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RESEARCH ARTICLE

Serum nectin-2 levels are diagnostic and prognostic in patients with colorectal carcinoma

M. Karabulut[1] · M. Gunaldi[2] · H. Alis[1] · C. U. Afsar[3] · S. Karabulut[4] · M. Serilmez[5] · C. Akarsu[1] · H. Seyit[1] · N. F. Aykan[4]

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Abstract
Introduction Nectins are a family of integral protein and immunoglobulin-like cell adhesion molecules involved in the formation of functioning adherence and tight junctions. Aberrant expression is associated with cancer progression, apoptosis and cell proliferation but little is known how these effects change in cell behavior. The objective of this study was to evaluate the serum levels of nectin-2 with regard to diagnostic, predictive and prognostic value in colorectal cancer (CRC) patients.
Materials and methods One-hundred and forty CRC patients were enrolled in this study. Serum nectin-2 levels were determined by enzyme-linked immunosorbent assay method. Age- and sex-matched 40 healthy controls were included in the analysis.
Results Median age of patients was 60 years old, range 24–84 years. The localization of tumor in majority of the patients was colon ($n = 81$, 58 %). Non-metastatic (stage II and III) and metastatic patients' baseline serum nectin-2 levels were significantly higher than those in the healthy control group ($p < 0.001$; for two group). However, known clinical variables including response to CTx (chemotherapy) were not found to be correlated with serum nectin-2 concentrations ($p > 0.05$). While non-metastatic group patients with elevated serum nectin-2 levels showed significant adverse effect on PFS, metastatic group patients with elevated serum nectin-2 levels showed no significant adverse effect on PFS ($p = 0.05$ and $p = 0.29$, respectively). On the other hand, our study results did not show statistically significant serum nectin-2 concentrations regarding overall survival rates.
Conclusion Serum levels of nectin-2 may have diagnostic roles for CRC patients. Moreover, our study results show the prognostic role of nectin-2 in non-metastatic group patients.

Keywords Nectin-2 · Serum · Diagnostic · Prognostic · Progression-free survival · Colorectal cancer

Introduction

Colorectal cancer (CRC) is ranked the third in men and women among all cancers and the occurrence of them ranks the 3rd in cancer-related deaths [1]. Most of the cancer-caused deaths depend on metastatic spread while 20–25 % of the patients are metastatic during diagnosis. Localized CRC (stage I–II) is curable by surgical excision, whereas only 70 % of stage III CRC cases with regional lymph node metastasis are curable by surgery and adjuvant chemotherapy. Despite the recent developments in chemotherapy, metastatic disease is often incurable [2, 3]. Therefore, in the development and progression of CRC, it is critically important to understand the prognostic and diagnostic markers and molecular changes to improve the survival of patients with CRC [4]. There are several prognostic and predictive markers reported in

✉ C. U. Afsar
cigdemusul@yahoo.com

[1] Clinic of General Surgery, Istanbul Bakırköy Dr. Sadi Konuk Education and Research Hospital, Istanbul, Turkey

[2] Clinic of Medical Oncology, Istanbul Bakirkoy Dr. Sadi Konuk Education and Research Hospital, Istanbul, Turkey

[3] Department of Medical Oncology, Ministry of Health, Istanbul Training and Research Hospital, Istanbul, Turkey

[4] Department of Medical Oncology, Institute of Oncology, Istanbul University, Istanbul, Turkey

[5] Department of Basic Oncology, Institute of Oncology, Istanbul University, Istanbul, Turkey

literature. MYBL2, DDX3, platelet to lymphocyte ratio, SPINK1, miR-1826, SIX1, glutamate dehydrogenase are some of the prognostic markers [5–11]. The most important predictive factor for colorectal carcinoma is the RAS status but the prognostic and predictive value of microsatellite instability (MSI) is still controversial [12].

Nectins are an important family of cell adhesion molecules similar to immunoglobulin in the formation and continuation of tight junctions and adherence connections. Four nectin proteins have been defined: nectin-1, -2, -3, -4. All of them have the same structure: extracellular loops similar to immunoglobulin, one transmembrane segment, and a short cytoplasmic domain that is capable of tying only to the transmembrane area and they are associated with the actin cytoskeleton through afadin [13–16]. They display function in homophilic and heterophilic structure on the cell surface. It is known that nectins with Ca^{2+} dependent cell adhesion molecules regulate the cell adhesion among epithelial cells by creating trans-dimmers with neighbor cells of nectin-2 member. It does this by acting as a mediator in increasing the cell adhesion by ensuring the formation of adherence connections based on e-cadherin after structuring of claudin-based connections [17–21]. Nectin binding site for each is different [15, 17, 18]. For example, nectin-4 and nectin-1 trans-homodimers and heterodimers trans-forms may be, but is not nectin-2 and nectin-3 [19]. They also vary in tissues; while nectin-1 and nectin-2 commonly found in immune tissues, nectin-3 is expressed in the testis and placenta mainly [17, 19, 21]. Nectins have been concerned in different diseases in humans where they assign as virus receptors, they are concerned in oral and facial malformations and currently they have been defined as markers, actors and potential therapeutic targets in cancer [21, 22]. Nectin-2 and nectin-4 are often overexpressed in cancer cells, and are associated with a poor prognosis [22]. Actually, nectin-2 has been found to be overexpressed in ovarian and breast cancer tissues using gene expression profile analysis and immunohistochemistry trials [23]. Nectin-2 was overexpressed in different tumor cell lines as well [21].

The nectin protein family is still little investigated in cancer [24]. The place and significance of serum nectin-2 in CRC have not been defined up to day. The purpose of this study is to determine the levels of nectin-2 in CRC and whether it has any diagnostic, prognostic and predictive role or not.

Materials and methods

Patients' characteristics

The serum samples of the 140 consecutive patients with CRC who referred to Istanbul University Institute of Oncology and Bakirkoy Dr. Sadi Konuk Training and Research Hospital from 2011 to 2014 were obtained. Median age of the patients was 60 years (range 24–84). All patients were staged using seventh editions of the American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) systems by radiologic and pathologic basis. All the patients were treated with multidisciplinary approach. Patients with colon cancer who were undergone surgery including segmental colon resection were treated with adjuvant chemotherapy according to their stages. Patients with rectum cancer who received neoadjuvant radiochemotherapy (RCTx) or radiotherapy (RT) were undergone low anterior resection or abdominoperineal resection Some patients were undergone palliative surgery and stage IV patients received palliative CTx with or without targeted therapy (bevacizumab or cetuximab). The pretreatment evaluation included detailed clinical history and physical examination with a series of biochemistry tests and complete blood cell counts. Selection for treatment required an Eastern Cooperative Oncology Group (ECOG) performance score (PS) of 0–2, and appropriate bone marrow (absolute neutrophil count >1500/μL, and platelet count >100,000/μL), cardiac, renal and hepatic function. Patients were treated with various CTx regimens including single agent or combination therapy. Regimens of single or combination CTx were selected according to the PS of patients and extension of disease. Patients received one of the following treatment regimens: simplified LV5FU2 (leucovorin 400 mg/m^2, followed by 5-fluorouracil as a 400 mg/m^2 bolus and a 2400 mg/m^2 infusion over 46 h every 2 weeks), capecitabine (1000 mg/m^2/b.i.d. p.o. for 14 days of each 21-day cycle), modified FOLFOX regimen (simplified LV5FU2 regimen plus oxaliplatin 85 mg/m^2 every 2 weeks), FOLFIRI (simplified LV5FU2 regimen plus irinotecan 180 mg/m^2 every 2 weeks), XELOX (capecitabine 1000 mg/m^2/b.i.d. p.o. for 14 days plus oxaliplatin 130 mg/m^2 every 3 weeks), or XELIRI (capecitabine 1000 mg/m^2/b.i.d. p.o. for 14 days plus irinotecan 240 mg/m^2 every 3 weeks). Bevacizumab was given at a dose schedule of either 5 mg/kg every 2 weeks or 7.5 mg/kg every 3 weeks. Cetuximab 500 mg/m^2 was administered intravenously every 2 weeks.

All patients had pretreatment imaging of primary tumors with magnetic resonance imaging (MRI) or computed tomography (CT). For patients with evaluable imaging studies before and after treatment, radiologic response was recorded according to Response Evaluation Criteria in Solid Tumors (RECIST) v. 1.1, and classified as follows: complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). The tumor response after 2 months of CTX was used for statistical analysis. Follow-up programs of metastatic disease consisted of clinical, laboratory, and CT or MRI depending on which

imaging methods were used at baseline and performed at 8-week intervals during CTx or every 12 weeks for no anticancer treatment. Patients with either CR or PR were classified as responders, and patients with SD or PD were considered as non-responders.

The study was approved by the Institutional Review Board of Istanbul University, Institute of Oncology. Baseline demographic, clinical, and laboratory data including age, gender, performance status, tumor marker levels, *KRAS* mutation status, and treatment details were collected retrospectively for all patients using uniform database templates to ensure consistent data collection. The comorbid diseases of patients were cardiac and metabolic diseases.

The control group consisted of age- and sex-matched 40 healthy people with no previous history of malignancy or autoimmune disorders. Blood samples were obtained from patients with CRC at first admission, 1 month after surgery, and 2 weeks before adjuvant or palliative CTx. Blood samples of healthy controls were taken into dry tubes and sera separated from cellular elements by centrifugation (at 4000 rpm for 10 min) within half an hour after blood samples were stored at −80 °C until analysis. All the samples were collected under the approval of the institutional review board and with adequate informed consents.

Measurement of serum nectin-2 levels

Nectin-2 levels were assessed using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). Serum samples and standards are added to the wells which are pre-coated with human nectin-2 monoclonal antibody. Following incubation, nectin-2 antibodies labeled with biotin and combined with Streptavidin-HRP are added to form immune complex and allowed to incubate for 1 h. Unbound material is washed away and then chromogen solution is added for the conversion of the colorless solution to a blue solution (20–30 min), the intensity of which is proportional to the amount of nectin-2 in the sample. As the effect of the acidic stop solution, the color has become yellow. The colored reaction product is measured using an automated ELISA reader (ChroMate® 4300 microplate awareness technology). The results were expressed as pg/mL.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) for Windows version 21.0 (SPSS Inc., Chicago, IL, USA) was employed for data analysis. Continuous variables were categorized using median values as cutoff point. For group comparison of categorical variables, Chi-square tests or One-Way Anova tests were used and for comparison of continuous variables, Mann–Whitney *U* test or Kruskall–Wallis tests were accomplished. Overall survival (OS) was calculated from the date of first admission to the clinics to disease-related death or date of last contact with the patient or any family member. Progression-free survival (PFS) was calculated from the date of admission to the date of first radiologic progression with/without elevated serum tumor marker. Kaplan–Meier method was used for the estimation of survival distribution and differences in PFS and OS were assessed by the log-rank statistics. All statistical tests were carried out two-sided and a p value ≤ 0.05 was considered statistically significant.

Results

One-hundred and forty patients who were pathologically diagnosed as CRC from May 2011 to August 2014 were included in the current study. Baseline demographic features and histopathological/laboratory characteristics of patients are shown in Table 1. Median age at diagnosis was 60 years old, range 24–84 years, where males constituted majority of the group ($n = 96$, 69 %). Forty-three of patients had family history of cancer including twelve lung cancer and fourteen CRC. The tumor localization was rectum in 42 % ($n = 59$) and colon in 58 % ($n = 81$) of patients (right colon, $n = 17$; hepatic flexura, $n = 5$; transverse colon, $n = 5$; descendent colon, $n = 13$; splenic flexura, $n = 1$; sigmoid colon, $n = 37$; multiple synchronous colon tumor, $n = 3$; rectosigmoid junction tumor, $n = 6$). The most frequent metastasis sites were liver ($n = 40$, 67.8 %) and peritoneum ($n = 17$, 28.8 %). The rate of synchronous ($n = 34$) and metachronous metastasis ($n = 25$) was 57.6 and 42.4 %, respectively. Of the 37 patients who had neoadjuvant treatment received with rectal cancer, 28 had fluoropyrimidine-based RCTx whereas 9 received short-course RT. Seventy-one patients who had adjuvant CTx received one of the following treatment regimens: simplified LV5FU2 or capecitabine ($n = 14$), mFOLFOX regimen ($n = 26$) or XELOX ($n = 31$). Palliative CTx was preferred oxaliplatin-based or irinotecan-based combination CTx regimens and single agent fluoropyrimidine in 24, 22, and 9 patients, respectively. Bevacizumab was given to 36 patients whereas 15 patients had cetuximab as targeted agents. Response to CTx was observed in 31 % of 55 metastatic patients who received palliative CTx.

The levels of serum nectin-2 of the whole group CRC patients and healthy controls are shown in Table 2. There was significant difference in baseline serum nectin-2 levels between the whole group patients and the healthy control group ($p < 0.001$; for all, non-metastatic (stage II or III), and metastatic patients) (Figs. 1, 2). Tables 3 and 4 show

Table 1 Characteristics of the patients and disease

Variables	*n*
No. of patients	140
Age (years)	
Median (range)	60 (24–84)
Gender	
Male/female	96/44
Performance status (PS)[b]	
0/1/2/3	68/61/7/1
Smoking[b]	
Yes/no	61/66
Alcohol intake[b]	
Yes/no	26/99
Comorbidity[b]	
Yes/no	56/79
Obstruction	
Yes/no	17/123
Surgery type	
Colectomy	56
Low anterior resection	36
Abdominoperineal resection	13
Palliative surgery	11
Pathologic tumor stage (T)[c]	
0/1/2/3/4	9/2/12/45/10
Pathologic node stage (N)[c]	
0/1/2	42/18/14
Pathologic stage	
2/3/4	17/64/59
Site of lesion	
Colon/rectum	81/59
Response to CTx[d]	
CR/PR/SD/PD/unknown	2/15/10/24/4
Targeted therapy	
Bevacizumab/cetuximab	36/15
Metastasis	
Yes/no[a]	59/81
Histology	
Adenocarcinoma/mucinous	129/11
Grade[b]	
1/2/3	8/56/6
Angio-lymphatic invasion[c]	
Yes/no	30/18
Vascular invasion[c]	
Yes/no	16/30
Perineural invasion[c]	
Yes/no	18/28
Regression score[e]	
1/2/3/4	1/12/4/8
KRAS mutation status[d]	
Mutant/wild	24/28
Lactate dehydrogenase (LDH)[b]	
Normal (<450 IU/L)/high (>450 IU/L)	97/16
Albumin[b]	
Normal (>4 g/dl)/low (<4 g/dl)	54/58
Carcinoembryonic antigen (CEA)[b]	
Normal (<5 ng/mL)/high (>5 ng/mL)	78/17
Carbohydrate antigen (CA) 19-9[b]	
Normal (<38 U/mL)/high (>38 U/mL)	81/28

[a] Stage II or III

[b] Patients with unknown data concerning the variables are not included in the analysis

[c] Non-metastatic patients with unknown data concerning the variables were not included in the analysis

[d] In 59 patients with metastatic CRC

[e] In 37 patients with rectal cancer who received neoadjuvant treatment

Table 2 The values of serum marker levels in CRC patients and healthy controls

	n	Nectin-2 level (pg/mL) Median (range)
All patients	140	1833.21 (846.31–2296.92)
Controls	40	1442.27 (789.71–1871.56)
P value		<0.001**
Non-metastatic patients[a]	81	1831.38 (1171.90–2296.92)
Controls	40	1442.27 (789.71–1871.56)
P value		<0.001**
Metastatic patients	59	1835.03 (846.31–2260.70)
Controls	40	1442.27 (789.71–1871.56)
P value		<0.001**

** $p \leq 0.05$

[a] Stage II or III

the correlation between the serum levels nectin-2 and clinico-pathological factors. Any clinical variables with the inclusion of response to CTx did not associate with serum assays ($p > 0.05$).

During the 14.0 months (range 1–34 months) follow-up period; forty-three (31 %) patients experienced disease progression and thirty-one (22 %) of the remaining patients died. Median PFS and OS of the whole group were 7.3 ± 1.0 months (95 % CI 5–9 months) and 26.9 ± 1.1 months (95 % CI 25–29 months), respectively. While 1-year PFS rates were 26.2 % (95 % CI 12.9–39.5), 1- and 2-year OS rates were 82.7 % (95 % CI 76.2–89.2) and 70.1 % (95 % CI 58.8–81.2), respectively. A significant relationship between other clinico-pathologic variables including the presence of metastasis ($p = 0.05$), no

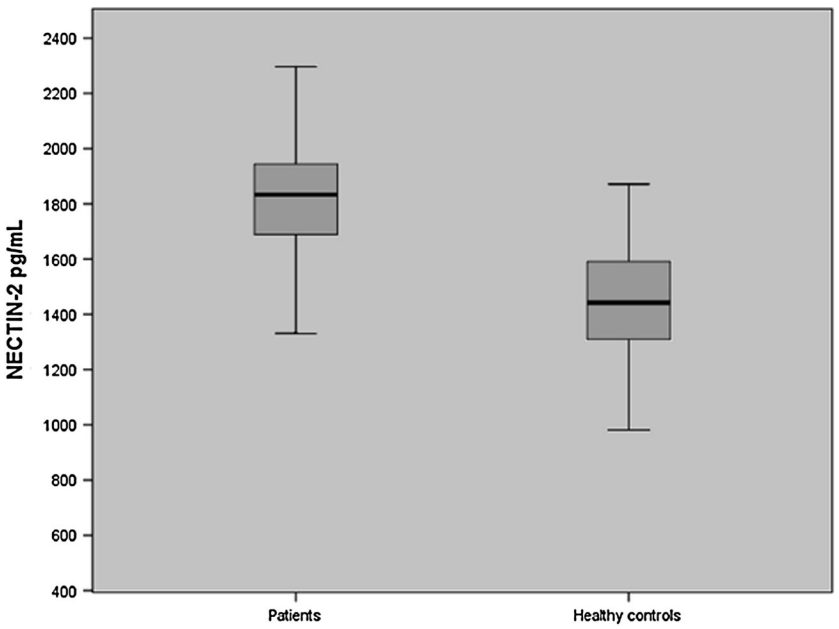

Fig. 1 The values of serum nectin-2 assays in CRC patients and controls ($p < 0.001$)

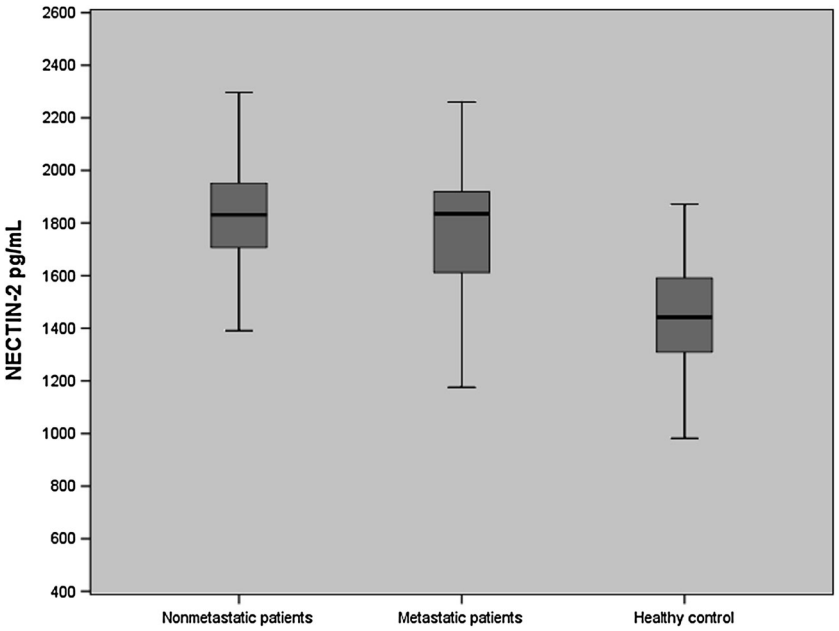

Fig. 2 The values of serum nectin-2 assays in non-metastatic (stage II or III), and metastatic CRC patients and controls ($p < 0.001$ and $p < 0.001$)

surgical resection ($p = 0.01$), CTx-unresponsiveness ($p = 0.001$), high serum levels carcino-embryonic antigen (CEA) ($p = 0.04$), and carbohydrate antigen (CA) 19-9 ($p = 0.03$) poorer PFS was determined. Moreover, all patients with elevated serum nectin-2 concentrations had significantly unfavorable PFS compared with those with lower levels (median 5.8 v 9.1 months, respectively, $p = 0.04$). While non-metastatic patients with elevated serum nectin-2 levels showed significant adverse effect on PFS (median 6.0 v 14.0 months, respectively, $p = 0.05$), metastatic patients with elevated serum nectin-2 levels showed no significant adverse effect on PFS ($p = 0.29$) (Tables 5, 6) (Figs. 3, 4). A significant relationship between other clinico-pathologic variables including localization of rectum ($p = 0.03$), presence of metastasis ($p < 0.001$), vascular invasion ($p = 0.02$), perineural invasion ($p = 0.03$), poor grade ($p = 0.02$), low PS ($p = 0.04$), no surgical resection ($p < 0.001$) CTx-unresponsiveness ($p = 0.002$), high serum levels of lactate dehydrogenase (LDH) ($p = 0.02$), CEA ($p < 0.001$), CA 19-9 ($p < 0.001$), low serum levels of albumin ($p = 0.02$) poorer OS was determined (Tables 7, 8). On the other

Table 3 Results of comparisons between the serum assays and various demographic and disease characteristics

Variables	*n*	Nectin-2 level (pg/mL) Median (range)	*P* value
Age (years)			
Young (<50)	22	1772.88 (1449.65–2035.57)	0.22
Older (≥50)	118	1855.13 (846.31–2296.92)	
Gender			
Male	96	1833.21 (846.31–2296.92)	0.92
Female	44	1836.86 (1331.43–2079.22)	
PS			
0	68	1824.07 (846.31–2296.92)	0.81
1–3	69	1844.17 (1175.62–2177.29)	
Smoking			
Yes	61	1871.56 (846.31–2296.92)	0.92
No	66	1814.94 (1449.65–2170.03)	
Alcohol intake			
Yes	26	1864.26 (646.31–2260.70)	0.62
No	99	1835.03 (1175.62–2296.92)	
Comorbidity			
Yes	56	1864.26 (846.31–2260.70)	0.34
No	79	1835.03 (1175.62–2296.92)	
Obstruction			
Yes	17	1842.34 (846.31–2296.92)	0.46
No	123	1787.51 (1578.52–2035.57)	
Surgery			
Yes	116	1772.87 (846.31–2126.46)	0.26
No	24	1844.17 (1171.90–2296.92)	
T stage			
0–2	23	1831.38 (1171.90–2079.22)	0.98
3–4	55	1842.34 (1457.03–2177.29)	
N stage			
0	42	1807.62 (1171.90–2177.29)	0.57
1–2	32	1838.69 (1309.59–2079.22)	
Metastasis			
Yes	59	1835.03 (846.31–2260.70)	0.24
No[a]	81	1831.38 (1171.90–2296.92)	
Response to CTx			
Yes (CR + PR)	17	1760.07 (1268.46–2260.70)	0.70
No (SD + PD)	34	1824.07 (846.31–1760.07)	
Targeted therapy			
Bevacizumab	36	1794.82 (1268.46–2260.70)	0.54
Cetuximab	15	1845.99 (846.31–2035.57)	
Site of lesion			
Colon	81	1816.76 (1268.46–2296.92)	0.90
Rectum	59	1856.95 (846.31–2170.03)	

[a] Stage II or III

Table 4 Results of comparisons between the serum assays and various histopathological features and laboratory parameters

Variables	*n*	Nectin-2 level (pg/mL) Median (range)	*P* value
Histology			
Adenocarcinoma	129	1813.11 (1541.74–2101.03)	0.69
Mucinous	11	1842.34 (846.31–2296.92)	
Grade			
Good	8	1805.79 (1171.90–2177.29)	0.67
Intermediate	56	1813.11 (1604.24–2170.03)	
Poor	6	1897.11 (1574.84–2035.57)	
Angio-lymphatic invasion			
Yes	30	1856.95 (1626.28–2101.03)	0.61
No	18	1838.69 (1390.59–2177.29)	
Vascular invasion			
Yes	16	1949.99 (1497.57–2079.22)	0.37
No	30	1824.07 (1390.59–2177.29)	
Perineural invasion			
Yes	18	1831.38 (1390.59–2177.29)	0.72
No	28	1822.50 (1497.57–2046.49)	
Regression score			
0–2	13	1816.76 (1453.03–1999.17)	0.30
3–4	12	1919.0 (1651.97–2170.03)	
KRAS mutation status			
Mutant	24	1814.93 (846.31–2126.46)	0.86
Wild	28	1809.45 (1175.62–2260.70)	
LDH			
Normal	97	1833.19 (1453.34–2126.46)	0.81
High	16	1842.34 (846.31–2260.70)	
Albumin			
Normal	54	1816.76 (846.31–2260.70)	0.93
Low	58	1856.95 (1175.62–2079.22)	
CEA			
Normal	78	1844.17 (846.31–2177.29)	0.43
High	17	1856.95 (1175.62–2028.29)	
CA 19-9			
Normal	81	1825.89 (1453.34–2028.29)	0.72
High	28	1842.34 (846.31–2177.29)	

hand, serum nectin-2 levels of patients showed no significantly adverse effect on OS ($p = 0.14$) (Table 8; Fig. 5). In addition, serum nectin-2 levels of metastatic and non-metastatic group patients showed no significantly adverse effect on OS ($p = 0.07$ and $p = 0.32$, respectively) (Table 8).

Discussion

Colorectal cancer (CRC) is an important disease that is observed in a substantial frequency in the world. Diagnosis and treatment at early stage have positive effects on general survival; however, prognosis of patients who are

Table 5 Univariate analyses of progression-free survival according to patient and disease characteristics

Variables	Survival (month)			
	N of events/total *N*	Survival Median (±SE)	1-year survival (%) (±SE)	*P* value
All patients	43/140	7.3 (1.0)	26.2 (6.8)	
Age (years)				
Young (<50)	6/22	8.3 (2.2)	NR	0.45
Older (≥50)	37/118	7.2 (1.1)	25.0 (7.2)	
Gender				
Male	29/96	7.5 (1.1)	28.6 (8.5)	0.46
Female	14/44	7.1 (2.1)	NR	
PS				
0	11/68	8.7 (2.1)	NR	0.30
1–3	32/69	6.9 (1.2)	24.1 (7.9)	
Obstruction				
Yes	6/17	6.3 (1.9)	NR	0.43
No	33/123	7.4 (1.1)	24.2 (7.5)	
Surgery				
Yes	32/116	8.3 (1.2)	31.3 (8.2)	0.01**
No	11/24	4.2 (1.3)	NR	
Tumor stage (T)				
0–2	2/23	11.0 (3.2)	NR	0.85
3–4	8/55	10.0 (6.0)	NR	
Node stage (N)				
0	4/42	6.5 (3.2)	NR	0.20
1–2	6/32	13.7 (3.7)	NR	
Metastasis				
Yes	33/59	6.3 (0.9)	21.9 (7.3)	0.05**
No[a]	10/81	10.8 (2.7)	NR	
Response to CTx				
Yes (CR + PR)	4/17	14.8 (2.3)	NR	0.001**
No (SD + PD)	27/34	4.1 (0.6)	NR	
Targeted therapy				
Bevacizumab	21/36	7.3 (1.2)	28.6 (9.9)	0.06
Cetuximab	4/15	3.5 (1.2)	NR	
Site of lesion				
Colon	19/81	8.3 (1.4)	33.3 (11.1)	0.18
Rectum	24/59	6.6 (1.3)	20.8 (8.3)	
Histology				
Adenocarcinoma	37/129	8.2 (2.6)	24.3 (7.1)	0.79
Mucinous	5/11	7.2 (1.1)	NR	
Grade				
Good	1/8	NR	9.0 (0.0)	0.79
Intermediate	13/56	NR	7.5 (2.2)	
Poor	2/6	NR	5.5 (2.5)	
Regression score				
0–2	2/12	9.5 (6.5)	NR	0.90
3–4	0/13	4.0 (0.0)	NR	
KRAS mutation status				
Mutant	14/24	4.9 (1.2)	NR	0.14
Wild	14/28	7.6 (1.7)	NR	

NR not reached

** $p \leq 0.05$

[a] Stage II or III

Table 6 Univariate analyses of progression-free survival according to laboratory parameters

Variables	Survival (month)			
	N of events/total *N*	Survival Median (±SE)	1-year survival (%) (±SE)	*P* value
LDH				
Normal	27/97	7.1 (1.1)	25.9 (8.4)	0.14
High	5/16	12.6 (5.0)	NR	
Albumin				
Normal	12/54	7.6 (1.6)	26.3 (10.7)	0.57
Low	19/58	8.9 (2.1)	41.7 (14.2)	
CEA				
Normal	16/78	8.9 (1.5)	43.8 (12.4)	0.04**
High	9/17	5.2 (2.1)	NR	
CA 19-9				
Normal	18/81	9.1 (1.3)	38.9 (11.5)	0.03**
High	19/28	6.5 (1.7)	21.1 (9.4)	
Nectin-2 of all patients				
Low (<median value)	21/70	9.1 (1.6)	35.0 (10.7)	0.04**
High (>median value)	22/70	5.8 (1.2)	18.2 (8.2)	
Nectin-2 of non-metastatic patients[a]				
Low (<median value)	6/40	14.0 (3.5)	NR	0.05**
High (>median value)	4/41	6.0 (3.3)	NR	
Nectin-2 of metastatic patients				
Low (<median value)	13/30	7.2 (1.4)	NR	0.29
High (>median value)	20/29	5.6 (1.2)	NR	

NR not reached

** $p \leq 0.05$

[a] Stage II or III

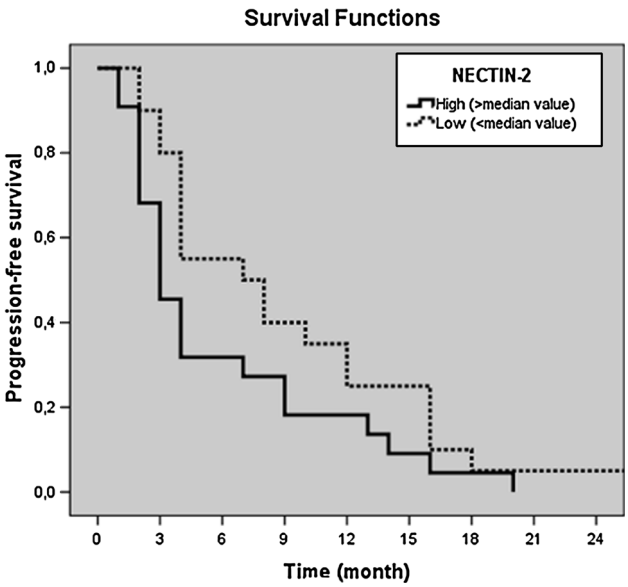

Fig. 3 Progression-free survival curves in all CRC patients according to serum nectin-2 levels ($p = 0.04$)

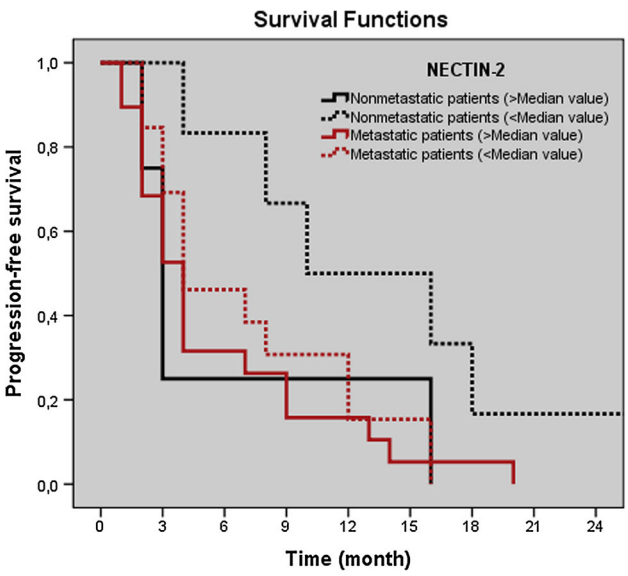

Fig. 4 Progression-free survival curves in non-metastatic (stage II or III), and metastatic CRC patients according to serum nectin-2 levels ($p = 0.05$ and $p = 0.29$)

diagnosed at advanced stage display unfavorable prognosis [25, 26]. The patients having positive prognosis and a long survival duration depend on the time when the tumor is detected. Colonoscopy, which is performed on patients whose occult blood test in the stool and tumor markers (such as carcinoembriogenic antigen) are

Table 7 Univariate analyses of overall survival according to patient and disease characteristics

Variables	Survival (month)			
	N of events/total *N*	Survival Median (±SE)	1-year survival (%) (±SE)	*P* value
All patients	31/140	26.9 (1.1)	82.7 (3.3)	
Age (years)				
Young (<50)	4/22	22.1 (1.4)	90.9 (6.1)	0.30
Older (≥50)	27/118	26.8 (1.2)	81.1 (3.8)	
Gender				
Male	20/96	26.3 (1.3)	83.3 (4.0)	0.76
Female	11/44	26.7 (1.9)	81.5 (5.9)	
PS				
0	9/68	25.4 (1.7)	87.5 (4.2)	0.02**
1–3	22/69	23.1 (0.9)	77.3 (5.2)	
Obstruction				
Yes	5/17	20.7 (2.0)	81.1 (9.9)	0.50
No	23/123	27.5 (1.3)	83.1 (3.6)	
Surgery				
Yes	20/116	28.6 (1.1)	88.0 (3.1)	<0.001**
No	11/24	13.3 (2.0)	56.9 (10.4)	
Tumor stage (T)				
0–2	0/23	NR	100.0 (0.0)	0.28
3–4	3/55	NR	98.2 (1.8)	
Node stage (N)				
0	1/42	32.3 (0.7)	97.6 (2.4)	0.43
1–2	2/32	32.3 (1.2)	100.0 (0.0)	
Metastasis				
Yes	27/59	15.9 (1.4)	61.1 (6.8)	<0.001**
No[a]	4/81	32.5 (0.7)	97.5 (1.7)	
Response to CTx				
Yes (CR + PR)	2/17	23.6 (1.6)	93.3 (6.4)	0.002**
No (SD + PD)	19/34	11.9 (1.4)	47.6 (9.4)	
Targeted therapy				
Bevacizumab	13/36	17.8 (1.7)	69.9 (8.6)	0.55
Cetuximab	7/15	15.2 (2.8)	52.5 (13.1)	
Site of lesion				
Colon	8/81	29.2 (1.2)	91.0 (3.8)	0.03**
Rectum	23/59	24.7 (1.6)	76.6 (4.9)	
Histology				
Adenocarcinoma	28/129	27.7 (1.1)	84.4 (3.3)	0.48
Mucinous	3/11	18.5 (2.7)	70.7 (14.3)	
Grade				
Good	0/8	NR	100.0 (0.0)	0.02**
Intermediate	6/56	NR	90.7 (4.0)	
Poor	3/6	NR	66.7 (19.2)	
Angio-lymphatic invasion				
Yes	3/30	NR	96.6 (3.4)	0.25
No	0/18	NR	100.0 (0.0)	
Vascular invasion				
Yes	3/16	NR	93.3 (6.4)	0.02**
No	0/30	NR	100.0 (0.0)	

Table 7 continued

Variables	Survival (month)			
	N of events/total *N*	Survival Median (±SE)	1-year survival (%) (±SE)	*P* value
Perineural invasion				
Yes	3/18	NR	94.1 (5.7)	0.03**
No	0/28	NR	100.0 (0.0)	
Regression score				
0–2	1/12	NR	91.7 (8.0)	0.30
3–4	0/13	NR	100.0 (0.0)	
KRAS mutation status				
Mutant	13/24	15.1 (2.0)	52.6 (10.3)	0.25
Wild	8/28	18.2 (2.1)	75.8 (9.7)	

NR not reached

** $p \leq 0.05$

[a] Stage II and III

Table 8 Univariate analyses of overall survival according to laboratory parameters

Variables	Survival (month)			
	N of events/total *N*	Survival Median (± SE)	1-year survival (%) (± SE)	*P* value
LDH				
Normal	21/97	21.5 (0.9)	84.6 (3.8)	0.02**
High	7/16	20.5 (3.8)	62.5 (12.1)	
Albumin				
Normal	7/54	23.2 (1.0)	89.8 (4.3)	0.02**
Low	20/58	23.4 (1.9)	73.7 (5.8)	
CEA				
Normal	7/78	24.4 (0.6)	95.7 (2.5)	<0.001**
High	6/17	17.9 (2.6)	68.0 (12.2)	
CA 19-9				
Normal	10/81	23.8 (0.7)	93.4 (2.9)	<0.001**
High	13/28	20.0 (2.8)	61.5 (9.7)	
Nectin-2 of all patients				
Low (<median value)	13/70	27.3 (1.7)	87.1 (4.3)	0.14
High (>median value)	18/70	25.5 (1.5)	78.1 (5.0)	
Nectin-2 of non-metastatic patients[a]				
Low (<median value)	1/40	33.3 (0.7)	100.0 (0.0)	0.32
High (>median value)	3/41	30.9 (1.2)	95.1 (3.4)	
Nectin-2 of metastatic patients				
Low (<median value)	16/30	17.1 (1.7)	70.8 (9.6)	0.07
High (>median value)	11/29	13.8 (2.0)	49.7 (9.6)	

NR not reached

** $p \leq 0.05$

[a] Stage II or III

positive, is used as the primary diagnostic tool. However, utilization of these tests is not very reliable due to their low sensitivity and specificity [27]. In making diagnosis in early stage tumors, there are no sensitive tests that can estimate chemotherapy sensitivity, relapse and long-term survival duration. In CRC, early diagnosis of primary and

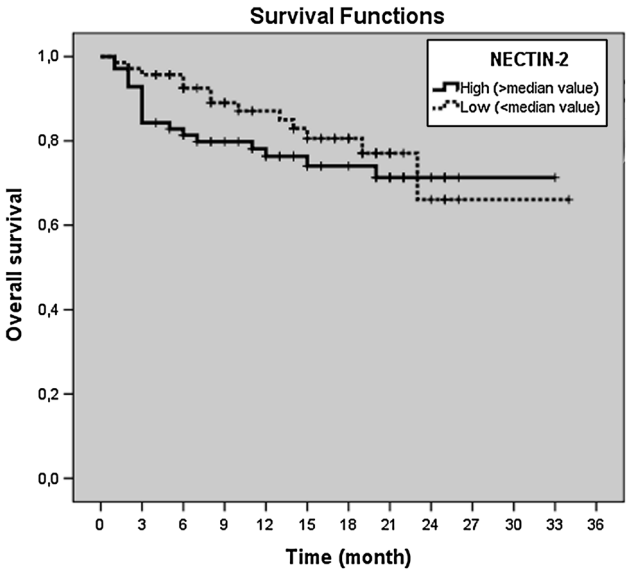

Fig. 5 Overall survival curves in all CRC patients according to serum nectin-2 levels ($p = 0.14$)

recurring disease is needed for recovery of the disease (Table 7).

Nectins are immunoglobulin-like cell adhesion molecules which have been indicated to play a key role in roles such as cell movement, differentiation, reproduction, cellular polarization and cell survival [28, 29]. It has been stated that each of them were expressed from various organs and the epithelial and endothelial cells of their tumors [13]. Relation of abnormal expression of nectins with cancer and the proof of this might be related to the integration of nectins with tight junctions. Increase of permeability in tight junctions as cell–cell adhesion in epithelium cells increases cellular permeability. This situation causes spread and metastasis of cancer cells [30] (Table 8).

It has been determined that nectin-2, which is a member of this family, is expressed less than lymphoblastic leukemia; however, nectin-2 has high expression in leukemic blasts and myeloma tissue. Nectin-2 was expressed in all acute myeloid leukemias (AML) consistently (except AML7 leukemia) [31].

In an immunohistochemical (IHC) study, nectin-2 protein was over-expressed in more than 80 % of breast cancer tissue samples and approximately 50 % of ovarian cancer tissues samples. Whereupon over-expression with IHC of nectin-2 in ovarian cancer and breast cancer has been indicated and authors have claimed that it could be the target treatment for antibody treatment in these types of cancer using flow cytometry in breast and ovarian cancer cells. As an additional test, nectin-2-expression was revealed in blood vessels in this trial [32]. We have evaluated the levels of serum nectin-2 of the CRC patients and healthy controls. There was a significant difference in baseline serum nectin-2 levels between the whole group patients and the healthy control group. This difference shows that it can be not only an evidence of nectin-2 expression in blood vessels but also expressed from tumor.

In previous studies, it has been indicated that in breast cancer high-level nectin-1 and nectin-2 are related to poor prognosis [31]. Nectin-2 expression plays a role in the invasion, metastasis and prognosis of gall bladder adenocarcinoma and carcinoma with squamous cell (ASC) and that in this role it is related to the aggressiveness of carcinomas with adenocarcinoma (AC) and squamous cell (SC) and their poor prognosis [33]. Increased levels of nectin-2 have been found as a biomarker for worse prognostic factor in metastatic gall bladder AC, ASC. No significant differences in clinico-pathological characteristics as well as the percentage of positive nectin-2 expression were observed between SC/ASC and AC patients. In literature, there is only one article about CRC and nectin which emphasizes that expression of both nectin-like receptor (necl)-1 and necl-4 was the most efficient in suppressing the tumorigenicity of colon cancer cells and this was associated with enhanced rates of apoptosis and change in several apoptosis-related markers [34].

In this study, we found no significant differences according to clinico-pathological features, including response to CTx and serum nectin-2 levels. We determined the prognostic significance of serum nectin-2 level in patients with CRC. High serum nectin-2 levels and PFS were found statistically insignificant. This significance is present in the non-metastatic group of patients with high nectin-2 level while a negative effect was not observed in metastatic CRC. Non-metastatic group patients, who have high serum nectin-2 levels, showed significant adverse effect on PFS; however, metastatic group patients, who have high serum nectin-2 levels, showed no significant adverse effect on PFS. On the other hand, our study results did not show a statistically significant relationship between serum nectin-2 concentration and OS.

As a result; in this study it was found that serum nectin-2 levels in patients with both metastatic and non-metastatic CRC cases have a diagnostic value. High serum nectin-2 levels have a bad prognostic impact on PFS of patients with early stage CRC while the impact on the general survival rate was not observed. Since the abnormal release of nectin-2 may change the cellular behavior, this may be related to cancer progression; therefore, we would recommend to increase the number of studies with the aim of determining their significance as predictive factors and for better treatment approaches in CRC.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest relating to the publication of this manuscript.

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Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical standard All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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