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Positive Peritoneal Lavage Cytopathology: Prevalence and Relation to Clinico-Pathological Characteristics of Colonic Adenocarcinoma: A Cohort Study

Mohamed A. Abousalama [a*], Ahmed I. Swelam [a], Hamdy S. Abdullah [a] and Osama H. Elkhadrawy [a]

[a] Department of General Surgery, Faculty Of Medicine, Tanta University, Tanta, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Positive peritoneal lavage cytopathology (PPLC) is an established poor prognostic factor in CRC that has been proven in many studies and was associated with an increase in peritoneal recurrence , overall recurrence and mortality. The incidence of PPLC among CRC patients varied in different studies between 2.1-52%. Similarly, different variables were reported to correlate with peritoneal lavage positivity in published studies. The aim of this trial was to study the prevalence of free intraperitoneal malignant cells at the time of radical resection of colon cancer and to assess its relation to different clinico-pathological characteristics.

Patients and methods: This cohort study was conducted at the gastrointestinal surgery unit, general surgery department and the emergency hospital at Tanta University during the period from January 2020 ending in December 2020. Forty patients with non-metastatic primary colonic carcinoma were included in the study. Peritoneal lavage fluid was collected before radical resection of the tumour. One hundred milliliters of warm normal saline solution was installed into the peritoneal cavity and at least 80 ml of the lavage fluid was collected for cytopathological examination. After preparation, the sample was stained with Haematoxylin and Eosin and examined under a light microscope by an experienced cytopathologist.

**Corresponding author: E-mail: mohamed.abdelreheem@med.tanta.edu.eg, Muhamedabdelrahimmd@gmail.com, mohamed146443_pg@med.tanta.edu.eg;*

Results: Eighteen smears (45%) were found positive for malignant cells. Among variables tested, a positive correlation was found between PPLC and a low BMI (< 25 kg/m^2), the histopathological tumour type, an advanced pT stage, a high lymph node ratio and an advanced overall TNM stage.
Conclusion: In non-metastatic AJCC stage II and III colon cancer, the prevalence of PPLC detected by conventional cytopathology was found to be 45%. A low BMI <25kg/m^2 , the presence of intraperitoneal free fluid at the time of the operation , a high lymph node ratio, an advanced T stage, histopathological type and an advanced overall TNM stage correlated positively with PPLC.

Keywords: Peritoneal carcinomatosis; colorectal cancer; free intraperitoneal cancer cells; peritoneal lavage cytopathology.

CRC : colorectal cancer
PM : peritoneal metastases
CA19.9 : Cancer Antigen 19,9
CEA : carcinoembryonic antige
PC : peritoneal carcinomatosis
PLC : peritoneal lavage cytopathology
PPLC : positive peritoneal lavage cytopathology
NPLC : negative peritoneal lavage cytopathology

1. INTRODUCTION

Colorectal cancer (CRC) is a leading cause of cancer-related death, with peritoneal recurrence occurring in 25-35% of patients [1]. Currently, patient selection for adjuvant therapy in CRC does not include routine evaluation of peritoneal lavage fluid for tumour cells [2].

Peritoneal lavage cytology (PLC) has been considered to be a useful tool for predicting an individual prognosis of some malignancies. *Keettel and Elkins* [3] introduced the technique of intraoperative peritoneal lavage cytology in ovarian cancer patients for the first time **.** The presence of free malignant cells in the peritoneal fluid in non-gynecological cancers, especially gastric, colorectal and pancreatic adenocarcinomas, is associated with a poor survival and peritoneal recurrence [4-6]. In a prospective study, Noura et al, [7] showed an improvement in peritoneal recurrence and overall survival in patients with positive lavage who underwent intraperitoneal chemotherapy with mitomycin C compared to those who did not**.**

Although free cancer cells can be detected in peritoneal fluid at the time of surgery, peritoneal lavage is not used in routine CRC surgery [8]. Several studies, including systematic reviews and meta-analyses, had proven that PPLC is a poor prognostic factor associated with a higher mortality, peritoneal recurrence and overall recurrence in CRC [5, 7, 9].

Testing for free intraperitoneal malignant cells involves examining peritoneal lavage for malignant cells or markers of such cells, using a variety of laboratory methods. Techniques used to date include histological examination (cytopathology), immunocytochemistry and molecular techniques polymerase chain reaction (PCR) [10]. The incidence of PPLC among COLONIC ADENOCARCINOMA patients varied in different studies between 2.1-52%. Similarly, different variables were reported to correlate with peritoneal lavage positivity in published studies. Factors most commonly associated with PPLC included the depth of tumour invasion (T stage) and the presence of metastases to lymph node, liver or the peritoneum. Controversial factors less commonly reported include lymphatic invasion, venous invasion, tumour stage, tumour grade, tumour necrosis, gross picture of the tumour, and elevated preoperative CA 19.9. No significant association was found between the age, gender or tumor site [5, 11]. The aim of this trial was to study the prevalence of free intraperitoneal malignant cells at the time of radical resection of colon cancer and to assess its relation to different clinico-pathological characteristics.

2. PATIENT AND METHODS

This cohort study was conducted on 40 patients with colonic carcinoma at the Gastrointestinal Surgery Unit, General Surgery Department and the Emergency Hospital at Tanta University during the period from January 2020 ending in December 2020.

2.1 Inclusion Criteria

Adult patients with primary non-metastatic colonic carcinoma eligible for radical resection,

either emergency or elective, were included in the study.

2.2 Exclusion Criteria

Patients with any of the followings were excluded from the study:

- Evidence of distant metastasis,
- Evidence of malignant ascites,
- Perforated colonic carcinoma,
- Recurrent colonic carcinoma,
- History of receiving neoadjuvant chemotherapy and/or radiotherapy for malignancy including cancer rectum,
- History of previous abdominal malignancy.

2.3 Technique of Peritoneal Lavage and Specimen Preparation

In the current study, peritoneal lavage was performed immediately after gaining access to the abdominal cavity through laparotomy incision and before touching the tumour. One hundred milliliter (ml) of warm normal saline solution (temp 37^{o}C) were installed into the peritoneal cavity including the tumor area with the table in the flat position. After gentle hand stirring, as much as possible of this fluid was collected from dependent areas at the Douglas and Morrison pouches. A minimum of 80 ml (80% retrieval) of the lavage fluid was adequate for analysis. If ascites was present, ascitic fluid was aspirated instead of performing a lavage. The specimen was immediately delivered to the pathology department for cytopathological examination**.** Once received by the pathology department, the sample was centrifuged in Cytospin 2® (Shanton; Athens, Greece; 1200 rotations/min) for 10 minutes. The cell pellet was then smeared on slides, fixed with cytospray, stained with Haematoxylin and Eosin stain (H&E) and examined under a light microscope by an experienced cytopathologist who was blinded to the clinical data. Smears were classified according to their cytopathological features, as follows:

1. *Positive for malignant cells (PPLC)*: malignant cells were recognized under light microscope using standard criteria **,** namely by presence of large, hyperchromatic nuclei, coarse granular nuclear chromatin, prominent nucleolus, distorted nuclear outline, increased nuclear/cytoplasmic ratio, intracytoplasmic mucin vacuoles, or heterogeneity of cells with clumping of groups. Cells are mainly arranged in loose clusters with the occasional presence of floating cells**.**
2. *Negative for malignant cells (NPLC):* normal cells present or cells showing only milder changes of chromatin. Cytopathological findings were graded from class I to V according to The International System (TIS) for reporting serous effusion cytopathology [12].

2.4 End Points

The primary endpoint was to study the prevalence of PPLC and its relation to the different clinico-pathological characteristic in selected colonic carcinoma patients.

2.5 Statistical Analysis

Collected data were tabulated and analyzed statistically using the appropriate tests, including the mean, standard deviation and were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using the number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The significance of the obtained results was judged at the 5% level. The used tests were:

1. Chi-square test for categorical variables, to compare between different groups.
2. Fisher's Exact or Monte Carlo correction for Chi-square when more than 20% of the cells have expected count less than 5.
3. Student t-test for normally distributed quantitative variables, to compare between two studied groups

3. RESULTS

3.1 Clinical Data

There were 18 males (45%) and 22 females (55%). The patients' ages ranged between 29 and 85 years with a median of 56.0 (49.0–65.0) years. The body mass index (BMI) ranged between 23 kg/m2 and 45 kg/m2 with a median of 30.0 kg/m2.Five patients (12.5%) had positive family history of malignancy and 2 of them had positive family history of CRC. Carcinoembryonic antigen (CEA) was elevated in 16 patients (40%) while Cancer antigen (CA19.9) was elevated in 14 patients (35%). All operations were performed

via open approach. Thirteen patients (32.5%) had malignant large bowel obstruction and were operated in the emergency hospital, while the remaining 27 patients (67.5%) had elective surgery Table 1.

3.2 Pathological Data

- ***Location of the tumor:*** 14 patients (35%) had their tumors located in the right side and 24 patients (60%) in the left side. Only one patient had his tumour located at the transverse colon (2.5%) while another patient had synchronous lesions at the ascending colon and splenic flexure (2.5%).
- ***Size of the tumours*****:** the maximunm tumor size ranged from 1 cm to 14 cm with a median size of 5.0 cm (4.0-7.50).
- ***Morphologic types:*** 14 patients (35%) had fungating mass, 12 patients (30%) had malignant ulcer and 8 patients (20%) had circumferential (annular) lesions and other 6 patients (15%) had mixed gross patterns.
- ***Histopathological types:*** conventional adenocarcinoma was found in 26 patients (65%), adenocarcinoma with mucoid differentiation in 10 patients (25%), adenocarcinoma with signet ring differentiation in 3 specimens (7.5%) and one patient had an adenocarcinoma with neuroendocrine differentiation.
- ***Tumour grade*****:** 18 patients (45%) had tumours with poor differentiation (grade III) and 22 patients (55%) had tumours of moderate differentiation (grade II).
- ***pT stage***: 27 patients (67.5%) had pT3 tumours, 9 patients (22.5%) had pT4 tumours and 4 patients (10%) had pT2 tumours.
- ***pN stage:*** the number of harvested LNs ranged from 14 to 31 with a median number of 17 lymph nodes. Sixteen cases (40%) has no LN metastasis (N0 stage**),** 10 patients (25%) had N1 nodal status, while 14 patients (35%) had N2 nodal status.
- ***The overall TNM stage:*** 16 patients (40%) had stage II tumours, 24 patients (60%) had stage III tumours Table (2).

3.3 Peritoneal Fluid Analysis

Variable amounts of intraperitoneal free fluid were found in the peritoneal cavity in 7 patients (17.5%) which weren't detectable preoperatively (no peritoneal lesions suspicious for metastases were detected during laparotomy). This fluid was aspirated and analyzed. Six out of these 7 patients (85.7%) had PPLC. The presence of intraperitoneal free fluid was found statistically significant with the positivity of peritoneal fluid for cancer cells in both univariate and multivariate analyses ($p<0.017$). Peritoneal lavage using normal saline was performed in the remaining 33 patients, which revealed PPLC in 12 (36.5%). Collectively, 18 smears (45%) were found positive for the presence of free malignant cells in the peritoneal fluid on cytopathologic examination (PPLC) while the remaining 22 specimens (55%) had no free malignant cells in the peritoneal fluid (NPLC) (Fig 1).

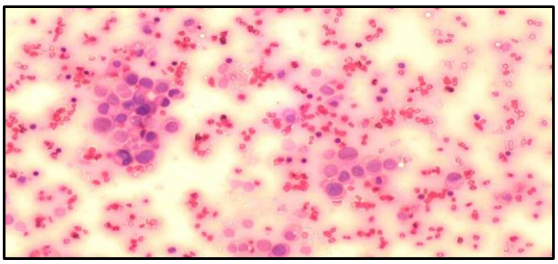

Fig. 1. Positive cytopathology. The cells are large columnar in shape, with moderate amount of vacuolated cytoplasm and have round, hyperchromatic nuclei with coarse chromatin (Low magnification - H&E stain)

3.3.1 Relation between peritoneal lavage cytopathology and different clinicopathological variables (Table 3, 4):

Comparison between patients who had PPLC and those with NPLC showed no statistically significant differences between the 2 groups regarding: gender, positive family history of CRC, clinical presentation, elevated preoperative tumor marker (CEA, CA19.9), tumor size, tumor site, morphologic type, tumour grade or lymph node involvements (pN stage).

On the other hand, comparison between both groups showed a statistically significant differences regarding the BMI, histopathological type, pT stage, the lymph node ratio and the overall TNM stage.

A. Relation between peritoneal lavage cytopathology and different clinical variables (Table 3):

(1) ***Gender:*** PPLC was found in 11 out of 18 male patients (61%), while 7 out of 22

females (31.8%) in our study were found to have positive result (38.9%). No statistically significant difference was found between both gender (p< 0.064).

(2) ***Family history of malignancy:*** Among 5 patients with positive family history of malignancy, 4 patients (80%) including the 2 patients with positive family history of CRC had PPLC and 1 patient (20%) had NPLC, no statistically significant difference was found (p<0.155).

(3) ***BMI:*** PPLC was found in 5 out of 6 patients (83.3%) with average BMI < 25kg/m^2. On the other hand, NPLC smears were found in 15 out 21 obese patients (71.4%) BMI >30 kg/m2, while PPLC was found in 7 out of 13 overweight patients. The difference in incidence of PPLC among the patients with different BMI was found statistically significant (P<0.044), with increased PPLC in patients with BMI <25kg/m2.

(4) ***Clinical presentation:*** Among the 27 patients with elective presentations, 11 patients (40.8%) had PPLC. On the other hand, 7 out of the 13 patients (59.3%) presented to the emergency department with intestinal obstruction had PPLC.

Of all 18 patients with PPLC, 11 patients (61.1%) presented as elective cases with iron deficiency anemia, change in bowel habits, bleeding per rectum, unexplained weight loss while the remaining 7 patients (38.9%) presented with intestinal obstruction. There was no statistically significant difference between both presentations (p< 0.435).

(5) ***Tumor markers:***

CEA**:** Sixteen patients had elevated serum CEA. Ten patients (62.5%) out of these 16 had PPLC while 6 patients (37.5%) had NPLC with no statistically significant difference (p< 0.069).

CA19.9: Fourteen patients were found to have elevated serum CA19.9 levels, 9 patients out of them (55.6%) had PPLC, while 5 (45.4%) patients had NPLC with no statistically significant difference (p< 0.072).

B. Relation between peritoneal lavage cytopathology and different pathological variables (Table 4):

(1) ***Tumour size:*** The median size of specimens in patients with PPLC was 6.0 cm while it was 5.0 cm in patients with NPLC. The difference in the tumour size between patients with PPLC and those with NPLC didn't attain statistical significance (p< 0.106).

(2) ***The morphologic type of the tumour:*** Among different morphologic patterns, the fungating growth pattern had a greater positivity of peritoneal lavage (8 out 14 patients (57.2%). On the other hand, 4 out 12 patients with ulcerating tumours (33.3%) had PPLC, while 4 out 8 patients with circumferential lesions (50%) had PPLC and 2 out of the 6 patients with a mixed growth pattern (33.3%) had PPLC. The difference in incidence of PPLC among the 4 growth patterns was found statistically insignificant (p<0.626).

(3) ***The Histopathological type of the tumor:*** Seven out of 10 peritoneal specimens (70%) in patients with mucoid adenocarcinoma were PPLC, while 2 out of 3 specimens (66.6%) of patients with signet ring carcinoma were found to have PPLC. On the other hand, 8 out 26 patients with adenocarcinoma (30.8%) had PPLC, while the patient with neuroendocrine differentiation had PPLC. The difference in incidence of PPLC among these 4 different histolopathological types was found statistically significant (p<0.047). The more aggressive the tumour histopathologically is, the higher the possibility of PPLC.

(4) ***Tumour grade:*** Eleven out of 18 patients (61.1%) with poorly differentiated adenocarcinomas (Grade 3) had PPLC, while 7 out of 22 patients (31.8 %) with moderately differentiated tumours (Grade 2) had a lower probability of PPLC. Although the possibility of PPLC increased with decreased tumour differentiation, the difference in incidence of PPLC between these 2 different grades didn't achieve statistical significance (p<0.064).

(5) ***pT-stage:*** Increasing the depth of invasion of malignant cell into the wall of the colon was associated with a higher possibility of PPLC. The four pT2 tumours had a NPLC. In pT3 tumours, on the other hand, 11 (40.8%) out of 27 specimens had a PPLC, while in pT4 tumours, 7 out of 9 specimens (77.8%) with were found to have a PPLC. The difference among the 3 categories regarding the positivity of specimen was found statistically significant (p< 0.025).

(6) ***pN- stage:*** Four out of 16 patients (25%) with no LN metastasis (pN0) had PPLC. Six out of 10 patients (60%) with pN1 had PPLC, while, 8 out of 14 patients (57.1%) with N2 had PPLC. The difference among the 3 categories regarding the positivity of peritoneal lavage was found statistically insignificant (p< 0.115).

(7) ***Lymph node ratio:*** In patients with PPLC, the range of lymph node ratio was 0-95.83% with a mean of 27.80 ± 23.55 (median 27.8). In patients with NPLC, the range of lymph node ratio was 0-50.94% with a mean of 12.40 ± 16.21 (median 10.0). The difference between PPLC and NPLC regarding the lymph node ratio was found statistically significant (p< 0.026).

(8) ***TNM stage:*** Fourteen out of 24 patients with stage III (58.3%) had PPLC, while only 4 out of 16 patients (25%) with stage II had positive cytology. The difference between the 2 stages was found statistically significant (p< 0.038). This meaning that the positivity of lavage analysis increased with increasing the overall TNM stage of the tumours.

(9) ***Lymphatic, venous and perineural invasion:*** Of 39 patients with lymphatic invasion, 18 patients (42.2%) had PPLC while the remaining 21 patients (57.8%) had NPLC. The difference was found statistically insignificant (p<1,000). Seventeen out of 37 patients with venous invasion (45.9%) had PPLC and 20 patients (54.1%) had NPLC. The difference was statistically insignificant (p<1.000). Finally, 16 out of 37 patients with perineural invasion (47.2%) had PPLC while the remaining 19 patients (52.8%) had NPLC. The difference was found statistically insignificant (p<0.613) Table (4).

Factors associated with positivity of peritoneal lavage cytopathology

We performed univariate and multivariate analyses including 20 different independent clinical and pathological variables which could have a potential correlation to PPLC. In the univariate analysis BMI, presence of intraperitoneal free fluid at exploration, lymph node ratio and depth of tumour invasion through colonic wall (pT stage) correlated positively with PPLC. In multivariate analysis, BMI, intraperitoneal free fluid and lymph node ratio were independent variables correlating with PPLC (Table 5).

4. DISCUSSION

The objective of our study was to detect those free intraperitoneal cancer cells (IPCC) by conventional cytopathologic examination of the peritoneal lavage fluid obtained from a select cohort of colonic carcinoma patients undergoing radical resection and to define both clinical and pathological variables that correlate positively with their presence.

Table 1. Patients' demographic data

Demographic data	No (40)	%
Sex		
Male	18	45.0
Female	22	55.0
Age (years)		
Range	29.0 – 85.0	
Mean ± SD.	57.90 ± 14.72	
Median (IQR)	56.0 (49.0–65.0)	
BMI (kg/m2)		
<25 (Normal)	6	15.0
>25 – <30 (Overweight)	13	32.5
>30 (Obese)	21	52.5
Min. – Max.	23.0 – 45.0	
Mean ± SD.	29.56 ± 5.42	
Median (IQR)	30.0 (25.0–31.0)	
Family history of malignancy		
Negative	35	87.5
Positive	5	12.5

Since the objective of our study was to detect the prevalence of PPLC in relation to clinical and pathological variables, we performed pre-resection peritoneal lavage before tumour manipulation, as described in literature [4, 13-17]. Conventional cytopathology was used in our study, because it is a universal and inexpensive method that can be easily performed at any institution [11]. Since no significant difference in the positivity of PLC was observed based on the type of stain used, H&E stain was the preferred stain employed in the current study.

Our study showed that the prevalence of PPLC was 45%. The reported prevalence of free IPCC on conventional cytopathology showed a wide range of positivity among published studies. Nishikawa et al, [11] published a meta-analysis of 18 studies, 15 studies employed conventional cytopathology as one of techniques used to detect free IPCC. In studies using conventional cytopathology as the sole method for detection of free IPCC, the detection rate ranged from 0 to 35.5 %. In the meta-analysis of Passot et al, [8], *the* yield rate of positive IPCC detection by conventional cytopathology varied from 4% to 35.5%.

Table 2. Postoperative Pathological data

Pathological data	No (40)	%
Tumor size (cm)		
Range	1– 14.0	
Mean	5.76 ± 2.89	
Median	5.0 (4.0–7.50)	
Morphologic type		
Fungating mass	14	35.0
Malignant ulcer	12	30.0
Circumferential lesion	8	20.0
Mixed	6	15.0
Histopathologic type		
Adenocarcinoma	26	65.0
Adenocarcinoma with mucoid differentiation	10	25.0
Adenocarcinoma with signet ring differentia tion	3	7.5
Adenocarcinoma with neuroendocrine differentiation	1	2.5
Grade		
II	22	55.0
III	18	45.0
pT stage		
T2	4	10.0
T3	27	67.5
T4	9	22.5
pN stage		
N0	16	40.0
N1	10	25.0
N2	14	35.0
Range	14-31	
Mean ± SD	16.7 ±6.23	
Median	17	
Overall TNM stage		
Stage II	16	(40%)
Stage III	24	(60%)
Intraperitoneal free fluid	33	82.5
Absent	7	17.5
Present		
Peritoneal lavage analysis	22	55
NPLC	18	45
PPLC		

Table 3. Peritoneal lavage cytopathology status and different clinical variables

	PLC				Test of Sig	P
	-ve(NPLC) (n=22)		+ve(PPLC) (n=18)			
	No.	%	No.	%		
Gender						
Male	7	38.9	11	61.1	χ^2=	0.064
Female	15	68.2	7	31.8	3.432	
BMI (kg/m^2)						
Normal	1	16.7	5	83.3	χ^2=	0.044*
Overweight	6	46.23	7	53.8	6.030*	
Obese	15	71.4	6	28.6		
Range	24.0 – 45.0		23.0 – 43.0		t= 1.464	0.153
Mean ± SD.	30.70 ± 4.80		28.17 ± 5.93			
Median	30.0		25.0			
Family history						
Positive	1	20	4	80	χ^2=	^{FE}p=
Negative	21	60	14	40	2.828	0.155
Clinical presentation						0.435
Elective	16	59.2	11	40.8	χ^2=	
Emergency	6	46.2	7	53.8	0.609	
Tumour Marker						
CEA						
Normal	16	66.7	8	33.3	χ^2=	0.069
Elevated	6	37.5	10	62.5	3.300	
CA19.9						
Normal	17	65.4	9	34.6	χ^2=	0.072
Elevated	5	35.7	9	64.3	3.237	

χ^2 Chi square test **MC Monte Carlo** **FE Fisher Exact**
t: Student t-test P: p value for comparing between the **two studied categories**
*: Statistically significant at p ≤ 0.05; PLC Peritoneal lavage cytopathology, CEA: carcinoembryonic antigen, CA19.9: Cancer Antigen, BMI: body mass index

Table 4. Peritoneal lavage cytopathology status and different pathological variables

	PLC				Test of Sig.	P
	-ve (NPLC) (n=22)		+ve(PPLC) (n=18)			
	No.	%	No.	%		
Morphologic type						
Fungating mass	6	42.8	8	57.2	χ^2=	^{MC}p=
Malignant ulcer	8	66.7	4	33.3	1.940	0.626
Circumferential lesion	4	50	4	50		
Mixed	4	66.7	2	33.3		
Tumor size cm (max)						
Range	1.0 – 12.0		1.80 – 14.0		U=	0.106
Mean ± SD.	5.10 ± 2.72		6.54 ± 2.97		131.50	
Median	5.0		6.0			
Grade						
II	15	68.2	7	31.8	χ^2=	0.064
III	7	38.8	11	61.2	3.432	
Histopathological type						
• Adenocarcinoma	18	69.2	8	30.8	χ^2=	^{MC}p=
• Adenocarcinoma with mucoid differentiation	3	30.0	7	70.0	6.317*	0.047*
• Adenocarcinoma with signet ring differentiation	1	66.6	2	33.3		
• Adenocarcinoma with neuroendocrine differentiation	0	0.0	1	100.0		
T-stage					χ^2=	^{MC}p=
T2	4	100.0	0	0.0	7.384*	0.025*
T3	16	59.2	11	40.8		
T4	2	22.2	7	77.8		
N stage					4.329	0.115
N0	12	75.0	4	25.0		
N1	4	40.0	6	60.0		
N2	6	42.9	8	57.1		
Lymph node ratio					U=	0.026*
Range	0 – 50.94		0 – 95.83		116. 0	
Mean	12.40 ± 16.21		27.80 ± 23.55			
Median	10		27.78			

	PLC				Test of Sig.	P
	-ve (NPLC) (n=22)		+ve(PPLC) (n=18)			
	No.	%	No.	%		
Stage						
II	12	75.0	4	25.0	χ^2=	0.038*
III	10	41.7	14	58.3	4.313	
Lymphatic invasion	21	53.8	18	46.2	0.839	1.000
Venous invasion	20	54.1	17	45.9	0.178	1.000
Perineural invasion	19	52.8	17	47.2	0.718	0.613

*χ^2: Chi Square test; MC: Monte Carlo U: Mann Whitney test; FE: Fisher Exact p: p value for comparing between two categories; *: Statistically significant at p ≤ 0.05; PLC Peritoneal lavage cytopathology*

Table 5. Factors associated with positive peritoneal lavage cytopathology

Independent variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Sex	3.367 (0.913–12.417)	0.109		
Age	0.554 (0.157–1.952)	0.356		
BMI	4.286 (1.135–16.182	0.022*	5.634 (2.547–11.647)	0.037*
Family history of COLONIC ADENOCARCINOMA	2.375(1.636-3.448)	0..109		
CEA level	0.300 (0.080–1.124)	0.114		
CA 19.9 level	0.294 (0.076–1.145)	0.126		
Free fluid	10.500 (1.126–17.908)	0.017*	8.625 (2.637–14.527)	0.024*
Tumour location	0.764 (0.215–2.708)	0.429		
Tumor size	0.589 (0.155–2.236)	0.457		
Morphologic type	8.521 (0.627–14.523)	0.671		
Histopathologic type	3.367 (0.913–12.5417)	0.217		
Tumour grade	0.297 (0.081–.095)	0.174		
pT stage	0.461 (0.103–0.617)	0.037*	0.685 (0.216–7.521)	0.327
pN stage	0.528 (0.167–4.521)	0.318		
Lymph node ratio	0.654(0.204-0.857)	0.019*	0.348(0.127-0.647)	0.041*
Overall TNM Stage	0.851 (0.318–7.521)	0.294		
Lymphatic invasion	0.526 (0.389–8.712)	0.317		
Venous invasion	1.700 (0.142–20.422)	0.719		
Perineural invasion	2.684 (0.254–28.311)	0.461		

This wide range of outcomes can be attributed to the marked heterogeneity among studies concerning different aspect of the technique including the volume of lavage fluid, the type of lavage fluid, timing of performing lavage, the volume of retrieved fluid, the different staining techniques employed for detection of free IPCC and the varied experience of cytopathologists involved in these studies. Also, the selection criteria of the included patients showed significant heterogeneity among studies. For these reasons, comparing our results with published results is difficult and should be interpreted cautiously.

Three studies employed technical steps very close to ours including pre-resection sample retrieval and using H&E in conventional cytopathology. The prevalence of PPLC reported in these studies was 35.5% (32/90), 7.9% (15/189) and 15.8% (23 /145) of patients reported by Vogel et al, [18] , Lee et al, [19] and Temesi et al, [20] respectively. Our results showed a higher prevalence of PPLC than these studies. Looking at the pathological results in our study may explain this higher outcome, as most of the patients had unfavorable pathology; about one third had mucinous and signet ring differentiation which behave less favorably than conventional adenocarcinoma. Also, with conventional adenocarcinoma, all patients have either moderately or poorly differentiated tumours. We have no T1 tumour and about one fourth of our patients has tumour reaching the serosal surface; T4. Moreover, 60% of our patients had advanced stage III cancer. All these pathological variables including; histopathological type, depth of tumour invasion, higher lymph node ratio and overall TNM staging revealed higher statistical significant correlation with PPLC in our study.

To the best of our knowledge, no prospective study had investigated the relation between the BMI and the presence of free IPCC in colonic carcinoma patients. Investigating this relation in our study, we found that 21 out of the 22 patients (95%) with NPLC were overweight or obese and only 1 patient (5%) had a normal BMI, while in patients with PPLC, 13 out of 18 patients (72.2%) were either overweight or obese and 5 patients (27.8%) had a normal BMI. The difference in incidence of PPLC among the patients with different BMI was found statistically significant (P<0.044), with increased PPLC in patients with BMI <25kg/m2. This outcome was confirmed in both univariate and multivariate analysis. Thus, a higher BMI seems to play a protective role against spread of free cancer cells within the peritoneal cavity. Recently in a retrospective analysis done by Aldaqal et al, [21] investigating the relation between obesity and clinicopathologic characteristics and prognosis of 233 patients with COLONIC ADENOCARCINOMA, they found that obese patients had the lowest incidence of stage IV disease (17 of 69 patients; 25.8%). Meanwhile, the percentage of stage IV disease increased by decreasing the BMI; 38.7%, 39.2% and 57.1% for overweight, normal and underweight patients respectively. The authors concluded that underweight patients may have the worst prognosis. The data of this study supported our observation that a higher BMI may have a protective role against spread of free cancer cells within the peritoneal cavity.

The presence of the malignant cells in the lavage fluid in the current study increased with increasing the depth of tumour invasion (pT stage) into the bowel wall (p =0.032). All 4 patients with pT2 tumors had NPC while, (7/9) of patients with pT4 tumours (78%) had PPLC**.** In the univariate analysis, the pT-stage showed a correlation with PPLC. Our results coincided with those reported widely in the literature [4, 13-15, 22, 23] in spite of using another stain in their conventional cytopathology. Also our results agree with the similar three clinical trials [18-20].

Concerning the histopathological types in our patients, 7 out of 10 peritoneal lavage specimens (70%) in patients with mucoid adenocarcinoma had PPLC, while 2 out of 3 specimens (66.6%) of patients with signet ring carcinoma were found to have PPLC. On the other hand, 8 out 26 patients with conventional adenocarcinoma (30.8%) had PPLC and the difference among these different histopathological types was found statistically significant (p<0.047). The more aggressive the tumour histopathologically is, the higher the possibility of PPLC. On studying the correlation between tumor grade and PPLC, we found that the possibility of PPLC increased with decreased tumour differentiation (poorly differentiated adenocarcinomas (Grade 3) was (61.1%) and moderately differentiated tumours (Grade 2) was (31.8 %)), although this difference in incidence of PPLC between these 2 different grades didn't achieve statistical significance (p<0.064). These outcomes in our

study coincided with Hara et al, [22], Nishikawa et al,[15]; Lee et al [19], who found a positive correlation between higher tumour grade and positive lavage .

Looking to the pN stage, no positive relation between this variable and PPLC was found in our study (p = 0.115). This was in agreement with what was reported by Hase et al,[24], Yamamoto et al,[2] and Lloyd et al, [25]. However, in multivariate analysis, Hara et al;[22] Noura et al, [4]; Katoh et al,[17] and Temesi et al, [20] reported a strong correlation between this variable and PPLC . It is noteworthy, however, that in the current study, a positive correlation was found between PPLC and lymph node ratio. Patients with PPLC had a lymph node ratio of 27.80±23.55 in comparison to 12.40 ± 16.21 in patients with NPLC and this difference was found statistically significant (p< 0.026) in both univariate and multivariate analysis. To the best of our knowledge, this variable wasn't investigated before in other studies.

In this study, 14 out of 24 patients with stage III (58.3%) had PPLC, while only 4 out of 16 patients (25%) with stage II had positive cytopathology. The difference between the 2 stages was found statistically significant (p< 0.038). This means that, the positivity of lavage analysis increased with increasing the overall TNM stage of the tumours. Lee et al, [19] reported similar results with 13.3% PPLC in stage II, 40% in stage III and 46.7% in stage IV which were statistically significant (p<0.001). Also, Kanellos et al,[13] and Noura et al, [4] found this positive correlation between tumor stage and PPLC.

In the current study, 6 out of the 7 patients (85.7%) who had free fluid at exploration were found to have PPLC . The presence of free fluid in the peritoneal cavity at the time of operation showed a positive correlation with PPLC in both univariate and multivariate analyses. Our results are supported by a retrospective study recently published by Sato et al, [26] who looked for free malignant cells in the minimal amount of free fluid found at surgery (n = 225) and in the peritoneal lavage fluid (n = 367) in 592 clinical stage II–IV /patients. The positivity rate was 17.8% versus 6.5% in peritoneal cytology and PLC groups respectively (p < 0.001). Patients with free fluid were younger, had more advanced and more aggressive disease with a higher incidence of emergency presentation. Our results were also quite similar to those reported by Hase et al, [24] and Gozalan et al,[14],who found that the presence of malignant cells in the peritoneal cavity can enhance fluid production by inducing peritoneal inflammation and hinder re-absorption by obstructing the stomata and lymphatic channels. Again, comparison here should be approached cautiously; we excluded patients with macroscopic metastasis discovered pre- or intra-operatively, while, these 2 cohorts included patients with gross peritoneal metastasis. Lee et al, [19], on the other hand, found a positive correlation between macroscopic peritoneal metastasis and PPLC, but, they didn't find correlation between peritoneal effusion and PPLC.

In our study, venous invasion, lymphatic invasion and perineural invasion didn't show correlation with PPLC. This was coincided with the results of Wind et al, [27] Gozalan et al, [14] and Fuiji et al, [16]. Hase et al, [24] found lymphatic invasion to be correlated with PPLC. Homma et al, [23] found venous invasion to be correlated with PPLC while lymphatic invasion didn't.

The detection of free IPCC by PLC at the time of surgery has been reported to be one of the most accurate prognostic factors in some gastrointestinal and gynaecologic malignancies. It has been especially well studied in gastric adenocarcinoma [18, 28]. According to the AJJC TNM classification, gastric cancer with PPLC is classified as Stage IV [28]. Moreover, PPLC has been shown as a useful prognostic marker for pancreatic, esophagogastric, and gynecological malignancies [29, 30]. However, in colonic carcinoma , the meaning of PPLC is still controversial [23], therefore, the technique of PLC till now is not a standard procedure during resection of colonic carcinoma and the presence of free IPCC does not currently influence the staging of colonic carcinoma or the decision regarding the use of adjuvant therapy [11].

Bae et al, [31] suggested that PLC must be a useful tool for selecting patients who are in need for IPC. Also Sato et al, [26] suggested that PLC should be a standard assessment modality for colonic carcinoma that may adopt intraperitoneal chemotherapy and hyperthermic intraperitoneal chemotherapy as mandatory additional treatment after radical resection.

5. CONCLUSION

In non-metastatic AJCC stage II and III colon cancer, the prevalence of PPLC detected by conventional cytopathology was found to be 45%. BMI < 25 kg/m^2, the presence of intraperitoneal free fluid at the time of the operation, a high lymph node ratio, an advanced T stage, the histopathological type and an advanced overall TNM stage correlated positively with PPLC.

6. LIMITATION OF OUR STUDY

Limited number of patients eligible for our inclusion criteria, PLC method lack the sensitivity and specificity of cytopathology and short period of follow up. So a large number of patients and a longer follow-up period are required to draw a definite conclusion.

ETHICAL APPROVAL AND CONSENT

An informed written consent was obtained from every patient before being enrolled in the study. The study was approved by the research ethics committee and quality assurance unit of the Faculty of medicine, Tanta University. The risk to participants and measures needed to minimize these risks.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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