

Original Article

Gut metabolite alterations in colorectal cancer: Fecal analysis of short-chain fatty acids and secondary bile acids

Anurag Mehta¹, Smreti Vasudevan², Neelam Sachdeva¹, Sneha Goel², Madhurarekha Chinnaboina³

Departments of ¹Laboratory and Transfusion Services, ²Research, Rajiv Gandhi Cancer Institute and Research Centre, New Delhi, ³Diagnostics, Vijayrekha Life Sciences, Hyderabad, Telangana, India.

*Corresponding author:

Smreti Vasudevan,
Department of Research, Rajiv
Gandhi Cancer Institute and
Research Centre, New Delhi,
India.

smreti@gmail.com

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ABSTRACT

Objectives: The gut metabolome of colorectal cancer (CRC) patients remains inadequately characterized. This study aimed to compare fecal short-chain fatty acid (SCFA) and secondary bile acid levels between patients with CRC and normal subjects.

Materials and Methods: Stool specimens were collected from patients with CRC and normal individuals. Fecal samples were extracted with isopropanol: acetonitrile:water (3:2:2 ratio), derivatized with ethyl chloroformate, and were separated and analyzed for acetate, propionate, butyrate, lithocholic acid (LCA), and deoxycholic acid (DCA) by gas chromatography–mass spectrometry. Calibration curves were established with reference standard solutions. Compounds were identified by running the raw data through the National Institute of Standards and Technology mass spectral library. Clinical details were obtained from medical records.

Statistical analysis: Statistical analyses were performed using the Statistical Package for the Social Sciences version 29. Statistical significance was assessed using Pearson's Chi-square, Mann–Whitney U, or Kruskal–Wallis tests. $P < 0.05$ was considered statistically significant.

Results: Higher levels of LCA and DCA were detected in the stool specimens of patients with CRC compared with the normal controls (median 3.56 vs. 1.67 pmol/kg, $P < 0.001$). The acetate, propionate, and butyrate levels did not significantly differ between the groups. The ratio of SCFAs to secondary bile acids was lower in the stool of patients with CRC than in the controls (median 20.64 vs. 49.61, $P < 0.001$).

Conclusions: A positive association between fecal LCA and DCA levels and CRC was observed in our cohort. These findings have implications for non-invasive screening and treatment of CRC.

Keywords: Colorectal cancer, Gas chromatography-mass spectrometry, Inflammation, Low-incidence countries, Stool metabolomics

INTRODUCTION

The incidence of colorectal cancer (CRC) has increased in India, noticeably among young adults and in urban areas. Intestinal inflammation is a major risk factor for CRC, and the mucosal immune system is orchestrated by the native gut microflora. Gut microbiota-derived metabolites, particularly short-chain fatty acids (SCFAs) and bile acids are putative players in CRC pathogenesis. SCFAs, such as acetate, propionate, and butyrate, are produced by the fermentation of indigestible dietary fibers by intestinal bacteria. These metabolites have anti-inflammatory

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effects and are considered beneficial for maintaining luminal health by mediating immune homeostasis. The second class of metabolites is bile acids, which are primarily synthesized from cholesterol in the liver, and subsequently undergo biotransformation by the intestinal flora into secondary bile acids. Lithocholic acid (LCA) and deoxycholic acid (DCA) are the two prominent secondary bile acids produced in the large intestine from bacterial dehydroxylation of cholic acid and chenodeoxycholic acid, respectively. Both LCA and DCA are cytotoxic and are considered to be involved in the development of CRC by promoting inflammation.^[1,2]

Despite the distinctive roles of gut microbial metabolites in disease pathogenesis, data supporting the relationship between CRC, SCFAs, and bile acids from different studies are conflicting. Some studies suggest that high fecal SCFA levels, especially increased levels of butyrate in the gut, attenuate the risk of CRC,^[3,4] whereas others failed to demonstrate a clear predictive pattern.^[5] Many studies propose that a high-fat diet and an elevated secondary bile acid concentration in the stool contribute to CRC development.^[6-10] However, some reports, including a meta-analysis, have failed to show an association between specific bile acid levels and CRC.^[11-13] Most studies have been conducted in Western countries, where the incidence of CRC is high and eating habits and lifestyles markedly differ from those in India. Studies on gut microbial metabolome in patients with CRC in countries with low incidence rates like India remain largely under-represented.^[14,15] There is a paucity of data on our part addressing the relationship between intestinal metabolites and CRC.

In this study, a targeted metabolomic approach using gas chromatography coupled with mass spectrometry (GC-MS) was used to investigate possible differences between fecal SCFA and secondary bile acid levels in patients with CRC and normal individuals. Furthermore, the relation between stool metabolites and clinical parameters in patients with CRC was assessed.

MATERIALS AND METHODS

Ethical approval

The study was initiated following ethical approval from the Institutional Review Board (RGCIRC/IRB-BHR/81/2023) and was conducted according to the ethical principles outlined in the Declaration of Helsinki. All participants provided consent before enrollment, and their clinical data were de-identified to protect their privacy.

Study design

Sample size

This study is exploratory, designed to assess the usefulness of fecal metabolomics in determining whether important fecal

SCFAs (acetate, propionate, and butyrate) and secondary bile acids (LCA and DCA) differ between our cohort of CRC patients and normal individuals. Fecal metabolite levels are influenced by geographic location, dietary habits, and microbiota composition, and these metabolites have not been previously characterized for our region. To address this, a pilot study was undertaken with approximately 18–20 participants in each group,^[16] considering the feasibility and constraints of available resources.

We collected thirty-nine freshly voided stool samples and assayed them by GC-MS. The initial two samples failed in quality measures and were excluded. Therefore, data from 37 participants were assessed.

Type of study

It is a case-control, observational study comprising CRC patients and normal individuals. Stool samples were prospectively obtained from patients with CRC undergoing treatment at our hospital. Age-matched normal volunteers were recruited from the hospital staff for the study.

Inclusion and exclusion criteria

CRC patients

The inclusion criteria for CRC cases were as follows: Age >18 years; confirmed diagnosis of CRC on histopathology; not received chemotherapy and/or radiotherapy in the previous 8 months; not on active antibiotic treatment; and not taking alternate medicines. The exclusion criteria were as follows: Patients with more than one malignancy; family history of CRC in first- or second-degree relatives; patients with inflammatory bowel disease, chronic kidney disease, or liver complaints; and patients who were not able/unwilling to provide stool samples.

Control group

The control belonged to Delhi or adjoining regions; did not suffer from any chronic illness like diabetes or cardiovascular disease, which can affect the gut microbiome; did not report gastrointestinal-related complaints or infections based on symptoms and routine laboratory tests; had a normal bowel habit; had not used antibiotics in the past 3 months; and were willing to be a part of the study and provide their stool sample.

The base population was North India (Delhi, Haryana, Punjab, and Uttarakhand regions).

Data collection

Demographic information about the participants was recorded at the time of sample collection. Clinical parameters

of the patients were retrieved from the hospital medical records.

Sample collection

Stool samples were collected from September 2023 to December 2023. Each participant was provided disposable gloves, a ziplock bag containing an icepack, and a sterile container, all of which were encased in a biohazard bag. The samples were transported within 2 h from collection to our laboratory. About 10–15 g of single passed stool specimen was collected as a whole sample in a single container, labeled, sealed with parafilm, and stored at -80°C . The subjects' compliance with the study was 64%.

The samples were shipped together on dry ice to the Vijayrekha Life Sciences Laboratory in Hyderabad, where fecal metabolite analysis was conducted blinded to the case or control status. All samples were assayed in January 2024.

Sample processing

For the extraction and quantitation of compounds from the stool and analysis by GC-MS, the following procedure was followed. First, the samples were thawed to room temperature, and specimen was taken from the two ends and the middle position of the stool and weighed. Then, approximately 40 mg of each sample was extracted using 1.5 mL of the extraction solvent isopropanol: acetonitrile:water (3:2:2 ratio) by vigorous shaking for 10 min. The extracted sample was derivatized with 80 μL of ethyl chloroformate.

Sample analysis

The samples were separated and analyzed using GC-MS (GC-MS-QP2020 NX, Shimadzu) with capillary column Nukol™ (30 m \times 0.25 mm, df 0.25 μm). The GC-MS instrument was calibrated with the octafluoronaphthalene (OFN) standard for mass accuracy. The samples were injected in splitless mode at 210°C injection port temperature and 250°C ion source temperature. The temperature program was started at 55°C with a holding time of 1 min, increased to 100°C at a rate of $10^{\circ}\text{C}/\text{min}$ with a holding time of 2 min, and increased to 190°C at $30^{\circ}\text{C}/\text{min}$ with a holding time for 1 min. The flow rate of helium gas was 2.5 mL/min, and ionization was at 80 eV. The compounds eluting from the column were ionized in the mass spectrometer for detection. The chromatogram contained the intensity and retention time of each eluting compound. The m/z s for acetate is 60.04, butyrate is 89.1, propionate is 75.08, LCA is 377.5, and DCA is 393.5 which were used in Selected Ion Monitoring (SIM) mode to quantitate using area under the curve as against the standard calibration curve run for each compound. The reference standard solution mix for SCFAs (10 mM, Sigma: CRM46975) was used to establish the calibration curve

ranging from 4 to 100 μM by serial dilution, and DCA and LCA were used for bile acids ranging from 0.001 to 100 nM. To identify the compounds, the raw data were run through the National Institute of Standards and Technology mass spectral library and were quantitated in the SIM mode.

Statistical analysis

The data were summarized using descriptive statistics. Pearson's Chi-square test was applied to determine the association between categorical variables. Non-parametric Mann-Whitney-U test was employed to compare the levels of different SCFAs and bile acids between CRC and control groups. The Kruskal-Wallis test was used to compare more than two categories. A $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences version 29.0 (IBM SPSS Inc., Chicago, IL, USA).

RESULTS

The characteristics of the study group are summarized in Table 1. The relative levels of fecal SCFAs and secondary bile acids in patients with CRC and normal subjects are presented in Figure 1a. As observed from the heat map, the concentration of acetate, propionate, and butyrate in patients with CRC tended to be lower than those in normal subjects; however, this difference did not reach statistical significance [Table 1 and Figure 1b]. Notably, higher levels of LCA and DCA were detected in the stool of patients with CRC compared with normal controls (median values of 3.56 vs. 1.67 pmol/kg, $P < 0.001$) [Table 1 and Figure 1b].

The ratio of the sum of SCFAs to secondary bile acids in the stool of patients with CRC was 20.64 and 49.61 in the control group ($P < 0.001$).

The clinicopathological features of patients with CRC are presented in Table 2. The majority were colon cancer cases (61.11%) with adenocarcinoma histology (94.44%). Eight patients (44.44%) had metastatic disease, and three tumors (16.67%) were mismatch repair (MMR) protein deficient [Table 2].

Next, we examined whether specific SCFAs or bile acids were associated with distinctive features of CRC. Table 3 summarizes the association between the fecal SCFA and secondary bile acid concentrations with clinical features including baseline CEA levels of the CRC patients. It was observed that butyrate levels were significantly higher in left-sided lesions than in right-sided lesions (median values of 15.00, range: 6.88–19.80 vs. 11.20, range: 9.03–14.00 mmol/kg, $P = 0.02$) [Table 3]. We found no significant variations in SCFAs or bile acids among patients with CRC in relation to age, tumor location, stage, MMR protein status, or baseline CEA levels.

Table 1: Characteristics of the study group (n=37).

Variable	Total	CRC patients	Normal controls	P-value
	n=37 (%)	n=18 (%)	n=19 (%)	
Age (years)				
Mean±SD	54.97±14.62	55.78±15.79	54.21±13.81	0.75 ^a
Gender				
Male	23 (62.16)	10 (55.56)	13 (68.42)	0.51 ^b
Female	14 (37.84)	8 (44.44)	6 (31.58)	
Hemoglobin (g/dL)				
Median (Range)	11.60 (8.20–15)	10.00 (8.20–11.90)	13.80 (8.70–15.00)	<0.001 ^c
Fecal short-chain fatty acid (mmol/kg)				
Acetate, Median (Range)	50.70 (23.10–88.60)	48.90 (28.70–63.50)	51.00 (23.10–88.60)	0.22 ^c
Propionate, Median (Range)	14.50 (11.20–19.80)	13.70 (11.80–17.60)	14.60 (11.20–19.80)	0.27 ^c
Butyrate, Median (Range)	13.50 (6.88–21.50)	14.20 (6.88–19.80)	13.50 (10.80–21.5)	0.87 ^c
Fecal secondary bile acids (pmol/kg)				
LCA+DCA, Median (Range)	2.66 (1.02–7.45)	3.56 (1.70–7.45)	1.67 (1.02–5.23)	<0.001 ^c

^aStudent's unpaired t-test; ^bPearson's Chi-square test; ^cMann-Whitney U-test, LCA: Lithocholic acid; DCA: Deoxycholic acid, SD: Standard deviation, CRC: Colorectal cancer

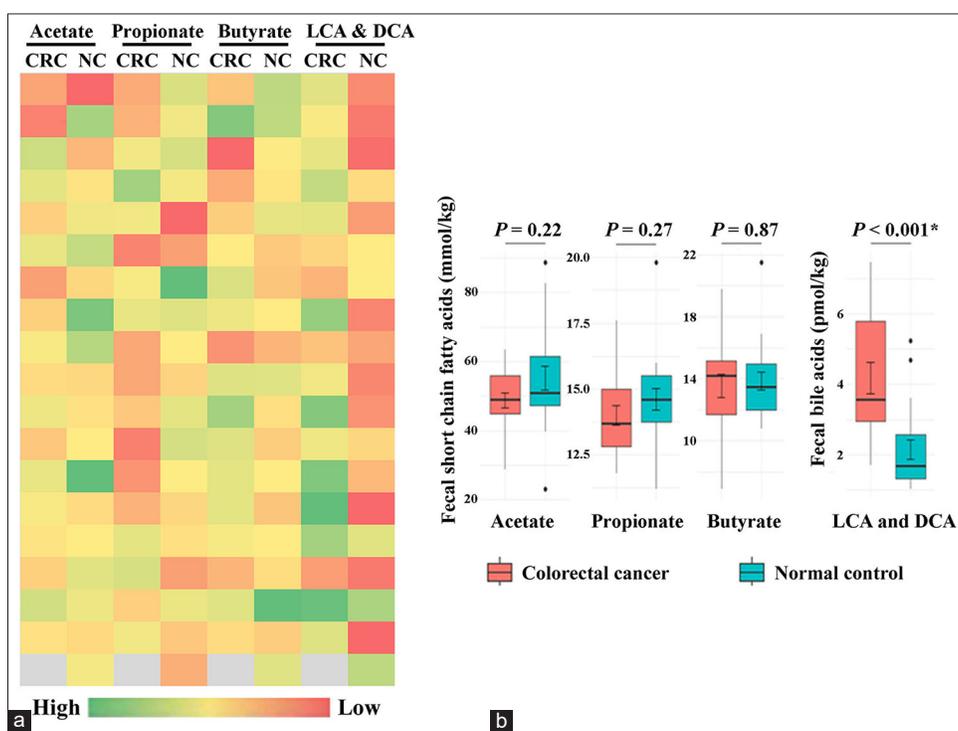


Figure 1: (a) Metabolic heat map and (b) Box and whisker plot comparing the levels of fecal acetate, propionate, butyrate, lithocholic acid (LCA), and deoxycholic acid (DCA) between colorectal cancer (CRC) patients and normal controls (NC). The levels of LCA and DCA are significantly increased in the stool of CRC patients than the NC group. * denotes statistical significance.

DISCUSSION

Gut health is considered important for overall health and disease prevention. The accumulation of harmful microbial metabolites or a reduction in beneficial metabolites in the gut can contribute to the development of CRC tumors. In

this study, we assessed the fecal levels of major SCFAs and bile acids and their implications for CRC. Specifically, we analyzed acetate, propionate, and butyrate, which are the most abundant SCFAs and major sources of energy for colonocytes, as well as LCA and DCA, which are secondary

Table 2: Clinical characteristics of colorectal cancer patients (n=18 colorectal cancer cases).

Variable	n=18 (%)
Tumor location	
Colon	11 (61.11)
Rectosigmoid	2 (11.11)
Rectum	5 (27.77)
*Tumor laterality	
Right-sided/ proximal tumors	6 (33.33)
Left-sided/ distal tumors	12 (66.67)
Anatomical site of the primary tumor	
Cecum	1 (5.55)
Ascending colon	4 (22.22)
Hepatic flexure	1 (5.55)
Splenic flexure	1 (5.55)
Descending colon	2 (11.11)
Sigmoid colon	2 (11.11)
Rectosigmoid	2 (11.11)
Rectum	5 (27.78)
Tumor histology	
Adenocarcinoma	17 (94.44)
Signet ring cell carcinoma	1 (5.55)
Tumor differentiation	
Well	6 (33.33)
Moderate	9 (50.00)
Poor	3 (16.67)
Tumor stage	
I	1 (5.55)
II	7 (38.89)
III	2 (11.11)
IV	8 (44.44)
*Metastasis	
Yes	8 (44.44)
No	10 (55.56)
Mismatch repair protein	
Deficient	3 (16.67)
Proficient	15 (83.33)
Main presenting symptom	
Abdominal pain	6 (33.33)
Altered bowel habits	2 (11.11)
Bloating	1 (5.55)
Blood in the stool	6 (33.33)
Chronic anemia	1 (5.55)
Constipation	2 (11.11)
Serum CEA (ng/mL)	3.13 (0.37–91.23)
Median (Range)	

(Contd...)

Table 2: (Continued)

Variable	n=18 (%)
Smoking history	
Yes	2 (11.11)
No	16 (88.89)
Alcohol consumption	
Yes	1 (5.55)
No	17 (94.44)
Food choice	
Vegetarian	9 (50.00)
Both vegetarian and non-vegetarian	9 (50.00)
Obesity	
Yes	2 (11.11)
No	16 (88.89)

*Left-sided: Distal CRC tumors located in the splenic flexure, descending colon, sigmoid colon, rectosigmoid, or rectum. Right-sided: Proximal CRC tumors located in the cecum, ascending colon, or hepatic flexure.
 *The metastatic site includes the lymph nodes, lung, or peritoneum.
 CEA: Carcinoembryonic antigen, CRC: Colorectal cancer

bile acids known to be released in the gut by microbial action and are linked to CRC pathogenesis.

Significantly higher levels of LCA and DCA were observed in patients with CRC than in the control group. LCA and DCA are among the most hydrophobic bile acids, and their chronic exposure is considered toxic to colonocytes. At physiologically high concentrations, they act as oncometabolites by inducing oxidative stress, DNA damage, apoptosis, and favoring harmful mutations.^[17,18] It is not clear whether bile acids act as tumor initiators or promoters. Previous studies have linked the consumption of a diet rich in animal fats and proteins to elevated levels of secondary bile acids such as LCA and DCA in feces, which have been associated with an increased risk of CRC.^[6-8] In contrast, SCFAs which are derived from the fermentation of dietary fibers in the gut are considered beneficial against CRC. Increased levels of stool SCFAs are associated with reduced risk of CRC.^[3,4] Table 4 compares the findings of the discussed studies.

In our study, the concentrations of acetate, propionate, and butyrate tended to be higher in the control group than in the CRC group, but the difference was not statistically significant. This could be due to the small sample size resulting in a lack of statistical power to detect differences. In addition, inter-individual variabilities in diet, SCFA production, or altered absorption by gut mucosa may also negate differences in SCFA levels between patients with CRC and the normal control group.^[19] Most of the patients in our CRC cohort had advanced disease, including two locally advanced stage II, two stage III, and eight stage IV cases. Advanced CRC is characterized by tumor hypoxia and enhanced glycolysis, leading to more lactate formation. The conversion of lactate

Table 3: Association of levels of fecal short-chain fatty acids and secondary bile acid levels with clinical parameters in colorectal cancer patients (n=18 cases).

Variable	n	Acetate	Propionate	Butyrate	DCA+LCA
		(mmol/kg)	(mmol/kg)	(mmol/kg)	(pmol/kg)
		Median (Range)	Median (Range)	Median (Range)	Median (Range)
Age group (years)					
≤40	4	50.55 (43.20–62.00)	13.05 (11.80–15.00)	15.00 (12.00–15.20)	5.01 (3.18–7.25)
>40	14	48.90 (28.70–63.50)	14.20 (11.90–17.60)	13.75 (6.88–19.80)	3.53 (1.70–7.45)
P-value ^a		0.75	0.24	0.46	0.24
Tumor location					
Colon	11	48.60 (34.80–57.10)	14.80 (11.90–17.60)	13.50 (9.03–15.40)	3.64 (1.70–7.45)
Rectosigmoid	2	59.85 (56.20–63.50)	13.65 (12.30–15.00)	10.89 (6.88–14.90)	4.98 (3.42–6.54)
Rectum	5	44.90 (28.70–62.00)	13.60 (11.80–15.00)	15.20 (12.00–19.80)	3.48 (2.89–7.25)
P-value ^b		0.14	0.66	0.11	0.61
*Tumor laterality					
Right-sided	6	50.80 (45.00–57.10)	14.15 (11.90–17.60)	11.20 (9.03–14.00)	3.01 (1.70–5.46)
Left-sided	12	47.85 (28.70–63.50)	13.70 (11.80–15.30)	15.00 (6.88–19.80)	3.59 (1.99–7.45)
P-value ^a		0.71	0.54	0.02	0.19
Tumor stage					
I and II	8	51.95 (35.80–63.50)	14.30 (11.90–17.60)	13.10 (6.88–18.20)	3.67 (2.38–6.43)
III and IV	10	47.10 (28.70–62.00)	13.45 (11.80–15.90)	14.90 (9.03–19.80)	3.33 (1.70–7.45)
P-value ^a		0.31	0.69	0.35	0.72
MMR protein status					
Deficient	3	35.80 (34.80–56.10)	12.90 (11.90–14.80)	13.50 (11.60–15.40)	2.38 (1.99–3.64)
Proficient	15	49.20 (28.70–63.50)	13.80 (11.80–17.60)	14.40 (6.88–19.80)	3.70 (1.70–7.45)
P-value ^a		0.26	0.25	0.95	0.14
*CEA level					
Moderately elevated	14	50.80 (28.70–63.50)	14.40 (11.80–17.60)	14.20 (6.88–19.80)	4.11 (1.70–7.45)
Highly elevated	4	41.50 (34.80–56.10)	12.85 (11.90–14.80)	14.35 (11.60–15.40)	2.75 (1.99–3.64)
P-value ^a		0.28	0.13	0.57	0.10

^aMann–Whitney–U test; ^bKruskal–Wallis test. ^{*}Left-sided: Distal CRC tumors located in the splenic flexure, descending colon, sigmoid colon, rectosigmoid, or rectum. Right-sided: Proximal CRC tumors located in the cecum, ascending colon, or hepatic flexure. ^{*}Moderately elevated CEA: ≤10 ng/mL and highly elevated CEA level: >10 ng/mL. MMR: Mismatch repair, CEA: Carcinoembryonic antigen, CRC: Colorectal cancer, LCA: Lithocholic acid, DCA: Deoxycholic acid

to SCFAs might further balance acetate, propionate, and butyrate levels in patients with CRC.^[20] Our results are similar to those of a large cohort study by Sze *et al.*, which reported a weak association between fecal SCFAs and tumor burden in patients with CRC.^[5]

While specific studies addressing the fecal metabolome of CRC patients in our region are scarce, a study from South India has identified CRC-associated fecal microbiome changes including enrichment of pathogenic taxa in the stool of CRC patients.^[15] Since the gut microbiome and microbiota-derived metabolites show region-specific variations, we restricted this study to the North Indian cohort of CRC patients and control. A previous study by Dhakan *et al.* (2019) involving healthy individuals from Central and South India reported that the Indian gut microbiome and downstream

metabolome are unique and are predominantly influenced by diet and regional factors.^[14] Adding to the study, Gupta *et al.* (2019) observed a distinct CRC-related microbiome profile, marked by differences in the fecal metabolites of CRC patients compared to healthy individuals.^[21] The authors have reported a strong association of *Bacteroides intestinalis* with the microbiome of Indian CRC patients. This gut bacterium is known to transform primary bile acids to secondary bile acids implicated in CRC and aligns with the present study's findings.

Interestingly, we observed a greater butyrate abundance in the feces of patients with distal than proximal CRC tumors. This may be due to differences in microbial distribution and tumor metabolism. It has been reported that right and left-sided CRC tumors harbor distinct microbiomes, leading

Table 4: Comparative table summarizing relationship of fecal SCFAs and secondary bile acid with CRC that were observed in different studies.

Study/Place	Cohort	SCFAs studied	Secondary bile acids	Key findings	Analytical method
Chen <i>et al.</i> , 2013 ^[3]	Advanced CRC, n=344	Acetic acid	Not assessed	High intake of fiber and increased fecal SCFA levels are associated with reduced risk of advanced CRC.	Gas chromatography
China	Healthy control, n=344	Propionic acid			
		Isobutyric acid			
		Butyric acid			
		Isovaleric acid			
Yusuf <i>et al.</i> , 2018 ^[4]	CRC patients, n=14	Acetate	Not assessed	CRC patients have lower fecal levels of acetate, propionate and butyrate than non CRC group.	Gas chromatography
Indonesia	Non CRC patients, n=14	Propionate			
		Butyrate			
Sze <i>et al.</i> , 2019 ^[5]	Adenoma cases, n=198	Acetate	Not assessed	No significant association between fecal SCFAs and CRC tumors.	High-performance liquid chromatography
USA	Colon carcinoma, n=120	Propionate			
	Healthy control, n=172	Butyrate			
Kamano <i>et al.</i> , 1999 ^[6]	CRC patients, n=63	Not assessed	DCA	DCA levels in stool were higher in CRC patients than healthy subjects.	Enzyme-linked immunosorbent assay
Japan	Healthy control, n=47				
Kawano <i>et al.</i> , 2010 ^[8]	CRC patients, n=366	Not assessed	DCA	Fecal DCA concentration was significantly higher in CRC patients and higher levels was associated with larger tumor and disease recurrence.	Enzyme-linked immunosorbent assay
Japan	Control, n=24				
Kim <i>et al.</i> , 2020 ^[9]	CRC patients, n=36	Not assessed	Secondary bile acids	Elevated fecal secondary bile acid levels in adenoma patients than the controls	Ultra-performance liquid chromatography-tandem mass spectrometry (untargeted metabolomics)
USA	Advanced adenoma, n=102				
	Matched control, n=102				
Little <i>et al.</i> , 2002 ^[12]	CRC patients, n=45	Not assessed	LCA	No significant association between fecal LCA and DCA levels and CRC. High fecal calcium level was associated with reduced risk of adenoma and CRC.	Gas chromatography
Nottingham	Adenoma, n=129		DCA		
	Fecal occult blood-negative controls, n=155				

CRC: Colorectal cancer; SCFA: Short-chain fatty acid, LCA: Lithocholic acid; DCA: Deoxycholic acid

to variations in microbial metabolites depending on tumor location.^[22] Future research exploring the composition and function of the microbiome with respect to tumor laterality is expected to provide additional insights into these differences.

Strengths and limitations of the study

The strengths of this study include its prospective design, the use of a sensitive analytical technique for metabolite detection, and the availability of complete clinical dataset.

To ensure sample stability, the samples were frozen immediately after collection. The containers were sterilized before use and sealed with parafilm after collection for storage. Transportation was done in dry ice to preserve sample integrity. Further, sample extraction was performed with precautions, like avoiding freeze-thaw cycles, and maintaining the samples at 4°C during extraction, following optimized protocols to ensure sample stability. All reagents used were of analytical grade. The GC-MS instrument was calibrated each time with the reference standard (OFN), and consistent retention time was ensured. At the post-analytical level, the spectra were subjected to baseline correction and normalized against peak intensity before detection and identification.

However, being an early report, there are some limitations to this study. First, we collected fecal samples at a single time point. This limits our ability to assess diurnal fluctuations in SCFA and bile acid levels. Second, the dietary habits of both patients with CRC and normal subjects were not controlled, which could have influenced the results. Third, the sample size was small, with a higher proportion of patients with advanced CRC.

CONCLUSIONS

The stool metabolite profile of patients with CRC was considerably altered compared with that of normal individuals. A positive association between fecal LCA and DCA levels and CRC was observed in our cohort. Future studies that consider the relationships of these metabolites with dietary patterns, lifestyle factors, and microbiome composition will further improve our understanding of these relationships.

Author contribution: AM: Concept and design, funding acquisition, critical revision of the manuscript and final approval; SV: Concept and design, funding acquisition, sample acquisition, project management, analysed data and wrote the manuscript; NS: Sample acquisition, data interpretation, analysis and final approval; SG: Statistical analysis and final approval; MC: Experimental studies, analysed results, manuscript writing, GC-MS supervision and final approval. The manuscript has been read and approved by all the authors.

Ethical approval: The research/study was approved by the Institutional Review Board at Rajiv Gandhi Cancer Institute and Research Centre, Institutional Review Board, number RGCIRC/IRB-BHR/81/2023, dated 28th August 2023.

Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest: There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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