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Research Article

Immunohistochemical Expression of CD44 in colorectal carcinoma

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Abstract

Background: Rectal cancer ranks eighth overall whereas colon cancer is the fourth most prevalent cancer worldwide. Incidence instances of colorectal cancer (CRC) were projected to reach 1.9 million in 2020, with 0.9 million deaths. Due to westernization, CRC is becoming more common in middle- and low-income countries, where it is more prevalent in developed nations. Colon cancer is the ninth most frequent cancer in Egypt and according to WHO figures, it accounts for 2.7% of all cancer cases and 2.4% of all cancer-related fatalities. Rectal cancer is the seventeenth most common cancer, accounting for 1.2% of all cancer cases and 0.98% of all cancer-related deaths. In Egypt, CRC is the sixth most prevalent cancer overall**.** Colorectal cancer spreads and relapses due in large part to stem cells (CSCs). A variety of cell surface markers, including CD44, which is a cell-surface trans-membrane glycoprotein and has been shown to activate several tumour biological behaviours including proliferation, differentiation, invasion, and motility, are used to identify colorectal CSCs. **Aim of the work**: the present study was conducted to study the relationship between CD44 expression and the clinicopathological characteristics of CRC. **Material and Methods:** 140 randomly chosen tissue blocks from primary colorectal adenocarcinomas and their lymph nodes were immunohistochemically stained for CD44. Of these, 53 (75.7%) cases were conventional adenocarcinomas (NOS), 7 (10%) were mucinous carcinomas, and 10 (14.3%) were Signet ring carcinomas. Institutional Review Board" IRB", Minya University has reviewed & approved submitted research proposal" **Approval No. 45:6/2021**. **Results**: CD44 high expression was detected in 86 (61.4%) of cases. A statistically significant association was detected between high CD44 expression and tumor size, tumor grade, poorly differentiated clusters (PDCs) grade, lymph node involvement, Lympho-vascular invasion (LVI) advanced tumor stage, tumor necrosis and tumor infiltrating lymphocytes (P value 0.005*, <0.001*, <0.001*, <0.001*, <0.001*, <0.001*, <0.011 and <0.001*). The association between CD44 high expression and different clinicopathological variables were further tested using univariate and multivariate regression analysis. This study detected that tumor grade, lympho-vascular invasion, modified Dukes staging and PDCs grade were independently associated with CD44expression (P value 0.008*, 0.009*, 0.007*, 0.001* respectively). **Conclusion**: CD44 high expression could be considered as poor prognostic marker in the evaluation of patients with Colorectal Carcinoma. CD44 can play an essential role in the pathogenesis, aggressiveness, invasion, and progression of CRC.

Key Words: Colorectal carcinoma, Clinicopathological Features, Immunohistochemistry, CD44.

Introduction

Rectal cancer is the eighth most common cancer overall, whereas colon cancer is the fourth most prevalent worldwide. Colorectal carcinomas (CRC) are the second most lethal cancer in the world, behind lung cancer, and the third most frequent cancer diagnosis worldwide. They

are responsible for 9.4% of cancer fatalities and 10% of cancer diagnoses overall[1]. Colon cancer is the ninth most frequent cancer in Egypt and according to WHO figures. It accounts for 2.7% of all cancer cases and 2.4% of all cancer-related fatalities. Rectal cancer is the seventeenth most common cancer, accounting for 1.2% of all cancer cases and 0.98% of all cancer-related deaths. (CRC) Both are the sixth most common cancer in Egypt. According to Bray et al., 2018[2]., colon cancer is more prevalent than rectal cancer, with high Age-standardized incidence rates (ASR) in females of 3.3 compared to males of 3.2.

Cancer stem cells (CSCs) are a class of cells that have the ability to self-renew, proliferate indefinitely, differentiate in multiple directions, and stimulate the growth of tumours. According to Munro et al., 2018[3], two mechanisms can cause the development of CSCs: the first is the carcinogenic mutation of normal stem cells, which results in unchecked cell proliferation, and the second is the dedifferentiation of regular cancer cells and their conversion into stem cell-like cells.

A number of cell surface markers, including CD44, CD133, CD24, EpCAM, LGR5, and ALDH, are used to identify colorectal CSCs. They have a crucial role in the metastasis and recurrence of colorectal cancer as well as disease-free survival because they are highly tumorigenic, chemo- and radio-resistant[4].

The functions of CD44 include lymphocyte homing, cell adhesion and aggregation, cell migration, leukocyte activation, lympho-poiesis and myelopoiesis, angiogenesis, and cytokine release. CD44 is highly expressed in lymphocytes, smooth muscle, fibroblasts, and different types of epithelia. Despite being expressed in several tissues, such as the liver, lung, pancreas, skin, and central nervous system, CD44s was initially isolated from hematopoietic cells [5].
When CD44 is knocked down in colon cancer cells, anti-apoptotic molecules like Bcl-2 and Bcl-xL are expressed less and apoptotic molecules like Bax and caspase-3/8/9 are expressed more. Following treatment with the anticancer agent etoposide, AKT phosphorylation, p21, and pRb were downregulated in cancer cells that had been transfected with CD44. This shows that the cell cycle regulators pRb and p21, as well as the pro-survival protein AKT2, are modulated by CD44 expression[6]. High CD44 expression was linked to CRC- lymph node metastases, poor differentiation, and distant metastasis[7].

Material and Methods

1. Tissue specimens

The present study comprised 140 randomly selected tissue blocks of primary colorectal adenocarcinoma and their lymph nodes, metastases if present. The available clinicopathological data included: patient age, sex, tumor size, tumor site, tumor grade, tumor histological subtypes, lymph-ovascular invasion, perineural invasion, poorly differentiated clusters (PDCs) grade, tumor necrosis, tumor infiltrating lymphocytes (TILs), lymph node status and Modified Dukes staging. Tumor type and grade were evaluated according to WHO criteria[8]. Central PDCs were graded by X20 objective lens into 3 grades; Grade 1 has less than 5 PDC clusters; Grade 2 has from 5 to 9 PDCs; Grade 3 has more than 9 PDCs within tumor stroma[9]. Tumor stage was estimated by Modified Dukes Staging[10]. (see table 1).

Table 1: Clinicopathological features for patients with CRC (n=140)

	CRC (N=140)	
	N	**%**
Age (y)		
≤ 45 y	36	25.7%
> 45 y	104	74.3%
Sex		
Male	70	50.0%
Female	70	50.0%
Tumor site		
Right Colon	90	64.3%
Left colon / Rectum	50	35.7%
Histological subtypes		
Conventional	106	75.7%
Signet ring cell carcinoma	20	14.3%
Mucinous carcinoma	14	10.0%
Tumor size		
<5 cm	62	44.3%
≥5 cm	78	55.7%
Nodal status		
Negative	46	32.9%
Positive	94	67.1%
Tumor's grade		
Grade I	24	17.1%
Grade II	60	42.9%
Grade III	56	40.0%
PDC Grade		
PDC Grade 1	26	18.6%
PDC Grade 2	46	32.9%
PDC Grade 3	68	48.6%
Modified Dukes Classification		
Stage A and B	46	32.9%
Stage C and D	94	67.1%
Tumor Necrosis		
Negative	64	45.7%
Positive	76	54.3%
Lymphovascular invasion		
Negative	56	40.0%
Positive	84	60.0%
Perineural Invasion (PNI)		
Absent	114	81.4%
Present	26	18.6%
Tumor infiltrating lymphocytes		
Absent	52	37.1%
Mild	38	27.1%
Moderate	26	18.6%
Marked	24	17.1%

The patients' ages ranged from 18-85y with the mean age was 52.2± 14.54 and median of 45 years. Thirty-six (25.7%) patients were ≤ 45 years and 104 (74.3%) patient were > 45 years. Seventy (50%) were males and 70(50%) were females. Concerning histological subtypes one hundred and six (75.7%) of cases were conventional adenocarcinomas, 14 (10%) cases were MA, and 20 (14.3%) cases were SRCC. The grades of conventional adeno-carcinoma cases were grade I in 24 (22%) of cases, grade II in 50 (47.5)**,** and grade III in 32 (30.3%) of cases, MA and SRCC were considered poorly differentiated tumors (grade III). Nighty (64.3%) of tumors were located primarily in the right colon, however 50 (35.7%) were located in the left colon and rectum.

Tumor size ranged between 2 and 9 cm, with a mean size 5.33 ± 2.15 and a median of 5 cm. sixty-two (44.3%) tumors were < 5 cm while 78 (55.7%) tumors which were ≥ 5 cm. Twenty-four (17.7%) of tumors were grade I, 60 (42.9%) were grade II tumors were, and grade III tumors were 58 (40%). According to poorly differentiated clusters grading, 26 (18.6%) of cases were PDC grade 1, 46 (32.9%) cases were PDC grade 2 and 68 (48.5%) cases were PDC grade 3. At the time of primary diagnosis, 94 (67.1%) patients had positive lymph node metastases. lymphovascular invasion and Peri-neural invasion were present in 84 (60%) and 26(18.6%) of cases respectively. Tumor necrosis was observed in (45.7%) of cases. Based on modified Dukes staging, forty-six of cases (32.9%) were stage A and B while 94 cases (67.1%) were stage C and D. Lymphocytic infiltrate was absent in 52 (37.1%) of cases, 38 (27.1%) of them showed mild lymphocytic infiltrate, 26 (18.6%) tumors displayed moderate lymphocytic infiltrate and 24 tumors (17.1%) had marked lymphocytic infiltrate. The proximal and distal surgical resection margins were free in all cases (100%).

2. Immunohistochemical (IHC) procedure

Five µm sections were prepared on positive charged slides for immunohistochemistry of CD44 primary antibodies utilizing the avidin biotin-peroxidase complex method with diaminobenzidine (DAB) chromogen detection system. Initially tissue sections on the positive charged slides were deparaffinized and rehydrated. Then the endogenous peroxidase was blocked by immersion in a 3% solution of hydrogen peroxide and incubated for 30 minutes. Antigen retrieval was performed by immersing the slides in citrate buffer solution (pH 6) for 2 times (10 minutes each) at 750-W. To block nonspecific background staining, the slides were treated by UV block. Primary antibody CD44 (100 µ, concentrated, ABclonal laboratories, China) were then added and tissue sections were incubated for 1 hour at room temperature (dilution 1:100). Excess reagent was thrown off and the slides were then rinsed gently with buffer solution for 5 minutes. After that Secondary biotinylated antibody was added for each slide for 30 minutes. DAB substrate and chromogen solutions were added to each slide and following that tissue sections were counter stained by Mayer's hematoxylin.

The Positive control for CD44 was normal rat kidney tissue while the negative control tissue sections was obtained by omitting the specific primary antibody from the staining procedure and replaced with PBS.

3. Scoring of Immunostaining

3.1. Scoring of CD44:

CD44 positive staining was detected in the cell membrane and/ or the cytoplasm of the tumor cells. Scoring of CD44 was based on both the intensity and the percentage of immunoreactive tumor cells. the staining intensity (scored from 0–3) multiplied by the percentage of positive cells within 5 high power fields (scored from 0–4). The intensity of CD44 protein expression was scored as: 0 (no staining); 1 (weak staining); 2 (moderate staining); or 3 (strong staining). The percentage of positive cells was scored as: 0 (absence of immunoreactivity); 1 (<10% immune-reactive tumor cells); 2 (10–50% immune-reactive tumor cells); 3(>50 immuno-

reactive tumor cells %). The final score ranges from 0-12. Total score ≤ 3 considered as low/negative expression, while score >3 was considered positive/ high expression [11].

4. Statistical analysis

The data analysis was performed using the IBM SPSS 28.0 statistical package software (IBM; Armonk, New York, USA). Data were expressed both number and percentage for qualitative data and were analyzed by the Chi-square test or Fisher's exact test. A binary logistic regression model was used to evaluate the predictive value of the different variables, using high expression of CD44 as dependent variables. A *p*-value < 0.05 is considered significant.

Results

The current study found that fifty-four (38.6%) of cases exhibited low/negative cytoplasmic CD44 expression, whereas 86 (61.4%) revealed high expression. No statistically significant association was found between CD44 expression and patient's age, sex, and tumor site, perineural invasion (P= 0.974, P= 0.728, P= 0.055, P= 0.365 respectively).

A statistically significant association was observed between CD44 high expression and larger tumor size, higher tumor grade **(Figure 1)**, poorly differentiated clusters (PDCs) grade **(Figure 2)**, regional lymph node involvement **(Figure 3)**, Lymphovascular invasion (LVI) **(Figure 4)**. advanced tumor stage, tumor necrosis **(Figure 5)** and tumor infiltrating lymphocytes **(Figure 6)** (P value 0.005*, <0.001*, <0.001*, <0.001*, <0.001*, <0.001*, <0.011 and <0.001*). see table (2)

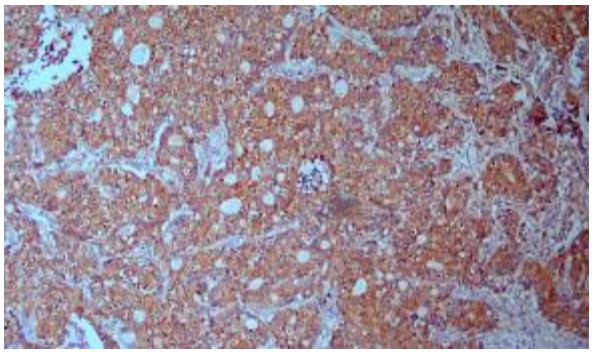

Figure 1: High membranous expression of CD44 in grade III conventional adenocarcinoma (IHC, X400).

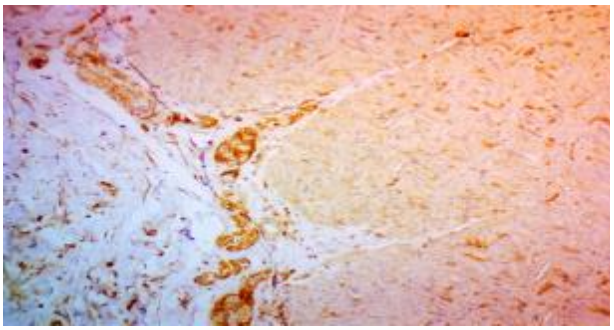

Figure 2: High membranous/cytoplasmic CD44 expression high grade PDCs grade III colorectal adenocarcinoma (IHC, X200)

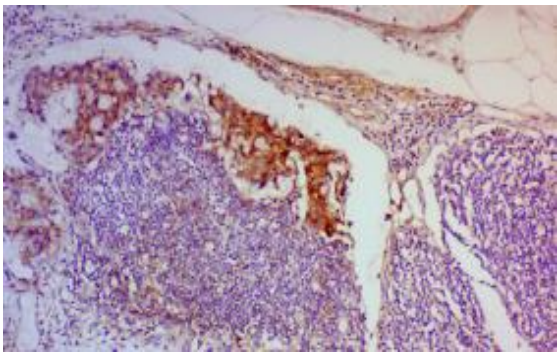

Figure 3: High membranous expression of CD44 in lymph node infiltrated by conventional adenocarcinoma (IHC, X200).

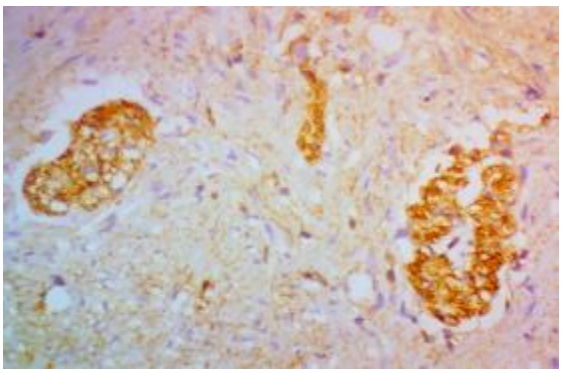

Figure 4: Vascular invasion by conventional adenocarcinoma showing high membranous expression of CD44 (IHC, X400).

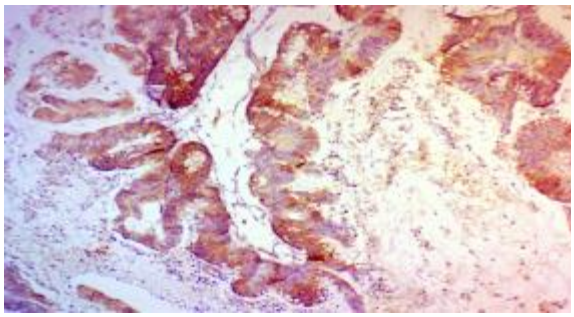

Figure 5: colorectal adenocarcinoma with tumor necrosis showing high membranous expression of CD44 (IHC, X400).

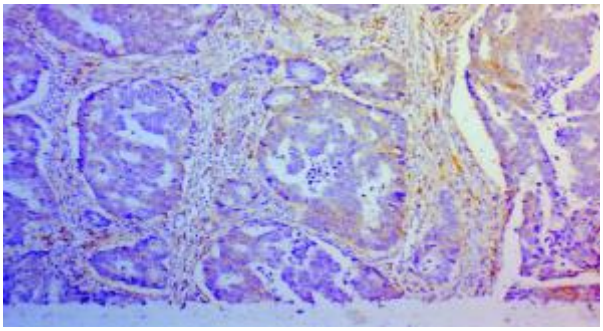

Figure 6: Low membranous expression of CD44 in conventional adenocarcinoma associated with tumor infiltrating lymphocytes (IHC, X200).

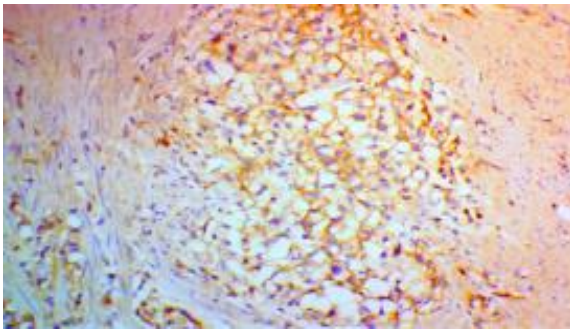

Figure 7: signet ring colorectal adenocarcinoma showing high membranous expression of CD44 (IHC, X400).

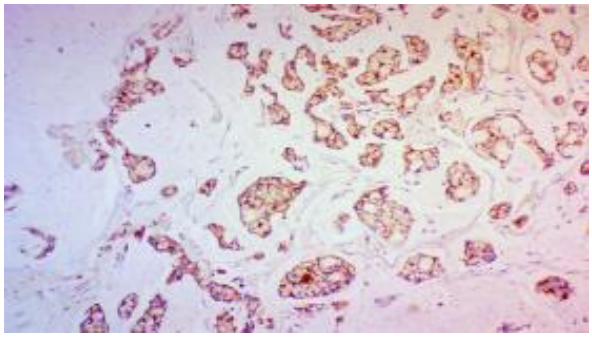

Figure 8: mucinous colorectal adenocarcinoma showing high membranous expression of CD44 (IHC, X400).

Table 2: Association between cytoplasmic CD44expression and clinicopathological features for patients with CRC (n=140)

	CD44		p value
	Low expression (N=54)	High expression (N=86)	
PDC Grade			
PDC Grade 1	22 (84.6%)	4 (15.4%)	<0.001*
PDC Grade 2	10 (21.7%)	36 (78.3%)	
PDC Grade 3	22 (32.4%)	46 (67.6%)	
Age (y)			
≤ 45 y	14 (38.9%)	22 (61.1%)	0.964
> 45 y	40 (38.5%)	64 (61.5%)	
Sex			
Male	28 (40.0%)	42 (60.0%)	0.728
Female	26 (37.1%)	44 (62.9%)	
Tumor site			
Colon	40 (44.4%)	50 (55.6%)	0.055
Rectum	14 (28.0%)	36 (72.0%)	
Histological subtypes			
Conventional	36 (34.0%)	70 (66.0%)	0.129

Signet ring cell carcinoma	10 (50.0%)	10 (50.0%)	
Mucinous carcinoma	8 (57.1%)	6 (42.9%)	
Tumor size			
<5 cm	32 (51.6%)	30 (48.4%)	0.005*
≥5 cm	22 (28.2%)	56 (71.8%)	
Nodal status			
Negative	30 (65.2%)	16 (34.8%)	<0.001*
Positive	24 (25.5%)	70 (74.5%)	
Tumor's grade			
Grade I	22 (91.7%)	2 (8.3%)	<0.001*
Grade II	18 (30.0%)	42 (70.0%)	
Grade III	14 (25.0%)	42 (75.0%)	
Modified Dukes Classification			
Stage A and B	32 (69.6%)	14 (30.4%)	<0.001*
Stage C and D	22 (23.4%)	72 (76.6%)	
Tumor Necrosis			
Negative	32 (50.0%)	32 (50.0%)	0.011*
Positive	22 (28.9%)	54 (71.1%)	
Lymphovascular invasion			
Negative	36 (64.3%)	20 (35.7%)	<0.001*
Positive	18 (21.4%)	66 (78.6%)	
Perineural Invasion (PNI)			
Absent	46 (40.4%)	68 (59.6%)	0.365
Present	8 (30.8%)	18 (69.2%)	
Tumor infiltrating lymphocytes			
Absent	14 (26.9%)	38 (73.1%)	<0.001*
Mild	10 (26.3%)	28 (73.7%)	
Moderate	12 (46.2%)	14 (53.8%)	
Marked	18 (75.0%)	6 (25.0%)	

*** P - value < 0.05 are considered statistically significant according to Chi-Square test and Fisher's exact test.**

Association of CD44 expression with different clinicopathological variables were further tested using univariate and multivariate analysis. The current study found that tumor grade, PDCs grade, modified Dukes staging and lymphovascular invasion were independently associated with CD44 expression (P value 0.008*, 0.009*, 0.007*, 0.001* respectively). See table 3.

Table 3: Univariate and Multivariate regression analysis of factors predicting high CD44 expression.

	CD44			
	crude OR (95% CI)	**p value**	**aOR (95% CI)**	**p value**
Age (y)				
≤ 45 y	1 (reference)			
> 45 y	1.02 (0.47-2.22)	0.964		
Sex				
Male	1 (reference)			
Female	1.13 (0.57-2.23)	0.728		
Tumor site				
Colon	1 (reference)			
Rectum	2.06 (0.98-4.33)	0.058		
Histological subtypes				
Conventional	1 (reference)			
Signet ring cell carcinoma	0.51 (0.20-1.35)	0.177		
Mucinous carcinoma	0.39 (0.12-1.20)	0.099		
Tumor size				
<5 cm	1 (reference)			
≥5 cm	2.72 (1.35-5.47)	0.005*		
Nodal status				
Negative	1 (reference)			
Positive	5.47 (2.55-11.74)	<0.001*		
Tumor's grade				
Grade I	1 (reference)			
Grade II	25.67 (5.45-120.84)	<0.001*	18.42 (2.13-158.97)	0.008*
Grade III	33.00 (6.87-158.43)	<0.001*	9.72 (1.38-68.45)	0.022*
PDC Grade				
PDC Grade 1	1 (reference)			
PDC Grade 2	19.80 (5.53-70.86)	<0.001*	15.19 (1.97-117.30)	0.009*
PDC Grade 3	11.50 (3.53-37.44)	<0.001*	1.43 (0.21-9.56)	0.713
Modified Dukes Classification				
Stage A and B	1 (reference)			
Stage C and D	7.48 (3.40-16.47)	<0.001*	6.74 (1.69-26.90)	0.007*
Tumor Necrosis				
Negative	1 (reference)			
Positive	2.46 (1.22-4.93)	0.012*		
Lymphovascular invasion				
Negative	1 (reference)			
Positive	6.60 (3.10-14.05)	<0.001*	15.50 (3.00-80.14)	0.001*
Perineural Invasion (PNI)				
Absent	1 (reference)			
Present	1.52 (0.61-3.79)	0.367		
Tumor infiltrating lymphocytes				
Marked	1 (reference)			
Absent	1.03 (0.40-2.66)	0.949		
Mild	0.43 (0.16-1.15)	0.093		
Moderate	0.12 (0.04-0.37)	<0.001*		

N.B. Dependent variable high CD44 expression, a OR adjusted odds ratio, CI confidence interval, NE not estimated R2= 0.431

Discussion

The current study included 140 cases of CRC with patients mean age 52.2 years ± SD 14.539 and the median was 45 years. This was in a line with several previous studies performed by Holah et al., 2017; Mohamed et al., 2019 and Sharaf El Din et al., 2022 [12.13.14]. on the other hand, other studies reported an older mean of age ranging from 60 to 93 years [15.16.17]. In this series 55.7% of tumours were ≥ 5 cm (median level) in size while 44.3% were < 5cm. Also, Zhu et al., 2022 and Sugiyama et al,2022 reported that 68.7% and 53.2% of case were ≥ 5cm respectively [18.19] on the other hand Mohamed et al., 2019 reported that 68.7 of cases were less than 5cm [13]. As regarding this study 64.3% of the tumors were located at right side of colon while, 35.7% were located at left side of the colon and rectum. This was close to finding reported by Said et al., 2022 who reported that 68.5% of the tumours were located at right side of colon while 32.5% were located at left side of colon and rectum [20]. however, Mohamed et al., 2019 and Sharaf El Din et al., 2022 reported more percentage of tumors in left side of colon and rectum [13.14]. With respect to tumor grades 17.7% of cases were low grade while 82.9% were high grade. This was in line with Said et al., 2022 who reported 10.5% of their cases were low grade [20]. On the other side, Rezaee et al., 2021 Reported 42.1 % of their cases were low grade[17]..

On current study PDC grade 18.6%% of cases PDC grade 1, 32.9% of cases were grade 2 and 48.6% cases were grade 3. PDC grade has an important prognostic impact on CRC prognosis [21]. Histological subtypes were adenocarcinoma, NOS (75.7%), mucinous adenocarcinoma (10%) and (14.3%) were signet ring carcinoma. This was in line with Said et al., 2022; Mohamed et al., 2022 and Sharaf El Din et al., 2022 who reported the predominance of adenocarcinoma, NOS subtype over the other two subtypes in their studies[20.13.14]. Regarding regional lymph node involvement 67.1% of cases had positive lymph node metastasis while 32.9 % were without lymph node metastasis. This was in accordance with Rezaee et al., 2021 who reported a slight lower percentage of cases with lymph node involvement [17]. The present study included 32.9 % of cases modified Dukes stage A and B and 67.1% modified Dukes stage C and D. this was in line with Sharaf El Din et al., 2022 and Said et al., 2022 who reported advanced tumor stage at 70.6% and 63.6 of their study cases [14.20]. On the other hand, Ji et al., 2014 and Rezaee et al., 2021 reported that 55% and 60% of their cases were early stage respectively [22.17]. This may be attributed to widely used screening programs that led to early detection of CRC.

Regarding lymphovascular invasion, 60% of cases showed lymphovascular invasion this was in agreement with Said et al., 2022 detect lymphovascular invasion in 78%[20]. However, Sharaf El Din et al., 2022 reported lymphovascular invasion in 40.2 of cases[14]. Lymphocytic infiltration was high in 17.1% of the tumors. This was in agreement with Sharaf El Din et al., 2022 who reported that 12.5% of tumors showed high lymphocytic infiltration[14]. In the current study, perineural invasion was present in about 18.6 % of cases. This was in a line with Ko and Pyo, 2019 and Rezaee et al., 2021 who noted presence of perineural invasion in about 16.7% and 20.1 of their cases respectively[23.17]. On the other hand, Bassam et al., 2021 reported presence of perineural invasion in 41 % of cases[24].

Tumor necrosis occur often in human solid cancers and is associated with unfavourable prognosis[25]. In this study, tumor necrosis was present in 54.3 % of tumors. This finding is close to Richards et al., 2012 who reported 42.3 % of tumors had necrosis respectively [25]. On the opposite side Väyrynen et al., 2016 reported tumor necrosis in 95.9 % in their studies [25].

The current study revealed that CD44 was positively expressed in 61.4 % of tumors. This finding was in agreement with Khelwatty et al., 2019 and Mohamed et al.,

2019 who reported positive CD44 expression in 58% and 64.5% of cases respectively [27.13]. However, Sadeghi et al., 2019 revealed a lower positivity 24% of cases [28].

In the current study CD44 positive expression was significantly associated with tumor size being more expressed in tumor size ≥5 cm. this finding was in agreement with Zhu et al., 2018; Mohamed et al., 2019 and Wang et al., 2019 [18.13.14]. On the other hand, Sadeghi et al., 2019 reported no significant association between CD44 expression and large tumor size[28].

In the present study CD44 positive expression was significantly associated with high tumor grade than lower tumor grade. This finding was similar to that reported by Zhu et al.,2018; Mohamed et al., 2019; Han et al., 2019 and Wang et al., 2019[18.13.29.4]. This finding was inconsistent with previous studies who reported no significant association between CD44 and tumor grade[28.27]. The possible explanation for this difference is the use of different scoring systems and different study sample size.

Concerning tumor stage, there was a significant association between CD44 and advanced tumor stage, CD44 was more expressed in modified Dukes stage C and D than modified dukes stage A and B. This result was in analogy with several previous studies[18.13.29.27.30]. However, Wang et al., 2019 showed no association between CD44 expression and tumors stage[4]. This may be due to different staging system and the use of different clones of antibodies.

Regarding lymphovascular invasion, CD44 expression was significantly associated with presence of lymphovascular invasion. This finding was compatible with Bhavikatti et al., 2023 who concluded the same association[30]. However, these finding were not in accordance with Sadeghi et al., 2019 who found no significant association between CD44 and lymphovascular invasion[28]. This difference may be due to the use of different clones of antibodies and lower percentage of cases who had lymphovascular invasion in their studies.

A statistically significant association p between CD44 expression and tumor necrosis. Finding also detected by Muys et al., 2021[31]. A statistically significant association between CD44 expression and degree of lymphocytic infiltration. Finding also detected by Mohamed et al., 2018 [13]. This is the first work that valued the association between CD44 expression and grade of PDCs. The current study detected a statistically significant association between CD44 and PDCs grade.

Multivariate regression analysis confirmed the independent association between positive CD44 expression and poor prognostic factors including high tumor grade, lymphovascular invasion, advanced tumor stage and PDCs grade suggesting the role of CD44 expression in tumor aggressive behavior.

All authors have no conflict of interest.

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