Original Article

A comparative analysis of fecal bile acid profiles in patients with ulcerative colitis and colorectal cancer

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Abstract

Ulcerative colitis is a chronic inflammatory disease of the colon in which mucosal inflammation, dysbiosis, and colorectal cancer risk are closely linked. Since intestinal bacteria convert primary bile acids into secondary bile acids, fecal bile acid composition may help describe metabolic differences between inflammatory and malignant colorectal disease. Therefore, the aim of this study was to compare fecal bile acid profiles in patients with ulcerative colitis and colorectal cancer, with colonoscopy-negative controls included as an additional comparator. This cross-sectional analytical study enrolled 100 individuals: 40 with ulcerative colitis, 40 with colorectal cancer, and 20 colonoscopy-negative controls without macroscopic colorectal abnormalities. The quantities of fecal cholic acid, chenodeoxycholic acid, deoxycholic acid, and lithocholic acid were measured by liquid chromatography-mass spectrometry (LC-MS). The parameter values between ulcerative colitis and colorectal cancer were compared using the Mann-Whitney test, while three-group analysis used the Kruskal-Wallis test followed by Bonferroni-corrected post-hoc Mann-Whitney tests. Fecal bile acid profiles differed significantly between ulcerative colitis and colorectal cancer. Patients with ulcerative colitis had higher levels of cholic acid (p<0.001), chenodeoxycholic acid (p=0.001), and total primary bile acid (p<0.001). Conversely, colorectal cancer group showed higher levels of deoxycholic acid, lithocholic acid, total secondary bile acids, total bile acids, and secondary-to-primary bile acid ratios (all p<0.001). In the three-group analysis, the overall significant differences (all had p<0.001 except chenodeoxycholic acid with p=0.002) were driven mainly by the colorectal cancer group. No statistically significant differences were detected between ulcerative colitis and colonoscopy-negative controls for any measured parameter, although this finding should not be interpreted as evidence of equivalent or normal fecal bile acid profiles. In conclusion, these distinct metabolic signatures suggest that fecal bile acids have potential as non-invasive biomarkers to differentiate colorectal malignancy from chronic inflammation.

Keywords: Fecal bile acid, colorectal cancer, ulcerative colitis, primary bile acid, secondary bile acid

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Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon that develops from the interaction between genetic susceptibility, environmental exposure, gut microbiota, and mucosal immune response [1]. The burden of this disease differs between regions. The highest prevalence is reported in North America and Europe, while epidemiological data in Indonesia are still limited

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and mostly come from hospital-based reports [2,3]. The Indonesian national consensus stated that the incidence of UC is still lower than in Western countries, although the increasing trend still needs attention along with changes in lifestyle, dietary pattern, and environmental exposure [3,4].

Colorectal cancer (CRC), which is a malignancy of the colon and/or rectum, remains one of the main contributors to cancer-related morbidity and mortality. In 2020, an estimated 1.93 million new cases and more than 935,000 deaths were reported globally [5]. The Asia-Pacific region gives a large contribution to this burden, and Indonesia is included as a country where CRC has become an important public health problem [6,7]. Given this significant clinical burden, exploring the unique metabolic characteristics of CRC is essential to help distinguish the luminal environment of a malignancy from other benign or inflammatory colorectal diseases.

Chronic inflammation becomes an important biological connection between UC and colorectal carcinogenesis. Patients with UC have a higher risk of CRC compared with the general population, especially when the inflammation is extensive, persistent, or occurs for a long duration. At the mucosal level, continuous inflammation can trigger oxidative stress, DNA damage, immune dysregulation, and dysbiosis, therefore creating a luminal and epithelial environment that can support neoplastic change [8,9]. However, UC and CRC are not the same clinical condition. Consequently, comparison of their metabolic profiles may help explain which changes are mainly related to chronic inflammation and which changes are more typical for malignancy. In UC, dysbiosis and inflammatory activity have been associated with impaired bile acid metabolism, including reduced concentrations of several secondary bile acids [10,11]. In CRC, higher levels of secondary bile acids, particularly DCA and LCA, have often been linked to procarcinogenic processes such as oxidative stress, DNA damage, inflammatory activation, impaired apoptosis, and increased epithelial proliferation [12-14].

Bile acid metabolism is one pathway that is strongly influenced by gut microbiota. Primary bile acids, including cholic acid (CA) and chenodeoxycholic acid (CDCA), are produced in the liver, secreted into the intestine, and mostly returned to the liver through enterohepatic circulation. A small part of bile acids reaches the colon, where gut bacteria convert them into secondary bile acids, especially deoxycholic acid (DCA) and lithocholic acid (LCA), through deconjugation and 7α-dehydroxylation [15-17]. However, direct comparisons between UC and CRC remain limited, particularly in Indonesian populations. Understanding these distinct metabolic profiles is crucial for explaining the biological differences between chronic colonic inflammation and colorectal malignancy. On that basis, this study compared fecal bile acid profiles in patients with UC and CRC, and included a colonoscopy-negative control group without macroscopic abnormalities as an additional comparator.

Methods

Study design and setting

This analytical observational study used a cross-sectional design and was conducted from May 2025 to March 2026. The study was carried out in the Inpatient Ward and/or Endoscopy Unit of Dr. Zainoel Abidin General Hospital, Banda Aceh, Indonesia. The primary analysis compared fecal bile acid profiles between patients with UC and patients with CRC. A colonoscopy-negative control group, defined as participants without macroscopic colorectal abnormalities, was included as an additional comparator.

Study population and sampling

The target population was adult patients with colorectal disease who underwent colonoscopic evaluation. During the study period, a total of 293 participants were initially screened for eligibility, comprising 74 patients with suspected or confirmed UC, 92 patients with CRC, and 127 colonoscopy-negative controls. Following the application of exclusion criteria, 89 patients were excluded. The specific reasons for exclusion were: malignancy outside the colorectal tract (n=16; 2 UC, 14 CRC), liver disease (n=4; 4 CRC), other autoimmune diseases (n=8; 7 UC, 1 control), and recent antibiotic or probiotic use (n=61; 13 UC, 17 CRC, 31 controls). From the remaining 204 eligible patients (52 UC, 57 CRC, and 95 controls), participants were enrolled consecutively until

the predefined target sample sizes were met. Consequently, 104 eligible patients (12 UC, 17 CRC, and 75 controls) were not included because the prespecified quotas had already been achieved. A total of 100 participants were ultimately included in the final analysis, comprising 40 with UC, 40 with CRC, and 20 colonoscopy-negative controls. The two main groups met the minimum sample size requirement for comparing independent groups, while the colonoscopy-negative control group was used as an additional reference group. The participant selection process is summarized in the study flow diagram (**Figure 1**).

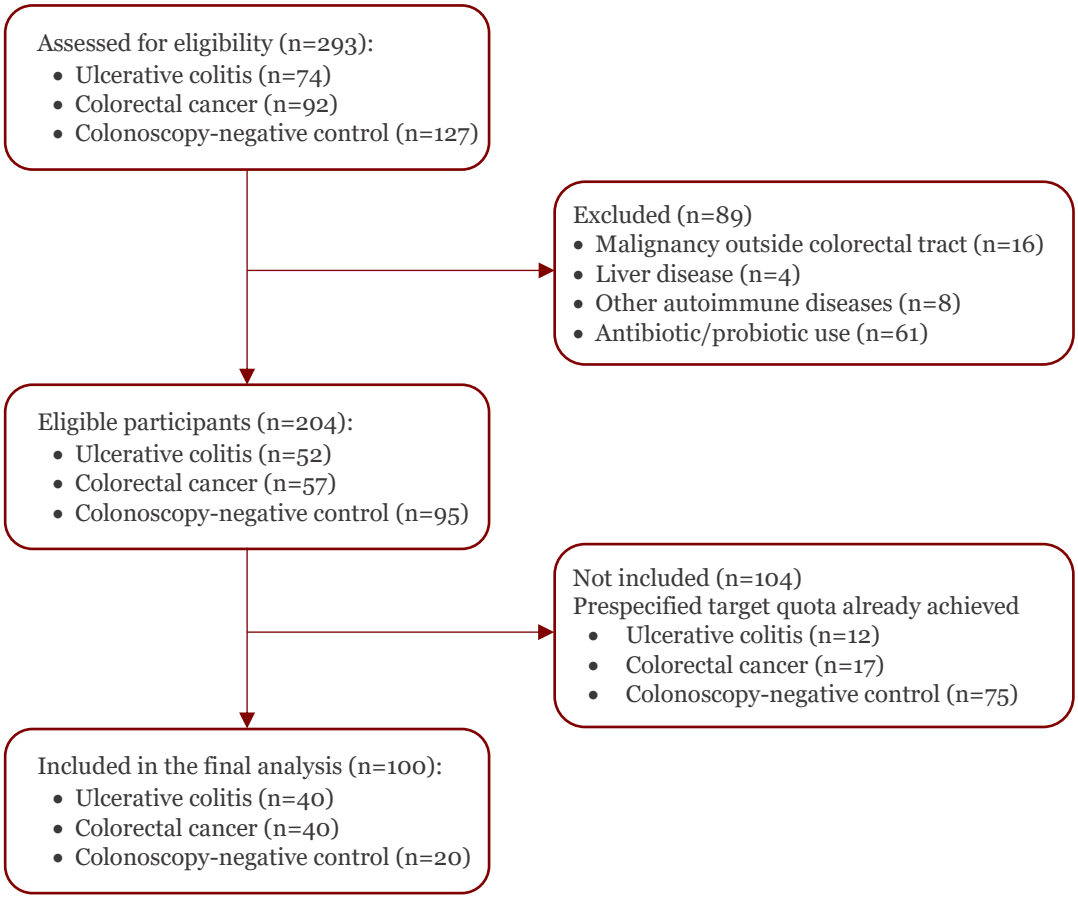

Figure 1. Flow diagram of participant recruitment and selection.

The sample size was calculated for the primary comparison between the UC and CRC groups using the formula for comparing two independent means. Because the absolute concentrations of individual bile acid components vary widely, the sample size was calculated based on an anticipated moderate-to-large standardized effect size (Cohen's d=0.65) to detect differences in our prespecified primary parameter of interest, deoxycholic acid (DCA). The calculation used a two-sided significance level of 5% and 80% power. This resulted in a minimum required sample size of 38 participants per group. To account for potential sample degradation, pre-analytical errors, or incomplete clinical data, the sample size was increased by approximately 5%, resulting in a final enrollment of 40 patients in each main group. The 20 colonoscopy-negative controls were included as an additional comparator and were not part of the primary sample-size calculation.

Eligibility criteria

The eligibility criteria in this study followed the recommendation from a previous study [18]. Eligible participants were 18 years of age or older and belonged to one of the following groups: UC diagnosed by clinical evaluation, colonoscopy, and/or histopathology according to available data; CRC confirmed by colonoscopy and histopathology; or colonoscopy-negative controls without macroscopically significant colorectal abnormalities. Participants were excluded if they had active malignancy outside the colorectal tract, chronic kidney disease, acute or chronic liver disease, pancreatic disease, hypothyroidism, other autoimmune diseases that could affect bile

acid metabolism, lower gastrointestinal inflammatory disease other than UC, prior liver transplantation, or had used probiotics or antibiotics within 12 weeks before sample collection.

Diagnostic definitions and disease characteristics

UC was diagnosed by a consultant gastroenterologist based on compatible clinical presentation and colonoscopic findings. All patients with UC underwent colonoscopy, but histopathological examination was not performed. Endoscopic severity was assessed using the Ulcerative Colitis Endoscopic Index of Severity (UCEIS) and was categorized as mild (scores 3-4), moderate (scores 5-6), or severe (scores 7–8). All patients with UC had not received UC-specific treatment before fecal sample collection. Data on disease duration, disease extent, and histological activity were not systematically available.

CRC was confirmed by colonoscopy and histopathological examination. All patients with CRC were newly diagnosed and had not received surgery, chemotherapy, or radiotherapy before fecal sample collection. Tumor stage was classified according to the eighth edition of the American Joint Committee on Cancer staging system based on imaging and histopathological findings [19]. Tumor location was categorized as right-sided or left-sided, with rectal tumors included in the left-sided group. Detailed histological subtype, tumor grade, intestinal obstruction, and specific metastatic sites were not collected.

Data collection and fecal bile acid measurement

Demographic, clinical, anthropometric, laboratory, colonoscopy, and histopathology data were obtained from interviews, clinical examinations, and medical records. The baseline variables included age, sex, hemoglobin, albumin, body mass index, and chief complaint at presentation.

All participants underwent a standardized bowel preparation using macrogol 4000 administered in a total volume of 2 L within 24 hours before colonoscopy. Fecal samples were collected 24 hours after colonoscopy from stool passed after completion of the bowel preparation. The same preparation and collection protocol was applied to the UC, CRC, and colonoscopy-negative control groups. The samples were stored at −70°C until shipment and laboratory analysis. Bile acid concentrations were calculated based on the wet weight of the fecal sample and expressed as nmol/g wet feces. No additional correction for fecal water content or stool dilution was performed. Fecal samples were collected in sterile containers and divided into two cryotubes. All samples were immediately stored at −70°C and transported under frozen conditions using dry ice. Bile acids were extracted from the fecal matrix using methanol, and insoluble particles were removed using a microporous membrane.

Fecal bile acids were quantified using an Agilent 6460 Series Triple Quadrupole LC-MS system (Agilent Technologies, Santa Clara, CA, USA). Approximately 0.5 g of wet fecal sample was used for extraction with 1 mL of cold methanol. Samples were vortex-mixed for 30 minutes, followed by centrifugation at 14,000 rpm for 30 minutes at 4 °C. The resulting extract was diluted (100 µL of supernatant mixed with 500 µL of methanol) before LC-MS analysis.

Chromatographic separation was performed using a Poroshell C18 column (2.1×75 mm) with 0.1% formic acid in water and acetonitrile as the mobile phases under isocratic conditions (68% and 32%, respectively). The injection volume was 2 µL, and analytes were detected using negative electrospray ionization (ESI-) mode with multiple reaction monitoring (MRM) tracking specific mass transitions (m/z 407.6 → 407.6 for CA, m/z 391.6 → 391.6 for DCA and CDCA, and m/z 375.6 → 375.6 for LCA). An isotope-labeled internal standard, deuterated deoxycholic acid (DCA-d4; monitored at m/z 395.3 → 395.3), was added to all samples for quantification.

Calibration curves for CA, CDCA, DCA, and LCA covered 52–200,000 nM and were constructed using ten calibration levels evaluated by linear regression of the peak-area ratio of each analyte to the internal standard. Although formal limits of quantification and analytical precision metrics were not independently reported by the external testing facility for this batch, the lower end of the calibration curve (52 nM) served as the working minimum boundary for detection. Final concentrations were calculated using Agilent MassHunter software from the measured extract concentrations after accounting for the dilution factor, and normalized directly to the wet fecal mass, with results expressed as nmol/g wet feces.

This sampling approach represents a methodological constraint; collecting samples 24 hours after bowel preparation may affect transit, microbiota, and metabolite concentrations, and wet-

weight normalization does not correct for water content or dilution. Information on internal standards, limits of detection or quantification, and analytical precision was not available to the investigators. LC-MS is a method with suitable analytical performance for bile acid measurement in biological matrices, including feces [20]. Bile acid concentrations were expressed in nmol/g. Total primary bile acids, total secondary bile acids, total bile acids, and the secondary-to-primary bile acid ratio were calculated from the measured concentrations and were not separately measured.

Statistical analysis

Data were analyzed using IBM SPSS Statistics software (version 26; IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test showed that fecal bile acid parameters were not normally distributed; therefore, these data were summarized as median and interquartile range. For graphical visualization purposes, given the wide concentration range and non-normal distribution, fecal bile acid values were log10(x+1) transformed prior to generating the boxplots. Baseline numerical variables, including age, hemoglobin, albumin, and body mass index, were compared using the Kruskal-Wallis test. Sex and chief complaint were analyzed using the Chi-square test. The four directly measured bile acids (CA, CDCA, DCA, and LCA) were defined as prespecified co-primary outcomes. For the primary comparison between UC and CRC groups using the Mann-Whitney test, a Bonferroni correction was applied to these four primary variables, setting the significance threshold at $p<0.0125$. The calculated total bile acid variables and the secondary-to-primary ratio were treated as secondary exploratory outcomes. Separately, an additional three-group analysis involving UC, CRC, and colonoscopy-negative controls was conducted using the Kruskal-Wallis test. For the post-hoc pairwise comparisons among these three groups, a distinct Bonferroni adjustment was applied for the three possible group pairings, resulting in a significance threshold of $p<0.017$. For all other analyses, $p<0.05$ was considered statistically significant. All bile acid comparisons were unadjusted. Multivariable adjustment was not performed because the calculated sample size was specifically powered for the primary unadjusted comparisons. Adding multiple covariates into a statistical model would risk overfitting, in addition to the absence of data on several important potential confounders.

Results

Characteristics of the study participants

A total of 100 participants were included in the analysis: 40 with UC, 40 with CRC, and 20 colonoscopy-negative controls. Baseline characteristics are presented in **Table 1**. Median age was highest in the CRC group (61 years), followed by the UC group (50 years) and the colonoscopy-negative control group (47 years), with a significant difference among groups ($p=0.003$). Albumin also differed significantly ($p=0.023$), with the lowest median value in UC and the highest in colonoscopy-negative controls. Hemoglobin and body mass index did not differ significantly, with p-values of 0.111 and 0.293, respectively.

Sex distribution was not significantly different among groups ($p=0.127$); overall, 56 participants were male, and 44 were female. Chief complaint at presentation differed significantly ($p<0.001$). In the UC group, chronic diarrhea was the most frequent complaint, reported by 27 participants (67.5%), followed by hematochezia in 13 participants (32.5%). In the CRC group, other complaints or absence of gastrointestinal symptoms formed the largest category, involving 20 participants (50.0%); hematochezia and chronic diarrhea were reported in 11 (27.5%) and 9 (22.5%) participants, respectively. Among colonoscopy-negative controls, other complaints or no gastrointestinal symptoms were recorded in 11 participants (55.0%), while chronic diarrhea was recorded in 9 participants (45.0%).

All patients with UC had not received UC-specific treatment before fecal sample collection. Based on UCEIS, endoscopic severity was categorized as mild in 10 patients (25.0%), moderate in 16 patients (40.0%), and severe in 14 patients (35.0%). All patients with CRC were newly diagnosed and had not received surgery, chemotherapy, or radiotherapy before sample collection. Among the 40 patients with CRC, 2 (5.0%) had stage I disease, 15 (37.5%) had stage II disease, 13 (32.5%) had stage III disease, and 10 (25.0%) had stage IV disease. Tumors were right-sided in 5

patients (12.5%) and left-sided in 35 patients (87.5%), with rectal tumors included in the left-sided category.

Table 1. Baseline characteristics of study participants

Characteristics	Colonoscopy-negative control (n=20)	Ulcerative colitis (n=40)	Colorectal cancer (n=40)	*p*-value
	n (%)	n (%)	n (%)	
Age (years), median (IQR)	47 (29)	50 (20)	61 (17)	0.003[a]*
Sex				0.127[b]
Male	15 (75.0)	22 (55.0)	19 (47.5)	
Female	5 (25.0)	18 (45.0)	21 (52.5)	
Hemoglobin (g/dL), median (IQR)	11.70 (4.15)	12.10 (2.12)	10.75 (1.95)	0.111[a]
Albumin, g/dL	3.61 (0.65)	3.15 (0.58)	3.49 (0.63)	0.023[a]*
BMI (kg/m²), median (IQR)	20.80 (2.70)	21.15 (3.48)	21.75 (3.00)	0.293[a]
Chief complaint at presentation				<0.001[b]*
Hematochezia	0 (0.0)	13 (32.5)	11 (27.5)	
Chronic diarrhea	9 (45.0)	27 (67.5)	9 (22.5)	
Other complaints/no GI symptoms[c]	11 (55.0)	0 (0.0)	20 (50.0)	

GI: gastrointestinal; IQR: interquartile range
[a] Analyzed using Kruskal-Wallis test
[b] Analyzed using Pearson's chi-square test
[c] Included constipation, unexplained weight loss, abdominal mass, and no GI symptoms
* Statistically significant at $p<0.05$

Fecal bile acid profiles in ulcerative colitis and colorectal cancer

The primary UC-versus-CRC analysis showed significant differences for every fecal bile acid parameter, as presented in **Table 2**. Primary bile acid levels were higher in UC. Median CA was 15.425 nmol/g in UC and 0.795 nmol/g in CRC ($p<0.001$). Median CDCA was 7.380 nmol/g in UC and 0.820 nmol/g in CRC ($p=0.001$). Total primary bile acids were also higher in UC than in CRC, with medians of 23.805 nmol/g and 1.450 nmol/g, respectively ($p<0.001$).

The opposite pattern was seen for secondary bile acids. Median DCA was higher in CRC than in UC (28.635 vs 4.665 nmol/g; $p<0.001$). LCA showed the largest numerical separation, with a median of 13,381.035 nmol/g in CRC compared with 190.725 nmol/g in UC ($p<0.001$). Total secondary bile acids and total bile acids were also higher in CRC. The secondary-to-primary bile acid ratio was higher in CRC but showed substantial variability. These findings show that CA and CDCA concentrations were higher in UC than in CRC, whereas DCA and LCA concentrations were higher in CRC than in UC.

Fecal bile acid profiles across the three study groups

The additional analysis that included colonoscopy-negative controls showed significant differences among the three groups for all fecal bile acid parameters. These differences, however, were driven mainly by the CRC group, which is presented in **Table 3**. UC and colonoscopy-negative controls did not differ significantly in any measured fecal bile acid parameter. Median CA was 15.425 nmol/g in UC and 10.580 nmol/g in colonoscopy-negative controls (post-hoc $p=0.520$), while median CDCA was 7.380 and 5.085 nmol/g, respectively (post-hoc $p=0.475$). For secondary bile acids, median DCA was 4.665 nmol/g in UC and 4.575 nmol/g in colonoscopy-negative controls (post-hoc $p=0.981$), while median LCA was 190.725 and 204.130 nmol/g, respectively (post-hoc $p=0.790$).

In the post-hoc analysis, UC and CRC differed significantly across all fecal bile acid parameters (all $p<0.001$) (**Table 3**). Furthermore, compared to colonoscopy-negative controls, the CRC group showed significantly different levels of CA ($p=0.012$), DCA ($p=0.003$), LCA ($p<0.001$), total primary bile acids ($p=0.011$), total secondary bile acids ($p<0.001$), total bile acids ($p<0.001$), and the secondary-to-primary bile acid ratio ($p<0.001$). However, after Bonferroni correction, CDCA did not differ significantly between CRC and colonoscopy-negative control groups ($p=0.025$).

The distribution of fecal bile acid components across the three groups is presented in **Figure 2**. Visually, DCA and LCA distributions were higher in CRC than in UC or colonoscopy-negative controls. In contrast, CA and CDCA tended to be higher in UC than in CRC, while the colonoscopy-negative control group generally showed a pattern closer to UC.

Table 2. Comparison of fecal bile acid profiles between ulcerative colitis and colorectal cancer

Parameter	Ulcerative colitis (n=40)	Colorectal cancer (n=40)	*p*-value[a]
	Median (interquartile range)	Median (interquartile range)	
Primary bile acid			
Cholic acid (CA), nmol/g	15.425 (51.510)	0.795 (12.170)	<0.001**
Chenodeoxycholic acid (CDCA), nmol/g	7.380 (45.220)	0.820 (2.560)	0.001*
Total primary bile acid, nmol/g	23.805 (81.830)	1.450 (18.500)	<0.001**
Secondary bile acid			
Deoxycholic acid (DCA), nmol/g	4.665 (14.080)	28.635 (92.410)	<0.001**
Lithocholic acid (LCA), nmol/g	190.725 (2036.250)	13381.035 (36259.850)	<0.001**
Total secondary bile acid, nmol/g	195.240 (2060.683)	13393.675 (36409.242)	<0.001**
Total bile acid, nmol/g	450.760 (2187.715)	13397.010 (36411.143)	<0.001**
Secondary-to-primary bile acid ratio	8.088 (154.236)	6382.478 (22682.906)	<0.001**

Total bile acid variables and the secondary-to-primary bile acid ratio were mathematically derived
[a] Analysis was performed using the Mann-Whitney test
* Statistically significant at $p<0.01$
** Statistically significant at $p<0.001$

Table 3. Fecal bile acid profiles in ulcerative colitis, colorectal cancer, and colonoscopy-negative controls

Parameter	Colonoscopy-negative control (n=20)	Ulcerative colitis (n=40)	Colorectal cancer (n=40)	*p*-value[a]	Post hoc	
	Median (IQR)	Median (IQR)	Median (IQR)		Pair	*p*-value[b]
Primary bile acid						
Cholic acid (CA), nmol/g	10.580 (88.330)	15.425 (51.510)	0.795 (12.170)	<0.001***	Control vs CRC	0.012†
					Control vs UC	0.520
					UC vs CRC	<0.001†
Chenodeoxycholic acid (CDCA), nmol/g	5.085 (33.440)	7.380 (45.220)	0.820 (2.560)	0.002**	Control vs CRC	0.025
					Control vs UC	0.475
					UC vs CRC	0.001†
Total primary bile acid, nmol/g	12.525 (126.665)	23.805 (81.830)	1.450 (18.500)	<0.001***	Control vs CRC	0.011†
					Control vs UC	0.530
					UC vs CRC	<0.001†
Secondary bile acid						
Deoxycholic acid (DCA), nmol/g	4.575 (37.560)	4.665 (14.080)	28.635 (92.410)	<0.001***	Control vs CRC	0.003†
					Control vs UC	0.981
					UC vs CRC	<0.001†
Lithocholic acid (LCA), nmol/g	204.130 (1544.240)	190.725 (2036.250)	13381.035 (36259.850)	<0.001***	Control vs CRC	<0.001†
					Control vs UC	0.790
					UC vs CRC	<0.001†
Total secondary bile acid, nmol/g	237.695 (1544.053)	195.240 (2060.683)	13393.675 (36409.242)	<0.001***	Control vs CRC	<0.001†
					Control vs UC	0.814
					UC vs CRC	<0.001†
Total bile acid, nmol/g	400.405 (1508.303)	450.760 (2187.715)	13397.010 (36411.143)	<0.001***	Control vs CRC	<0.001†
					Control vs UC	0.863
					UC vs CRC	<0.001†
Secondary-to-primary bile acid ratio	9.424 (2163.286)	8.088 (154.236)	6382.478 (22682.906)	<0.001***	Control vs CRC	<0.001†
					Control vs UC	0.888
					UC vs CRC	<0.001†

CRC: colorectal cancer; IQR: interquartile range; UC: ulcerative colitis
[a] Overall comparisons were performed using the Kruskal–Wallis test
[b] Pairwise comparisons were performed using the Mann–Whitney U test with Bonferroni correction; † $p<0.017$ was considered statistically significant
Total bile acid variables and the secondary-to-primary bile acid ratio were mathematically derived
* Statistically significant at $p<0.05$
* Statistically significant at $p<0.01$
** Statistically significant at $p<0.001$

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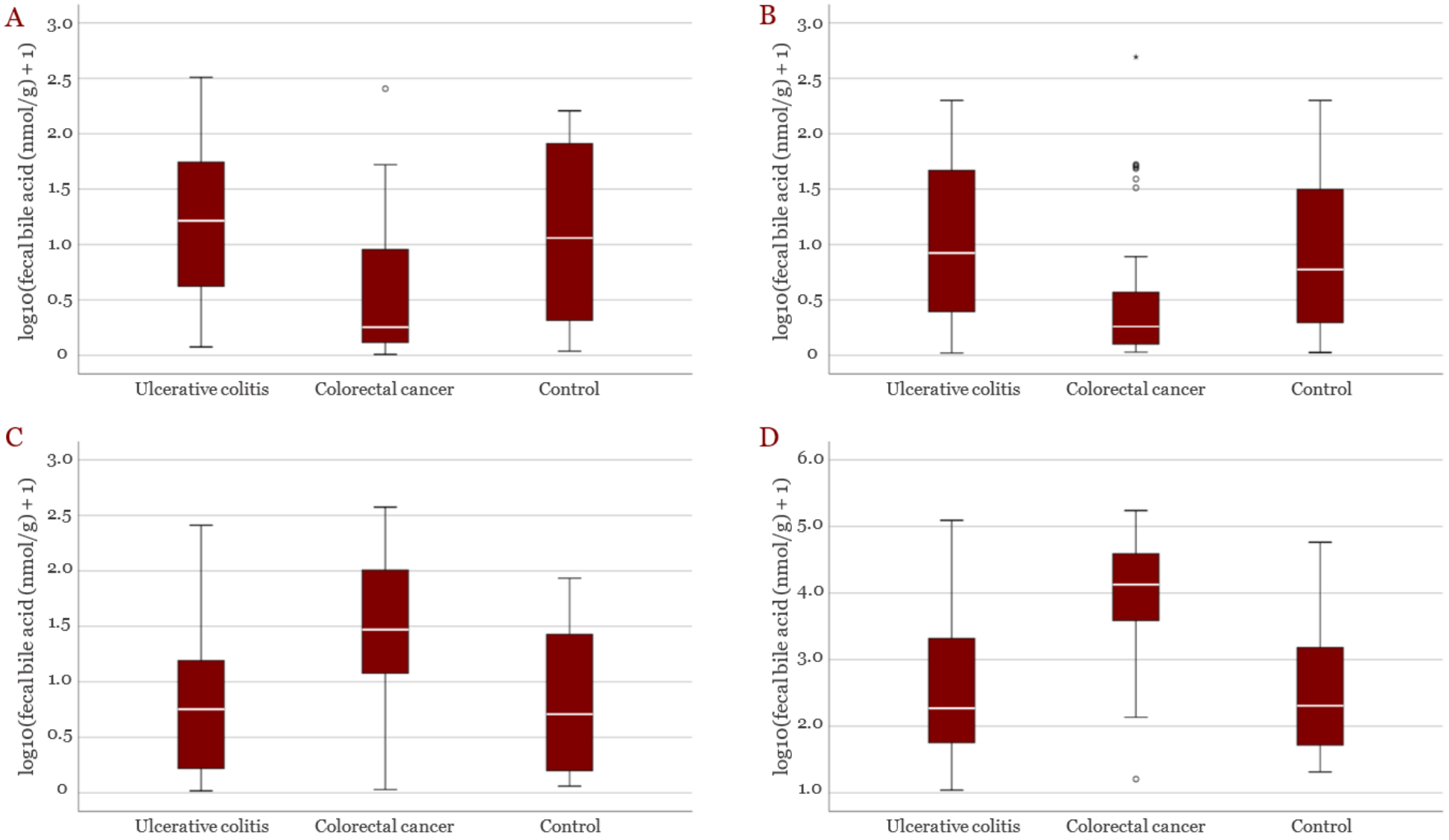

Figure 2. Distribution of fecal bile acid components in the ulcerative colitis, colorectal cancer, and colonoscopy-negative control groups. Boxplots show log10(x+1) transformed levels of (A) cholic acid (CA), (B) chenodeoxycholic acid (CDCA), (C) deoxycholic acid (DCA), and (D) lithocholic acid (LCA). The data were transformed for graphical visualization due to their wide numerical range.

Discussion

Findings from the present study revealed clear differences in the fecal bile acid profile between patients with UC and patients with CRC. UC was related to higher levels of CA, CDCA, and total primary bile acids. In contrast, CRC showed higher levels of DCA, LCA, total secondary bile acids, total bile acids, and the secondary-to-primary bile acid ratio. These findings show that UC and CRC are not only different clinically and histopathologically, but also have different luminal metabolic patterns. The inclusion of a colonoscopy-negative control group also showed that the biggest difference was mainly related to CRC.

In the present study, the CRC group was older than the UC group, and presenting complaints differed across the study groups. Although these findings reflect differences in the clinical characteristics of the included populations, they were not the primary focus of this study. As established by previous literature, demographic variables such as age can inherently alter metabolic profiles [5,8]. Therefore, it is possible that these underlying clinical differences influenced the unadjusted bile acid comparisons observed in the current study.

In contrast, according to a previous study, UC is more often diagnosed in young and productive-age populations [9]. Although the UC group in this study was younger than the CRC group, the relationship between UC and colorectal malignancy remains biologically relevant because the risk of cancer increases with longer disease duration, wider disease extent, and persistent inflammatory activity.

Based on the findings of the present study, the higher CA and CDCA concentrations in UC than in CRC may be compatible with differences in the microbial conversion of primary into secondary bile acids. In line with a previous report, this conversion involves bacterial deconjugation and 7α-dehydroxylation and may be affected by intestinal inflammation and dysbiosis [21]. Previous studies have reported altered bile acid metabolism and microbial metabolic function in inflammatory bowel disease [10]. However, microbiota composition and bile acid-transforming enzyme activity were not measured in the present study; therefore, the mechanism underlying the observed differences cannot be determined.

The higher fecal DCA and LCA concentrations observed in the CRC group of the present study are consistent with previous evidence linking these secondary bile acids to colorectal carcinogenesis. DCA and LCA have been associated with DNA damage, oxidative stress, activation of inflammatory pathways, impaired apoptosis, and increased proliferation of colonic epithelial cells [12,13]. However, the present cross-sectional findings do not establish that these bile acids caused or promoted CRC. The observed concentrations may also have resulted from changes associated with the tumor, intestinal transit, diet, or gut microbiota.

In the present study, the secondary-to-primary bile acid ratio was markedly higher in CRC, but it also showed wide variability. This ratio may become unstable when the total primary bile acid concentration in the denominator is very low. Therefore, the ratio was interpreted as an exploratory derived measure and not as an independent finding. No post-hoc exclusion threshold was applied because a minimum denominator value had not been prespecified. However, the simultaneous increase of DCA, LCA, total secondary bile acids, and total bile acids shows that this ratio finding was not a separate statistical finding, but part of a wider metabolic pattern. Existing literature indicates that gut bacterial metabolites can influence CRC risk in either protective or procarcinogenic directions, depending on the type of metabolite and its biological context [22].

Herein, the three-group analysis provides important additional context. The absence of statistically significant differences between UC and colonoscopy-negative controls should not be interpreted as evidence of metabolic equivalence or normal bile acid metabolism. The control participants underwent colonoscopy for clinical indications and were not healthy volunteers. Some participants had gastrointestinal symptoms, including chronic diarrhea, which may affect intestinal transit and fecal bile acid concentrations. In addition, routine histological assessment was not performed in this group.

UC itself is also a heterogeneous disease. Previous studies have shown that inflammatory activity, disease duration, lesion extent, treatment exposure, dietary pattern, nutritional status, and microbiota composition can independently influence fecal bile acid profiles [11,23]. The presence of these unmeasured confounding factors may explain why group-level differences were difficult to detect in our current colonoscopy-negative control group.

On the other hand, the CRC group showed consistent differences compared with both the UC and colonoscopy-negative control groups. This finding is in line with a previous report that demonstrated changes in fecal metabolites in CRC, including increased secondary bile acids compared with healthy controls [24]. Building upon those prior findings, the present study further highlights that the increase in secondary bile acids is substantially more prominent in colorectal malignancy than in chronic inflammation represented by UC. These results support fecal bile acids as candidates for further biomarker research, especially when integrated with clinical variables, microbiota analysis, and broader metabolomic profiling. These findings should be considered exploratory and hypothesis-generating.

Several limitations should be considered. First, this was a single-center cross-sectional study; therefore, temporal or causal relationships between fecal bile acid concentrations and disease development could not be determined. Second, fecal samples were collected only once, 24 hours after colonoscopy and bowel preparation. Although the same protocol was applied to all groups, bowel cleansing may have affected intestinal transit, microbiota composition, fecal water content, and metabolite concentrations. Concentrations were normalized to wet fecal weight without additional correction for water content or dilution. Third, the control group was small and consisted of colonoscopy-negative participants rather than healthy volunteers. Some had gastrointestinal symptoms, including chronic diarrhea, and routine histological assessment was not performed. Fourth, UC duration, disease extent, and histological activity, as well as detailed CRC histological characteristics, obstruction, and metastatic sites, were not systematically available. Finally, all comparisons were unadjusted. Important potential confounders, including age, albumin, diet, medication exposure, intestinal transit, and microbiota composition, may have influenced the findings. Multivariable adjustment was not performed because of the limited sample size and incomplete availability of these variables.

Conclusion

Fecal bile acid concentrations, specifically CA, CDCA, DCA, and LCA, differed between the UC and CRC groups. Compared with UC, CRC showed lower CA and CDCA concentrations and higher DCA and LCA concentrations. In the three-group analysis, the statistically significant differences mainly reflected the distinct profile of the CRC group. UC and colonoscopy-negative controls had similar fecal bile acid concentrations in stool samples collected after standardized bowel preparation. However, this similarity should not be interpreted as evidence that patients with UC have a normal fecal bile acid profile, particularly because the measurements were obtained from stool samples collected after bowel preparation. Larger prospective studies using samples collected before bowel preparation and with better control of potential confounding factors are needed.

Ethics approval

The study was approved by the Health Research Ethics Committee of Dr. Zainoel Abidin General Hospital (270/ETIK-RSUDZA/2025). Each subject received information about the study objectives, procedures, benefits, and risks, and written informed consent was obtained before participation. Subject identities were protected using study codes.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized during the data collection, analysis, or visualization stages of this study. During the preparation of this manuscript, the authors utilized Google Gemini solely for the purposes of English translation and grammatical correction. After using this tool, the authors reviewed and edited the manuscript and take full responsibility for the final content of the publication.

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