

# The role of preoperative serum levels for Dickkopf-related protein 1 as a potential marker of tumor invasion in patients with stage II and III colon cancer

E. GURLULER, L.V. TUMAY<sup>1</sup>, O.S. GUNER<sup>1</sup>, N.T. KUCUKMETIN<sup>2</sup>,  
B. HIZLI<sup>3</sup>, A. ZORLUOGLU<sup>1</sup>

Department of General Surgery, Acibadem University School of Medicine, Istanbul, Turkey

<sup>1</sup>Department of General Surgery, Acibadem Bursa Hospital, Bursa, Turkey

<sup>2</sup>Department of Gastroenterology, Acibadem University School of Medicine, Istanbul, Turkey

<sup>3</sup>Department of Biochemistry, Acibadem Bursa Hospital, Bursa, Turkey

**Abstract. – OBJECTIVES:** To determine and correlate preoperative serum levels of CEA and Dickkopf-related protein 1 (DKK-1) in stage II and III colon cancer and so identify the patients at high risk for early relapse.

**PATIENTS AND METHODS:** A total of 100 patients with stage II (n = 50; median age: 53 years, 31 males) and III (n = 50; median age: 57 years, 32 males) colon cancer and 50 controls (median age: 53.9 years, 25 males) were included. Serum levels for CEA and DKK-1 were recorded in each subject.

**RESULTS:** Mean (SD) levels for serum CEA and DKK-1 were significantly higher in stage III patients than in stage II patients and controls ( $p < 0.001$  for each). There was a significant correlation between serum levels of CEA and DKK-1 in stage II ( $r = 0.71$ ,  $p < 0.001$ ) stage III ( $r = 0.62$ ,  $p < 0.001$ ) patients.

**CONCLUSIONS:** Preoperative serum levels for DKK-1 seem to be a potential marker in prediction of tumor invasion and relapse in stage II-III colon cancer.

*Key Words:*

CEA, DKK-1, Stage II, Stage III, Colon cancer.

## Introduction

Ranking among the most frequent malignancies, colon cancer is the fourth leading cause of cancer-related death worldwide<sup>1</sup> and is the third leading cause of cancer-related death in Turkey<sup>2</sup>. Tumors state counts on fecal occult blood tests<sup>3</sup> with colonoscopy considered as the reference standard for diagnostic confirmation while it is an invasive method that causes major morbidity in 0.3% of subjects<sup>4,5</sup>. Diagnosis, treatment and

survival of colon cancer at an early stage fairly further the probability of survival, while patients diagnosed at an advanced stage have a rather poor prognosis. Approximately 50% of colon cancer patients will improve advanced disease excessive time, though treatment attempts<sup>6</sup>, while screening therapy in advanced disease has also been considered to be beneficial<sup>7,8</sup>.

Surgical resection is the recommended treatment for many colon cancer patients and advance in 5-year survival probability was reported in Stage III patients who receive adjuvant chemotherapy following surgery<sup>9</sup>, whereas the benefit of adjuvant chemotherapy in stage II patients is still subject to discuss with consideration of its use in not for overall but only in a subgroup of patients with stage II disease<sup>10</sup>. In this regard, the need for a biomarker with a potential to identify stage II patients who may utilize from adjuvant chemotherapy has been emphasized.

Carcinoembryonic antigen (CEA) is a large family of 36 different, but related, glycoproteins, which are part of the immunoglobulin superfamily. Identified in the gastrointestinal during fetal life and being expressed at low concentrations in adults, CEA was first reported to be quite specific for tumors of the GI tract, but further investigations demonstrated high levels of CEA also in several other malignant and benign diseases<sup>11</sup>. Today, after 30 years of clinical research, it is well retained that CEA should not be used for screening or early detection of colon cancer, and in association with clinical history<sup>12</sup>.

This marker may provide benefit to determine colon cancer, but high-strong, controlled studies

are still needed to justify its value. Indeed, serum markers have been considered to be more appropriate for screening purposes, to have better acceptability and to ensure higher patient consistency<sup>13</sup>.

Dickkopf-related protein 1 (DKK-1), a secreted protein, is known as a negative regulator of the Wnt signaling pathway which plays an important role in development and in regulating adult stem cell systems. A variety of cellular processes are mediated by Wnt signaling, including proliferation, differentiation, survival, apoptosis, and cell motility<sup>14,15</sup>.

In the present study, we aimed to determine and correlate preoperative serum levels of CEA and DKK-1 in stage II and III colon cancer and so identify the patients who are at high risk for early relapse.

## Patients and Methods

### Study Population

A total of 100 patients diagnosed with stage II (n = 50; median age 53 (ranged 30-70) years, 31 males and 19 females) and stage III (n = 50; median age 57 (ranged 39-70) years, 32 males and 18 females) colon cancer and underwent tumor resection after colonoscopy were included in this study conducted between 2010 and 2013. Control group was composed of age and sex-matched healthy volunteers (n = 50; 25 males and 25 females, median age: 53.9 (ranged 40-70) years). All patients were classified according to the Union for International Cancer Control (UICC) stage classifications using resected specimens. Patients who received chemotherapy and radiotherapy prior to sampling were excluded from the study. Preoperative levels for serum CEA and DKK-1 were recorded in each patient and also in control subjects.

Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the Institutional Ethics Committee.

### Determination of CEA

A 3 ml peripheral blood sample was collected in 100 patients < 3 days before operation. Preoperative serum CEA levels were determined an enzyme immunoassay test kit DPC (Diagnostic

Product Co., Los Angeles, CA, USA) with the upper limit of 5 ng/ml<sup>-1</sup> defined as normal according to the manufacturers of the kits used.

### Determination of DKK-1

Serum levels of DKK-1 were measured by commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA). A total of 96-well plates were coated overnight at room temperature with monoclonal mouse antihuman DKK-1 capture antibodies in phosphate-buffered saline (PBS). The plates were washed with PBS/Tween and blocked with 1% bovine serum albumin in PBS for 1 hour at room temperature. The samples were added to the plates and incubated for 2 hours. Goat antihuman detection antibodies were added, and the plates were incubated for another 2 hours. Streptavidin-horseradish peroxidase was added and incubated for 20 minutes. After the plates were washed with PBS, the substrate reagent was added for another 20 minutes. The substrate reaction was stopped upon addition of 1 mol/l sulfuric acid, and extinction was measured at 450 nm wave-lengths using a multiple ELISA reader (Anthos Microsysteme, GmbH, Krefeld, Germany). All measurements were performed in duplicate for each sample, and the mean value was calculated<sup>16</sup>.

### Statistical Analysis

Statistical analysis was made using computer software (SPSS version 21.0, SPSS Inc. Chicago, IL, USA). Shapiro Wilk normality control and histogram charts were drawn. One-way analysis of variance (one way ANOVA) was used for analysis of quantitative variables. Binary comparisons (post hoc) were made via Tukey HSD in homogeneous and Tamhane tests in non-homogeneous variances. Nominal variables were compared by Chi-square ( $\chi^2$ ) test. Correlations between the variables in each group were performed with Pearson's correlation test. Data were expressed as "mean (standard deviation; SD)", median (minimum-maximum) and percent (%) where appropriate.  $p < 0.05$  was considered statistically significant.

## Results

Patient and control groups were homogenous in terms of age and gender distribution (Table I, Figure 1).

**Table I.** Demographic characteristics and serum levels of CEA (ng/ml) and DKK-1 (ng/ml) in study groups.

		Controls (n = 50)	Patients (n = 100)		Total
			Stage II (n = 50)	Stage III (n = 50)	
Age (years)		53.9 (40-70)	53 (30-70)	57 (39-70)	150 (30-70)
Gender			n (%)		
Female		25 (50.0)	19 (38.0)	18 (36.0)	37 (37.0)
Male		25 (50.0)	31 (62.0)	32 (64.0)	63 (63.0)
CEA (ng/ml)	Mean (SD)	4.0 (1.2)	9.1 (1.3)	13.2 (1.6)*+	8.8 (4.0)*
	Median (min-max)	1.2 (1-7)	9.0 (6-11)	13.2 (10-16)	8.7 (1-16)
DKK-1 (ng/ml)	Mean (SD)	3.9 (0.8)	4.8 (0.6)	6.3 (0.6)*+	5.0 (1.2)*
	Median (min-max)	3.9 (2-6)	4.7 (3-6)	6.2 (5-8)	4.9 (2-8)

\* $p < 0.001$  compared to controls; + $p < 0.001$  compared to Stage II patients.

There were no significant difference between males and females.

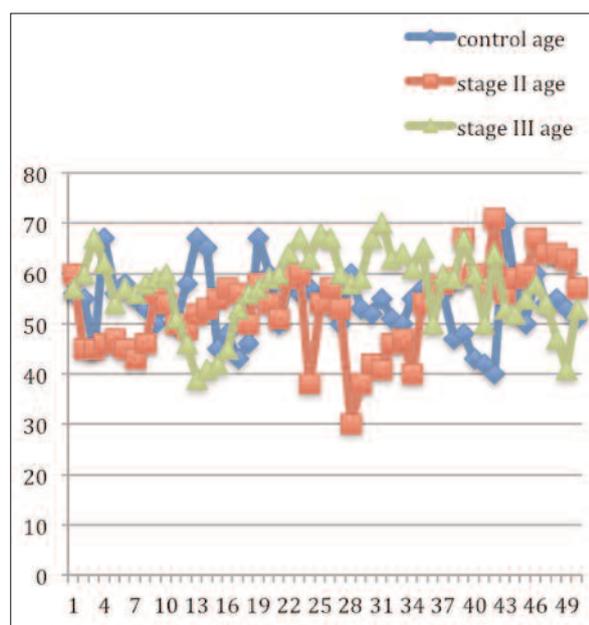
Mean (SD) serum levels for CEA (ng/ml) were significantly higher in overall (8.8(4.0)) and Stage III (13.2(1.6)) patients compared with controls (4.0(1.2)) and in Stage III than Stage II patients (9.1(1.3)) ( $p < 0001$  for each; Table I, Figure 2).

Mean (SD) serum levels for DKK-1 (ng/ml) were significantly higher in overall (5.0(1.2)) and Stage III patients (6.3(0.6)) compared with controls (3.9(0.8)) and in Stage III than Stage II patients (4.8(0.6)) ( $p < 0001$  for each; Table I, Figure 3).

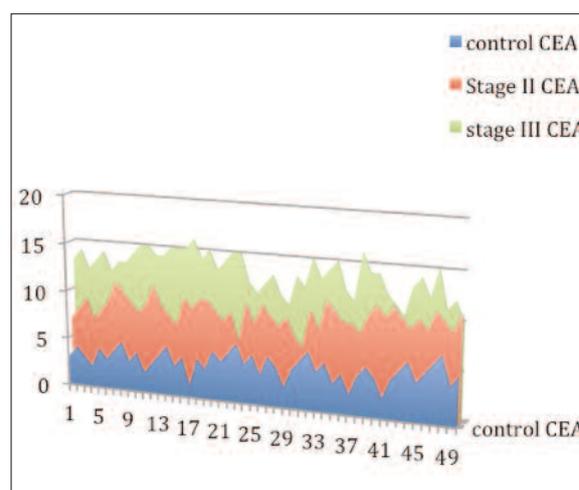
Age was not significantly correlated to serum levels of CEA ( $r = 0.26$ ;  $p = 0.42$ ) or DKK-1 ( $r = 0.27$ ;  $p = 0.34$ ) while there was a significantly correlation between serum levels of CEA and DKK-1 in stage II ( $r = 0.71$ ,  $p < 0.001$ ) stage III ( $r = 0.62$ ,  $p < 0.001$ ) patients (Table II).

### Discussion

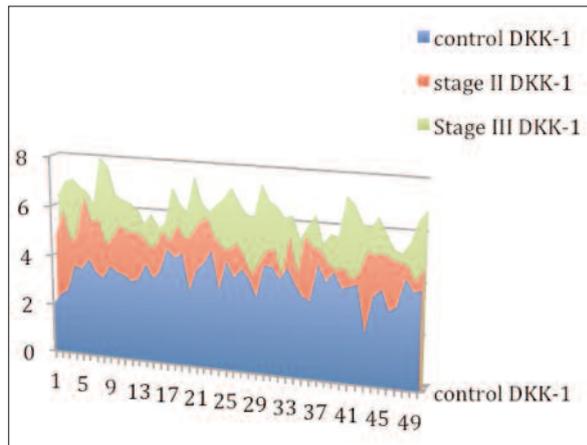
Our data are consistent with the data from several studies that reported the significantly higher preoperative serum CEA levels with respect to postoperative serum levels in patients with colon cancers and in stage III patients, in particular<sup>17,18</sup>. To our knowledge, this is the first study on DKK-1 levels in stage II and III colon cancer. Higher



**Figure 1.** The histogram of age in the control, stage II and stage III groups.



**Figure 2.** The histogram of the CEA levels in the control, stage II and stage III groups.



**Figure 3.** The histogram of the DKK-1 levels in the control, stage II and stage III groups.

DKK-1 levels in Stage III than Stage II patients with colon cancer in the present study may be explained by increased invasion, differentiation and metastasis caused by cancer and/or increased expression of serum DKK-1.

Based on the documented association of tumor invasion with tumor grade and stage<sup>19</sup>, pronounced increase in preoperative serum DKK-1 levels in stage III colon cancer patients seems to indicate the potential of DKK-1 levels to predict tumor invasion and therefore earlier detection of advanced tumor.

Increased expression of Wnt inhibitor DKK-1 by stage III colon cancers in our study at levels

systemically detectable in humans seems to indicate its usefulness in diagnostic evaluation of colon cancer patients.

As an onco-protein up-regulation of which has been considered as a frequent and important feature of the malignant nature of human cancers, DKK-1 seems likely to be involved in critical steps of tumor growth/metastasis<sup>20</sup>. Albeit no clear-cut evidence is available on the significance of DKK-1 expression in tumors, possible contribution to tumor pathogenesis and expansion along with the likelihood of acting in an autocrine manner on the tumor cells have been suggested<sup>21</sup>.

DKK-1 can solely act as a mitogen, but its presence at a critical threshold may serve a checkpoint in the tumor proliferation<sup>21</sup>. One hypothesis is that DKK-1 has to be present to prevent inappropriate differentiation during a rapid burst of mitosis and collateral effect<sup>22</sup>.

Although CEA normal function(s) and its relevance to malignant transformation are not clear, the secretion of CEA by many colorectal tumors has been associated with a worse prognosis and a greater likelihood of metastasis<sup>23</sup>. Given its positive correlation to CEA levels and limitations of immunohistochemical owing to poor objectivity and low quantitative analytic power, determination of higher levels serum DKK-1 concentration in patients with colon cancer and remarkably higher levels in Stage III vs. II patients in our study emphasize that DKK-1 offers an inexpensive and easy to use

**Table II.** Correlations between age, CEA (ng/ml) and DKK-1 (ng/ml) in the overall and Stage II/III patient population.

			Age (years)	CEA (ng/ml)	DKK-1 (ng/ml)
Age (years)	Overall	r	–	0.26	0.27
		p	–	0.42	0.34
	Stage II	r	–	0.217	0.843
		p	–	< 0.001	< 0.001
	Stage III	r	–	1	0.279
		p	–	< 0.001	< 0.001
CEA (ng/ml)	Overall	r	0.26	–	0.571
		p	0.42	–	< 0.001
	Stage II	r	0.217	–	r = 0.71
		p	< 0.01	–	p < 0.001
	Stage III	r	0.116	–	0.62
		p	< 0.01	–	p < 0.001
DKK-1 (ng/ml)	Overall	r	0.27	0.812	–
		p	0.34	< 0.001	–
	Stage II	r	0.029	r = 0.71	–
		p	< 0.001	p < 0.001	–
	Stage III	r	0.279	0.62	–
		p	< 0.001	p < 0.001	–

alternative to immunohistochemical assays with potential to predict tumor invasion and therefore disease at advanced stage associated with high risk of occult metastases.

## Conclusions

Our findings revealed correlated increase in preoperative values for DKK-1 and CEA in Stage III than Stage II patients with colon cancer. Screening of patients with colon cancer in terms of preoperative serum levels for DKK-1 seems to offer a less expensive and easier to use alternative to immunohistochemical assays with potential to predict tumor invasion and relapse of stage II-III colon cancer.

## Conflict of Interest

The Authors declare that there are no conflicts of interest.

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