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Prognostic value of EUS combined with MSCT in predicting the recurrence and metastasis of patients with gastric cancer

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Abstract

Objective: This study aims to explore the prognostic value of endoscopic ultrasonography combined with multi-slice spiral computed tomography in predicting the recurrence and metastasis of gastric cancer, as well as investigate the correlation of fragile histidine triad protein expression with the tumor–node–metastasis stage of gastric cancer patients.

Methods: A total of 81 gastric cancer patients were selected in our study. All patients were examined by endoscopic ultrasonography and multi-slice spiral computed tomography before operation, and gastric cancer tissues and adjacent normal tissues were obtained after operation. Immunohistochemistry was performed to detect fragile histidine triad expression. All patients were followed up for 3 years after operation. Univariate and multivariate analysis of risk factors were conducted for the prognosis of gastric cancer patients.

Results: Endoscopic ultrasonography combined with multi-slice spiral computed tomography could increase the accuracy of preoperative tumor–node–metastasis stage of gastric cancer patients. In gastric cancer tissues, fragile histidine triad expression was mostly weakly positive with a positive rate of 60.5%. In gastric cancer adjacent normal tissues, the positive fragile histidine triad expression was mostly moderate with a positive rate of 79.0%. The fragile histidine triad expression was negatively correlated with tumor–node–metastasis stage of gastric cancer patients. The fragile histidine triad expression decreased along with the increase of T-stage, N-stage and M-stage of gastric cancer patients. Univariate and multivariate analysis showed that T-stage and N-stage were risk factors for the recurrence/metastasis and 3-year mortality of gastric cancer patients, while fragile histidine triad expression was a protective factor.

Conclusion: Our study demonstrated that endoscopic ultrasonography combined with multi-slice spiral computed tomography may be more accurate in assessing the preoperative tumor–node–metastasis stage of gastric cancer patients.

Key words: endoscopic ultrasonography, fragile histidine triad, gastric cancer, metastasis, multi-slice computed tomography, prognosis, recurrence

Introduction

Gastric cancer (GC) is one of the most common malignant tumors in the world with an incidence rate ranking fourth among all malignant tumors. About 650 000 people die of GC each year with a death rate only second to lung cancer (1). Genetic and environmental factors may contribute to the occurrence of GC, including the genetic background of the patients, infections and dietary habits (2). In Japan, a mature system to detect GC by endoscopic examination has been established, which has improved diagnosis and cure rates of early GC, but in many western countries, most GC patients are presented with advanced stages at the initial diagnosis (3,4). Therefore, a reliable method to determine the tumor–node–metastasis (TNM) stage and lymph node metastasis would be of great significance for the treatment and prognosis of GC.

Endoscopic ultrasonography (EUS) is the first-choice imaging modality for predicting the invasion depth of early GC (5). In recent years, multi-slice spiral computed tomography (MSCT) has become a useful tool for tumor detection owing to its ability to determine non-calcified nodules of small size (6). On the other hand, EUS has been found to be useful in detecting ascites, indicating a more precise determination for peritoneal metastasis (7). Therefore, combination of MSCT with EUS may avoid the risk of inaccurate staging and significantly increase the preoperative sensitivity to detect GC metastasis. As fragile histidine triad (FHIT) gene has non-activated tumor suppressor function in many kinds of tumors (8), it is located on human chromosome 3p14.2 and its expression is absent in many human cancers, indicating that its protein product might have tumor suppressor functions (9). Although the exact mechanisms of FHIT remain unclear, FHIT as a tumor suppressor gene has been widely supported by experiments as well as on a cellular level, while it also induces apoptosis and delays the metastasis of cancer cells (10,11). In this study, we aim to explore the prognostic value of EUS combined with MSCT in predicting the recurrence and metastasis of GC patients, as well as investigate the correlation of FHIT expression with the TNM stage of GC patients.

Materials and methods

Ethics statement

This study was performed in accordance with the guidelines established by the Ethics Review Committee of Xiangnan University, and all patients have signed the forms of consent.

Study subjects

A total of 81 GC patients undergoing surgical treatments were collected at Xiangnan University from 1 February 2010 to 1 January 2013, consisting of 58 male and 23 female cases, with the age 56.80 ± 11.51 years. The baseline characteristics of 81 patients with GC are presented in Table 1. The inclusion criteria were: (i) with upper gastrointestinal symptoms and clinical suspicions of stomach diseases; (ii) GC confirmed by gastroscopy and biopsy diagnosis; (iii) received GC removal operations at Xiangnan University; (iv) with complete follow-up data and (v) received no chemotherapy or other anti-tumor therapies before the surgery. The exclusion criteria were: (i) patients with a history of abdominal surgeries; (ii) patients with other malignancies or history of malignancy; (iii) incomplete pathological data; (iv) contraindications to the use of contrast agents and (v) incomplete follow-up information. Surgical treatment was conducted, with 81 GC tissues and 81 adjacent normal tissues obtained.

Table 1. Baseline characteristics of 81 patients with gastric cancer

Characteristic	Case ($n = 81$)	Proportion (%)
Age (years)		
<60	63	77.78
≥60	18	22.22
Gender		
Male	58	71.60
Female	23	28.40
Pathological type		
Ulcer type	16	19.75
Infiltrative type	53	65.43
Lump type	12	14.81
Lymph node metastasis		
Yes	51	62.96
No	30	37.04
Distant metastasis		
Yes	13	16.05
No	78	83.95

EUS and MSCT examinations

All patients underwent EUS and abdominal MSCT examinations 2 weeks prior to operation, and all results were assessed by two experts manually. EUS was conducted using a Fujinon SU-7000 ultrasound system (Fujifilm, Japan) with a circular scanning electron EUS EG-530UR. The scanning frequencies were 5, 7.5, 10 and 12 MHz. A small ultrasonic probe (Fujinon SP-702, Fujifilm, Japan) was used at the scanning frequency of 12, 15, 20 and 25 MHz, respectively. Patients fasted for 12 h before the examination. During the examination, patients were placed in the left lateral decubitus position, and the EUS probe was inserted into the stomach. In the initial inspection, conventional endoscopy was performed to assess the general situation in the stomach, and to clear as much gastric mucus and food debris as possible. Subsequently, EUS probe was inserted to the descending part of the duodenum (except for patients with pyloric obstruction) and observed with a reversal back mirror method. During the inspection, the air was sucked out and 300–800 ml of degassed water was injected into the stomach. The regions along the descending part of the duodenum and the path back to the cardia were scanned continuously. EUS was used for larger lesions and lymph nodes, while the depth of invasion of smaller lesions was determined by EUS combined with a smaller probe.

MSCT examination was conducted by using a Brilliance 16 MSCT machine (Philips, the Netherlands). Before the examination, patients fasted for at least 8 h and ingested 800–1200 ml warm water before checking and 200–300 ml water during the examination to fill the stomach and stretch the gastric walls, thus making the lesion images more prominent and clearer. During the inspection, the lesion sites underwent plain scan first, followed by an enhanced scan. The thickness of the scanning slice was 5 mm, with the same slice scanned 40 times continuously. The tube voltage was 120 kV and the tube current was 100 mA, with the vision range and reconstruction interval time adjusted as desired. During the scanning, 50 ml of the contrast agent was injected at a rate of 3 ml/s. The upper bound of the scanning area was the dome of the diaphragm, and the lower bound was a lower renal pole. The scanning included the arterial and portal phases.

Preoperative and postoperative TNM stage

The TNM stage of GC was evaluated according to the fifth edition of the UICC TNM stage standard (12). EUS and MSCT stages were in accordance to the following criteria: T referred to the depth of invasion: T_1 indicated the tumor had invaded mucosa and/or mucosa muscle or submucosa; T_2 indicated the tumor had invaded muscular or subserosal; T_3 indicated the tumor had invaded serosa and T_4 indicated the tumor had invaded adjacent structures.

Tumor in EUS image was a hypoechoic mass of five-layer structure damage of normal gastric wall, and EUS staging was carried out according to the damage level to determine the GC depth of invasion; while GC in MSCT had thickened stomach wall and/or abnormal enhancement of gastric wall. The criteria for judging the depth of invasion were as follows: T1 indicated the lesion of gastropathy similar to the normal gastric wall performance; T2 indicated multi-layer structures in the lesion of gastropathy with low density zone of submucosa; T3 indicated surface polishing and finishing of the thickened stomach wall serosal and T4 indicated the fat layer disappearance between thickening gastric wall and the adjacent organs. N referred to lymph node metastasis: N_0 indicated no lymph node metastasis; N_1 indicated 1–6 sites of regional lymph node metastasis; N_2 indicated 7–15 regional sites of lymph node metastasis and N_3 indicated more than 15 regional sites of lymph node metastasis. M referred to distant metastasis: M_0 indicated that there was no distant organ metastasis and M_1 indicated that there was distant organ metastasis (including liver, lung, peritoneum, kidney, lymph nodes and peritoneal ascites).

Definition of lymph node metastasis

In EUS, hypoechoic, circular and well-defined lymph nodes were referred to as metastatic lymph nodes, while high-level echo, oval and lymph nodes with fuzzy boundaries were identified as non-metastatic lymph nodes. In MSCT, short range of lymph nodes around the stomach more than 6 and 8 mm was referred to as lymph node metastasis.

Immunohistochemistry

Immunohistochemistry was performed with the streptavidin-perosidase (SP) three-step method. All pathological specimens were fixed in 4% formalin and embedded in paraffin. The specimens were sliced continuously with a slice thickness of 2 μm. After microwave repair of the antigen and endogenous oxidase blocking, non-immune sera were added on the slides drop-wise to remove impurities in the antigen. The slides were then incubated at room temperature for 30 min and washed three times with phosphate-buffered saline (PBS). The primary antibody was dropped onto the slides (mouse anti-human polyclonal FHIT antibody, Zhongshan Biotech, Beijing, China) and incubated overnight at 4°C. After being washed repeatedly with PBS, the slides were added with secondary antibody. After the 10 min incubation, the reaction was terminated by adding anti-biotin-labeled peroxidase solution, colorizing the slides with diaminobenzidine. Following the re-staining with hematoxylin, the nucleus of a cell was revealed using PBS. Finally, after dehydrated

Table 2. Determination of TNM stage of 81 patients with gastric cancer by EUS and MSCT

Perioperative stage	Pathological stage										Accuracy (%)
	T_1	T_2	T_3	T_4	N_0	N_1	N_2	N_3	M_0	M_1	
EUS											
T_1	14	2	0	0							87.5
T_2	0	9	3	0							75
T_3	0	3	25	4							78.2
T_4	0	0	5	16							76.2
N_0					26	3	0	0			89.6
N_1					4	16	3	0			69.6
N_2					0	10	14	0			58.3
N_3					0	0	3	2			40
M_0									65	2	97
M_1									9	5	35.7
MSCT											
N_0					24	5	0	0			82.8
N_1					6	14	3	0			60.9
N_2					0	6	16	2			66.7
N_3					0	0	2	3			60
M_0									67	0	100
M_1									1	13	92.8
EUS + MSCT											
T_1	14	2	0	0							87.5
T_2	0	9	3	0							75.0
T_3	0	3	25	4							78.2
T_4	0	0	5	16							76.2
N_0					26	3	0	0			89.6
N_1					4	16	3	0			69.6
N_2					0	6	16	2			66.7
N_3					0	0	2	3			60.0
M_0									67	0	100.0
M_1									1	13	92.8

EUS, endoscopic ultrasonography; MSCT, multi-slice spiral computed tomography; TNM, tumor–node–metastasis.

with anhydrous ethanol and dried, the slides were mounted with neutral gum and observed under a 400× microscope.

The results were determined according to tissue staining intensity and the number of cells: cells with no staining were determined as negative, while the brown-stained cells on a light blue background were judged as positive. Expression is showed according to grid counting method (13). In the measurement, five non-overlapping view fields were chosen and the cells were counted. The expression was determined as: no positive staining (negative, –), 1–25% of cells showed positive staining (weakly positive, +), 26–50% of the cells showed positive staining (moderate positive, 2+), >50% cells showed positive staining (strongly positive, 3+).

Follow-up

Follow-up dates were calculated based on the dates of the surgeries, and the follow-ups were done by telephone communications, clinical revisits or mails. The follow-ups were completed 3 years after operation.

Statistical analysis

All data were processed using the SPSS 21.0 (SPSS, Inc., Chicago, IL, USA) statistical software. All measurement data were presented as mean ± standard deviations, and were verified by the *t*-tests. All enumeration data were presented using percentages or rates, and were verified using the chi-square tests. The correlation coefficients (r and P values) between the FHIT expression and the TNM stage of GC patients were obtained using the Spearman test. A P value <0.05 was considered statistically significant.

Results

Determination of TNM stage of GC patients

As presented in Table 2, the accuracies of MSCT and EUS examinations for the determination of TNM stage of GC patients were evaluated by the comparison with pathological examination (gold standard). The accuracies of EUS for T_1, T_2, T_3 and T_4 stages were 87.5, 75.0, 78.2 and 76.3%, respectively. EUS is relatively accurate in determining the T-stage of GC patients, but its accuracy was poor for N-stage (N_{2-3}). The mean diameters of metastatic and non-metastatic lymph nodes were 10.4 ± 5.1 and 6.2 ± 1.7 mm, respectively. For N-stage, the accuracies of EUS were 89.6% (N_0), 69.6% (N_1), 58.3% (N_2) and 40.0% (N_3), while the accuracies of MSCT were 82.8% (N_0), 60.9% (N_1), 66.7% (N_2) and 60.0% (N_3). The MSCT showed obvious advantages over EUS in N_3 stage ($P < 0.05$). The accuracies of EUS and MSCT for M_0 were 97.0 and 100.0%, respectively ($P > 0.05$). However, the accuracies of EUS and MSCT were, respectively, indicating MSCT was superior to EUS for the determination of M_1 stage (92.8% vs. 35.7%, $P < 0.05$). EUS combined with MSCT had high accuracy, which could increase the accuracy of preoperative TNM stage of GC patients. The T, N_0 and N_1 staging was mainly determined based on EUS, while the N_2, N_3 and M staging was mainly determined based on MSCT.

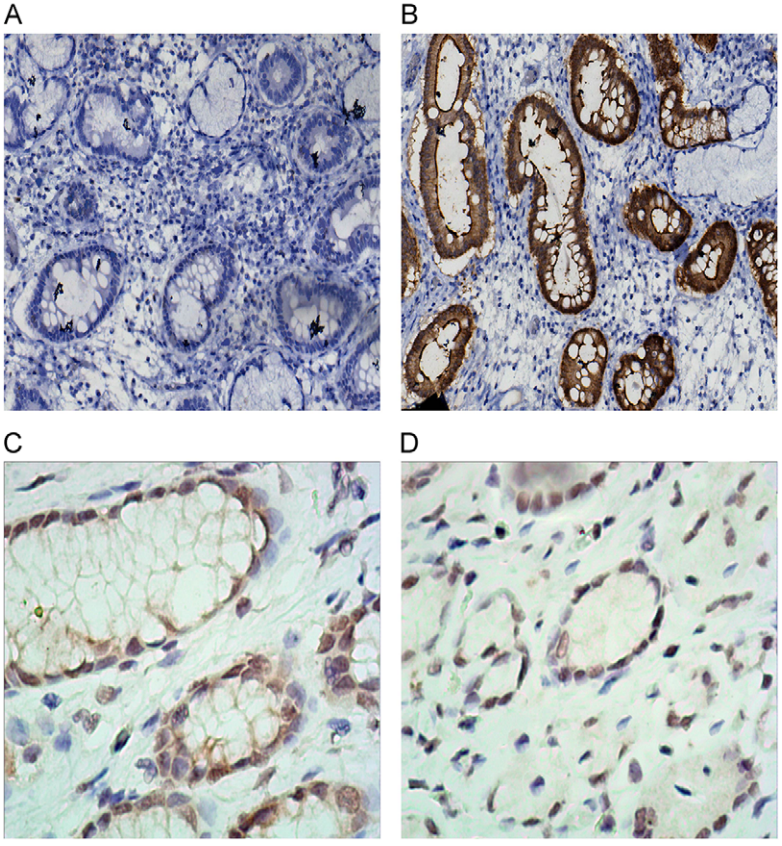

Figure 1. Comparison of FHIT expression between gastric cancer tissues and adjacent normal tissues detected by immunohistochemistry. Note: (A) PBS negative control; (B) positive FHIT expression in adjacent normal tissues (×100); (C) positive FHIT expression in gastric cancer tissues (×400) and (D) weakly positive FHIT expression in gastric cancer tissues (×400). FHIT, fragile histidine triad; PBS, phosphate-buffered saline.

Table 3. Comparison of FHIT expression between gastric cancer tissues and adjacent normal tissues

	FHIT expression				Positive rate (%)
	−	+	++	+++	
Gastric cancer tissues	32	36	13	0	60.5*
Adjacent normal tissues	17	21	36	7	79.0

*Compared with adjacent normal tissues, $P < 0.05$; FHIT, fragile histidine triad.

Comparison of FHIT expression between GC tissues and adjacent normal tissues

FHIT specific staining was located in the cytoplasm. No staining aggregation was seen inside or around the nucleus, and no FHIT expression was seen within the stroma (Fig. 1). As presented in Table 3, in GC tissues, FHIT expression was mostly weakly positive and was restricted in flaky regions, with a positive rate of 60.5%; but in adjacent normal tissues, FHIT expression was mostly moderate and showed a diffusive pattern, with a positive rate of 79.0%. The positive rate of FHIT expression in GC tissues was significantly lower than that in the adjacent normal tissues ($P < 0.05$).

Correlation of FHIT expression with TNM stage of GC patients

In all 81 GC patients, the positive rates of FHIT expression for T_1, T_2, T_3 and T_4 stages were 87.5, 75.0, 50.0 and 47.6%, respectively; the positive rates of FHIT expression for N_0, N_1, N_2 and N_3 stages were 79.3, 69.6, 41.7 and 40.0%, respectively; and the positive rates of FHIT expression for M_0 and M_1 stages were 65.7 and 35.7%, respectively. It was discovered that FHIT expression was negatively correlated with TNM stage of GC patients ($r = -0.410$, $P = 0.007$). As presented in Table 4, the FHIT expression decreased along with the increase of T-stage, N-stage and M-stage of GC patients (Table 4) (all $P < 0.05$).

Univariate and multivariate analysis of risk factors for the recurrence, metastasis and mortality of GC patients

Among these 81 GC patients, 25 patients had cancer recurrence (30.9%), and 20 patients died (24.7%). As presented in Table 5, univariate analysis found that age, T-stage and N-stage were risk factors for the recurrence/metastasis and 3-year mortality of GC patients (all $P < 0.05$). Multivariate analysis showed that age, T-stage and N-stage were independent risk factors for the recurrence/metastasis and 3-year mortality of GC patients, while FHIT expression was an independent protective factor (all $P < 0.05$) (Table 6).

Discussion

Although in most developed countries, GC morbidity and mortality are significantly reduced, it still ranks second in cancer death worldwide (14). Currently, EUS and MSCT are major methods to determine the stages of cancers. In addition, FHIT is considered to have a tumor suppressing function and loss of FHIT expression has been found in many human cancer cases (9). Therefore, it may be of significance in the timely treatment and prognosis of GC by using EUS, MSCT and FHIT expression.

Table 4. Correlation of FHIT expression with TNM stage of patients with gastric cancer

TNM stage	*n*	FHIT expression			*P*
		−	+	++	
T-stage					0.015
T1	16	2	12	2	
T2	12	3	4	5	
T3	32	16	13	3	
T4	21	11	7	3	
N-stage					0.040
N0	29	6	15	8	
N1	23	8	12	3	
N2	24	14	8	2	
N3	5	4	1	0	
M-stage					0.041
M0	67	23	34	10	
M1	14	9	2	3	

Table 5. Univariate analysis of risk factors for the recurrence/metastasis and 3-year mortality of patients with gastric cancer

Factor	*n*	Recurrence/metastasis	*P*	3-Year mortality	*P*
Age (years)			0.019		0.011
<60	63	15		11	
≥60	18	10		9	
Gender			0.606		0.253
Male	58	19		12	
Female	23	6		8	
T-stage			0.026		0.038
T1	16	1		0	
T2	12	3		2	
T3	32	10		10	
T4	21	11		8	
N-stage			0.026		<0.001
N0	29	6		2	
N1	23	5		6	
N2	24	10		7	
N3	5	4		5	
FHIT			0.010		0.022
−	32	16		13	
+	36	7		6	
++	13	2		1	

This study has found that after combining MSCT and EUS in GC inspection, the accuracy of TNM stage would be significantly improved. EUS has become an indispensable diagnostic method that pairs up the conventional endoscopy with the high-frequency cavity ultrasound endoscopy, thus achieving the high resolution required to determine the depth of tumor invasion and gastrointestinal tract lesions (15). Also, accurate preoperative staging is important for determining a reasonable GC therapy, and it has been confirmed that EUS is quite accurate to determine the T-stage of GC, which is consistent with our study (16). In addition, it has been shown that the accuracy of EUS in determining the N-stage of GC was 71.3% (17), similar to the accuracy of EUS in determining the occurrence of lymph node metastasis in our study. Consistently, the development of MSCT has shown substantial progresses in the CT technology, whose impressive imaging speeds can make the inspection more

Table 6. Multivariate analysis of risk factors for the recurrence/metastasis and 3-year mortality of patients with gastric cancer

Factor	Recurrence/metastasis			3-Year mortality		
	Sig.	Exp (*B*)	95% CI for Exp (*B*)	Sig.	Exp (*B*)	95% CI for Exp (*B*)
Age	0.001	33.718	4.371–260.116	0.496	1.761	0.345–8.976
T-stage	0.023	10.295	1.379–76.853	0.002	16.866	2.730–104.194
N-stage	0.047	9.736	1.031–91.960	0.001	19.399	3.150–119.45
FHIT expression	0.001	0.12	0.033–0.432	0.008	0.065	0.009–0.489

Sig., significance; CI, confidence interval.

comfortable and perform sophisticated three-dimensional (3D) angiographic rendering. Therefore, it can be expected that the combination of EUS and MSCT may further improve the accuracy in determining the TNM stages of GC.

Our study has proven that the higher the TNM stage is, the lower the FHIT expression is. It has been shown that the TNM stage of GC is the most important independent prognostic factor and is indispensable in determining an appropriate treatment. Consistently, a previous study has demonstrated that the pT-stage is an independent factor that affects the prognosis of GC patients: the later the pN-stage is, the worse the prognosis is and the lower the 5-year survival rates are (18).

In addition, FHIT gene contains 10 exons with 1.8 Mb genomic regions, of which only exons 5–9 can code for proteins that may be involved in regulation of cell proliferation and apoptosis (19). Similarly, FHIT is a tumor suppressor gene that spans the most common fragile site in the human genome, FRA3B (20). Hu et al. (21) has pointed out that the weakening tumor suppressor FHIT expression is closely related to human tumor progression, which is consistent with our study. In addition, the multivariate analysis has indicated that T-stage and N-stage are independent risk factors affecting tumor recurrence, metastasis and overall survival, while FHIT protein is a protective factor for cancer recurrence, metastasis and overall survival of GC.

In conclusion, our findings provide stronger evidence that EUS combined with MSCT may be more accurate in assessing the preoperative TNM stage of GC patients. Furthermore, the FHIT expression decreased along with the increase of T-stage, N-stage and M-stage of GC patients. T-stage and N-stage are risk factors for the prognosis of GC patients, but FHIT expression may be a protective factor. However, the anti-tumor mechanisms of FHIT protein in living organisms and whether FHIT expression could combine with EUS and MSCT for the diagnosis of GC still remain unclear. Furthermore, the main limitation of this study is the small sample size. Therefore, more researches would be further conducted to provide reference for the clinical diagnosis of MSCT combined with EUS for GC.

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Conflict of interest statement

None declared.

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