Incidence of positive exfoliative peritoneal lavage before and after tumor resection for colorectal cancer

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Background
Colorectal cancer (CRC) represents the seventh commonest cancer in Egypt. Early detection of peritoneal metastasis is a major challenge in CRC management. The aim of this study was to assess the incidence of positive malignant cells in peritoneal lavage before and after resection of CRC.
Patients and methods
This prospective study was conducted from May 2020 to June 2022 and included 50 patients who underwent colorectal tumor resection. Intraoperative peritoneal lavage before and after tumor resection was done to detect intraperitoneal free cancer cells by conventional cytology.
Results
Preresection cytology was positive in four (8%) patients and negative in 46 (92%) patients. Postresection cytology was positive in five (10%) patients and negative in 45 (90%) patients. Positive preresection cytology was significantly prevalent in cases with mucinous carcinoma ($P<0.031$) and in cases with positive lymph node metastases ($P<0.008$). Positive postresection cytology in originally negative preresection one was significantly prevalent in the younger age group ($P=0.046$). There was more incidence of change of negative preresection cytology to positive postresection cytology whenever the tumor was located in the rectum than in cases of left or right tumor location.
Conclusions
Positive intraperitoneal free cancer cells are a prognostic factor of recurrence for patients treated for CRC, and it may be used as a criterion for selecting patients for further management.

Keywords:
colorectal cancer, cytology, peritoneal, recurrence

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Background

Worldwide, colorectal cancer (CRC) causes more than 8% of all annual fatalities. It ranks as the third cancer-related death in women and the fourth in men [1]. Almost 1.2 million new cases of CRC are identified each year [2]. It accounts for 3.47% of male cancers and 3% of female cancers in Egypt, making it the seventh most prevalent cancer in Egypt [3].

Patients with advanced-stage CRC frequently get peritoneal metastases. It stands for the second-most typical CRC metastatic location after hepatic metastases [4]. The prognosis for individuals with metastatic CRC has improved owing to the development of new chemotherapeutic and targeted biologic therapies [5]. Currently, patients with CRC can choose between hyperthermic intraperitoneal chemotherapy and cytoreductive surgery, which is performed after determining whether the initial tumor can be surgically removed [6].

Peritoneal lavage fluid cytology is considered a useful method to early identify the cytologic result during surgery and take the decision of intraperitoneal chemotherapy immediately after resection of the tumor [7]. Prevention of peritoneal recurrence with early intraoperative chemotherapy is a reasonable alternative treatment modality.

Therefore, this study was conducted to assess the incidence of positive malignant cells in peritoneal lavage preresection and postresection of CRC to detect high-risk patients for peritoneal metastases and for further treatment.

Patients and methods

This prospective pilot study was conducted at the Department of General Surgery, Menoufia University hospitals, from May 2020 to June 2022 and included 50 patients who underwent colorectal

tumor resection. Patients who had peritoneal carcinomatosis, ascites, or clinically evident metastatic disease were excluded. All participants provided their informed consent, and the study was registered and authorized by the regional ethics council of the Menoufia University Faculty of Medicine (approval number: 42020SURG). Before surgery, all patients underwent a thorough clinical examination, the necessary laboratory testing (complete blood count, bleeding profile, liver function tests, renal function tests, and tumor markers including CEA and CA 19-9), and imaging studies as needed (computed tomography on chest, abdomen, and pelvis and MRI on abdomen and pelvis for patients with rectal cancer).

Surgical procedure

The patient was placed in the supine position, the abdomen was opened, and just before manipulating the tumor, 100 ml of physiological saline solution (37°C) was injected into the peritoneal cavity surrounding the tumor location. The patient was then placed into the Trendelenburg position. After gentle stirring and with the patient in the anti-Trendelenburg posture, two to three 50-ml syringes were used to drain the fluid accumulation at the Douglas Pouch. The procedure was repeated after colorectal resection. Preoperative and postoperative samples were collected in two labeled syringes that were sent to the pathological examination for processing. Each sample was subjected to centrifugation. The supernatant was smeared on several slides and immediately fixed in a jar containing absolute alcohol (100%). The pellet was put in gauze for preparation of cell block. The gauze containing the sediment of the fluid was fixed in formalin 10% for a night, then was subjected to dehydration by immersion in jars with ascending grades of alcohol from 70% passing to 95% till absolute alcohol (100%), and then it was immersed in xylene and hot paraffin where a cell block was ready for cutting. Several sections were cut from the prepared cell block by a microtome, 5-μm thickness, and loaded on slides. Finally, the prepared smears as well as the prepared cell block were subjected to hematoxylin and eosin staining.

Statistical analysis

The data analysis was conducted using SPSS (statistical package for social science; IBM, Chicago, Illinois, USA) program, version 13 for Windows. Descriptive statistics were used in which qualitative data were presented in the form numbers and percentages and quantitative data were presented in the form of SD, mean, and range. Statistical significance was demonstrated for results ($P<0.05$) using Student *t* test. χ^2 test, Fisher exact test, and post-hoc test were used to study the association between two qualitative variables.

Results

A total of 50 patients were included in this study, comprising 22 (44%) males and 28 (56%) females, with a median age of 55.5 years. Regarding surgical procedure, 37 (74%) patients had open technique and 13 (26%) patients had laparoscopic approach. A total of 25 (50%) patients had right colon cancer, 15 (30%) patients had left colon cancer, and 10 (20%) patients had rectal cancer. Preresection cytology was positive in four (8%) patients and negative in 46 (92%) patients. Postresection cytology was positive in five (10%) patients and negative in 45 (90%) patients. Generally, six (12%) patients had positive cytology and 44 (88%) patients had negative cytology. According to T status, two (4%) patients were pT1, nine (18%) were pT2, 35 (70%) were pT3, and four (8%) were pT4. Regarding lymph nodes status, 19 (38%) patients were positive lymph nodes to malignancy and 31 (62%) patients were negative lymph nodes. The overall tumor stage was as follows: 10 (20%) patients were stage I, 20 (40%) were stage II and 20 (40%) were stage III. Pathological examination revealed that well-differentiated and moderate-differentiated adenocarcinoma had been encountered in 43 (86%) patients, in comparison with seven (14%) patients who had mucinous carcinoma (Table 1).

Cytological interpretation

All slides were reviewed and diagnosed by an experienced pathologist with a specialization in gastrointestinal oncology. Fluids were cytologically diagnosed as 'negative for malignancy,' 'suspicious for malignancy,' or 'positive for malignancy' based on the standard cyto-morphologic criteria for diagnosis of serous effusion according to Koss and Melamed (Koss Diagnostic Cytology and Its Histopathologic Bases) [8]. Figures 1–3 show different results under a microscope.

The included patients within this study were divided into two groups according to peritoneal lavage fluid conventional cytology performed immediately after opening abdomen and before manipulation or resection tumor. Group I with positive cytology from start (positive preresection cytology) (four patients) and group II with negative cytology (negative preresection cytology) (46 patients). On comparison between the two groups, negative

Table 1 Sociodemographic characteristics, operative data, and pathological data of the participants (*N*=50)

Variables	*n* (%)
Sex	
Male	22 (44.0)
Female	28 (56.0)
Age (years)	
Range	28–86
Median	55.50
Mean±SD	53.40±13.915
Age groups (classified according to median)	
<55.5 years	25 (50.0)
>55.5 years	25 (50.0)
Operative data	
Surgical procedure	
Open	37 (74.0)
Laparoscopic	13 (26.0)
Tumor resection part	
Right colon	25 (50.0)
Left colon	15 (30.0)
Rectum	10 (20.0)
Pathological data	
Preresection cytology	
Positive	4 (8.0)
Negative	46 (92.0)
Postresection cytology	
Positive	5 (10.0)
Negative	45 (90.0)
Total cytology	
Positive	6 (12.0)
Negative	44 (88.0)
T classification	
T1	2 (4.0)
T2	9 (18.0)
T3	35 (70.0)
T4	4 (8.0)
N staging	
Positive	19 (38.0)
Negative	31 (62.0)
Tumor type	
Adenocarcinoma	43 (86.0)
Mucinous	7 (14.0)

preoperative cytology was highly statistically significant less than positive cytology ($P<0.001$). Regarding pathological tumor criteria, positive preresection cytology was significantly prevalent in cases with mucinous carcinoma ($P<0.031$) and in cases with positive lymph node metastases ($P<0.008$). On the contrary, T stage did not show statistically significant difference between the two groups ($P=1.00$). Regarding sociodemographic criteria, operative procedure (open or laparoscopic), and tumor location and resection type, there was no statistically significant difference between the two groups (Table 2).

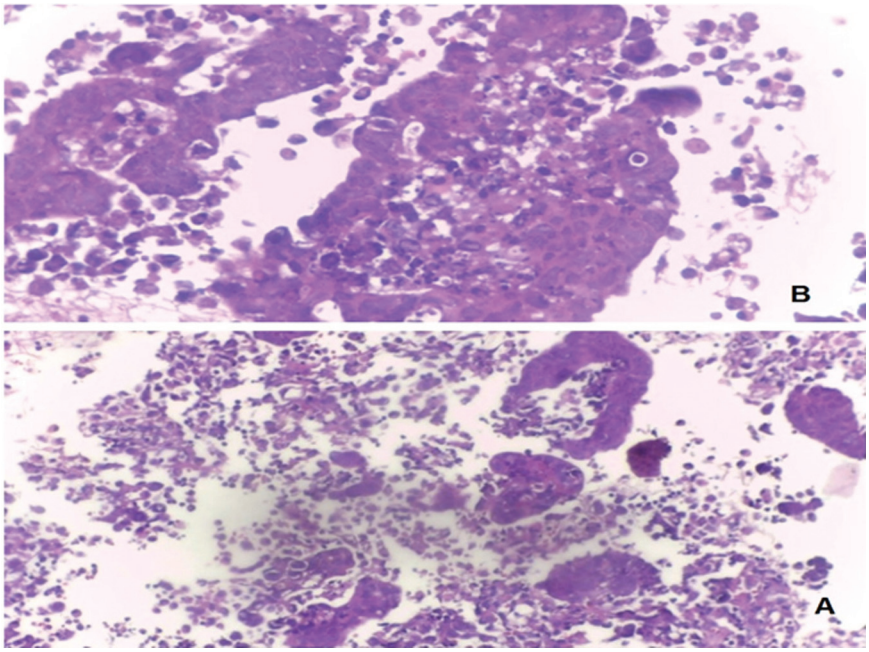
All patients have been subjected to postresection cytology that has been cytologically examined for malignant cells. All the patients in group I with positive preresection cytology had positive postresection cytology except one case where the postresection cytology was negative for malignant cells. Patients with negative preresection cytology (group II) (46 patients) were further subdivided into two groups according to the status of postresection cytology: group IIA (two patients) with positive postresection cytology and group IIB (44 patients) with negative one. Comparison between the two groups showed significantly less prevalence of positive postresection cytology in cases of negative preresection cytology ($P=0.001$). Positive postresection cytology in originally negative preresection one was significantly prevalent in the younger age group. However, sex did not show significant difference between the two groups. However, there was more incidence of change of negative preresection cytology to positive postresection whenever the tumor was located in the rectum than in cases of left or right tumor location. Difference was statistically significant, with P value of 0.033. A comparison between both groups regarding nodal status showed significant prevalence of positive postresection cytology in cases of positive nodal metastases ($P=0.038$). Although there was more prevalence of higher (T) status in group IIA, with T3 and T4 more than T1 and T2, compared with group IIB, this difference did not reach the statistical power of significance, with P value of 0.264. There was no statistically significant difference between the two groups regarding type of tumor (adenocarcinoma or mucinous) ($P=0.614$). Regarding operative procedures (open or laparoscopic), there was no statistically significant difference between the two groups (Table 3).

Discussion

In the current study, intraperitoneal exfoliated cancer cells were examined in patients with CRC by intraoperative lavage cytology before and after tumor resection. The conventional cytology is the most popular method because it is relatively inexpensive and requires neither the preservation of RNA nor the implementation of a complex technique [9]. The specificity and sensitivity of tumor cells detected as positive in peritoneal lavage fluid were 83–100% [10,11], and 86%, respectively [12].

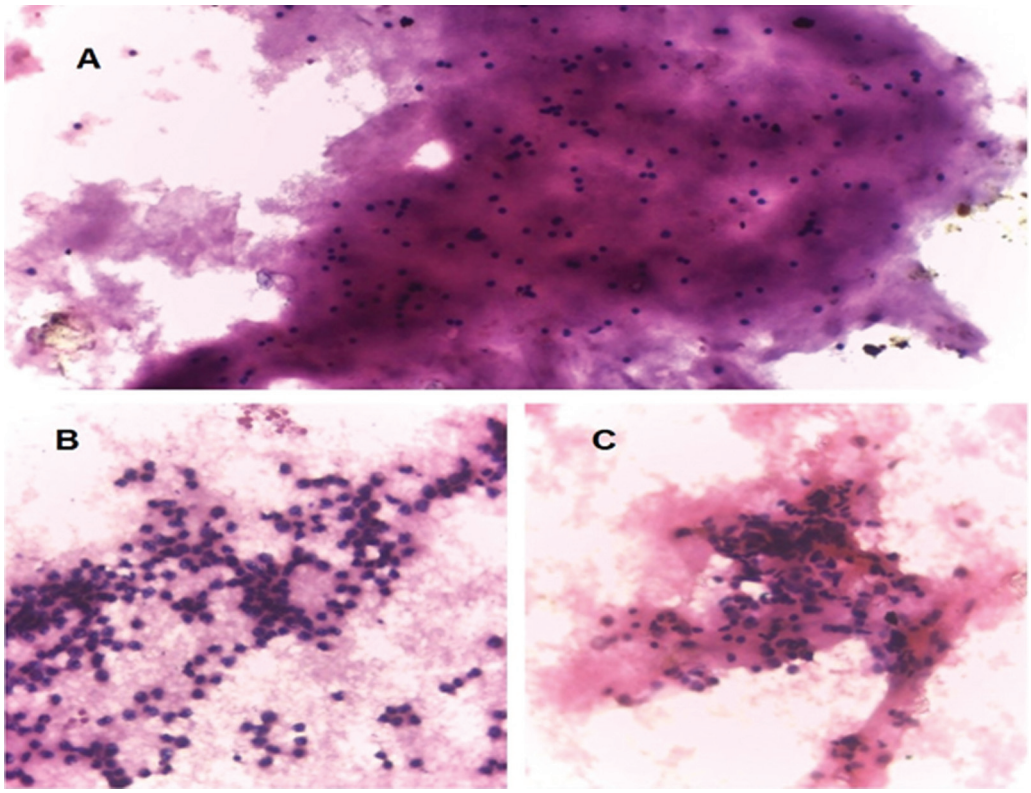
This study found 12% of peritoneal fluid lavages with positive cytology, 8% with positive preresection lavages, and 4.4% with positive postresection lavages with cytology that was initially negative. According to

Figure 1



(a) Prepared smear shows malignant epithelial cells arranged in sheets, occasional acini and individually (H&E, ×200), and (b) higher power view demonstrating sheets of malignant epithelial cells.

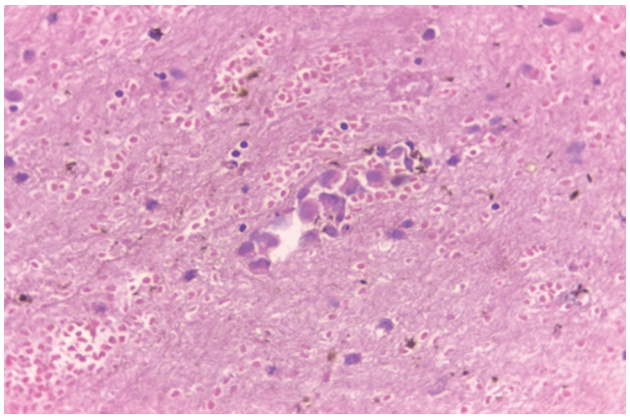
Figure 2



(a) Smear revealed potential material included inflammatory cells, that is, lymphocytes and neutrophils (H&E, ×400), (b) inflammatory cells mainly neutrophils and pus cells, and (c) inflammatory cells, mainly lymphocytes.

earlier research, the incidence ranged from 2.1 to 52% in patients with colon cancer and positive cytology [13,14], from 3.7 to 13.3% in preresection and from 13.3 to 26.7% in postresection washings [15]. The disparity in detection rates of positive cytology can be attributed to the variety of methods employed to identify cancerous cells in peritoneal lavage fluid [13,16].

Figure 3



Prepared smear shows mesothelial cells manifested by eccentric nuclei and vacuolated cytoplasm (H&E, ×400).

The most common mechanisms of metastasis in large bowel are lymphatic spread to regional lymph nodes and hematogenous spread to the liver via the portal vein, and these are thought to occur owing to undetected local, peritoneal, lymphatic, or hematogenous micrometastases present before surgical resection [14]. The presence of malignant cells in the preresection washings may be owing to the fact that the tumor had invaded through the full thickness of the bowel wall and involved the serosa. However, the origin of malignant cells in patients with only postresection positive washings is less certain. The source of these tumor cells may include severed lymphatic vessels, disrupted tissue interstices at the lateral margins of tumor dissection, venous blood that is heavily contaminated with tumor cells, or cancer cell spillage by surgical manipulation [14]. These findings are matched with the results of the current study, which has shown that there was a statistically significant prevalence of positive cytology

Table 2 Comparison between the two studied groups of the participants regarding sociodemographic characteristics, the total cytology results, operative data, and the pathological data of the tumor

Variables	Group I (*N*=4) [*n* (%)]	Group I (*N*=46) [*n* (%)]	Test of significance	*P* value
Sex				
Male	2 (50.0)	20 (43.5)	FE=1.000	0.801
Female	2 (50.0)	26 (56.5)		
Age				
Range	35–72	28–86	*t*=0.874	0.386
Mean±SD	59.25±16.500	52.89±13.762		
Cytology				
Positive	4 (100.0)	2 (4.3)	FE=0.000065	<0.001**
Negative	0	44 (95.7)		
Surgical procedure				
Open	4 (100.0)	33 (71.7)	FE=0.561	0.216
Laparoscopic	0	13 (28.3)		
Tumor resection part				
Right colon	2 (50.0)	23 (50.0)	χ^2=0.956	1.000
Left colon	1 (25.0)	14 (30.4)		
Rectum	1 (25.0)	9 (19.6)		
T classification				
T1	0	2 (4.3)		
T2	1 (25.0)	8 (17.4)	χ^2=0.884	1.000
T3	3 (75.0)	34 (69.6)		
T4	0	4 (8.7)		
N staging				
Positive	4 (100.0)	15 (32.6)	FE=0.017	0.008*
Negative	0	31 (67.4)		
Tumor type				
Adenocarcinoma	2 (50.0)	41 (89.1)	FE=0.089	0.031*
Mucinous	2 (50.0)	5 (10.9)		

Group I: positive cytology from the start. Group II: negative cytology from the start. FE, Fisher exact test; *t*, Student *t* test; χ^2, χ^2 test.
**Highly statistically significant. *Statistically significant.

Table 3 Comparison between the participants regarding the preresection and postresection cytology results, sociodemographic characteristics, operative data, and pathological data of the tumor (*N*=46)

Variables	Group IIA: negative pre and positive post (*N*=2) [*n* %)]	Group IIB: negative pre and positive post (*N*=44) [*n* (%)]	Test of significance	*P* value	Post-hoc test
Postresection cytology					
Positive	2 (100.0)	0	FE=0.000966	0.001**	
Negative	0	44 (100.0)			
Sex					
Male	1 (50.0)	19 (43.2)	FE=1.00	0.849	
Female	1 (50.0)	25 (56.8)			
Age					
Range	28–40	33–86	*t*=2.055	0.046*	
Mean±SD	34.00±8.485	53.75±13.385			
Surgical procedure					
Open	2 (100.0)	31 (70.5)	FE=1.00	0.364	
Laparoscopic	0	13 (29.5)			
Tumor resection part					
Right colon	0	23 (52.3)	χ^2=8.596	0.033*	*P*1=0.01*
Left colon	0	14 (31.8)			*P*2=0.064
Rectum	2 (100.0)	7 (15.9)			*P*3=0.999
T classification					
T1	0	2 (4.5)			
T2	0	8 (18.2)	χ^2=4.672	0.264	
T3	1 (50.0)	31 (70.5)			
T4	1 (50.0)	3 (6.8)			
N staging					
Positive	2 (100.0)	13 (29.5)	FE=0.101	0.038*	
Negative	0	31 (70.5)			
Tumor type					
Adenocarcinoma	2 (100.0)	39 (88.6)	FE=1.000	0.614	
Mucinous	0	5 (11.4)			

FE, Fisher exact test; *t*, Student *t* test; χ^2, χ^2 test. *P*1 between rectum and right colon. *P*2 between rectum and left colon. *P*3 between right colon and left colon. **Highly statistically significant less than 0.001. *Statistically significant.

(either before or after resection) in case of positive lymph nodal metastases.

The pathologic TNM stage, particularly T stage, is one of the important criteria that greatly influences the prognosis of CRC. Similar investigations have shown that the deeper the intestinal wall invasion (pT3/4), the more advanced the disease, and the higher the incidence of tumor-positive peritoneal lavage [17,18]. The current study showed that the majority of patients with positive preresection cytology were T3 75%, whereas T2 was present in 25%. Moreover, patients who had preresection negative cytology and were converted to positive postresection cytology were only T3/T4. However, the difference could not reach a statistically significant level. This can be attributed to the small number of included cases.

Studies have shown that the incidence of malignant cells in peritoneal lavage before tumor resection was higher in colon cancer than rectal cancer. The authors of these investigations have linked this to the high occurrence of implants in the ileocecal region, which is fixed to the retroperitoneum, and organs with peritoneal fluid resorption (omentum and omental appendages) [19].

In the current study, tumor location did not show significant effect on the detection of positive preresection cytology. However, positive postresection cytology in cases of negative preresection one was significantly more prevalent in cases of rectal cancer. This could be explained by inclusion of originally extraperitoneal rectal tumor into the peritoneal cavity during resection, hence, more liability for tumor cell spillage within the surgically opened peritoneal space.Studies have documented that mucinous subtype of CRC is associated with a higher incidence of free intraperitoneal free cancer cell [20–22]. This finding matches the results of the current study, which revealed that patients with the mucinous histologic variant of CRC were significantly associated with higher incidence of free intraperitoneal free cancer cell in

the preresection cytology. This can be attributed to that this tumor variant tends to have higher ratio of lymph node infiltration [21,22]. Moreover, mucinous CRCs are more frequently diagnosed when they are already in advanced stages especially in younger patients. This explains their preferential tendency to metastasize to peritoneum [20].

Conclusion

Adding to the existing facts, the use of lavage cytology, along with other prognostic factors, will identify high-risk patients for recurrence like positive lymph nodes for malignancy or mucinous carcinoma and for further management, even after curative surgery. Moreover, the site of cancer infiltration of the serosa should be covered during the operation to prevent the exfoliation of tumor cells, and it is necessary to exercise care to prevent injury to the bowel by surgical manipulations.

Authors' contributions

Surgical practices: S.A.E., M.A.A.B, M.M.A., and O. S.A.E. Concept: S.A.E, M.A.A.B, M.M.A., O.S.A. E., and A.G.A. Pathological report: A.G.A. Design: M.M.A., O.S.A.E, and A.G.A. Data collection or processing: M.M.A. and O.S.A.E. Analysis or interpretation: M.M.A., O.S.A.E., and A.G.A. Writing and revision: S.A.E., M.A.A.B., M.M.A., and O.S.A.E.

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Conflicts of interest

There are no conflicts of interest.

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