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Original Research

Clinical significance of cell-free DNA concentration and integrity in serum of gastric cancer patients before and after surgery

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Abstract: Early onset of gastric cancer (GC) is almost asymptomatic, thereby making early diagnosis and early treatment difficult. Blood samples were taken from 90 GC patients who had not undergone surgery, and from another set of 110 GC patients who had undergone surgery. The control consisted of 90 healthy individuals. Cell-free DNA (cfDNA) and its integrity were assayed using qPCR. The association between cfDNA levels and clinical presentations of GC was analyzed. In addition, cfDNA, carcino-embryonic antigen (CEA), carbohydrate antigen 724 (CA724), carbohydrate antigen 125 (CA125) and carbohydrate antigen 199 (CA199) were subjected to specificity and sensitivity analyses using ROC. The levels of cfDNA of GC patients before surgery were markedly higher than corresponding values in patients with GC after surgery. Post-surgery, the two indices were also significantly higher in GC patients than in the healthy group. The correlation between cfDNA concentration/integrity and gender, age, TNM stage, tumor differentiation, tumor location, neuronspecific enolase (NSE), or alpha feto-protein (AFP) expression, was not significant in GC patients before or after surgery. However, the correlation between cfDNA and concentrations of CEA/CA125 was significant. The CA199 expression level was significantly correlated with cfDNA integrity. The AUC values of cfDNA concentration and integrity were higher than other tumor markers. Measurement of cfDNA concentration and integrity may be an ideal tumor screening method with higher sensitivity and specificity than traditional tumor biomarkers. The cfDNA concentration and integrity are significantly increased in plasma of GC patients, and may serve as promising indicators for GC.

Key words: Gastric cancer; Cell-free DNA; Quantitative PCR; ROC curve.

Introduction

Gastric cancer (GC) is a cancer derived from gastric mucosal epithelium (1). It is one of the most common tumors in the world, and the second leading cause of cancer-related death (2). It is also among the prevalent malignancies in China (3). Patients with gastric cancer show no specific symptoms: the usual symptoms at the time of presentation include weight loss, upper abdominal pain, vomiting, and nausea (4). Early onset of gastric cancer is almost asymptomatic. Thus, it is difficult to achieve early diagnosis and treatment. In most cases, gastric cancer patients are already in middle and late stages of the disease by the time they contact their doctor, thereby missing the best treatment opportunity (5, 6). Studies have confirmed that the 5-year survival of patients with early gastric cancer who received effective surgery was greater than 90%, while the 5-year survival of patients with advanced gastric cancer who received effective surgery was approximately 20%. The diagnosis methods of gastric cancer involve upper gastrointestinal barium meal imaging, and gastroscopy and histopathological examination called "gold standard". The former has a low degree of diagnosis for cancer, and it is not suitable for patients with gastrointestinal obstruction symptoms. Moreover, patients experience a lot of pain during gastroscopy. Indeed, some patients cannot tolerate the procedure. In addition, apart from the high cost of the examination, gastroscopy is associated with certain complications. Thus, this method is not suitable

for early tumor screening and dynamic monitoring of gastric cancer.

Serological examination is simple and convenient, and it is highly operable. It is suitable for large-scale screening and diagnosis of gastric cancer. This method provides a scientific basis for the diagnosis of gastric cancer patients through determination of serum levels of tumor markers (7). At present, the clinically common gastric cancer-associated tumor markers are CEA, CA724, CA125, CA199, NSE and AFP (8-10). Limitations of pathological biopsy and upper gastrointestinal barium meal imaging in clinical practice, such as difficulty in obtaining tumor tissue, difficulty in re-taking specimen, and tumor heterogeneity can be avoided through liquid biopsy. Liquid biopsy refers to non-invasive or micro-invasive methods which involve the determination of circulating tumor cells (CTC), circulating tumor DNA (ctDNA), circulating cfDNA, and exosomes from body fluids such as sputum, blood, and urine, thereby obtaining biological information from tumor tissues (11). Compared with tissue biopsy, liquid biopsy is minimally invasive, reproducible, and high level of patient acceptance (12). The cfDNA is a free self-DNA fragment in the patient's plasma. It enters the peripheral blood circulation via necrosis, apoptosis or active secretion of normal cells and tumor cells, and it carries biological information with high consistency with the primary tumor. Plasma cfDNA detection is a suitable alternative to histopathological examination when tumor tissue is difficult to obtain (13). Obviously, blood biopsy is more convenient in terms of follow-up and monitoring. The aim of the present study was to investigate the importance of cfDNA in the diagnosis of gastric cancer.

Materials and Methods

Sample selection

Ninety preoperative gastric cancer patients were selected as study subjects from June 2017 to June 2018. They comprised 55 (61.11%) male and 35 (38.89%) female, with age ranging from 28 to 77 years (mean age = 55.3±6.8 years). In addition, 110 postoperative gastric cancer cases at the same period were selected, including 58 (52.73%) male and 52 (47.27%) female, with ages ranging from 25 to 78 years (mean age = 49.2±5.7 years). Age and gender were comparable between the two groups.

Inclusion criteria: (1) all patients who were initially diagnosed with gastric cancer through gastroscopy and/or histopathology; (2) patients whose diagnosis criteria were consistent with the "Guidelines for the Standardization of Diagnosis and Treatment of Gastric Cancer" formulated by the National Health and Family Planning Commission of the People's Republic of China in 2013; and (3) subjects who did not have a history of special medication within 1 week before their blood samples were taken.

Exclusion criteria: (1) patients with incomplete clinical data; (2) patients who had gastric tumor removal before their blood samples were taken; (3) patients treated with chemotherapy or other related anti-tumor treatments; and (4) patients with other tumors. A set of 90 healthy subjects was used as healthy control group. They comprised 49 (54.44%) males and 41 (45.56%) females, with ages ranging from 33 to 70 years (mean age = 49.9±7.6 years). This study received approval from the Ethics Committee of Nantong Tumor Hospital, and it was implemented in line with the Helsinki Declaration. All subjects gave written informed consent prior to blood collection.

Plasma separation and cfDNA extraction

Plasma samples were obtained after centrifugation of venous blood samples taken in EDTA bottles. Aliquots (200 µl) of the plasma were subjected to DNA extraction and purification using QIAamp DNA Blood Mini Kits (Qiagen, Valencia, CA) in line with the kit protocol. The purified DNA was used immediately or preserved at -20°C freezer prior to use.

Quantitative Polymerase Chain Reaction

The purified DNA was subjected to q-PCR using a LightCycler LC480 PCR machine (Roche Molecular Systems, CA, USA). The plasma cfDNA, DNA integrity index and total DNA were determined using the procedure outlined previously (14). The qPCR reaction mixture (20 µl) comprised 1µl DNA template, 0.5 µl each of forward and reverse primers, 10 µl UltraSYBR Mixture, and 8 µl double-distilled water. The conditions used were: 1 minute at 95°C, and 35 cycles of 95°C for 8 seconds, and 60°C for 15 seconds. Each plate comprised plasma DNA sample, negative control (water) and seven serial dilutions of DNA standard.

Determination of tumor biomarkers

Electrochemiluminescence was used for the determination of tumor biomarkers. Serum were obtained after centrifugation of fasting venous blood, using fully automated Electrochemiluminometer E170and assorted kits (Roche, Switzerland). The reference ranges for the various biomarkers are: cancer embryonic antigen (CEA): < 3.5 ng/mL, cancer antigen CA724: < 6.9 U/ml, cancer antigen CA199: < 39 U/ml, cancer antigen CA125: < 35 U/ml, neuron-specific enolase (NSE): < 16.3 ng/mL, and alpha- fetoprotein (AFP): < 7 ng/mL.

Statistical analysis

Data from cfDNA quantification are expressed as mean ± SD. Group values were statistically compared using Kruskal-Wallis rank sum test. Comparison of count data was carried out with *r*-test, while measurement data comparison was effected with *t*-test. The ROC curve was used as an index for assessing the accuracy critical values as indices for distinguishing stages of the disease. All statistical analyses were carried out with SPSS software version 21.0. Values of $p < 0.05$ were assumed statistically significant.

Results

Clinicopathological characteristics of patients

Based on the TNM staging of the Union for International Cancer Control (UICC), 39 of the gastric cancer patients (43.33%) were categorized as stages I and II, while 51 (56.67%) patients were in stages III and IV. There were 30 cases (33.33%) of poorly differentiated adenocarcinoma, 39 cases (43.33%) of moderately

differentiated adenocarcinoma, and 21 cases (23.33%) of highly differentiated adenocarcinoma. Moreover, 8 of the 90 patients (8.89%) had tumor in the cardia, 30 patients (33.33%) had tumor in the corpus, 48 patients (53.33%) had tumor in the antrum, while there were 4 cases (4.44%) with stomach tumor. In the postoperative gastric cancer group, 61 patients (55.45%) were categorized as stages I and II, while 49 patients (44.55%) were in stages III and IV. There were 35 cases (31.82%) of poorly differentiated adenocarcinoma, 46 cases (41.82%) of moderately differentiated adenocarcinoma, and 29 cases (26.36%) of highly differentiated adenocarcinoma. Ten patients (9.09%) had tumor in the cardia, while 36 patients (32.73%) had tumor in the corpus. Moreover, there were 59 cases (53.64%) with tumor in the antrum, and 5 cases (4.55%) with stomach tumor. The results from assay of the 6 tumor biomarkers showed that among the 90 patients with preoperative gastric cancer, 38 (42.22%) had positive expression of CEA, while 52 (57.78%) were CEA-negative. There were 40 cases (44.44%) of positive expression of CA724, and 50 cases (55.56%) of negative expression of CA724; 53 patients (58.89%) had positive expression of CA125, while 37 patients (41.11%) had negative expression of CA724. There were 43 cases (47.78%) with positive expression of CA199, and 47 cases (52.22%) with negative expression of CA199. There were 7 cases (7.78%) with positive expression of NSE, and 83 cases (92.22%) with negative NSE expression. Six patients (6.67%) were positive for AFP, while 84 patients (93.33%) were AFP-negative. There were 20 cases (18.18%) with positive expression of CEA, while 90 cases (81.82%) were CEA negative; 23 patients (20.91%) had positive expression of CA724, while 87 patients (79.09%) were CA724-negative; 32 patients (29.09%) had positive expression of CA125, while 78 patients (70.91%) were CA125 negative; 17 patients (15.45%) had positive expression of CA199, while 93 patients (84.55%) were CA125 negative. There were 9 cases (8.18%) of positive expression of NSE, and 101 cases (91.82%) of negative expression of NSE. The expression of AFP was positive in 8 cases (7.27%), but negative in 102 cases (92.73%). There were significant differences in the expressions of CEA, CA724, CA199 and CA125 between gastric cancer patients before and after surgery, while NSE, AFP expressions were not statistically significant between the 2 populations. These results are shown in Table 1.

Table 1. Demographic and clinical features of GC patients.

Variables	Before surgery	After surgery	*p*
Gender			
Male	55	58	0.2341
Female	35	52	
Age			
≥65	26	39	0.3642
<65	64	71	
TNM stage			
I /II	39	61	0.0881
III/IV	51	49	
Tumor differentiation			
Low	30	35	
Medium	39	46	0.8854
High	21	29	
Tumor location			
Cardia	8	10	
Gastric body	30	36	0.9997
Gastric antrum	48	59	
Full stomach	4	5	
CEA			
≥3.5ng/ml	38	20	<0.01
<3.5ng/ml	52	90	
CA724			
≥6.9U/ml	40	23	<0.01
<6.9U/ml	50	87	
CA125			
≥35 U/ml	53	32	<0.0001
<35 U/ml	37	78	
CA199			
≥39 U/ml	43	17	<0.0001
<39 U/ml	47	93	
NSE			
≥16.3ng/ml	7	9	1.000
<16.3ng/ml	83	101	
AFP			
≥7ng/ml	6	8	1.000
<7ng/ml	84	102	

Plasma cfDNA levels GC patients and controls

Figure 1 shows that the cfDNA level in healthy control subjects was 7.11±3.73 ng / mL, while cfDNA integrity was 1.52±0.62. The cfDNA concentration of gastric cancer patients before surgery was 30.19 ± 30.25 ng/mL, and the cfDNA integrity was 6.33±10.25. In operated GC patients, the cfDNA was 19.04±25.48 ng/mL, with cfDNA integrity of 4.25±2.64. The cfDNA concentration and cfDNA integrity of patients with gastric cancer before surgery were markedly increased, when compared with those of GC patients after surgery. Moreover, the two indices of gastric cancer patients after surgery were significantly higher than the corresponding values in healthy control group (p <0.05).

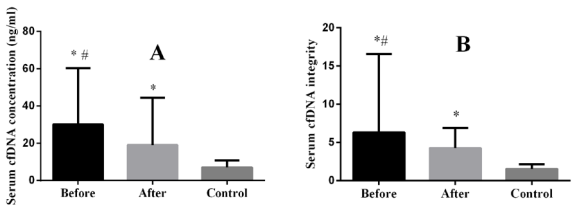

Figure 1. QPCR results for quantification of cfDNA concentration and integrity (*p < 0.05, compared to control group, #p< 0.05, compared to after surgery group).

Correlation between cfDNA concentrations/integrity and patients' clinicopathological characteristics

Table 2 shows analysis of the correlation between cfDNA and clinicopathological features of gastric cancer patients, while the results of the correlation analysis are shown in Table 2 (cfDNA concentration), and in Table 3 (cfDNA integrity). No significant correlation

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

Variables	Before surgery	After surgery
Gender		
Male	32.15±20.22	20.49±15.24
Female	35.47±44.16	18.86±32.46
P	0.6299	0.7325
Age		
≥65	33.18±47.16	22.47±19.75
<65	29.46±36.49	19.66±16.02
P	0.6888	0.4202
TNM stage		
I /II	29.58±41.26	18.99±16.41
III/IV	35.46±50.17	24.18±30.45
P	0.5510	0.2562
Tumor differentiation		
Low	40.19±29.44	30.42±20.59
Medium	35.79±33.57	26.51±12.35
High	32.16±25.81	22.49±25.31
P	0.5713	0.2914
Tumor location		
Cardia	34.18±49.11	20.58±25.43
Gastric body	32.59±45.26	18.46±23.57
Gastric antrum	36.48±29.41	19.69±20.54
Full stomach	33.58±36.51	23.14±22.45
P	0.9313	0.8057
CEA		
≥3.5ng/ml	67.29±39.16	46.28±30.82
<3.5ng/ml	35.57±28.52	20.86±15.23
P	<0.01	<0.01
CA724		
≥6.9U/ml	69.18±42.51	45.56±20.84
<6.9U/ml	39.41±26.35	26.53±18.33
P	<0.01	<0.01
CA125		
≥35 U/ml	65.59±54.47	43.42±20.16
<35 U/ml	26.34±18.95	18.36±15.23
P	<0.01	<0.01
CA199		
≥39 U/ml	45.31±33.84	36.25±24.16
<39 U/ml	30.06±30.74	22.48±16.18
P	0.1744	0.1053
NSE		
≥16.3 ng/mL	37.16±26.24	26.99±33.64
<16.3 ng/mL	30.67±15.67	21.28±13.81
P	0.3234	0.3114
AFP		
≥7 ng/mL	40.91±36.49	22.34±30.51
<7 ng/mL	35.04±20.81	19.23±13.88
P	0.5295	0.5861

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

Variables	Before surgery	After surgery
Gerder		
Male	6.34±5.26	4.11±3.21
Female	6.08±4.15	4.52±2.94
P	0.8052	0.4880
Age		
≥65	5.96±5.24	3.59±3.41
<65	6.48±5.61	4.30±2.12
P	0.6857	0.1811
TNM stage		
I /II	6.25±5.14	5.20±4.23
III/IV	8.64±6.21	6.24±4.61
P	0.2908	0.5747
Tumor differentiation		
Low	8.29±6.57	7.41±3.58
Medium	8.06±5.16	6.91±4.21
High	6.48±3.97	6.02±3.49
P	0.8711	0.5742
Tumor location		
Cardia	6.29±4.10	5.22±3.18
Gastric body	7.19±4.91	4.91±3.47
Gastric antrum	6.43±5.64	6.49±5.48
Full stomach	7.05±5.27	5.74±3.81
P	0.6378	0.8001
CEA		
≥3.5ng/ml	9.65±7.41	5.69±7.16
<3.5ng/ml	7.25±4.41	4.27±4.21
P	0.0583	0.2399
CA724		
≥6.9U/ml	10.54±8.63	5.16±6.55
<6.9U/ml	8.19±6.84	3.67±4.20
P	0.1530	0.1859
CA125		
≥35 U/ml	9.29±5.94	5.01±4.83
<35 U/ml	7.35±4.81	4.25±3.87
P	0.1036	0.3870
CA199		
≥39 U/ml	13.58±6.49	6.59±10.16
<39 U/ml	7.84±5.13	2.74±3.78
P	<0.01	<0.05
NSE		
≥16.3 ng/mL	9.16±8.14	5.29±4.17
<16.3 ng/mL	7.35±4.96	4.31±2.66
P	0.3824	0.3165
AFP		
≥7 ng/mL	8.54±6.34	4.82±5.16
<7 ng/mL	6.18±4.19	3.16±2.97
P	0.2016	0.1552

was seen between cfDNA concentration/integrity and gender, age, TNM stage, tumor location, and tumor differentiation and expressions of NSE, AFP expressions in GC patients before or after surgery ($p > 0.05$). However, there was a significant correlation between the expression levels of CEA/CA125/CA724 and cfDNA concentration ($p < 0.05$. In addition, these parameters were not significantly correlated with cfDNA integrity ($p > 0.05$). However, there was no significant correlation between CA199 expression level with cfDNA concentration, but

there was a significant correlation between these parameters and cfDNA integrity ($p < 0.05$).

ROC curves for cfDNA concentrations in GC patients

The specificities and sensitivities of plasma cfDNA concentration and tumor biomarkers (CEA, CA724, CA125, CA199) in GC diagnosis patients were calculated, and then the corresponding ROC curves were drawn. The results are shown in Figure 2. For gastric cancer patients before surgery, the area under curve (AUC) values for CEA, CA724, CA125, cfDNA concentration, and cfDNA integrity were 0.8017 (95% CI: 0.7418 to 0.8616), 0.7516 (95% CI: 0.6720 to 0.8312), 0.6025 (95% CI: 0.4914 to 0.7136), 0.7156 (95% CI: 0.6218 to 0.8094), 0.8124 (95% CI: 0.7396 to 0.8851), and 0.8596. (95% CI: 0.8126 to 0.9066), respectively. For gastric cancer patients after surgery, the AUC values for CEA, CA724, CA125, CA199, cfDNA concentration, and cfDNA integrity were 0.7810(95% CI: 0.7025 to 0.8594), 0.7622 (95% CI: 0.6841 to 0.8403), 0.5626 (95% CI: 0.4764 to 0.6488), 0.5659 (95% CI: 0.4874 to 0.6443), 0.8564 (95% CI: 0.8065 to 0.9063), and 0.8869 (95% CI: 0.8424 to 0.9314), respectively. These data indicate that determination of cfDNA level and integrity constitute an effective method for screening tumor, and their sensitivity and specificity are higher than those of traditional tumor biomarkers.

Discussion

More and more patients have achieved complete relief through tumor resection, radiotherapy and chemotherapy, thereby returning to normal lives. However, the road to cancer rehabilitation is not smooth, especially in view of the risk of recurrence and metastasis which threaten the lives of patients always. Therefore, cancer patients should be monitored scientifically to understand the risk of tumor recurrence and metastasis, so that doctors and patients can understand the physiological signals as much as possible. This will have important clinical value and broad application prospects.

Tumor liquid biopsy based on urine, saliva, and cerebrospinal fluid is only applicable to certain types of cancer. Traditional peripheral blood biomarkers such PSA, CEA, AFP, and CA can only detect one or a few types of cancer, but cannot support the whole process of tumor onset, treatment, metastasis, and recurrence. Therefore, it is important to evolve new indicators and new methods for diagnosing gastric cancer. A simple, non-invasive, and easy-to-use dynamic monitoring method is important for the early diagnosis of gastric cancer.

A tumor marker is an antigen or biologically active substance produced during carcinogenesis that reflects the degree of disease progression. These antigens are rarely produced in normal tissues, but they can be detected in tumor tissue, body fluids and excretions. These attributes make them qualify as markers of tumorigenesis. The aim of detecting tumor markers in the serum of patients with gastric cancer is to find a diagnostic marker with high sensitivity and specificity so as to improve the diagnosis of gastric cancer, and to provide a theoretical reference for the clinical diagnosis and treatment of gastric cancer.

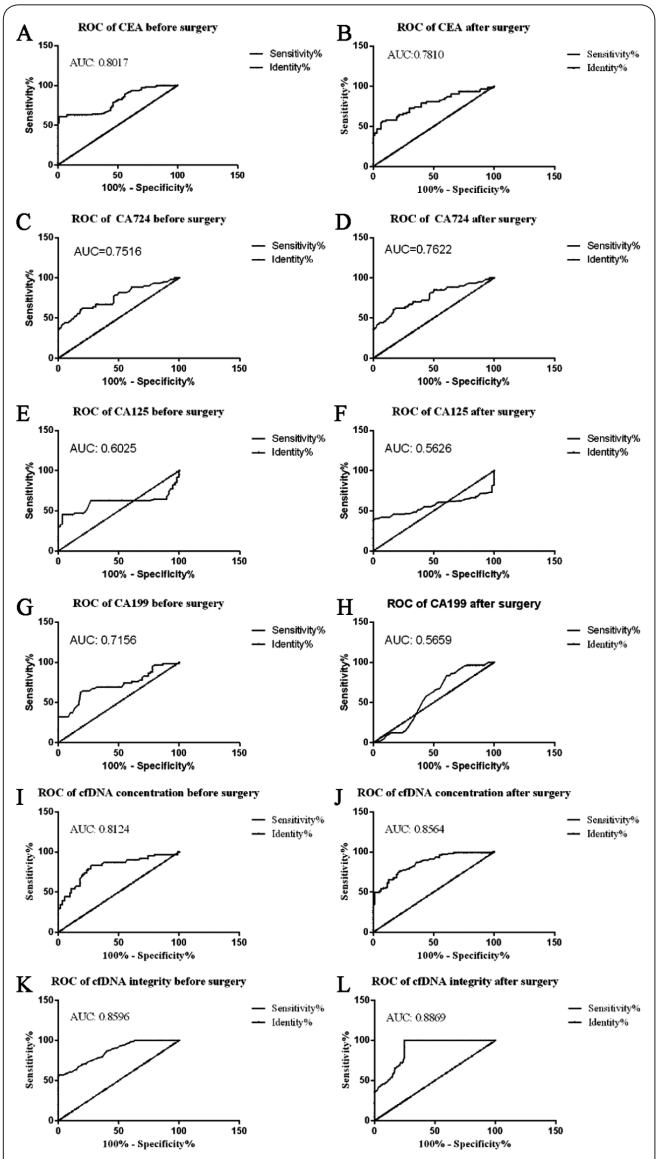

Figure 2. ROC curve analysis of cfDNA and traditional tumor markers. A. ROC curve of CEA in GC patients before surgery. B: ROC curve of CEA in GC patients after surgery. C. ROC curve of CA724 in GC patients before surgery. D. ROC curve of CA724 in GC patients after surgery. E. ROC curve of CA125 in GC patients before surgery. F. ROC curve of CA125 in GC patients after surgery. G. ROC curve of CA199 in GC patients before surgery. H. ROC curve of CA199 in GC patients after surgery. I. ROC curve of cfDNA concentration in GC patients before surgery. J. ROC curve of cfDNA concentration in GC patients after surgery. K. ROC curve of cfDNA integrity in GC patients before surgery. L. ROC curve of cfDNA integrity in GC patients after surgery.

Cell-free DNA (cfDNA) is extracellular free DNA present in body fluids such as serum and plasma. It was discovered in the blood of healthy people in 1948 by French scientists Mendel and Metais. Studies by Leon *et al*. (15) reported that the plasma cfDNA content of tumor patients was higher than that of healthy people. Moreover, Stroun *et al*. (16) found that the cfDNA in the blood has characteristics similar to those of cancer cell DNA. The peripheral blood cfDNA of tumor patients may have some molecular/biological characteristics which are consistent with the DNA of primary tumor cells, such as p53 gene mutation and gene microsatellite instability. These findings laid the foundation for the determination of serum DNA in patients with gastric cancer. In recent years, more and more experimental re-

sults have shown that tumor cells release DNA into the surrounding blood during growth, increasing the circulating DNA concentration in peripheral blood of tumor patients. The phenomenon of high cfDNA concentration in the blood of cancer patients relative to that of healthy people has been repeatedly confirmed in a large number of scientific studies (17). Therefore, when the concentration of cfDNA in the blood is significantly increased, it is likely to imply tumorigenesis (18). Many studies have simultaneously demonstrated the prognostic value of cfDNA for tumor recurrence and patient survival, as well as its monitoring value for treatment response. A study by Gautschi *et al*. showed a significant correlation between the survival time of patients before chemotherapy and the amount of serum DNA. In another study, Hsieh *et al*. found that in patients with colorectal cancer, there was better prognosis in those with low plasma circulating DNA levels. In patients with esophageal cancer, an increase in the concentration of cfDNA usually indicates that the tumor will recur early (19).

Multiple studies have shown that the accuracy of cfDNA detection can be further improved by combining it with determination of cfDNA integrity (20-23). In normal populations, cfDNA is mainly derived from cell apoptosis, and the cfDNA fragment released under the action of enzymatic hydrolysis is generally 185-200 bp in length, while the high concentration of cfDNA in cancer patients is mostly derived from necrosis of tumor cells which release large amounts of free DNA with long fragments. Therefore, the cfDNA integrity in the serum of cancer patients is significantly higher than that of healthy people. Tumor burden can be estimated more accurately on a concentration basis through biological analysis. The results of this study showed that the expressions of CEA, CA724, CA125, CA199 were significantly correlated with clinico-pathological characteristics of gastric cancer patients, but their specificities were not high enough. Therefore, it is clinically necessary to find serological indicators that are specific and sensitive.

The present study is the first report on the quantitative determination of cfDNA in normal subjects and GC patients before and after surgery. The results showed that the cfDNA level of gastric cancer patients differed markedly from corresponding values for normal people. Interestingly, there were no significant associations between cfDNA and gender, age, TNM stage, tumor differentiation, tumor location, and NSE, AFP and CA199 expressions. However, cfDNA was significantly correlated with CEA, CA724 and CA125 expressions. In addition, CA199 expression was significantly correlated with cfDNA integrity, indicating that cfDNA is an index of GC proliferative activity. The marked differences in cfDNA level and integrity before receiving surgery and after surgery suggest the potential of cfDNA as an index of effectiveness of GC therapy.

The role cfDNA in gastric cancer screening was assessed using ROC curve research to calculate the specificity and sensitivity of cfDNA and other tumor biomarkers (CEA, CA724, CA125, and CA199). The findings revealed that patients with gastric cancer before or after surgery had greater AUC for cfDNA than AUCs for CEA, CA724, CA125 and CA199. This indicates the effectiveness of cfDNA as a tumor marker for the prognosis of gastric cancer.

This study provides a new method for GC diagnosis using plasma cfDNA, thereby providing a theoretical basis for the application of cfDNA in tumor diagnosis. The merits cfDNA application include simplicity, stability and non-invasiveness. Measurement of changes in cfDNA allow for early detection of cancer recurrence which provides a basis for judging the effect of therapy, guiding the treatment plan and prognosis, and finally providing assistance for individualized medical treatment of the tumor.

List of abbreviations

cfDNA = cell free DNA; qPCR = quantitative polymerase chain reaction; CEA = carcinoembryonic antigen; CA = cancer antigen; NSE = neuron specific enolase; AFP = alpha fetoprotein; ROC = receiver operating characteristic curve; GC = gastric cancer; AUC = area under the curve.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

Yuejiao Zhong and Xianfeng Cheng conceived and designed this study. Xueliang Zhang, Zhenfeng Wu and Qian Shen performed the experiments and analyzed the data. Rong Li and Xiaohui Jiang wrote the manuscript. Jindong Wu, Ding Li, Ding Wang and Chang Zou revised the manuscript.

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