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Prognostic Value of IMP3 and Cyclin D1 Expression in Patients with Colorectal Cancer

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Abstract

Background: Colorectal cancer (CRC) is known to be the third most frequently diagnosed cancer and the fourth leading cause of cancer death worldwide. In Egypt, colorectal carcinoma is considered the 7th prevalent cancer, accounting for 3.47% of male cancers and 3% of female malignancies. A localized CRC can be entirely cured via surgical resection. Metastasis remains the leading cause of cancer mortality. IMP3 is an independent prognostic biomarker that expects metastasis and poor prognosis in the CRC. The upregulation of nuclear cyclin D1 plays an essential role in pathogenesis and metastases of CRC. We aimed to investigate the expression of IMP3 and cyclin D1 in colorectal carcinoma and their correlation with other clinicopathological features.
Method: In this retrospective cohort study, 80 formalin-fixed and paraffin-embedded blocks of CRC were obtained from the subjects. The immunohistochemical expression of IMP3 and cyclin D1 were examined and found to be correlated with clinical-pathological parameters and the outcome of the patients.
Results: Overexpression of IMP3 and cyclin D1 was noted in 68.75% and 56.25%, respectively. IMP3 expression was significantly correlated with tumor grade (P <0.001), TNM stage (P =0.040), and LVI (P= 0.005); cyclin D1 was significantly associated with TNM stage (P<0.001), LN metastasis (P<0.001), and DM (P =0.004); cyclin D1 was significantly correlated with TNM stage (P<0.001), LN metastasis (P<0.001), and DM (P= 0.004).
Conclusion: IMP3 and cyclin d1 were associated with poor prognosis in CRC, which makes them attractive targets for anticancer drug development.
Keywords: IMP3, Cyclin D1, Colorectal cancer, Recurrence, Survival

Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer and the fourth top cause of cancer death worldwide.[1]

In Egypt, colorectal carcinoma is considered to be the 7th prevalent cancer, representing 3.47% of male cancers and 3% of female malignancies.[2]

Once the disease is localized, the surgical resection of the primary tumor could be enough for a complete cure. Distant metastasis remains the main reason behind treatment failure and cancer fatality.[3]

CRC commonly metastasizes to the liver as most of the GIT drainage pass to the hepatic portal venous circulation. More than 50% of patients with CRC have liver metastasis, which is the leading cause of cancer mortality in more than two-thirds of cases.[4]

Insulin-like growth factor (IGF) II mRNA-binding protein 3 (IMP3) is one of the IGF mRNA binding protein (IMP) family members containing IMP1, IMP2, and IMP3.[5]

IGF2 messenger RNA binding protein 3 (IGF2BP3, also known as IMP3) has an essential role in the stabilization and trafficking of RNA, cellular growth, and cell migration during embryogenesis.[6]

A range of malignancies as pancreatic, lung, esophageal, thyroid, and melanomas showed IMP3 expression.[7]

Several studies have suggested that IMP3 promotes tumor cell proliferation, adhesion, and invasion.[5]

Furthermore, it has been shown that IMP3 expression, determined via immunohistochemistry, has prognostic significance in a variety of cancers, including colorectal cancer.[8]

Cyclin D1 plays an essential role in the cell cycle as it controls the shift of the cell cycle from phase G1 to S phase through pRb mediation. Cyclin D1/Cyclin-Dependent Kinase (CDK) 4–6 complexes initiate the phosphorylation of pRb and cyclin E/CDK2 complex completes the process in the late G1 phase. Alterations in cyclin and CDK expression result in increased cellular proliferation and participate in malignancy.[9]

The expression of cyclin D1 is of great importance in colorectal pathogenesis and metastasis.[10]

Thus, we aimed to investigate the expression of IMP3 and cyclin D1 in colorectal carcinoma and their correlation with other clinicopathological features.

Material and Methods

A retrospective cohort study that includes sections from formalin-fixed and paraffin-embedded samples from 80 CRC patients was enrolled in this work.

The local Ethics Committee of the faculty of medicine, Zagazig University, approved this study (ethics code: ZU-IRB#6352/24-8-2020). Written approved consent was taken from the patients or their relatives.

This work was done in the pathology department in collaboration with internal medicine, surgical, tropical, and clinical oncology groups, and university hospitals from January 2015 to June 2019. At the pathology department, the research included sections from formalin-fixed and paraffin-embedded samples from 80 CRC patients' specimens that were sent and processed. The patients' data were collected from their records approved by the local Ethical Committee.

All the participants underwent a proper history and examination. Investigations were performed in the form of lab profile and radiological studies, such as pelviabdominal ultrasonography, chest x-ray, and chest and pelviabdominal computed tomography. Bone scan and PET scan were carried out according to clinical scenarios. All the patients underwent colonoscopic diagnostic biopsy. Chemotherapy protocols were proposed for those with functional performance status and adequate organ function in the form of XELOX, Folfox, and single-agent capecitabine was offered as 1st line chemotherapy while the FOLFIRI regimen was mostly as the 1st proposed protocol for metastatic and progressed CRC patients. Fifteen cases were diagnosed with rectal cancer out of 80 patients who received neoadjuvant chemoradiation. The patients' follow-up was to detect disease recurrence and survival outcome.

Immunohistochemical staining

We utilized the streptavidin-biotin technique.[11] Paraffin-embedded blocks were cut into Four-micron thick sections; deparaffinization was done in a sequence of xylene and rehydration was done in descending grades of alcohol and endogenous peroxidase activity was blocked by placing the part in 0.5% hydrogen peroxide in methanol for 10 min of microwave antigen retrieval.

Primary rabbit polyclonal antibody was directed against IMP3 (Thermo Fisher Scientific/Lab Vision Corporation, Rockford, USA. Used in a dilution of 1-100) and rabbit monoclonal anti-Anti-cyclin D1 antibody [SP4] (ab16663) dilution 1:50). Furthermore, diaminobenzidine (DAB), as the chromogen, was added for 30 minutes at room

temperature. The secondary antibody was added to sections for 30 minutes. Following the incubation, the reaction product was observed with diaminobenzidine. Ultimately, the sections were counterstained with Mayer's hematoxylin. The negative controls had the primary antibody replaced by a buffer.

Immunohistochemical evaluation of both markers

For Imp3

Cytoplasmic IMP3 expression was evaluated following the method previously designated in a previous study.[12] The percentage of positively stained cells was assessed via microscopic examination of the whole stained section. We defined positive IMP3 expression, as there was considerable brown cytoplasmic staining in more than 5% of tumor cells, regardless of the staining intensity.

For Cyclin D1

We recorded the proportion of positive tumor cells (0 = 0 to 1%, 1 = 2 to 25%, 2 = 26 to 50%, 3 = 51 to 75%) and 4 = > 75%. For further statistical analyses, cyclin D1 expression was dichotomized into negative, with no expression, and positive, without any expression, fraction, and intensity.[13]

Statistical Analysis

Continuous variables were expressed as the mean ± SD and median (range) and the categorical variables as a number (percentage). Continuous variables were checked for normality using the Shapiro-Wilk test. Independent samples Student's t-test was employed to compare the two groups of normally distributed variables while the Mann Whitney U test was used for non-normally distributed variables. The percentages of categorical variables were compared with Pearson's Chi-square test or Fisher's exact test once it was appropriate. The trend of changes in the distribution of relative frequencies between ordinal data was analyzed using the Chi-square test for trend. The correlation between immunohistochemical markers was analyzed via Spearman correlation. A *P*-value <0.05 was considered to be significant. All the statistics were performed with SPSS 22.

Results

Clinicopathological results

We had 80 cases of CRC, 56 (70%) of whom were male and 24 (30%) were female, with age ranges from 39 to 72 years with a median (58). 70 cases were conventional adenocarcinoma and10 were Mucoid carcinoma (Table 1).

Immunohistochemical results

IMP3 Results

IMP3 was expressed in the cytoplasm of cancer cells. In our study, IMP3 was found in 55 (68.75%) cases. IMP3 expression was significantly correlated with tumor grade (*P*<0.001), TNM stage (*P*=0.040), and LVI (*P*= 0.005). Meanwhile, we found no significant correlations between IMP3 expression and age, gender, tumor location, and tumor size (Table 2, Figure 2).

Cyclin D1 results

Cyclin D1 was detected in the nucleus of tumor cells. Herein, cyclin D1 was expressed in 45 (56.25%) cases; cyclin D1 was significantly correlated with TNM stage (*P*<0.001), LN metastasis (*P*<0.001), DM (*P*=0.004). Nevertheless, we found no significant correlations between cyclin D1expression and age, gender, tumor location, tumor size, and grade (Table 2, Figure 3).

IMP3 positive expressed CRC patients had an inadequate treatment response with *P*=0.007 considered to be significant (Table 3).

Cyclin D1 and IMP3 positive expressed patients had shown significantly worse 3-year DFS (*P* =0.002 and *P* <0.001, respectively) (95% CI) (Table 3, Figure 1A, 1B, and 1C).

Negative cyclin D1 and IMP3 were associated with a better 3- year -OS (*P*=0.003 and *P* <0.001, respectively) (95% CI) (Table 3, Figure 1D, E, and F).

Discussion

Colorectal cancer is believed to be one of the deadliest cancers worldwide. Tumor markers are one of the most important factors, which can detect tumor aggressiveness and subsequently the appropriate method of treatment.

A localized CRC can be thoroughly cured with surgical resection. Metastases remain the leading cause of cancer mortality.[14]

IMP3 is an independent prognostic biomarker that expects metastasis and poor prognosis in CRC.[15]

IMP3 is considered a novel biomarker that could differentiate normal from malignant tissue in a variety of organ systems.[7]

Herein, we had 80 cases of colorectal carcinoma, 15 of whom were GI, 45 were GII, and 20 were GIII. IMP3 was expressed in 55 patients. In the study by Huang et al.,[16] IMP3 was abnormally expressed in (72.3%) of tumor cells.[16] However, in the survey conducted by Wei et al., IMP3 was shown in (83.1%) of cases. Lochhead et al.[15] observed IMP3

expressed in 35% of CRC cases. This variation in the percentages of expression may be due to the use of different primary antibodies. We found that IMP3 expression was significantly correlated with the grade of the tumor (P <0.001) since the expression increased with grade progression as it was expressed in 26.7% of GI, 73.3% of GII, and 90% of GIII; this is in agreement with the work by Wei et al.[17] who found IMP3 to be significantly correlated with the grade in a resection specimen (P=0.043). Moreover, Lochhead et al.[15] found a significant correlation between Imp3 expression and the degree of the tumor (P=0.0003); This is contrary to the findings of Huang et al.,[16] who failed to find a significant correlation between IMP3 expression and grade of the tumor (P= 0.122).

In this study, we also found a significant correlation between IMP3 expression and TNM stage (P<0.001) and LVI (P= 0.005). This is following the findings of Wei et al.,[17] who found a substantial correlation between IMP3 expression and TNM stage (P= 0.007), LN metastases (P= 0.023), and T classification (P= 0.035). This is also in agreement with the study by Yuan et al.,[8] who found a significant correlation between IMP3 expression and high-stage tumor (P= 0.0417) and lymph node metastasis (P= 0.0232).

In our study, we found no meaningful relationships between IMP3 expression and age, gender, tumor location, and tumor size. The obtained results were similar to those of both Wei et al.[17] and Huang et al.;[16] meanwhile, they were contrary to the results of Yuan et al., who found a significant correlation between IMP3 expression and large tumor size (P= 0.0452).[8]

The authors found that cyclin D1 was expressed in 45 (56.25%) cases. However, in the survey by Bahnassy et al.[18] cyclin, D 1 was shown at 68.3%. In addition, in the review performed by Salem et al., it was revealed in 48.3%. We found that cyclin D1 expression was significantly correlated with the TNM stage (P<0.001), LN metastasis (P<0.001), and DM (P=0.004).[19] This is in line with the study by Salem et al.,[19] who found a significant correlation between cyclin D1 expression and the presence of lymph node metastases (P= 0.000) and distant metastasis (P= 0.029). Furthermore, Van Wangenheim et al. proved that cyclin D1 was associated with unlucky clinicopathological features of CRC.[20] However, Al-Maghrabi et al. found no associations between cyclin D1 expression and clinicopathological characteristics, except with lymphovascular invasion.[21] Nevertheless, the results of both Holland et al.[22] and Ogino et al.[23] were contrary to our findings since they reported that cyclin D1overexpression is associated with a good prognosis; moreover, they found that cyclin D1-expressing tumors are less destructive than tumors with reduced cyclin D1 expression.

In this work, we found no significant correlations between the expressions of both markers despite the increase in the expression of both markers in the high stage. This may be due to different numbers of positive cases in each pen as IMP3 expressed in 55 cases, yet cyclin D appeared only in 45.

Consistent with our study, positively expressed IMP3 patients showed significantly worse 3 -year- DFS= P<0.001 and associated with worse 3- year- OS with significance [8,24]. Yang et al. observed worse 5 -year- survival, P=0.0012, in IMP3 positive patients and Li et al. demonstrated poor prognosis and colon cancer progression and poor survival prediction in CRCs patients with a positive IMP3 expression in tumor cells (P=0.02).[8,24]

Lochhead et al. indicated that IMP3 was an independent prognostic biomarker as it predicted metastasis and poor prognostic CRC outcome.[15]

Huang et al. observed that patients with positive IMP3 expression in tumor cells had a more mediocre survival rate in comparison with those with negative IMP3 expression in tumor cells. Meanwhile, they found no statistically significant associations between stromal expression of IMP3 and survival rate and demonstrated reduced survival rates for patients with a stromal expression of IMP3 (P=0.06).[16]

Salem et al. reported worse 3-year DFS and OS with significance P<0.001 and P<0.003, respectively, in positive cyclin D1 expression, which is utterly consistent with our results regarding cyclin D1; we also demonstrated worse 3-year- DFS and OS with significance P=0.002 and P=0.003, respectively[19].

Bahnassy et al.[18] and Maeda et al.[27] observed that cyclin D1 overexpression is associated with poor prognosis 8-9, yet Wangefjord et al.[13] disagreed with it since they found that cyclin D1 expression was associated with a more favorable outcome for CRC male patients, but not for female; this may be due to the larger sample size of the studied patients and different patient characteristics.[13]

In a meta-analysis, which investigated cyclin D1 expression significance among Asian and non-Asian CRC patients, Li et al showed that high

cyclin D1 overexpression was associated with poor OS in 21 studies and associated with worse DFS in 10 studies.[24]

The present research is in agreement with other investigators who demonstrated that the multidisciplinary expansion in the use of preoperative chemotherapy, radiation, and target therapy advances are in favor of disease recurrence reduction and high-risk disease survival benefits in positive expressed IMP3.[25,26]

The limitations of this work were the small sample size. Furthermore, serial estimation of IMP3 after treatment wasn't done. Therefore, we could recommend further studies with bigger sample sizes for studying IMP3 in diagnostic biopsy as well as after management for further clarification of more beneficial justifications of treatment strategies.

Conclusion

IMP3 and cyclin D1 were associated with poor prognosis in the CRC; accordingly, they could be taken into account as targets for anticancer drug development.

Conflicts of Interest

None declared.

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Table 1. Clinicopathological features, immunohistochemical markers, treatment, and outcome

Characteristics	All patients (N=80) No.	%
Gender		
Male	56	70%
Female	24	30%
Age		
<50 years	20	25%
≥50 years	60	75%
Site		
Rt colon	18	22.5%
Lt colon	32	40%
Transverse	8	10%
Rectum	22	27.5%
Gross pattern		
Fungating	46	57.5%
Annular	10	12.5%
Ulcerative	24	30%
Tumor size		
≤5cm	34	42.5%
>5cm	46	57.5%
Pathological type		
Conventional	70	87.5%
Mucoid	10	12.5%
Grade		
Grade I	15	18.8%
Grade II	45	56.3%
Grade III	20	25%
LVI		
Absent	30	37.5%
Present	50	62.5%
T		
T1	9	11.3%
T2	20	25%
T3	39	48.8%
T4	12	15%
LN		
Negative	30	37.5%
Positive	50	62.5%
DM		
Negative	70	87.5%

Characteristics	All patients (N=80) No.	%
Surgery		
No surgery	10	12.5%
Hemicolectomy	51	63.7%
	19	23.8%
Neoadjuvant treatment	(N=69)	
No	54	78.3%
Chemoradiation	15	21.7%
Adjuvant chemotherapy	(N=70)	
No	10	14.3%
Yes	60	85.7%
Chemotherapy for Stage IV	(N=10)	
FOLFOX	1	10%
XELOX	1	10%
FOLFIRI	8	80%
Response	(N=10)	
PR	4	40%
SD	2	20%
PD	4	40%
Follow-up duration (months)		
Mean±SD	30.37	±7.10
Median (Range)	34	(10 – 36)
Relapse	(N=70)	
Absent	30	42.9%
Present	40	57.1%
First-line chemotherapy	(N=40)	
5fu+leucovorin	4	10%
Xeloda	3	7.5%
FOLOFOX	10	25%
XELOX	20	50%
FOLFIRI	3	7.5%
Second-line chemotherapy	(N=50)	
No	1	2%
Xeloda	1	2%
FOLOFOX	2	4%
XELOX	12	24%
FOLFIRI	34	68%

Positive	10	12.5%

Stage		
Stage I	12	15%
Stage II	18	22.5%
Stage III	40	50%
Stage IV	10	12.5%
Cyclin D1		
Negative	35	43.8%
Positive	45	56.2%
IMP		
Negative	25	31.2%
Positive	55	68.8%

Third line chemotherapy	(N=50)	
No	46	92%
Xeloda	1	2%
FOLFIRI	3	6%
Mortality	(N=80)	
Alive	38	47.5%
Died	42	52.5%

Categorical variables were expressed as number (percentage); Continuous variables were expressed as mean ± SD & median (range).DM (distant metastasis), LVI (lymphovascular invasion), APR (abdominoperineal resection).

Table 2. Relationship between the clinicopathological features and immunohistochemical staining of cyclin D1 and IMP

Characteristics	All patients (N=80)		Cyclin D1				IMP				*P* -value
			Negative (N=35)		Positive (N=45)	*P* -value	Negative (N=25)		Positive (N=55)		
	No.	(%)	No.	(%)	No.		No.	(%)	No.	(%)	
Gender											
Male	56	(70%)	23	(41.1%)	33	(58.9%)	0.461‡	18	(32.1%)	38	(67.9%)	0.792‡
Female	24	(30%)	12	(50%)	12	(50%)		7	(29.2%)	17	(70.8%)	
Age											
<50 years	20	(25%)	10	(50%)	10	(50%)	0.515‡	7	(35%)	13	(65%)	0.676‡
≥50 years	60	(75%)	25	(41.7%)	35	(58.3%)		18	(30%)	42	(70%)	
Site											
Rt colon	18	(22.5%)	8	(44.4%)	10	(55.6%)	0.467‡	6	(33.3%)	12	(66.7%)	0.784‡
Lt colon	32	(40%)	15	(46.9%)	17	(53.1%)		11	(34.4%)	21	(65.6%)	
Transverse	8	(10%)	5	(62.5%)	3	(37.5%)		3	(37.5%)	5	(62.5%)	
Rectum	22	(27.5%)	7	(31.8%)	15	(68.2%)		5	(22.7%)	17	(77.3%)	
Gross pattern											
Fungating	46	(57.5%)	20	(43.5%)	26	(56.5%)	0.563‡	12	(26.1%)	34	(73.9%)	0.324‡
Annular	10	(12.5%)	3	(30%)	7	(70%)		5	(50%)	5	(50%)	
Ulcerative	24	(30%)	12	(50%)	12	(50%)		8	(33.3%)	16	(66.7%)	
Tumor size											
≤5cm	34	(42.5%)	16	(47.1%)	18	(52.9%)	0.608‡	10	(29.4%)	24	(70.6%)	0.760‡
>5cm	46	(57.5%)	19	(41.3%)	27	(58.7%)		15	(32.6%)	31	(67.4%)	
Pathological type											
Conventional	70	(87.5%)	30	(42.9%)	40	(57.1%)	0.741‡	23	(32.9%)	47	(67.1%)	0.494‡
Mucoid	10	(12.5%)	5	(50%)	5	(50%)		2	(20%)	8	(80%)	
Grade											
Grade I	15	(18.8%)	11	(73.3%)	4	(26.7%)	0.154§	11	(73.3%)	4	(26.7%)	<0.001§
Grade II	45	(56.3%)	15	(33.3%)	30	(66.7%)		12	(26.7%)	33	(73.3%)	
Grade III	20	(25%)	9	(45%)	11	(55%)		2	(10%)	18	(90%)	
LVI											
Absent	30	(37.5%)	13	(43.3%)	17	(56.7%)	0.954‡	15	(50%)	15	(50%)	0.005‡
Present	50	(62.5%)	22	(44%)	28	(56%0		10	(20%)	40	(80%)	
T											
T1	9	(11.3%)	3	(33.3%)	6	(66.7%)	0.496§	3	(33.3%)	6	(66.7%)	0.602§
T2	20	(25%)	11	(55%)	9	(45%)		9	(45%)	11	(55%)	
T3	39	(48.8%)	18	(46.2%)	21	(53.8%)		8	(20.5%)	31	(79.5%)	
T4	12	(15%)	3	(25%)	9	(75%)		5	(41.7%)	7	(58.3%)	
LN											
Negative	30	(37.5%)	22	(73.3%)	8	(26.7%)	<0.001‡	9	(30%)	21	(70%)	0.852‡
Positive	50	(62.5%)	13	(26%)	37	(74%)		16	(32%)	34	(68%)	
DM											

Negative	70	(87.5%)	35	(50%)	35	(50%)	0.004‡	19	(27.1%)	51	(72.9%)	0.063‡
Positive	10	(12.5%)	0	(0%)	10	(100%)		6	(60%)	4	(40%)	
Stage												
Stage I	12	(15%)	10	(83.3%)	2	(16.7%)	<0.001§	6	(50%)	6	(50%)	.040§
Stage II	18	(22.5%)	12	(66.7%)	6	(33.3%)		3	(16.7%)	15	(83.3%)	
Stage III	40	(50%)	13	(32.5%)	27	(67.5%)		10	(25%)	30	(75%)	
Stage IV	10	(12.5%)	0	(0%)	10	(100%)		6	(60%)	4	(40%)	
Cyclin D1												
Negative	35	(43.8%)						12	(34.3%)	23	(65.7%)	0.605‡
Positive	45	(56.2%)						13	(28.9%)	32	(71.1%)	
IMP												
Negative	25	(31.2%)	12	(48%)	13	(52%)	0.605‡					
Positive	55	(68.8%)	23	(41.8%)	32	(58.2%)						

Categorical variables were expressed as number (percentage); ‡ Chi-square test; § Chi-square test for trend; $P<0.05$ is significant. DM (distant metastasis), LVI (lymphovascular invasion),

Table 3. Relationship between immunohistochemical staining of cyclin D1 and IMP and the outcomes among the studied patients (N=80)

Outcome	All Patients No. (%)	Cyclin D1 Negative No. (%)	Cyclin D1 Positive No. (%)	*P*-value	IMP Negative No. (%)	IMP Positive No. (%)	*P*-value
Response	(N=10)		(N=10)		(N=6)	(N=4)	
PR	4 (40%)		4 (40%)	-----	4 (66.7%)	0 (0%)	0.007‡
SD	2 (20%)		2 (20%)		2 (33.3%)	0 (0%)	
PD	4 (40%)		4 (40%)		0 (0%)	4 (100%)	
Relapse	(N=70)	(N=35)	(N=35)		(N=19)	(N=51)	
Absent	30 (42.9%)	21 (60%)	9 (25.7%)	0.004‡	19 (100%)	11 (21.6%)	<0.001‡
Present	40 (57.1%)	14 (40%)	26 (74.3%)		0 (0%)	40 (78.4%)	
DFS							
Mean DFS	20.98months	25.65months	16.31months	0.002†	36months	15.39months	<0.001†
(95%CI)	(17.89-24.07)	(21.42-29.88)	(12.37-20.25)			(12.34-18.44)	
Median DFS	14months	NR	10months		NR	10months	
(95%CI)	(11.77-16.22)		(7.83-12.16)			(10-12)	
6month DFS	88.5%	94.3%	82.9%		100%	84.3%	
12month DFS	51.4%	65.7%	37.1%		100%	33.3%	
24month DFS	42.9%	65.7%	25.7%		100%	21.6%	
36month DFS	42.9%	60%	25.7%		100%	21.6%	
Mortality	(N=80)	(N=35)	(N=45)		(N=25)	(N=55)	
Alive	38 (47.5%)	23 (65.7%)	15 (33.3%)	0.004‡	22 (88%)	16 (29.1%)	<0.001‡
Died	42 (52.5%)	12 (34.3%)	30 (66.7%)		3 (12%)	39 (70.9%)	
OS							
Mean OS	30.37months	32.97months	28.35months	0.003†	33.92months	28.76months	<0.001†
(95%CI)	(28.82-31.92)	(31.24-34.69)	(26.12-30.58)		(31.64-36.19)	(26.91-30.61)	
Median OS	34months	NR	28months		NR	28months	
(95%CI)			(25.37-30.62)			(25.58-30.41)	
12month OS	96.3%	100%	93.3%		100%	94.5%	
24month OS	78.8%	88.6%	71.1%		88%	74.5%	
36month OS	47.5%	65.7%	33.3%		88%	29.1%	

Continuous variables were expressed as mean (95%CI) and median (95%CI); categorical variables were expressed as number (percentage); NR: not reached yet; ‡ Chi-square test; † Log-rank analysis; $P<0.05$ is significant.PR (partial response), SD (stable disease), PR (progressive disease).

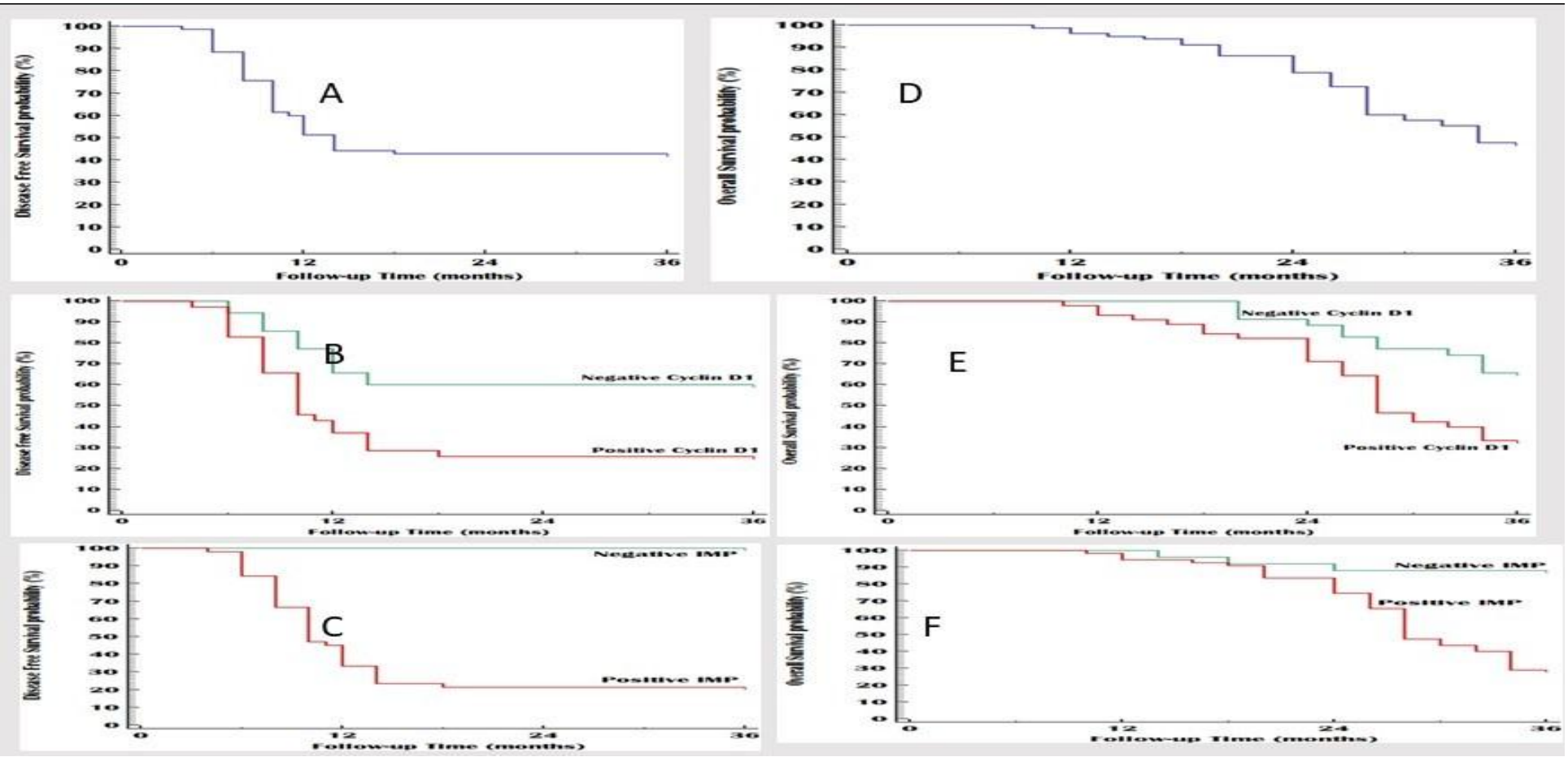

Figure 1. Kaplan Meier plot of the colorectal carcinoma patients (N=80), Left panel: disease-free survival (DFS); Right panel: overall survival (OS); (A and D) All the studied patients, (B and E) Stratified with cyclin D1 and (C and F) Stratified with IMP.

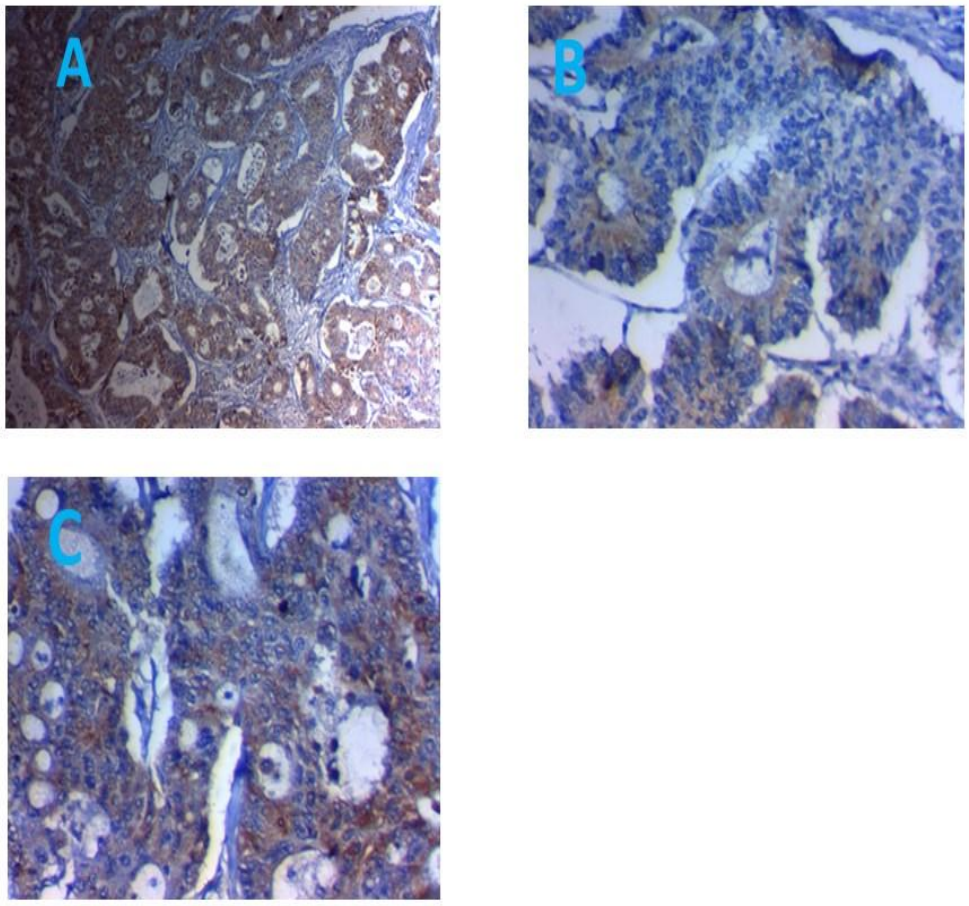

Figure 2. IMP3 expression in colon cancer: A) Positive strong cytoplasmic IMP3 expression in moderate differentiated colonic carcinoma (ABC, DABx200); B) Positive strong cytoplasmic IMP3 expression in moderate differentiated colonic carcinoma (ABC, DABx400); C) Positive strong cytoplasmic IMP3 expression in high-grade colonic carcinoma (ABC, DABx400).

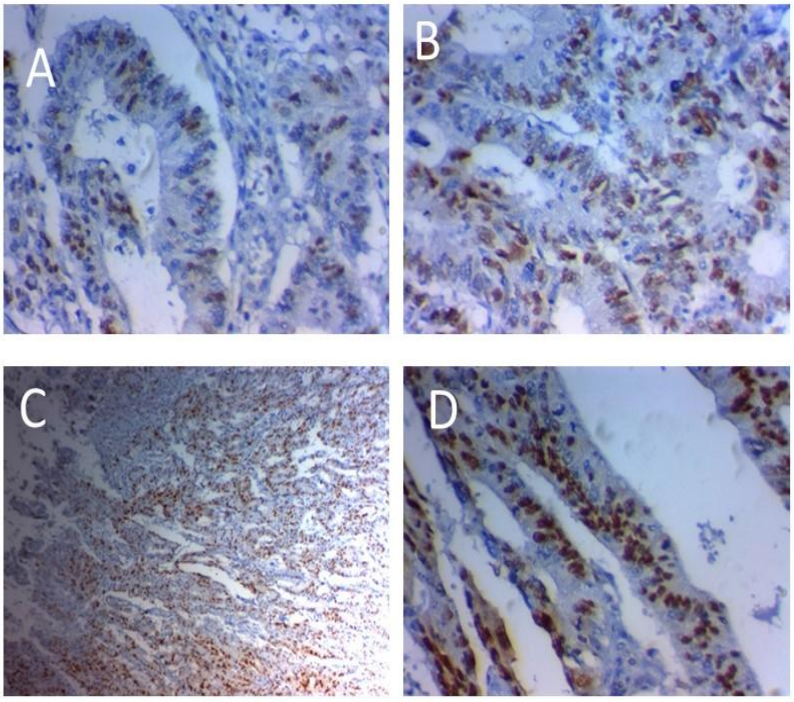

Figure 3. Cyclin D1 expression in cancer colon: A) Positive moderate nuclear cyclin D1 expression in moderate differentiated colonic carcinoma (ABC, DABx400); B) Positive moderate nuclear cyclin D1 expression in moderate differentiated colonic carcinoma (ABC, DABx400); C) Positive strong nuclear cyclin D1 expression in moderate differentiated colonic carcinoma (ABC, DABx100); D) Positive strong nuclear cyclin D1 expression in high-grade colonic carcinoma (ABC, DABx400).