Rapid Communication

Relationship between preoperative staging by endoscopic ultrasonography and MMP-9 expression in gastric carcinoma

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AIM: To investigate the relationship between the staging by endoscopic ultrasonography (EUS) and the expression of carcinoma metastasis associated gene in the patients with gastric carcinoma.

METHODS: Sixty-three patients with gastric cancer were diagnosed by electric gastroscopy and EUS. The preoperative staging of gastric cancer was measured by EUS and compared with pathologic staging and MMP-9 expression. Peripheral serum level of MMP-9 was measured with enzyme-linked immunosorbent assay (ELISA), while the expression of MMP-9 protein was tested with immunohistochemistry and hybridization in situ in the gastric carcinoma tissues.

RESULTS: The total accuracy of EUS in estimating invasive depth of gastric cancer was 80.95%, while that in estimating lymphatic metastasis was 73.02%. Serum MMP-9 levels were consistent with the expression of MMP-9 protein and MMP-9 mRNA in tissue, a result closely correlated with invasive degree, staging with EUS and lymphatic metastasis in gastric cancer (P < 0.05). The total accuracy of estimating invasive depth in gastric cancer was 95.22% using both EUS and MMP-9.

CONCLUSION: The MMP-9 level of preoperative serum presents the reference value for preoperative staging by EUS in the patients with gastric cancer. When serum MMP-9 level in gastric cancer is significantly high, physicians should pay closer attention to the metastasis which reaches the serosa or beyond. Combining EUS and MMP-9 improves the accuracy in deciding the invasion and metastasis in the patients with gastric carcinoma.

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a week after surgery. The supernatant liquid of blood specimens was taken and quickly stored in a refrigerator of -80℃ for preparation after blood clotting. A superficial gastritis group comprised 30 subjects (aged between 26 and 72 years), among whom 18 were males and others were females; the mean age was 56.8 ± 14.2 years. Of the 30 normal control subjects (aged between 28 and 74 years), 19 subjects were males and others were females; the mean age was 57.5 ± 13.8 years.

Methods

**Main instruments:** Olympus GF-240 electronic gastroscope; GF-UMQ 240 electro-ultrasonic gastroscope, 360-degree circular scan, frequency range: 7.5 MHz to 20 MHz.

**Main reagents:** MMP-9 mouse monoclonal antibody, S-P Kit (Fuzhou Maixin biotechnology Co, Ltd.); MMP-9 probe, hybridization *in situ* kit (Boster Biotechnology Co. Ltd, Wuhan, China); MMP-9 ELISA Kits (Jingmei Company, Shenzhen, China).

**EUS inspection:** Such methods as the water-filled balloon method, water immersion or both were adopted to inspect different foci. The hierarchical structure of foci and perifoci was scanned with a ultrasonic transducer, and lymph nodes around stomach were inspected from the greater curvature to a lesser curvature in sequence.

**Histopathological staging of gastric cancer:** all patients in question were operated within two weeks after the EUS inspection; examinations performed include detailed pathologic examinations on the depth of gastric wall invasion, the invasion of surrounding tissue and lymphatic metastasis. The postoperative pathologic TN staging was taken as the gold standard, the accuracy with EUS for each stage was: T1 87.50% (7/8), T2 73.33% (11/15), T3 85.19% (23/27), T4 76.92% (10/13) respectively. Seven cases were under-staged while 5 cases were over-staged; the total accuracy was 80.95% in the judgment of invasion depth in gastric cancer. The accuracy of lymphatic metastases was 80.00% (20/25) and 68.4% (26/38) respectively. The staging results of 46 cases staged with EUS were in line with pathologic staging. The total accuracy of lymphatic metastasis was 73.02% (46/63).

**Relationship between MMP-9 level and pathologic staging**

The MMP-9 serum level of 63 preoperative patients with gastric carcinoma [(380.20 ± 295.76) ng/L] were obviously higher than that of normal group [(98.79 ± 86.43) ng/L] and that of chronic gastritis group [(110.28 ± 90.35) ng/L] (P < 0.01); the MMP-9 serum level [(145.17 ± 133.84) ng/L] of postoperative patients dropped dramatically within one week (P < 0.01), approaching normal levels. Compared with the normal control, the discrepancy was of no statistical significance (P > 0.05). The positive expression rate of MMP-9 protein was 63.49% (40/63) in 63 cases with gastric carcinoma (Figure 3A). Twenty-nine cases (78.38%) were MMP-9 positive in the tissue among and hybridization *in situ*. Each stained slice was reviewed in a double-blind manner.

**Statistical analysis**

Statistical analysis was made using the software package SPSS10.0 for Windows. The results of immunochemistry and *in situ* hybridization were examined by χ² test. The results of ELISA were expressed as mean ± SD, and examined by t test. Probability value (P) below 0.05 was considered statistically significant.

**RESULTS**

**Comparison between EUS staging and pathologic staging**

Combining gastroscope and biopsy histopathology, the diagnostic accuracy reached 93.7% (59/63) in this group (Figure 1). The diagnostic accuracy of EUS was 92.1% (58/63) (Figure 2); with gastroscope and EUS inspection combined, the diagnostic accuracy reached 100%. The preoperative stagings with EUS for 63 patients with gastric carcinoma found 9 cases (T1), 14 cases (T2); 28 cases (T3); 12 cases (T4), respectively. As for lymphatic metastases (N), 32 cases were N0, and 31 cases were N+. When pathologic staging was taken as the gold standard, the accuracy with EUS for each stage was: T1 87.50% (7/8), T2 73.33% (11/15), T3 85.19% (23/27), T4 76.92% (10/13) respectively. Seven cases were under-staged while 5 cases were over-staged; the total accuracy was 80.95% in the judgment of invasion depth in gastric cancer. The accuracy with EUS for cases staged N0 and N+ was 80.00% (20/25) and 68.4% (26/38) respectively. The staging results of 46 cases staged with EUS were in line with pathologic staging. The total accuracy of lymphatic metastasis was 73.02% (46/63).

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The correlation between preoperative EUS staging and MMP-9 expression of gastric carcinoma was concluded (Table 1). MMP-9 and MMP-9 mRNA positive expression was not detected in 30 cases of normal gastric mucosa, while weakly positive staining was detected in 63 cases of peri-tumor tissue. In the tissues of gastric carcinoma, MMP-9 mRNA was mainly found in the matrix of cancer cells, and the positive expression rate was 63.48%. The discrepency is also of statistical significance ($P < 0.05)$. Of 40 cases with confirmed invasion of serous membrane or further, 30 cases were MMP-9 positive (75.00%); however, among 23 cases without invaded serous membrane, only 10 cases were reported positive (43.48%). The discrepency is statistically significant ($P < 0.05$). With respect to lymphatic metastasis, 29 cases (76.32%) were MMP-9 positive among 38 cases which had lymphatic metastasis; and 11 cases (44.00%) were MMP-9 positive among 25 cases without lymphatic metastasis. The discrepency is also of statistical significance ($P < 0.05$) (Table 1).

MMP-9 and MMP-9 mRNA positive expression was detected in 30 cases of normal gastric mucosa, while weakly positive staining was detected in 63 cases of peri-tumor tissue. In the tissues of gastric carcinoma, MMP-9 mRNA was mainly found in the matrix of cancer cells, and the positive expression rate was 63.49% (40/63), which was consistent with the results of MMP-9 immunohistochemistry (Figure 3B). The serum level and tissue expression of MMP-9 was also the highest among all stages. Although the mean serum level and tissue expression of MMP-9 in stage $T_1$ and stage $T_2$ were different, the discrepency was of no statistical significance ($P > 0.05$); however, compared with stage $T_1$ and $T_2$, increment was observed in MMP-9 serum level in stage $T_3$ and $T_4$; and the MMP-9 serum levels were much higher in stage $N_3$ than that in stage $N_0$, which was of statistical significance ($P < 0.05$).

There was a positive correlation between MMP-9 expression and EUS staging. In the 12 cases which were not correctly staged, 2 cases of stage $T_1$ were staged $T_3$, 2 cases of stage $T_2$ and 3 cases of stage $T_3$ staged $T_4$, 1 case of stage $T_4$ staged $T_5$, 2 cases of stage $T_3$ staged $T_4$, and 2 cases of stage $T_4$ staged $T_5$. Among 7 cases which were under-staged, 5 cases were turned out to be stage $T_3$ or $T_4$; the serum level of MMP-9 reached 410 ng/mL, though they were confirmed as stage $T_2$ by EUS. Among 5 cases which were over-staged, 4 cases were pathologically confirmed stage $T_2$ or $T_3$; the MMP-9 serum level was 220 ng/L. Combining EUS inspection and detection of MMP-9 level, the total accuracy for the determination of invasion depth of gastric cancer reached 95.22%.

**DISCUSSION**

The correct staging for carcinoma is significant for reaching a reasonable treatment option and evaluating the prognosis; therefore, preoperative staging plays an important role in the diagnostic model of modern medicine. In the preoperative diagnosis of gastric cancer, EUS is greatly superior to other methods available in that the ultrasound transducer installed on the tip of the endoscope can get closer to foci inside the body, presenting a more accurate and detailed resultant ultrasound image than those obtained with traditional ultrasound. Moreover, high consistency can be identified between results of EUS and that of postoperative pathology. It is indicated in our research that the diagnostic accuracy with EUS was 87.50% ($T_1$), 73.33% ($T_2$), 85.19% ($T_3$), 76.92% ($T_4$), respectively; and the diagnostic accuracy for lymphatic metastasis was 80.00% ($N_0$), and 68.42% ($N_1$), respectively. The results were basically consistent with current literature. However, further efforts are expected to avoid overstaging or understaging and to reflect the

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biological characteristics of gastric cancerous tissue through preoperative staging.

Matrix metalloproteinases (MMPs) are a group of proteinases that can digest extra-cellular matrix and basal membrane. In the process of invasion and metastasis of cancer cells, it is necessary for them to break the barrier formed by extra-cellular matrix and basal membrane.\cite{6,7} MMP-9 is an important member of MMP-9 family; it is widely regarded as proteinase that correlates closely with invasion and metastasis of tumor. MMP-9 can degrade extra-cellular matrix and basal membrane and promote invasion and metastases of cancer cells. In our research, the serum levels of MMP-9 in 63 preoperative patients with gastric carcinoma was remarkably higher than that of normal control and that of chronic gastritis group (P < 0.01). The MMP-9 serum levels rose gradually along with the depth of cancerous invasion in patients with gastric carcinoma; the MMP-9 serum levels in patients with invaded serous membrane (T1, T2) were much higher than that of those without suffered (T1, T3). Furthermore, the MMP-9 serum levels are obviously higher in patients with lymphatic metastasis than those without. Our research identifies the consistency between MMP-9 serum levels and MMP-9 protein expression in tissue of our subjects. There is a high MMP-9 protein expression in the tissue of subjects with high MMP-9 serum levels; the MMP-9 serum levels are much higher in subjects with positive expression of MMP-9 protein than those without. It indicates that gastric cancer tissue can secrete much MMP-9, which participates in the degradation of collage IV of basal membrane. Not only can MMP-9 make invasion in situ but break extracellular membrane (ECM) barrier and vessel wall, and finally prepare for successful metastasis.

Preoperative EUS reflects the extent of invasion of gastric cancer; it is not yet satisfactory, even if the total accuracy of depth of cancerous invasion and lymphatic metastases by EUS was 80.95% and 73.02% respectively. It is worthwhile mentioning that 7 cases were understaged and 5 cases were over-staged with EUS. Combining preoperative EUS and the detection of MMP-9 serum level combined, the accuracy of judgment on the depth of invasion will be significantly raised up to 95.22%. This combination can also make up for insufficiencies of EUS staging. Possible causes for unsatisfactory EUS staging are as follows: with respect to early gastric cancer, routine EUS is not satisfactory in diagnostic accuracy of invasion depth, especially when scanning the gastric antrum or gastric notch. This setback may be caused by the ultrasonic transducer placed too close to the gastric wall, preventing ultrasound from efficiently focusing on foci. However, a water-filled space between the transducer and gastric wall will press gastric wall directly, which obscures the display of the inner three layers of gastric wall. It is difficult to come to a precise judgment on the depth of the invasion. Besides, the overstaging of early gastric cancer is correlated with deep-seated fibrosis. It seems that EUS with a small ultrasonic transducer is superior to the routine EUS when scanning superficial or small foci. In evaluation of the invasion depth, EUS is optimal for progressive gastric cancer, as the mean accuracy is over 80%. In addition, it is also effective in the evaluation of peri-gastric lymphatic metastases. Failure to detect the tiny invasive foci outside the sub-mucosa should be responsible for the understaging of progressive stage gastric cancer. Combining EUS with detection of preoperative MMP-9 serum level will help improve the accuracy of evaluation on the invasion and metastases of gastric cancer.

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COMMENTS

Background

In gastric carcinoma, the expression of MMP-9 was related to invasion and lymph node metastasis, but not to age and gender. High expression of MMP-9 promotes tumor metastasis. Patients with high MMP-9 expression are found with a low survival rate. However, there exists no significant relationship between MMP-9 and stage of bladder cancer.

EUS is one of the most accurate methods for assessing the locoregional extent of neoplasia in alimentary tract; it plays an important role in preoperative T and N staging of gastric carcinoma, but further efforts are expected to avoid overstaging or understaging, and to identify metastatic lymph nodes.

Research frontiers

MMP-9 was reported to be associated with tumor biological characteristic. Inhibition of the enzyme activity of MMP-9 might become a novel therapeutic strategy of cancer. EUS and EUS-guided techniques are found superior not only in diagnosis but also in treatment of pathological changes.

Innovations and breakthroughs

MMP-9 was reported as playing an important role in the development and metastasis in such cancers as hepatocellular carcinoma, gastric carcinoma, and bladder cancer. Some reports found MMP-9 level in serum or tissue likewise associated with tumor stage. EUS was widely used in diagnosing and judging the location and resectability of carcinoma; however, few reports probe into the combination of EUS and MMP-9 level. Our research found improved accuracy of EUS in staging tumors when used together with MMP-9 level detection.

Applications

Endoscopic color Doppler ultrasonography (ECDUS) can further increase the accuracy of diagnosis by clearly presenting blood signal of tissue. High resolution EUS (HREUS) can display distinctly the five-layer structure of the alimentary tract, which is superior to MRI and CT. Among mushrooming new techniques, EUS emerges as a superior technique in clinical practice, such as EUS-guided pancreatic pseudocyst drainage, EUS-guided celiac plexus neurolysis (EUS-CPN), endoscopic mucosal resection (EMR), EUS-guided radio frequent ablation (EUS-RFA), EUS-guided needle injection (EUS-FNI), to name a few.

Terminology

Endoscopic Ultrasonography (EUS): A procedure that combines endoscopy and ultrasound to obtain images and information on the digestive tract and the surrounding tissue and organs. In EUS, a small ultrasound transducer is installed on the tip of the endoscope, allowing the transducer to get closer to the organs inside the body. Therefore, the resultant ultrasound images are often more accurate and detailed than those obtained by traditional ultrasound; Matrix Metalloproteinase: A member of a group of enzymes that can break down proteins, such as collagen, that are normally found in the spaces between cells in tissues. Because these enzymes need zinc or calcium atoms to work properly, they are called metalloproteinases. They are involved in wound healing, angiogenesis, and tumor cell metastasis; Enzyme-linked Immunosorbert Assay (ELISA): A sensitive laboratory test method that relies on an enzymatic conversion reaction and is used to detect the presence of specific substances (such as enzymes or viruses or antibodies or bacteria); Hybridization in situ: A laboratory test that locates a gene or gene product by adding specific radioactive or chemically tagged probes for the gene and locates the radioactivity or chemical on the chromosome or in

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the cell after hybridization.

**Peer review**

The approach of combining EUS and a MMP-9 as biomarker is an innovative way of studying prognosis in gastric cancer by means of translational research; it gives a clear delineation of the research background, objectives, materials and methods, results and conclusions. The design of the controls is rational. Tables and figures reflect the major findings of the study and they are appropriately presented, and the references are appropriate.

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