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Bile acid profiles and classification model accuracy for inflammatory bowel disease diagnosis

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Abstract

To investigate the utility of serum bile acid profiling for the diagnosis of inflammatory bowel disease (IBD). We analyzed 15 specific bile acids in the serum of 269 IBD patients, 200 healthy controls (HC), and 174 patients with other intestinal diseases (OID) using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Serum bile acid levels were compared between IBD group, HC group, and OID group. Binary logistic regression-based models were developed to model the bile acids and diagnose IBD. Furthermore, receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic accuracy of each bile acid and the model. Compared to HC group, IBD group exhibited significantly lower levels of chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TDCA), lithocholic acid (LCA), glycolithocholic acid (GLCA), taurolithocholic acid (TLCA), and an elevated primary-to-secondary bile acid ratio. DCA had an area under the curve (AUC) of 0.860 for diagnosing IBD, with a sensitivity of 80.67% and a specificity of 82.50%. A model Y_0 combining DCA and CDCA to distinguish between IBD group and HC group further improved accuracy (AUC = 0.866, sensitivity = 76.28%, specificity = 89.37%). Compared to non-IBD group (which combined healthy controls and those with other intestinal diseases), IBD group had significantly lower levels of DCA, GDCA, TDCA, LCA, GLCA, and TLCA, and elevated levels of glycocholic acid (GCA) and glycochenodeoxycholic acid (GCDCA). A model Y_1 incorporating GCDCA, DCA and TLCA to distinguish between IBD group and non-IBD group yielded an AUC of 0.792, with a sensitivity of 77.67% and specificity of 71.91%. IBD patients exhibit decreased serum secondary bile acid levels and an elevated primary-to-secondary bile acid ratio. Serum bile acid alterations are associated with the onset of IBD. A model consisting of CDCA and DCA has potential for distinguishing between IBD group and HC group, while a model incorporating GCDCA, DCA and TLCA may be suitable for distinguishing between IBD group and non-IBD group.

Abbreviations: AUC = area under the curve, CA = cholic acid, CD = Crohn disease, CDCA = chenodeoxycholic acid, DCA = deoxycholic acid, GCA = glycocholic acid, GCDCA = glycochenodeoxycholic acid, GDCA = glycodeoxycholic acid, GLCA = glycolithocholic acid, GUDCA = glycoursodeoxycholic acid, HC = healthy controls, IBD = inflammatory bowel disease, LCA = lithocholic acid, LC-MS/MS = liquid chromatography-tandem mass spectrometry, OID = other intestinal diseases, ROC = receiver operating characteristic, TCA = taurocholic acid, TCDCA = taurochenodeoxycholic acid, TDCA = taurodeoxycholic acid, TLCA = taurolithocholic acid, TUDCA = tauroursodeoxycholic acid, UC = Ulcerative colitis, UDCA = ursodeoxycholic acid.

Keywords: bile acid profile, classification model, inflammatory bowel disease

1. Introduction

Inflammatory bowel disease (IBD) is a chronic condition with a complex etiology involving genetics, environment, immune dysfunction, and gut microbiota alterations.[1] While its incidence in China is rising.[2,3] IBD diagnosis currently lacks a gold standard, relying on a combination of clinical evaluation, endoscopy, histology, and imaging.[4,5] The absence of reliable

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The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

This study received approval from the Ethics Committee of Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine and adhered to the principles outlined in the Declaration of Helsinki. Prior to their inclusion in the study, all patients provided informed consent.

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serological markers necessitates the identification of new diagnostic tools.

Recent research suggests that IBD pathogenesis begins with intestinal mucosal dysfunction.[6] Disruption of the epithelial barrier, immune cell stimulation by gut microbes, and dysbiosis contribute to mucosal injury.[1,7,8] Moreover, IBD patients exhibit a less diverse and more unstable gut microbiome compared to healthy individuals, highlighting its potential role in disease development.[9] Given their role as key gut microbiota metabolites, bile acids may be intricately linked to IBD.

Bile acid profiling, encompassing a group of structurally similar 24-carbon molecules, was traditionally challenging due to limitations in separation and detection. Advancements in liquid chromatography tandem mass spectrometry (LC-MS/MS) have overcome these hurdles, enabling separation and quantification based on molecular weight, structure, and polarity. This technological leap opens new avenues for exploring individual bile acids in disease pathogenesis and progression.

In this study, we utilized LC-MS/MS to analyze the serum bile acid profiles of 269 IBD patients, 200 healthy controls, and 174 patients with other intestinal diseases. The analyzed bile acids include cholic acid (CA), glycocholic acid (GCA), taurocholic acid (TCA), chenodeoxycholic acid (CDCA), glycochenodeoxycholic acid (GCDCA), taurochenodeoxycholic acid (TCDCA), deoxycholic acid (DCA), glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TDCA), lithocholic acid (LCA), glycolithocholic acid (GLCA), taurolithocholic acid (TLCA), ursodeoxycholic acid (UDCA), glycoursodeoxycholic acid (GUDCA), and tauroursodeoxycholic acid (TUDCA). Our objective is to elucidate the correlation between bile acid profiles and IBD, as well as, explore their potential diagnostic value.

2. Materials and methods

2.1. Population under study and inclusion criteria

A total of 269 patients diagnosed with IBD and treated at Renji Hospital affiliated to Shanghai Jiao Tong University School of Medicine, between June 2018 and January 2022, were included in the IBD group. The study also incorporated 174 patients with other intestinal diseases (OID), such as colorectal cancer, colorectal polyps, and acute enteritis, constituting the OID group. Additionally, 200 healthy volunteers who underwent health checkups at the same hospital from December 2020 to March 2021 were selected as the healthy control (HC) group. Inclusion criteria for the IBD group were based on clinical symptoms, radiology, endoscopy, and histopathological diagnosis standards.[4,5] Patients with abnormal liver function, concurrent autoimmune liver diseases, gallstones, or a history of intestinal resection were excluded. OID group comprised patients clinically diagnosed to exclude IBD and without other significant diseases. The healthy volunteers were recruited from people that had their medical checkup in our center. After excluding IBD and other gastrointestinal diseases through colonoscopy, as well as excluding malignant tumors, inflammation, and cardiovascular diseases through thoracic CT scans, electrocardiograms and abdominal ultrasounds, they were defined as healthy. No statistically significant differences in age and gender were observed among the groups ($P > .05$). This study was reviewed and approved by the Ethics Committee of Renji Hospital affiliated to Shanghai Jiao Tong University School of Medicine.

2.2. Method

2.2.1. Sample preprocessing. Four milliliters of fasting whole blood were collected from each group using a serum separator tube (Guangdong, China). The samples were centrifuged at 2685 × g for 10 minutes to separate the serum. We utilized approximately 0.8 mL of serum for testing various parameters, strictly following the manufacturer instructions throughout the testing process.

2.2.2. Instruments and reagents. The bile acid profile was detected using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with the API3200MD triple quadrupole mass spectrometer (ABSciex, USA) and Shimadzu series liquid chromatograph (Shimadzu, Japan). The reagent kits were purchased from Shanghai ClinMeta Co., Ltd. The assay comprised 15 components: CA, GCA, TCA, CDCA, GCDCA, TCDCA, DCA, GDCA, TDCA, LCA, GLCA, TLCA, UDCA, GUDCA, TUDCA. The reagent kit was equipped with 12 isotope internal standards. Among them, due to isomer variant, DCA and CDCA share the same internal standard DCA-d4, GDCA and GCDCA share the same internal standard GDCA-d4, and TDCA and TCDCA share the same internal standard TDCA-d4. The remaining analytes are each equipped with a separate internal standard.

2.2.3. Bile acid metabolic spectrum analysis pre-sample processing. A 100 µL serum sample was combined with 500 µL extraction liquid containing internal standards, vortexed (2500 rpm, 5 minutes), and subsequently centrifuged (13,000 rpm, 10 minutes). The supernatant (400 µL) was transferred to a 96-well plate and dried under nitrogen gas at 60°C. The dried sample was reconstituted in 100 µL of reconstitution solution and mixed on a thermostatic shaker for 10 minutes at 700 rpm. Subsequently, the reconstituted solution was transferred to a dedicated filter plate positioned over a new 96-well plate. Both components were centrifuged together in a multi-tube rack automatic balance centrifuge (4000 rpm, 1 minute) to collect the filtrate for sample injection. Chromatographic conditions utilized an XbridgeC18 column with water as mobile phase A and methanol as mobile phase B, employing gradient elution. The total analysis time was 13 minutes, with a flow rate of 0.5 mL/min and an injection volume of 8 µL. Mass spectrometry conditions involved an electrospray ionization source, negative ion scanning, nebulizer pressure set at 40 psi, auxiliary heater pressure at 60 psi, curtain gas pressure at 20 psi, collision gas pressure at 6 psi, ion source voltage of −4500 V, and an ion source temperature of 600°C.

2.3. Statistical analysis

Statistical analysis was conducted using SPSS 22.0 and GraphPad 6.0 software. Normality of distribution was assessed with the Kolmogorov-Smirnov test. Non-normally distributed quantitative data were presented as median (Q1, Q3). Kruskal Wallis test was used for comparison between multiple groups, and Mann–Whitney *U* test was used for comparison between 2 groups. *P* value <.05 was considered statistically significant.

The bile acids demonstrating statistically significant variances across the groups were chosen for the development of a stepwise binary logistic regression algorithm (employing the backward likelihood ratio method), used for model construction. Samples were stratified based on their receiving dates, allocating 80% to form the training set and 20% for internal validation. At each step, the bile acid with the least contribution to the model likelihood was iteratively removed. The process continued until the likelihood ratio test indicated that further removal of bile acids would significantly alter the model fit ($P < .05$). The remaining bile acids were considered the best subset, which composed the optimal model. Values exceeding optimal threshold were classified as IBD. The diagnostic accuracy of individual bile acids and the model was assessed using receiver operating characteristic (ROC) curves.

3. Results

3.1. Baseline characteristics of the study participants

Table 1 summarizes the baseline characteristics of the study participants. The median age was similar across the 3 groups: 60 years old for IBD group, 61 years old for HC group, and 65 years old for OID group. The gender distribution was also comparable, with males constituting approximately 60% of each group (59.5% in IBD, 61.0% in HC, and 60.3% in OID). Within the IBD group, Crohn disease (CD) was the predominant diagnosis (69.9% of cases), followed by ulcerative colitis (UC) at 30.1%.

3.2. Comparison of 15 bile acids across the groups

In comparison with the HC group, the IBD group displayed significantly lower serum levels of CDCA, DCA, GDCA, TDCA, LCA, GLCA, TLCA ($P < .05$).

Compared to the OID group, the IBD group exhibited decreased serum levels of DCA, GDCA, TDCA, GLCA, and TLCA ($P < .05$), and higher levels of CA, CDCA, GCDCA, UDCA, GUDCA, and TUDCA ($P < .05$).

Notably, the study revealed a significant reduction in secondary bile acids within the IBD group. This resulted in an increased ratio of primary to secondary bile acids. Additionally, the ratio of glyco-conjugated to tauro-conjugated bile acids experienced an upsurge. Detailed findings are presented in Table 2.

3.3. Using combined bile acids to diagnose IBD

3.3.1. Development of the model Y_0 to distinguish between IBD group and HC group. Significant differences were observed in 7 bile acids (CDCA, DCA, GDCA, TDCA, LCA, GLCA, TLCA) between the IBD and HC groups. To improve diagnostic accuracy, we combined these 7 bile acids and performed stepwise binary logistic regression to develop a model Y_0. The training set, comprising 80% of the samples, was chosen by receiving date, including 215 cases from the IBD group (151 with CD and 64 with UC) and 160 cases from the HC group. The established model was expressed as $Y_0 = 1/(1 + e^{-\mathrm{Logit(P0)}})$, where Logit($P$) = –0.0003 × CDCA + 0.0354 × DCA-0.920, as presented in Table 3.

3.3.2. Development of the model Y_1 to distinguish between IBD group and non-IBD group. Compared to the non-IBD group (which combined healthy controls and those with other intestinal diseases), patients with IBD exhibited significantly higher serum levels of GCA and GCDCA ($P < .05$). Conversely, serum levels of DCA, GDCA, TDCA, LCA, GLCA, and TLCA were all significantly lower in the IBD group ($P < .05$) (Fig. 1). We combined these 8 bile acids and performed stepwise binary logistic regression to develop a model Y_1. The training set, comprising 80% of the samples, including 215 cases from the IBD group (151 with CD and 64 with UC) and 299 cases from the non-IBD group (160 with HC and 139 with OID). The established model was expressed as $Y_1 = 1/(1 + e^{-\mathrm{Logit(P)}})$, w here Logit($P$) = 0.0003 × GCDCA-0.0014 × DCA-0.3558TLCA-0.0986, as presented in Table 4.

3.3.3. Analysis of model diagnostic accuracy. ROC curve analysis revealed that model Y_0 had an area under the curve (AUC) of 0.866 (95% CI: 0.827–0.898). The optimal threshold value was determined to be 0.32, resulting in a sensitivity of 76.28% and a specificity of 89.37%. The model Y_1 had an AUC of 0.792 (95% *CI*: 0.754–0.827). The optimal threshold value was determined to be 0.45, resulting in a sensitivity of 77.67%, a specificity of 71.91%. As shown in Figure 2.

3.3.4. Model validation. The remaining samples constituted the validation set. Model Y_0 achieved a sensitivity of 81.48%, a specificity of 80.00% and a diagnostic accuracy of 80.85%, correctly classifying 44 cases in IBD group and 32 cases in HC group. While model Y_1 showed a sensitivity of 74.07%, a specificity of 70.67% and a diagnostic accuracy of 72.09%, correctly classifying 40 cases in IBD group and 53 cases in non-IBD group. These results mirrored the diagnostic concordance observed in the training set.

3.4. Comparing the accuracy of bile acids and models in diagnosing IBD

Both individual bile acids and the models were evaluated using ROC curves. Among the bile acids with statistically significant differences, DCA emerged as the most promising biomarker. It achieved an AUC of 0.860 for distinguishing IBD from HC and 0.790 for distinguishing IBD from non-IBD. Despite showing promise, individual bile acids, like DCA, were slightly outperformed by models Y_0 (AUC: 0.866) and Y_1 (AUC: 0.792) in

Table 1

Clinical characteristics of the participants.

Group	IBD	HC	OID
Total (%)	269 (100.0)	200 (100.0)	174 (100.0)
Age distribution			
<55 (%)	73 (27.1)	46 (23.0)	38 (21.8)
55–64 (%)	93 (34.6)	79 (39.5)	71 (40.8)
>65 (%)	103 (38.3)	75 (37.5)	65 (37.4)
Median (yr)	60.00	61.00	65.00
Mean (yr)	60.40	59.33	58.86
SD	10.60	9.65	11.35
Gender distribution			
Male (%)	160 (59.5)	122 (61.0)	105 (60.3)
Female (%)	109 (41.5)	78 (39.0)	69 (39.7)
Classification distribution			
CD (%)	188 (69.9)	—	—
UC (%)	81 (30.1)	—	—
Colorectal cancer (%)	—	—	91 (52.3)
Colorectal polyps (%)	—	—	53 (30.5)
Acute enteritis (%)	—	—	30 (17.2)

CD = Crohn's disease, HC = healthy control, IBD = inflammatory bowel disease, OID = other intestinal diseases, UC = ulcerative colitis.

Table 2

Comparison of 15 bile acid levels across 3 groups.

Bile acid (nmol/L)	IBD group (269 cases)	HC group (200 cases)	OID group (174 cases)	*P* value
CA	83.88 (36.50~193.31)	90.35 (41.17~288.07)	50.82 (21.43~128.28)**	<.001
GCA	184.79 (72.06~410.82)	125.33 (68.60~262.49)	137.58 (57.18~352.39)	.065
TCA	16.60 (4.81~43.47)	15.73 (7.36~37.46)	15.01 (5.77~45.51)	.711
CDCA	390.44 (113.77~1234.39)	552.20 (257.16~1120.66)*	137.62 (41.87~584.50)**	<.001
GCDCA	906.54 (426.62~1878.92)	661.93 (382.91~1278.35)	687.86 (259.20~1217.26)**	.009
TCDCA	54.35 (20.18~168.22)	63.58 (34.36~131.05)	64.45 (26.11~163.62)	.604
DCA	6.26 (0.01~76.07)	388.41 (168.02~655.34)**	78.53 (13.74~205.03)**	<.001
GDCA	3.48 (0.01~57.08)	216.88 (92.23~419.44)**	63.84 (18.31~194.66)**	<.001
TDCA	0.45 (0.79~5.64)	27.20 (10.54~50.24)**	7.87 (1.54~24.78)**	<.001
LCA	6.54 (0.01~18.33)	28.21 (18.44~41.96)**	6.53 (1.43~16.23)	<.001
GLCA	0.52 (0.01~2.87)	6.85 (2.74~16.52)**	2.17 (0.01~5.40)**	<.001
TLCA	0.14 (0.01~0.51)	1.06 (0.41~1.98)**	0.46 (0.22~1.13)**	<.001
UDCA	65.03 (9.78~350.51)	92.51 (33.19~186.72)	22.91 (5.22~107.90)**	<.001
GUDCA	113.28 (30.25~365.37)	119.70 (65.10~297.37)	61.25 (18.94~189.50)**	<.001
TUDCA	2.90 (0.79~10.41)	3.94 (1.56~8.08)	1.68 (0.01~5.70)**	<.001
Bile acid	**IBD group (269 cases)**	**HC group (200 cases)**	**OID group (174 cases)**	***P* value**
Primary (nmol/L)	2297.50 (1071.81~4451.29)	1826.44 (1052.20~3328.28)	1447.27 (592.42~3161.65)**	<.001
Secondary (nmol/L)	35.93 (8.92~231.91)	818.57 (340.20~1202.61)**	199.17 (49.99~463.38)**	<.001
Primary/ Secondary	49.27 (7.36~284.10)	2.34 (1.45~5.67)**	5.76 (2.67~18.52)**	<.001
DCA/(DCA + CA)	0.07 (0.00~0.56)	0.77 (0.51~0.90) **	0.54 (0.26~0.81) **	<.001
LCA/(LCA + CDCA)	0.01 (0.00~0.06)	0.05 (0.02~0.13) **	0.03 (0.00~0.16) **	<.001
Glyco/Tauro	15.03 (8.27~26.30)	11.2 (7.15~16.14)**	9.92 (5.73~16.52)**	<.001
Total (nmol/L)	3160.76 (1561.47~6271.75)	2977.34 (1959.59~5192.56)	1962.78 (916.62~4190.22)**	<.001

Paired comparison after multiple comparisons show statistically significant differences: Compared to IBD group,
*$P < .05$,
**$P < .01$; CA = cholic acid, CDCA = chenodeoxycholic acid, DCA = deoxycholic acid, GCA = glycocholic acid, GCDCA = glycochenodeoxycholic acid, GDCA = glycodeoxycholic acid, GLCA = glycolithocholic acid, GUDCA = glycoursodeoxycholic acid, HC = healthy controls, IBD = inflammatory bowel disease, LCA = lithocholic acid, OID = other intestinal diseases, TCA = taurocholic acid, TCDCA = taurochenodeoxycholic acid, TDCA = taurodeoxycholic acid, TLCA = taurolithocholic acid, TUDCA = tauroursodeoxycholic acid, UDCA = ursodeoxycholic acid.

Table 3

Performance of the stepwise binary logistic regression models in distinguishing between IBD group and HC group.

Model (Step)	Indicators in model	Removing indicator	AUC	*P* value (vs next model)
1	CDCA, DCA, GDCA, TDCA, LCA, GLCA, TLCA	TDCA	0.868	.742
2	CDCA, DCA, GDCA, LCA, GLCA, TLCA	GDCA	0.868	.523
3	CDCA, DCA, LCA, GLCA, TLCA	GLCA	0.868	.485
4	CDCA, DCA, LCA, TLCA	TLCA	0.867	.091
5	CDCA, DCA, LCA	LCA	0.866	.067
6	**CDCA, DCA**	**—**	**0.866**	**.002**

The bolded terms CDCA and DCA represent the optimal components of the model Y0. Removing indicator: The indicator would be removed in next step; *P* value: According to Likelihood ratio test. AUC = area under the curve; CDCA = chenodeoxycholic acid; DCA = deoxycholic acid; GDCA = glycodeoxycholic acid; GLCA = glycolithocholic acid; LCA = lithocholic acid; TDCA = taurodeoxycholic acid; TLCA = taurolithocholic acid.

terms of diagnostic accuracy. These findings are presented in Tables 5 and 6.

3.5. Comparison of 15 bile acids between CD and UC

In comparison to the CD group, the UC group showed lower levels of CA, CDCA, UDCA, and GUDCA ($P < .05$). Conversely, the level of TCDCA was higher in the UC group compared to the CD group ($P < .05$). These findings are presented in Table 7. We evaluated the diagnostic accuracy of these bile acids for differentiating between CD and UC using ROC curves (Table 8). Unfortunately, none of the individual bile acids achieved an AUC exceeding 0.7, suggesting insufficient accuracy for distinguishing between the 2 diseases.

4. Discussion

Bile acid metabolism is a sophisticated process that extends through the enterohepatic circulation, involving various stages such as bile acid synthesis in the liver, bile secretion, intestinal absorption, metabolic conversion, and hepatic reabsorption.[10,11] Emerging research has demonstrated that the bile acid profile undergoes specific changes in response to diverse disease states,[12] highlighting its potential as a biomarker for diagnosing complex conditions. In contrast to traditional total bile acid measurement, a thorough examination of the bile acid profile offers deeper insights into pathology, paving the way for accurate diagnosis and personalized treatment strategies.[13]

Numerous studies have observed changes in the bile acid metabolome to understand the progression of associated diseases. Mousa et al conducted a comprehensive study with a large sample size, exploring variations in the serum bile acid profile of patients with primary sclerosing cholangitis.[14] Our study observed that alterations in the serum bile acid metabolome of IBD patients are marked by a notable reduction in secondary bile acids, and an elevated primary-to-secondary bile acid ratio, consistent with the outcomes reported by Duboc et al.[15] It may be linked to the following mechanisms: Recent studies have highlighted noteworthy alterations in the gut microbiome and its metabolic byproducts in patients with IBD.[16,17] Dysbiosis in IBD patients, characterized by reduced microbial diversity, especially a decline in the Clostridium leptum group representing the Firmicutes phylum, leading to a diminished ability to convert primary bile acids to secondary bile acids.[18–20] Monma et al described a decrease in the DCA/(DCA + CA) and LCA/

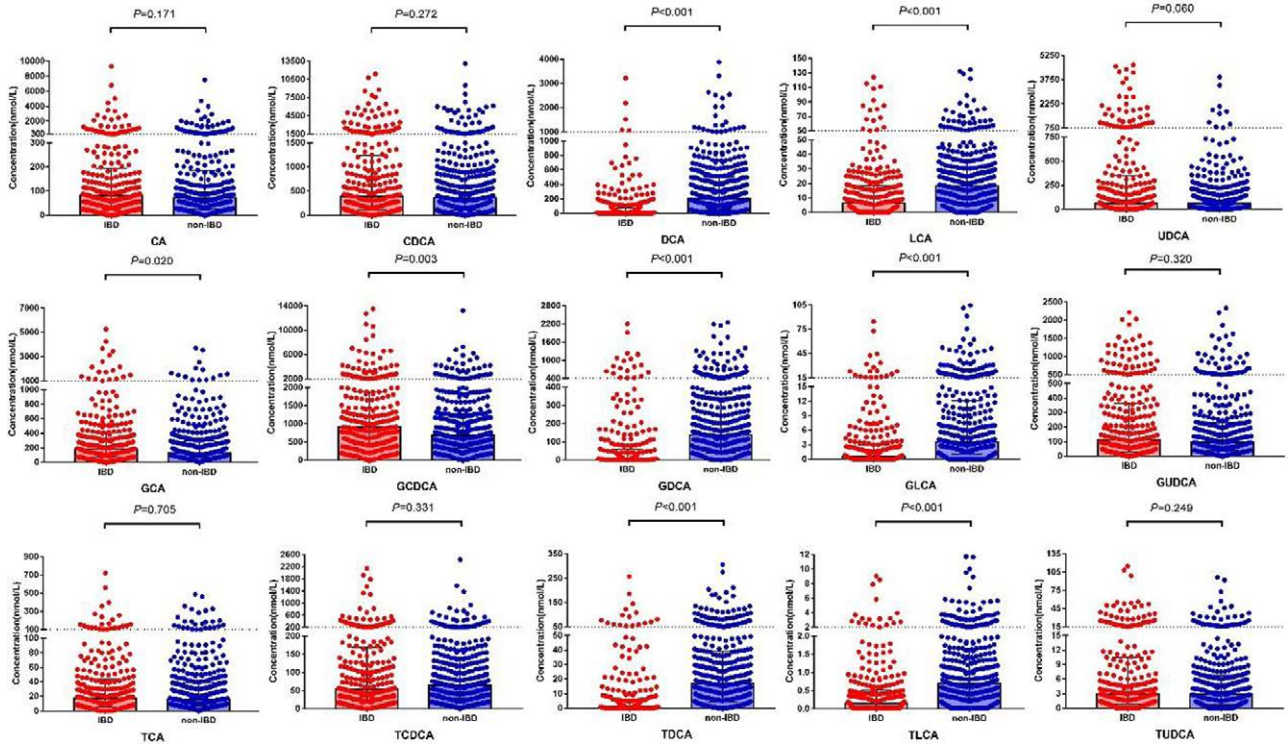

Figure 1. Comparison of 15 bile acids between IBD group and non-IBD group. IBD: inflammatory bowel disease group (n = 269); non-IBD: non-inflammatory bowel disease group (n = 374); CA = cholic acid; CDCA = chenodeoxycholic acid; DCA = deoxycholic acid; GCA = glycocholic acid; GCDCA = glycochenodeoxycholic acid; GDCA = glycodeoxycholic acid; GLCA = glycolithocholic acid; GUDCA = glycoursodeoxycholic acid; LCA = lithocholic acid; TCA = taurocholic acid; TCDCA = taurochenodeoxycholic acid; TDCA = taurodeoxycholic acid; TLCA = taurolithocholic acid; TUDCA = tauroursodeoxycholic acid; UDCA = ursodeoxycholic acid.

Table 4

Performance of the stepwise binary logistic regression models in distinguishing between IBD group and non-IBD group.

Model (Step)	Indicators in model	Removing indicator	AUC	*P* value (vs next model)
1	GCA, GCDCA, DCA, GDCA, TDCA, LCA, GLCA, TLCA	GCA	0.793	.866
2	GCDCA, DCA, GDCA, TDCA, LCA, GLCA, TLCA	TDCA	0.793	.807
3	GCDCA, DCA, GDCA, LCA, GLCA, TLCA	GDCA	0.793	.812
4	GCDCA, DCA, LCA, GLCA, TLCA	GLCA	0.793	.526
5	GCDCA, DCA, LCA, TLCA	LCA	0.792	.315
6	**GCDCA, DCA, TLCA**	—	**0.792**	**<.001**

The bolded terms GCDCA, DCA, and TLCA are the optimal components of model Y1. Removing indicator: The indicator would be removed in next step; *P* value: According to Likelihood ratio test. AUC = area under the curve, DCA = deoxycholic acid, GCA = glycocholic acid, GCDCA = glycochenodeoxycholic acid, GDCA = glycodeoxycholic acid, GLCA = glycolithocholic acid, LCA = lithocholic acid, TDCA = taurodeoxycholic acid, TLCA = taurolithocholic acid.

(LCA + CDCA) ratios in IBD patients, linking it to impaired secondary bile acid conversion due to reduced Clostridium subcluster XIVa abundance in the gut.[21] We confirm these findings, as our calculations of these ratios in our study yielded consistent results (Table 2). Reduced farnesoid X receptor (FXR) activity, a key bile acid receptor in IBD, disrupts bile acid homeostasis, leading to the accumulation of primary bile acids in hepatocytes and intestinal epithelial cells. This occurs because decreased FXR activity hinders the conversion of primary bile acids into secondary bile acids.[22] Suppression of the hepatic bile acid-FXR-hepatic stellate cell growth factor negative feedback loop disrupts bile acid homeostasis, leading to increased re-synthesis of primary bile acids in the liver and a subsequent decrease in the proportion of secondary bile acids.[23]

This study utilized LC-MS/MS to quantitatively analyze the bile acid profile in serum and explore their potential value in diagnosing IBD. Two classification models were constructed: model Y_0 to differentiate IBD patients from HC group, and model Y_1 to differentiate IBD patients from non-IBD patients. The results demonstrated that bile acid profile-based classification models achieved higher AUC values and overall diagnostic accuracy compared to using single bile acid. Notably, model Y_0 demonstrated superior performance compared to model Y_1, suggesting greater specificity of bile acid profiles in distinguishing IBD patients from healthy individuals. However, the model performed a little poorly in differentiating IBD from non-IBD, it likely due to the similarity in serum bile acid level observed in IBD patients and those with other intestinal diseases such as colorectal cancer or acute enteritis. These findings highlight the limitations of using solely bile acid profiles for IBD diagnosis. Therefore, incorporating additional specific markers into a multi-marker model for differential diagnosis is warranted to improve diagnostic accuracy and specificity. Furthermore, the bile acid profile exhibited poor performance in differentiating between CD and UC, 2 subtypes of IBD.

The detection of the bile acid spectrum, facilitated by mature reagent kits, is relatively commonplace in clinical settings and could serve as a valuable complement to imaging studies such as colonoscopy and immunological tests, thereby enhancing diagnostic accuracy. However, the range of bile acid indicators is somewhat limited, and their diagnostic accuracy has a certain ceiling. To further advance in diagnostic accuracy, the inclusion of indicators like apolipoproteins,[24] amino acids,[25] and microRNAs[26]—already proven to be abnormally expressed in the blood of IBD patients—could be considered. We will delve deeper into this aspect. Moreover, the combined use of blood

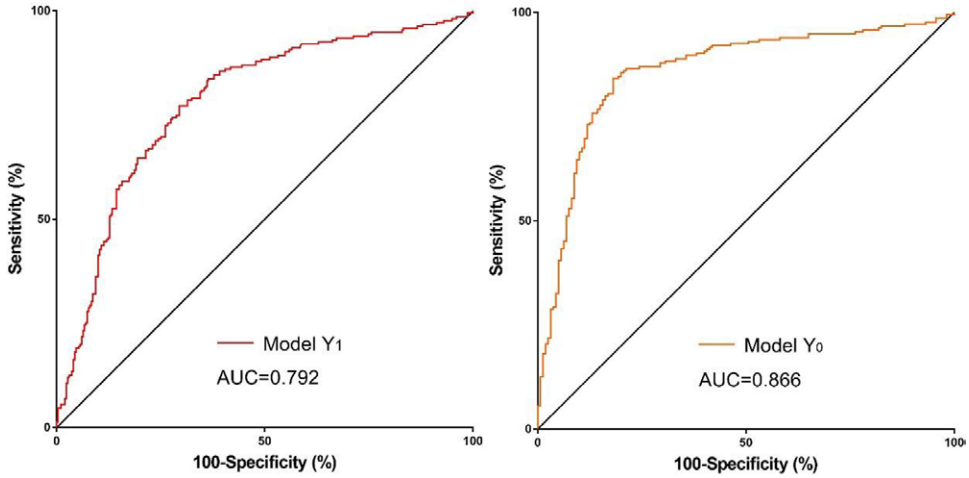

Figure 2. ROC of the model Y_0 and model Y_1. AUC = area under the curve.

Table 5

Performance of the bile acids and the model in distinguishing between IBD group and HC group

Bile Acid	AUC (95% *CI*)	Optimal Threshold	Sensitivity (%)	Specificity (%)	Youden index
CDCA	0.566 (0.519~0.611)	285.52 nmol/L	44.61	73.50	0.18
DCA	0.860 (0.824~0.890)	123.24 nmol/L	80.67	82.50	0.63
GDCA	0.823 (0.785~0.856)	47.72 nmol/L	74.35	83.50	0.59
TDCA	0.839 (0.803~0.871)	5.67 nmol/L	75.46	84.50	0.60
LCA	0.811 (0.772~0.845)	11.29 nmol/L	63.57	90.00	0.54
GLCA	0.789 (0.749~0.825)	2.57 nmol/L	74.35	77.50	0.52
TLCA	0.772 (0.731~0.809)	0.48 nmol/L	74.72	70.50	0.45
Model Y_0	0.866 (0.827~0.898)	0.32	76.28	89.37	0.66

Model Y_0 : $1/(1+e^{-\text{Logit(P)}})$, where Logit(P) = 0.0003 × GCDCA-0.0014 × DCA-0.3558TLCA-0.0986; CI = confidence interval; CDCA = chenodeoxycholic acid; DCA = deoxycholic acid; GDCA = glycodeoxycholic acid; GLCA = glycolithocholic acid; LCA = lithocholic acid; TDCA = taurodeoxycholic acid; TLCA = taurolithocholic acid.

Table 6

Performance of the bile acids and the model in distinguishing between IBD group and non-IBD group.

Bile acid	AUC (95% CI)	Optimal threshold	Sensitivity (%)	Specificity (%)	Youden index
GCA	0.554 (0.514~0.593)	152.30 nmol/L	55.39	57.22	0.13
GCDCA	0.568 (0.528~0.606)	1172.98 nmol/L	40.89	71.93	0.13
DCA	0.790 (0.757~0.821)	19.86 nmol/L	62.83	83.69	0.47
GDCA	0.774 (0.739~0.806)	13.20 nmol/L	62.08	86.01	0.48
TDCA	0.789 (0.756~0.820)	5.73 nmol/L	76.21	71.76	0.48
LCA	0.673 (0.635~0.709)	8.85 nmol/L	59.85	68.72	0.29
GLCA	0.703 (0.667~0.738)	1.77 nmol/L	66.17	70.05	0.36
TLCA	0.718 (0.681~0.752)	0.36 nmol/L	66.17	69.52	0.36
Model Y_1	0.792 (0.754~0.827)	0.45	77.67	71.91	0.50

Model Y : $1/(1+e^{-\text{Logit(P)}})$, where Logit(P) = 0.0003 × GCDCA-0.0014 × DCA-0.3558TLCA-0.0986;
CI = confidence interval, DCA = deoxycholic acid, GCA = glycocholic acid, GCDCA = glycochenodeoxycholic acid, GDCA = glycodeoxycholic acid, GLCA = glycolithocholic acid, LCA = lithocholic acid, TDCA = taurodeoxycholic acid, TLCA = taurolithocholic acid.

indicators for diagnosing IBD is still in the exploratory stage both domestically and internationally; therefore, the conclusions drawn in this paper require validation through large-scale, multi-center studies. On the other hand, it is important to note that our study provides a snapshot of bile acid metabolism at a single point in time. Longitudinal studies are needed to fully elucidate the dynamic changes in bile acid composition and their relationship to IBD progression and outcomes.

This study assessed the potential of bile acid profiles as a diagnostic tool for IBD. The findings revealed that the model based on DCA and CDCA showed promise in differentiating IBD patients from healthy controls. Meanwhile, the model combining GCDCA, DCA and TLCA exhibited slight limitations in distinguishing IBD from non-IBD patients with other intestinal diseases like colorectal cancer. This highlights the need for incorporating additional markers to improve diagnostic accuracy for differentiating IBD from various non-IBD conditions.

Author contributions

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Table 7

Comparison of the levels of 15 bile acids between CD group and UC group.

Bile acid (nmol/L)	HC group (200 cases)	CD group (188 cases)	UC group (81 cases)	*P* value
CA	90.35 (41.17~288.07)	92.02 (42.28~249.71)	64.58 (24.62~136.07)*#	.012
GCA	125.33 (68.60~262.49)	170.91 (68.79~393.21)	232.18 (87.87~515.20)*	.040
TCA	15.73 (7.36~37.46)	12.97 (4.43~43.71)	21.11 (7.07~47.79)	.133
CDCA	552.20 (257.16~1120.66)	557.08 (151.63~1473.66)	209.40 (42.70~629.00)**##	<.001
GCDCA	661.93 (382.91~1278.35)	898.96 (423.41~1858.30)	995.10 (437.56~1986.28)	.094
TCDCA	63.58 (34.36~131.05)	48.56 (15.84~151.26)	80.33 (33.80~195.76)#	.020
DCA	388.41 (166.51~655.34)	4.90 (0.01~117.37)**	7.77 (0.01~40.15)**	<.001
GDCA	216.88 (92.23~419.44)	5.74 (0.01~81.09)**	2.09 (0.01~27.38)**	<.001
TDCA	27.20 (10.54~50.24)	0.36 (0.01~6.74)**	0.78 (0.01~5.15)**	<.001
LCA	28.21 (18.44~41.96)	4.50 (0.01~16.60)**	8.70 (0.37~22.56)**	<.001
GLCA	6.85 (2.74~16.52)	0.36 (0.01~3.57)**	0.55 (0.01~2.31)**	<.001
TLCA	1.06 (0.41~1.98)	0.06 (0.01~0.50)**	0.18 (0.01~0.56)**	<.001
UDCA	92.51 (33.19~186.72)	138.32 (17.08~602.62)	14.24 (0.01~84.38)**##	<.001
GUDCA	119.70 (65.10~297.37)	154.84 (44.27~466.03)	48.37 (23.95~208.65)**##	<.001
TUDCA	3.94 (1.56~8.08)	2.88 (0.77~11.70)	2.99 (0.88~8.25)	.228

Paired comparison after multiple comparisons show statistically significant differences: Compared to HC group,

*$P < .05$,

**$P < .01$; Compared to CD group,

#$P < .05$,

##$P < .01$; CA = cholic acid; CD = crohn's disease; CDCA = chenodeoxycholic acid; DCA = deoxycholic acid; GCA = glycocholic acid; GCDCA = glycochenodeoxycholic acid; GDCA = glycodeoxycholic acid; GLCA = glycolithocholic acid; GUDCA = glycoursodeoxycholic acid; HC = healthy controls; LCA = lithocholic acid; TCA = taurocholic acid; TCDCA = taurochenodeoxycholic acid; TDCA = taurodeoxycholic acid; TLCA = taurolithocholic acid; TUDCA = tauroursodeoxycholic acid; UC = ulcerative colitis; UDCA = ursodeoxycholic acid.

Table 8

Performance of the bile acids in differentiating CD and UC.

Parameter	AUC (95% CI)	Optimal threshold	Sensitivity (%)	Specificity (%)	Youden index
CA	0.609 (0.547~0.667)	70.64 nmol/L	62.77	58.02	0.21
CDCA	0.655 (0.595~0.712)	500.65 nmol/L	53.19	74.07	0.27
TCDCA	0.596 (0.534~0.655)	25.12 nmol/L	34.04	83.95	0.18
UDCA	0.698 (0.648~0.759)	59.39 nmol/L	63.83	72.84	0.37
GUDCA	0.647 (0.587~0.704)	77.99 nmol/L	64.89	61.73	0.27

CA = cholic acid, CDCA = chenodeoxycholic acid, CI = confidence interval, GUDCA = glycoursodeoxycholic acid, TCDCA = taurochenodeoxycholic acid, UDCA = ursodeoxycholic acid.

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