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The prognostic Value of Cancer Stem Cell Marker, Cluster of Differentiation 133 Expression in Stage III Colorectal Carcinoma

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ABSTRACT

Background: Colorectal cancer (CRC) is the 3rd most prevalent diagnosed cancer and the 4th leading cause of cancer-related deaths globally. Nodal staging is significantly more essential in rectal cancer that detects most the therapy options.

Objectives: This study aimed to evaluate CD133 expression in the colorectal carcinoma stage III using immunohistochemistry as well as its relationship to clinicopathological characteristics and patient outcomes.

Patients and Methods: The study includes 60 cases with stage III CRC five years after surgical removal of the tumor and regular follow-up with the Departments of Medical and Clinical Oncology where clinicopathological and prognostic data are collected from the archives. The patients were classified into two groups, GI includes 22 CRC patients with relapse, and GII includes 38 CRC patients without relapse. Sixty archive paraffin blocks of primary resection and metastatic lymph nodes were extracted from the archives of the Pathology Department processed for CD133 immunohistochemistry. CD133 expression levels were assessed, analyzed, and correlated with clinicopathological and prognostic criteria.

Results: Positive CD133 expression was significantly linked with old age (P=0.034), large tumor size ($p < 0.001$), perineural invasion ($p = 0.0017$), lympho-vascular invasion ($P < 0.001$), high-grade ($p < 0.001$), resistance to chemotherapy ($p = 0.011$), lymph nodes metastasis and relapse ($p = 0.005$*) and DFS ($p = 0.005$)

Conclusion: CD133 expression in colorectal carcinoma is related to tumour progression and is considered a marker of poor prognosis and a strong indicator of relapse and poor survival. Moreover, CD133 stem cell marker may act as a targeted therapy in chemotherapeutic resistance patients with colorectal carcinoma.

Keywords: Cancer stem cell marker, CD133, Stage III, Colorectal carcinoma.

INTRODUCTION

Colorectal cancer (CRC) is the 3rd most commonly diagnosed cancer and the 4th major cause of cancer-related deaths worldwide. Its deadly consequences are anticipated to rise by 60% to more than 2.2 million new cases and 1.1 million cancer deaths by 2030 [(1)].

Cancer stem-like cells (CSC) were discovered first by John Dick in acute myeloid leukemia (AML) in the late 1990s. They are a group of highly tumorigenic cells that can repopulate tumours following apparent curative treatment. They have characteristics similar to normal intestinal stem cells, such as the ability to self-renew and long-term repopulation, which leads to tumour heterogeneity. They can also give rise to all types of cells found in a cancer sample [(2)].

prominin-1, also known as CD133 is a transmembrane glycoprotein found in various tissues, including hematopoietic cells, neuroepithelial cells, and endothelial cells. CD133 is a clear marker of primary colorectal cancer stem cells (CSC), and its expression has been associated with differentiation and tumour growth in colorectal cancer [(3)]. CD133 could identify a group of tumor cells with characters of stem cells. Those CD133-positive tumor-initiating cells can self-renewal, differentiate, regenerate, and form tumors when injecting them into immunodeficient mice. CD133 expression has a crucial influence in CRC development [(4)].

The proper preoperative staging of lymph nodes underpins several CRC therapeutic approaches. Nodal staging is vital in rectal cancer since clinically node-positive CRC patients are frequently treated with neo-adjuvant radiation-based therapy. The first appearance of LN metastasis in CRC is stage IIIa, which has spread to 1 to 3 nearby lymph nodes (N1) [(5, 6)].

This study aimed to assess CD133 expression in stage III colorectal carcinoma using immunohistochemistry as well as its relationship to clinicopathological characteristics and patient outcomes.

PATIENTS AND METHODS:

The study includes 60 cases with stage III CRC(50colon ,and 10 rectal cancer) five years after surgical removal of the tumor at the Department of General Surgery, Faculty of Medicine, Zagazig University, and after receiving planned treatment according guidelines for stage III colorectal cancer they were on regular follow-up with the Departments of Medical and Clinical Oncology, Faculty of Medicine, Zagazig University, where clinicopathological and prognostic parameters were collected from their archives, including (age, sex, tumor type, tumor size, tumor grade, drug resistance, and relapse after therapy).

The patients were classified into 2 groups, the 1st group (GI) includes 22 patients with relapse, and the 2nd group (GII) includes 38 patients without relapse. Sixty archive paraffin blocks of metastatic lymph nodes of primary resection were extracted from the Pathology Department's archives and processed for CD133 immunohistochemistry. CD133 expression levels were assessed, analyzed, and correlated with clinicopathological

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and prognostic criteria to detect whether there is a relationship between CD133 expression with relapse, drug resistance, and poor clinicopathological parameters.

Ethical approval:

Approval was obtained from Zagazig University's Faculty of Medicine's Institutional Review Board (IRB), Egypt, (no. ZU-IRB#/9988) to collect data and samples from relevant departments. The research was carried out in compliance with the declaration of Helsinki of the World Medical Association. Before participating in the study, all patients or their legal representatives signed informed permission forms.

Immunohistochemistry:

Using IGg antibody against CD133 (CD133/Prominin-1 (PROM1) Concentrated, Code: RM0202, Clone: MD49R, Source: Rabbit, The EnVision TM FLEX + system (DAKO, North America Inc, CA, USA) was used for immunohistochemical staining, which was a two-step polymer-enhanced IHC detection method. Recommended 3-in-1 specimen preparation procedure using pretreatment procedure: Deparaffinization, rehydration, and heat-induced epitope recovery were performed in paraffin-embedded tissue sections[7, 8]. The staining steps with the recommended incubation time were reprogrammed in the Auto Stainer Link 48 software. At first, endogenous peroxidase activity was reduced by incubating the sample for 5 minutes [9]. The sample was then incubated with a monoclonal antibody against CD133 for 20 minutes at room temperature, followed by incubation with a polymer-immunoglobulin enzyme complex for 30 minutes. The staining was completed at room temperature for ten minutes by incubation with EnVisionTM FLEX Substance Working solution (DAB + substrate-chromogen) (DM 827) that resulted in a brownish discoloration at the antigen site. After each incubation, the slide was dipped in a buffer for 5 minutes. EnVision™ FLEX Hematoxylin did the counterstaining, then dehydration by deionized water, and finally the slides were mounted with a coverslip using DPX (BDH Clinical, Merck, USA) [10, 11]. The use of kidney tissue provided positive control. The negative control included sections incubated without primary antibodies.

Interpretation of CD133 expression:

The CD133 immunostaining was assessed as membrane staining of the luminal surface of the malignant glands as well as luminal cellular debris staining, while other tumor masses showed cytoplasmic expression of the malignant cells. CD133 staining intensity was classified semi-quantitatively as low and high CD133 expression, low expression was considered when < 50% CD133-positive glands were found, while high CD133 expression was considered high when ≥ 50% CD133-positive tumor glands were found, examining five medium-power fields of each tumour tissue. Positivity was defined either luminal membrane staining, staining of shed cellular debris in the lumen of the tumour glands or cytoplasmic expression of the malignant cells [12].

Analyses of statistical data:

The mean, standard deviation, and median (range) of continuous data were used, whereas categorical variables were reported as a number (percentage). The Shapiro-Wilk test was used to ensure that continuous variables were normal. Samples that are unrelated to compare two sets of normally distributed dat

a, the Student's t-test was utilised. Whereas, Mann-Whitney U test was used for non-normally distributed variables. The disease free survival (DFS) was calculated as the time from surgery to the date of local recurrence or distant metastasis discovery or the most recent follow-up in which no local recurrence or distant metastasis was diagnosed (censored). Overall survival (OS) was calculated using the time from diagnosis to death or the most recent follow-up contact. All clinical and immunohistochemical variables were used to stratify patients with OS and DFS. Kaplan-Meier plot was used to estimate the time-to-death distributions and the two-sided exact log-rank test was used for comparison. All tests were carried out in pairs. Significant was defined as a P-value of less than 0.05. For all statistics, SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium) were used [13].

RESULTS

Features of the patients

This waws a retrospective study that included 60 cases of stage III colorectal carcinoma(50 colon cancer and 10 rectal cancer) that were regularly followed up by the Departments of Medical and Clinical Oncology, Faculty of Medicine Zagazig University. The clinicopathological characteristics and demographic data are summarized in table (1). 34 (56.6%) of the cases were under 60 years of age and 26 (43.3%) were over 60 years of age. Male patients constituted 55%, while the females were 45%. A small tumor size below 5 cm was found in 28 (46.6%) and large tumor size in 32 (53.3) patients. High CEA levels were reported in 27 (53.1%) cases while 33 (46.9%) showed low CEA levels. Patients with perineural invasion constituted 18.3%, while 81.7% were without. In terms of lymphovascular invasion, 60% of patients showed its absence. A high grade of colorectal carcinoma was recorded in 48.3% of the patients, while the rest showed low grades. 35 (58.3%) successfully responded to chemotherapy, while 25 (41.6%) showed resistance to chemotherapy treatment. 51 (85%) grade III colorectal carcinoma samples showed positive expression of CD133, while 9 (15%) were negative. Among 51 (85%) positive cases, 20 (39.2%) showed high expression and 31 (60.7) showed CD133 low expression. Regarding the survival analysis, among the 60 patients, 22 (36.3%) showed recurrence after initial treatment, 22.7% of them showed local recurrence, 68.1 showed distant recurrence, and 9 % showed both types of recurrence. After 60 months of follow-up, among 60 patients 19 (31.6%) patients died (Table 1).

CD133 Expression

The expression was recorded as three patterns of expression of brown discoloration that was summarized in table (1). The first pattern was the luminal surface membranous staining with luminal cellular debris staining of the malignant glands. This pattern was found in 27 (52.9%) patients, whereas 10 (19.6%) of patients showed cytoplasmic expressions of cancer cells, both patterns of expression were found in 14 (27.4%) of patients. Regarding the expression of CD133 in metastatic lymph nodes of grad III CRC cases, among 60 lymph node blocks, CD133 was expressed in 48 (80%) while 12 (20%) cases showed negative expression of CD133 immunostaining (Table 1).

Table (1) : Clinicopathological characters of the studied colorectal carcinoma cases (N= 60)

Clinicopathological feature		No.	(%)
Age group	<60	34	56.6
	≥ 60	26	43.3
Sex	Male	33	55
	Female	27	45
Tumor size	≤ 5 cm	28	46.6
	> 5 cm	32	53.3
CEA, ng/ml	≤ 5 ng/ml	33	46.9
	> 5 ng/ml	27	53.1
Perineural invasion	Absent	49	81.7
	Present	11	18.3
Lymphovascular invasion (LVI)	Absent	36	60
	Present	24	40
Grade	Low grade	31	51.6
	High grade	29	48.3
Treatment resistance	Absent	35	58.3
	Present	25	41.6
CD133 expression	Negative	9	15
	Positive	51	85
CD133 scoring	Low	31	60.7
	High	20	39.2
CD133 pattern of expression	Luminal	27	52.9
	Cytoplasmic	10	19.6
	Both	14	27.4
CD133 expression in lymph node	Absent	12	20
	Present	48	80
Relapse	Absent	38	63.3
	Present	22	36.3
Type of recurrence	Local recurrence	5	22.7
	Distant recurrence	15	68.1
	Both	2	9.09
Mortality	Alive	41	68.3
	Died	19	31.6

The association between the expression of CD133 and clinicopathological characteristics in colorectal carcinoma:

Table (2) showed the relationship between the expression of CD133 and the clinicopathological characters. A statistically significant relationship existed between positive CD 133 expression and old age (p = 0.034). Moreover, among those positive cases of CD133, cases of high expression of CD133 showed a strong association with old age (p=0.002).

There was no statistically significant link between either total CD133 positive expressed cases or high CD133 expressed cases and patient gender (p = 0.489 & p = 0.426 respectively). The large tumor size was highly statistically significant in relation to high CD133 expression (p < 0.001) and not significant in relation to only positive CD133 expression (p = 0.884). The expression of CD133 was not related to CEA (ng/ml) level (p=0.303 and p=0.156).

The Perineural invasion and CD133 expression had a strong statistically significant connection (p=0.0017), whereas the extent of CD133 expression was not related to perineural-invasion (p = 0.564). High CD133 expression was highly significant in patients with lymphovascular invasion (LVI) (P < 0.001). CD133 positive expression and high expression of CD133 were highly significantly related to patients with high-grade colorectal carcinoma compared to cases with low grade (p =0.015 & p < 0.001 respectively).

High expression of CD133 was significantly expressed in cases of resistance to chemotherapy (p=0.011). Different CD133 patterns were not related to the expression's extent or intensity. Expression of CD133 expression in the lymph nodes was highly significant concerning both positive expression and high expression of CD133 in surgical tumor biopsies in colonic tissues (p < 0.001 & p = 0.036 respectively).

Table 2:The relationship between the expression of CD133 and clinicopathological parameters													
Parameters	**CD 133 in all cases** (n=60)				p-value	**CD 133 positive cases** (n=51)				p-value
	Negative (N=9)		Positive (N=51)				Low (N=31)		High (N=20)		
	No	(%)	No.	(%)			No.	(%)	No.	(%)	
Age						**Age**					
<60 years (N=34)	8	(88.8%)	26	(50.9%)	0.034*	<60 years N=26	21	(67.7%)	5	(25.0%)	0.002*
=>60 years (N=26)	1	(11.1%)	25	(49.0%)		>60 years N=25	10	(32.2%)	15	(75.0%)	
						Sex					
Male (N=33)	4	(44.4%)	29	(56.8%)	0.489	Male N=29	19	(61.2%)	10	(50%)	0.426
Female (N=27)	5	(55.5%)	22	(43.1%)		Female N=22	12	(38.7%)	10	(50%)	
Tumor size						**Tumor size**					
≤ 5 cm (N=28)	4	(76.7%)	24	(47.0%)	0.884	≤ 5 cm (N=24)	21	(67.7)	3	(15%)	<0.001*
> 5 cm (N=32)	5	(73.5%)	27	(52.9%)		> 5 cm (N=27)	10	(32.2)	17	(85%)	
CEA, ng/ml						**CEA, ng/ml**					
≤ 5 cm (N=33)	3	(33.3%)	30	(58.8%)	0.156	≤ 5 cm (N=30)	20	(64.5%)	10	(50%)	0.303
> 5 cm (N=27)	6	(66.6%)	21	(41.4%)		> 5 cm (N=21)	11	(35.4%)	10	(50%)	
Perineural invasion						**Perineural invasion**					
Absent (N=49)	4	(44.4%)	45	88.2%)	0.0017*	Absent (N=45)	28	(90.3%)	17	(85%)	0.564
Present (N=11)	5	(55.5%)	6	11.7%)		Present (N=06)	3	(16.1%)	3	(15%)	
Lymphovascular invasion (LVI)						**Lymphovascular invasion (LVI)**					
Absent (N=36)	8	(88.8%)	28	(54.9%)	0.055	Absent (N=28)	26`	(83.8%)	2	(10.0%)	<0.001*
Present (N=24)	1	(11.1%)	23	(45.0%)		Present (N=23)	5	(16.1%)	18	(90.0%)	
Grade						**Grade**					
Low (N=31)	8	(88.8%)	23	(54.9%)	0.015*	Low (N=23)	22	(81.2%)	1	(18.8%)	<0.001*
High (N=29)	1	(11.1%)	28	(45.0%)		High (N=28)	9	(0.0%)	19	(100.0%)	
Treatment resistance						**Treatment resistance**					
Absent (N=35)	6	(66.6%)	29	(56.8%)	0.582	Absent (N=29)	22	(70.9%)	7	(35%)	0.011*
Present (N=25)	3	(33.3%)	22	(43.1%)		Present (N=22)	9	(29%)	13	(65%)	
CD133 pattern of expression						**CD133 pattern of expression**					
						Luminal (N=27)	18	(58%)	9	(45%)	0.560
						Cytopla (N=10)	5	(16.1%)	4	(20%)	
						Both (N=14)	7	(22.5%)	7	(35%)	
CD133 expression in lymph node						**CD133 expression in lymph node**					
Absent (N=12)	6	(55.5%)	6	(17.6%)	<0.001*	Absent (N=6)	6	(29.0%)	0	(5.4%)	0.036*
Present (N=48)	3	(44.4%)	45	(82.3%)		Present (N=45)	25	(71. %)	20	(100%)	
Relapse						**Relapse**					
Absent (N=38)	8	(88.8%)	30	(58.8%)	0.084	Absent (N=30)	23	(74.1%)	7	(35%)	0.005*
Present (N=22)	1	(11.1%)	21	(41.1%)		Present (N=21)	8	(25.8%)	13	(65%)	
Mortality											
Alive (N=41)	6	(66.6%)	35	(68.6%)	0.907	Alive (N=35)	26	(83.8%)	9	(45%)	0.003*
Died (N=19)	3	(33.3%)	16	(31.3%)		Died (N=16)	5	(16.1%)	11	(55%)	
DFS						**DFS**					
Mean (months)(95%CI)	60		52			Mean (95%CI)	54.7		48.2		0.003*
Median DFS	NR		NR		0.101	Median DFS	NR		44		
5-year DFS	83.8%		58.8%			5-year DFS	74.2%		35%		
OS						**OS**					
Mean (months)(95%CI)	60		56			Mean(95%CI)	58.1		55.1		0.009*
Median OS	NR		NR		0.829	Median OS	58		39		
5-year OS	83%		68..6%			5-year OS	66.7%		45%		

The link between the expression of CD133 and the outcome survival results in 60 colorectal carcinoma patients:

Relapse was significantly more common in patients with high positive expression of CD133 than in those with low expression (p=0.005*). The median DFS in patients with high expression was 44 months, which was significantly poor than the median DFS in low CD133 expressed cases (p=0.005) 95% CI. Overall survival was considerably higher in patients with low CD133 expression, with a mean of 58 months compared to 39 months in those who expressed high expression of CD133 (p0.009) at 95% CI.

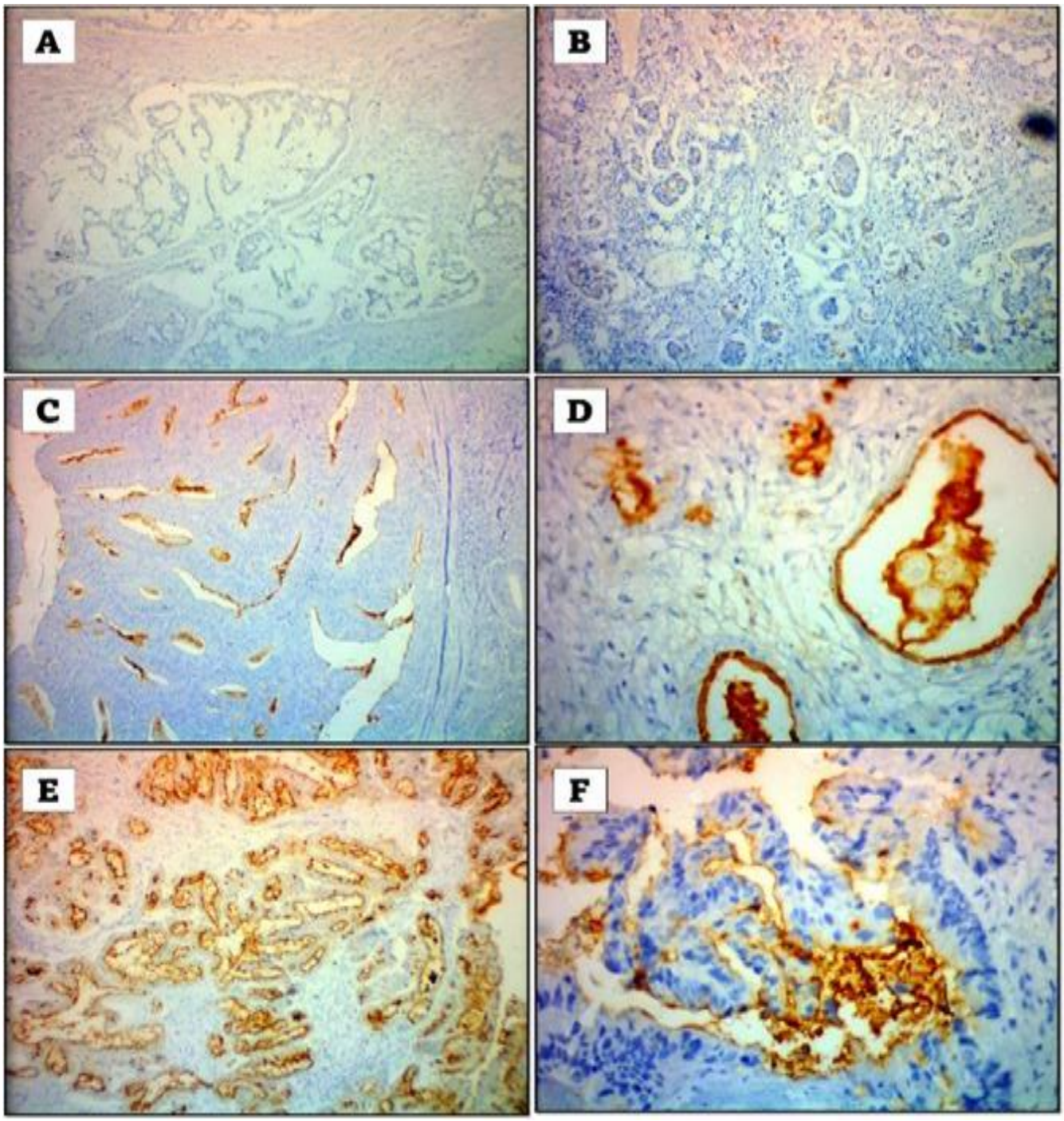

Figure (1): Immunohistochemistry of CD133 expression in CRC showing luminal surface expression pattern.CD133 appeared as brown membranous staining of the luminal surface and cellular debris staining in the lumen of the malignant glands. (A): Showed negative expression of CD133 (100X). (B): Showed mild positive expression of CD133 (100X). (C): Showed moderate positive expression of CD133 (100X). (D): Showed a high power of moderate positive expression of CD133 (400X). (E):
Showed strong positive expression of CD133 (100X). (F): Showed high power of strong positive expression of CD133 (100X) (Figure 1).

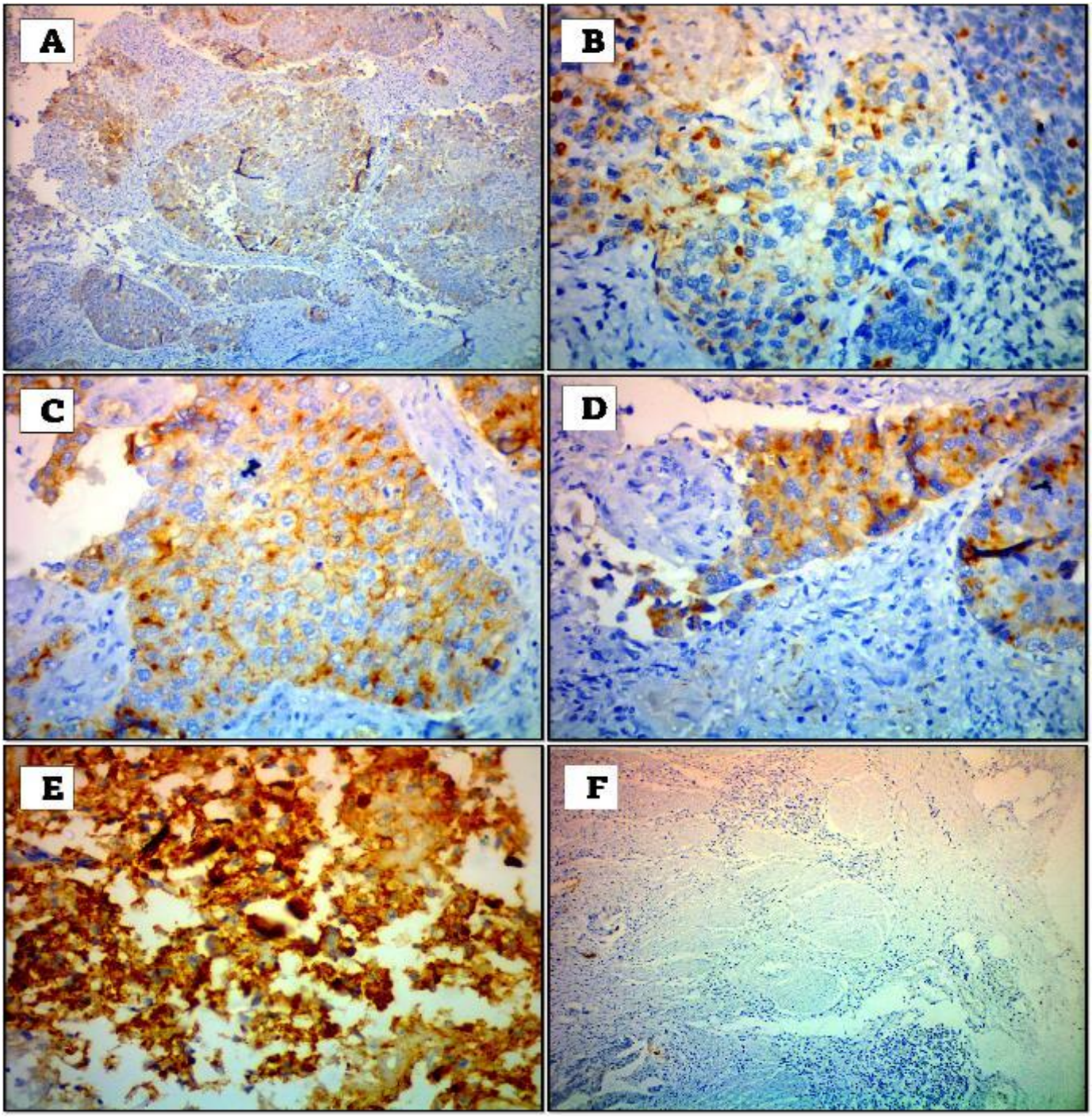

Figure (2): Immunohistochemistry of CD133 expression in colorectal carcinoma showing a cytoplasmic expression pattern. CD133 appeared as brownish cytoplasmic staining of tumor cells in non-glandular masses. (A): Showed mild expression of CD133 (100X). (B): Showed a high power of mild positive expression of CD133 (400X). (C=D): Showd moderate positive expression of CD133 (400X). (D): Showed a high power of moderate positive expression of CD133 (400X). (E): Showed strong positive expression of CD133 (400X). (F): Showed negative expression of CD133 (100X) (Figure 2).

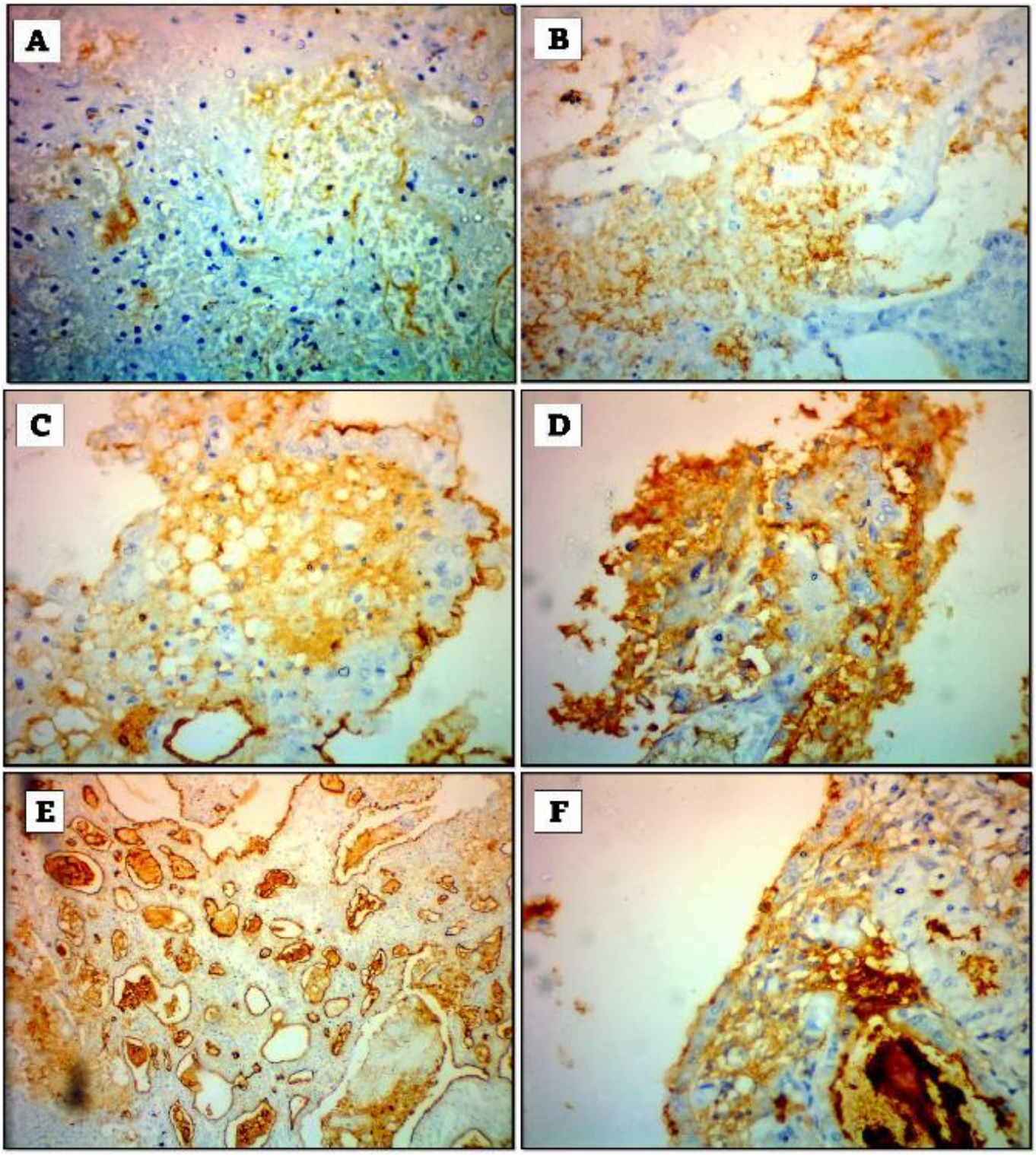

Figure (3): Immunohistochemical expression of CD133 in CRC showing a combined cytoplasmic and luminal surface expression pattern. (A, B): Showed mild expression of CD133 (400X). (C, D): Showed a high power of moderate positive expression of CD133 (400X). (E): Showed strong positive expression of CD133 (100X). (F): Showed a high power of strong positive expression of CD133 (400X) (Figure 3).

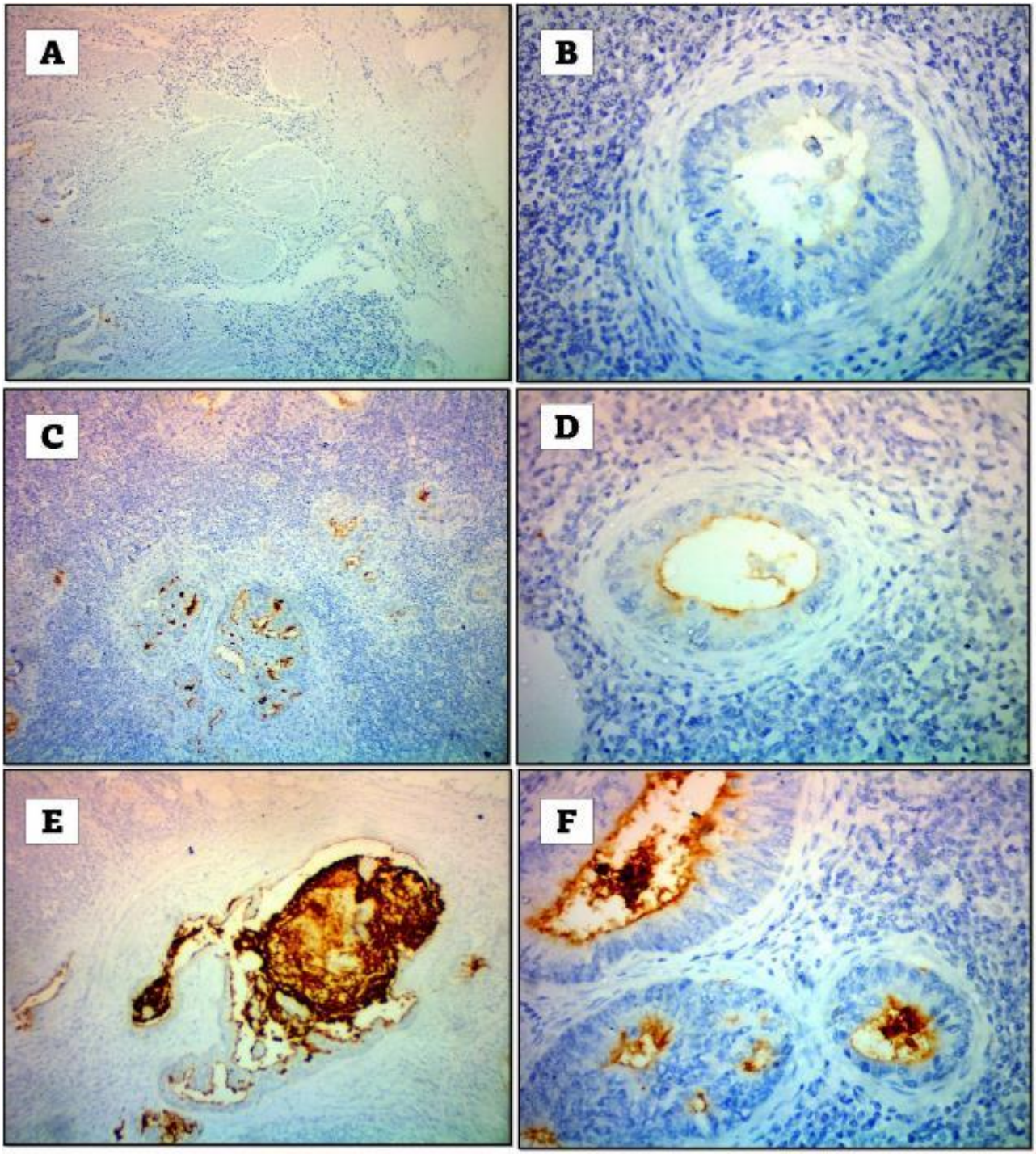

Figure (4): Immunohistochemical expression of CD133 in a metastatic lymph node of colorectal carcinoma showing luminal surface pattern of expression.(A): Showed negative expression of CD133 (100X). (B): Showed a high power of negative CD133 expression (400X). (C): Showed mild positive expression of CD133 (100X). (D): Showed a high power of mild positive expression of CD133 (400X). (E): Showed strong positive expression of CD133 (100X). (E): Showed a high power of strong positive expression of CD133 (400X) (Figure 4).

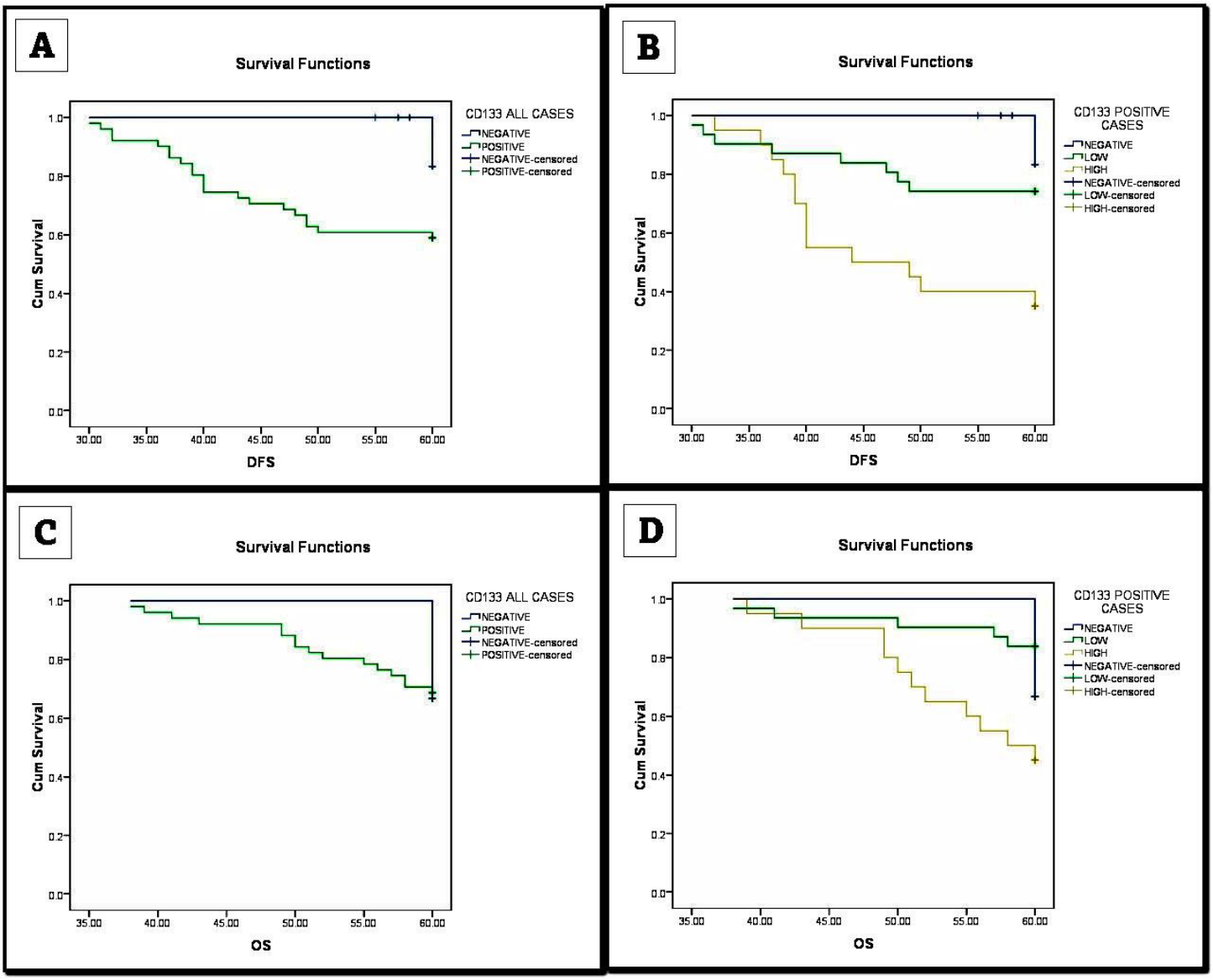

Figure (5): Disease free survival.A: Kaplan Meier plot of disease free survival, (N=60). (B): Stratified by positive CD133 expression; (C): Kaplan Meier graph of overall-survival (OS) (N=60) (D): Stratified by positive CD133 expression (Figure 5).

DISCUSSION

Colorectal cancer (CRC) is the world's 3rd most frequent cancer and the 4th major cause of cancer-related deaths. Its prevalence and death rate are rising worldwide. Approximately 20% of CRC patients have distant metastases at the time of diagnosis, and only 10% to 30% of them are eligible for surgical excision of primary and metastatic tumors. The prognosis is determined by the stage of cancer, with a 5-year survival rate of 90% in patients with carcinoma in situ, 67% in patients with local lesions, and just 10% in patients with distant metastases [14, 15]. In Egypt, colorectal cancer is seen in 5.1 percent of males and 4.7 percent of females. It has been shown that Egyptian patients with colorectal cancer under the age of 30 had a threefold increase in mortality rate within 5 years compared to those with colorectal cancer over 50 [16]. Chemotherapy, combined with effective cytotoxic anticancer drugs has recently been used to treat advanced CRC and cytoreductive surgery for peritoneal dissemination (PD). But after all of these lines of treatment, the prognosis is still not reaching the expected cure rate. So, to improve the outcome of the treatment for colorectal cancer, a powerful biomarker is required [17]. CD133 was identified among cancer stem cells of a variety of cancers like ovarian, kidney, pancreas, lung, and brain cancers, but its role was highlighted in colorectal carcinoma [3, 18]. CD133 positive tumor-initiating cells are capable of differentiation, self-renewal and regeneration [4, 19].

Our study evaluated CD133 expression in stage III colorectal carcinoma with different clinicopathological parameters. 51 (85%) of 60 stage III colorectal cases showed positive expression for CD133. From these positive cases, 20/60 (39.2%) showed high

CD133 expression. This is in alignment with the results of **Rey *et al.*** [20], who found that the correlated percent of CRC patients with high expression of CD133 to be 37.3%. One of the most significant prognostic factors in colorectal cancer is tumour grade. In studying the relation between CD133 and tumor grade, we found that high-grade colorectal carcinoma had a high statistically significant correlation with CD133 expression and its high expression. Accordingly, cancer stem cells may play a vital part in the progression of colorectal cancer. **Rosiq *et al.*** [21] mentioned in their study that CD133 was linked with poorer tumor differentiation (P<0.05). **Chaitra *et al.*** [22] highlighted the significant difference between CD133 expression and moderately differentiated colorectal carcinomas (P=0.03). Furthermore, **Wang *et al.*** [23] found a statistically significant association between poor tumor differentiation and CD133 expression (P = 0.04). On the other hand, **Zhou *et al.*** [24] declared that tumor grading is independent of expression levels of CD133 (P>0.005). This divergence might result from the fact that these researches were conducted in a divergent set of patients, various research designs, and the use of different commercial antibodies.

Our results revealed a positive relation between high CD133 expression and tumor size ≥ 5cm (p < 0.001). This is not in line with **Fathi *et al.*** [25], **Huang *et al.*** [26], and **Rezaee *et al.*** [27], who noticed that the tumor size was independent of CD133 expression levels.

A correlation that can be supported by statistical analysis was shown between high CD133 expression and lymphatic invasion (p<0.001). This may denote the link between CD133 expression and epithelial mesenchymal transition status. **Huang *et al.*** [26] pointed to the connection that exists between the expression of CD133 and lymphatic and vascular invasion. Emphasizing these results, **Rezaee *et al.*** [27] stated that LN and vascular invasion were more prevalent in patients with higher expression of CD133 (P<0.05).

We found that the presence of perineural invasion in grade III colorectal carcinoma was significantly related to CD133 expression (P=0.0017). This is in accordance with **Akbari *et al.*** [3], who found that the high CD133 expression was significantly correlated with perineural invasion (P=.002).We found that lymph node metastasis revealed a statistically significant relationship with CD133 expression (P<0.001). **Park *et al.*** [12] explained the role of CD133 in epithelial mesenchymal transition, in facilitating the dissemination process. In support of our results, **Sun *et al.*** [28] and **Li *et al.*** [29] found a significant association between the expression of CD133 and LN. The cancer stem cell tumorogenicity concept could elucidate the reasons why traditional chemotherapies fail to eradicate cancer [30]. In the course of this research, we found that high expression of CD133 was highly correlated with chemotherapeutic resistance, which made a high expression of CD133 to be a strong prognostic and predictive marker for conventional chemotherapeutic failure in stage III colorectal carcinoma patients. This result is parallel to the results by **Ong *et al.*** [31] and **Ren *et al.*** [32], who found that CD133+ CRC is more resistant to chemotherapy based on 5-fluorouracil. This opens the door to targeting and treating a big problem that has always claimed the lives of a large segment of colon cancer patients. **Yuan *et al.*** [33] and **Akbari *et al.*** [34] found that CD133 targeting reverses drug resistance via the AKT/NF-B/MDR1 pathway, which could offer a viable therapeutic target to reverse MDR in CRC.

There were no statistically significant differences between the expression of CD133 and the sex of the patients, CEA level, and pattern of CD133 expression. While, the category of old age category > 60 years was significantly correlated with high expression of CD133. These results are in accordance with **Okada *et al.*** [35], **Park *et al.*** [12], and **Fathi *et al.*** [25]. Although overall survival (OS) in CRC patients has improved significantly, the median survival time in individuals with metastatic CRC remains less than two years. Despite intensive surgery and postoperative treatment, approximately 1/3 of patients with stage III CRC had tumour recurrence. The presence of tumor-initiating cells, which can proliferate, self-renew, and differentiate into a variety of distinct cancer cell types, is one putative mechanism giving treatment resistance [36]. These cells may be responsible for primary resistance to chemotherapy and relapse after initial treatment. So, to improve adequate management from the start, for CRC a powerful biomarker is required to predict relapse in CRC patients. In our research, we discovered that high expression of CD133 is significantly associated with relapse, mortality, poor disease-free survival, and low overall survival. These results are in agreement with **Huang *et al.*** [26], **Ren *et al.*** [32], **Lim *et al.*** [36], **Horst *et al.*** [37] and **Chen *et al.*** [38] who found that CD133 is a marker for relapse and low survival rates in colorectal cancer.

CONCLUSION

CD133 expression in colorectal carcinoma is associated with tumor progression and is considered a poor prognostic marker and a strong indicator of relapse and poor survival. Moreover, CD133 stem cell marker may act as a targeted therapy in chemotherapeutic resistance patients with colorectal carcinoma.

DECLARATIONS

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