Study on Blood Metabolomics of Early Gastric Cancer Based on Liquid Chromatography-Mass Spectrometry Technology

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Research

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Abstract

Background: Metabolomics is widely used to accurately find the basic characteristics and material basis of life activities. The purpose of this study is to use metabolomics to discover biomarkers for the diagnosis of early gastric cancer.

Methods: We collected the blood samples and clinical data of 63 patients with gastric cancer from the First Hospital of Jilin University, including 26 patients with advanced gastric cancer (group A), 37 patients with early gastric cancer (group B), and 18 healthy volunteers (group C). Chromatography-mass spectrometry (LC-MS) is used for detect metabolites and obtain metabolic profile. Support vector machine (SVM) is used to screen the differential metabolites with a weight of 100% from the blood sample. Total ion current diagram, principal component analysis and analysis of variance (ANOVA) are used to identify differential metabolites. PCA and the quadratic discriminant analysis were used to evaluate the similarity between samples. The receiver characteristic curve (ROC) is used to evaluate the diagnostic ability of metabolites. After the nuclear ratio of the selected metabolites is imported into the Human Metabolome Database (HMDB), the structure is identified to determine the corresponding substances, and then the verification group is used to test the accuracy of the metabolites.

Results: Through LC-MS, TIC, ANOVA and PCA, differential metabolites were found in different blood samples. Cluster analysis showed similar metabolites in the three groups A, B, and C. ROC curve represented the diagnostic ability of metabolites. The different metabolites between group A and C were spermine, enterostatin, heparin sulfate, and triacylglycerol. The difference metabolites between group A, group B and group C were same as those between group A and C. The cluster analysis and ROC also showed that all four metabolites had high specificity and sensitivity in the verification group. And the results of verification group were consistent with the experimental group.

Conclusion: Spermine, enterostatin, heparin sulfate, and triacylglycerol may be potential biomarkers for the diagnosis of early gastric cancer.

Introduction

Gastric cancer, one of the most common upper gastrointestinal tract cancers, ranks fourth in the incidence of malignant tumors and second in mortality¹. Surgery is the main treatment for gastric cancer currently while chemotherapy and radiotherapy are adjuvant treatments². However, the survival of gastric cancer after surgery vary in different stages (stage I, 93%; stage II, 76%; stage III 49%; stage IV 19%). Therefore, early diagnosis is vital to improve the prognosis.

At present, the diagnosis of early gastric cancer mainly depends on endoscopic technology³. The diagnosis rate of early gastric cancer by endoscopy is about 50–70%⁴, although the diagnostic rate of magnifying endoscopy (ME) and narrow band imaging (NBI) is significantly higher than that of traditional endoscopy⁵. However, endoscopy is an invasive examination, which has limitations because of pain, poor medical compliance, and high cost. Therefore, it is urgent to find a low-invasive, simple, efficient, and low-cost early gastric cancer screening method.

Metabolomics is an emerging discipline to study the types and quantity of molecules in organisms. It can amplify small changes in the genome and proteome, reflect the end point of gene function activities and changes in biochemical phenotypes⁶⁻¹⁰. Recently, many studies use metabolomics to explore biomarkers from body fluids for disease¹¹, such as breast cancer, liver cancer, prostate cancer, lung cancer, and brain tumors. However, the role of metabolomics in the diagnosis of early gastric cancer is not yet clear.

In this study, we collected blood samples and clinical data of 63 patients with gastric cancer from the First Hospital of Jilin University. And liquid chromatography-mass spectrometry (LC-MS) was used to detect blood sample and obtain metabolic profile. Composition analysis and variance analysis were used to identify different metabolites. Cluster analysis and partial least square discriminant analysis were used to evaluate the similarity between samples. ROC curve was used to evaluate the diagnostic ability of metabolites and identify differential metabolites with strong diagnostic ability. The verification group to test the accuracy of the determined metabolites. Finally, we found that spermine, enterostatin, heparin sulfate, and triacylglycerol serve as specific biomarkers for the diagnosis of early gastric cancer.

Methods

Patient Source and Sample Collection

63 gastric cancer patients diagnosed from October 2019 to January 2020 in the First Hospital of Jilin University including 26 cases of advanced gastric cancer, 37 cases of early gastric cancer, and 18 healthy people. The inclusion criteria for patients with gastric cancer are as follows: 1. Pathologically diagnosed as gastric cancer; 2. New-onset and single-onset malignant tumor; 3. No treatment other than surgery such as traditional Chinese medicine treatment or chemotherapy; 4. Clinical pathological data for patients over 70-year-old. The exclusion criteria are: 1. known congenital diseases; suffering from metabolic diseases, severe heart, lung, liver, kidney and nerve, mental diseases, etc.; 2. alcoholics, drug addicts, long-term use of proton pump inhibitors, Hormones or non-steroidal anti-inflammatory drugs; 3. acute symptoms or severe stress response (such as mental trauma, burns) in the past 2 weeks; 4. blood diseases, including various leukemias and various anemia. The venous blood samples and related clinical data were obtained. The informed consent was obtained from the patients or their family members. The study was approved by the ethics committee of the First Hospital of Jilin university.

Collection and processing of blood samples:

The patient had a light diet the day before collection, and immediately centrifuged at 3500 r/min for 5 minutes after collecting the blood sample. The supernatant was stored at -80°C. After collecting the specimens, take them out of the refrigerator and dissolve them at room temperature. Take 100ul of each sample and add 500ul of acetonitrile, vortex to mix, and centrifuge at 4°C, 14000r/min for 5min. Take the supernatant and add it to a C8 chromatographic column (2.1×150mm, 3.5µm Thermo Fisher Eclipse Plus C18, Thermo Fisher, USA). The column temperature is maintained at 45°C, and the injection volume is 20ul each time. The mobile phase A for positive ions is water plus 0.1% formic acid, and the mobile phase B is acetonitrile plus 0.1% formic acid; the mobile phase A for negative ions is water, and the mobile phase B is

acetonitrile. Before the experiment, the mobile phase was ultrasonicated in a numerically controlled ultrasonic cleaner to exhaust the gas.

Data analysis

Analysis of variance (ANOVA) was used to analyze the data of the cation mode and the anion mode respectively. And the metabolites with statistical differences were screened with F value > 1 and $P < 0.05$ as the standards. Partial least square discriminant analysis (PLS-DA) and support vector machine analysis (SVM) respectively use VIP value > 1 and weight value equal to 100% to screen out highly specific metabolites. Cluster analysis determined the distribution of metabolites in different groups. ROC curve showed the true positive rate on the abscissa and the false positive rate on the ordinate. Through calculating the area under the curve (AUC value), the accuracy of grouping metabolites is judged. Then the metabolic products were selected and the nucleus ratio is imported into the Human Metabolome Database (HMDB) for structural identification to determine the corresponding substance. Finally, the verification group is used to verify the differential metabolites and test their reliability and sensitivity.

Results

Different levels of metabolites in blood samples of patients with early gastric cancer

According to clinical data, the samples were divided into 3 groups. Group A included 26 patients with advanced gastric cancer, group B included 37 patients with early gastric cancer, and group C included 18 healthy people. The mass spectrum data of the metabolites were obtained after LC-MS analysis. PCA found significant differences in the classification of metabolites among there groups (Fig. 1A-B). Total ion current (TIC) showed significant differences in the peak values among the three groups (Fig. 1C-E). Analysis of variance (ANOVA) used F value > 1 and $P < 0.05$ were the screening criteria, and 316 statistically significant metabolites were obtained (Table 1).

Table 1
Results of ANOVA in experimental group

	f.value	p.value	time	FDR	Fisher's LSD
144.04	66.081	2.84E-17	16.547	5.03E-13	3 - 1; 3 - 2
126.05	63.028	8.72E-17	16.06	7.73E-13	3 - 1; 3 - 2
154.98	49.806	1.73E-14	13.763	1.02E-10	1-2; 3 - 1; 3 - 2
186.97	44.349	1.94E-13	12.712	8.60E-10	3 - 1; 3 - 2
346.02	41.497	7.34E-13	12.134	2.60E-09	3 - 1; 3 - 2
144.03	40.776	1.04E-12	11.985	3.06E-09	1-2; 1-3
144.98	37.714	4.62E-12	11.335	1.17E-08	1-2; 3 - 1; 3 - 2
154.01	35.503	1.42E-11	10.849	3.14E-08	2 - 1; 3 - 1; 3 - 2
154.02	34.484	2.40E-11	10.62	4.72E-08	1-2; 1-3
231.02	34.229	2.74E-11	10.562	4.86E-08	3 - 1; 3 - 2
127.06	32.883	5.57E-11	10.254	8.98E-08	1-2; 3 - 1; 3 - 2
173.02	32.386	7.27E-11	10.138	1.07E-07	1-2; 1-3
172.99	31.716	1.04E-10	9.9817	1.34E-07	3 - 1; 3 - 2
83.21	31.693	1.06E-10	9.9761	1.34E-07	3 - 1; 3 - 2
111.05	29.665	3.22E-10	9.4917	3.81E-07	3 - 1; 3 - 2
225.03	29.53	3.48E-10	9.459	3.85E-07	3 - 1; 3 - 2
484.36	29.1	4.42E-10	9.3542	4.46E-07	3 - 1; 3 - 2
143.03	29.057	4.53E-10	9.3437	4.46E-07	1-2; 3 - 1; 3 - 2
224.01	28.916	4.91E-10	9.3091	4.58E-07	1-2; 3 - 2
149.01	28.481	6.28E-10	9.202	5.57E-07	3 - 1; 3 - 2
181.99	28.011	8.21E-10	9.0856	6.93E-07	1-2; 3 - 1; 3 - 2
222.95	27.279	1.25E-09	8.9026	1.01E-06	3 - 1; 3 - 2
274.15	26.557	1.91E-09	8.72	1.47E-06	3 - 1; 3 - 2
169.96	25.827	2.93E-09	8.5333	2.09E-06	3 - 1; 3 - 2
169.97	25.818	2.94E-09	8.531	2.09E-06	1-2; 1-3; 3 - 2
147	25.287	4.04E-09	8.3939	2.75E-06	1-2; 1-3
478.25	24.403	6.87E-09	8.163	4.51E-06	3 - 1; 3 - 2

	f.value	p.value	time	FDR	Fisher's LSD
113.05	24.058	8.47E-09	8.072	5.36E-06	3 - 1; 3 - 2
180.03	23.841	9.67E-09	8.0144	5.91E-06	1-2; 3 - 1; 3 - 2
196.05	23.37	1.29E-08	7.8889	7.63E-06	1-2; 3 - 2
142.02	22.595	2.09E-08	7.6804	1.19E-05	3 - 1; 3 - 2
590.38	22.449	2.29E-08	7.6407	1.27E-05	3 - 1; 3 - 2
158.98	22.278	2.55E-08	7.5942	1.37E-05	3 - 1; 3 - 2
428.33	22.159	2.74E-08	7.5618	1.43E-05	3 - 1; 3 - 2
93.28	21.899	3.23E-08	7.4907	1.64E-05	3 - 1; 3 - 2
163.04	21.417	4.39E-08	7.3578	2.16E-05	1-2; 3 - 1; 3 - 2
251.98	20.762	6.67E-08	7.1758	3.20E-05	1-2; 3 - 1; 3 - 2
164.08	20.704	6.93E-08	7.1595	3.23E-05	1-2; 3 - 1; 3 - 2
180	20.62	7.31E-08	7.136	3.32E-05	2 - 1; 2-3
151.03	20.397	8.45E-08	7.0734	3.68E-05	1-2; 3 - 1; 3 - 2
451.29	20.386	8.51E-08	7.0703	3.68E-05	3 - 1; 3 - 2
136.07	19.845	1.21E-07	6.9174	5.10E-05	1-2; 3 - 1; 3 - 2
504.33	19.775	1.27E-07	6.8975	5.22E-05	3 - 1; 3 - 2
163.02	19.448	1.57E-07	6.8042	6.32E-05	2 - 1; 2-3
521.34	19.274	1.76E-07	6.7543	6.94E-05	3 - 1; 3 - 2
95.1	19.105	1.97E-07	6.7059	7.59E-05	3 - 1; 3 - 2
180.97	18.772	2.46E-07	6.6099	9.26E-05	1-2; 3 - 1; 3 - 2
152.03	18.485	2.97E-07	6.5266	0.00010985	3 - 1; 3 - 2
358.27	17.814	4.68E-07	6.3302	0.00016914	3 - 1; 3 - 2
171.04	17.668	5.16E-07	6.287	0.00018309	1-2; 3 - 1; 3 - 2
79.24	17.07	7.77E-07	6.1095	0.00027014	1-2; 1-3
196.06	16.762	9.61E-07	6.0173	0.00032755	3 - 1; 3 - 2
199.07	16.692	1.01E-06	5.9965	0.0003324	1-2; 1-3
275.15	16.686	1.01E-06	5.9946	0.0003324	3 - 1; 3 - 2
175.97	16.208	1.41E-06	5.8502	0.00045507	1-2; 1-3; 3 - 2
174.99	15.984	1.65E-06	5.7822	0.00052265	3 - 1; 3 - 2

	f.value	p.value	time	FDR	Fisher's LSD
272.96	15.856	1.81E-06	5.7431	0.00055437	3 - 1; 3 - 2
103.09	15.819	1.85E-06	5.7319	0.00055437	3 - 1; 3 - 2
180.04	15.785	1.90E-06	5.7216	0.00055437	3 - 1; 3 - 2
135.05	15.727	1.98E-06	5.7037	0.00055437	3 - 1; 3 - 2
130.21	15.726	1.98E-06	5.7034	0.00055437	3 - 1; 3 - 2
195.07	15.707	2.01E-06	5.6977	0.00055437	3 - 1; 3 - 2
156.99	15.698	2.02E-06	5.6948	0.00055437	3 - 1; 3 - 2
264.06	15.689	2.03E-06	5.6922	0.00055437	3 - 1; 3 - 2
402.35	15.681	2.04E-06	5.6898	0.00055437	3 - 1; 3 - 2
588.38	15.639	2.10E-06	5.6769	0.00055437	3 - 1; 3 - 2
502.27	15.639	2.11E-06	5.6767	0.00055437	3 - 1; 3 - 2
456.32	15.624	2.13E-06	5.6723	0.00055437	3 - 1; 3 - 2
598.41	15.553	2.24E-06	5.6505	0.00057441	3 - 1; 3 - 2
547.35	15.422	2.45E-06	5.6101	0.00062145	1-2; 3 - 1; 3 - 2
696.46	15.373	2.54E-06	5.595	0.00063442	3 - 1; 3 - 2
612.44	15.344	2.59E-06	5.5861	0.0006385	3 - 1; 3 - 2
224.02	15.274	2.73E-06	5.5645	0.00066186	3 - 1; 3 - 2
61.37	15.192	2.89E-06	5.5392	0.00069216	1-2; 1-3; 2-3
457.26	15.096	3.09E-06	5.5094	0.00073136	3 - 1; 3 - 2
161.04	15.068	3.15E-06	5.501	0.0007355	1-2; 3 - 1; 3 - 2
286.09	15.051	3.20E-06	5.4955	0.0007355	3 - 1; 3 - 2
404.24	14.991	3.33E-06	5.477	0.00075777	3 - 1; 3 - 2
400.31	14.883	3.60E-06	5.4435	0.00080592	3 - 1; 3 - 2
503.29	14.869	3.64E-06	5.4392	0.00080592	3 - 1; 3 - 2
157.01	14.817	3.78E-06	5.423	0.00082633	1-2; 1-3; 2-3
392.33	14.571	4.51E-06	5.3461	0.00097422	3 - 1; 3 - 2
467.28	14.549	4.58E-06	5.3394	0.00097752	3 - 1; 3 - 2
479.28	14.438	4.96E-06	5.3044	0.0010469	3 - 1; 3 - 2
166.97	14.311	5.44E-06	5.2645	0.0011222	3 - 1; 3 - 2

	f.value	p.value	time	FDR	Fisher's LSD
270.11	14.309	5.44E-06	5.2641	0.0011222	3 - 1; 3 - 2
199.08	14.062	6.51E-06	5.1861	0.0013274	3 - 1; 3 - 2
253.06	14.02	6.72E-06	5.1729	0.0013528	3 - 1; 3 - 2
252.01	13.72	8.36E-06	5.0777	0.0016654	2 - 1; 2 - 3
655.36	13.702	8.47E-06	5.0719	0.0016689	3 - 1; 3 - 2
600.44	13.56	9.40E-06	5.0269	0.0018308	3 - 1; 3 - 2
157.97	13.43	1.03E-05	4.9852	0.0019934	3 - 1; 3 - 2
327.05	13.403	1.06E-05	4.9766	0.0020113	3 - 1; 3 - 2
405.22	13.059	1.36E-05	4.8664	0.0025648	3 - 1; 3 - 2
172.03	13.022	1.40E-05	4.8542	0.0025927	3 - 1; 3 - 2
148.01	13.017	1.40E-05	4.8526	0.0025927	1-2; 3 - 1; 3 - 2
362.24	12.936	1.49E-05	4.8267	0.0027239	3 - 1; 3 - 2
107.11	12.76	1.70E-05	4.7697	0.0030742	3 - 1; 3 - 2
194.08	12.703	1.77E-05	4.7512	0.0031754	3 - 1; 3 - 2
251.99	12.627	1.88E-05	4.7267	0.0033263	1-2; 1-3
229	12.587	1.93E-05	4.7136	0.0033936	3 - 1; 3 - 2
225.98	12.481	2.09E-05	4.6789	0.0036405	2 - 1; 2 - 3
60.34	12.379	2.26E-05	4.6457	0.0038633	1-2; 3 - 2
174.98	12.369	2.28E-05	4.6425	0.0038633	3 - 1; 3 - 2
126.07	12.363	2.29E-05	4.6405	0.0038633	1-2; 1-3
144.97	12.323	2.36E-05	4.6275	0.0039125	2 - 1; 2 - 3
293	12.321	2.36E-05	4.6268	0.0039125	3 - 1; 3 - 2
156.03	12.266	2.46E-05	4.6088	0.0040402	1-2; 3 - 1; 3 - 2
84.24	12.144	2.70E-05	4.5687	0.0043904	3 - 1; 3 - 2
140.02	12.055	2.89E-05	4.5397	0.0046508	3 - 1; 3 - 2
525.38	12.009	2.99E-05	4.5245	0.0047735	3 - 1; 3 - 2
216.02	11.976	3.06E-05	4.5136	0.0048506	1-2; 3 - 2
468.3	11.932	3.17E-05	4.4991	0.0049711	3 - 1; 3 - 2
162.06	11.913	3.21E-05	4.4928	0.0049987	3 - 1; 3 - 2

	f.value	p.value	time	FDR	Fisher's LSD
198.13	11.869	3.32E-05	4.4784	0.0051227	1-2; 3 - 1; 3 - 2
525.37	11.787	3.54E-05	4.4514	0.0054042	3 - 1; 3 - 2
345.05	11.761	3.61E-05	4.4428	0.005466	3 - 1; 3 - 2
144.99	11.697	3.79E-05	4.4216	0.0056898	1-2; 1-3
404.21	11.617	4.03E-05	4.3949	0.0060003	1-2; 1-3
432.19	11.605	4.06E-05	4.391	0.0060032	3 - 1; 3 - 2
518.29	11.505	4.39E-05	4.3577	0.0063764	3 - 1; 3 - 2
520.3	11.501	4.40E-05	4.3564	0.0063764	3 - 1; 3 - 2
142.03	11.494	4.42E-05	4.3541	0.0063764	3 - 1; 3 - 2
73.36	11.457	4.55E-05	4.3419	0.0065059	3 - 1; 3 - 2
350.26	11.396	4.77E-05	4.3214	0.006765	3 - 1; 3 - 2
251.97	11.379	4.83E-05	4.3158	0.0067842	3 - 1; 3 - 2
186.98	11.371	4.86E-05	4.3133	0.0067842	1-2; 3 - 2
166.02	11.355	4.92E-05	4.3079	0.0068149	3 - 1; 3 - 2
157.98	11.281	5.21E-05	4.2831	0.0071597	1-2; 3 - 1; 3 - 2
236	11.237	5.39E-05	4.2684	0.0073497	1-2; 3 - 2
225.99	11.216	5.48E-05	4.2616	0.0073738	1-2; 3 - 2
214.01	11.203	5.53E-05	4.2571	0.0073738	1-2; 3 - 2
97.1	11.203	5.53E-05	4.2571	0.0073738	3 - 1; 3 - 2
90.19	11.173	5.66E-05	4.2473	0.007486	1-2; 3 - 2
392.31	11.148	5.77E-05	4.2386	0.0075798	3 - 1; 3 - 2
248.99	11.006	6.44E-05	4.1912	0.0083924	3 - 1; 3 - 2
561.32	10.969	6.63E-05	4.1787	0.0085748	3 - 1; 3 - 2
286.14	10.954	6.70E-05	4.1738	0.0086091	1-2; 1-3
496.3	10.829	7.39E-05	4.1316	0.0094177	3 - 1; 3 - 2
61.36	10.811	7.49E-05	4.1255	0.0094847	3 - 1; 3 - 2
64.34	10.672	8.34E-05	4.0786	0.010489	2 - 1; 1-3; 2-3
218.98	10.636	8.58E-05	4.0664	0.010578	3 - 1; 3 - 2
208.03	10.574	9.00E-05	4.0456	0.010578	3 - 1; 3 - 2

	f.value	p.value	time	FDR	Fisher's LSD
130.22	10.573	9.01E-05	4.0451	0.010578	3 - 1; 3 - 2
437.15	10.571	9.02E-05	4.0446	0.010578	3 - 1; 3 - 2
55.4	10.571	9.03E-05	4.0444	0.010578	3 - 1; 3 - 2
103.1	10.57	9.04E-05	4.044	0.010578	3 - 1; 3 - 2
196.07	10.566	9.06E-05	4.0428	0.010578	3 - 1; 3 - 2
404.25	10.563	9.08E-05	4.0419	0.010578	3 - 1; 3 - 2
331.05	10.559	9.11E-05	4.0404	0.010578	3 - 1; 3 - 2
363.22	10.558	9.12E-05	4.04	0.010578	3 - 1; 3 - 2
248.97	10.556	9.13E-05	4.0394	0.010578	3 - 1; 3 - 2
424.3	10.556	9.14E-05	4.0392	0.010578	3 - 1; 3 - 2
464.32	10.548	9.19E-05	4.0367	0.010578	3 - 1; 3 - 2
166.98	10.533	9.30E-05	4.0315	0.010635	1-2; 1-3
500.3	10.52	9.40E-05	4.0271	0.010673	3 - 1; 3 - 2
348.08	10.508	9.48E-05	4.0231	0.010673	3 - 1; 3 - 2
608.45	10.502	9.52E-05	4.0212	0.010673	3 - 1; 3 - 2
595.4	10.49	9.61E-05	4.0171	0.010673	3 - 1; 3 - 2
74.31	10.487	9.64E-05	4.016	0.010673	3 - 1; 3 - 2
500.31	10.48	9.69E-05	4.0135	0.010673	3 - 1; 3 - 2
111.06	10.458	9.86E-05	4.006	0.01075	3 - 1; 3 - 2
388.34	10.447	9.94E-05	4.0025	0.01075	3 - 1; 3 - 2
462.34	10.444	9.97E-05	4.0013	0.01075	3 - 1; 3 - 2
173	10.439	0.00010007	3.9997	0.01075	3 - 1; 3 - 2
420.3	10.424	0.00010125	3.9946	0.010809	3 - 1; 3 - 2
402.33	10.417	0.00010184	3.9921	0.010809	3 - 1; 3 - 2
430.31	10.408	0.0001025	3.9893	0.010815	3 - 1; 3 - 2
360.16	10.393	0.00010373	3.9841	0.01088	3 - 1; 3 - 2
223.99	10.374	0.00010532	3.9775	0.010982	2 - 1; 2-3
586.31	10.365	0.00010601	3.9746	0.010989	3 - 1; 3 - 2
396.31	10.355	0.00010687	3.9711	0.011014	3 - 1; 3 - 2

	f.value	p.value	time	FDR	Fisher's LSD
658.45	10.329	0.00010905	3.9624	0.011174	3 - 1; 3 - 2
479.3	10.308	0.00011087	3.9552	0.011237	3 - 1; 3 - 2
449.25	10.301	0.00011147	3.9528	0.011237	3 - 1; 3 - 2
486.3	10.3	0.00011157	3.9525	0.011237	3 - 1; 3 - 2
444.35	10.289	0.00011253	3.9487	0.011269	3 - 1; 3 - 2
348.97	10.253	0.00011579	3.9363	0.0114	3 - 1; 3 - 2
194.05	10.252	0.00011586	3.936	0.0114	2 - 1; 2 - 3
518.31	10.248	0.0001162	3.9348	0.0114	3 - 1; 3 - 2
407.24	10.246	0.00011641	3.934	0.0114	3 - 1; 3 - 2
543.31	10.234	0.00011754	3.9298	0.011422	3 - 1; 3 - 2
675.53	10.23	0.00011791	3.9284	0.011422	3 - 1; 3 - 2
412.36	10.214	0.00011943	3.9229	0.011505	3 - 1; 3 - 2
390.3	10.202	0.00012052	3.9189	0.011548	3 - 1; 3 - 2
218.01	10.185	0.00012216	3.9131	0.011595	1 - 2; 3 - 2
180.02	10.183	0.00012232	3.9125	0.011595	1 - 2; 1 - 3
481.3	10.169	0.00012369	3.9077	0.011662	3 - 1; 3 - 2
544.32	10.153	0.00012527	3.9022	0.011748	3 - 1; 3 - 2
545.35	10.128	0.0001277	3.8938	0.011908	3 - 1; 3 - 2
474.33	10.122	0.00012834	3.8916	0.011908	3 - 1; 3 - 2
232.95	10.101	0.00013048	3.8845	0.011908	3 - 1; 3 - 2
117.09	10.083	0.00013231	3.8784	0.011908	2 - 1; 2 - 3
345.04	10.083	0.00013233	3.8784	0.011908	3 - 1; 3 - 2
506.35	10.083	0.00013237	3.8782	0.011908	3 - 1; 3 - 2
745.58	10.08	0.00013261	3.8774	0.011908	3 - 1; 3 - 2
498.35	10.08	0.0001327	3.8771	0.011908	3 - 1; 3 - 2
270.98	10.077	0.00013301	3.8761	0.011908	3 - 1; 3 - 2
431.25	10.04	0.00013689	3.8636	0.012112	3 - 1; 3 - 2
362.25	10.037	0.00013722	3.8626	0.012112	3 - 1; 3 - 2
477.31	10.036	0.00013735	3.8622	0.012112	3 - 1; 3 - 2

	f.value	p.value	time	FDR	Fisher's LSD
400.29	9.9863	0.00014284	3.8452	0.01248	3 - 1; 3 - 2
481.31	9.9856	0.00014292	3.8449	0.01248	3 - 1; 3 - 2
528.36	9.9609	0.00014573	3.8364	0.012663	3 - 1; 3 - 2
286.1	9.9308	0.00014925	3.8261	0.012905	3 - 1; 3 - 2
113.06	9.9038	0.00015247	3.8168	0.013119	3 - 1; 3 - 2
466.32	9.8682	0.00015682	3.8046	0.013406	3 - 1; 3 - 2
477.3	9.8644	0.0001573	3.8033	0.013406	3 - 1; 3 - 2
448.35	9.8476	0.0001594	3.7975	0.01352	3 - 1; 3 - 2
414.27	9.8356	0.00016093	3.7934	0.013584	3 - 1; 3 - 2
134.11	9.8207	0.00016284	3.7882	0.01368	3 - 1; 3 - 2
523.38	9.7981	0.00016578	3.7805	0.01386	3 - 1; 3 - 2
452.31	9.7923	0.00016654	3.7785	0.01386	3 - 1; 3 - 2
386.27	9.7832	0.00016775	3.7753	0.013895	3 - 1; 3 - 2
305.17	9.7748	0.00016887	3.7725	0.013922	3 - 1; 3 - 2
422.32	9.7651	0.00017018	3.7691	0.013966	3 - 1; 3 - 2
522.34	9.7476	0.00017256	3.7631	0.014093	3 - 1; 3 - 2
699.48	9.7408	0.00017349	3.7607	0.014093	3 - 1; 3 - 2
202	9.732	0.00017471	3.7577	0.014093	1-2; 3 - 2
565.35	9.7264	0.00017548	3.7558	0.014093	3 - 1; 3 - 2
555.81	9.7248	0.0001757	3.7552	0.014093	3 - 1; 3 - 2
274.13	9.7132	0.00017734	3.7512	0.01416	1-2; 1-3
462.35	9.669	0.00018368	3.7359	0.0146	3 - 1; 3 - 2
442.33	9.6552	0.0001857	3.7312	0.014695	3 - 1; 3 - 2
290.11	9.6442	0.00018734	3.7274	0.014759	3 - 1; 3 - 2
564.37	9.6272	0.00018988	3.7215	0.014893	3 - 1; 3 - 2
361.11	9.5677	0.00019909	3.701	0.015546	3 - 1; 3 - 2
270.99	9.5259	0.00020584	3.6865	0.016003	3 - 1; 3 - 2
250.99	9.5013	0.00020993	3.6779	0.01625	3 - 1; 3 - 2
492.39	9.4716	0.00021495	3.6677	0.016479	3 - 1; 3 - 2

	f.value	p.value	time	FDR	Fisher's LSD
260.05	9.4714	0.00021498	3.6676	0.016479	1-2; 1-3
615.47	9.4605	0.00021687	3.6638	0.016479	3 - 1; 3 - 2
243.04	9.4585	0.00021722	3.6631	0.016479	1-2; 1-3
299.05	9.4566	0.00021754	3.6625	0.016479	3 - 1; 3 - 2
283.11	9.4442	0.0002197	3.6582	0.016511	2 - 1; 2-3
737.48	9.4436	0.00021982	3.6579	0.016511	3 - 1; 3 - 2
169	9.4295	0.0002223	3.6531	0.016626	3 - 1; 3 - 2
556.43	9.3765	0.00023192	3.6347	0.017273	3 - 1; 3 - 2
394.28	9.3654	0.00023401	3.6308	0.017356	3 - 1; 3 - 2
117.1	9.3529	0.00023635	3.6264	0.017456	1-2; 3 - 2
464.36	9.2924	0.00024808	3.6054	0.018202	3 - 1; 3 - 2
239.04	9.2876	0.00024904	3.6037	0.018202	3 - 1; 3 - 2
194.06	9.2852	0.00024952	3.6029	0.018202	1-2; 3 - 1; 3 - 2
142.07	9.2735	0.00025187	3.5988	0.018298	2 - 1; 2-3
62.35	9.2351	0.00025974	3.5855	0.018793	1-2; 1-3; 2-3
126.09	9.2295	0.00026093	3.5835	0.018802	2 - 1; 2-3
318.22	9.2089	0.00026527	3.5763	0.018987	3 - 1; 3 - 2
357.71	9.2072	0.00026564	3.5757	0.018987	3 - 1; 3 - 2
469.33	9.2015	0.00026685	3.5737	0.018997	3 - 1; 3 - 2
390.33	9.1887	0.0002696	3.5693	0.019116	3 - 1; 3 - 2
89.21	9.1709	0.0002735	3.563	0.019315	1-2; 3 - 2
255.02	9.1621	0.00027544	3.56	0.019352	3 - 1; 3 - 2
114.89	9.1532	0.00027742	3.5569	0.019352	3 - 1; 3 - 2
227.98	9.1527	0.00027751	3.5567	0.019352	3 - 1; 3 - 2
213.07	9.1488	0.00027839	3.5553	0.019352	3 - 1; 3 - 2
421.21	9.1281	0.00028306	3.5481	0.019599	3 - 1; 3 - 2
305.09	9.1207	0.00028475	3.5455	0.01964	2 - 1; 2-3
188.04	9.0912	0.00029159	3.5352	0.020034	1-2; 3 - 2
255.03	9.0802	0.0002942	3.5314	0.020135	3 - 1; 3 - 2

	f.value	p.value	time	FDR	Fisher's LSD
466.33	9.0694	0.00029676	3.5276	0.020232	3 - 1; 3 - 2
368.16	8.9879	0.00031689	3.4991	0.021522	3 - 1; 3 - 2
304.21	8.9637	0.00032316	3.4906	0.021864	3 - 1; 3 - 2
510.31	8.9501	0.00032673	3.4858	0.022021	2 - 1; 2 - 3
649.4	8.9387	0.00032974	3.4818	0.02214	3 - 1; 3 - 2
232.01	8.9241	0.00033364	3.4767	0.022317	1 - 2; 3 - 2
639.38	8.8842	0.00034458	3.4627	0.022962	3 - 1; 3 - 2
235.99	8.8199	0.000363	3.4401	0.024099	1 - 2; 3 - 2
206.94	8.8088	0.00036626	3.4362	0.024183	3 - 1; 3 - 2
369.26	8.8063	0.00036699	3.4353	0.024183	3 - 1; 3 - 2
97.11	8.7978	0.00036954	3.4323	0.024261	3 - 1; 3 - 2
520.32	8.7725	0.00037718	3.4235	0.024671	3 - 1; 3 - 2
131.05	8.6669	0.00041095	3.3862	0.026781	3 - 1; 3 - 2
441.24	8.5853	0.00043911	3.3574	0.028512	3 - 1; 3 - 2
450.28	8.5531	0.0004508	3.346	0.029164	3 - 1; 3 - 2
141.02	8.5059	0.00046846	3.3293	0.030157	3 - 1; 3 - 2
747.57	8.503	0.00046955	3.3283	0.030157	3 - 1; 3 - 2
363.19	8.498	0.0004715	3.3265	0.030172	1 - 2; 1 - 3
217.99	8.4797	0.00047857	3.3201	0.030515	2 - 1; 2 - 3
146.98	8.4299	0.0004984	3.3024	0.031665	2 - 1; 3 - 1; 3 - 2
197.02	8.3825	0.00051808	3.2856	0.032798	1 - 2; 3 - 1; 3 - 2
444.34	8.3726	0.0005223	3.2821	0.032947	3 - 1; 3 - 2
359.2	8.3234	0.00054375	3.2646	0.034179	3 - 1; 3 - 2
213.13	8.3107	0.00054942	3.2601	0.034414	1 - 2; 1 - 3
241.01	8.3002	0.00055416	3.2564	0.034588	3 - 1; 3 - 2
182.94	8.2891	0.00055922	3.2524	0.034754	2 - 1; 2 - 3
480.31	8.2858	0.00056073	3.2512	0.034754	3 - 1; 3 - 2
448.33	8.2768	0.00056492	3.248	0.034891	3 - 1; 3 - 2
126.08	8.2191	0.00059225	3.2275	0.036452	2 - 1; 2 - 3

	f.value	p.value	time	FDR	Fisher's LSD
154.99	8.1959	0.00060365	3.2192	0.037025	1-3; 2-3
231.03	8.1712	0.00061598	3.2104	0.03763	1-2; 1-3
320.16	8.1646	0.00061935	3.2081	0.03763	3-1; 3-2
548.35	8.1603	0.00062156	3.2065	0.03763	3-1; 3-2
116.11	8.1594	0.00062201	3.2062	0.03763	3-1; 3-2
341.1	8.1421	0.00063088	3.2001	0.038002	3-1; 3-2
401.26	8.136	0.0006341	3.1978	0.038002	3-1; 3-2
729.56	8.135	0.00063458	3.1975	0.038002	3-1; 3-2
138.03	8.1007	0.00065275	3.1853	0.038958	3-1; 3-2
460.26	8.0696	0.00066968	3.1741	0.039835	3-1; 3-2
118.12	8.0517	0.00067961	3.1677	0.04029	1-2; 3-2
177.03	8.0183	0.00069854	3.1558	0.041204	2-1; 2-3
84.25	8.0163	0.00069968	3.1551	0.041204	1-3; 2-3
583.24	8.001	0.0007086	3.1496	0.041592	3-1; 3-2
142.04	7.9634	0.00073091	3.1361	0.04276	1-2; 1-3
99.09	7.9571	0.0007347	3.1339	0.04284	3-1; 3-2
533.28	7.9479	0.00074027	3.1306	0.042943	3-1; 3-2
458.31	7.9462	0.00074132	3.13	0.042943	3-1; 3-2
690.55	7.8895	0.00077687	3.1097	0.044856	3-1; 3-2
325.03	7.8432	0.00080716	3.093	0.046453	3-1; 3-2
174.01	7.8374	0.00081103	3.091	0.046525	1-2; 1-3
184.98	7.8269	0.00081816	3.0872	0.046783	1-2; 1-3
299.07	7.8214	0.00082188	3.0852	0.046844	3-1; 3-2
744.54	7.8168	0.00082502	3.0835	0.046873	3-1; 3-2
472.29	7.7804	0.00085022	3.0705	0.04815	3-1; 3-2
263.05	7.7721	0.00085607	3.0675	0.048319	3-1; 3-2
190.95	7.7685	0.00085865	3.0662	0.048319	3-1; 3-2
543.32	7.7529	0.00086985	3.0606	0.048794	3-1; 3-2

Screening of differential metabolites in blood samples of gastric cancer patients with a weight of 100%.

Partial least squares discriminant analysis and analysis of variance were used to further analyze the metabolites, and the score map (Fig. 2A) and VIP value map (Fig. 2B) were obtained. According to the VIP map, there are 5 metabolites with VIP value greater than 1. The support vector machine in the BRB-array software was used for further analysis, and a total of 4 different metabolites with a weight of 100% were found (Table 2). Sensitivity and specificity were calculated among the groups of overall gastric cancer (group A and B), early gastric cancer (group B), and healthy people (group C) (Table 3 and Table 4). The cluster analysis of the above four substances showed that there are different metabolites between each group (Fig. 2C). The four metabolites were imported into the human metabolomics database (HMDB), and the mass-to-charge ratio (M/Z) was used to identify their structure to determine the corresponding substances (Table 5).

Table 2
Support vector machine results

	ParametricP-value	T-value	CVsupport(%)	Geom mean of intersities in class 1	Geom mean of intersities in class 2	Geom mean of intersities in class 3	Unique ID	Clone ID
1	< 1e-07	1.00E+07	100	< 1e-07	< 1e-07	10	83.21	83.21
2	3.80E-06	15.885	100	< 1e-07	5.30E-07	2.88	496.3	496.3
3	3.80E-06	14.642	100	2.67	4.00E-05	3.04	214.01	214.01
4	5.63E-05	11.792	100	< 1e-07	8.70E-07	0.0078	521.34	521.34

Table 3
The sensitivity and specificity of 4 metabolites for distinguishing gastric cancer from healthy people

Predicting Outcomes	Actual outcomes		summation	Sensitivity	Specificity
	gastric cancer group	Normal group			
gastric cancer group	45	2	47		
Normal group	0	10	10	100%	83.3%
summation	45	12	57		

Table 4

The sensitivity and specificity of 4 metabolites in distinguishing early gastric cancer from healthy people

Predicting Outcomes	Actual outcomes		summation	Sensitivity	Specificity
	gastric cancer group	Normal group			
gastric cancer group	28	1	29		
Normal group	0	10	10	100%	90.9%
summation	28	11	39		

Table 4

The names and formulas of the four metabolites

nucleo-cytoplasmic ratio	English ID	Molecular formula
83.21	Spermine	$C_{10}H_{26}N_4$
496.3	APGPR Enterostatin	$C_{21}H_{36}N_8O_6$
214.01	Heparan sulfate	$C_{14}H_{25}NO_{21}S_3$
521.34	2,3-Diacetoxypopyl stearate	$C_{25}H_{46}O_6$

Differential metabolites have good diagnostic ability for patients with early gastric cancer.

ROC curve analysis shows that spermine, enterostatin, heparin sulfate, and triacylglycerol had good diagnostic ability in overall gastric cancer (group A and B), healthy people (group C), and early gastric cancer (Fig. 3A, Table 6, Table 7). Further the bar graph showed the mean values of 4 different metabolites in the three groups, and the results show that: 4 metabolites have significant statistical differences in each group (Fig. 3B).

Table 6

ROC curve area value (AUC) of 4 metabolites in gastric cancer patients and healthy people

Test variable results	Area	Standard error	Gradual Sig	Asymptotic 95% confidence interval	
				upper limit	inferior limit
83.21	.962	.024	.000	.914	1.000
214.01	.857	.050	.000	.760	.955
496.3	.962	.027	.000	.909	1.000
521.34	.874	.086	.000	.706	1.000

Table 6
ROC curve area value (AUC) of 4 metabolites in early gastric cancer patients and healthy people

Test variable results	Area	Standard error	Gradual Sig	Asymptotic 95% confidence interval	
				upper limit	inferior limit
83.21	.883	.083	.000	.720	1.000
214.01	.895	.079	.000	.741	1.000
496.3	.966	.034	.000	.899	1.000
521.34	.862	.092	.001	.681	1.000

Verification of 4 metabolites

The samples of the verification group were divided into three groups: A (advanced gastric cancer), B (early gastric cancer), and C (healthy volunteers). The blood samples of 24 patients in the three groups were processed by LC-MS to obtain mass spectrum data and then clustered analysis. The analysis results were consistent with the experimental grouping (Fig. 4A). To ensure the reliability of the verification experiment results, the experimental results were tested for sensitivity, specificity (Table 8, Table 9) and ROC curve analysis (Fig. 4B-C, Table 10, Table 11). The results proved that the verification group experiment has good reliability.

Table 8
To verify the sensitivity and specificity of 4 metabolites for distinguishing gastric cancer from healthy people

Predicting Outcomes	Actual outcomes		summation	Sensitivity	Specificity
	gastric cancer group	Normal group			
gastric cancer group	15	1	16		
Normal group	2	6	8	88.2%	85.7%
summation	17	7	24		

Table 9

The sensitivity and specificity of the 4 metabolites in the verification group for distinguishing early gastric cancer from healthy people

Predicting Outcomes	Actual outcomes		summation	Sensitivity	Specificity
	gastric cancer group	Normal group			
gastric cancer group	7	1	8		
Normal group	2	6	8	77.8%	85.7%
summation	9	7	16		

Table 10

ROC curve table of 4 metabolites in gastric cancer and healthy human samples was verified

Test variable results	Area	Standard error	Gradual Sig	Asymptotic 95% confidence interval	
				Upper limit	inferior limit
83.21	.938	.052	.001	.836	1.000
214.01	.853	.047	.000	.860	1.000
496.3	.805	.101	.017	.606	1.000
521.34	.930	.075	.001	.783	1.000

Table 11

ROC curve table of 4 metabolites in the validation group in early gastric cancer and healthy human samples

Test variable results	Area	Standard error	Gradual Sig	Asymptotic 95% confidence interval	
				upper limit	inferior limit
83.21	.938	.072	.003	.836	1.000
214.01	.883	.111	.111	.860	1.000
496.3	.922	.069	.069	.606	1.000
521.34	.931	.071	.001	.783	1.000

Discussion

Gastric cancer is one of the common malignant tumors with high morbidity. According to statistics in 2018, there were 1.034 million new cases worldwide, and about 783,000 patients died, accounting for 8.2% of the total deaths¹². However, the prognosis of advanced gastric cancer and early gastric cancer are completely different. Due to the lack of specificity symptoms of early gastric cancer and insufficient screening methods,

more than two-thirds of patients have been at the middle and advanced stages of gastric cancer at the first diagnosis, resulting in only 30% of 5-year survival rate¹³. However, the 5-year overall survival rate after accurate diagnosis at an early stage and timely radical resection of gastric cancer exceeds 90%. At present, the diagnosis of gastric cancer mainly relies on imaging examination, gastrointestinal endoscopy, and histopathology. However, early gastric cancer has no obvious symptoms or signs. Conventional ultrasound, CT and other imaging detection methods cannot accurately diagnose. Besides, there are no obvious general changes in the early gastric cancer lesions. Therefore, conventional endoscopy is less sensitive to early gastric cancer, with a detection rate of about 7.3%¹⁴. At the same time, endoscopy is invasive, prone to cross-infection and relatively expensive. Due to its high limitations, it cannot be used as a tool for large-scale gastric cancer screening. Therefore, it is imperative to find more sensitive early gastric cancer diagnostic markers. Metabolomics is an important method for the screening of biomarkers. Compared with the problems of gene complexity and post-transcriptional modification faced by other omics research, metabolomics can directly and comprehensively reflect the body's endogenous metabolic information^{15,16}. Many studies have reported the application of metabolomics in the discovery of early biomarkers for breast cancer, liver cancer, rectal cancer, and other tumors. For example, Xu et al. (show) adopted Mendelian randomized method to perform 27 case-control studies on the relationship between blood homocysteine levels and the risk of gastric cancer, which proved that the blood homocysteine level has a significant impact on the risk of gastric cancer. The purpose of this experiment is to use liquid chromatography-mass spectrometry (LC-MS) to detect serum samples of advanced gastric cancer, early gastric cancer, and healthy people. Spermine, enterostatin, Heparan sulfate and 2,3-Diacetoxypentyl stearate have good diagnostic ability for early gastric cancer, and the verification group have proved that the results are reliable and sensitive.

Spermine is a more common type of polyamines (PAs). It is widely involved in many cell activities, including cell growth, proliferation, differentiation, amino acid and protein synthesis, and DNA damage^{17,18}. Studies have found that changes in the content of polyamines are accompanied by the occurrence and development of tumors, which proved that polyamines are involved in tumor transformation and the regulation of tumor microenvironment¹⁹. The content of polyamines in proliferating cells is significantly higher than that in slow proliferation. Therefore, the content of polyamines is higher in many tumor tissues than that in corresponding normal tissues^{20,21}. Besides, the biosynthesis, catabolism, and membrane transport of polyamines in cells are strictly regulated. Therefore, changes in polyamine content also represent abnormal cell membrane functions. Rupesh haturvedi found that *Helicobacter pylori* strains increase the level of spermine oxidase, which decomposes spermine to increase the level of its metabolite hydrogen peroxide, resulting in DNA damage, and ultimately leads to gastric cancer occur²². In his study, we found significant different levels of spermine in blood samples between early gastric cancer and healthy people. The results are the same as previous studies, providing a new scientific basis for biomarker screening and drug targets for early gastric cancer.

Heparan sulfate, also called heparan sulfate, is composed of glucosamine (GLcN) and glucuronic acid (GLcA) or iduronic acid (IdocA). Its function is modified by some enzymatic reactions in the synthesis process²³. Heparan sulfate combines with the core protein on the cell membrane to form the heparan sulfate

proteoglycan (HSPG) in the tumor extracellular matrix and basement membrane. It plays an important role in the regulation of inflammation, cell growth, apoptosis, infection, tumor development and other different pathophysiological processes²⁴. At the same time, heparin sulfate can also be used as a carrier of cell nutrition and growth factors, which can transport cellular nutrients into cells, and can also transport growth factors without membrane receptors, thereby affecting the signal activation of downstream transduction pathways²⁵, Barbara Bartolini systematically described the role of heparin sulfate in the cancer occurrence and development²⁶. Other studies have shown that heparin sulfate plays an important role in the embryonic development of the stomach: HS is necessary for the differentiation of the front stomach squamous epithelium and the basal cells. HS activates the FGF in epithelial cells by promoting the combination of FGF and FGFR, which controls the development of glands. HS can also limit the proliferation of gastric interstitial smooth muscle²⁷⁻²⁹. This shows that heparin sulfate is involved in the embryonic development of the stomach and has a potential connection with gastric diseases, which supports the results of this study. In this study, it was found that the level of heparin sulfate in patients with gastric cancer first decreased and then significantly increased, which is consistent with previous studies. There is also evidence that heparin sulfate as a carrier can participate in the process of transporting polyamines from outside of the cell to the inside of the cell³⁰. Whether it is related to the change in spermine content is worthy of our further study.

Triacylglycerol is a component of lipids, which is a lipid formed by glycerol and 3 fatty acids. Under normal circumstances, the triglycerides in the plasma maintain a dynamic balance. However, the lipid consumption and lipid metabolite levels of gastric cancer patients increase. Therefore, the plasma lipid level of gastric cancer patients decreases and the lipid level can be used as a diagnostic biomarker of gastric cancer^{31,32}. In this study, we found that the decrease of lipid metabolites such as triacylglycerol in patients with gastric cancer indicates that the lipid metabolism of patients with early gastric cancer has changed significantly. Although there are no reports about the relationship between gastric cancer and triacylglycerol, triacylglycerol serves as a kind of Important ester compounds, may involved in life metabolism, and are likely to have some potential connection with gastric cancer. Therefore, the results of this study provide new insights in this regard. In addition, studies have found that the lipid metabolism process of tumor cells affects the oncogenic signal transduction pathway. And lipids participate in the immune regulation of tumor cells, preventing tumor cells from the destruction of the body's immune system³³. All the above need to be further studied.

Enterostatin is a pentapeptide released by proenzyme during fat digestion. In addition to the pancreas, intestinal statin immune response cells are also present in the gastric antrum and the proximal small intestine. Enterostatin can selectively reduce fat intake, reduce insulin secretion, and increase energy expenditure by activating brown adipose tissue during high-fat feeding. At present, there are many studies about enterostatin in the treatment of diabetes, but there are few reports related to the relationship between enterostatin and gastric cancer^{34,35}. Enterostatin is an important part of the normal metabolism of the human body. In this study, we found that enterostatin in patients with gastric cancer is lower than that in healthy people, indicating that enterostatin may affect gastric cancer occurrence and development, which providing a new way for the diagnosis and therapy of gastric cancer.

This study has discovered and verified the different expressions of the four statistically significant metabolites in blood samples of early gastric cancer. The changes in their content may be related to the cancer occurrence and development. And the metabolic mechanism can be continued to be explored. However, to obtain more ideal results, a larger sample size is required. Metabolomics is a research that requires high-throughput support. If the sample size is sufficient, more different metabolites may be screened. At the same time, metabolomics can find the differences between compounds. To fully understand the mechanism of disease occurrence and development, it is necessary to combine genomics and proteomics to conduct research from multiple perspectives.

Conclusion

In this experiment, we found that there are metabolomics differences between patients with early gastric cancer and healthy people. Among them, spermine, enterostatin, and triacylglycerol showed a decreasing trend in tumor development; heparin sulfate showed a trend of first decreasing and then increasing in tumor development. Therefore, spermine, enterostatin, heparin sulfate, and triacylglycerol were potential biomarkers for early gastric cancer diagnosis. In the future, we will study the further molecular mechanisms of these four metabolites specifically in tumor occurrence and development.

Abbreviations

LC-MS, Chromatography-mass spectrometry; SVM, Support vector machine; ANOVA, analysis of variance; PCA, principal component analysis; ROC, receiver operating characteristic; HMDB, Human Metabolome Database.

Declarations

Ethics statements

The patient sample comes from the First Affiliated Hospital of Jilin University. All patients have the right of written informed consent, and compliance with the declaration of Helsinki.

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Conflict of Interest:

The authors declare that they have no conflict of interest.

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Figures

Figure 1

The mass spectrum data of the metabolites were obtained after LC-MS analysis. PCA found significant differences in the classification of metabolites among these groups (Figure 1A-B). Total ion current (TIC) showed significant differences in the peak values among the three groups (Figure 1C-E).

Figure 2

Partial least squares discriminant analysis and analysis of variance were used to further analyze the metabolites, and the score map (Figure 2A) and VIP value map (Figure 2B) were obtained. According to the VIP map, there are 5 metabolites with VIP value greater than 1. The support vector machine in the BRB-array software was used for further analysis, and a total of 4 different metabolites with a weight of 100% were found (Table 2). Sensitivity and specificity were calculated among the groups of overall gastric cancer (group A and B), early gastric cancer (group B), and healthy people (group C) (Table 3 and Table 4). The cluster analysis of the above four substances showed that there are different metabolites between each group (Figure 2C).

Figure 3

ROC curve analysis shows that spermine, enterostatin, heparin sulfate, and triacylglycerol had good diagnostic ability in overall gastric cancer (group A and B), healthy people (group C), and early gastric cancer (Figure 3A, Table 6, Table 7). Further the bar graph showed the mean values of 4 different metabolites in the three groups, and the results show that: 4 metabolites have significant statistical differences in each group (Figure 3B).

Figure 4

The blood samples of 24 patients in the three groups were processed by LC-MS to obtain mass spectrum data and then clustered analysis. The analysis results were consistent with the experimental grouping (Figure 4A). To ensure the reliability of the verification experiment results, the experimental results were tested for sensitivity, specificity (Table 8, Table 9) and ROC curve analysis (Figure 4B-C, Table 10, Table 11). The results proved that the verification group experiment has good reliability.