Original Article

Incidence of Free Colorectal Cancer Cells in Peritoneal Cavity and Correlation with Clinicopathologic Variables: An Analysis of 275 Patients Undergoing Curative Intent Resection for Colorectal Cancer

Weerapat Suwanthanma MD[1], Chakrapan Euanorasetr MD[1], Panuwat Lertsithichai MD[1], Atcharaporn Pongtippan MD[2], Ekkapob Sirachainan MD[3], Napaphat Poprom MPh[1]

[1] Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[2] Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[3] Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Background: Positive peritoneal cytology has impact on prognosis of several intra-abdominal malignancies. There was no consensus on whether we should routinely perform peritoneal cytology in operated-colorectal cancer patients because previous studies showed very low yield.

Objective: The present study was conducted to investigate the incidence of free cancer cells in the peritoneal lavage cytology of patients underwent curative resection for colorectal cancer.

Materials and Methods: Between January 2006 and December 2012, intraoperative peritoneal lavage cytology was performed in 275 patients underwent curative resection for colorectal cancer. Immediately after exploration of abdomen, 100 ml of normal saline solution was instilled into peritoneal cavity over the tumor site. Peritoneal lavage was then aspirated and sent for cytological examination.

Results: Six (2.18%) of the 275 specimens examined were found to have positive peritoneal lavage cytology. Demographic variables, site of tumor, degree of differentiation, mucinous component, perineural invasion, angiolymphatic invasion, depth of tumor penetration, staging, and level of serum CEA were found not to affect the incidence of free cancer cells. Positive nodal status was the only factor found to be associated with incidence of positive peritoneal cytology (p = 0.04).

Conclusion: Our study demonstrates low incidence of positive free colorectal cancer cell in lavage fluid obtained before resection in patients that underwent curative intent resection for colorectal cancer. The factor associated with positive peritoneal cytology is lymph node involvement.

Keywords: Free colorectal cancer cell, Peritoneal lavage cytology, Colorectal cancer

J Med Assoc Thai 2018; 101 (2): 203-8
Website: http://www.jmatonline.com

Complete removal of colorectal cancer is the most effective treatment for colorectal cancer. However, 20% to 45% of patients will eventually have the tumor spreading at the tumor bed or outside its primary origin[1]. Liver, lung, pelvis, and peritoneum are the most common sites of recurrence and metastasis. Various clinical, biological, and pathological features appear to relate to the prognosis of colorectal carcinoma, and these prognostic factors are used for prognostic classification in the Dukes staging and the TNM classification[2].

Several published reports showed that peritoneal cytology is useful for predicting the prognosis of gastric[3-8], pancreatic[9], and gynecologic malignancies[10,11]. Recently, studies that analyzed the incidence of free colorectal cancer cells in peritoneal cavity at the time of surgery have been done. While the positive yield was quite low, they showed some prognostic significance[10-15].

For this reason, we intended to evaluate the incidence of free colorectal cancer cell in peritoneal cavity at the time of surgery in the Thai colorectal cancer patients. Additionally, we identify specific

Correspondence to:

Suwanthanma W. Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Mahidol University 270 Rama VI Road, Ratchathewi, Bangkok 10400, Thailand.
Phone: +66-2-2011315, **Fax**: +66-2-2011316
Email: weerapat.suw@mahidol.ac.th

How to cite this article: Suwanthanma W, Euanorasetr C, Lertsithichai P, Pongtippan A, Sirachainan E, Poprom N. Incidence of free colorectal cancer cells in peritoneal cavity and correlation with clinicopathologic variables: an analysis of 275 patients undergoing curative intent resection for colorectal cancer. J Med Assoc Thai 2018;101:203-8.

clinicopathologic features that could predict its presence in the present study.

Materials and Methods

Patients

We conducted the study between January 2006 and December 2012. The Institutional Review Board approved for the study. Inclusion criteria were Thai patient with operable colorectal cancer, age 15 years and older, stable hemodynamic, and no emergency conditions such as bleeding or obstruction. The exclusion criteria were patients younger than 15 years, unresectable tumor, distant metastases, unstable hemodynamic, previous neoadjuvant therapy, no resection, and pregnancy. Four hundred fifty-four Thai patients with colorectal cancer that underwent resection at our institution were included in the present study.

For the sample size were used in this study, we collected all patients diagnosed as operable colorectal cancer between January 2006 and December 2012 according to inclusion criteria.

Sixty-four patients were excluded from the study because intraoperative peritoneal washing was not performed, 76 patients because of distant metastases and/or recurrence, 17 patients because of having received neoadjuvant therapy, and 17 patients because of the lack of resection, leaving 275 patients included for analysis.

Cytology specimens from intraperitoneal lavage were prospectively collected from 275 Thai patients with colorectal cancer. No neoadjuvant therapy was provided to any patient. All patients underwent open curative oncologic resection performed by two colorectal surgeons. There was no postoperative mortality.

The method of cytology collection was standardized as follow, immediately after opening the peritoneal cavity, one hundred milliliters of sterile normal saline solution was poured into the peritoneal cavity over the tumor site. The patient was raised head up and the peritoneal washing fluid collected in the pelvic cul-de-sac was aspirated until reaching the volume of at least 50 ml. If ascites were present, the ascites were removed at the same volume. The peritoneal washing was sent for examination by an experienced and well-trained cytologist at Ramathibodi Hospital. After finishing specimen collection, surgeons continued the operation until completion.

A positive result was defined as detection of free tumor cells in the lavage fluid by conventional cytology methods.

Clinicopathologic variables such as age, gender, tumor site, cell type, depth of tumor invasion, nodal involvement, tumor differentiation, angiolymphatic invasion, perineural invasion, and operation performed were analyzed for association with the results of peritoneal lavage cytology using t-test ranked, and Fisher's exact test as appropriate. Associations were considered significant if *p*-value was 0.05 or less. Stata program version 14 was used to perform the analysis.

Results

Among the 275 patients studied, intraperitoneal free cancer cells were found in six patients (2.18%). The demographic variables of patients were shown in

Table 1. Clinicopathologic variables of patients

Variable	Data (total = 275)
Age, n (%)	
<40 years	8 (2.91)
≥40 years	267 (97.09)
Sex, n (%)	
Male	131 (47.64)
Female	144 (52.40)
T stage, n (%)	
T1	10 (3.64)
T2	50 (18.20)
T3	194 (70.55)
T4	20 (7.30)
Unknown	1 (0.40)
N stage, n (%)	
N0	148 (53.82)
N1	66 (24.00)
N2	61 (22.20)
Number of lymph node harvested, mean ± SD	20.15±9.90
M stage, n (%)	
M0	274 (99.64)
M1	1 (0.36)
AJCC stage, n (%)	
1	49 (17.82)
2	98 (35.64)
3	128 (46.55)
4	0 (0.00)
Site, n (%)	
Colon	162 (58.91)
Rectum	113 (41.10)
Operation, n (%)	
Right colectomy	95 (34.55)
Left colectomy	18 (6.55)
Sigmoidectomy	43 (15.64)
Anterior/low anterior resection	103 (37.45)
APR	16 (5.82)
Preoperative serum CEA (ng/ml), n (%)	
Normal	143 (52.00)
High (>4.6 ng/ml)	131 (47.64)

AJCC = American Joint Committee on Cancer; APR = abdominoperineal resection; CEA = carcinoembryonic antigen

Table 1. The results of univariate analysis of potential prognostic variables are shown in Table 2.

The majority of the patients were older than 60 years. The most common tumor location was in the colon (58.9%). One-hundred ninety-four patients (70.65%) had T3 tumor. The average number of lymph nodes harvested was 20.1 nodes (18.9 to 21.3 nodes). Fifty-three percent of patients had negative nodal status. Most of our patients (46.5%) had stage III cancer according to the American Joint Committee on Cancer

Table 2. Incidence of positive cytology and correlation analysis

Variable	Positive cytology (n = 6)	95% confidence interval	Negative cytology (n = 269)	95% confidence interval	*p*-value
Age (year), n (%)		2.00 to 2.00		1.95 to 1.99	0.67
<40	0 (0.00)		8 (2.97)		
≥40	6 (100)		261 (97.03)		
Age (year), mean ± SD	67.33±15.77	50.78 to 83.88	64.93±12.05	63.48 to 66.38	0.63
Sex, n (%)		0.79 to 1.88		1.47 to 1.59	0.43
Male	4 (66.67)		127 (47.21)		
Female	2 (33.33)		142 (52.79)		
Site, n (%)		1.12 to 2.21		1.35 to 1.46	0.20
Colon	2 (33.33)		160 (59.48)		
Rectum	4 (66.67)		109 (40.52)		
Serosal invasion, n (%)		2.00 to 2.00		1.94 to 1.98	0.61
Present	0 (0.00)		11 (4.10)		
Absent	6 (100)		258 (95.91)		
T stage, n (%)		2.12 to 3.21		2.75 to 2.90	0.83
T1	0 (0.00)		10 (3.72)		
T2	2 (33.33)		48 (17.84)		
T3	4 (66.67)		190 (70.63)		
T4	0 (0.00)		20 (7.43)		
Unknown	0 (0.00)		1 (0.37)		
N stage, n (%)		1.34 to 2.66		1.59 to 1.77	0.04
N0	1 (16.67)		147 (54.65)		
N1	4 (66.67)		62 (23.10)		
N2	1 (16.67)		60 (22.30)		
M stage, n (%)		1.00 to 1.00		1.00 to 1.01	0.88
M0	6 (100)		268 (99.63)		
M1	0 (0.00)		1 (0.37)		
AJCC stage, n (%)		1.81 to 3.52		2.19 to 2.37	0.14
1	1 (16.67)		48 (17.84)		
2	0 (0.00)		98 (36.43)		
3	5 (83.33)		123 (45.72)		
Degree of differentiation, n (%)		0.74 to 1.60		1.02 to 1.10	0.08
Well or moderately	5 (83.33)		258 (95.91)		
Poorly	1 (16.67)		6 (2.23)		
Not reported	0 (0.00)		5 (1.86)		
Mucinous characteristics, n (%)		1.00 to 1.00		1.00 to 1.02	0.83
Present	6 (100)		267 (99.26)		
Absent	0 (0.00)		2 (0.74)		
Angiolymphatic invasion, n (%)		1.13 to 3.20		2.21 to 2.41	0.90
Present	2 (33.33)		67 (24.91)		
Absent	1 (16.67)		51 (18.96)		
Not reported	3 (50.00)		151 (56.13)		
Perineural invasion, n (%)		1.62 to 3.38		2.70 to 2.83	0.52
Present	1 (16.67)		17 (6.32)		
Absent	1 (16.67)		30 (11.15)		
Not reported	4 (66.67)		222 (82.53)		
Serum CEA, mean ± SD	17.41±26.99	-10.92 to 45.74	13.02±34.82	8.84 to 17.20	0.76

AJCC = American Joint Committee on Cancer; CEA = carcinoembryonic antigen

[AJCC] staging system.

The demographic variables including gender, age, and sex were not associated with peritoneal lavage cytology. In addition, tumor depth of penetration, tumor location, serosal invasion, AJCC stage, degree of differentiation, presence or absence of mucinous characteristics, presence or absence of angiolymphatic invasion, presence or absence of perineural invasion, and level of serum carcinoembryonic antigen [CEA] were not significantly related to positive peritoneal lavage cytology.

Lymph node involvement (overall 46.2%) was found to be significantly related to positive peritoneal lavage cytology. This association was statistically significant ($p = 0.04$).

Discussion

Peritoneal metastasis in colorectal cancer occurs in 25% to 35% of patients[16,17]. Mechanisms of peritoneal metastasis include a cascade of tumor cells shedding from primary tumor, cell adhesion to peritoneal surface, and eventually invasion into the peritoneum and lymphatic channels[18]. Peritoneal lavage is a useful tool for staging in gastric[3-8], pancreatic[9] and gynecologic malignancies[10,11]. It also has a prognostic significance[10-15]. However, the role of peritoneal cytology in colorectal cancer has not been well-established. Identifying patients who are at risk for developing peritoneal metastasis may help early detection and selection of appropriate treatment. It may also provide prognostic information for physicians.

Previous studies reported on the incidence of positive peritoneal lavage cytology (Table 3). The present study found a positive lavage cytology rate of 2.18%, which was comparable to other studies that used conventional cytology method (2.2% to 26.3%).

Numerous factors have been reported to be associated with positive peritoneal lavage (Table 4). Overall, factors most commonly associated with positive peritoneal lavage were depth of tumor invasion (three studies) and lymph node metastasis (two studies). In our study, the factor associated with positive peritoneal lavage was positive lymph nodes ($p = 0.04$).

The wide range of incidence of positive peritoneal lavage may reflect the difficulty in comparing studies with different methods of peritoneal lavage. The volume of lavage fluid used ranged from 50 to 2,000 ml. There was one study using Ringers Lactate solution

Table 3. Incidence of positive lavage cytology in studies using conventional cytology method, which included only patients underwent curative resection for colorectal cancer

Study	Year	n	Lavage fluid	Timing of specimen retrieval (relative to resection)	Incidence of positive cytology (%)
Wind et al.[19]	1999	88	50 ml 0.9% NaCl	Before	28.0
Bosch et al.[14]	2003	53	700 ml Ringers lactate	Before and after	18.8
Yamamoto et al.[2]	2003	189	50 ml 0.9% NaCl	Before	5.8
Kanellos et al.[10]	2006	110	100 ml 0.9% NaCl	Before	26.3
Katoh et al.[20]	2009	226	100 ml 0.9% NaCl	Before	14.6
Nishikawa et al.[21]	2009	410	200 ml 0.9% NaCl	Before	7.6
Noura et al.[22]	2009	697	100 ml 0.9% NaCl	Before	2.2
Present study	2013	275	100 ml 0.9% NaCl	Before	2.18

Table 4. Factors associated with positive peritoneal lavage

Study	Year	n	Incidence of positive cytology (%)	Factors associated with positive peritoneal lavage
Wind et al.[19]	1999	88	28.0	NS
Bosch et al.[14]	2003	53	25.0	NR
Yamamoto et al.[2]	2003	189	5.8	NR
Kanellos et al.[10]	2006	98	26.3	Depth of tumor invasion
Katoh et al.[20]	2009	226	14.6	Positive nodes, elevated preoperative CA 19-9
Nishikawa et al.[21]	2009	410	7.6	Non-R0 resection, depth of tumor invasion, peritoneal dissemination, high tumor grade
Noura et al.[22]	2009	697	2.2	Depth of tumor invasion, positive nodes, pathologic stage, lymphatic invasion, venous invasion
Present study	2013	275	2.18	Positive nodes

CA = carbohydrate antigen; NR = not reported; NS = not statistically significant

while others used 0.9% NaCl. In our study, we used conventional cytology methods. The advantages of this method are that it is inexpensive, does not require other complex techniques, and is reproducible in many institutions.

Conclusion

Our study estimated the incidence of positive free colorectal cancer cell in lavage fluid obtained before resection in patients that underwent curative intent resection for colorectal cancer. The only factor associated with positive peritoneal cytology was lymph node involvement. Patients with positive result should have long-term follow-up, to see whether this will impact with their local recurrence, peritoneal metastasis, and distant metastasis.

What is already known on this topic?

Positive peritoneal cytology in colorectal cancer patients will eventually associate with poor prognosis. Until now, peritoneal cytology in colorectal cancer was not routinely performed due to its low incidence.

What this study adds?

This study is, to the best of our knowledge, the first study of peritoneal cytology of colorectal cancer in Thai patients. We emphasized the small impact of its presence due to its low incidence.

Acknowledgement

The authors would like to thank Faculty of Medicine, Ramathibodi Hospital for their funding support of the present study.

Potential conflicts of interest

The authors declare no conflict of interest.

References

1. Guller U, Zajac P, Schnider A, Bosch B, Vorburger S, Zuber M, et al. Disseminated single tumor cells as detected by real-time quantitative polymerase chain reaction represent a prognostic factor in patients undergoing surgery for colorectal cancer. Ann Surg 2002;236:768-75.
2. Yamamoto S, Akasu T, Fujita S, Moriya Y. Long-term prognostic value of conventional peritoneal cytology after curative resection for colorectal carcinoma. Jpn J Clin Oncol 2003;33:33-7.
3. Bando E, Yonemura Y, Takeshita Y, Taniguchi K, Yasui T, Yoshimitsu Y, et al. Intraoperative lavage for cytological examination in 1,297 patients with gastric carcinoma. Am J Surg 1999;178:256-62.
4. Suzuki T, Ochiai T, Hayashi H, Nakajima K, Yasumoto A, Hishikawa E, et al. Importance of positive peritoneal lavage cytology findings in the stage grouping of gastric cancer. Surg Today 1999;29:111-5.
5. Hayes N, Wayman J, Wadehra V, Scott DJ, Raimes SA, Griffin SM. Peritoneal cytology in the surgical evaluation of gastric carcinoma. Br J Cancer 1999; 79:520-4.
6. Kodera Y, Yamamura Y, Shimizu Y, Torii A, Hirai T, Yasui K, et al. Peritoneal washing cytology: prognostic value of positive findings in patients with gastric carcinoma undergoing a potentially curative resection. J Surg Oncol 1999;72:60-4.
7. Suzuki T, Ochiai T, Hayashi H, Hori S, Shimada H, Isono K. Peritoneal lavage cytology findings as prognostic factor for gastric cancer. Semin Surg Oncol 1999;17:103-7.
8. Bentrem D, Wilton A, Mazumdar M, Brennan M, Coit D. The value of peritoneal cytology as a preoperative predictor in patients with gastric carcinoma undergoing a curative resection. Ann Surg Oncol 2005;12:347-53.
9. Merchant NB, Conlon KC, Saigo P, Dougherty E, Brennan MF. Positive peritoneal cytology predicts unresectability of pancreatic adenocarcinoma. J Am Coll Surg 1999;188:421-6.
10. Kanellos I, Demetriades H, Zintzaras E, Mandrali A, Mantzoros I, Betsis D. Incidence and prognostic value of positive peritoneal cytology in colorectal cancer. Dis Colon Rectum 2003;46:535-9.
11. Baskaranathan S, Philips J, McCredden P, Solomon MJ. Free colorectal cancer cells on the peritoneal surface: correlation with pathologic variables and survival. Dis Colon Rectum 2004;47:2076-9.
12. Horattas MC, Evasovich MR, Topham N. Colorectal carcinoma and the relationship of peritoneal cytology. Am J Surg 1997;174:334-7.
13. Solomon MJ, Egan M, Roberts RA, Philips J, Russell P. Incidence of free colorectal cancer cells on the peritoneal surface. Dis Colon Rectum 1997;40:1294-8.
14. Bosch B, Guller U, Schnider A, Maurer R, Harder F, Metzger U, et al. Perioperative detection of disseminated tumour cells is an independent prognostic factor in patients with colorectal cancer. Br J Surg 2003;90:882-8.
15. Huguet EL, Keeling NJ. Distilled water peritoneal lavage after colorectal cancer surgery. Dis Colon Rectum 2004;47:2114-9.

16. Gellad ZF, Provenzale D. Colorectal cancer: national and international perspective on the burden of disease and public health impact. Gastroenterology 2010;138:2177-90.
17. Brodsky JT, Cohen AM. Peritoneal seeding following potentially curative resection of colonic carcinoma: implications for adjuvant therapy. Dis Colon Rectum 1991;34:723-7.
18. Ceelen WP, Bracke ME. Peritoneal minimal residual disease in colorectal cancer: mechanisms, prevention, and treatment. Lancet Oncol 2009;10: 72-9.
19. Wind P, Norklinger B, Roger V, Kahlil A, Guin E, Parc R. Long-term prognostic value of positive peritoneal washing in colon cancer. Scand J Gastroenterol 1999;34:606-10.
20. Katoh H, Yamashita K, Sato T, Ozawa H, Nakamura T, Watanabe M. Prognostic significance of peritoneal tumour cells identified at surgery for colorectal cancer. Br J Surg 2009;96:769-77.
21. Nishikawa T, Watanabe T, Sunami E, Tsuno NH, Kitayama J, Nagawa H. Prognostic value of peritoneal cytology and the combination of peritoneal cytology and peritoneal dissemination in colorectal cancer. Dis Colon Rectum 2009;52:2016-21.
22. Noura S, Ohue M, Seki Y, Yano M, Ishikawa O, Kameyama M. Long-term prognostic value of conventional peritoneal lavage cytology in patients undergoing curative colorectal cancer resection. Dis Colon Rectum 2009;52:1312-20.