



The value of ultrasound contrast for assessing cancer cell proliferation and invasion function as well as angiogenesis in lesions of in patients with gastric cancer

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ABSTRACT

Objective: To study the value of ultrasound contrast for assessing cancer cell proliferation and invasion as well as angiogenesis in lesions of in patients with gastric cancer. **Methods:** A total of 39 patients with gastric cancer and 48 patients with gastric ulcer who were treated in our hospital between August 2012 and May 2016 were included in gastric cancer group and gastric ulcer group respectively, and 50 healthy subjects who accepted gastroscopy in our hospital during the same period were included in normal control group. The day after admission, color Doppler diasonograph was used to test the gastric ultrasound contrast parameters; fluorescence quantitative PCR method was used to detect the proliferation and invasion gene mRNA expression in stomach tissue; enzyme-linked immunosorbent assay (ELISA) was used to detect the serum angiogenesis index levels. **Results:** Ultrasound contrast parameters ET and TTP levels of gastric cancer group and gastric ulcer group were significantly lower than those of normal control group, and ultrasound contrast parameters ET and TTP levels of gastric cancer group were significantly lower than those of gastric ulcer group; Stat3, Survivin, Bcl-2, β -catenin, eIF4E, CD44, UHRF1 and c-met mRNA expression in tissue as well as VEGF, EGFR, HIF- α and Ang-2 levels in serum of gastric cancer group were higher than those of gastric ulcer group and normal control group while E-cadherin mRNA expression in tissue was lower than those of gastric ulcer group and normal control group; Spearman correlation analysis showed that ultrasound contrast parameters ET and TTP levels were correlated with the cancer cell proliferation and invasion function as well as angiogenesis indexes in lesions. **Conclusion:** Ultrasound contrast parameters can accurately assess the malignant degree of gastric cancer, and is expected to become the reliable means for early diagnosis and treatment guidance of gastric cancer in the future.

1. Introduction

Gastric cancer is the most common clinical malignant digestive system tumor that is mostly developed from chronic gastritis, gastric ulcer, etc. Lesion biopsy and pathological examination under gastroscopy is the gold standard for diagnosis of gastric cancer, but the gastroscopy is invasive and difficult to become a routine method for tumor screening[1,2]. Looking for noninvasive and effective

means to screen gastric cancer is the key of the current clinical research, ultrasound contrast technology uses fluorescent contrast to reflect tissue organ perfusion, and it has been successfully applied in the diagnosis of liver cancer, lung cancer and colorectal cancer. At present, some scholars recommend contrast-enhanced ultrasonography in early diagnosis of gastric cancer[3], but there is no clear report about the correlation of contrast-enhanced ultrasound parameters with gastric cancer cell proliferation and invasion function as well as angiogenesis. In the following study, the value of ultrasound contrast for assessing cancer cell proliferation and invasion as well as angiogenesis in lesions of in patients with gastric cancer was analyzed.

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2. Information and methods

2.1 General information

A total of 39 patients with gastric cancer and 48 patients with gastric ulcer who were treated in our hospital between August 2012 and May 2016 were included in gastric cancer group and gastric ulcer group respectively, and 50 healthy subjects who accepted gastroscopy in our hospital during the same period were included in normal control group. Inclusion criteria were as follows: (1) diagnosed after histopathology; (2) without gastroscopy history three months prior to inclusion; (3) not associated with malignant tumor diseases of any other tissues and organs; (4) with normal blood coagulation function; (5) the research subjects signed informed consent themselves. Exclusion criteria: (1) couldn't cooperate with examination due to cognitive dysfunction; (2) pregnant or breastfeeding women; (3) allergic to contrast agents; (4) dropping out of the research and with clinical data missing. Gastric cancer group included 21 male cases and 18 female cases, they were 43-78 years old, and the body weight was 48-79 kg and (59.23±8.17) kg in average; gastric ulcer group included 25 male cases and 23 female cases, they were 39-76 years old, and the body weight was 47-81 kg and (60.27±8.95) kg in average; normal control group included 27 male cases and 23 female cases, they were 37-71 years old, and the body weight was 47-80 kg and (61.43±8.65) kg in average. Three groups of research subjects were not statistically different in gender, age and body weight distribution ($P>0.05$), and the research process was approved by the hospital ethics committee.

2.2 Ultrasound contrast parameters

The day after admission, color Doppler diasonograph (Shantou Institute of Ultrasonic Instruments, model Apogee 800 Plus) was used to detect gastric ultrasound images of three groups of subjects. After fasting for solids for 8 h and fasting for liquids for 4 h, the subjects drank 500-1 000 mL of water prior to the inspection to fill the gastral cavity and took supine position for gastric fundus, gastric body and gastric antrum scanning in turn to identify the sites of lesions. Contrast agent Sono Vue (from Bracco Company in Italy) was selected and mixed with 5 mL of saline, 2.4 mL of mixture was injected through the median cubital vein, and then 5mL of saline was injected. The timer was started after the subjects held the breath, and the contrast agent perfusion and echo change in lesion areas were observed. Imaging process lasted for 5-8 min, the images were stored in the hard drives, and the built-in time-intensity curve (TIC) analysis software was used to calculate enhancement time (ET) and time to peak (TTP).

2.3 Proliferation and invasion gene mRNA expression

Lesion tissue samples were collected from gastric cancer and gastric ulcer group under gastroscopy, tissue samples were collected from the corresponding parts of normal control group, and the samples were added in Trizol reagent (Shanghai Enzyme Research Biotechnology Co., LTD., the article number 5301100) to break cells, then added in 0.2 mL of chloroform (Shanghai Xinfan Biotechnology Co., LTD., the article number 0757) and centrifuged under 4 °C at 12 000 rpm for 15 min, the upper colorless aqueous phase was collected and mixed with same volume of isopropyl alcohol (Sigma Company in the United States, the article number HT662), the total RNA in it was precipitated to form gel block. 75% ethanol (Sigma Company in the United States, the article number YN091) was used to clean the RNA precipitation, the ethanol solution was absorbed, and then the precipitation was dried at room temperature for 5-10 min. UV absorption method was used to detect RNA purity and concentration, the reverse transcription kit (Thermo Fisher Company, the article number 4368814) instructions were followed to synthesize sample cDNA, and the reaction system was as follows: buffer 2 μL, 0.2 μL each of upstream and downstream primers, dNTP 0.1 μL, MMLV 0.5 μL, DEPC water 5 μL and RNA template 2 μL. Fluorescence quantitative PCR kit (Thermo Fisher Company, the article number 444556) instructions were followed for the mRNA amplification of proliferation genes Stat3, Survivin, Bcl-2 and β-catenin as well as invasion genes eIF4E, CD44, E-cadherin, UHRF1 and c-met. The corresponding PCR amplification curves were obtained from computer software, and the target gene mRNA expression was also calculated.

2.4 Serum angiogenesis indexes

Immediately after admission, 2 mL of peripheral venous blood was extracted from the three groups, let stand at room temperature for 30 min and centrifuged at low-speed to get supernatant, and enzyme-linked immunosorbent assay kit (from the Sigma Company in the United States) instructions were followed to detect the levels of angiogenesis indexes, including vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), hypoxia-inducible factor-1 α (HIF-1 α) and angiogenin-2 (Ang-2).

2.5 Statistical methods

SPSS 18.0 software was used for statistical processing, measurement data in the study was in terms of (Mean ± SD), comparison among three groups was by variance analysis, comparison between two groups was by routine t test, correlation

was by Spearman correlation analysis and $P < 0.05$ indicated statistical significance in differences.

3. Results

3.1 Ultrasound contrast parameters

Comparison of ultrasound contrast parameters ET and TTP among three groups of subjects was as follows: differences in ET and TTP levels were statistically significant among three groups of subjects ($P < 0.05$). ET and TTP levels of gastric cancer group and gastric ulcer group were significantly lower than those of normal control group, ET and TTP levels of gastric cancer group were significantly lower than those of gastric ulcer group, and differences in pair-wise comparison of ultrasound contrast parameters ET and TTP levels were statistically significant among gastric cancer group, gastric ulcer group and normal control group ($P < 0.05$), shown in Table 1.

Table 1.

Comparison of ultrasound contrast parameters among three groups of subjects (s).

Groups	<i>n</i>	ET	TTP
Gastric cancer	39	9.26±0.97 ^{##}	23.15±2.47 ^{##}
Gastric ulcer	48	12.74±1.85 [†]	26.77±2.94 [†]
Normal control	50	15.37±1.89	29.61±3.27
<i>F</i> value		9.221	7.293
<i>P</i> value		<0.05	<0.05

Note: compared with normal control group, [†] $P < 0.05$; compared with gastric ulcer group, ^{##} $P < 0.05$.

3.2 Proliferation gene mRNA expression

Comparison of proliferation genes Stat3, Survivin, Bcl-2 and β -catenin mRNA expression in tissue among three groups of subjects

Table 2.

Comparison of proliferation gene mRNA expression among three groups of subjects.

Groups	<i>n</i>	Stat3	Survivin	Bcl-2	β -catenin
Gastric cancer	39	213.85±25.47 ^{##}	198.25±22.17 ^{##}	231.27±28.38 ^{##}	201.74±25.36 ^{##}
Gastric ulcer	48	132.74±15.93 [†]	135.82±14.28 [†]	125.93±13.29 [†]	140.38±16.83 [†]
Normal control	50	99.27±10.18	101.73±12.18	100.64±11.28	98.36±10.15
<i>F</i> value		12.184	14.392	18.293	12.184
<i>P</i> value		<0.05	<0.05	<0.05	<0.05

Note: compared with normal control group, [†] $P < 0.05$; compared with gastric ulcer group, ^{##} $P < 0.05$.

Table 3.

Comparison of invasion gene mRNA expression among three groups of subjects.

Groups	<i>n</i>	eIF4E	CD44	E-cadherin	UHRF1	c-met
Gastric cancer	39	215.38±25.49 ^{##}	223.64±25.49 ^{##}	23.27±2.84 ^{##}	243.17±28.95 ^{##}	191.65±24.08 ^{##}
Gastric ulcer	48	120.17±15.38 [†]	132.54±15.39 [†]	71.28±8.09 [†]	135.37±15.41 [†]	122.18±14.32 [†]
Normal control	50	97.23±9.51	98.25±10.17	99.84±10.13	103.24±11.57	98.27±10.17
<i>F</i> value		15.834	17.973	8.291	11.674	10.982
<i>P</i> value		<0.05	<0.05	<0.05	<0.05	<0.05

Note: compared with normal control group, [†] $P < 0.05$; compared with gastric ulcer group, ^{##} $P < 0.05$.

was as follows: differences in proliferation genes Stat3, Survivin, Bcl-2 and β -catenin mRNA expression in tissue were statistically significant among three groups of subjects ($P < 0.05$). Stat3, Survivin, Bcl-2 and β -catenin mRNA expression in tissue of gastric cancer group and gastric ulcer group were significantly higher than those of normal control group, Stat3, Survivin, Bcl-2 and β -catenin mRNA expression in tissue of gastric cancer group were significantly higher than those of gastric ulcer group, and differences in pair-wise comparison of Stat3, Survivin, Bcl-2 and β -catenin mRNA expression in tissue were statistically significant among gastric cancer group, gastric ulcer group and normal control group ($P < 0.05$), shown in Table 2.

3.3 Invasion gene mRNA expression

Comparison of invasion genes eIF4E, CD44, E-cadherin, UHRF1 and c-met mRNA expression in tissue among three groups of subjects was as follows: differences in eIF4E, CD44, E-cadherin, UHRF1 and c-met mRNA expression in tissue were statistically significant among three groups of subjects ($P < 0.05$). eIF4E, CD44, UHRF1 and c-met mRNA expression in tissue of gastric cancer group and gastric ulcer group were significantly higher than those of normal control group while E-cadherin mRNA expression were significantly lower than that of normal control group, eIF4E, CD44, UHRF1 and c-met mRNA expression in tissue of gastric cancer group were significantly higher than those of gastric ulcer group while E-cadherin mRNA expression was significantly lower than that of gastric ulcer group, and differences in pair-wise comparison of invasion genes eIF4E, CD44, E-cadherin, UHRF1 and c-met mRNA expression in tissue were statistically significant among gastric cancer group, gastric ulcer group and normal control group ($P < 0.05$), shown in Table 3.

Table 4.

Comparison of serum angiogenesis index levels among three groups of subjects.

Groups	<i>n</i>	VEGF	EGFR	HIF- α	Ang-2
Gastric cancer	39	653.78±58.98 [#]	95.75±9.87 [#]	64.13±6.76 [#]	2038.38±241.57 [#]
Gastric ulcer	48	293.32±25.75 [*]	43.11±5.64 [*]	50.65±6.82 [*]	912.87±9.65 [*]
Normal control	50	74.82±8.95	18.23±2.71	11.26±1.54	201.75±24.64
<i>F</i> value		12.193	9.272	8.938	16.384
<i>P</i> value		<0.05	<0.05	<0.05	<0.05

Note: compared with normal control group, ^{*}*P*<0.05; compared with gastric ulcer group, [#]*P*<0.05.

3.4 Serum angiogenesis indexes

Comparison of serum angiogenesis indexes VEGF (pg/mL), EGFR (ng/mL), HIF- α (ng/L) and Ang-2 (pg/mL) levels among three groups of subjects was as follows: differences in serum VEGF, EGFR, HIF- α and Ang-2 levels were statistically significant among three groups of subjects (*P*<0.05). Serum VEGF, EGFR, HIF- α and Ang-2 levels of gastric cancer group and gastric ulcer group were significantly higher than those of normal control group, serum VEGF, EGFR, HIF- α and Ang-2 levels of gastric cancer group were significantly higher than those of gastric ulcer group, and differences in pair-wise comparison of serum VEGF, EGFR, HIF- α and Ang-2 levels were statistically significant among gastric cancer group, gastric ulcer group and normal control group (*P*<0.05), shown in Table 4.

3.5 Correlation analysis

Spearman correlation analysis showed that gastric ultrasound contrast parameters ET and TTP levels in patients with gastric cancer were negatively correlated with proliferation genes Stat3, Survivin, Bcl-2 and β -catenin mRNA expression; they were negatively correlated with invasion genes eIF4E, CD44, UHRF1 and c-met mRNA expression and positively correlated with E-cadherin mRNA expression; they were negatively correlated with serum angiogenesis indexes VEGF, EGFR, HIF- α and Ang-2 levels (*P*<0.05).

4. Discussion

Under physiological state, gastric cavity contains a large number of gases and fluids, the stomach wall can't form a clear reflective surface, so ultrasound cannot become a routine inspection method for stomach disorders. With the progression of imaging technology, intravenous contrast agent injection to fill the gastric cavity can eliminate the interference from intragastric gases and mucous, clearly display the lining of gastric wall and make contrast-enhanced ultrasonography for gastric lesions possible[4]. ET and TTP can accurately reflect the malignant degree of tissue, the much vascular bed blood flow and fast velocity within malignant lesion tissue shorten the enhancement time of contrast agent, so the more the new blood vessels in tumor tissues, the greater the malignant degree, and the shorter the contrast agent ET and TTP[5]. It was found in the study that compared with normal control group, gastric cancer

group and gastric ulcer group were with smaller ET and TTP values, it indicates that there is a certain degree of angiogenesis in both groups, ET and TTP value of gastric cancer group were significantly smaller than those of gastric ulcer group, this is consistent with the tissue characteristics, and it indicates that ultrasound contrast parameter levels are significantly different between patients with different gastric lesions.

It has been confirmed that gastric cancer patients are with smaller local ET and TTP values, but the correlation of ET and TTP value change with the malignant degree of stomach tissue is still not clear. Abnormal expression of proliferation and invasive genes is the root cause of tumor progression, and the pro-proliferation and pro-invasion gene expression activity directly determines the malignant degree of tumor[6]. It has been reported in different studies that Stat3, Survivin, Bcl-2 and β -catenin are related to gastric cancer cell proliferation, and Stat3 signaling pathway activation can lead to abnormal proliferation and malignant transformation of cells; Survivin is a member of the anti-apoptosis protein family, and it is highly expressed in most of the cancer tissues; Bcl-2 is an important proto-oncogene that can inhibit cell apoptosis and is commonly abnormally expressed in leukemia and myeloma[7]. The study of WANG Yan-li[8] confirms that β -catenin is highly expressed in tissue of rats with gastric cancer, and it can induce gastric cancer cell proliferation and inhibit its apoptosis. In the study, stomach tissues were obtained from the three groups to detect the above proliferation gene mRNA expression, and it was found that compared with normal control group, gastric cancer group and gastric ulcer group were with different degree of high Stat3, Survivin, Bcl-2 and β -catenin mRNA expression, and Stat3, Survivin, Bcl-2 and β -catenin mRNA expression in tumor tissue of gastric cancer group were significantly higher than those of gastric ulcer group, which confirms that pro-proliferation gene are highly expressed in gastric cancer tissue, and directly indicates that proliferation gene detection is the reliable index to judge the malignant transformation of tumor.

Abnormal invasion gene expression is the necessary foundation for malignant transformation of tumor cells, the study of ZHAO Shi-qiao[9] has confirmed that there are high eIF4E and CD44 mRNA expression as well as low E-cadherin mRNA expression in gastric cancer tissue; the study of ZHOU Lin[10] shows that UHRF1 regulates RB1 methylation to promote the invasion and metastasis of gastric cancer cells; it has been pointed out in the study of SHEN Hao[11] that silencing c-met gene can inhibit the distant metastasis of gastric cancer cells. In the study, the above invasion gene mRNA expression levels in gastric tissue samples were detected, and it was

found that there were different levels of high eIF4E, CD44, UHRF1 and c-met mRNA expression as well as low E-cadherin mRNA expression in tissue of gastric cancer group and gastric ulcer group, and eIF4E, CD44, UHRF1 and c-met mRNA expression of gastric cancer group increased sharply while E-cadherin mRNA expression further reduced. This means that abnormal invasion gene expression is the important mechanism of the occurrence of gastric cancer, and can also be the accurate indicator to judge benign and malignant disease.

Tumor angiogenesis is the common basis of malignant cell proliferation and invasion, there is exuberant angiogenesis in most malignant tumor tissues, and this is mainly based on the massive pro-angiogenesis molecule expression in it[12,13]. The study of FANG Yu[14] confirms that invasion and metastasis are more likely to occur in gastric cancer cells with positive VEGF and EGFR expression; the study of YANG Feng[15] shows that dendritic cell infiltration density is higher in gastric cancer tissue with high VEGF expression, and the cells are more prone to escape and metastasis. HIF- α and Ang-2 are also the factors that can strongly promote angiogenesis, and their high expression has been found in liver cancer, lung cancer and colorectal cancer[16,17]. It was found in the study that compared with normal control group, gastric cancer group and gastric ulcer group were with different degree of higher serum pro-angiogenesis indexes VEGF, EGFR, HIF- α and Ang-2 levels, and gastric cancer group were with even higher VEGF, EGFR, HIF- α and Ang-2 levels, which is consistent with previous studies and indicates the positive value of angiogenesis index levels for differentiating benign and malignant gastric lesions.

In the study, Spearman correlation analysis was finally adopted to define the correlation between ultrasound contrast parameters and the above gastric cancer illness-related index levels, and the results showed that the ultrasound contrast parameters ET and TTP levels were directly correlated with cancer cell proliferation and invasion function as well as angiogenesis in lesions. Therefore, ultrasound contrast parameters can accurately assess the malignant degree of gastric cancer, and are expected to become the reliable means for early diagnosis and treatment guidance of gastric cancer in the future.

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