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Original Research

Serum cell-free DNA concentrations and integrity analysis of colorectal cancer patients before and after surgery

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Abstract: This study was carried out to investigate cell-free DNA (cfDNA) as a potential biomarker for colorectal cancer diagnosis.Patients with colorectal cancer (n = 25) who had not undergone surgery, and 35 patients with postoperative colorectal cancer were enrolled. Peripheral blood samples were collected from the colorectal cancer subjects (experimental group), and also from 30 healthy volunteers (control group). Quantitative PCR (qPCR) was used to determine cfDNA concentration and integrity in each group. The cfDNA levels of the two groups were analyzed to determine the relationship between the cfDNA and the clinical features of colorectal cancer patients. The receiver operator curve (ROC) was used to analyze sensitivity and specificity of cfDNA, carcinoembryonic antigen (CEA), cancer antigen 199 (CA199) and cancer antigen 125 (CA125). cfDNA concentration and cfDNA integrity in patients with colorectal cancer before surgery were significantly higher than those in patients with colorectal cancer after surgery, and cfDNA concentration of colorectal cancer patients after surgery was also significantly higher than that of the healthy control group, but the integrity was not significantly different from the control group. There was no significant correlation between cfDNA concentration/integrity and gender, age, disease stage, tumor location, tumor differentiation, and expressions of cancer antigen 153 (CA153), neuron specific enolase (NSE) and alpha fetoprotein (AFP) in colorectal cancer patients before or after surgery. However, there was a significant correlation between the expression levels of CEA/CA125 and concentration of cfDNA. The CA199 expression level was significantly correlated with cfDNA integrity. The sensitivity and specificity of cfDNA and integrity were higher than those used for traditional tumor biomarker detection. cfDNA concentration is significantly increased in serum of colorectal cancer patients. Thus, it may serve as a potential indicator of colorectal cancer.

Key words: Cell-free DNA; Colorectal cancer; CA199; CA125; CEA; ROC curve.

Introduction

Colorectal cancer is one of the most common gastrointestinal tumors in clinics. In terms of morbidity and mortality of cancers in the world, colorectal cancer is ranked third and fourth, respectively (1). In China, there are more than 250,000 new cases of colorectal cancer and 140,000 deaths each year. The new and death cases account for 20% of the global colorectal cancer cases. Colorectal cancer imposes a heavy medical burden on the society and the patient. Regular screening, early diagnosis and early treatment can effectively prevent or even cure colorectal cancer. However, 60-70% of patients are usually at the advanced stage at the time of diagnosis, and only 11.8% of cases are diagnosed at an early stage (2).

Currently, screening methods used for colorectal cancer include imaging examination, pathological biopsy and tumor markers. Indeed, imaging examination and pathological biopsy are the gold standards for diagnosis of colorectal cancer (3). Accurate diagnosis can be obtained under conditions of adequate bowel preparation, good patient cooperation, and skilled operation of endoscopic physicians. However, there are many limitations in clinical practice, such as invasive procedures, difficulty in obtaining pathological tissues, and tumor heterogeneity. Tumor markers usually used to screen for colorectal cancer in clinical practice include cancer antigen 199 (CA199), cancer antigen 125 (CA125), carcinoembryonic antigen (CEA), cancer antigen 153 (CA153), neuron specific enolase (NSE) and alpha fetoprotein (AFP) , but they are associated with poor specificity, and their concentrations are also increased in other malignant tumors, inflammatory bowel disease (4), and autoimmune diseases. The use of a single indicator for diagnosis of colorectal cancer is of low value. Therefore, there is a need for an examination-based method that is simpler, has a high diagnostic value, and is easily accepted by the examinee, so as to improve the early detection of colorectal cancer.

In recent years, research on cell-free DNA (cfDNA) has attracted more and more attention. Cell-free DNA

(cfDNA) is a newly discovered biomarker in cancer research. The cfDNA enters the peripheral blood circulation through necrosis or apoptosis of normal cells and tumor cells, and it carries biological information that is highly consistent with the primary tumor (5, 6). Due to its simple, rapid and non-invasive collection, cfDNA can be used to monitor tumor patients in real time and dynamically too, and evidence for its prospects in tumor diagnosis and prognosis are increasing. Spindler KL et al. studied the prognostic value of cfDNA in patients with second-line chemotherapy for colorectal cancer, and determined that cfDNA concentration can be used as an effective tool to predict the outcome of chemotherapy for colorectal cancer (5). To further understand the value of cfDNA concentration and integrity in colorectal cancer, we used real-time quantitative PCR to determine the content of cfDNA, and compared the specificity and sensitivity of cfDNA concentration and integrity with other tumor markers to study the implication of cfDNA in colorectal cancer screening.

Materials and Methods

Sample selection

Twenty-five primary colorectal cancer patients who had not undergone surgery between June 2018 and November 2018 were selected as study subjects. Out the 25 cases, 14 (56%) were male and 11 (44%) were female. Their ages ranged from 31 to 76 years (mean age = 49.4±4.5 years). In addition, 35 colorectal cancer patients undergoing surgery at the same period were enrolled, including 18 (51.43%) males and 17 (48.57%) females. Their ages ranged from 37 to 75 years (mean age = 57.6±11.1 years). There was no significant difference in gender and age between the two groups ($p > 0.05$).

Inclusion criteria: (1) patients diagnosed using histopathology examination, digestive tract barium meal and B-ultrasound; (2) patients with complete medical records; and (3) patients with KPS score > 60 points. *Exclusion criteria*: (1) colorectal patients with other malignant tumors at the same time; (2) those with serious diseases such as heart, liver and kidney diseases; (3) patients with acute or chronic infectious diseases; and (4) those with mental illness who were unable to cooperate in normal medical activities. In addition, 25 healthy people served as a control group, including 11 (44%) males and 14 (56%) females, aged between 41 and 69 years (mean age = 51.1±2.8 years). All participants signed informed consent prior to enrollment in the study. This clinical trial was performed following approval by the Clinical Research Ethics Committee of Nantong Tumor Hospital.

Serum separation and cfDNA extraction

Venous blood sample was collected from each patient in non-EDTA-coated tubes and serum was separated by centrifuging at 1600g for 10 minutes. The supernatant was transferred to a new tube and centrifuged at 16000g for 10 minutes. The serum was carefully removed without disturbing the lower residual layer. A minimum aliquot of 200 µl serum was used for DNA extraction immediately or stored at -80°C freezer. Serum samples were thawed on ice and spun at 10,000g for 3 minutes before DNA purification. The DNA was purified from 200 µl of serum and eluted with 50 µl elution buffer using QIAamp DNA Blood Mini Kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. The DNA samples were quantified or stored at -20°C in a freezer.

Quantitative Polymerase Chain Reaction

Quantitative polymerase chain reaction (QPCR) was performed on a LightCycler LC480 PCR machine (Roche Molecular Systems, Inc. Pleasanton, CA, USA). To measure the concentration of serum cfDNA, the repetitive LINE 1 (Long interspersed nuclear element 1) 97 bp (both for short and long) and LINE1 259bp (only for long) DNA fragments were amplified as described previously (6). The LINE1 97bp primer amplified apoptotic and non-apoptotic DNA fragments, while the LINE1 259bp primer amplified non-apoptotic DNA fragments only. The total amount of serum DNA was represented by the QPCR result with LINE1 97bp primer. The DNA integrity index was calculated as the ratio of results of LINE1 259 and LINE1 97 QPCR. A serial diluted standardized solution of human genomic DNA (Thermo Fisher Scientific, Waltham, MA, USA) was used as a standard curve reference, and the concentration of cfDNA in each sample was calculated from the standard curve. The QPCR reaction was performed in triplicate and mean values from triplicates assays were used for further analysis. The QPCR mixture (20 µl) contained 1 µl DNA template, 0.5 µl of each forward and reverse primer (LINE1 97 or LINE1 259), 10 µl UltraSYBR Mixture (Cwbiotech, Beijing, China), and 8 µl of double-distilled water. Cycling conditions were 1 minute at 95°C, and 35 cycles of 95°C for 8 seconds, and 60°C for 15 seconds. Each plate consisted of a serum DNA sample and a negative control (water template) and 7 serial diluted standard DNA solutions.

Detection of tumor biomarkers

Electro-chemiluminescence was used for the determination of tumor biomarkers. Serum was obtained by centrifugation of fasting venous blood, using fully automated Electrochemiluminometer E170 and assorted kits (Roche, Switzerland). The reference ranges for the various parameters were: cancer antigen (CA)153 < 25 U/ml, CA199: < 39 U/ml, CA125: < 35 U/ml, carcinoembryonic antigen (CEA): < 3.5 ng/mL, neuron-specific enolase (NSE): < 16.3 ng/mL, and alpha- fetoprotein (AFP): < 7 ng/mL.

Statistical analysis

The results of cfDNA quantification are expressed as mean ± standard deviation (x ± SD). Kruskal-Wallis rank sum test was used for comparison between groups. Count data were compared using *r*-test, while measurement data were compared using Student's *t*-test. The area under the ROC curve was used to determine the degree of accuracy in differentiating between two different diseases for different critical values. All statistical analyses were done with SPSS 21.0 software. Values of $p < 0.05$ were considered statistically significant.

Results

Clinical characteristics of patients

Table 1 shows the clinicopathological features of the patients before and after surgery. Among the 25 patients with colorectal cancer who had not undergone surgery, there were 10 cases (40%) of primary colon tumors and 15 cases (60%) in tumor in the rectum. Disease grading was performed for all enrolled patients according to the criteria of the World Health Organization grading standard for colorectal cancer (7). Nine patients (36%) were categorized as stage I/II, while 16 patients (64%) were in stage III/IV. There were 19 cases (76%) with moderately/poorly differentiated colorectal cancer, and 6 cases (24%) with highly differentiated colorectal cancer. Among the 35 colorectal cancer patients receiving surgery, 15 patients (42.86%) had primary tumor in the colon, 20 patients (57.14%) had tumor in the rectum; 20 cases (57.14%) were categorized as stage I/II, while 15 cases (42.86%) were in stage III/IV. In addition, 22 patients (62.86%) had moderately/poorly differentiated colorectal cancer, while 13 cases (37.14%) had highly differentiated colorectal cancer. There were no statistically significant differences in clinicopathological features in patients with colorectal cancer before and after surgery ($p > 0.05$).

The results of assay for 6 tumor biomarkers in 25 patients with preoperative colorectal cancer showed that 4 cases (16%) were positive for CA153 and 21 cases (84%) were CA153 negative. There were 12 cases (48%) with positive expression of CA199, and 13 cases (52%) with negative CA199 expression; 15 cases (60%) had positive expression of CA125, while 10 cases (40%) had negative expression. Eight cases (32%) were positive for CEA expression, while 17 cases (68%) had negative expression of CEA; 4 patients (16%) had positive expression of NSE, while 21 patients (84%) had negative expression of NSE. With respect to AFP expression, 2 cases (8%) were positive, while 23 cases (92%) were AFP negative. Among the 35 patients with colorectal cancer after surgery, there were 6 cases (17.14%) with positive expression of CA153, and 29 cases (82.86%) with negative expression of CA153; 9 cases (25.71%) were CA199 positive, while 26 cases (74.29%) had negative expression of CA199. Eleven patients (31.43%) were with positive expression of CA125, while 24 patients (68.57%) were CA125 negative. There were 10 cases (28.57%) with positive expression of CEA, and 25 cases (71.43%) with negative expression of NSE. Five patients (14.29%) had positive expression of NSE,

Table 1. Clinical features of colorectal cancer patients.

Variables	Before surgery	After surgery	*P*
Gender			
Male	14	18	0.7964
Female	11	17	
Age			
≥65	8	10	0.7832
<65	17	25	
Tumor site			
Colon	10	16	0.7928
Rectum	15	19	
Disease stage			
I/II	11	20	0.4326
III/IV	14	15	
Tumor differentiation			
Low-medium	19	22	0.3997
High	6	13	
CA153			
≥25 U/ml	8	6	0.2232
<25 U/ml	17	29	
CA199			
≥39 U/ml	12	7	0.0273
<39 U/ml	13	28	
CA125			
≥35 U/ml	15	11	0.0364
<35 U/ml	10	24	
CEA			
≥3.5 ng/mL	13	8	0.0285
<3.5 ng/mL	12	27	
NSE			
≥16.3 ng/mL	10	11	0.5865
<16.3ng/mL	15	24	
AFP			
≥7 ng/mL	9	7	0.2375
<7 ng/mL	16	28	

while 30 patients (85.71%) had negative expression of NSE. Four cases (11.43%) of positive expression of AFP were seen, while 31 cases (88.57%) were with negative expression. The expressions of CA199, CA125 and CEA in serum of patients with colorectal cancer before and after surgery differed significantly, while the expressions of CA153, NSE and AFP did not differ significantly, when pre- and post-operative values were compared.

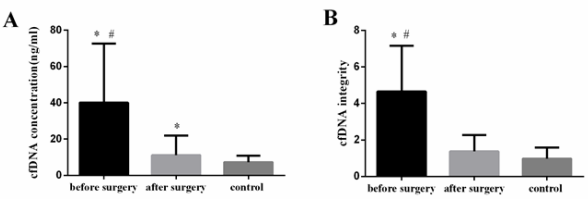

Figure 1. Concentration and integrity of cfDNA in serum of patients with colorectal cancer. **p*<0.05, compared with the control group; #*p*<0.05, compared with the post-surgery group.

cfDNA concentration and integrity in serum of healthy individuals and colorectal cancer patients

The results of Q-PCR for determination of cfDNA concentration and integrity are shown in Figure 1. The cfDNA concentration in healthy people was 7.31 ± 3.48 ng / mL, and the cfDNA integrity was 0.97 ± 0.55. In contrast, the cfDNA concentration of colorectal cancer patients before surgery was 39.55±31.21 ng/mL, and the cfDNA integrity was 4.71±2.44. However, after surgery, the cfDNA concentration of patients with colorectal cancer was 11.21±10.62 ng/mL, while the cfDNA integrity was 1.36±0.87. The cfDNA concentration and cfDNA integrity of patients with colorectal cancer before surgery were significantly higher than those of patients with colorectal cancer after surgery, and both indices of colorectal cancer patients after surgery were also significantly higher than those of healthy control group (p <0.05).

Relationship between cfDNA concentration/integrity and clinical features of colorectal cancer patients

Analysis of the correlation between cfDNA and clinical features of colorectal cancer patients was performed after measuring cfDNA concentration and integrity. The results of the correlation analysis are shown in Table 2

Table 2. Correlation between serum cfDNA concentration and clinical characteristics.

Variables	Before surgery	After surgery
Gender		
Male	41.22±30.75	11.48±10.55
Female	38.25±26.15	14.04±11.22
P	0.8012	0.4812
Age		
≥65	35.32±23.50	12.89±11.03
<65	39.57±28.66	10.34±10.23
P	0.7115	0.4715
Disease site		
Colon	28.58±25.28	13.59±12.67
Rectum	34.32±30.24	11.59±10.62
P	0.6317	0.6177
Disease stage		
I/II	35.21±25.75	19.61±9.57
III/IV	43.59±38.49	15.65±16.11
P	0.5378	0.3782
Tumor differentiation		
Low-medium	45.55±43.63	11.56±9.22
High	33.66±23.24	15.73±13.78
P	0.5833	0.2865
CA153		
≥25 U/ml	47.57±32.51	20.51±16.56
<25 U/ml	38.86±23.21	15.87±13.78
P	0.4463	0.4866
CA199		
≥39 U/ml	53.61±50.25	30.57±29.48
<39 U/ml	26.48±18.58	16.86±12.52
P	0.0852	0.0672
CA125		
≥35 U/ml	70.35±53.77	27.62±18.71
<35 U/ml	31.86±21.55	13.52±10.22
P	< 0.05	< 0.01
CEA		
≥3.5 ng/mL	70.21±52.89	38.55±31.32
<3.5 ng/mL	30.91±23.51	15.82±13.53
P	< 0.05	< 0.01
NSE		
≥16.3 ng/mL	43.86±26.72	13.11±12.73
<16.3 ng/mL	40.81±28.52	11.49±10.53
P	0.7844	0.6960
AFP		
≥7 ng/mL	46.58±43.92	14.16±10.55
<7 ng/mL	40.22±28.57	10.75±8.27
P	0.6575	0.3762

Table 3. Correlation between integrity of cfDNA and clinical characteristics.

Variables	Before surgery	After surgery
Gender		
Male	4.28±2.58	1.41±0.87
Female	4.69±5.18	1.22±0.86
P	0.8121	0.6823
Age		
≥65	5.41±7.31	1.58±1.17
<65	4.03±3.56	1.32±0.68
P	0.5252	0.3614
Tumor site		
Colon	6.35±7.85	1.49±2.11
Rectum	5.66±4.76	1.68±3.24
P	0.8113	0.8182
Diease stage		
I/II	4.79±3.48	1.32±1.25
III/IV	6.41±5.32	1.48±1.02
P	0.4091	0.6179
Tumor differentiation		
Low-medium	4.28±5.11	1.52±0.92
High	5.32±4.26	1.79±1.13
P	0.6875	0.3315
CA153		
≥25 U/ml	7.03±5.28	1.59±1.01
<25 U/ml	5.11±4.58	1.31±0.62
P	0.3766	0.2633
CA199		
≥39 U/ml	11.28±8.16	4.71±2.72
<39 U/ml	7.11±5.78	2.42±1.31
P	<0.05	<0.01
CA125		
≥35 U/ml	13.72±10.44	4.13±3.18
<35 U/ml	8.32±5.52	2.72±1.40
P	0.1513	0.0938
CEA		
≥3.5ng/mL	9.21±8.55	4.72±3.55
<3.5ng/mL	6.39±4.56	3.05±2.01
P	0.3466	0.1044
NSE		
≥16.3 ng/mL	4.91±2.63	1.76±1.32
<16.3 ng/mL	3.49±2.87	1.28±1.18
P	0.2565	0.2768
AFP		
≥7 ng/mL	5.12±3.58	1.91±0.89
<7 ng/mL	4.07±3.48	1.58±0.59
P	0.5501	0.0577

(cfDNA concentration) and Table 3 (cfDNA integrity). There was no significant correlation between cfDNA concentration/integrity and gender, age, TNM stage, tumor location, tumor differentiation and expressions of CA153, NSE and AFP in patients with colorectal cancer before or after surgery ($p > 0.05$). However, there was a significant correlation between the expression levels of CA125/CEA and cfDNA concentration ($p< 0.05$), but they were not significantly correlated with cfDNA integrity ($p > 0.05$). The expression level of CA199 was not significantly correlated with cfDNA concentration ($p > 0.05$), but it was significantly correlated with cfDNA integrity ($p < 0.05$).

Receiver operator characteristic curve analysis of cfDNA levels in colorectal cancer patients

The cfDNA concentrations in the serum of patients with colorectal cancer before and after surgery, and the serum levels of tumor biomarkers (CA199, CA125, CEA) were used to determine their specificity and sensitivity in colorectal cancer diagnosis. Then, corresponding ROC curves were drawn. The results are shown in Figure 2. For colorectal cancer patients before surgery, the area under curve (AUC) of CA199 was 0.7442 (95% CI: 0.6423 - 0.8515); the AUC for CA125 was 0.6589 (95% CI: 0.5601 - 0.7672), while AUC for CEA was 0.7328 (95% CI: 0.6619 - 0.8041). The AUC for cfDNA concentration was 0.7810 (95% CI: 0.7028 - 0.8558), and AUC for cfDNA integrity was 0.8061. (95% CI: 0.7492 - 0.8628). For colorectal cancer patients after surgery, the AUC for CA199 was 0.6940 (95% CI: 0.6241 - 0.7651); the AUC for CA125 was 0.6043 (95% CI: 0.5188 - 0.6879); the AUC for CEA was 0.7755 (95% CI: 0.6982 - 0.8553), while the AUC for cfDNA concentration was 0.8638 (95% CI: 0.8148 to 0.9133). The area under curve (AUC) for cfDNA integrity was 0.8923 (95% CI: 0.8461 - 0.9375). These results suggest that cfDNA concentration and integrity are ideal tumor screening parameters, and their sensitivity and specificity are higher to those of traditional tumor biomarker detections.

Discussion

In 1948, Mendel and Metais, two French scientists discovered cfDNA in the serum of healthy people. In 1977, Lemon *et al*. (8) discovered for the first time that the cfDNA content in the serum of patients with tumors was higher than that of normal people. However, at that time, research on cfDNA was limited, and it was not

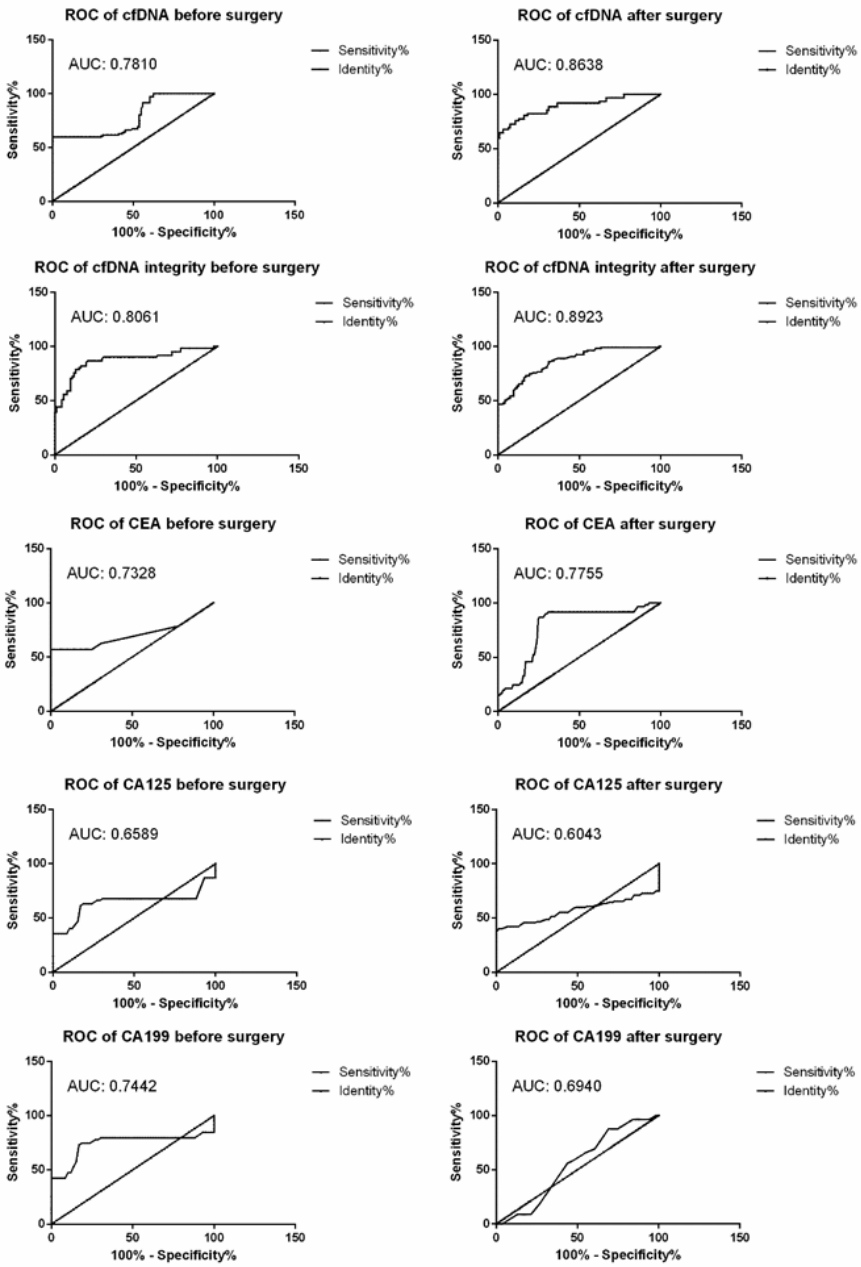

Figure 2. ROC curve analysis of tumor markers and cfDNA before and after surgery in colorectal cancer patients.

clear whether these DNAs were derived from normal tissues or tumor cells. Researchers first determined that some of the DNA found in the serum of tumor patients came from tumor cells, through comparison of thermodynamic stability of DNA strands, and proposed the concept of "liquid biopsy", which replaced tissue biopsy with blood tests (9). The emergence of such liquid biopsy overcame the inconvenience of tumor tissue acquisition. Due to its non-invasiveness, convenience and repeated material selection of liquid biopsy, cfDNA has gained importance in early screening and tumor diagnosis (10,11).

Healthy human peripheral blood may also contain cfDNA, but usually in very small amounts, with fragment sizes between 180-220 bp (12). The cfDNA content of peripheral blood of patients with tumors is significantly higher than that of healthy people, and they are mostly long-chain DNA fragments (13). In the 1980s, some scholars detected cfDNA levels in patients with gastrointestinal malignant tumors at average level of 412 ng/mL, and 118 ng/mL in benign patients, which was about four times higher than the corresponding levels in normal people. However, the quantitative analysis of cfDNA levels as index of risk of tumors remains controversial (14). Many studies have suggested that cfDNA levels have early diagnostic value for tumors, while some studies have indicated that cfDNA levels are not related to patient age, gender, tumor site, tumor differentiation, and disease staging (15). At present, there are limited studies on the diagnostic value of colorectal cancer in China and abroad, and there is dearth of clinical data on colorectal cancer patients before and after surgery. Systematic analysis of the relevant clinicopathological characteristics of preoperative and postoperative colorectal cancer is important for determining whether cfDNA can be used as an early diagnostic indicator for tumors.

A large number of studies have repeatedly confirmed that the concentration of cfDNA in the blood of cancer patients is higher than that of healthy people (16).

On the other hand, many studies have simultaneously confirmed the prognostic value of cfDNA for tumor recurrence and patient survival, as well as its value in monitoring of treatment response. It has been reported that cfDNA is an independent risk factor for disease survival and is elevated in many cancer patients (17). For example, Hsieh *et al.* reported that in patients with esophageal cancer, if the concentration of cfDNA increases after surgery, it usually indicates early recurrence of tumor (18). Therefore, it is possible to evaluate cancer by measuring changes in cfDNA levels in the blood.

Multiple studies have shown that the inclusion of cfDNA integrity can further improve the accuracy of cfDNA detection (19-22). Not only does the content of cfDNA in blood of tumor patients increase, the structure and sequence of cfDNA are changed in the course of disease progression and treatment (23). A study of serum cfDNA concentration, combined with cfDNA biological information can more accurately assess tumor burden.

The present study showed that the expressions of CEA, CA199, and CA125 were significantly associated with clinical features of colorectal cancer patients, but their specificity was not high enough. Histopathology does not help early diagnosis, although it is the gold standard for the diagnosis of colorectal cancer. Therefore, it is necessary to find serological indicators with specificity, sensitivity and availability.

This study is the first to quantify the concentration and integrity of cfDNA in healthy individuals and colorectal cancer patients before and after surgery. The results showed that the concentration and integrity of cfDNA in patients with colorectal cancer were significantly different from those in healthy individuals. The concentration and integrity of serum cfDNA before surgery in patients with colorectal cancer were significantly higher than those after surgery. The possible reason is that the local blood supply of the tumor tissue was insufficient before the surgical treatment, resulting in the hypoxic necrosis of the tumor tissue. The necrotic or apoptotic tumor cells are then phagocytized by macrophages, releasing a large number of long-chain DNA fragments, thereby significantly increasing the cfDNA concentration and integrity. The results of this study suggest that the concentration and integrity of cfDNA are of diagnostic value in colorectal cancer. They are expected to function as non-invasive molecular diagnostic method for screening high-risk groups of colorectal cancer.

In the systematic analysis of the clinicopathological features of colorectal cancer before and after surgery, serum cfDNA concentration was not significantly correlated with patient age, gender, disease location, disease stage, degree of differentiation, and expressions of CA153, CA199, AFP, NSE. However, it was significantly correlated with CEA and CA125 expressions, and CA199 expression was significantly correlated with cfDNA integrity, suggesting that cfDNA reflected active disease proliferation.

To evaluate the specificity and sensitivity of cfDNA, we established ROC curves with three other tumor markers (CA199, CA125 and CEA). The ROC curve analysis confirmed that the AUC of cfDNA was greater than those of CEA, CA199 and CA125 for colorectal cancer before and after surgery, suggesting that the diagnostic effect of cfDNA as a diagnostic criterion for colorectal cancer is better than those of CEA, CA199 and CA125. Thus, cfDNA may be a reliable auxiliary method for the diagnosis of colorectal cancer in the future.

The clinical application of cfDNA is an emerging and rapidly developing field of research (24). If it is used as a routine testing program in clinical practice, there are still significant challenges. For example, there are no standardized procedures for the analysis of cfDNA. In addition, the use of anticoagulants, and the standardization of storage time and conditions which may affect the qualitative and quantitative analysis of cfDNA, need to be addressed. Therefore, if large-scale clinical trials are to be conducted, uniform standards need to be established for all aspects of cfDNA testing, and strict positive and negative controls should be established to reduce the occurrence of false positive and false negative results.

This study provides a new idea for the diagnosis of colorectal cancer using serum cfDNA. It provides a theoretical basis for the application of cfDNA in tumor diagnosis. Being non-invasive and easy to acquire, cfDNA and can be used as an ideal indicator of tumor diagnosis and monitoring. However, this study only retrospectively examined 2 different groups of colorectal cancer patients at the same time period. Further studies are planned to prospectively trace a group of patients from their initial diagnosis stage to the time after surgery, in order to monitor surgery-induced changes in cfDNA and other tumor biomarkers.

Ethical approval and informed consent

All trial participants signed informed consent prior to enrollment in the study. This clinical trial was performed following approval by the Clinical Research Ethics Committee of Nantong Tumor Hospital.

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Competing interests

The authors declare no conflict of interest.

Authors' contributions

YS conceived and designed this study. YX and XJ performed the experiments and analyzed the data. XT collected the patient data. GC wrote the manuscript, while DW revised it.

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