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Prognostic value of combining E-cadherin, p53, Bcl-2 and Bcl-xL expression and survival in Tunisian colorectal adenocarcinoma patients

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ABSTRACT

Colorectal cancer (CRC) is a common health issue worldwide with an extremely low survival rate after relapse. This study aims to evaluate the immunohistochemical expression of p53, E-cadherin, Bcl-2 and Bcl-xL and find a potential correlation between these markers, clinicopathological factors and overall survival of colorectal cancer patients. Marker expression was immunohistochemically determined in 105 patients with colorectal adenocarcinoma from southern Tunisia, followed by statistical analysis. Positivity rate of nuclear p53, membranous E-cadherin and cytoplasmic Bcl-2 - Bcl-xL was 85.71%, 76.47%, 59.8%, and 85.71% respectively. Spearman correlation showed that p53 was significantly and positively related to E-cadherin, Bcl-2, Bcl-xL and distant metastasis. A positive significant correlation between E-cadherin and anti-apoptotic proteins was also seen. Membranous E-cadherin expression was significantly and negatively associated to poor prognosis factors including lymph node metastasis, lymph invasion, venous invasion and distant metastasis. Bcl-2 expression was significantly correlated to distant metastasis. Multivariate analysis showed a significant association between dependent variable E-cadherin and covariates including differentiation, lymph invasion, venous invasion, distant metastasis, Bcl-2 and Bcl-xL. Poor 3-years OS and 5-years OS were significantly related to p53, Bcl-2 expression and E-cadherin loss. Positive E-cadherin combined with negative p53 and Bcl-2 as well as double-positive for E-cadherin and Bcl-xL were associated to best overall survival. Although each protein can be an independent prognostic factor, Simultaneous E-cadherin, p53, Bcl-2, Bcl-xL expression could be a crucial prognostic and overall survival marker to CRC patients. Multivariate analysis confirmed a positive correlation between membranous E-cadherin loss and colorectal cancer severity.

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Introduction

Colorectal cancer (CRC) is a challenging health problem worldwide. It presents the second deadliest cancer in the world. An alarming rise in CRC incidence was observed especially in developing countries (1). With lifestyle change and the absence of effective strategies, Khiari *et al* estimated that the CRC age-standardized incidence rate will still grow for men and women until 2024 in Tunisia (2).

Apoptosis disruption is considered as a hallmark of colorectal malignancy (3). Tow pathways involved in apoptosis regulation: extrinsic and intrinsic pathways (4). The first is activated by the death receptor Fas and other members of the tumor necrosis factor (TNF) receptor family, whereas the second was governed by the Bcl-2 protein family (5).

Bcl-2 expression was frequently observed in various types of cancers but its prognostic role remains controversial. Some reports showed that overexpression of Bcl-2 promoted cancer metastasis although others identified this protein as a good prognostic marker (6,7). Current studies displayed that targeting Bcl-2 was involved in reducing progression and invasion as well as promoting apoptosis and chemotherapy sensibility of colorectal cancer cells (8,9).

Bcl-xL is another essential anti-apoptotic member of the Bcl-2 family. Many studies had proved the oncogenic potential of Bcl-xL in tumor proliferation. It was overexpressed in various human cancer (10,11).

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In colorectal cancer, Bcl-xL expression was related to a worse prognosis. It was identified as a strong modulator in colorectal tumorigenesis and progression (12).

The tumor suppressor p53 plays a key role in apoptosis regulation. Therefore, it orchestrates a cascade of reactions involving in cell proliferation and death. *P53* gene mutation is the most frequent and can be found in approximately 60 % of patients with colorectal cancer. Mutant p53 protein accelerates tumor progression and stimulates cancer invasiveness and metastasis (13). Altered p53 protein is associated to chemoresistance. Then, the restoration of wild-type p53 function in mouse models limits cancer development (14). Recently, several investigations have proved the prognostic value of p53 in many types of cancer including breast cancer (15), lung cancer (16) and colorectal cancer (17).

Cell adhesion is a key mechanism in maintaining coherent primary cancer. Disruption of cell adhesiveness promotes cancer cell invasion and metastasis. E-cadherin presents the essential structure for maintaining intercellular adhesion stability. Adhesion molecule was down-regulated in many types of cancer (18). In a colorectal tumor, invasion and metastasis were frequently associated with the reduction of E-cadherin expression (19). The elevated level was also observed in CRC patients compared to healthy subjects (20).

Frequent studies have evaluated the expression of p53, Bcl-2 Bcl-xL and E-cadherin in colorectal adenocarcinoma separately while there are few studies analyzing the combined impact of these biomarkers in CRC. Hence, we try to explore the potential relationship between these immunohistochemistry markers, clinicopathological factors and survival in Tunisian CRC patients.

Materials and methods

Patients and samples

A total of one hundred-five patient with colorectal adenocarcinoma (CRA) from southern Tunisia were enrolled in this study. Archival formalin-fixed and paraffin-embedded tumor from each patient was collected from the Department of Pathology, Habib Bourguiba University Hospital, Medicine, Tunisia, Department of Pathology, Habib Bourguiba University Hospital, Sfax, Tunisia and the Laboratory of Pathology of Djerba, Tunisia. Samples (105) were from forty-three females and sixty-two male patients. Clinical and histopathological and survival information were collected for each case.

Immunohistochemical staining

The immunohistochemistry technique was performed using the indirect avidin-biotin-peroxidase amplification method to detect Bcl-2, Bcl-xL, p53 and E-cadherin in tumor tissues. Paraffin-embedded TMA and tissue sections were deparaffinised and rehydrated in ethanol baths of decreasing degree. Slides were subjected to antigen retrieval, followed by an incubation in H_2O_2 to block endogenous peroxidase. Sections were washed in PBS, then incubated in β blocking to avoid non-specific binding in a humid chamber. The primary antibody was applied to the sections with the appropriately diluted primary antibody (1/100). After PBS washing, slides were incubated 25 min with the secondary antibody and then with post-primary antibody. After the revelation with 3.3′ - diaminobenzidine and the hematoxylin counter-staining, the sections were dehydrated and then mounted. A semiquantitative score was used starting from negative (0) to intense (3).

Statistical analysis

We analysed the normal distribution using the Shapiro-Wilk test, then in our study, variables have not normal distribution; and we use non-parametric tests. Comparative analysis for clinicopathological parameters between female and male groups was assessed using the Chi-squared test. The Spearman analysis was used to evaluate the correlation between clinicopathological factors and protein expression. For quantitative variables, a comparison between mean age tumor size and survival was done using Mann-Whitney. Binary logistic regression analysis was performed to predict the impact of clinicopathological factors on the dependent variable. Survival analysis was conducted using the Kaplan-Meier method and survival curves were compared using the Log-rank test. The accepted level of significance was $P \leq 0.05$.

The statistical analyses were done using the SPSS program (version IBM SPSS Statistics 22).

Results and discussion

Clinicopathological factors in the study population

The clinicopathological parameters and gender differences are summarized in Table 1.

One hundred five patients diagnosed with colorectal adenocarcinoma were included in this study. Sixty-tow patients (59%) were men and forty-three (41%) were women. The mean age of the study population was 63 ± 14.40 years (65.40 ±13.56 years for men and 59.53 ± 15.02 years for women). 78.09 % of all patients were older than 50 years. Liberkuhnian adenocarcinoma is the predominant histological type in this study group; in a witch, 68.57 % were well differentiated. 92 tumors were located in the colon whereas 13 were located in the rectum. Most of the patients were diagnosed with CRC measuring more than 5 cm. The mean tumor size was 4.85 ± 2 .12 cm (5.04 ± 2.47 cm for men and 4.58 ± 1.45 cm for women). The primary tumor staging (T) revealed that most of the cases were in advanced stage T3-T4 (94.28%). 61 patients had lymph node metastasis. The majority of patients had no vascular and venous invasion. 54.84 % of patients had metastatic adenocarcinoma at the time of diagnosis.

Regarding gender differences, there was no significant difference in the most of clinicopathological features: origin (P = 0.864), tumor size (P = 0.282), differentiation (P = 0.836), lymph node metastasis (P = 0.875), lymph invasion (P = 0.991), vascular invasion (P = 0.484), perineural invasion (P = 0.362), and venous invasion (P = 0.517). Female patients were significantly younger at diagnosis (P = 0.028). Women represent the highest proportion of patients aged 50 years or younger. However, 64.63% of patients older than 50 were men. Compared to women, men exhibited more aggressive tumor features. Hence, advanced stage (T3-T4) tumor (P = 0.030) and distant metastasis were significantly ascribed to men (P = 0.023). The liver is the most common site of distant metastasis in both men and women (Table 1).

Expression of tumor biomarkers (p53, Bcl-2, Bcl-xL and E-cadherin)

Status expression of biomarkers (p53, Bcl-2, Bcl-xL and E-cadherin) in colorectal adenocarcinoma patients is presented in Table 1 and immunohistochemistry (IHC) staining in Figure 1.

85.71% of CRC patients expressed the tumor suppressor marker p53. Out of ninety p53 positive tumors, 32 (30.5%) demonstrated strong staining, 31 (29.5%) showed moderate expression and 27 (25.7 %) revealed weak staining. Positive membranous E-cadherin staining was identified in 76.47% of patients. Intense E-cadherin staining was observed in 17.1 % while moderate and weak expressions were detected in 30.5 % and 24.8 % respectively.

Cytoplasmic expression of Bcl-2 was shown in 59.8%. Thus, 23.8% present weak Bcl-2 expression, 25.7% present moderate expression and 8.6% show strong staining. Bcl-xL positivity was seen in 85.71 % of CRC patients. Low and moderate staining was observed in more than 30% of patients while strong expression was seen in 17.1% of CRC patients.

Table 1. Clinicopathological factors and expression status of p53, Bcl-2, Bcl-xL and E-cadherin in colorectal adenocarcinoma (comparison between men and women)

	Factor		N	%	Men	Women	*P*
Clinicopathological factors	All cases		105	100	62	43	
	Age (105)	≤50	23	21.90	9	14	0.028*
		>50	82	78.09	53	29	
	Origin (105)	Rural	60	57.14	35	25	0.864
		Urban	45	42.85	27	18	
	Adenocarcinoma (105)	Liberkhunian	90	94.29	59	40	
		Mucinous	6	5.71	3	3	
	Anatomic site (105)	Colon	92	87.62	57	35	0.107
		Rectum	13	12.38	5	8	
	T Stage (105)	T1-T2	6	5.71	1	5	0.030*
		T3-T4	99	94.28	61	38	
	Tumor size (105)	≤5	72	68.57	40	32	0.282
		>5	33	31.43	22	11	
	Differentiation (105)	Well	72	68.57	33	29	0.836
		Moderate - poor	33	31.43	19	14	
	Lymph node metastasis (105)	No	43	40.95	25	18	0.875
		Yes	62	59.05	37	25	
	Lymph invasion (105)	No	39	37.14	23	16	0.991
		Yes	66	62.86	39	27	
	Vasculair invasion (105)	No	58	55.24	36	22	0.484
		Yes	47	44.76	26	21	
	Perineural invasion (105)	No	53	50.48	29	24	0.362
		Yes	52	49.52	33	19	
	Venous invasion (105)	No	72	68.57	41	31	0.517
		Yes	33	31.43	21	12	
	Distant Metastasis (93)	No	42	45.16	19	23	0.023*
		Yes	51	54.84	35	16	
IHC	P53 (105)	Negative	15	14.29	8	7	0.627
		Positive	90	85.71	54	36	
	E-cadherin (102)	Negative	24	23.53	14	10	0.867
		Positive	78	76.47	47	31	
	Bcl-2 (105)	Negative	44	41.90	26	18	0.994
		Positive	61	58.1	36	25	
	Bcl-xL (105)	Negative	15	14.29	7	8	0.292
		Positive	90	85.71	55	35	

Analysis using the Chi-square test; *$P \leq 0, 05$: significant.

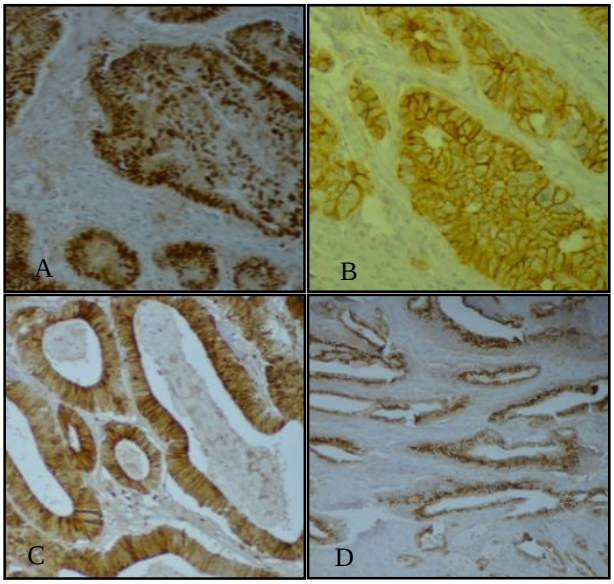

Figure 1. Immunohistochemical staining for p53, E-cadherin, Bcl-2 and Bcl-xL in colorectal adenocarcinoma; A: Strong nuclear staining for p53; B: Strong membranous staining for E-cadherin; C: Strong cytoplasmic staining for Bcl-2; D: Moderate cytoplasmic staining for Bcl-xL (Magnification 400x).

Correlation between p53, Bcl-2, Bcl-xL and E-cadherin expression

Spearmen correlation showed a strong association between p53, Bcl-2, Bcl-xL, and E-cadherin expression in colorectal adenocarcinoma. P53 was positively and significantly correlated to Bcl-2 (P = 0.018), Bcl-xL (P = 0.008) and E-cadherin (P = 0.028). Additionally, a closely positive association between E-cadherin and both anti-apoptotic proteins was found ($P \leq 0.001$, $P \leq 0.001$). There is also a strong positive relationship between Bcl-2 and Bcl-xL expression ($P \leq 0.001$).

Correlation between p53, Bcl-2, Bcl-xL and E-cadherin expression and clinicopathological factors

The correlation between p53 expression and clinicopathological parameters are presented in Table 2.

The result of our study indicated no correlation among p53 staining and the majority of clinicopathological characteristics. Importantly, positive p53 was significantly related to distant metastasis (P = 0.013). Thus, p53 staining was detected in 48 out of 51 metastatic colorectal adenocarcinomas. In the male group, tumors not exceeding 5cm had a clear tendency to express p53 (P = 0.090). Wen in the female group, p53 expression was significantly more seen in patients aged 50 years or younger. Also, it was positively related to distant metastasis (P = 0.014). Furthermore, tumors displaying lymph node metastasis (P = 0.087) and perineural invasion (P = 0.085) showed a trend towards significance with positive staining for p53.

The association between E-cadherin staining and clinicopathological characteristics are illustrated in Table 2.

Our analysis revealed a negative correlation between membranous E-cadherin staining and tumor location (P = 0.028). Therefore, it was more seen in tumor located in the colon than in the rectum. More interesting, positive membranous E-cadherin expression was inversely correlated to poor prognosis factors including lymph node metastasis ($P \leq 0.001$), lymph invasion (P = 0.007), venous invasion (P = 0.035) and distant metastasis (P = 0.002). CRC patients with E-cadherin negative tended to display vascular (P = 0.066) and perineural invasion (P = 0.080).

There was no significant interaction between this adhesion molecule and others clinicopathological factors such as age, gender, origin, differentiation, and stage (T).

In men patients, E-cadherin expression was negatively correlated to lymph node metastasis (P = 0.003), venous invasion (P = 0.042) and distant metastasis (P = 0.008) (Table 2).

In women, E-cadherin expression was significantly and inversely associated with the anatomic site (P = 0.027) and lymph node metastasis (P = 0.031). Insignificant increase in E-cadherin immunoexpression was observed in relation to lymph invasion (P = 0.067) and distant metastasis (P = 0.109) (Table 2).

The relationship between cytoplasmic Bcl-2 and Bcl-xL expression and clinicopathological features are presented in Table 3.

The immunohistochemical staining of these pro-survival proteins showed no association with most clinicopathological factors. Whereas, a significant positive correlation between distant metastasis and Bcl-2 was noted (P = 0.011). Bcl-2 staining was significantly higher in patients with metastatic CRA than those without distant metastasis. Regarding the male group, the only significant inverse correlation we found was between Bcl-2 expression and perineural

invasion (P = 0.033). In the female group, Positive immunostaining for Bcl-2 was associated with women displaying distant metastasis (P = 0.008).

Despite the expression of Bcl-xL in over 87% of colorectal adenocarcinoma, there was no significant relationship between Bcl-xL staining and the majority of clinicopathological parameters, while it was more expressed in tumor located in the colon than in the rectum. In male patients, a negative significant correlation was seen between Bcl-xL and tumour size. Therefore, it was less identified in colorectal adenocarcinoma exceeding 5 cm (P = 0.035).

In women, tumors located in the colon tended significantly to display positive Bcl-xL expression ($P \leq 0.001$). Also, a positive significant association was observed between lymph node metastasis and Bcl-xL staining (P = 0.036), suggesting a positive expression in patients with lymph node involvement.

With regard to the prognostic impact of simultaneous expression of these biomarkers, E-cadherin- / p53+ appeared to have a positive predictive value to lymph node metastasis ($P \leq 0.001$), vascular invasion (P = 0.033) and distant metastasis ($P \leq 0.001$). Moreover, E-cadherin- / Bcl-2 + co-expression was positively correlated to lymph node (P = 0.027) and distant metastasis ($P \leq 0.001$). Also, double-negative tumor for E-cadherin and Bcl-xL was significantly associated to well differentiated (P = 0.031) and distant metastatic cancer (P = 0.05) (Table 4).

Table 2. Correlation between nuclear p53 and membranous E-cadherin expression and clinicopathological factors in colorectal adenocarcinoma.

Factor		Nuclear p53 expression (105)						Membranous E-cadherin expression (102)					
		All cases (105)		Men (62)		Women (43)		All cases (102)		Men (61)		Women (41)	
		+ %	R / P	+ %	R / P	+ %	R / P	+ %	R / P	+ %	R / P	+ %	R / P
Age	≤50	91.30	R = -0.085	77.78	R = 0.115	100	R = -0.306	69.57	R = 0.040	66.67	R = 0.103	71.43	R = 0.070
	>50	84.15	P = 0.391	88.68	P = 0.375	75.86	P = 0.046*	78.48	P = 0.968	78.85	P = 0.431	77.78	P = 0.663
Origin	Rural	83.33	R = 0.079	85.71	R = 0.047	80	R = 0.119	70.69	R = 0.156	71.43	R = 0.155	69.57	R = 0.159
	Urban	88.89	P = 0.426	88.89	P = 0.717	88.89	P = 0.448	84.09	P = 0.116	84.62	P = 0.233	83.33	P = 0.320
Adenocarcinoma	Liberkhunian	86.87	-	86.44	-	87.5	-	76.29	-	77.59	-	74.36	-
	Mucinous	66.67	-	100	-	33.33	-	80	-	66.67	-	100	-
Anatomic site	Colon	85.87	R = -0.012	85.96	R = 0.114	88.24	R = -0.238	80	R = -0.228	78.57	R = -0.121	82.35	R = -0.346
	Rectum	84.62	P = 0.905	100	P = 0.378	66.67	P = 0.125	50	P = 0.028*	60	P = 0.352	42.86	P = 0.027*
T stage	T1 – T2	83.33	R = 0.017	100	R = -0.049	80	R = 0.037	100	R = -0.226	100	R = -0.070	100	R = -0.187
	T3 - T4	85.86	P = 0.865	86.89	P = 0.704	84.21	P = 0.816	75.26	P = 0.207	76.67	P = 0.589	72.97	P = 0.242
Tumor size	≤5	87.5	R = -0.075	92.5	R = -0.217	81.25	R = 0.114	75.36	R = 0.038	86.11	R = -0.077	70	R = 0.216
	>5	81.82	P = 0.445	77.27	P = 0.090	90.91	P = 0.466	78.79	P = 0.706	72.73	P = 0.554	90.90	P = 0.176
Differentiation	Well	87.5	R = -0.075	88.10	R = -0.53	89.29	R = -0.144	81.43	R = -0.172	83.33	R = -0.228	78.57	R = -0.101
	Moderate - poor	81.82	P = 0.445	84.21	P = 0.683	78.57	P = 0.362	65.62	P = 0.082	64.71	P = 0.078	69.23	P = 0.529
Lymph node metastasis	No	81.40	R = 0.103	88.46	R = -0.035	72.22	R = 0.264	95.12	R = -0.360	96	R = -0.376	93.75	R = -0.338
	Yes	88.71	P = 0.297	86.11	P = 0.790	92	P = 0.087	63.93	$P \leq 0.001$*	63.89	P = 0.003*	64	P = 0.031*
Lymph invasion	No	84.62	R = 0.024	91.30	R = -0.096	75	R = 0.182	91.67	R = -0.265	90.90	R = -0.248	92.86	R = -0.289
	Yes	86.36	P = 0.807	84.62	P = 0.456	88.89	P = 0.243	68.18	P = 0.007*	69.23	P = 0.054	66.67	P = 0.067
Vascular invasion	No	81.03	R = 0.149	83.33	R = 0.132	77.27	R = 0.179	83.64	R = -0.182	82.86	R = -0.160	85	R = -0.213
	Yes	91.49	P = 0.130	92.30	P = 0.306	90.48	P = 0.251	68.09	P = 0.066	69.23	P = 0.217	66.67	P = 0.180
Perineural invasion	No	84.91	R = 0.023	93.10	R = -0.168	75	R = 0.265	84	R = -0.174	85.71	R = -0.190	81.82	R = -0.156
	Yes	86.54	P = 0.813	81.82	P = 0.192	94.74	P = 0.085	69.23	P = 0.080	69.70	P = 0.143	68.42	P = 0.331
Venous invasion	No	84.72	R = 0.042	87.80	R = -0.030	80.65	R = 0.134	82.60	R = -0.290	85	R = -0.261	79.31	R = -0.134
	Yes	87.88	P = 0.671	85.71	P = 0.820	91.67	P = 0.392	63.64	P = 0.035*	61.90	P = 0.042*	66.67	P = 0.404
Distant metastasis	No	76.19	R = 0.257	84.21	R = 0.110	69.57	R = 0.390	90	R = -0.319	94.74	R = -0.359	85.71	R = -0.268
	Yes	94.12	P = 0.013*	91.43	P = 0.430	100	P = 0.014*	62	P = 0.002*	61.76	P = 0.008*	62.5	P = 0.109

Analysis using Spearman correlation; R: correlation coefficient; *$P \leq 0.05$: significant; ** $P \leq 0.001$: highly significant.

Multivariate analysis

Multivariate analysis by binary logistic regression showed that E-cadherin expression was an independent predictor for CRC prognosis (differentiation (P = 0.05), lymph invasion (P = 0.05), venous invasion (P = 0.048), distant metastasis (P = 0.016). Also, a significant association between E-cadherin and Bcl-2 (P = 0.004) and Bcl-xL (P = 0.020) was seen. This result indicated that these proteins might act synergistically in colorectal cancer development. Membranous CDH1 expression had a trend towards association with overall survival (P = 0.063) and nuclear p53 expression (P = 0.064) (Table 5).

Table 3. Correlation between cytoplasmic Bcl-2 and Bcl-xL expression and clinicopathological factors in colorectal adenocarcinoma.

Factor		Cytoplasmic Bcl-2 expression (105)						Cytoplasmic Bcl-xL expression (105)					
		All cases		Men		Women		All cases		Men		Women	
		+ %	*R* / *P*	+ %	*R* / *P*	+ %	*R* / *P*	+ %	*R* / *P*	+ %	*R* / *P*	+ %	*R* / *P*
Age	≤50	56.52	*R* = 0.017	55.56	*R* = 0.021	57.14	*R* = 0.014	82.61	*R* = 0.047	77.78	*R* = 0.142	85.71	*R* = -0.077
	>50	58.54	*P* = 0.864	58.49	*P* = 0.872	58.62	*P* = 0.929	86.59	*P* = 0.634	90.57	*P* = 0.270	79.31	*P* = 0.623
Origin	Rural	65	*R* = -0.062	62.86	*R* = -0.111	68	*R* = -0.236	83.33	*R* = 0.079	91.43	*R* = - 0.098	72	*R* = 0.285
	Urban	48.49	*P* = 0.100	51.85	*P* = 0.392	44.44	*P* = 0.128	88.89	*P* = 0.426	85.19	*P* = 0.449	94.44	*P* = 0.064
Adenocarcinoma	Liberkhunian	59.60	-	57.63	-	62.5	-	86.87	-	89.83	-	82.5	-
	Mucinous	33.33	-	66.67	-	0	-	66.67	-	66.67	-	66.67	-
Anatomic site	Colon	60.87	*R* = -0.150	61.40	*R* = -0.228	61.76	*R* = -0.143	89.13	*R* = -0.260	89.47	*R* = -0.082	91.18	*R* = -0.489
	Rectum	38.46	*P* = 0.128	20	*P* = 0.074	44.44	*P* = 0.361	61.54	*P* = 0.007*	80	*P* = 0.529	44.44	*P* ≤0.001*
T stage	T1 – T2	66.67	*R* = -0.043	100	*R* = -0.109	60	*R* = -0.014	66.67	*R* = 0.134	100	*R* = -0.046	60	*R* = 0.199
	T3 - T4	57.58	*P* = 0.665	57.38	*P* = 0.400	57.89	*P* = 0.931	86.87	*P* = 0.173	88.52	*P* = 0.724	84.21	*P* = 0.200
Tumor size	≤5	59.72	*R* = -0.049	62.50	*R* = -0.121	56.25	*R* = 0.065	87.5	*R* = -0.075	95	*R* = -0.268	78.13	*R* = 0.143
	>5	54.55	*P* = 0.622	50	*P* = 0.348	63.64	*P* = 0.677	81.82	*P* = 0.445	77.27	*P* = 0.035*	90.90	*P* = 0.359
Differentiation	Well	58.33	*R* = -0.007	57.14	*R* = -0.019	60.71	*R* = -0.034	88.89	*R* = -0.134	90.48	*R* = -0.114	89.29	*R* = -0.226
	Moderate - poor	57.58	*P* = 0.942	57.89	*P* = 0.887	57.14	*P* = 0.829	78.79	*P* = 0.173	88.24	*P* = 0.382	71.43	*P* = 0.150
Lymph node metastasis	No	62.79	*R* = -0.079	69.23	*R* = -0.192	55.56	*R* = 0.044	79.07	*R* = 0.158	88.46	*R* = 0.007	66.67	*R* = 0.321
	Yes	54.84	*P* = 0.422	50	*P* = 0.134	60	*P* = 0.777	90.32	*P* = 0.107	88.89	*P* = 0.959	85.19	*P* = 0.036*
Lymph invasion	No	61.54	*R* = -0.054	69.57	*R* = -0.192	50	*R* = 0.127	79.49	*R* = 0.137	86.96	*R* = 0.043	68.75	*R* = 0.250
	Yes	56.06	*P* = 0.587	51.28	*P* = 0.134	62.96	*P* = 0.417	89.39	*P* = 0.164	89.74	*P* = 0.743	88.89	*P* = 0.106
Vascular invasion	No	63.79	*R* = -0.128	69.44	*R* = -0.179	54.55	*R* = 0.075	82.76	*R* = 0.094	86.11	*R* = 0.097	77.27	*R* = 0.064
	Yes	51.06	P = 0.192	42.31	*P* = 0.164	61.90	*P* = 0.634	89.36	*P* = 0.341	92.31	*P* = 0.455	85.71	*P* = 0.682
Perineural invasion	No	56.60	*R* = 0.031	62.07	*R* = -0.271	50	*R* = 0.185	83.02	*R* = 0.078	86.21	*R* = 0.074	79.17	*R* = 0.108
	Yes	59.62	*P* = 0.757	54.55	*P* = 0.033*	68.42	*P* = 0.234	88.46	*P* = 0.430	90.91	*P* = 0.567	84.21	*P* = 0.489
Venous invasion	No	58.33	*R* = -0.007	60.98	*R* = -0.076	54.84	*R* = 0.108	83.33	*R* = 0.101	85.37	*R* = 0.148	80.65	*R* = 0.035
	Yes	57.58	*P* = 0.942	52.38	*P* = 0.557	66.67	*P* = 0.492	90.90	*P* = 0.308	95.24	*P* = 0.567	83.33	*P* = 0.844
Distant metastasis	No	40.48	*R* = 0.262	42.11	*R* = -0.08	39.13	*R* = 0.418	85.71	*R* = -0.019	89.47	*R* = -0.053	82.61	*R* = -0.017
	Yes	66.67	*P* = 0.011*	60	*P* = 0.524	81.25	*P* = 0.008*	84.31	*P* = 0.853	85.71	*P* = 0.701	81.25	*P* = 0.916

Analysis using Spearman correlation; *R*: correlation coefficient; *$P \leq 0.05$: significant; ** $P \leq 0.001$: highly significant.

Survival analysis

The Relationship between prognosis factors, protein expression and survival time is presented in Table 6 and Figure 2.

Among 94 patients, 55 were men and 36 were women. The mean survival time of all included cases was 26.16 ± 15.75 months and the median was 24 months (1-75). The mean survival time for men was 23.27 ± 13.72 compared to 30.58 ± 17.74 for women. This gender difference in survival time was statistically significant ($P = 0.005$).

According to Kaplan-Meier analysis, there is no significant correlation between global survival and most of the clinicopathological parameters such as stage (T), lymph-vascular invasion and lymph node metastasis. Similarly, no significant relationship between survival time and lymph node metastasis in the male group. However, survival was significantly longer in women without lymph node metastasis ($P = 0.045$).

Then, we found a significant association between survival and perineural invasion ($P \leq 0.001$) and distant metastasis ($P \leq 0.001$). In both men and women, distant metastasis contributed to lower overall survival ($P \leq 0.001$).

Next, we conducted the survival analysis to explore the relationship between p53, Bcl-2, Bcl-xL and E-cadherin levels, and the survival time of CRC patients (Table 6).

Statistical analysis showed a significant association between p53 expression and global survival ($P = 0.025$). Patients whose colorectal adenocarcinoma stained positive for p53 had a shorter survival time than those whose colorectal cancer scored negatively. However, the survival time was not significantly different according to p53 expression in both women and men ($P = 0.055$ and $P = 0.424$ respectively) (Figure 3).

Moreover, membranous E-cadherin staining was closely related to survival time ($P = 0.006$). Thus, patients with positive E-cadherin expression had a longer survival time. In contrast, loss of this adhesion molecule was associated with a short survival time. The CDH1 positive male patients exhibited the best overall survival ($P = 0.008$) while no survival difference between E-cadherin positive group and E-

cadherin negative group in female patients (*P = 0.208*) (Figure 4).

Additionally, Bcl-2 expression was significantly associated to poor overall survival (*P* = 0.014). In fact, a longer survival time was observed in the Bcl-2-negative group. Women whose Bcl-2 negative presented higher survival (*P = 0.010*). In contrast, there was no significant difference in the men's group (*P = 0.171*) (Figure 5).

For Bcl-xL, there was an insignificant association between this protein and survival time. Hence, positive Bcl-xL patients tended to survive longer than those whose Bcl-xL was negative. Similar findings were observed in both men and women (Figure 6).

Regarding 3-years OS and 5-years OS, the survival period was significantly higher in patients with perineural invasion (*P = 0.004, P ≤ 0.001*) and distant metastasis (*P ≤ 0.001*). Further, the presence of p53 and Bcl-2 was associated with a shorter survival time. Inversely, loss of E-cadherin was related to poor overall survival (Table 6).

Global Survival was significantly influenced by the simultaneous expression of E-cadherin, p53, Bcl-2 and Bcl-xL. E-cadherin +/ p53- group had the best survival time comparing to E-cadherin-/p53+ group *(P = 0.003*). Moreover, the survival rate significantly differed according to the combination of E-cadherin and Bcl-2 (*P ≤ 0.001).* Therefore, the worst survival time was observed in E-cadherin- / Bcl-2+ patients. The co-expression of E-cadherin and Bcl-xL was significantly associated with a good prognosis (*P = 0.036*). Thus, patients who expressed both CDH1 and Bcl-xL had a longer survival time than patients whose tumors stained negative for E-cadherin and Bcl-xL (Figure 7).

Table 4. Correlation between combining E-cadherin / p53, E-cadherin / Bcl-2, E-cadherin / Bcl-xL and prognosis factors.

Factors	E-cadherin / p53 (29)		E-cadherin / Bcl-2 (36)		E-cadherin / Bcl-xL (82)	
	R	*P*	*R*	*P*	*R*	*P*
Differentiation	0.051	0.791	0.269	0.113	0.239	0.031*
Lymph node metastasis	0.587	*P≤0.001***	0.368	0.027*	0.179	0.107
Perineural invasion	0.193	0.316	0.283	0.094	0.084	0.455
Vascular invasion	0.396	0.033*	0.069	0.688	0.102	0.361
Distant metastasis	0.713	*P≤0.001***	0.713	*P≤0.001***	0.230	0.05*

Analysis using Spearman correlation; *R*: correlation coefficient; *$P \leq 0.05$: significant; **$P \leq 0.001$: highly significant.

Table 5. Binary logistic regression analysis of the association between clinicopathological factors and E-cadherin expression in colorectal adenocarcinoma.

Covariables	B	E.S	Wald	df	*Sig*	Exp(B)
Gender	1.198	1.029	1.355	1	0.244	3.312
Age	1.068	1.274	0.703	1	0.402	2.910
Anatomic site	-1.607	0.786	4.175	1	0.041*	0.200
Tumor size	0.524	1.102	0.226	1	0.634	1.689
Lymph invasion	-3.394	1.736	3.820	1	0.05*	0.034
Venous invasion	-2.257	1.142	3.905	1	0.048*	0.105
Vascular invasion	0.818	0.973	0.707	1	0.400	2.266
Overall survival	0.107	0.057	3.468	1	0.063	1.113
Origin	3.807	1.360	7.836	1	0.005*	45.013
Differentiation	-2.192	1.123	3.811	1	0.05*	0.112
Distant metastasis	-4.113	1.715	5.754	1	0.016*	0.016
Nuclear p53 expression	2.804	1.517	3.418	1	0.064	16.514
Cytoplasmic Bcl-2 expression	4.706	1.612	8.525	1	0.004*	110.639
Cytoplasmic Bcl-xL expression	4.213	1.816	5.283	1	0.020*	67.530

Multivariate analysis using Binary logistic regression; *$P \leq 0.05$: significant.

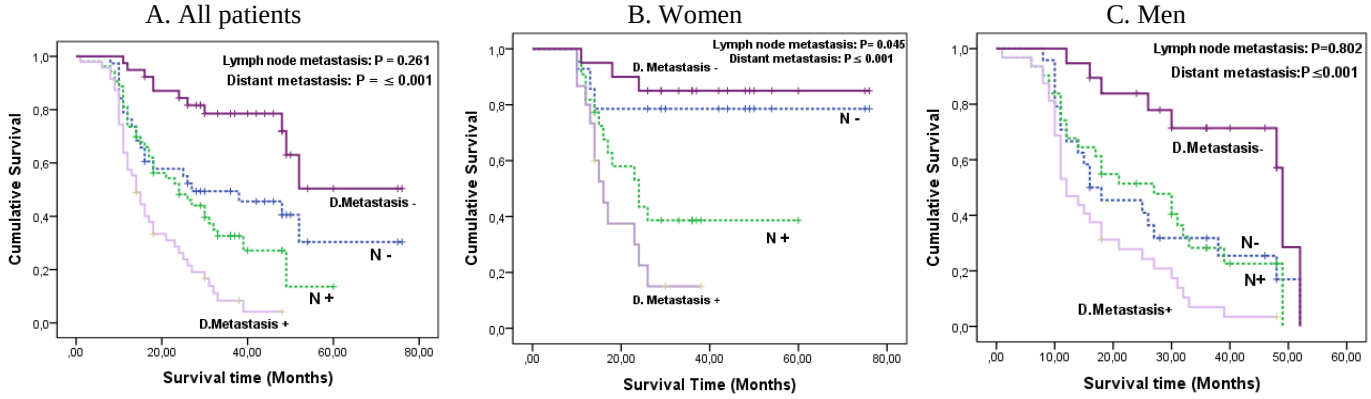

Figure 2. Overall survival curves of colorectal adenocarcinoma patients in relation to lymph node metastasis and distant metastasis; A: Overall survival curves in all patient; B: Overall survival curves in women; C: Overall survival curves in men.

Table 6. Survival analysis, clinicopathological factors and markers expression in colorectal adenocarcinoma.

Factor		Survival analysis					
		3 years OS (85)			5 years OS (61)		
		Survival (Death) %	95 % CI	*P*	Survival (Death) %	95 % CI	*P*
T Stage	T1-T2	33.33 (66.67)	16.90 - 36.69	0.483	33.33 (66.67)	21.01 - 61.39	0.396
	T3-T4	36.59 (63.41)	21.66 - 26.49		5.17 (94.83)	26.67 - 35.50	
Anatomic site	Colon	36.49 (63.51)	21.94 - 26.94	0.736	5.88 (94.12)	26.89 - 36.22	0.943
	Rectum	36.36 (63.64)	15.44 - 29.88		11.11 (88.89)	18.64 - 43.63	
Lymph node metastasis	No	45.71 (54.29)	21.46 - 28.91	0.377	11.45 (88.55)	27.80 - 41.77	0.261
	Yes	30 (70)	20.48 - 26.60		2.86 (97.14)	23.39 - 34.27	
Lymph invasion	No	45.16 (54.84)	22.03 - 29.78	0.176	13.64 (86.36)	28.93- 43.97	0.156
	Yes	31.48 (68.52)	20.31 - 20.31		2.56 (97.44)	23.81- 34.13	
Vascular invasion	No	35.42 (64.58)	21.26 - 27.44	0.795	10.52 (89.48)	25.91 - 37.31	0.981
	Yes	37.84 (62.16)	20.37 - 26.56		0 (100)	32.77 - 34.63	
Perineural invasion	No	52.27 (47.73)	24.02 - 30.42	0.004*	16 (84)	31.76 - 44.14	*P*≤0.001**
	Yes	19.51 (80.49)	17.89 - 24,35		0 (100)	18.40 - 25.39	
Distant metastasis	No	76.47 (23.53)	30.07 - 34.93	*P*≤0.001**	28.57 (71.43)	43.00 - 54.13	*P*≤0.001**
	Yes	8.70 (91.30)	14.80 - 20.34		0 (100)	15.13 - 21.47	
p53 expression	Negative	72.73 (27.27)	27.49 - 37.10	0.017*	25 (75)	35.68 - 54.12	0.025*
	Positive	31.08 (68.92)	20.71 - 25.69		5.26 (94.74)	25.07 - 34.01	
E- cadherin expression	Negative	10.53 (89.47)	14.56 - 23.69	0.010*	0 (100)	14.56 - 23.69	0.006*
	Positive	43.75 (56.25)	23.03 - 28.30		6.82 (93.18)	29.55 - 39.56	
Bcl-2 expression	Negative	52.78 (47.22)	24.09 - 31.39	0.016*	17.39 (82.61)	31.26 - 44.64	0.014*
	Positive	24.49 (75.51)	18.73 - 24.6		0 (100)	21.13 - 30.64	
Bcl-xL expression	Negative	25 (75)	15.95 - 28.04	0.355	10 (90)	16.31 - 35.68	0.383
	Positive	38.36 (61.64)	22.02 - 27.1		5.88 (94.12)	27.52 - 36.93	

Survival analysis using Kaplan Meier; 95% CI: confidence interval; *$P \leq 0.05$: significant; **$P \leq 0.001$: highly significant.

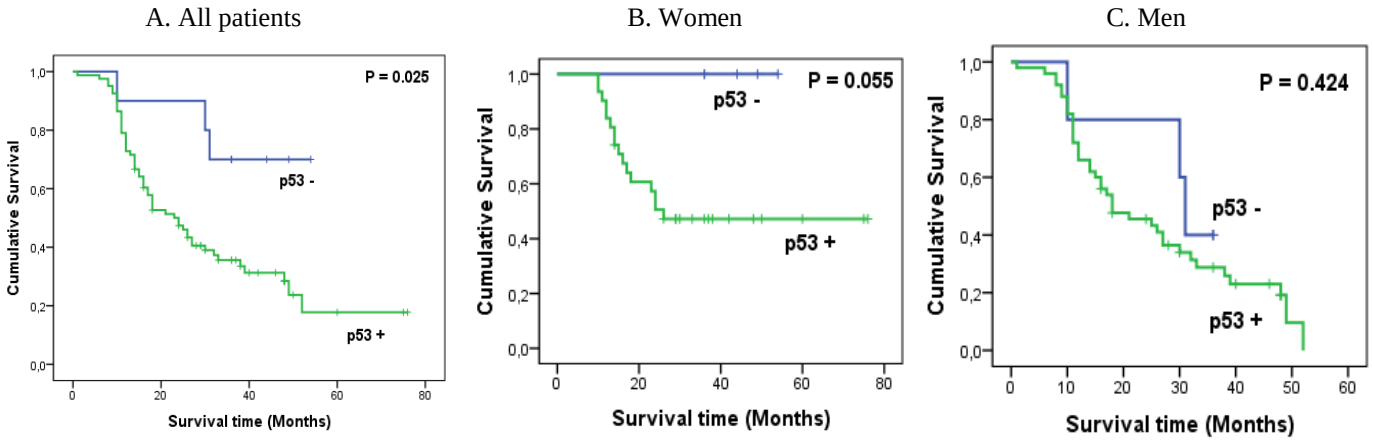

Figure 3. Overall survival curves of colorectal adenocarcinoma patients in relation to nuclear p53 expression; A: Overall survival curves in all patients, B: Overall survival curves in women; C: Overall survival curves in men.

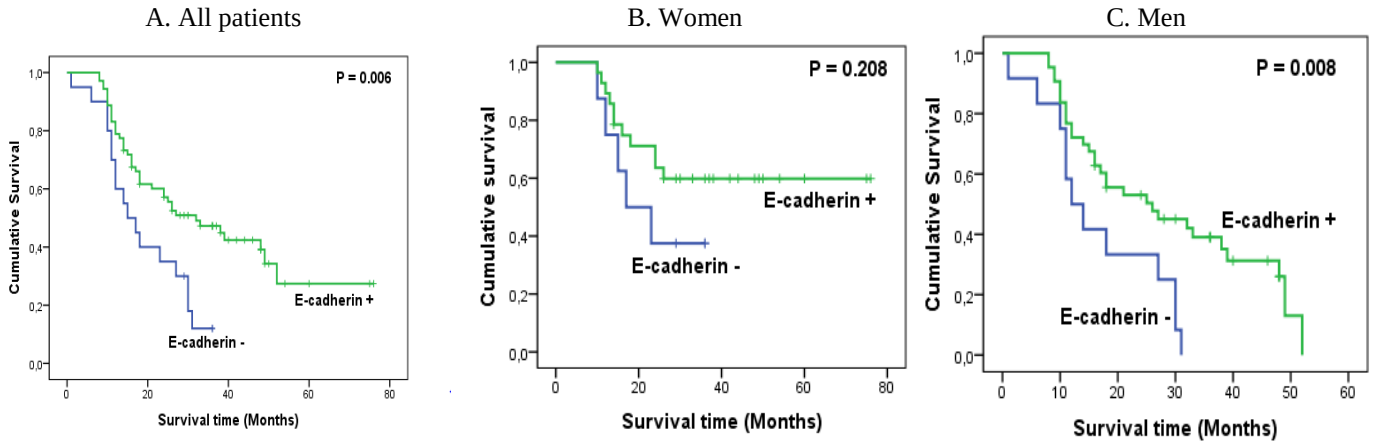

Figure 4. Overall survival curves of colorectal adenocarcinoma patients in relation to membranous E-cadherin expression; A: Overall survival curves in all patients, B: Overall survival curves in women; C: Overall survival curves in men.

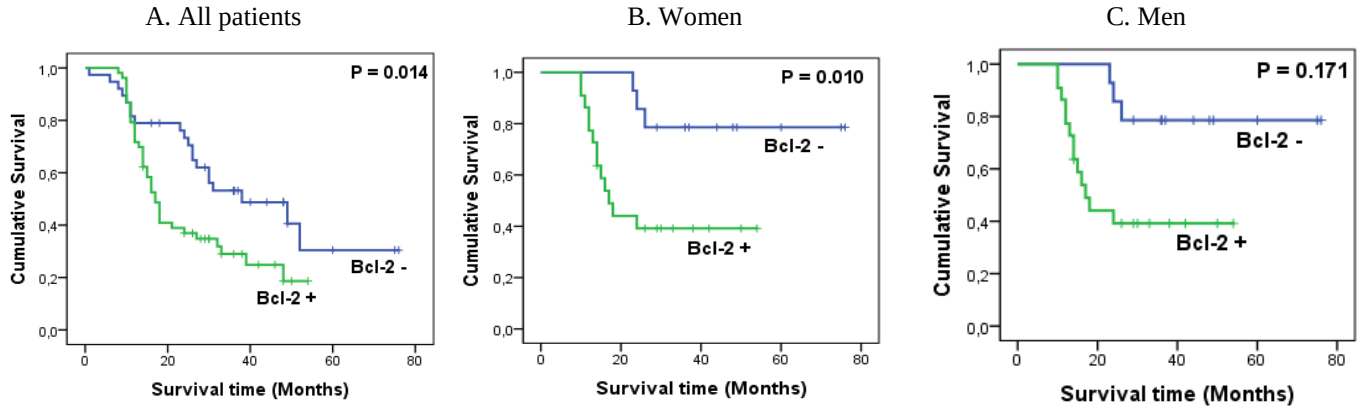

Figure 5. Overall survival curves of colorectal adenocarcinoma patients in relation to cytoplasmic Bcl-2 expression; A: Overall survival curves in all patients, B: Overall survival curves in women; C: Overall survival curves in men.

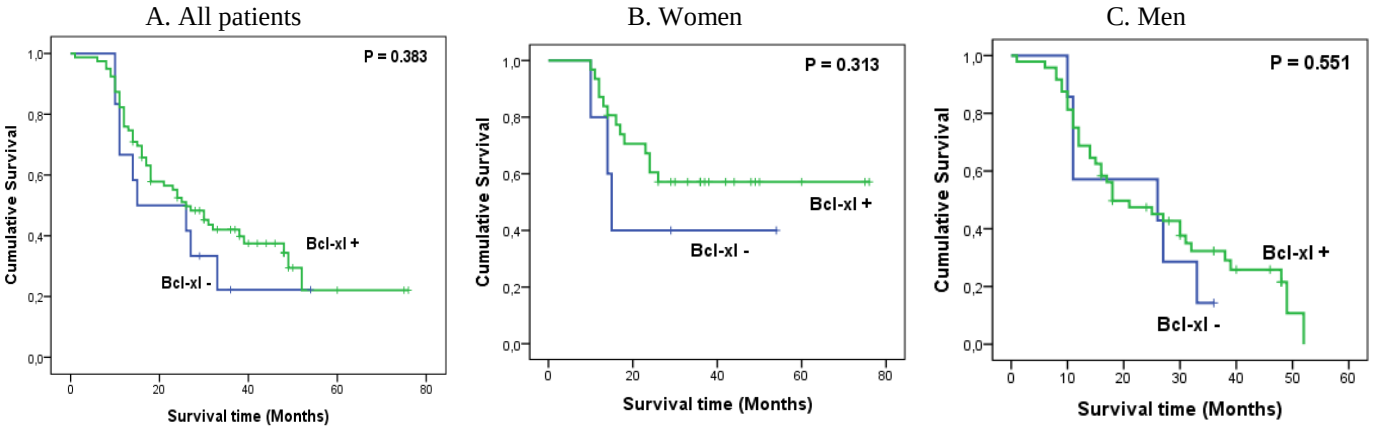

Figure 6. Overall survival curves of colorectal adenocarcinoma patients in relation to cytoplasmic Bcl-xL expression; A: Overall survival curves in all patients, B: Overall survival curves in women; C: Overall survival curves in men.

Table 7. Expression of p53, Bcl-2, Bcl-xL and E-cadherin in patients with colorectal cancer from different populations.

Biomarker	Population	Samples (n)	Positive (%)	Method	Reference
Nuclear expression of p53	South Tunisia	105	85.71	HIC	Our study
	Morocco	16	87	HIC	(Amsaguine et al., 2015)
	Korea	621	80.19	HIC	(Oh et al., 2019)
	India	30	70	HIC	(Chithrra et al., 2018)
	Romania	31	65	HIC	(Melincovici et al., 2016)
	China	124	58.06	HIC	(Wang et al., 2017)
	Iran	95	58.9	HIC	(Azarhoush et al., 2018)
	Iraq	104	46.15	HIC	(Mohammed et al., 2020)
Membranous expression of E-cadherin	South Tunisia	102	76.47	HIC	Our study
	Tunisia	70	74.3	HIC	(Miladi-Abdennadher et al., 2012)
	Egypt	50	74	HIC	(Hegazy, 2014)
	Romania	65	72.3	HIC	(Palaghia et al., 2016)
	Tunisia	40	57.5	HIC	(Triki et al., 2020)
	China	108	51.9	HIC	(Gao et al., 2016)
	Croatia	40	95	HIC	(Jurčić et al., 2019)
Cytoplasmic expression of Bcl-2	South Tunisia	105	58.1	HIC	Our study
	Romania	31	39.13	HIC	(Melincovici et al., 2016)
	France	226	36	HIC	(Poincloux et al., 2009)
	Poland	60	80	HIC	(Brzozowa-Zasada et al., 2018)
	Poland	14	92	HIC	(Sadowska et al., 2014)
Cytoplasmic expression of Bcl-xL	South Tunisia	105	85.71	HIC	Our study
	Korea	81	97.5	HIC	(Han et al., 2006)
	China	56	38.78	HIC	(Zhang et al., 2008)
	Poland	14	14	HIC	(Sadowska et al., 2014)

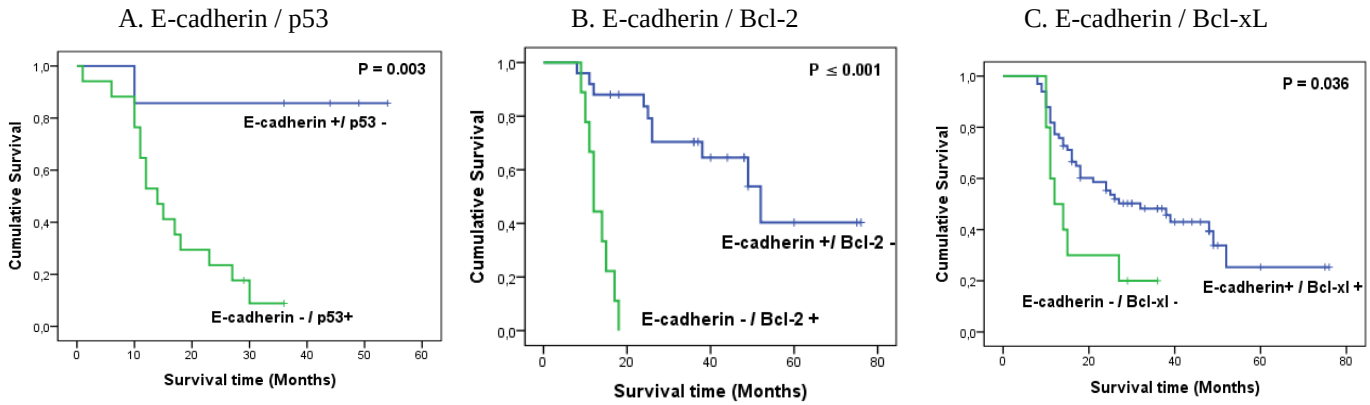

Figure 7. Overall survival curves of colorectal adenocarcinoma patients in relation to combined expression of E-cadherin, p53, Bcl-2 and Bcl-xL; A: Overall survival curves in relation to E-cadherin / p53 co-expression; B: Overall survival curves in relation to E-cadherin / Bcl-2 co-expression; C: Overall survival curves in relation to E-cadherin /Bcl-xL co-expression.

P53, Bcl-2, Bcl-XL and E-cadherin are involved in the control of apoptotic cell death and invasive potential, which are important hallmarks of cancer development.

Immunohistochemical staining of p53, Bcl-2, Bcl-xL and E-cadherin in CRC patients

Our study showed that positive nuclear p53 expression was detected in 85.71 % of cases of population. These observations were in accordance with others studies which p53 expression was shown in more 80% (21,22). When some authors found positive p53 staining only in half of the patients (17,23,24).

In our study group, membranous E-cadherin was expressed in 76.47% of CRC patients. Our observations confirm those noted by Jurcic and his collaborators (25). Membranous E-cadherin was detected in 72.3% of Romanian patients with colorectal cancer (26). Additionally, an Egyptian study reported a highly positive expression of this protein in more than 70% (27). While Cytoplasmic and parcial membranous E-cadherin expression was observed in 52% of CRC Chinese patients (28). Triki *et al* identified that only 57.5% of Tunisian patients with mucinous colorectal adenocarcinoma had positive membranous E-cadherin expression. These paradoxical findings may be explained by the difference between study groups as well as by the small number of cases of this study (29).

In this present study, cytoplasmic Bcl-2 expression was positive in 58.1% of CRC patients. Poincloux *et al* and Melincovici *et al* found similar results (23,30). Additionally, Sadowska *et al* demonstrated that Bcl-2 was detected in 92.85% (31).

Cytoplasmic staining of Bcl-xL was found in 85.71% of CRC patients. Han et al (32) confirmed this observation. Many investigations revealed that Bcl-xL was up-regulated whereas others affirmed the lack of this protein in colorectal tumor. Sadawska and his collaborators noted that most specimens were Bcl-xL negative (86%) (31).

Correlation between p53, Bcl-2, Bcl-xL and E-cadherin expression

The correlation between p53 and anti-apoptotic proteins (Bcl-2 and Bcl-xL) in CRC was very controversial in the literature. Previous studies revealed that p53 was not correlated to Bcl- 2 (32,33). A disputable finding reported by Georgiou *et al* affirmed a negative correlation between these markers (34). The opposite result was noted by Zalata *et al* who confirmed a significant positive association between these two proteins (35). Our data also revealed that the expression status of p53 was positively related to Bcl-2. Han *et al* noted a negative association between p53 and Bcl-xL (32). On the contrary, we found a positive interaction between them.

Regarding the correlation between anti-apoptotic proteins (Bcl-2 and Bcl-xL) in colorectal cancer, strong positive correlation was obtained *($P \leq 0.001$)*. Some authors affirmed that these proteins was insignificantly correlated (31) while others demonstrated the lack of connection between Bcl-2 and Bcl-xL (32). There are relatively few studies

assessing the correlation between p53, Bcl-2, Bcl-xL in CRC.

Our analysis showed a strong association between p53, Bcl-2, Bcl-xL, and E-cadherin expression in colorectal adenocarcinoma. P53 was positively correlated to E-cadherin (P = 0.028). Additionally, closely positive association between E-cadherin and anti-apoptotic proteins was found ($P \leq 0.001$, $P \leq$ 0.001). Concerning E-cadherin, there is a paucity of information regarding its correlation with p53, Bcl-2 and Bcl-xL in colorectal adenocarcinoma. Further studies evaluating the interaction between these markers was needed.

Correlation between p53, Bcl-2, Bcl-xL and E-cadherin expression and clinicopathological factors

The assessment of the relationship between p53 immunostaining and clinicopathological factors in our study demonstrated a positive correlation between this protein and distant metastasis. Besides its role in early tumorigenesis of colorectal cancer, our results demonstrate that p53 was also involved in cancer spread and progression. Further studies have shown that p53 did not correlate to clinicopathological characteristics (23,26). Inversely, the Indian study found that p53 staining was significantly associated with histologic types, grade, tumor location, T4 stage and lymph node metastasis (37). Also, other study demonstrated a significant correlation between positive p53 and differentiation, tumor size, pTNM stage, nerve invasion and vessel invasion (17). Azarhoush *et al* noted a significant connection between this protein and vascular involvement, lymph node involvement, round neural invasion, depth of invasion, tumor size and grade (38).

Statistical analysis revealed negative correlation between positive membranous staining of E-cadherin and tumor location (P = 0.028), lymph node metastasis ($P \leq 0.001$), lymph invasion *($P = 0.007$)*, venous invasion ($P = 0.035$) and distant metastasis (P = 0.002). Another similar study from Tunisia showed controversial results. Triki *et al* revealed a significant association between CDH1 expression and distant metastases and venous invasion in mucinous colorectal cancer while Miladi-abdennadher *et al* noted a significant correlation between this protein and age at diagnosis and tumor size (29,39). The observations shown by kim and his team reported that CDH1 expression was associated to positive lymph nodes but they failed to demonstrate the correlation between this protein and distant metastasis (40). Our findings was also supported by a Chinese study that confirmed the association between E-cadherin staining and prognosis factors including differentiation, the depth of tumor invasion, lymph node metastasis and clinical stage (28). Our results were contrasted with the study of Juracic *et al* demonstrating the lack of association between this protein and clinicopathological features in primary colorectal cancer (25).

The immunohistochemical staining of Bcl-2 did not associate with the majority of clinicopathological factors. Interestingly, metastatic colorectal adenocarcinomas had a strong tendency to express Bcl-2 (P = 0.011). Several studies have shown a significant correlation between positive staining of this protein and many clinicopathological factors. Therefore, Brzozowa-Zasada *et al* noticed the association between Bcl-2 and grade, tumor size, lymphovascular invasion, and regional lymph node involvement (41). Moreover, Poincloux and his collaborators reported a significant correlation between this anti-apoptotic protein and tumour size, the extension of parietal invasion pT, the invasive nature of the tumour and the extent of the circumference (30). Contrasting findings were noted by the immunohistochemical study of Sadowska *et al*. Thus, no significant correlation was revealed between Bcl-2 and clinicopathological characteristics (31).

The result of our study demonstrated that Bcl-xL, expressed in over 87% of colorectal adenocarcinoma, did not significantly correlate to the majority of clinicopathological parameters. This protein was more identified in tumor located in the colon than in the rectum (P = 0.007). More interesting, Bcl-xL was positively related to lymph node metastasis in women (P = 0.036).

Contrasting to our study, Sadawska *et al* noted the lack of interaction between Bcl-xL and clinicopathological characteristics (31). Other studies showed that this anti-apoptotic regulator was significantly related to prognostic factors. Jin-Song *et al* reported that the level of Bcl-xL protein expression was closely correlated with tumor differentiation, lymph node metastasis, venous permeation, and Duke's classification (42). The study carried out by

Zang and his collaborators confirmed the association between Bcl-xL, pathological grade, lymph node metastasis and Duke's stage of colorectal carcinoma (43).

Spearmen correlation revealed that E-cadherin- / p53+ group was associated to lymph node metastasis ($P \leq 0.001$), vascular invasion ($P = 0.033$) and distant metastasis ($P \leq 0.001$). Moreover, a significant positive correlation was seen between E-cadherin-/Bcl-2+ combination, lymph node involvement ($P = 0.027$) and distant metastasis ($P \leq 0.001$). Also, simultaneous expression of E-cadherin and Bcl-xL was significantly associated with poor-moderate differentiation ($P = 0.031$) and absence of distant metastasis ($P = 0.05$).

Multivariate analysis

Binary logistic regression analysis showed an independent prognostic impact of E-cadherin expression in CRC (differentiation ($P = 0.05$), lymph invasion ($P = 0.05$), venous invasion ($P = 0.048$), distant metastasis ($P = 0.016$). Also, E-cadherin was clearly associated to Bcl-2 ($P = 0.004$) and Bcl-xL ($P = 0.020$). Cadherin 1 might act synergistically with Bcl-xL and antagonistically with Bcl-2 to promote CRC progression. Membranous CDH1 expression had only a tendency towards overall survival ($P = 0.063$) and nuclear p53 expression ($P = 0.064$). The current result cannot confirm those presented by Kim *et al* (40). Multivariate analysis configured by Lee *et al* showed a significant association between aberrant expression of E-cadherin and overall survival (44).

Survival analysis

In this study, we assessed the prognostic role of clinicopathological factors and the expression of markers (p53, Bcl-2, Bcl-xL and E-cadherin) in patients with colorectal adenocarcinoma from southern Tunisia. Kaplan Maier analysis revealed that colorectal adenocarcinoma patients with perineural invasion ($P \leq 0.001$) and distant metastasis ($P \leq 0.001$) had a significantly lower 3-year overall survival and 5-years overall survival than those without perineural invasion and distant metastasis. This study failed to demonstrate the significant connection between overall survival and other clinicopathological features. We found also a significant relationship between markers (p53, E-cadherin, Bcl-2) expression and survival time. As the results shown by Wang *et al*, we noted that CRC patients with p53 positive had significantly shorter OS than those with p53 negative ($P = 0.025$) (17). Others have previously confirmed the presence of p53 in colorectal adenocarcinoma was related to poor prognosis (24). Further reports showed no association between p53 expression and global survival (23,45).

Regarding the prognostic role of E-cadherin, our study demonstrated that the lack of this protein was significantly related to poor survival ($P = 0.006$). Previous studies demonstrated that loss of membranous E-cadherin is strongly associated with short survival time (28). Besides, a meta-analysis configured by He *et al* confirmed a strong relation between loss of E-cadherin and worse survival time (46). Further Tunisian studies suggested no relationship between this protein and overall survival (29,39). These findings correlated with the report of a similar study from Egypt, demonstrating the lack of association between E- cadherin expression and survival time (27,47). Consistent with the recent study of Chang *et al*, loss of membranous E-cadherin expression could be of clinical usefulness in assessing colorectal cancer progression and survival (48).

Our present finding suggests that Bcl-2 positivity was significantly associated to survival time ($P = 0.014$). Therefore, patients who expressed Bcl-2 had shorter survival than those whose Bcl-2 was negative. Our results were in contrast with those reported by Torsello *et al* (49). Some authors did not show any significant effect of Bcl-2 protein on the survival rate (30).

The present study showed that neither 3-years OS nor 5-years OS was influenced by Bcl-xL expression. In contrast, many investigations revealed that this protein was significantly associated to overall survival. In fact, Song *et al* reported that patients with high Bcl-xL expression showed poorer overall survival than those with low Bcl-xL expression (42). Biroccio *et al* also revealed that the risk of relapse and death in patients whose tumor specimens displayed Bcl-xL positivity was raised (51).

A recent study identified Bcl-xL as a highly active survival factor and a promising therapeutic target in colorectal cancer (52).

Although each protein has a significant effect by itself, their combined expression may have prognostic

value in colorectal adenocarcinoma. E-cadherin+/p53- combination was significantly improved global survival (P = 0.003). E-cadherin+ / Bcl-2- combination was also increased overall survival ($P \leq$ 0.001). Furthermore, double-positive patients for E-cadherin and Bcl-xL had a longer survival time than those double negative for these proteins (P = 0.036).

Combining E-cadherin with p53, Bcl-2 and Bcl-xL, we could identify CRC patients at high risk of cancer progression and death.

Conclusions

In conclusion, p53 and Bcl-2 expression are independent factors predicting distant metastasis and poor survival in CRC patients. Furthermore, lack of membranous E-cadherin expression was strongly related to poor prognostic factors and short overall survival. Multivariate analysis confirmed a positive correlation between membranous E-cadherin loss and colorectal cancer severity. Combined expression of E-cadherin with p53, Bcl-2 and Bcl-xL could be considered as an important predictor for prognosis and survival of colorectal cancer patients. These biomarkers may also be a potential drug target for protocol treatment in colorectal cancer.

Interest conflict

The authors declare that they have no conflict of interest.

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