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Biomarkers of unstable angina pectoris and yangxin decoction intervention

An exploratory metabonomics study of blood plasma

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Abstract

Background: This study aimed to explore the related metabolic biomarkers and to observe the effects of Yangxin Decoction (YXD) on plasma metabolism of patients with unstable angina (UA).

Methods: In total, 10 patients with UA (intervention group) and 10 healthy participants (control group) were recruited for this study from January 2009 to December 2010. Plasma samples from both groups were analyzed using liquid chromatography mass spectrometry (LC-MS). Principle component analysis (PCA) and partial least squares (PLS) were used to explore the correlations between metabolic markers in patients with UA.

Results: The LC-MS results indicated that the serum levels of 5 potential metabolic markers, namely, ceramide, glycocholic acid, allocholic acid, lithocholic acid, and leukotriene (LT) B4, were significantly higher in the intervention group than those in the control group.

Conclusion: The results of this study demonstrated potential metabolic markers that can be used to distinguish and diagnose patients with UA.

Abbreviations: 5-LO = 5-lipoxygenase, LC-MS = liquid chromatography mass spectrometry, LC-MS = liquid chromatography-mass spectrometry, LTs = leukotrienes, PCA = Principle component analysis, PLS = partial least squares, PLS-DA = partial least squares method, TCM = traditional Chinese medicine, UA = unstable angina, YXD = Yangxin Decoction.

Keywords: metabolic biomarker, metabonomics, unstable angina, yangxin decoction

1. Introduction

Unstable angina (UA) is a very common complication in patients with coronary heart disease,[1,2] which annually accounts for more than 1 million hospitalizations.[3] In addition, it also affects approximately one-third of the population before the age of 70 years, and has been regarded as the major cause of mortality and morbidity in the developed countries.[3,4]

The pathogenesis of many diseases is associated with abnormalities in the metabolism of both body fluids and tissue.[5] A previous study demonstrated that metabolomics can be used to measure the dynamic metabolic response of living systems to biological stimuli or genetic manipulation.[6] It allows physiological investigation, disease diagnosis, biomarkers exploration, and identification of perturbed pathways, through the evaluation of a range of endogenous and exogenous molecules.[7,8]

According to traditional Chinese medicine (TCM) theory, syndromes often reflect and manifest the sickness of patients. In these cases, metabolomics can confirm and help diagnose the TCM syndrome. In the current study, according to the guidance of TCM theory and correlation between metabolomics and TCM syndromes, we aimed to explore the effect of Yangxin Decoction (YXD) on the plasma metabolic profile of patients with UA. The YXD formula consists of multiple herbs, including astragalus, ginseng, poria with hostwood, angelica, ligusticum, zizyphus jujube, platycladi seed, schisandra, ginger, red jujube, dried tangerine peel, pinellia, polygalaceae, licorice, and spicy cinnamon.

Our previous studies demonstrated that YXD increased the plasma level of 6-Keto-PGF 1α,[9] improved myocardial function and aortic abnormalities,[10] and increased the levels of high-density lipoprotein cholesterol and apolipoprotein A.[11] In addition, YXD also reduced TXB2 production, thereby protecting ischemic myocardium, and preventing the increase in arterial thickness caused by a high-fat diet.[12] It further protected ischemic myocardium by regulating vascular endothelial function and increasing nitric oxide and nitric oxide synthase production.[12]

In the current study, we evaluated the correlated metabolic biomarkers to observe the effects of YXD on the plasma

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metabolism of patients with UA, using liquid chromatography-mass spectrometry (LC-MS).

2. Methods

2.1. Study design and participants

In the present study, serum samples were collected from 10 UA patients (intervention group) and 10 healthy participants (control group). All participants were recruited from the Second Department of Cardiovascular of The First Affiliated Hospital of Heilongjiang University of Chinese Medicine from January 2009 to December 2010. This study was approved by the Ethics Committee of The First Affiliated Hospital of Heilongjiang University of Chinese Medicine and written informed consent was obtained from all participants. The diagnosis of patients with UA was made and confirmed using the "Guideline Update for the Management of Patients with Chronic Stable Angina" (ACC/AHA, 2002) and "Diagnosis and treatment recommendations of UA" (Chinese Society of Cardiology, 2000).[13] In addition, all the general information, such as past medical history, family history, and individual history, were collected within 12 hours after the participants were admitted. Basic characteristics of the participant cohort are presented in detail in Table 1.

The inclusion criteria included patients aged between 40 and 70 years, and a positive resting electrocardiogram with ST segment depression ≥0.05 mV and/or an R wave in major manifestation and inversion T wave >0.1 mV. The exclusion criteria included patients who had acute myocardial infarction, stable exertional angina, pericardial disease, cardiac neurosis and other heart diseases, intercostal neuralgia, menopausal syndrome, hyperthyroidism, cervical spondylosis, esophageal hiatus hernia, aortic dissection caused by chest pain, uncontrolled hypertension disease (systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥100 mm Hg), severe complications of diabetes mellitus, cardiopulmonary dysfunction, arrhythmia, renal and liver diseases, disability, history of drug abuse, or allergies (more than 2 kinds of food or drug allergy), and pregnant or lactating women, or those who had participated in other clinical trials 1 month before the current study.

Table 1

Basic clinical characteristics of UP patients and healthy control.

Indexes	Intervention group (n=10)	Control group (n=10)	*P*
Age, y	58.33±11.24	56.96±10.55	.78
Sex (F/M)	5/5	5/5	1.00
BMI, kg/m^2	23.84±3.57	24.11±3.93	.87
Risk factors			
Smokers	3 (30.00)	2 (20.00)	.61
Triglycerides, mmol/L	1.92±1.13	0.83±0.25	.003
Cholesterol, mmol/L	4.57±1.06	3.84±0.82	.08
HDL, mmol/L	1.11±0.26	1.22±0.30	.38
LDL, mmol/L	3.03±0.83	2.34±0.52	.03
Medical history			
Antiplatelet drug	10 (100.00)	N/A	—
Nitrate esters drug	4 (40.00)	N/A	—
Statins	7 (70.00)	N/A	—
ACEI/ARB	1 (10.00)	N/A	—
β-blocker	6 (60.00)	N/A	—
Ca^{2+} antagonist	10 (100.00)	N/A	—

ACEI/ARB = angiotensin-converting enzyme inhibitors/angiotensin antibody, BMI = body mass index, HDL = high-density lipoprotein, LDL = low-density lipoprotein.

2.2. Intervention

All patients in the intervention group received oral YXT (Produced by the Pharmacy of First Affiliated Hospital of Heilongjiang University), 150 mL/bag, twice daily, in the morning and evening, respectively, for 28 days. Healthy participants in the control group did not receive any kind of treatment.

2.3. Serum sample collection and preparation

Serum samples from all the patients with UA patients (intervention group) were collected before and after treatment, while samples from the healthy participants (control group) were only collected at the beginning of the study. The serum sample was collected from each participant in the morning, after fasting for at least 10 hours or more, from elbow vein blood, and promptly anticoagulated with eathylene diamine tetraacetic acid and centrifuged at 3000 rpm for 15 minutes. The plasma was first stored in a 1.5 mL Eppendorf tube at −20°C and then transferred to −80°C 1 week later.

For analysis, the serum sample was thawed naturally, mixed with a liquid mixer for 30 seconds, and transferred to a high-speed centrifuge at 10,000 rpm for 2 minutes. From this sample, 20 μL was transferred into a new labeled Entrance potential (EP) tube, to which 180 μL of methanol was added, which diluted it down by a factor of 10. It was then mixed with a liquid rapid mixer for 30 seconds, before being centrifuged at 13,200 rpm for 5 minutes. Approximately 100 to 150 μL of this sample was analyzed.

2.4. Liquid chromatography mass spectrometry measurement of serum

Serum samples were analyzed using an LC-MS/MS 3200 Q1 (API 3200 LC/MS/MS, MDS Inc., Ontario, Canada) full scan test with flow phase B (10 mmol/L, ammonium acetate, and 0.10% formic acid) and flow phase C (acetonitrile column: Eclip XDB C18 (Agilent Technologies, Inc. Santa Clara), 46 × 150 mm, 5 μm), with an Aglient sample (Agilent Technologies, Inc. Santa Clara) volume of 5 μL, and the following mass spectrometry parameters: Curtain Gas (CUR), 25.00; temperature (TEM), 550.00; Ion Source Gas (GS1), 50.00; GS2, 60.00; ihe turn on (ON); IS, −4500.00; declustering potential (DP), −50.00; and EP, −10.00. The gradient of LC-MS/MS is summarized in Table 2. In addition, the detection conditions of MS were as follows: scan range, 50 to 1000; CUR, 20.00; IS, 5500.00; TEM, 580.00; GS1, 55.0; GS2, 60.00; CAD, Medium; DP, 50.0; EP, 10.0; Flow rate, 0.75 mL/min; Column temperature, 30°C; flow phase A, water; and flow phase B, nitrile. The gradient of LC-MS is summarized in Table 3.

2.5. Statistical analysis

Total ion chromatograms that were obtained from the Q1 scan were first converted into the required data format using MZmine software, before being analyzed using SIMCA-P (Version11.5;

Table 2

The gradient of LC-MS/MS.

Retention, min	Flow, mL/min	B%	C%
0	0.75	90	10
0	0.75	90	10
1.5	0.75	90	30
7	0.75	72	80
12	0.75	10	95
15	0.75	5	95
15	0.75	90	10
20	0.75	90	10

Table 3

The gradient of LC-MS.

Time, min	A%	B%	Time, min
0	90	10	0
0	90	10	0
1	70	30	1
5.5	20	80	5.5
8	5	95	8
15	5	95	15
15.1	90	10	15.1
20	90	10	20

Umetrics AB, Umea, Sweden) software, with principal components analysis (PCA) and partial least squares method (PLS-DA). In addition, *t* tests were used to analyze the data and make comparisons between the 2 groups.

3. Results

Serum is one of the most readily available biological fluids and contains a large number of metabolites (pertaining to a series of biochemical processes). Thus, it can provide valuable bio-information about an organism's metabolism. Representative spectra of the LC-MS analysis of serum samples from patients with UA and healthy control patients are shown in Figs. 1 to 3.

Two-dimensional and three-dimensional maps of LC-MS results are shown in Figs. 4 and 5. The expression of metabolites in the intervention group and control group was significantly different at the preservation time of 6 to 10 minutes, with M/Z 300–400 (within virtual frame) and M/Z 700–800 (within solid frame) (Figs. 4 and 5). The results of PCA are shown in Fig. 6.

Results of potential marker analysis showed that the scores in the 2 groups were mainly distributed in the elliptical scatter plot (95% confidence interval) (Fig. 7, Table 4). Samples 8, 9, and 10 in the intervention group, and samples 1 and 10 in the control group were separated from the concentrated area. The results of the potential markers are summarized in Table 4.

4. Discussion

UA often manifests as chest pain, pressure, or squeezing, and is caused by heart muscle ischemia due to the obstruction or spasm of the coronary arteries. Although it is often life-threatening, it

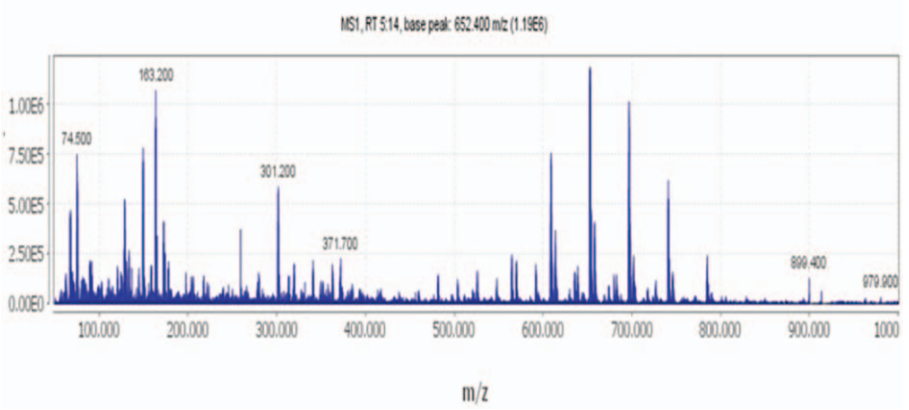

Figure 1. General scan map by LC-MS/MS.

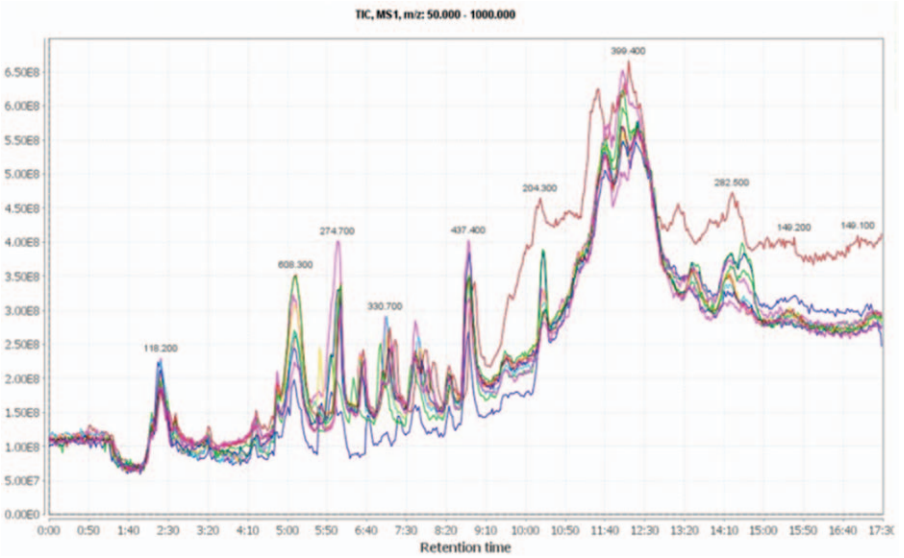

Figure 2. Scan map by LC-MS/MS in healthy participants.

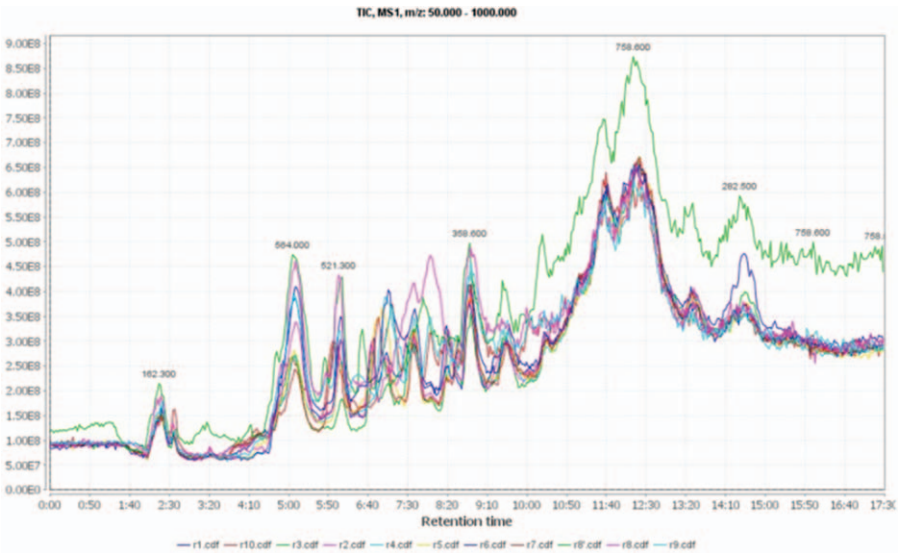

Figure 3. Scan map by LC-MS/MS in UA patients.

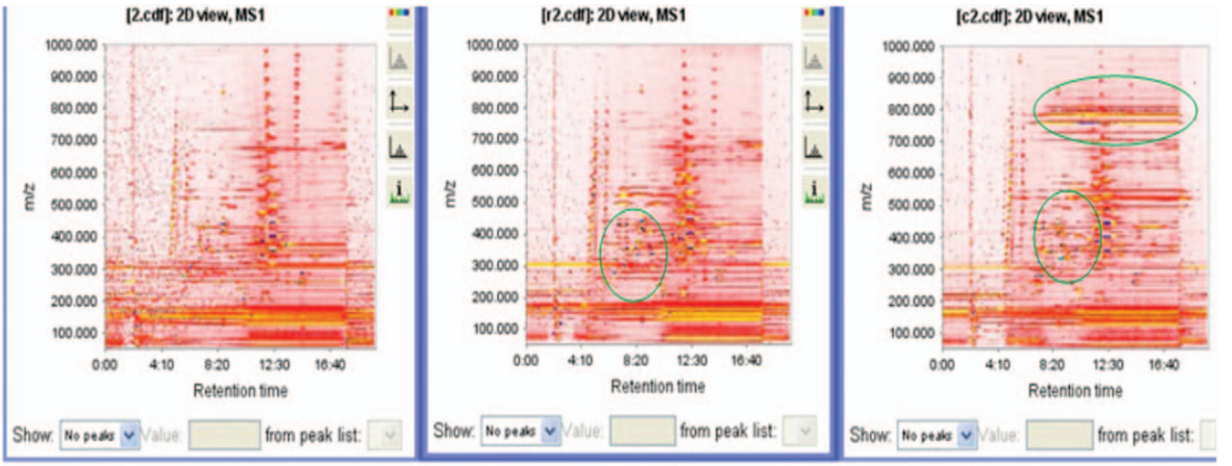

Figure 4. Two-dimensional maps of LC-MS/MS in UA patients.

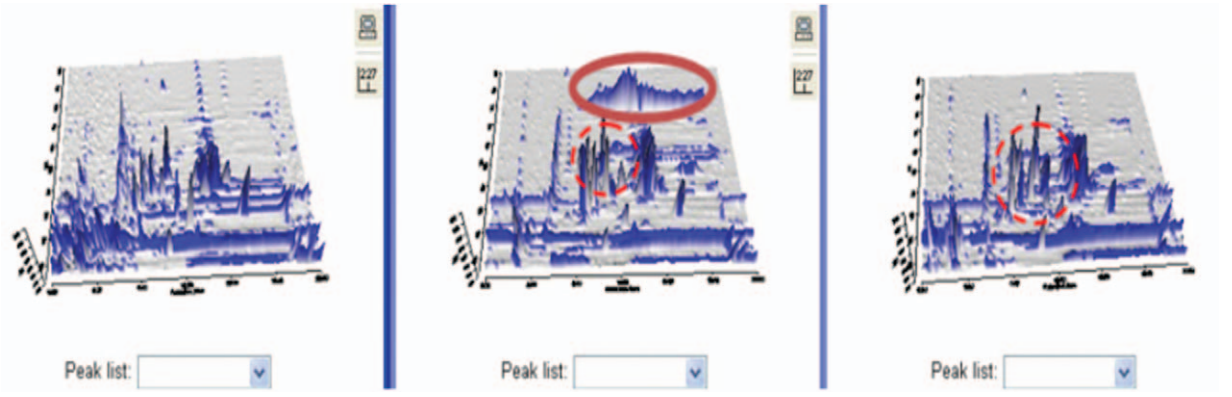

Figure 5. Three-dimensional maps of LC-MS/MS in UA patients.

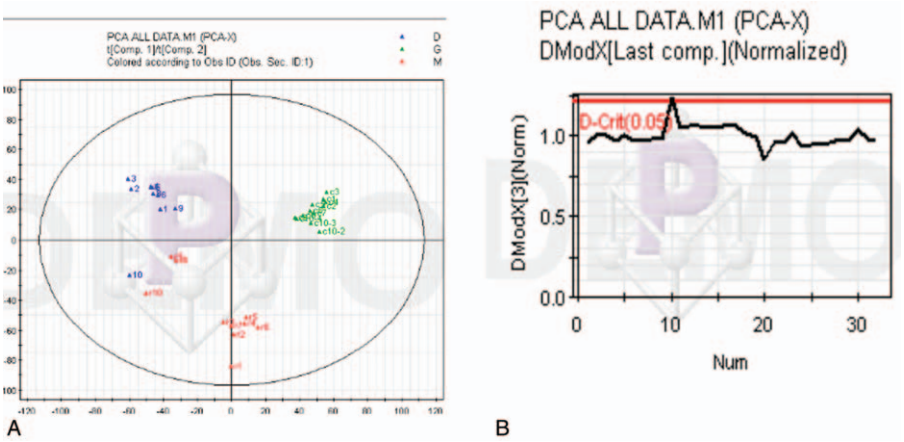

Figure 6. The results of 3 groups by PCA analysis with elliptical figure (A) and distance figure (B).

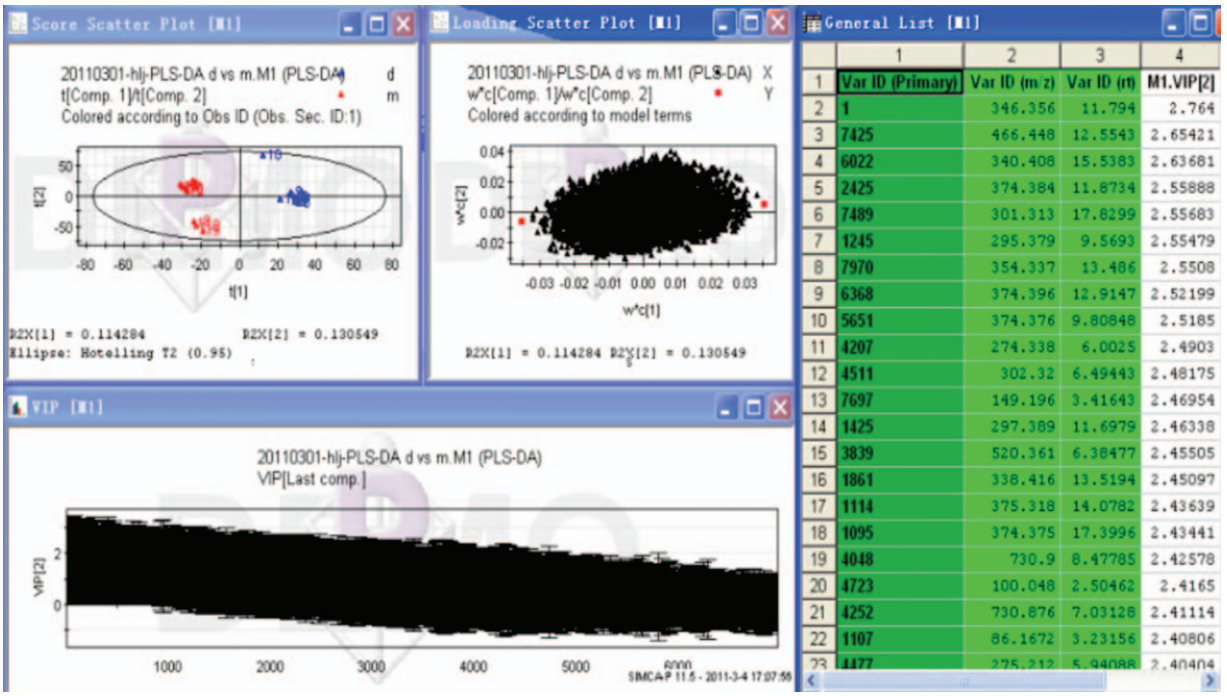

Figure 7. Potential markers in the intervention and control groups.

can be controlled if it is diagnosed in a timely, accurate, and definitive fashion. Although previous studies have identified several markers from patients with UA, including C-reactive protein, interleukin (IL)-6, IL-10, IL-18, and CD-40L, those markers had limited use for the diagnosis and risk stratification in patients with UA.[14–16] Therefore, the putative markers of UA should still be explored and, at least partly, be reflected in serum.

Table 4

Potential markers.

Mass, M/Z	ID	VIP	Retention time, min	Peak height in d VS m	Name
330.46	11869	2.17	8.34	↑	Dihydroceramide
466.45	7425	2.65	12.55	↑	Glycocholic acid
377.44	5	2.03	12.50	↑	Allolithocholic acid
	7970			↑	6,7-dihydro-12-epi-LTB4;
				↑	10,11-dihydro-leukotriene B4

In the current study, LC-MS was used to demonstrate metabolic differences between patients with UA and healthy participants. On subsequent analysis, the metabolite profiles of serum samples of patients with UA patients could be distinguished from healthy participants, which highlights the potential of metabolomic analysis in disease evaluation, especially in accurately and reliably diagnosing UA. The 5 potential metabolic markers that were identified in patients with UA were ceramide, glycocholic acid, allocholic acid, lithocholic acid, and LT B4.

The significantly higher level of LT B4 in the serum of patients with UA, which has also been reported in previous studies, can promote the transformation of monocytes to foam cells during atherosclerosis development and strongly induce Monocyte chemoattractant protein-l expression and its interaction with early human monocytes.[17–22] This result confirms previous reports of the presence of inflammation in the coronary artery endothelium of patients with UA patients, and the formation of atherosclerotic plaque instability, which results in coronary artery spasm and finally leads to recurrent UA. In addition, 5-lipoxygenase (5-LO), a member of the lipoxygenase family, is a key enzyme that catalyzes the conversion of arachidonic acid to LTs.[17–22] In present study, the high-expression of LT B4 in serum of patients with UA suggests the possible existence of 5-LO metabolic pathways in patients with UA too. Moreover, the significantly higher levels of glycocholic acid, cholic acid, and lithocholic acid in patients with UA indicate metabolic transformation and lipoprotein secretion of fatty acids, which exceeds the ability of liver uptake.[23] This may impair liver cell function, decreasing the ability to synthesize bile acids, and reducing bile acid volume pool.[23] Maintenance of normal bile acid enterohepatic circulation requires an increase in enterohepatic circulation, which in turn results in a loss of bile acid. At the same time, impaired liver function increases the bile acid content via the liver sinusoidal. In addition, the increase in enterohepatic circulation increase results in higher levels of blood bile acid levels, which reflects that a metabolism disorder happened in patients with UA. Furthermore, the significantly increased level of ceramides in patients with UA patients indicates the activation of ceramide stress-activated protein kinase and Jun-N-terminal kinase pathway, and the initiation of the Fas system, which results in the apoptosis of vascular smooth muscle cells.[24,25] The results from the current study corroborate with our previous findings.[9]

A limitation of the current study is the small sample size. Thus, further research, with a larger sample size, is required to validate the results of the current study.

5. Conclusion

The current study found 5 potential markers that can be used to distinguish patients with UA from healthy people and to help diagnose this disease.

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