

Research Paper

Serum Unsaturated Free Fatty Acids: A Potential Biomarker Panel for Early-Stage Detection of Colorectal Cancer

Yaping Zhang¹, Chengyan He², Ling Qiu³, Yanmin Wang⁴, Xuzhen Qin³, Yujie Liu¹, Zhili Li¹✉

1. Department of Biophysics and Structural Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & School of Basic Medicine, Peking Union Medical College, Beijing, China;
2. Clinical Lab Diagnosis, China-Japan Union Hospital, Jilin University, Changchun, China;
3. Department of Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China;
4. Department of Clinical Laboratory, Heze Municipal Hospital, Heze, China.

✉ Corresponding author: Zhili Li, Department of Biophysics and Structural Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & School of Basic Medicine, Peking Union Medical College, 5 Dongdan San Tiao, Beijing 100005, P.R. China. Tel/Fax: +86-10-69156479; E-mail: lizhili@ibms.pumc.edu.cn.

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Abstract

Background: To screen biomarkers to differentiate early-stage colorectal cancer (CRC) from benign colorectal disease (BCD) and healthy controls.

Materials & Methods: Quantitative and qualitative analysis of C_{16:1}, C_{18:3}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6} in 185 healthy controls, 55 patients with BCD, and 139 patients with CRC was performed. Comparisons of their levels in between CRC patients, BCD patients, and healthy controls were performed using Mann–Whitney U test.

Results: Serum levels of C_{16:1}, C_{18:3}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6} in CRC patients were significantly decreased compared with healthy controls and BCD patients. A combination of C_{16:1}, C_{18:2}, C_{20:4}, and C_{22:6} has excellent diagnostic performance to differentiate early-stage CRC patients from healthy controls plus BCD patients, with an AUC of 0.926, a sensitivity of 84.6%, and a specificity of 89.8%.

Conclusions: Serum levels of C_{16:1}, C_{18:2}, C_{20:4}, and C_{22:6} could be diagnostic indicators of early-stage CRC patients.

Key words: unsaturated free fatty acids; biomarker panel; early detection; serum; colorectal cancer.

Introduction

Colorectal cancer (CRC) is the third most common cancer and the fifth most frequent cause of cancer deaths in China [1]. Clinical data show that 5-year survival rate of early-stage CRC postoperative patients is around 90% [2]. However, most of CRC patients were diagnosed at advanced-stage due to its asymptomatic and poor diagnostic techniques. Early screening is an effective way to reduce the morbidity and mortality [3]. Colonoscopy is a primary screening tool for diagnosing CRC because of its excellent diagnostic accuracy. However, invasive nature limits its

effectiveness. The fecal occult blood test is an economical, noninvasive, and widely available screening method for diagnosing CRC, with low sensitivity [4, 5]. Stool DNA test [6] has better sensitivity and specificity than the fecal occult blood test, but the high cost has limited its use. Fecal immunochemical test is more sensitive in detecting both CRC and adenomas than the fecal occult blood test, with the sensitivities of 25% to 100% and the specificities of >90% [7]. So, it is necessary to develop low-cost, less invasive, high-sensitivity, and high-specificity screening

methods for early diagnosis of CRC.

Cancer involves in numerous metabolic pathways such as glycolysis and tricarboxylic acid cycle [8]. Recent studies have shown that metabolite profile could be a diagnostic tool to differentiate CRC from healthy controls [9, 10], but most of these studies failed to quantify the levels of metabolites and to evaluate their diagnostic accuracies to detect CRC. Study on cancer cells also displayed specific changes in varied facets of lipid metabolism [11]. As the main source of energy in body, free fatty acids (FFAs) are the vital substrates for lipid synthesis, which could better interpret the mechanisms of disease and physiological processes. Unsaturated FFAs provide a large energy during cellular proliferation which is closely associated with malignancy [12]. Previous studies mainly focused on the effects of dietary unsaturated fatty acids on cancer cells, which still remains controversial [13]. Our previous study has indicated that the level ratios of serum $C_{18:2}/C_{18:1}$ and $C_{18:3}/C_{18:1}$ have excellent diagnostic ability to differentiate early-stage pancreatic cancer from pancreatitis plus controls, with an area under receiver operating characteristic (ROC) curve (AUC) of 0.912, a sensitivity of 86.7%, and a specificity of 88.6%, and that the panel of $C_{16:1}$, $C_{18:3}$, $C_{18:2}$, $C_{18:1}$, $C_{20:4}$, and $C_{22:6}$ has excellent diagnostic ability to differentiate advanced-stage pancreatic cancer from controls plus pancreatitis, with an AUC value of 0.989, a sensitivity of 91.7%, and a specificity of 98.6% [14] and to differentiate early-stage breast cancer from healthy controls, with an AUC value of 0.953, a sensitivity of 83.3%, and a specificity of 87.1% [15]. Our previous studies have also found that serum FFA levels are closely correlated with lung cancer [16] and gastric cancer [17].

In this study, we performed simultaneous quantitative and qualitative analysis of serum $C_{16:1}$, $C_{18:3}$, $C_{18:2}$, $C_{18:1}$, $C_{20:4}$, and $C_{22:6}$ in 379 participants using chip-based direct-infusion nanoESI-Fourier transform ion cyclotron resonance mass spectrometry (CBDI-nanoESI-FTICR MS) in the negative ion mode. The linearity and stability test were performed to evaluate feasibility of the platform. The results indicate that a combination of $C_{16:1}$, $C_{18:2}$, $C_{20:4}$, and $C_{22:6}$ has excellent diagnostic accuracy for differentiating early-stage CRC patients from healthy controls plus patients with BCDs, with an AUC value of 0.926, a sensitivity of 84.6%, and a specificity of 89.8%.

Materials & methods

Chemicals & reagents

HPLC-grade methanol, ethanol, and acetonitrile were supplied by Fisher Scientific (Pittsburg, PA, USA). The ultrapure water was purified by a Milli-Q

system (Millipore, USA). Palmitoleic acid ($C_{16:1}$), heptadecenoic acid ($C_{17:1}$), linolenic acid ($C_{18:3}$), linoleic acid ($C_{18:2}$), oleic acid ($C_{18:1}$), arachidonic acid ($C_{20:4}$), heneicosanoic acid ($C_{21:0}$), docosahexaenoic acid ($C_{22:6}$), and ammonium acetate (all with purity of > 99%, except $C_{22:6}$, purity of > 98%) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA).

Participants and study design

139 CRC patients were recruited from China-Japan Union Hospital (n=59) and Peking Union Medical College Hospital (n=80). Evaluations of hematochemical parameters were performed in both hospitals. Tumors were further classified into early stage (stage I or II, n = 39) and advanced stage (stage III or IV, n = 75) based on the Union for International Cancer Control (UICRC) tumor-node metastasis (TNM) classification. 55 BCD patients were from Peking Union Medical College Hospital. 185 healthy controls were enrolled from Heze Municipal Hospital (n=69) and China-Japan Union Hospital (n=116), and clinical records were reviewed to make sure that these individuals were in health status. All samples were remaining sera after the clinical laboratory examination which were drawn in the morning after about 10 hours overnight fast without bowel preparation. The investigation was divided into a training study and a validation study. CRC patients (n=59) from China-Japan Union Hospital and the age- and gender-matched healthy controls (n=69) from Heze Municipal Hospital were included in the training set. Other 80 CRC patients and 55 BCD patients from Peking Union Medical College Hospital and 116 healthy controls from China-Japan Union Hospital were included in the validation set. The characteristics of all participants are summarized in **Table 1**. The study was approved by the Ethics Review Board at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

Table 1. Characteristics of participants in the study.

	Training Set		Validation Set		
	Controls (n=69)	CRC (n=59)	Controls (n=116)	BCD (n=55)	CRC (n=80)
Male/Female	36/33	34/25	72/44	34/21	36/44
Age(years) Mean±SD	57.9±10.4	59.1±11.4	58.9±10.4	58.2±10.9	59.5±10.3
Range	35-73	34-75	42-78	42-77	42-78
Stage I & II (Early Stage)		1/3			21/14
III & IV (Advanced Stage)		23/15			23/14

SD: standard deviation; CRC: colorectal cancer; BCD: benign colorectal disease.

Standard solutions and sample preparation

Standard solutions were prepared as our own previous study [14]. Briefly, $C_{17:1}$ and $C_{21:0}$ were used

as internal standards (ISs) at the final concentrations of 83.3 nM and 33.3 nM in ethanol. The standard solutions used for generating the calibration curves were prepared by mixing C_{16:1}, C_{18:3}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6} at six different concentrations in ethanol, along with the fixed concentrations of ISs (83.3 nM C_{17:1} and 33.3 nM C_{21:0}). Six different concentrations are as follows: the first mixture: 684.0 nM C_{16:1}, 163.3 nM C_{18:3}, 1137.7 nM C_{18:2}, 1671.7 nM C_{18:1}, 365.7 nM C_{20:4}, and 112.3 nM C_{22:6}; the second: 342.0 nM C_{16:1}, 81.7 nM C_{18:3}, 568.8 nM C_{18:2}, 835.8 nM C_{18:1}, 182.8 nM C_{20:4}, and 56.2 nM C_{22:6}; the third: 68.4 nM C_{16:1}, 16.3 nM C_{18:3}, 113.8 nM C_{18:2}, 167.2 nM C_{18:1}, 36.6 nM C_{20:4}, and 11.2 nM C_{22:6}; the fourth: 34.2 nM C_{16:1}, 8.2 nM C_{18:3}, 56.9 nM C_{18:2}, 83.6 nM C_{18:1}, 18.3 nM C_{20:4}, and 5.6 nM C_{22:6}; the fifth: 13.7 nM C_{16:1}, 3.3 nM C_{18:3}, 22.8 nM C_{18:2}, 33.4 nM C_{18:1}, 7.3 nM C_{20:4}, and 2.3 nM C_{22:6}; and the sixth: 8.6 nM C_{16:1}, 2.0 nM C_{18:3}, 14.2 nM C_{18:2}, 20.9 nM C_{18:1}, 4.6 nM C_{20:4}, and 1.4 nM C_{22:6}. Each of these mixtures was analyzed three times, and the results are expressed as mean ± standard deviation (SD). The calibration equations were constructed between the concentration ratios of fatty acids to ISs (C_{16:1}, C_{18:3}, C_{18:2}, C_{18:1} to C_{17:1} and C_{20:4}, C_{22:6} to C_{21:0}) and their respective corresponding intensity ratios. The third mixture was selected as quality control sample to evaluate the experimental stability. The quality control sample was analyzed once every 10 test samples. Relative SD was calculated based on the intensity ratios of C_{16:1}, C_{18:3}, C_{18:2}, or C_{18:1} to C_{17:1} and C_{20:4} or C_{22:6} to C_{21:0}, respectively.

Sample preparation was also performed as our own previous study[14]. Briefly, 50 μL of each serum sample was spiked with 950 μL of methanol/acetonitrile (3/2, v/v) to precipitate serum proteins. The resulting mixture was vortexed for 30 s and then stored at -20 °C overnight. After the mixture was centrifuged at 19000 g for 30 min at 4 °C, the supernatant was transferred into a new tube. Accurate pipetting of the above supernatant of 20 μL was mixed with 1 μL of the ISs solution, 500 μL of hexane, and 500 μL of water, and then the resulting solution was vortexed and centrifuged at 1500 g for 10 min. After the centrifugation, top layer was transferred to another vial and air-dried. The dried residue was re-dissolved in 1 mL of methanol/acetonitrile/5 mM ammonium acetate (42/28/30, v/v/v) for analysis.

Mass Spectrometry

Extracted FFAs were analyzed using a 9.4 T Apex-ultra™ hybrid Qh-FTICR MS (Bruker Daltonics, Billerica, MA, USA) equipped with a NanoMate system (Advion BioSciences, Ithaca, NY, USA) in the

negative ion mode. 0.1 μL of the sample followed by 0.5 μL of air was introduced directly into the nanoESI source, with a voltage of -1.8 kV and a head pressure of 0.7 psi at a flow rate of 100 nL/min. Each spectrum was accumulated 10 full scans over the mass range of 150–400 Da and the resolution was 200,000 at *m/z* 400. A mixture of C_{15:0} (molecular weight = 242.22458 Da), C_{17:0} (270.25588 Da), and C_{21:0} (326.31848 Da) was employed to calibrate the instrument before analysis.

Serum unsaturated FFAs in this study were identified on the basis of their observed accurate molecular masses and reliable isotope distributions detected by FTICR MS. Their mass error was ≤ 0.00025 Da and the relative intensity error of their isotopic peaks was < 2%. For the missing levels of unsaturated FFAs, the baseline intensity in each spectrum was adopted for the following statistical analysis.

Statistical analysis

Comparisons of the levels of these unsaturated FFAs between healthy controls, patients, and patients with different CRC stages in the training study or the validation study were performed by Mann-Whitney U test. Significantly changed unsaturated FFAs were subjected to ROC analysis. The AUC, sensitivity, and specificity were calculated to evaluate the diagnostic ability. All statistical analyses were performed by SPSS software (version 16.0, Chicago, IL, USA). The results are expressed as mean ± SD. *p* values of <0.05 were considered to be statistically significant.

Results

Simultaneous qualitative and quantitative analysis of serum FFAs

As shown in Table 2, the calibration equations of C_{16:1}, C_{18:3}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6} were constructed with correlation coefficients (*R*²) of >0.99 on the basis of their mixture standard working solutions. 39 mass spectra of the quality control sample were analyzed with relative SD of <16% for each analyte, indicating that the experimental reproducibility is acceptable for complex biological samples. Representative mass spectra of serum FFAs in one healthy control, one BCD patient, and one CRC patient are shown in Fig. 1. The levels of these six unsaturated FFAs were calculated on the basis of their respective corresponding calibration equations listed in Table 2. These unsaturated FFAs were identified via comparing the observed accurate molecular masses and reliable isotope distributions with their respective theoretical values, with the absolute mass error of ≤ 0.00025 Da and the relative intensity error of their isotopic peaks of < 2%.

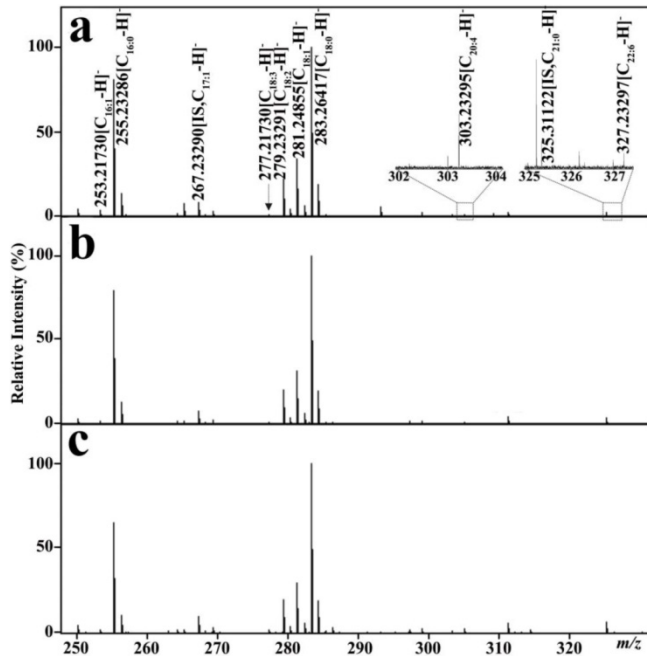


Figure 1. Representative mass spectra of serum FFAs from one healthy control (a), one BCD patient (b), and one CRC patients (c) in the negative ion mode.

Table 2. Calibration equations, correlation coefficient (R²), and experimental stability.

FAs	FA (nM)	Linearity (n=3)		QC (%)
		Equation	R ²	
C _{16:1}	8.5-684.0	Y=0.518(±0.028)X+0.161(±0.030)	0.996	<16
C _{18:3}	2.0-163.5	Y=1.365(±0.195)X-0.054(±0.043)	0.993	<12
C _{18:2}	14.2-1138.0	Y=1.521(±0.072)X+0.121(±0.099)	0.996	<12
C _{18:1}	20.9-1672.0	Y=1.646(±0.032)X+1.249(±0.076)	0.994	<16
C _{20:4}	4.6-366.0	Y=3.164(±0.100)X-0.350(±0.298)	0.997	<12
C _{22:6}	1.4-112.3	Y=2.006(±0.208)X-0.016(±0.008)	0.995	<13

X: Concentration ratios of individual fatty acids to ISs (83.3 nM C_{17:1} and 33.3 nM C_{21:0}); Y: respective corresponding intensity ratios of fatty acids to ISs; QC: quality control.

Comparison of changes in the levels of FFAs between healthy controls and patients

In the training study, Mann-Whitney U test indicated that the levels of C_{16:1}, C_{18:3}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6} in CRC patients were significantly decreased compared with healthy controls ($p < 0.001$). Their scatter plots are shown in Fig. 2. As shown in Table 3, monounsaturated free fatty acids (MUFA, *i.e.*, C_{16:1} and C_{18:1}), polyunsaturated free fatty acids (PUFA, *i.e.*, C_{18:3}, C_{18:2}, C_{20:4}, and C_{22:6}), and panel a (a combination of C_{16:1}, C_{18:3}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6}) displayed good diagnostic ability to differentiate CRC patients from healthy controls, with the AUC values of > 0.90, the sensitivities of > 81%, and the specificities of > 82%. Representative ROC curve for panel a is shown in Fig. 3A.

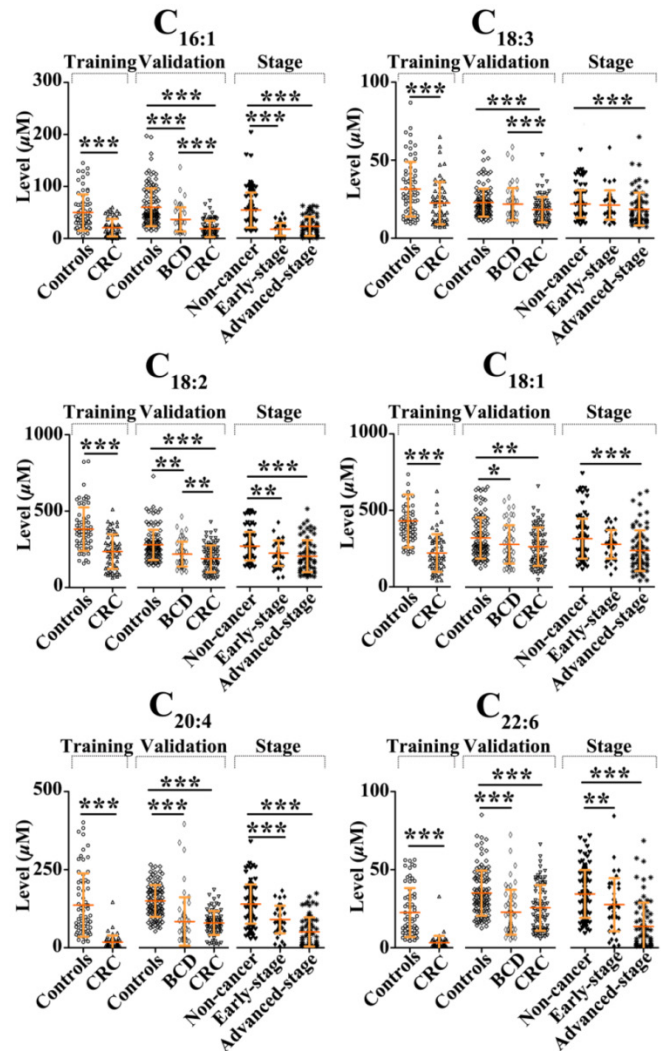


Figure 2. Scatter plots of serum levels of C_{16:1}, C_{18:3}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6} in the training set, the validation set, and different CRC stages. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

To validate the above-mentioned findings, we further performed an independent validation study, including 55 BCD patients, 80 CRC patients, and 116 healthy controls. ROC analysis indicated that C_{16:1}, C_{20:4}, MUFA, PUFA, and panel a had high diagnostic ability to differentiate CRC patients from healthy controls, with the AUC values of >0.86, the sensitivities of >74%, and the specificities of >70%. Representative ROC curve for panel a is shown in Fig. 3B.

We also performed the comparisons between healthy controls, BCD patients, and CRC patients using Mann-Whitney U test. Statistical results indicate that significant decrease in the levels of C_{16:1}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6} in BCD patients or CRC patients were observed compared with healthy controls ($p < 0.05$). It should be noted that the levels of C_{16:1}, C_{18:2}, and C_{18:3} in BCD patients were significantly increased compared with CRC patients ($p < 0.01$). ROC analysis indicated that only C_{20:4} and panel b (a combination of

C_{16:1}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6}) has high diagnostic ability to differentiate BCD patients from healthy controls, with the AUC values of > 0.82. Representative ROC curve for panel b is shown in Fig. 3C. It is worth noting that panel c (a combination of C_{16:1}, C_{18:3}, and C_{18:2}) has high diagnostic ability to differentiate BCD patients from CRC patients, with the AUC of 0.78, the sensitivity of 70%, and the specificity of 81%. Representative ROC curve for panel c is shown in Fig. 3D.

Association of changes in the levels of FFAs with CRC stages

Comparisons of non-cancer participants (healthy controls plus BCD patients) with different stages of CRC showed that significant decrease in the levels of FFAs was observed in both early-stage patients (except C_{18:1} and C_{18:3}) and advanced-stage patients (*p* < 0.01, Fig. 2). ROC analysis showed that panel d (a combination of C_{16:1}, C_{18:2}, C_{20:4}, and C_{22:6}) and panel a provide excellent diagnostic performance to differentiate early-and advanced-stage patients from non-cancer participants, respectively, with the AUC values of >0.92, the sensitivities of > 84%, and the specificities of >83% (Table 4). ROC curves for panels d and a are shown in Fig. 3E-F, respectively.

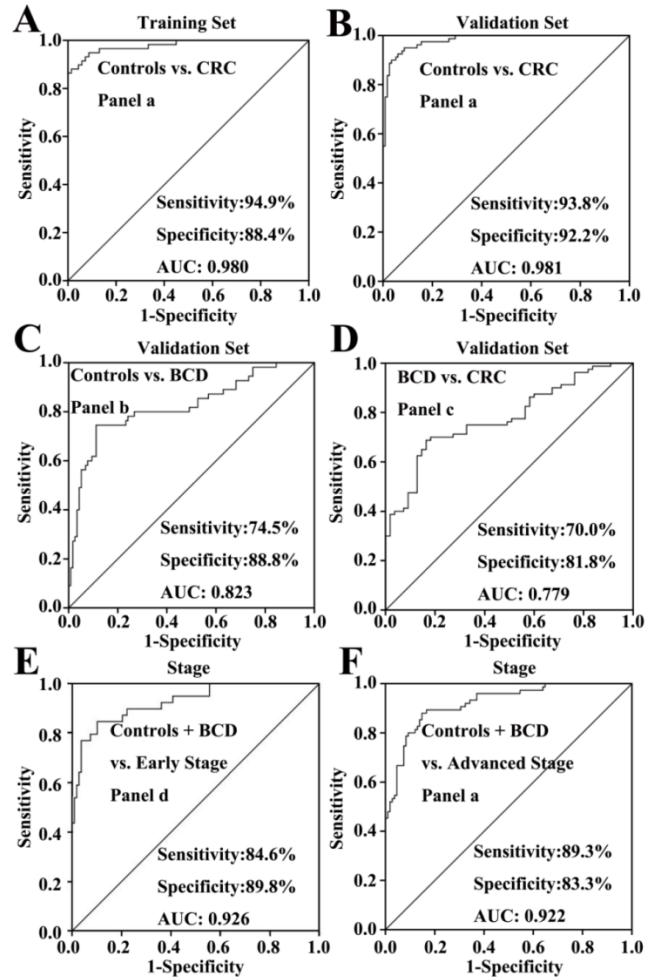


Figure 3. Representative ROC curves of different biomarker panels. (A) the panel a (a combination of C_{16:1}, C_{18:3}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6}) differentiates CRC patients from healthy controls in the training set. (B) the panel a differentiates CRC patients from healthy controls in the validation set. (C) the panel b (a combination of C_{16:1}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6}) differentiates BCD patients from healthy controls. (D) The panel c (a combination of C_{16:1}, C_{18:3}, and C_{18:2}) differentiates CRC patients from BCD patients. (E) The panel d (a combination of C_{16:1}, C_{18:2}, C_{20:4}, and C_{22:6}) differentiates early-stage CRC patients from non-cancer participants (healthy controls plus BCD patients). (F) the biomarker panel a differentiates advanced-stage CRC patients from non-cancer participants.

Table 3. The AUC values, cut-off values, sensitivity, and specificity of significantly changed unsaturated FFAs between controls and patients.

FFAs	Training set				Validation set											
	Controls vs. CRC				Controls vs. CRC				Controls vs. BCD				BCD vs. CRC			
	AUC (95% CI)	Sens (%)	Spec (%)	cut-off	AUC (95% CI)	Sens (%)	Spec (%)	AUC (95% CI)	Sens (%)	Spec (%)	cut-off	AUC (95% CI)	Sens (%)	Spec (%)	cut-off	
C _{16:1}	0.790(.714-.867)	79.7	55.9	20.7	0.907(.865-.949)	95.7	70.7	0.732(.650-.813)	73.3	67.3	38.7	0.776(.699-.852)	72.7	70.0	20.7	
C _{18:3}	0.664(.571-.758)	92.8	25.4	11.4	0.661(.582-.740)	99.1	21.2					0.610(.515-.705)	98.2	21.2	11.4	
C _{18:2}	0.790(.713-.868)	98.6	30.5	160.4	0.753(.681-.825)	97.4	42.5	0.701(.612-.789)	64.7	69.1	240.6	0.602(.507-.696)	72.7	42.5	160.4	
C _{18:1}	0.878(.816-.940)	98.6	57.6	208.6	0.622(.539-.704)	81.0	43.8	0.599(.505-.692)	66.4	52.7	247.2					
C _{20:4}	0.963(.933-.993)	46.4	98.3	113.6	0.865(.815-.915)	74.1	83.8	0.845(.769-.921)	79.3	81.8	107.2					
C _{22:6}	0.973(.944-.999)	26.1	98.3	32.0	0.686(.607-.764)	50.0	70.0	0.757(.674-.841)	81.0	61.8	23.0					
MUFFA	0.908(.854-.962)	81.4	87.0	0.6	0.914(.874-.954)	75.0	90.5	0.735(.654-.816)	72.7	66.4	0.4					
PUFFA	0.976(.951-1.001)	98.3	82.6	0.3	0.904(.860-.948)	87.5	73.3									
Panel a	0.980(.959-1.000)	94.9	88.4	0.4	0.981(.966-.996)	93.8	92.2									
Panel b								0.823(.750-.896)	74.5	88.8	0.4					
Panel c												0.779(.702-.855)	70.0	81.8	0.7	

Note: CRC: colorectal cancer; BCD: benign colorectal diseases; Sens: sensitivity; Spec: specificity; MUFFA: monounsaturated free fatty acid; PUFFA: polyunsaturated free fatty acid; Panel a: C_{16:1}, C_{18:3}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6}; Panel b: C_{16:1}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6}; Panel c: C_{16:1}, C_{18:3}, and C_{18:2}.

Table 4. The AUC values, cut-off values, sensitivity and specificity of significantly changed unsaturated FFAs between controls plus BCD patients and CRC patients with different stages.

FFAs	Controls plus BCD vs. Early stage			Cut-off	Controls plus BCD vs. Advanced stage		
	AUC (95% CI)	Sens (%)	Spec (%)		AUC (95% CI)	Sens (%)	Spec (%)
C _{16:1}	0.906 (.851-.960)	95.4	71.8	20.7	0.820 (.757-.883)	95.4	56.0
C _{18:3}				11.4	0.655 (.569-.742)	88.9	46.7
C _{18:2}	0.730 (.622-.838)	93.5	30.8	160.4	0.703 (.621-.785)	84.3	50.7
C _{18:1}				208.6	0.690 (.608-.772)	83.3	54.7
C _{20:4}	0.766 (.672-.811)	64.8	76.9	113.6	0.874 (.825-.923)	75.0	81.3
C _{22:6}	0.626 (.522-.729)	50.9	69.2	32.0	0.852 (.791-.913)	81.5	74.7
MUFFA				0.6	0.821 (.759-.883)	58.7	93.5
PUFFA	0.785 (.704-.866)	66.7	82.4	0.3	0.877 (.828-.926)	70.7	89.8
Panel a				0.4	0.922 (.883-.961)	89.3	83.3
Panel d	0.926 (.876-.975)	84.6	89.8	0.4			

Note: CRC: colorectal cancer; BCD: benign colorectal diseases; MUFFA: monounsaturated free fatty acid; PUFFA: polyunsaturated free fatty acid; Panel a: C_{16:1}, C_{18:3}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6}; Panel d: C_{16:1}, C_{18:2}, C_{20:4}, and C_{22:6}.

Discussion

In the present study, we employed CBDInanoESI-FTICR MS platform to perform simultaneous quantitative and qualitative analysis of six serum unsaturated FFAs in 379 participants, with high throughput. Compared with liquid chromatography or gas chromatography coupled to MS, this CBDInanoESI-FTICR MS strategy is less time-consuming, with about 30 seconds per sample. As shown in **Fig. 2**, significant decrease in the levels of C_{16:1}, C_{18:3}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6} was observed in CRC patients in both the training and validation study, which are not totally and positively correlated with those in CRC tissues. Previous study showed a decrease in the levels of C_{18:3} and C_{18:2} and a increase in the levels of C_{18:1} and C_{20:4} in human CRC tissues [18]. These change trends between serum and tissue may be due to renal and/or hepatic clearance, which play important roles in the metabolites [19]. Significant decrease in the levels of C_{14:0}, C_{15:0}, C_{18:3}, and C_{18:0} and increase in the levels of C_{24:0}, C_{25:0}, C_{26:0}, C_{28:0}, and C_{30:0} in CRC patients were also observed compared with healthy controls [20]. Recent tissue study showed that significant decrease in the levels of C_{16:1} and C_{18:1} in CRC tissue were also observed compared with adjacent normal tissue [21], suggesting that these fatty acids may involve in colorectal cancer. It was found that C_{18:2}, C_{18:1}, and C_{16:1} could induce the generation of lipid droplets and trigger efficient lipid droplet accumulation in melanoma cells [22]. Previous prospective studies have also showed that unsaturated fatty acids inhibited some mechanism pathways in colorectal carcinogenesis [21, 23, 24]. In addition, the associations between PUFFA intake and risk of CRC remains inconclusive. Some epidemiological studies suggested that consumption of n-3 PUFFA or lower ratio of n-6 to n-3 fatty acids may decrease CRC risk [25-27]. However, recent study showed that there were no statistically significant associations between

CRC risk and dietary intake of any of the vitamins, minerals or essential fatty acids [28].

Statistically significant decrease in the levels of C_{16:1}, C_{18:2}, C_{18:1}, C_{20:4}, and C_{22:6} were observed in BCD patients compared with healthy controls, and significant increase in the levels of C_{16:1}, C_{18:3}, and C_{18:2} in BCD patients were detected compared with CRC patients. ROC analysis showed that panel b can discriminate BCD patients from healthy controls, with the AUC value of 0.823, the sensitivity of 74.5%, and the specificity of 88.8%. More importantly, panel c has excellent diagnostic accuracy to differentiate CRC patients from BCD patients, with the AUC value of 0.779, the sensitivity of 70.0%, and the specificity of 81.8%. It is worth noting that panels a and d have shown excellent diagnostic ability to differentiate advanced-stage and early -stage CRC from non-cancer participants, respectively, with the AUC values of >0.92, the sensitivities of >84%, and the specificities of >83%, which are the similar to or better than other tumor biomarkers, such as lipid profiling [29], the combination of 2-hydroxybutyrate, aspartic acid, kynurenine and cystamine [30], the serum dermokine level [31], the methylation of serum NEUROG1 [32], and the combination of 3 protein mass peaks for discriminating CRC, BCD, and healthy controls with accuracy of >80% [33].

Conclusions

In this study, we used CBDInanoESI-FTICR MS platform to perform the simultaneously quantitative and qualitative analysis of multiple targeted serum unsaturated FFAs with high resolution, high mass accuracy, and high throughput. Change trends in the levels of serum unsaturated FFAs in all participants including 185 healthy controls, 55 BCD patients, and 139 CRC patients were closely correlated with the pathological status of participants. A combination of C_{16:1}, C_{18:2}, C_{20:4}, and C_{22:6} as a biomarker panel has

shown excellent diagnostic ability to differentiate early-stage CRC from healthy controls plus BCD. It is worth noting that the development of colorectal diseases are accompanied with decreased levels of serum unsaturated FFAs, indicating that the detection of serum unsaturated FFAs might have important clinical significance for early detection of CRC.

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Competing Interests

All authors disclose any affiliations that are consideration to be relevant and important with any organization that to our knowledge has any direct interest in the subject matter discussed.

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