydroxy-5β-cholest-24-enoyl-CoA
3α,7α,12α-Trihydroxy-5β-cholest-24-enoyl-CoA
4.2.1.107
4.2.1.107
3α,7α-24-Trihydroxy-5β-cholestanoyl-CoA
3α,7α,12α-24-Tetrahydroxy-5β-cholestanoyl-CoA
1.1.1.35
1.1.1.35
3α,7α-Dihydroxy-5β 24-oxocholestanoyl-CoA
3α,7α,12α-Trihydroxy-5β-24-oxocholestanoyl-CoA
2.3.1.176
2.3.1.176
3α,7α-Dihydroxy-5β-cholenoyl-CoA
3α,7α,12α-Trihydroxy-5β-24-cholanoyl-CoA
2.3.1.65
2.3.1.65
2.3.1.65
2.3.1.65
Taurocheno-deoxycholate
3.1.2.27
Glycocheno-deoxycholate
Tauro-cholate
3.1.2.27
Glyco-cholate
Taurine
6.2.1.7
Glycine
Taurine
6.2.1.7
Glycine
3.5.1.24
3.5.1.24
3.5.1.24
3.5.1.24
Chenodeoxycholate
Cholate
Secondary bile acid biosynthesis
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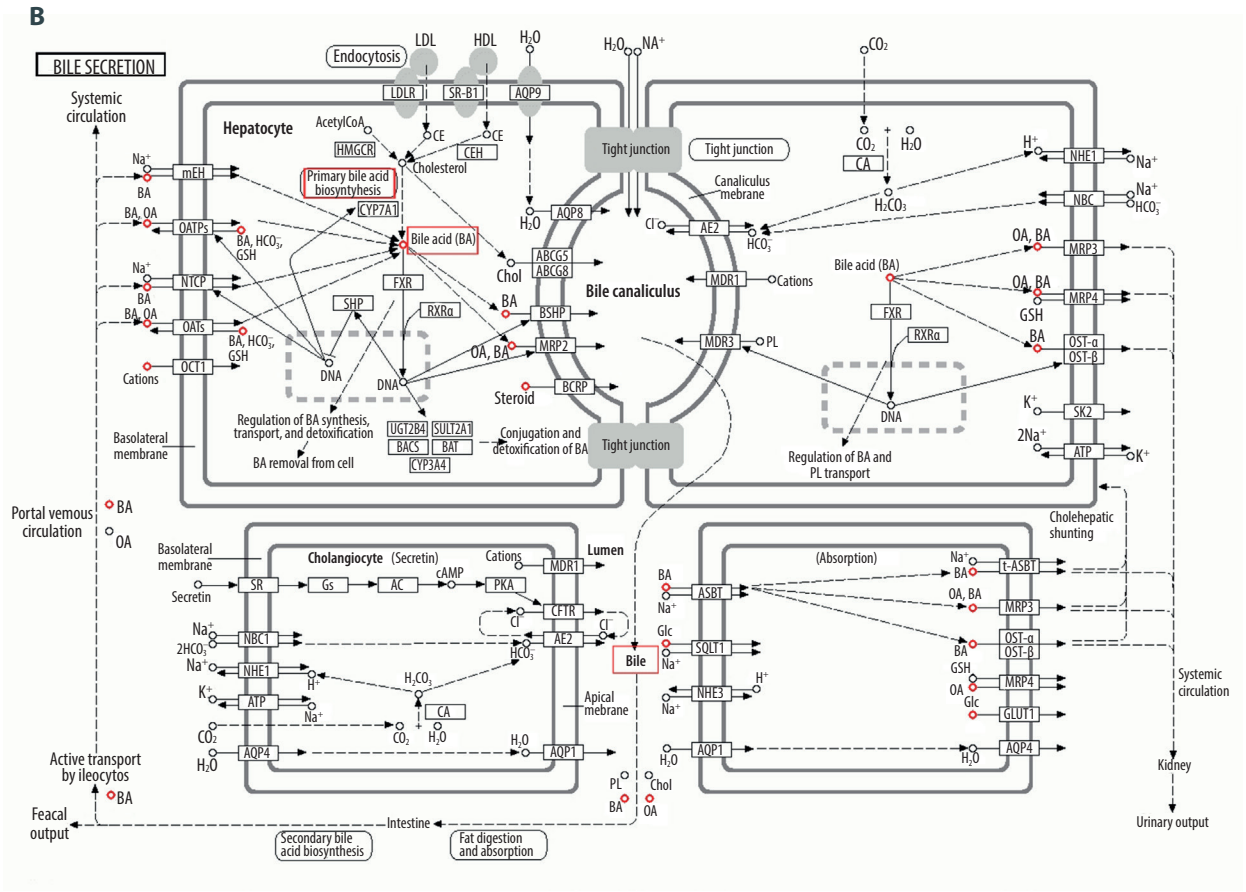

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Figure 4. KEGG pathway analysis: summary of the metabolites in the bile acid pathway shown in the red box and described in this article. (**A**) The primary and secondary bile acid biosynthesis pathways. (**B**) The bile secretion pathway.

Previous reports have suggested that the delayed recovery of hepatic bile secretion observed in donor livers may hamper graft bile flow recovery in recipients, which is an early sign of proper liver function [9]. A recent study also suggested that taurocholic acid and taurochenodeoxycholic acid are significantly increased after transplantation and that this effect might be linked to liver regeneration and *de novo* bile acid synthesis [26]. In the present study, we also found that taurocholic acid and taurochenodeoxycholic acid were increased on postoperative day 3 compared with postoperative day 1 and showed a decrease on postoperative day 7. This result showed that increased taurocholic acid and taurochenodeoxycholic acid were involved in liver regeneration and *de novo* bile acid biosynthesis. The metabolic levels after 7 postoperative days were gradually becoming closer to those of NC, which may suggest that grafts recover normal function by normal bile secretion through the bile acid pathway. KEGG pathway analysis also showed that taurocholic acid and taurochenodeoxycholic acid are involved in the biosynthesis of primary bile acid biosynthesis in the bile acid pathway. Although chenodeoxycholic acid glycine conjugate and glycocholic acid are also involved in the biosynthesis of primary bile acid biosynthesis in the bile acid pathway, their expression levels are not similar to those of taurocholic acid and taurochenodeoxycholic acid, which first increased and then stabilized. This effect may be because the number of specimens was small, which could introduce errors into the experimental results. There may also be other alternative pathways that affect chenodeoxycholic acid glycine conjugate and glycocholic acid expression. Therefore, we will consider lengthening the follow-up time and increasing the collection of specimens in future related studies. We also plan to study the regulatory networks of metabolomics, gene expression, and proteins in perioperative liver transplantation.

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Conclusions

Taurocholic acid and taurochenodeoxycholic acid may be important metabolites to use in evaluating liver function recovery after liver transplantation. Our research provides a new concept for studying dynamic changes in the metabolites of metabolic pathways to identify biomarkers during perioperative liver transplantation.

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