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Original Article

Pathology Section

Clinical and Prognostic Significance of PD-L1, Beclin 1 and Fascin 1 Immunohistochemical Expression in Colorectal Carcinoma

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ABSTRACT

Introduction: Colorectal Cancer (CRC) is a leading cause of death worldwide, and its incidence has been rising. It is important to identify novel markers that could predict the prognosis of the disease.

Aim: To identify the impact of Programmed cell death-ligand 1 (PD-L1), Beclin 1, and Fascin 1 immunohistochemical expression on CRC behaviour and disease prognosis. Also, to assess the different surgical options (laparoscopic versus open) in the management of these patients.

Materials and Methods: This cohort study included 72 cases of CRC, conducted in the Pathology, General Surgery, Internal Medicine, Clinical Oncology, and Medical Oncology Departments of the Faculty of Medicine, Zagazig University Hospitals, Egypt between June 2018 and June 2022. PD-L1, Beclin 1, and Fascin 1 expression were assessed immunohistochemically, and their prognostic significance were evaluated. Data were analysed using IBM Statistical Package for the Social Sciences (SPSS) 23.0 for Windows (SPSS Inc., Chicago, IL, USA) and NCSS 11 for Windows (NCSS LCC., Kaysville, UT, USA) in the Community Medicine Department, Faculty of Medicine, Zagazig University, Egypt.

Results: A significant association was found between PD-L1 expression and grading (p-value=0.02), lymphovascular invasion, distant metastasis (p-value=0.001), peritoneal spread (p-value=0.002), tumour budding (p-value=0.005), lymph node metastasis, and American Joint Committee on Cancer (AJCC) stage (p-value <0.001). A significant association was also found between Beclin 1 expression and distant metastasis (p-value= 0.001), lymphovascular invasion (p-value=0.004), lymphocytic infiltration (p-value=0.006), perineural invasion (p-value=0.01), peritoneal spread (p-value=0.002), and AJCC stage (p-value= 0.007). A highly significant association between Fascin 1 expression and grading, lymphovascular invasion, tumour budding, lymph node metastasis, lymphocytic infiltration, perineural invasion, metastasis, peritoneal spread, and AJCC stage (p-value <0.001) was detected. In patients with negative PD-L1 or high Fascin 1 expression, survival rates were lower. Beclin 1 expression was not associated with a favourable prognosis (p-value >0.05).

Conclusion: PD-L1, Beclin 1, and Fascin 1 expression are significantly elevated in CRC tissues and closely associated with adverse prognostic factors. PD-L1 and Fascin 1 expression have important prognostic value and may be of great help in identifying high-risk patients who will benefit the most from treatment.

Keywords: Colorectal cancer, Laparoscopic, Programmed cell death-ligand 1

INTRODUCTION

CRC is the third most common cancer in males and females in the United States [1]. The American Cancer Society estimated that for 2021, there were 104,270 new cases of colon cancer and 45,230 new cases of rectal cancer [2]. It is the second most common cause of worldwide mortality, with 930,000 deaths and 1.9 million new cases registered in 2020. In Egypt, the number of CRC patients was about 3,430 in 2020 [3], representing approximately 33.8% of total gastrointestinal tumours and 6.2% of all malignancies. Recently, immune checkpoint blockers, cancer vaccines, and adoptive T cell therapy have achieved significant success in cancer treatment [4]. Immune checkpoints, such as PD-1 and its ligand PD-L1, allow malignant cells to evade antitumour immunity. PD-L1 is a transmembrane molecule belonging to the B7 family [5]. It acts through the PD-1/PD-L1 transduction pathway, blocking the proliferation and differentiation of T cells and inhibiting the secretion of many cytokines, thereby promoting the invasion and metastasis of cancer cells [6]. PD-L1 is upregulated in many cancers, including lung, breast, and pancreatic cancers [7,8].

Autophagy is a catabolic and homeostatic mechanism in which cytoplasmic organelles are sequestered in autophagosomes and subsequently degraded through lysosomes to maintain normal metabolism [9]. It plays an important role in cellular resistance to stress [10]. Studies have shown that inhibiting autophagy in cancer cells can increase chemotherapy-induced tumour cell death, making autophagy a potential therapeutic target [11]. Autophagy is controlled by specific genes, including Beclin 1 [12]. Overexpression of Beclin 1 stimulates autophagy and inhibits tumour development [13]. Fascin actin-bundling protein 1 (Fascin 1) is a filamentous actin-binding protein that belongs to the fascin family. It has recently been identified as a therapeutic target for metastatic or aggressive types of many carcinomas. It promotes tumour cell migration, invasion, and metastasis [14].

The objectives of this study are to assess PD-L1, Beclin 1, and Fascin 1 expression in CRC and associate their immunohistochemical expressions with clinicopathological parameters to identify their impact on tumour behaviour and disease prognosis. Additionally, the study aims to assess different surgical options (laparoscopic versus open) in the management of CRC based on the site, stage of the tumour, and the general condition of the patients according to the American Society of Anaesthesiology (ASA) staging.

MATERIALS AND METHODS

This is a cohort study conducted in the Pathology, General Surgery, Internal Medicine, Clinical Oncology, and Medical Oncology

Departments of the Faculty of Medicine, Zagazig University Hospitals, Egypt, between June 2018 and June 2022. The study included 72 cases of CRC, excluding cases previously treated with chemotherapy or radiotherapy. The research was approved by the ethical committee of the Faculty of Medicine, Zagazig University (Zu-IRB: 9905/28). CRC cases were graded according to the World Health Organisation (WHO) 2019 classification [15], and TNM staging was applied according to AJCC [16]. Tumour budding was assessed according to Guzińska-Ustymowiczk [17], while inflammatory infiltrate, blood and lymphatic embolism were assessed according to Jass JR et al., [18]. Lymphocytic infiltration was evaluated as present or absent [19]. Tumour borders were classified as either pushing or infiltrating. Perineural invasion was defined by the presence of tumour cells within the perineural space or tumour cells infiltrating the endoneurium, according to Batsakis JG [20]. Most patients had follow-up records for four years. Patients were followed-up until their most recent medical examination or death, with the follow-up period ending at the completion of the study.

Immunohistochemistry: Sections cut at 4-5 µm were deparaffinised in xylene and rehydrated in graded alcohol. Then, they were incubated for 10 minutes in 0.3% hydrogen peroxide in absolute methanol to block endogenous peroxidase activity. Antigen retrieval was performed using pH 6.0 Dako target recovery solution (Dako, CA, USA). The sections were then incubated for 60 minutes at room temperature with antibodies against PD-L1 (monoclonal, rabbit, Novus Bio, USA, dilution 1:200), Beclin 1 (rabbit monoclonal, EPR1733Y, ab51031, Abcam, Cambridge, UK, dilution 1:50), and mouse anti-Fascin 1 antibody (clone #833223, R&D Systems, Minneapolis, MN, USA, dilution 1:50). The stained slides were independently examined by two pathologists, and there was no interobserver variability.

Assessment of Immunohistochemical Staining

PD-L1 immunohistochemistry: PD-L1 was visualised as cytoplasmic and membranous brown granules. The percentage of positive cells was scored as follows: <5%: 0, 5% to 25%: 1, 26% to 50%: 2, 51% to 75%: 3, and >75%: 4. The staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). A total score was calculated by multiplying the percentage and intensity scores. Further classification was done as follows: Negative group (0 to 1), weak positive (+) (2-4), medium positive (++) (5-8), strong positive (+++) (9-12). The expression was considered positive when scored as '+-+++' [21].

Beclin 1 immunohistochemistry: Beclin 1 expression was mainly cytoplasmic, with occasional nuclear staining. The intensity was scored as follows: 0 (pale yellow or no staining), 1 (yellow), 2 (deep yellow), and 3 (brown). The percentage of positive cells was scored as follows: 0 (0%-10%), 1 (10%-25%), 2 (25%-50%), and 3 (50%-100%). A mean score was calculated as [(intensity reader 1×percentage reader 1)+(intensity reader 2×percentage reader 2)]/2. Finally, Beclin 1 expression was scored as low expression (=0) and high expression (>0) [22].

Fascin 1 immunohistochemistry: Fascin 1 expression was scored semi-quantitatively. It was localised in the cytoplasm of tumour cells and cell membrane. A low expression of Fascin 1 staining was defined as less than 10% of the tumour. Patients with expression between 10% and 35% were considered to have moderate expression, while patients exhibiting ≥35% were considered to have high expression [23].

STATISTICAL ANALYSIS

Data was analysed by using IBM SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA) and NCSS 11 for Windows (NCSS LLC., Kaysville, UT, USA). The quantitative data were reported as mean±Standard Deviation (SD), while for qualitative data, we used frequency and percentage. The following tests were performed:

- Independent sample t-test: Used for normally distributed data.
- Mann-Whitney test: Used for not normally distributed data.
- Chi-square test and Fisher's exact test: Used for the analysis of qualitative data.
- Kaplan-Meier test: Used for survival analysis.

A p-value <0.05 was considered significant, a p-value <0.001 was considered highly significant, while a p-value >0.05 was considered insignificant.

RESULTS

Clinicopathological parameters are presented in [Table/Fig-1,2]. The mean age was 58.9±10.5 years, with 54 cases being males (75%) and 18 cases being females (25%).

Parameters		CRC cases N=72
Age (years) M±SD		58.9±10.5
		n (%)
Gender	Male	54 (75.0)
	Female	18 (25.0)
Co-morbidity	Absent	23 (31.9)
	Diabetes mellitus	25 (34.7)
	Obesity	20 (27.8)
	Hypertension	4 (5.6)
Previous history of CRC		22 (30.6)
History of other cancer (gastric, endometrial)		9 (12.5)
Familial adenomatous polyposis		17 (23.6)
Family history		17 (23.6)
Smoking		35 (48.6)
IBD		2 (2.8)
First presentation	Obstruction	10 (13.9)
	Perforation	4 (5.5)
	Bleeding	58 (80.5)
Fascin	Low	36 (50.0)
	Intermediate	21 (29.2)
	High	15 (20.8)
PDL-1	Negative	38 (52.8)
	Positive	34 (47.2)
BECLIN	Low	32 (44.4)
	High	40 (55.6)

[Table/Fig-1]: Demographic and basic characteristics of the studied groups.

Variables		n (%)
Size	<5 cm	27 (39.7)
	>5 cm	45 (60.3)
HP subtypes	Adenocarcinoma	55 (76.4)
	Mucinous	17 (23.6)
Initial site	RT colon	62 (86.1)
	LT colon	8 (11.1)
	Rectum	2 (2.8)
Tumour border	Bushing	27 (37.5)
	Infiltrating	45 (62.5)
Polyps	Present	25 (34.7)
Necrosis	Present	42 (58.3)
Tumour budding	Present	27 (37.5)
Distant metastasis		14 (19.4)
Perineural invasion		7 (9.7)
Lymphovascular invasion		13 (18.1)
Lymphocytic infiltrate		30 (41.7)
Peritoneal spread		31 (43.1)

Grade	I	28 (38.8)
	II	22 (30.6)
	III	22 (30.6)
T stage	T1	5 (7.0)
	T2	9 (12.5)
	T3	32 (44.4)
	T4	26 (36.1)
N stage	N0	31 (43.1)
	N1	28 (38.9)
	N2	13 (18.0)
AJCC stage	I	9 (12.5)
	II	23 (32.0)
	III	26 (36.1)
	IV	14 (19.4)

[Table/Fig-2]: Tumour characteristics of the studied groups.

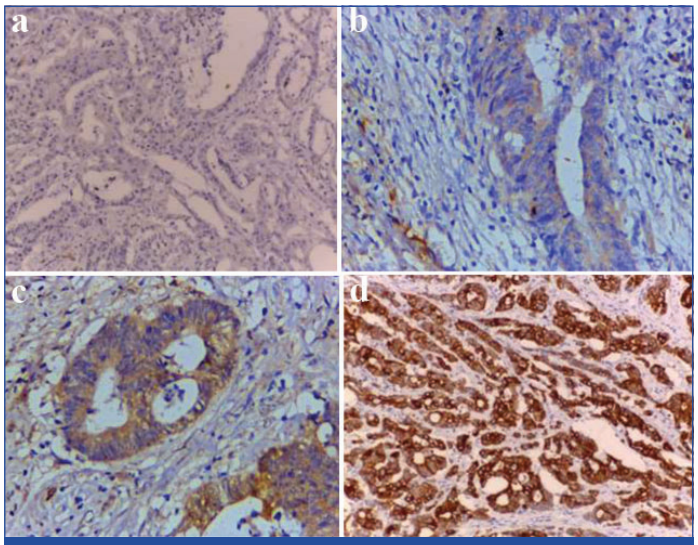
Immunohistochemical Results

PDL-1 expression: Thirty-eight cases (52.8%) were negative for PD-L1, while 34 cases (47.2%) showed positive PD-L1 expression. A significant association was found between PD-L1 expression and grading (p-value=0.02), lymphovascular invasion, distant metastasis (p-value=0.001), peritoneal spread (p-value=0.002), and tumour budding (p-value=0.005). A highly statistically significant association was found between PD-L1 expression and lymphocytic infiltration, lymph node metastasis, and AJCC stage (p-value <0.001). No statistically significant relationship was found between PD-L1 expression and both T stage (p-value=0.29) and perineural invasion (p-value=0.11) [Table/Fig-3,4].

Beclin 1 expression: Forty cases (55.6%) showed high Beclin 1 expression, while 32 cases (44.4%) showed low expression. There was a significant association between Beclin 1 expression and distant metastasis (p-value=0.001), lymphovascular invasion (p-value=0.004), lymphocytic infiltration (p-value=0.006), perineural invasion (p-value=0.01), peritoneal spread (p-value=0.002), and AJCC stage (p=0.007). No association was found between Beclin 1 expression and grade (p-value=0.75), tumour stage (p-value=0.09), tumour stage (p-value=0.22), or lymph node metastasis (p-value=0.05) [Table/Fig-5,6].

Fascin	Low	23 (67.6)	13 (34.2)	13.9	0.001^{S}
	Intermediate	10 (29.4)	11 (28.9)		
	High	1 (2.9)	14 (36.8)		
BECLIN	Low	15 (44.1)	17 (44.7)	0.003	0.96^{NS}
	High	19 (55.9)	21 (55.3)		

[Table/Fig-3]: Relation between PDL-1 marker level and tumour characteristics of the studied groups.



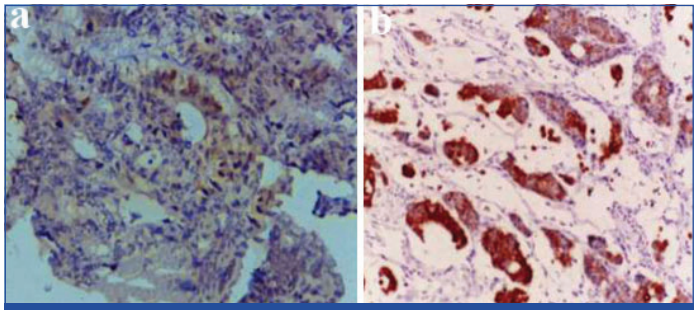
[Table/Fig-4]: Representatve samples of PD-L1 Immunohistochemical expression in colonic carcinoma: (a) Negative PD-L1 immunoreactivity (Avidin biotin complex X20); (b) WeakPD-L1 immunoreactvity (Avidin Biotin Complex X20; (c) Moderate PD-L1 immunoreactvity (Avidin Biotin Complex X200) &; (d) Strong PD-L1 immunoreactvity (Avidin Biotin Complex X200).

Variables		Positive N=34 n (%)	Negative N=38 n (%)	χ^2	p-value
Tumour border	Bushing (n=27)	18 (66.7)	9 (33.3)	6.55	0.01^{S}
	Infiltrating (n=45)	16 (35.6)	29 (64.4)		
Necrosis (n=42)		15 (35.7)	27 (64.3)	5.32	0.02^{S}
Tumour budding (n=27)		7 (25.9)	20 (74.1)	7.86	0.005^{S}
Distant metastasis (n=14)		1 (7.1)	13 (92.9)	Fisher	0.001^{S}
Perineural invasion (n=7)		1 (14.3)	6 (85.7)	Fisher	0.11^{NS}
Lymphovascular invasion (n=13)		1 (7.7)	12 (92.3)	F	0.001^{S}
LN metastasis (n=27)		5 (18.5)	22 (81.5)	14.6	<0.001^{HS}
Lymphocytic infiltrate (n=30)		22 (73.3)	8 (26.7)	14.1	<0.001^{HS}
Peritoneal spread (n=31)		9 (29)	22 (71.0)	7.25	0.002^{S}
Grade	I (n=28)	19 (67.9)	9 (32.1)	7.82	0.02^{S}
	II (n=22)	7 (31.8)	15 (68.2)		
	III (n=22)	8 (36.4)	14 (63.6)		
T stage	T1 (n=5)	2 (40.0)	3 (60.0)	3.67	0.29^{NS}
	T2 (n=9)	4 (44.4)	5 (55.6)		
	T3 (n=32)	19 (59.4	13 (40.6)		
	T4 (n=26)	9 (34.6)	17 (65.4)		
N stage	N0 (n=31)	22 (70.9%)	9 (29.1%)	13.8	0.001^{S}
	N1 (n=28)	10 (35.7%)	18 (64.3%)		
	N2 (n=13)	2 (15.3%)	11 (84.7%)		
AJCC stage	I (n=9)	4 (44.4)	5 (55.6)	18.3	<0.001^{HS}
	II (n=23)	18 (78.3)	5 (21.7)		
	III (n=26)	11 (42.3)	15 (57.7)		
	IV (n=14)	1 (7.1)	13 (92.9)		

Variable		High N=40 n (%)	Low N=32 n (%)	χ^2	p-value
Tumour border	Bushing (n=27)	9 (33.3)	18 (66.7)	8.54	0.003^{S}
	Infiltrating (n=45)	31 (68.9)	14 (31.1)		
Necrosis (n=42)		28 (66.7)	14 (33.3)	5.02	0.03^{S}
Tumour budding (n=27)		17 (63.0)	10 (37.0)	0.96	0.33^{NS}
Distant metastasis (n=14)		13 (92.9)	1 (7.1)	Fisher	0.001^{S}
Perineural invasion (n=7)		7 (100)	0	Fisher	0.01^{S}
Lymphovascular invasion (n=13)		12 (92.3)	1 (7.7)	F	0.004^{S}
LN metastasis (n=27)		19 (70.4)	8 (29.6)	3.84	0.05^{NS}
Lymphocytic infiltrate (n=30)		11 (36.7)	19 (63.3)	7.43	0.006^{S}
Peritoneal spread (n=31)		24 (77.4)	7 (22.6)	10.5	0.002^{S}
Grade	I (n=28)	14 (50.0)	14 (50.0)	0.57	0.75^{NS}
	II (n=22)	13 (59.1)	9 (40.9)		
	III (n=22)	13 (59.1)	9 (40.9)		
T stage	T1 (n=5)	1 (20.0)	4 (80.0)	3.43	0.22^{NS}
	T2 (n=9)	6 (66.7)	3 (33.3)		
	T3 (n=32)	16 (50.0)	16 (50.0)		
	T4 (n=26)	17 (65.4)	9 (34.6)		
N stage	N0 (n=31)	11 (35.4%)	20 (64.6%)	10.4	0.006^{S}
	N1 (n=28)	18 (64.3%)	10 (35.7%)		
	N2 (n=13)	11 (35.4%)	20 (64.6%)		
AJCC stage	I (n=9)	3 (33.3)	6 (66.7)	12.3	0.007^{S}
	II (n=23)	9 (39.1)	14 (60.9)		
	III (n=26)	15 (57.7)	11 (42.3)		
	IV (n=14)	13 (92.9)	1 (7.1)		

PDL-1	Negative	21 (52.5)	15 (46.9)	0.003	0.96^{NS}
	Positive	19 (47.5)	17 (53.1)		
Fascin	Low	14 (35.0)	22 (68.8)	10.3	0.006^{S}
	Intermediate	13 (32.5)	8 (25.0)		
	High	13 (32.5)	2 (6.2)		

[Table/Fig-5]: Relation between Beclin 1 marker level and tumour characteristics of the studied groups.



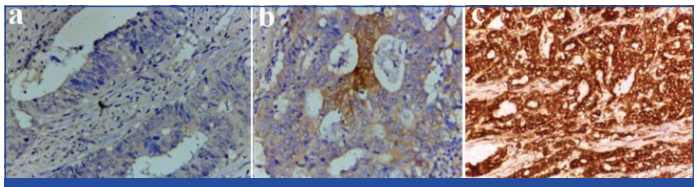
[Table/Fig-6]: Representatve samples of Beclin 1 Immunohistochemical expression in colonic carcinoma: (a) Low Beclin 1 immunoreactivity (Avidin Biotin Complex X200); (b) High Beclin 1 immunoreactvity (Avidin Biotin Complex X200).

Fascin 1 expression: Thirty-six cases (50%) showed low expression, 21/72 (29.1%) showed moderate expression, and 15/72 (20.9%) showed high expression. There was a highly significant relation between Fascin 1 expression and lymphovascular invasion, grading, lymphocytic infiltration, nodal metastasis, peritoneal spread, and tumour budding (p-value <0.001). A highly significant association was found between Fascin 1 expression, perineural invasion, metastatic disease, and AJCC stage (p-value <0.001). Fascin 1 expression was significantly higher in patients with clinical Stage III and IV compared with Stage I and II (p-value=0.03) [Table/Fig-7,8].

Variable		Low N=36 n (%)	Intermediate N=21 n (%)	High N=15 n (%)	χ^2	p-value
Tumour border	Bushing (n=45)	25 (69.4)	1 (4.8)	1 (6.7)	31.4	<0.001^{HS}
	Infiltrating (n=45)	11 (30.6)	20 (95.2)	14 (93.3)		
Necrosis (n=42)		9 (25.0)	19 (90.5)	14 (93.3)	32.9	<0.001
Tumour budding (n=27)		4 (14.8)	13 (48.1)	10 (37.0)		<0.001
Distant metastasis (n=14)		2 (14.3)	1 (7.1)	11 (78.6)	35.1	<0.001
Perineural invasion (n=7)		1 (14.3)	0	6 (85.7)	19.9	<0.001
Lymphovascular invasion (n=13)		2 (15.4)	1 (7.7)	10 (76.9)	30.3	<0.001
LN metastasis (n=27)		7 (25.9)	7 (25.9)	13 (48.1)	20.6	<0.001
Lymphocytic infiltrate (n=30)		25 (83.3)	5 (16.7)	0	24.9	<0.001
Peritoneal spread (n=31)		4 (12.9)	12 (38.7)	15 (48.4)	36.5	<0.001
Grade	I (n=28)	24 (85.7)	3 (10.7)	1 (3.6)	34.8	<0.001^{HS}
	II (n=22)	9 (40.9)	4 (18.2)	9 (40.9)		
	III (n=22)	3 (13.6)	14 (63.6)	5 (22.7)		
T stage	T1 (n=5)	5 (100)	0	0	13.7	0.03^{S}
	T2 (n=9)	5 (55.6)	2 (22.2)	2 (22.2)		
	T3 (n=32)	19 (59.4)	6 (18.8)	7 (21.9)		
	T4 (n=26)	7 (26.9)	13 (50.0)	6 (23.1)		
N stage	N0 (n=31)	30 (96.7%)	1 (3.3%)	0	64.8	<0.001^{HS}
	N1 (n=28)	4 (14.2%)	18 (64.2%)	6 (21.6%)		
	N2 (n=13)	2 (15.3%)	2 (15.3%)	9 (69.4%)		
AJCC stage	I (n=9)	9 (100)	0	0	83.8	<0.001^{HS}
	II (n=23)	21 (91.3)	1 (4.3)	1 (4.3)		
	III (n=26)	4 (15.4)	19 (73.1)	3 (11.5)		
	IV (n=14)	2 (14.3)	1 (7.1)	11 (78.6)		

PDL-1	Negative	13 (36.1)	11 (52.4)	14 (93.3)	13.9	0.001^{S}
	Positive	23 (63.9)	10 (47.6)	1 (6.7)		
BECLIN	Low	14 (38.9)	13 (61.9)	13 (86.7)	10.3	0.006^{S}
	High	22 (61.1)	8 (38.1)	2 (13.3)		

[Table/Fig-7]: Relation between Fascin 1 marker level and tumour characteristics of the studied groups.

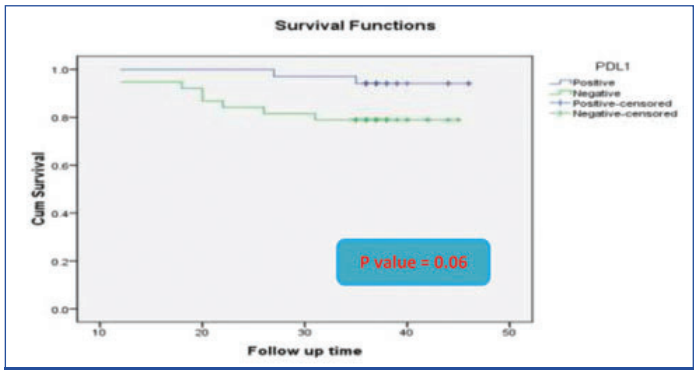


[Table/Fig-8]: Representatve samples of Fascin 1 immunohistochemical expression in CRC: (a) Low fascin immunoreactivity (Avidin Biotin Complex X400); (b) Intermediate fascin immunoreactvity (Avidin Biotin Complex X 400); (c) High Fascin immunoreactivity (Avidin Biotin Complex X200).

Relation between PD-L1, Beclin 1 and Fascin 1 expression: There were significant associations between PD-L1 and Fascin 1 expression (p-value=0.001) and between Beclin 1 and Fascin 1 expression (p-value=0.006), while no significant association was found between Beclin 1 expression and PD-L1 (p-value=0.96) [Table/Fig-3,5,7].

Association of PD-L1, Beclin 1 and Fascin 1 expression and survival: Three-year Progression-Free Survival (PFS) and four-year Overall Survival (OS) were used as the primary outcomes.

Correlation between the expression of PD-L1 and the prognosis of CRC patients: The 4-year cumulative survival rate of patients positive for PD-L1 (n=34) was 94.1%, whereas that of patients negative for PD-L1 (n=38) was 78.9% with a near statistically significant association (p-value=0.06) [Table/Fig-9].

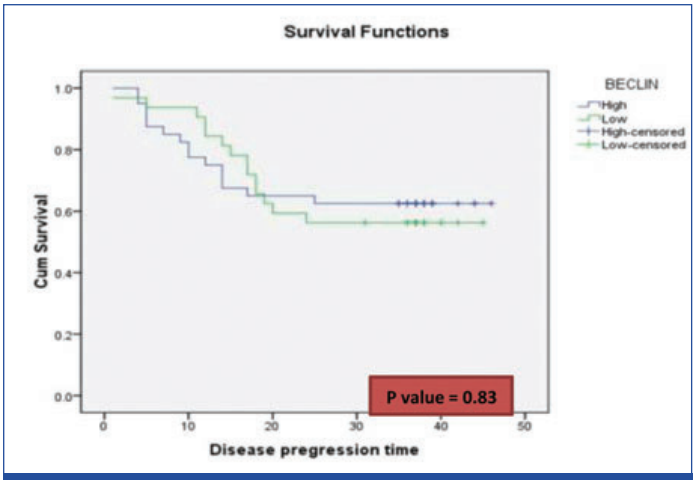


[Table/Fig-9]: Overall survival of CRC cases in relation to PDL-1 expression.

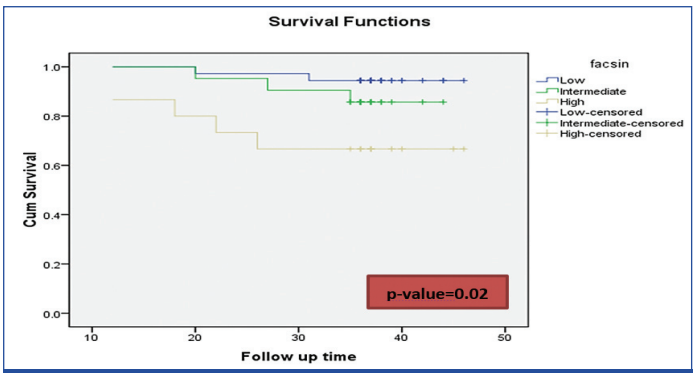
The progression-free survival for patients positive for PD-L1 was 61.8% during follow-up, with an estimated mean event-free survival of 12.7 months. For patients negative for PD-L1, the progression-free survival was 57.9% with a mean time of 12.1 months. However, there was no significant difference in PFS between the two groups (p-value=0.64).

The relationship between survival and Beclin 1 marker: The four-year follow-up, 34 out of 40 patients with high Beclin 1 expression were still alive, resulting in an 85% survival rate. For patients with low Beclin 1 expression, the overall survival was 87.5% (28 out of 32 patients were alive). However, there was no significant difference in overall survival between the two groups (p-value=0.7). Univariate analysis using the Kaplan-Meier method showed no correlation between Beclin 1 expression and progression-free survival (p-value=0.8) [Table/Fig-10].

Prognostic significance of Fascin 1 in relation to progression-free and OS: The four-year overall survival was 85.7% for patients with moderate expression of Fascin 1 and 66.7% for patients with high expression, compared to 94.4% for patients with low expression (p-value=0.02) [Table/Fig-11]. In terms of progression-free survival, patients with high and intermediate expression had worse PFS (53.3% and 52.4%) compared to patients with low expression (66.7%), but this difference was not statistically significant (p-value=0.45).



[Table/Fig-10]: Progression free survival of CRC cases in relation to Beclin 1 expression.



[Table/Fig-11]: Overall survival of CRC cases in relation to Fascin 1.

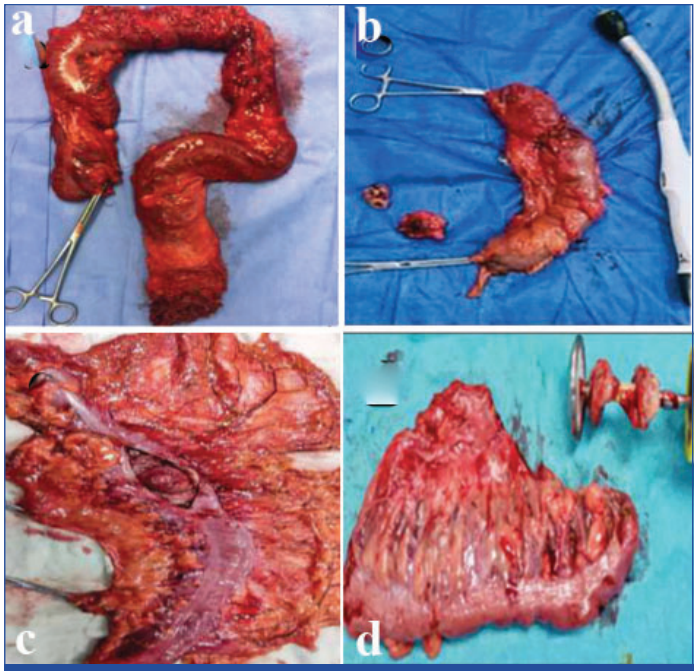
Surgical results: 18 (25%) patients underwent laparoscopic resection, with half of them having a covering stoma and the other half undergoing primary anastomosis. Open resection was performed in 40 cases (55%), with 50% of them having a stoma, and the remaining cases were managed by resection and primary reconstruction. Fourteen patients (20%) were considered inoperable and underwent palliative surgery with a converting stoma. When comparing open and laparoscopic surgery, laparoscopic surgery was found to be safer, with less intraoperative blood loss and postoperative complications, especially when combined with protecting proximal stoma [Table/Fig-12,13].

Postoperative outcomes	n (%)	Resection and primary anastomosis (29)	Resection and covering or end stoma (29)	Stoma without Resection (Stage IV-Inoperable) (14)
Type of procedure				
Laparoscopic resection	18 (25)	9	9	-
Open resection	54 (75)	20	20	14
Postoperative anastomotic leak	9 (12.5)	9	0	
Postoperative hospital stay (days)	-	7-11	1-2	1-2
Postoperative (30 days) mortality	10 (13.9)	5	3	2

[Table/Fig-12]: Surgical management options and postoperative outcomes.

DISCUSSION

Worldwide, colon cancer-related mortality is mainly attributed to distant spread [24]. PD-L1 can induce inhibitory signals when it binds to its receptor, leading to T cell activation, cytokine secretion, and promoting immune tolerance and immune escape of tumour cells. The effect of PD-1/PD-L1 inhibitors has been widely recognised [25]. In present study, PD-L1 expression was significantly associated



[Table/Fig-13]: (a) Laparoscopic total colectomy; (b) Laparoscopic left hemicolectomy; (c) Open extended right hemicolectomy for proximal transverse colon cancer; (d) Open sigmoid colectomy with colorectal anastomosis.

with tumour grade (p-value=0.02), lymphovascular invasion, distant metastasis (p-value=0.001), peritoneal spread (p-value=0.002), lymphocytic infiltration, and lymph node metastasis (p-value <0.001). These findings are consistent with previous studies by Shan T et al., except for lymph node metastasis [26]. Furthermore, in terms of Overall Survival (OS), PD-L1 expression showed a near significant association with favourable clinical outcomes (p-value=0.06). This finding was in line with Sabatier R et al., who reported that positive PD-L1 expression was significantly associated with better OS in breast cancer cases, as well as better metastasis-free survival and response to chemotherapy. However, our results contradict those of Li Y et al., who found that PD-L1 was associated with an unfavourable outcome [27,28]. This discrepancy may be attributed to the larger number of cases in present study. Present study results demonstrate that PD-L1 is significantly associated with poor tumour outcomes, supporting the findings of Shan T et al., [26]. This was consistent with poorer outcomes observed in non small cell lung cancer, renal cell carcinoma (Wang A et al.,), and osteosarcoma (Lussier DM et al.,) [29,30]. These findings can be attributed to the complex functions of PD-L1 in the initiation and growth of colon cancer.

Beclin 1 plays a role in regulating both apoptosis and autophagy, and the balance between these processes determines the efficacy of anticancer treatments [31]. Present study found a statistically significant relationship between Beclin 1 expression and distant metastasis (p-value=0.001), which was consistent with the findings of Zhang B et al., who also reported a close association between Beclin 1 and distant metastasis in CRC [32]. However, Zhang B et al., did not find a relationship between Beclin 1 expression and venous invasion or lymph node metastasis, which contradicts present study results as authors found a statistically significant relationship between Beclin 1 expression and lymphovascular invasion (p-value=0.004) as well as the degree of lymphocytic infiltration (p-value=0.006). In present study, there was no significant association between Beclin 1 expression and tumour grade (p-value=0.75) or stage (p-value=0.09), which was consistent with the findings of Zhang B et al., who also did not find an association between Beclin 1 and tumour grade or stage. However, in present study, Beclin 1 expression was not associated with favourable prognostic outcomes in CRC patients (p-value >0.05). This contradicts the findings of Park JM et al., who found that high expression of Beclin 1 in primary tumours was associated with reduced survival in patients receiving 5-FU as adjuvant therapy [33].

Fascin 1 expression in tumour cells is associated with characteristics of progenitor cells in colon cancer, and it is considered a stem cell marker for metastatic cancers [34]. In present study, Fascin 1 expression showed a highly statistically significant relationship with lymph node metastasis, tumour grade, and the presence of metastatic disease (p-value <0.001), which was in line with the findings of Tampakis A et al., who also found an association between Fascin 1 expression and lymph node metastasis (p-value <0.001), distant metastasis (p-value=0.002), and high-grade tumours (p-value=0.002) [23].

Machesky LM and Li A proposed that the upregulation of Fascin 1 in the EMT pathway facilitates the motility and invasion of tumour cells [35]. Previous studies have found a significant association between overexpression of Fascin 1 and low E-cadherin, indicating the progression of cells through the Epithelial to Mesenchymal Transition (EMT). As cells acquire Fascin 1, they lose E-cadherin [36]. However, Oh SY et al., did not find a significant relationship between Fascin 1 expression and lymph node metastasis, which contradicts present study findings. Present study results were similar as present study did not find a significant association between Fascin 1 expression and clinicopathological factors such as tumour size [37].

In present study, patients with low levels of Fascin 1 had better survival, and there was a significant difference (p-value=0.02). This was consistent with the findings of Tampakis A et al., who reported that patients with high expression of Fascin 1 had significantly higher rates of disease recurrence or death [23].

When comparing laparoscopic and open surgeries in patients with CRC, present study observed that postoperative complications were significantly reduced in patients who underwent laparoscopic surgery. The most serious complication, postoperative leak, was observed in nine patients, with only one of them undergoing laparoscopic resection. Present study findings are in line with the studies conducted by Ma K et al., and Benson AB et al., who found that laparoscopic procedures are highly effective in reducing ileus, infection, hospital stay, and postoperative leak compared to open surgery [38,39].

Limitation(s)

Unfortunately, due to a lack of financial support, additional prognostic markers or microRNA assessment of these or other markers were not included.

CONCLUSION(S)

Positive PD-L1 expression and low Fascin 1 expression in CRC are favourable for patients' survival. PD-L1, Beclin 1, and Fascin 1 expression can be considered reliable biomarkers in the development of CRC and distant metastasis. These three markers are significantly associated with each other. Present study data could serve as a foundation for further investigating the value of these markers in targeted therapy for CRC. Postoperative surgical outcomes are better among patients who undergo laparoscopic surgery, with or without a covering stoma.

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