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Correlation of CDX2 Protein Expression with Clinicopathologic Features and Survival Rate in Iraqi Patients with Colorectal Cancer

Muntadher J. Jahil, Karrar S. Zayed*

Department of Laboratory Investigations, Faculty of Science, University of Kufa, Najaf, Iraq

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ABSTRACT

Predicting the prognosis of colorectal cancer (CRC) is challenging since these tumors exhibit a wide range of biological behaviors. It has been hypothesized that caudal-related homeobox gene 2 (CDX2), which is vital for intestinal growth and maintenance, has a tumor-suppressing effect and promising role in CRC prognosis but studies are still controversial. This study used the immunohistochemical (IHC) staining method to determine the expression of the CDX2 protein in mucinous and non-mucinous CRC adenocarcinoma, as well as in normal colorectal tissues as a control, and correlate this expression with clinicopathological features such as grade, tumor distant metastasis, tumor site, histological type, lymph node metastasis, tumor invasion, sex, age, and rate of 4 years Overall survival (OS) after diagnosis. A total of sixty three tissue samples were obtained from CRC patients (58.90±14.94) years and embedded in wax and thirty-seven normal non-tumoural colorectal tissue samples with (56.43±12.28) years as a control group. CDX2 protein expression decreased significantly ($p<0.05$) in CRC patients than control, advanced age, mucinous pattern of CRC, moderate and poorly differentiated grades, lymph node metastasis, advanced tumor invasion (T3, T4), and organs metastasis. Moreover, the (OS) for patients with low CDX2 expression was (17.943±1.7) months compared to (33.431±2.7) months for those with high CDX2 expression ($p = 0.0001$). This study concluded that protein expression of CDX2 is regarded as a prognostic and diagnostic marker for CRC patients.

1. Introduction

Colorectal cancer (CRC) is the main cause of mortality and morbidity in the world. Approximately 1,148,515 new cases will be detected in 2020, and 576,858 deaths will be related to it, making it is the world's third most prevalent cancer after lung and breast cancer (Sung *et al.* 2021). In, Iraq it accounts for 4.6%, and 8.4% of all cancer diagnoses in women and men respectively, making it the eighth most prevalent disease overall (Bray *et al.* 2020). Although the diagnosis of CRC is apparent in the primary site, it may constitute a diagnostic difficulty in a metastatic tumor of uncertain primary origin (Bayrak *et al.* 2012). Since adenocarcinomas of an unclear source site are among the most confusing clinical problems, it is imperative that reliable diagnostic indicators are established, which approve or exclude colorectal origin (Su *et al.* 2008). Furthermore, typical the prognosis of CRC is commonly estimated using prognostic factors such as histopathological type, histologic grade, and stage; however, patients with the same stage or histologic grade often demonstrate inhomogeneous biological behaviour. In addition, there currently exist no effective predictive biomarkers for CRC (Slik *et al.* 2019).

The caudal-related homeobox 2 (CDX2) transcription factor is required for normal intestinal differentiation, development, cell growth, migration, and tumor development (Simmini *et al.* 2014). The paraHox gene cluster on chromosome 13q12 contains the CDX2 gene, which codes for this protein (Olsen *et al.* 2014). Only the nuclei of intestinal epithelial cells (which found in intestines from duodenum to rectum) contain CDX2 (Slik *et al.* 2019). CRC patients with CDX2 positivity have significant nuclear immunoreactivity, however in 10 to 30% of

* Corresponding Author
E-mail Address: karrars.alshebly@uokufa.edu.iq

patients, CDX2 expression decreases or is complete loss (Bae *et al.* 2015). In addition, classical prognostic indicators including histological grading and stage are correlated with decreased CDX2 expression (Matsuda *et al.* 2010; Xu *et al.* 2019). Despite the fact that the tumor suppressor CDX2 has been associated with colon cancer, mutations in the CDX2 gene and the loss of CDX2 expression in cancer tissue are extremely rare (Slattery *et al.* 2007).

CDX2 expression in CRC patients is still up for debate, as various studies suggest both high (Salari *et al.* 2012; Shigematsu *et al.* 2018; Subtil *et al.* 2007) and low expression in CRC patients (Asgari-Karchekani *et al.* 2019; Sjoerd *et al.*2021; Xu *et al.* 2019). Besides, there was a study that revealed CDX2 expression is correlated with less histological differentiation, higher staging, and invasive tumoral growth (Oslen *et al.* 2014), while a few conflicting studies showed no association (Hong *et al.* 2013; Kim *et al.* 2013). For CRC patients, clinical and pathological variables, such as stage and tumor grade, are commonly used to predict clinical outcomes and prognosis, therefore new biomarkers with prognostic value should be developed and employed in follow-up (Asgari-Karchekani *et al.* 2019). Downregulation of CDX2 expression has been correlated with CRC progression, and the results of various studies suggest this gene may have tumor suppressor activity, though the extent to which this activity correlates with prognosis, patient survival, and clinicopathologic characteristics is still debatable (Cecchini *et al.* 2019; Xu *et al.* 2019). Previous researches have demonstrated that a low CDX2 expression is associated with a worse prognosis and shorter survival in CRC patients (Çalik *et al.* 2020; Zheng *et al.* 2017). Therefore, the purpose of this study was to determine whether or not CDX2 expression was associated with better outcomes for people with CRC. Classical prognostic indicators were also studied in regards to CDX2 expression.

2. Materials and Methods

2.1. Collection of Samples

Sixty-three CRC patients were examined for this study, using data acquired retrospectively from the archives of the Middle Euphrates unit of cancer research. All of the patients had undergone surgical resection between 2015 and 2020 and the cancer research unit collected the samples in conformation to standard ethics and with written informed consent. Each pathological specimen was reevaluated histologically by two different pathologists. Data of the age, sex, tumor site, grade, lymph node metastasis, invasion stage, and distant metastasis of patients with mucinous and non-mucinous CRC adenocarcinoma, as well as normal colorectal tissue subjects as controls, were obtained from the electronic medical records of these laboratories. All these data were used to detect the level of CDX2 protein expression using IHC. After getting the CRC paraffin blocks, it has been followed up the data of death for these blocks of CRC patients revealed 20 patients' death (event), 7 patients' survival (censored), and 36 patients missing. OS can be determined by monitoring patients from the time of surgery until the death occurs, excluding those who are still alive at the time of the last follow-up which called censoring patients (Asgari-Karchekani *et al.* 2019). On the other hand, thirty-seven blocks of normal, colorectal tissue were randomly selected during the collection of malignant tissue blocks as a control group and were then diagnosed by the same two pathologists to ensure they're perfectly normal.

2.2. CDX2 Immunohistochemistry Staining

The Dako K8002 kit was used to detect IHC (Dako, Glostrup, Denmark). FLEX IHC Microscope Slides (Dako K8020) was used to dry tissue sections (4 µm thick) for one hour at 60°C. Retrieval Solution, pH 9, 0.01%, was used for deparaffinization and antigen demasking for 20 minutes at 97 degrees Celsius. EnVision FLEX Peroxidase-Blocking Reagent was used for 5 minutes to inhibit the activity of endogenous peroxidase. Tissue slides were treated for 30 minutes of incubation with a mouse monoclonal antibody diluted 1:75 in antibody diluent. EnVision FLEX+, Mouse Linker, and EnVision FLEX/HRP were used to amplify the antibody signal for 20 minutes and 30 minutes, respectively to detect secondary antibodies. Each stage was followed by a wash in Buffer of EnVision FLEX. Tris-buffered saline (pH 7.6) with 0.5% copper sulfate (pH 7.6) was used for 10 minutes to amplify the signal from diaminobenzidine (DAB). DAB+Chromogen diluted in substrate buffer was used for the visualization. Hematoxylin was used to counterstain all of the sections. CDX2-stained cases were included in all IHC staining batches to serve as positive controls for the CDX2 IHC stain, and primary antibody-free negative controls were also included.

2.2.1. Result of Staining

Tumor cells only had to show positive nuclear staining to be considered. Staining intensity was taken in to account with staining ratio (the percentage of stained cells). The results of the staining ratio scoring method were 0(0%), 1(larger than 0 to 25%), 2(larger than 25 to 50%), 3(larger than 50 to 75%), and 4(larger than75%), cells with no staining were assigned a score of 0; cells with a very light staining intensity were assigned a score of 2; cells with a moderate staining intensity were assigned a score of 3; and cells with a very high staining intensity were assigned a score of 4. We calculated the total staining score by multiplying the intensity score by the percentage score. Samples with a staining score of 4 or lower belonged to the low expression group, whereas those with a score of 5 or more belonged to the high expression group (Çalik *et al.* 2020).

2.3. Statistical Analyses

Analyses of data were performed in SPSS 21, numerical variables represented by mean ± SE, and nominal variables expressed by number and percentage. Student t-test was used for comparing the averages of two groups while to compare frequency distributions, the chi-square test was employed, and risk assessment was performed using an odd ratio with a 95% confidence interval. Kaplan-Meier analysis was used to examine the correlations between CDX2 expression and OS (log-rank test). A P-value of less than 0.05 was considered to indicate statistical significance.

3. Results

3.1. Clinicopathologic Features in the CRC Patients

The current study was achieved on 63 CRCs with 36 (57.1%) males and 27 (42.9%) females with mean age (58.90±14.94) years, (68.3%) of patients with >50 years while patients with ≤50 years were (31.7%). Majority of cases were Non-mucinous adenocarcinoma accounting for 50 patients (79.4%) while Mucinous adenocarcinoma was seen in 13 (20.6%) of patients. Regarding CRC histological grades, 13 patients (20.6%) had well differentiated CRC, 37 patients (58.8%) had moderate differentiated CRC and 13 patients (20.6%) had poorly differentiated CRC. Furthermore, it was obvious that the right colon is the most common site for CRCs in patients accounting for 27 (43%) of CRCs cases than the left colon 19 (30%) and rectum 17 (27%). Also, tumor invasion 5(7.9%) of CRC patients were T1 while 11(17.5%), 25(39.7%), and 22(34.9%) were T2, T4, and T3 respectively. The assessment of lymph node metastasis in CRC patients revealed that 26(41.3%), 10(15.9%) of patients had lymph node metastasis and distant metastasis while 37(58.7%), 53(84.1%) of patients missing lymph node and organs metastasis respectively. According to available data for CRC, 20 patients (31.7%) with CRC died while 7(11.1%) and 36(57.1%) patients were censored (survived) and missing from follow up respectively as shown in Table 1.

3.2. Nuclear Expression of CDX2 in CRC Patients and Controls

Low expression of CDX2 in control and patient groups were 0(0.00%) and 30(47.6%), respectively, on the other hand, high expression of this protein were 37(100%) and 33(52.4%) in control and patient groups respectively, so the high expression decreased significantly in CRC patients when compared with control (p = 0.001), Odd ratio (95%CI) = 2.12(1.65-2.71) as illustrated in Table 2, also, the CDX2 expression

Table 1. Clinicopathologic features of CRC patients

Clinicopathologic characteristics	No.	%
Age:		
≤ 50	20	31.7
>50	43	68.3
Sex:		
Male	36	57.1
Female	27	42.9
Histological types:		
Non-mucinous adenocarcinoma	50	79.4
Mucinous adenocarcinoma	13	20.6
Grade:		
Well-differentiated	13	20.6
Moderate differentiated	37	58.8
Poorly differentiated	13	20.6
Site of tumor:		
Right colon	27	43.0
Left colon	19	30.0
Rectum	17	27.0
Depth of invasion:		
T1	5	7.9
T2	11	17.5
T3	25	39.7
T4	22	34.9
Lymph node metastasis:		
Present	26	41.3
Absent	37	58.7
Distant metastasis:		
Present	10	15.9
Absent	53	84.1
Rate of survival:		
Event (death)	20	31.7
Censored	7	11.1
Missing	36	57.1

Results expressed as ratio and analysis by Chi-square test

of patients with CRC in weak, moderate and strong staining shown in Figure 1. Furthermore, patients revealed a statistically significant increase in CDX2 negative expression compared to controls (p = 0.001), three cases(4.8%) had weak expression(+1), two cases(3.2%) had moderate expression(+2), seventeen cases(27%) and sixteen cases (25.4%) had strong expression(+3) and (+4) respectively, in contrast, all control subjects had high CDX2 expression 4 cases(10.8%) and 33 cases(89.2%) were +3 and +4 respectively as seen in Table 3. Moreover, with regards to the staining intensity of CRC cells, patients showed a statistically significant increase in IHC intensity staining of cells, twenty five cases (39.7%) compared to controls 0 cases (0.0%) (p = 0.001) as shown in Table 4. So, staining scores were calculated by multiplying the intensity score by the percentage score, with a final staining value of 4 indicating low CDX2 expression and a score of >4 indicating strong CDX2 expression, as shown in Table 2.

Table 2. Nuclear CDX2 expression in CRC patients compared to healthy controls

Nuclear CDX2 expression	Control		Patients		P. value	Odd ratio (95%CI†)
	No.	%	No.	%		
Low expression	0	0.00	30	47.60	0.001	2.12(1.65-2.71)
High expression	37	100.00	33	52.40		
Total	37	100.00	63	100.00		

Results expressed as ratio and analysis by Chi-square test, CDX2: caudal-related homeobox2, CRC: colorectal cancer, CI†: confidence interval

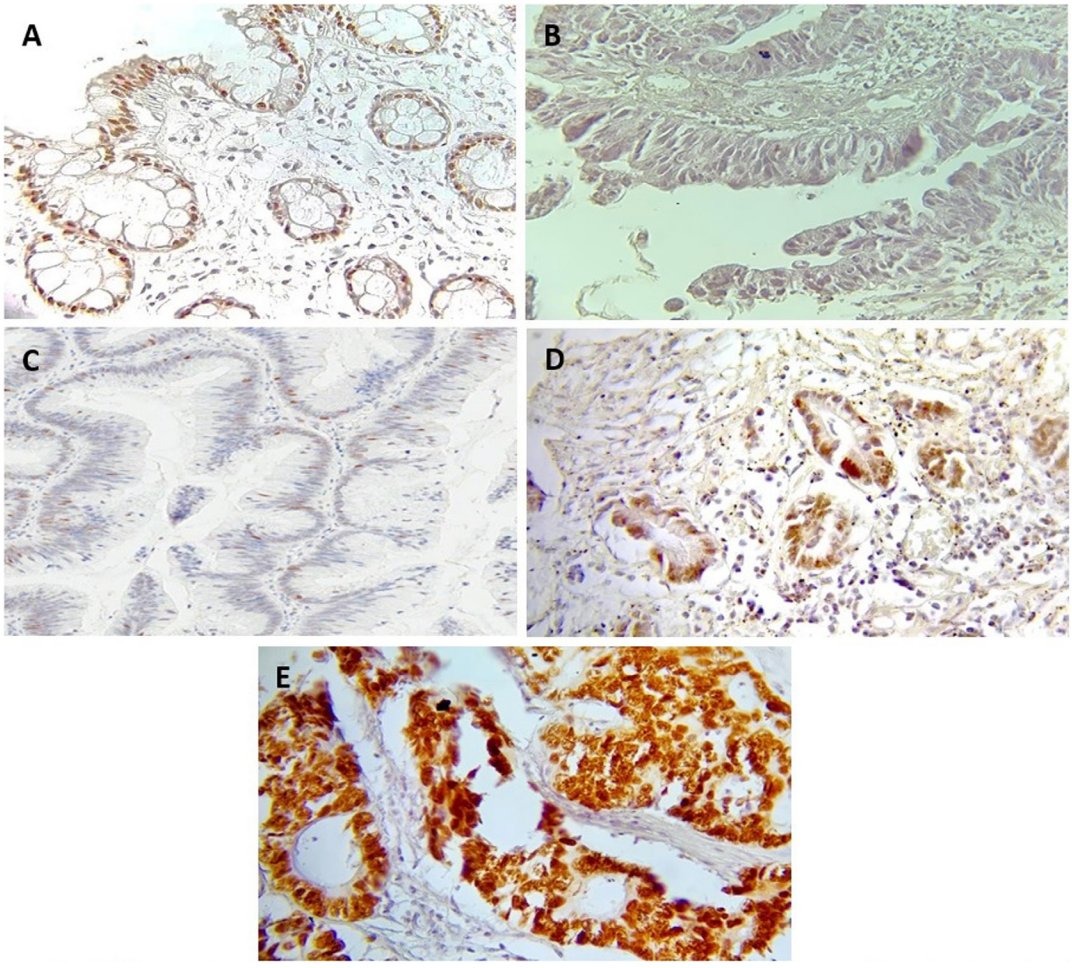

Figure 1. The CDX2 expression status of patients with CRC. (A) Normal epithelial cells in the mucosa of the colon showed positive nuclear staining with CDX2 (400X), (B) adenocarcinoma of the colon showed negative nuclear staining of tumor cell for CDX2 (400X), (C) adenocarcinoma of the colon showed weak nuclear positive staining of tumor cell for CDX2 (400X), (D) adenocarcinoma of the colon showed moderate nuclear positive staining of tumor cell for CDX2 (400X), (E) adenocarcinoma of the colon showed strong nuclear staining of tumor cell for CDX2 (400X)

3.3. Association between CDX2 Protein Expression and Clinicopathologic Features

CDX2 expression was decreased significantly (p = 0.014) in CRC patients with >50 years, distant metastasis, non-mucinous pattern of tumor, lymph node metastasis, poorly differentiated CRC and T3 and T4 stage, and (p<0.05) as presented in Table 5.

3.4. Differences in OS between Positive CDX2 and Negative CDX2 Expression in BC Patients

Kaplan-Meier analysis revealed that the OS was (61.77%) in patients with low CDX2 expression and (79.12%) in patients with high CDX2 expression; furthermore, patients with low CDX2 expression had a shorter OS (17.9431.7 months) than those

Table 3. CDX2 protein expression in CRC patients and control subjects according to a scoring system

Scoring	Control		Patients		Total	
	No.	%	No.	%	No.	%
0	0	0.0	25	39.7	25	25.0
+1	0	0.0	3	4.8	3	3.0
+2	0	0.0	2	3.2	2	2.0
+3	4	10.8	17	27.0	21	21.0
+4	33	89.2	16	25.4	49	49.0

Results expressed as ratio and analysis by Chi-square test, CDX2: caudal-related homeobox2, CRC: colorectal cancer p = 0.001

Table 4. IHC Intensity staining in cells of CRC patients and control subjects

Intensity	Control		Patients		Total	
	No.	%	No.	%	No.	%
0	0	0.0	25	39.7	25	25.0
1	0	0.0	3	4.8	3	3.0
2	0	0.0	12	19.0	12	12.0
3	37	100.0	23	36.5	60	60.0

Results expressed as ratio and analysis by Chi-square test, CDX2: caudal-related homeobox2, CRC: colorectal cancer p = 0.001

Table 5. Protein expression of CDX2 in CRC patients and clinicopathologic features

Clinicopathologic features	CDX2 expression				P. value
	Low		High		
	No.	%	No.	%	
Age:					
≤ 50	5	25.0	15	75.0	0.014
>50	25	58.1	18	41.9	
Sex:					
Male	17	47.2	19	52.8	0.94
Female	13	48.1	14	51.9	
Histological types:	27	54.0	23	46.0	
Non-mucinous adenocarcinoma	3	23.1	10	76.9	0.047
Mucinous adenocarcinoma					
Grade:	1	7.7	12	92.3	
Well-differentiated	19	51.4	18	48.6	
Moderate differentiated	10	76.9	3	23.1	0.002
Poorly differentiated					
Site of tumor:	13	48.1	14	51.9	
Right colon	10	52.6	9	47.4	
Left colon	7	41.2	10	58.8	0.788
Rectum					
Depth of invasion:	0	0.0	5	100.0	
T1	3	27.3	8	72.7	
T2	15	60.0	10	40.0	0.04
T3	12	54.5	10	45.5	
T4					
Lymph node metastasis:		73.1	7	26.9	
Present	19	29.7	26	70.3	0.01
Absent	11				
Distant metastasis:		80.8	2	20.0	
Present	8	41.5	31	58.5	0.025
Absent	22				

Results are expressed as ratio and analysis by Chi-square test. CDX2: caudal-related homeobox 2, CRC: colorectal cancer

with high CDX2 expression (33.4312.7 month) (P = 0.0001), Odds ratio (95%CI) = 2.18(1.29-3.70), as shown in Table 6 and Figure 2.

4. Discussion

CDX2 encodes a homeodomain transcription factor essential for proper intestinal epithelium maintenance and development (Beck 2002). Since CDX2 expression is a nearly specific marker for gastrointestinal neoplasms, notably CRC (Kaimaktchiev *et al.* 2004). A significant decrease in high CDX2 protein expression was observed in tissues from CRC patients compared to control tissues that have no one with low expression of CDX2 while approximately half of CRC patients enrolled in this study had low expression of CDX2. Consistent with the current findings, prior studies has shown that the CDX2 expression level in CRC patients was decreased when compared with CDX2 expression in control with normal colorectal mucosa (Choi *et al.* 2006; Hinoi *et al.* 2001; Knösel *et al.* 2012; Matsuda *et al.* 2010; Winn *et al.* 2009). As well as playing a crucial role in intestinal growth and differentiation, CDX2 is also known to exert a tumor-suppressor role in CRCs. Evidence for the tumor-suppressor activity of CDX2 in CRCs includes an increase in tumor susceptibility in heterozygous Cdx2+/- mice, rapid progression from G1 to S phase, and decreased CDX2 levels are associated with increased chromosomal instability in colon cancer cell lines (Bonhomme *et al.* 2003; Saandi *et al.* 2013).

Table 6. OS in low and high CDX2 protein expression in CRC patient

CDX2 protein expression	Mean OS Survival ± SE (Months)	Event (death)		OS Percentage %	P. value	Odd (95%CI†)
		N	%			
Low Expression	17.943±1.7	12	60	61.77	0.0001	2.18
High Expression	33.431±2.7	8	40	79.12		(1.29-3.70)

Results expressed as mean ± SE and analysis by independent T-test, CDX2: caudal-related homeobox 2, CRC: colorectal cancer, OS: overall survival, CI†: confidence interval

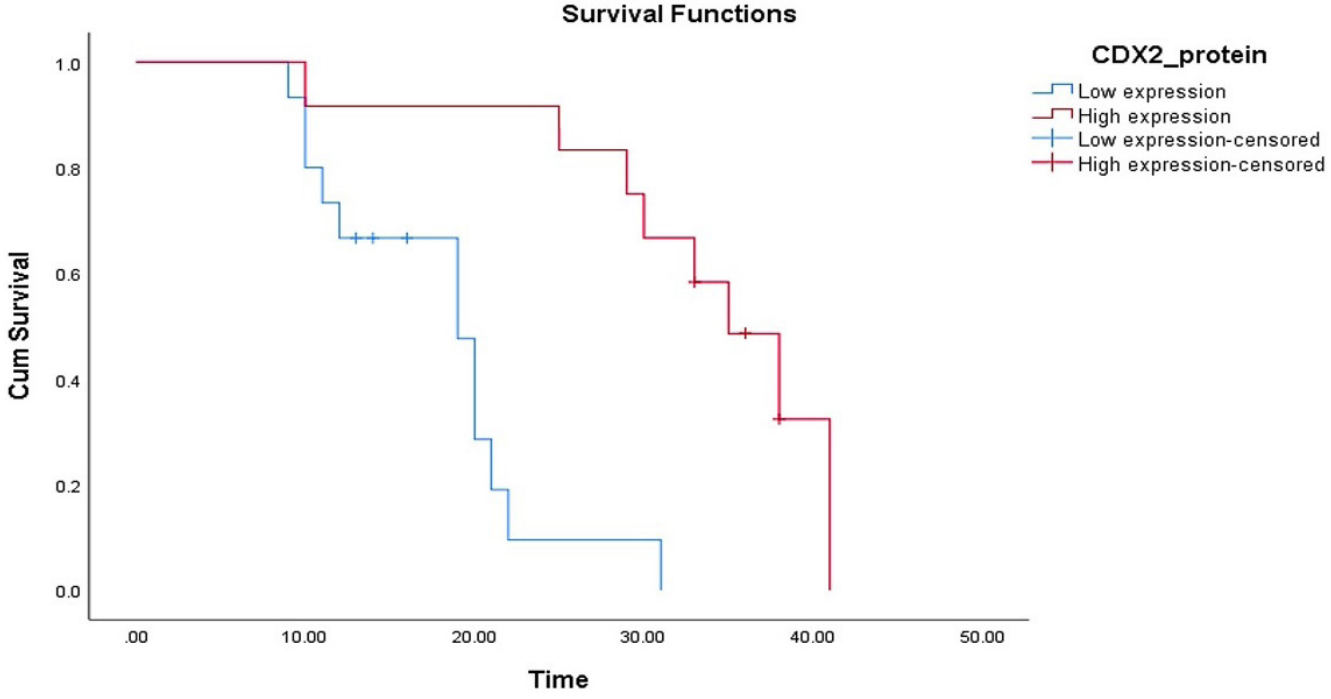

Figure 2. Kaplan-Meier survival analysis of patients with CRC based on their CDX2 expression status. (p = 0.0001). Red: high expression, Blue: low expression, CDX2: caudal-related homeobox 2, CRC: colorectal cancer, OS: overall survival

Other studies didn't compare CDX2 expression levels to that found in the normal colon but they focused only on the differences between low and high expression of CDX2 in CRC patients, so the current finding is consistent with these studies have been recorded by Knösel *et al.* (2012), Sjoerd *et al.* (2021), Xu *et al.* (2019), Asgari-Karchekani *et al.* (2019), Dawson *et al.* (2013), Baba *et al.* (2009) and Asmaa *et al.* (2019) who observed that CRC patients typically have low CDX2 expression detected by IHC were (65.4%), (55.5%), (52.2%), (39.7%), (35.3%), (34.5%), (29.0%) and (22.0%) respectively which clarified that this low expression was higher in tissues of CRC patients. By contrast, other findings by Bae *et al.* (2015), Dalerba *et al.* (2016), Ryan *et al.* (2018) and Shigematsu *et al.* (2018) showed that the low expression of this protein in CRC patients was lower than (20%) as follows (15.7%), (9.1%), (5.9%), (4.1%) consequently, which differs from the percentage of CDX2 expression in the present study. However, it was not yet known what causes CDX2 expression to decrease throughout the development of CRC. Rarely, CRCs that have defective DNA mismatch repair will have a mutation in the CDX2 gene (i.e., MSI-high), also, reducing CDX2 expression isn't related to CDX2 polymorphisms (Sullivan *et al.* 2008). Loss of heterozygosity at the CDX2 locus (13q12-13) may account for the loss of CDX2 expression in some cases of CRC, but this was unlikely to be a major cause (Sivagnanasundaram *et al.* 2001). Research with colon cancer cell lines uncovered evidence for a dominant transcriptional repressor of CDX2, suggesting that CDX2 silencing may be the result of an epigenetic modification such as promoter CpG island methylation (Hinoi *et al.* 2003).

Importantly, this study also found that low CDX2 expression is associated with high CRC grades. Similarly, other studies observed low CDX2 expression is significantly correlated with poor differentiation grade. Besides, other studies found the same result regarding the association between low expression of this protein and high grade of CRC (Bonetti *et al.* 2017; Çalik *et al.* 2020; Dalerba *et al.* 2016; Oslen *et al.* 2014; Shigematsu *et al.* 2018). On the contrary, the current result was in disagreement with that reported by (Hong *et al.* 2013; Sjoerd *et al.* 2021). Loss of cell differentiation and increased cell proliferation may result from downregulated CDX2 expression, potentially through interactions with other genes (Brabletz *et al.* 2004). Elevation of this protein in human colon cancer cells also causes the cells to induce genes associated with goblet and enterocyte differentiation (Brabletz *et al.* 2004). However, low CDX2 expression led to cancerous growth in human colon cells to be more vulnerable to apoptosis and loss of colon cell differentiation (Mallo *et al.*1998). Besides, lymph node metastasis was inversely correlated with CDX2 expression. This finding was in accord with other recent studies (Asgari-Karchekani *et al.* 2019; Asmaa *et al.* 2019; Ryan *et al.* 2018;). Also, Bae *et al.* (2015) and Platet *et al.* (2017), reported that knockdown CDX2 expression contributed to the invasion of tumor cells. These findings make sense, while a tumor is forming, its initial cells are constantly being shed into the blood and lymphatic systems (Valera *et al.* 2005).

Furthermore, tumors with a deeper invasion (T) were found to have lower CDX2 expression. This result matches those observed in other studies (Dawson *et al.* 2013; Kim *et al.* 2013; Lugli *et al.* 2008). In addition, the finding of the current study didn't support the previous finding (Asgari-Karchekani *et al.* 2019; Sjoerd *et al.* 2021). CRC is more likely to invade the serosa and spread to other organs when CDX2 expression is low, suggesting that this protein may promote tumor invasion (Xu *et al.* 2019). CRC specimens included in this study revealed that CDX2 expression was generally low throughout all subtypes of CRC, from adenocarcinoma to mucinous. A later study by Asgari-Karchekani *et al.* (2019) and Çalik *et al.* (2020) emphasized that low CDX2 expression increased in adenocarcinoma than in mucinous CRC. Another valuable finding was that CDX2 low expression was statistically associated with advanced age. A comparison of this finding with those of other studies confirms the same obtained result (Dalerba *et al.* 2016; Kim *et al.* 2013; Xu *et al.* 2019). This result may be clarified by the fact that CDX2 mutations increased in colon cells over time which leads to decreased CDX2 expression (Çalik *et al.* 2020). Conversely, the results of the present study didn't support the prior results (Bae *et al.* 2015; Shigematsu *et al.* 2018; Sjoerd *et al.* 2021). Also, this study has been unable to demonstrate that the CDX2 level is affected by the location of the tumor in the colon or rectum. On the other hand, prior research has shown that a lack of CDX2 expression didn't correlate with tumor site (Baba *et al.* 2009).

Conversely, Olsen *et al.* (2014) and Bae *et al.* (2015) presented that the low CDX2 level was associated with right-sided tumors. Regarding sex, the current study in accordance with Shigematsu *et al.* (2018) and Sjoerd *et al.* (2021) showed that there was no correlation between CDX2 expression and type of patient's gender. On the other hand, the existing findings seem to be inconsistent with other studies (Asgari-Karchekani *et al.* 2019; Ryan *et al.* 2018).

CRC Four-year (OS) was lower for patients with low CDX2 expression (17.94±1.7 months; 61.77% survival rate) compared to those with high CDX2 expression (33.43±2.7 months; 79.12% survival rate). These results consistent with previous research showed that patients with low CDX2 expression have a shorter OS (34.77±2.22 months) compared to those with high CDX2 expression (56.20±0.73 months) (Çalik *et al.* 2020). Ryan *et al.* (2018) revealed low and high CDX2 in CRC were correlated with low 5-year OS (51.0% versus 70.1%) respectively. Also, Yu *et al.* (2016) reported a poor prognosis of OS in cases where CDX2 expression was low. Besides, Bae *et al.* (2015) clarified that reduced of CDX2 expression showed worse OS in CRC patients with 34.7 months and the Odd ratio obtained from IHC evaluation of CDX2 reached approximately (2.4) at best, this was in accordance with those reported in the current study (2.18). Patients with CRC continue to face difficulties in prognostic assessment because of the disease's variability. Low CDX2 expression may result from aberrant changes like high MSI, high CIMP, or point mutations in CRC patients, all of which contribute to increased tumor invasion and a poor prognosis. (Xu *et al.* 2019). In contrast to this finding, Knösel *et al.*(2012), Asgari-Karchekani *et al.* (2019) and Cecchini *et al.* (2019) showed that CDX2 expression didn't correlate with CRC patient survival. CDX2 loss appears to have a different effect on prognosis depending on the presence or absence of a prior CRC diagnosis in the family. In particular, when CDX2 is lost, the prognosis for patients with a family history of CRC is worse than for patients without a family history of CRC (Hinoi *et al.* 2003). This study concluded that CDX2 expression is regarded as a prognostic and diagnostic marker for non-mucinous CRC adenocarcinoma, also it is a sensitive indicator for intestinal-type differentiation and valuable in establishing the origin site for some intestinal type tumor metastasis.

Conflicts of Interest

The authors report no conflicts of interest.

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