Original Article

Use of peritoneal washing cytology for the detection of free peritoneal cancer cells before and after surgical treatment of gastric adenocarcinoma

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ABSTRACT

Aim: Cytological detection of peritoneal-free gastric cancer cells is considered as the gold standard with variable sensitivity. Seeding of cancer cells after radical surgery for gastric cancer is a controversial issue. In this study, it was aimed to detect the rate of positive peritoneal washing cytology and the incidence of spreading of tumor cells after radical surgery.

Materials and Methods: Patients with pathologically proven and surgically treated gastric adenocarcinoma were enrolled. Three peritoneal washing samples were examined cytologically: at the beginning, after completion of resection, and before closure of the abdomen. Identification of peritoneal-free gastric cancer cells was regarded as the main outcome.

Results: Thirty-four patients with a mean age of 60.7 ± 12 years were enrolled. T3 and N0 were the most common stages seen in 16 (47%) and 12 patients (35.3%), respectively. There were two positive results (5.9%) as the first peritoneal sample. Considering T3- or N-positive patients, the incidence increased to 9.1%. There was no conversion of negative to positive cytology. Cytological positivity remained only in one case (2.9%) after the second and the third peritoneal samples.

Conclusion: Rate of positive peritoneal washing cytology in patients with gastric cancer is influenced by clinicopathological findings and the technique used. Use of cytology alone is thought to be failed to detect free cancers cells within the peritoneal cavity.

KEY WORDS: Cytology, dissemination, gastric cancer, peritoneal lavage/washing, radical gastrectomy

INTRODUCTION

Although cytological examination of peritoneal washes is considered as the gold standard for assessing the presence of free cancer cells in the peritoneal cavity, its sensitivity ranges from 21% to 35% depending on the tumor (T) and node (N) stages of gastric cancer.[1,2] Recently, molecular approaches using reverse-transcriptase polymerase chain reaction (RT-PCR) techniques have made it possible to increase the sensitivity to detect micrometastasis in the peritoneal cavity.[1,3,4] However, lack of immediate intraoperative results, relatively high false-positive results, higher cost, and lack of this technology in each center are the main limitations, which prevent the common use of such techniques.[1,3,5]

Occurrence of peritoneal recurrences of gastric adenocarcinoma after radical gastrectomy with lymph node dissection remains to be a controversial issue.[1,4-7] Among the routes for spreading of gastric cancer cells, direct seeding of tumor cells through gastric wall, via blood vessels, and perigastric lymphatic channels are thought to be the major causes for such recurrences.[1,4-6] However, peritoneal recurrences cannot be explained by tumoral infiltration through gastric wall or spillage of tumor cells from lympho-vascular vessels in T1 and N0 cases, respectively.[5] Therefore, a clear and definite reason for the presence of free cancer cells in the peritoneal cavity before and after radical surgery for gastric cancer has not been fully established yet.[1,3]

Conversion of negative preoperative cytology to positive postoperative cytology has been reported previously after the surgical procedures performed

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for colonic and esophageal cancers.[8-13] There have been many reports about the spreading of gastric cancer cells after surgical manipulation.[3,5,6,14] However, the exact incidence of tumor cell implantation during surgery and the factors which are directly related with this issue has been unclear.

In this study, it was aimed to detect the rate of positive peritoneal washing cytology in patients with gastric cancer and the incidence of spreading of tumor cells after radical surgery via cytological analysis of peritoneal washings.

MATERIALS AND METHODS

Patients

Patients in whom gastric cancer was histologically confirmed by endoscopic biopsy, preoperative staging revealed nonmetastatic disease, and preoperative workup excludes comorbidity obviating major surgery between June 2014 and June 2015 were enrolled into this prospective study. Informed consent was obtained before the surgery. Patients with extensive intraperitoneal metastases or unresectable disease at laparotomy were excluded. Patients were not excluded if they had received preoperative neoadjuvant chemotherapy which was planned for resectable but locally advanced disease.

This study was performed according to the Declaration of Helsinki, approved by the local Ethical Committee (71306642-050.01.04/03.12.2014) and was registered to Clinical Trials with an identifier number of NCT02287168.

A worksheet listing patient's demographics (age, gender), past history with regard to neoadjuvant treatment, operative findings, and pathologic features including tumor node metastasis (TNM) staging, grade and quality of lymph node dissection was used.

Surgical procedure and sampling methods

The surgical tumor stage was carefully examined immediately after the laparotomy before the manipulation of the tumor. After thorough examination of the peritoneal cavity revealing the absence of peritoneal dissemination, the peritoneal cavity was washed with 200 ml of saline, and at least one-third was aspirated from several regions of the peritoneal cavity, including near the primary tumor, the left and right subphrenic areas, and the pouch of Douglas with suction tubes to a clean bottle and designated as the first peritoneal sample (Cyt1). Radical subtotal distal or total gastrectomy plus D2 lymphadenectomy was performed according to the Japanese Classification of Gastric Carcinoma (third English edition).[15] For the lymphatic dissection and all the named vessels of the stomach except the left gastric artery and the right gastroepiploic arteries, the electrothermal bipolar vessel sealer (LigaSure, Valleylab, Boulder, CO, USA) was used. The left gastric and the right gastroepiploic arteries were ligated by 2/0 vicryl sutures. During the surgical procedures, the distal transection line (i.e., duodenum) was transected via linear cutting staples. For subtotal gastrectomy, the proximal resection line of the stomach was transected via linear cutting staples at first [Figure 1]; the anastomosis was performed through the controlled opening of the transection line at the left lateral border after the placement of occlusive intestinal clamps throughout the resection line [Figures 2 and 3]. For total gastrectomy, occlusive L-shaped intestinal clamps were used to close the esophageal lumen before the transection of the distal esophagus.

After the completion of the resection, the peritoneal cavity was washed and aspirated with 200 ml of saline as described above as the second peritoneal sample (Cyt2). Following reconstruction via Roux-en-Y gastro- or esophago-jejunostomy, the peritoneal cavity was washed with 1 L saline three times, which was then gently and completely aspirated. The peritoneal cavity was cleaned with abdominal packing to remove all lavage fluid. Before the closure of the abdomen, the peritoneal cavity was washed and aspirated with 200 ml of saline as described above as the third peritoneal sample (Cyt3).

Cytologic examination

Each saline wash sample (Cyt1, Cyt2, and Cyt3) was centrifuged at 1500 rpm for 10 min to collect intact cells. The remaining precipitate was smeared onto four slides, fixed with acetone, and stained with hematoxylin and eosin (H and E). The specimens were examined and interpreted by an experienced cytopathologist.

Pathological examination

After completion of the surgery, the specimens were sent to the pathology laboratory immediately. H and E staining was used for the evaluation of the paraffin blocks from each patient. T and lymph N staging was evaluated. The seventh American Joint Committee on Cancer/International Union Against Cancer TNM system was used for the staging of gastric cancer.[16]

Statistics

All statistics were performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Normally distributed continuous

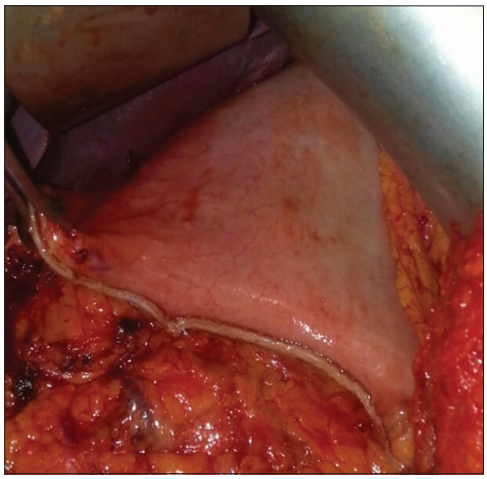

Figure 1: For subtotal gastrectomy, complete closure of the proximal resection line of the stomach via linear cutting staples

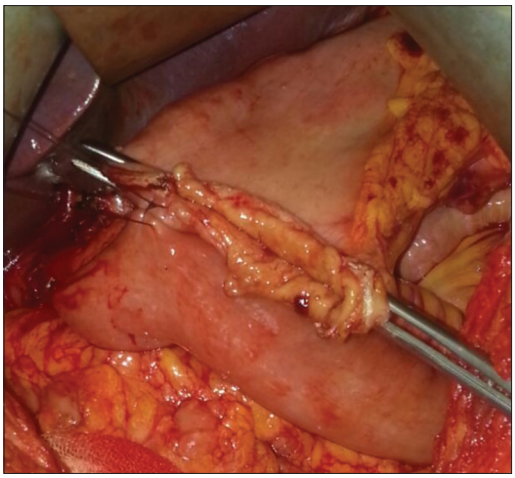

Figure 2: Controlled opening of the transection line at the left lateral border after the placement of an occlusive intestinal clamp

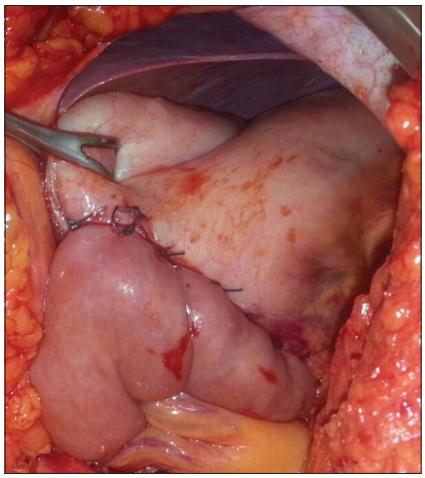

Figure 3: For subtotal gastrectomy, completion of gastrojejunostomy anastomosis

variables were expressed as mean ± standard deviation and variables without a normal distribution were expressed as median and ranges. Categorical variables were expressed as frequencies and percentages.

RESULTS

Patients

A total of 34 patients with pathologically proven and surgically treated gastric adenocarcinoma were enrolled. The mean age of the patients was 60.7 ± 12 years. Clinicopathological features of the patients were shown in Table 1. Neoadjuvant chemotherapy was performed in 10 patients (29.4%). According to T and N stages, T3 and N0 were the most common stages seen in 16 (47%) and 12 patients (35.3%), respectively. M1 patients (8.8%) were due to solitary <1 cm liver metastasis in one and positive peritoneal washing cytology in two patients. The mean number of total lymph nodes in all patients and the metastatic lymph nodes in all N positive (N1, N2, and N3) patients were 35.1 ± 12.9 and 8.0 ± 8.9, respectively.

Cytologic examination of peritoneal washing fluids (Cyt1, Cyt2, and Cyt3)

There were two positive peritoneal washing cytology (2 out of 34, 5.9%) as the first peritoneal sample (Cyt1). Considering T3- or N-positive patients, the incidence increased to 9.1% (2 out of 22 for both T3- and N-positive cases). Cytologic examination of the second and the third peritoneal samples revealed that the positive result remained positive only in one case (2.9%). There was no conversion of negative to positive cytology in the study group.

Table 1: Clinicopathological features of the patients

Feature	*n* (%)
Age (years)	60.7±12
Gender (male/female)	24/10
Operation	
Distal subtotal gastrectomy	18 (53)
Total gastrectomy	16 (47)
Tumor grade	
Well-differentiated	5 (14.7)
Moderately differentiated	10 (29.4)
Undifferentiated/signet ring cell	19 (55.9)
T stage	
Tis	1 (2.9)
1a	3 (8.8)
1b	4 (11.8)
2	4 (11.8)
3	16 (47.1)
4a	5 (14.7)
4b	1 (2.9)
N stage	
0	12 (35.4)
1	8 (23.5)
2	6 (17.6)
3a	3 (8.8)
3b	5 (14.7)
Tumor node metastasis stage	
1a	7 (20.6)
1b	1 (2.9)
2a	7 (20.6)
2b	7 (20.6)
3a	2 (5.9)
3b	4 (11.8)
3c	3 (8.8)
4	3 (8.8)

DISCUSSION

In this study, it could not be possible to show the positivity of peritoneal washing cytology by cytologic examination after radical surgery for gastric adenocarcinoma, although there has been sufficient data in the literature. However, the use of cytologic techniques only and inclusion of the cases with all stages and neoadjuvant treatment might affect the results negatively. Controversy between the present study and the others necessitates future studies to clarify the conflicting points.

For the detection of free cancer cells, cytological analysis has been regarded as the gold standard during operation besides its low sensitivity and specificity.[1,2,17-20] However,

it has also been shown that combination of both cytology and RT-PCR for carcinoembryonic antigen (CEA) and CK-20 mRNA increases the sensitivity comparing the techniques alone.[1,3,21] The positivity of free cancer cells within the peritoneal cavity by cytologic examination has been reported between 16% and 43%.[17,22,23] In addition, the detection of free cancer cells by the peritoneal lavage cytology in about 20–33.3% of curatively resected serosa-involved cases was shown in previous studies.[6,24] However, almost 50–90% of the cases in these studies had peritoneal carcinomatosis implying a positive bias. Therefore, it has been thought that detection of a small number of cancer cells by cytology can be difficult.[6] In this study, the peritoneal positivity was detected in 5.9% and 9.1% of all patients and the patients with T3 or N3 stages, respectively. This low rate can be explained by the use of cytology alone, inclusion of all stages of gastric cancer, and of the patients with neoadjuvant treatment. To overcome this difficulty, the use of sensitive methods such as the immunocytochemical method, with a combination of selected monoclonal antibodies or RT-PCR can be offered, and it is generally accepted that use of such innovative techniques helps to increase the sensitivity to detect free cancer cells in gastric adenocarcinoma patients.[5,6,24,25]

In a study by Hao,[3] it has been shown that depth of tumor invasion, area of invaded serosa, nodal involvement, and pathological stage are directly related with the presence of free cancer cells after laparoscopic or open gastrectomy groups. However, the size and histological type of the tumor were not associated with positive results from peritoneal washings. The incidence of positive peritoneal-free cancer cells was the highest as 80.95% in cases with serosa and adjacent organ invasion.[3] Serosal invasion can be regarded as a prerequisite for transperitoneal invasion. In a study by Ikeguchi,[26] it was also shown that the area of serosal invasion is directly related with the free cancer cells. It has been reported that only 17.3% of the patients has free cancer cells if the area of serosal invasion was <10 cm^2. However, the similar results could not be produced in the present study, even in cases with T3 stages. Use of cytology alone or wide inclusion criteria including all T and N stages might be important for this issue. However, the shedding of cancer cells from both the serosal surface and angio-lymphatic system for the development of peritoneal carcinomatosis might be questioned based on the findings of the present study.

Although conversion of negative peritoneal cytology to positive result after the surgery could be an expected event, especially in high-grade or locally advanced gastric tumors, this conversion has been reported as 2.6% by both cytology and CEA RT-PCR in the study by Hao.[3] It has been believed that it can be difficult to detect the malignant cells among the large number of nucleated cells after postresectional peritoneal lavage cytology by cytology alone.[6] However, it has also been found that free cancer cells after lymph node dissection were detected in 14.3% and 26.7% of the cases with submucosal and muscle involved tumors, respectively.[1] It has been thought that free cancer cells can be introduced during the surgical maneuvers including opening the gastric wall and lympho-vascular channels.[5] Authors explained this low rate by performing an optimum surgical technique, adequately controlling the spillage of cancer cells from the gastric lumen or the lympho-vascular channels.[2,24]

In the study by Han,[5] it was speculated that the free cancer cells in the gastric lumen can be the source of peritoneal dissemination in serosa noninfiltrating node-negative gastric cancers. For the surgical technique used in the present study, spillage of the gastric and esophageal secretions was prevented by using the preventive measures such as closed transection of the stomach and the esophagus. In addition, widespread use of energy-based devices to close lympho-vascular channels during the lymph node dissection could be another important factor to prevent shedding of the tumor cells in accordance with the study by Han.[5] Therefore, application of such surgical techniques may be an important measure to prevent peritoneal seeding.

Limited number of the cases, use of cytologic techniques only and inclusion of the cases with all stages, and neoadjuvant treatment were regarded as the limitations of the study. Prospective studies revealed that more gastric cancer cases with specifically T3 or more tumors with positive lymph node involvement may be useful to clarify these controversial issues.

CONCLUSION

Rate of positive peritoneal washing cytology in patients with gastric cancer is influenced by clinicopathological findings and the technique used for this purpose. Use of cytology alone is thought to be failed to detect free cancers cells within the peritoneal cavity. Therefore, the use of more sensitive molecular approaches including the immunocytochemical methods, with a combination of selected monoclonal antibodies or RT-PCR may improve the detection rate of free intraperitoneal cancer cells.

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Conflicts of interest

There are no conflicts of interest.

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