Correlation of vascular endothelial growth factor (VEGF) and CEA with clinicopathological variables in colorectal cancer patients

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The aim of the presented study is to analyze VEGF levels and its correlation with the clinicopathological characteristics of patients with colorectal carcinoma. Thirty-three patients with colorectal adenocarcinoma and 10 healthy controls were evaluated by estimation of VEGF and CEA levels and correlation with clinicopathological features. The serum VEGF and CEA concentrations of colorectal patients were higher than the healthy controls ($p<0.05$). Patients in advanced stage had high levels of both markers but these differences were not statistically significant. There was a positive correlation between both markers and, tumor size and peritumoral vascular invasion (PVI) but when compared VEGF with CEA, VEGF had a stronger correlation. Diagnostic sensitivity of VEGF for colorectal carcinoma was higher than the sensitivity of CEA and combining both markers the sensitivity to predict colorectal carcinoma was higher than of each marker alone. Our study indicated that VEGF compared to CEA had a higher diagnostic sensitivity for colorectal carcinoma and might provide even additional information about tumor features.

Key words: vascular endothelial growth factor, VEGF, colorectal cancer

The most life-threatening aspects of the neoplastic process are invasion and metastasis. Many studies have shown that angiogenesis, which is a dynamic and complex process that involves new blood vessel formation from established vasculature, is essential for solid tumor growth, invasion and metastasis [7, 15, 18, 22]. Angiogenesis is controlled by several angiogenetic and angiostatic factors. Among these vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is one of the most effective angiogenetic proteins known [5–7, 22]. VEGF is a 34–42 kDa dimeric, heparin-binding glycoprotein that function as active mitogen of vascular endothelial cells, providing opportunity for their migration and organization for the neovascularization of micrometastasis. It is expressed in four isoforms derived by alternative mRNA splicing, $VEGF^{121}$, $VEGF^{165}$, $VEGF^{189}$ and $VEGF^{206}$ [7]. Among these the larger forms, $VEGF^{189}$ and $VEGF^{206}$ are bound to the cell surface, and the smaller forms $VEGF^{121}$ and $VEGF^{165}$ are soluble proteins so they can be detected in the serum with an immunoassay [2]. VEGF has been reported to be synthesized and secreted by a variety of cultured tumor cells and various human cancers [3, 9, 14, 20]. Recent studies have exposed a relationship between increased expression of VEGF and tumor growth, distant metastasis and poor prognosis [11, 16, 21]. In the present study the VEGF and CEA levels were correlated with the clinicopathological characteristics of patients with colorectal carcinoma.

Material and methods

Patients. The study sample consists of two separate consecutive populations. The first population of thirty-three patients with histopathologically confirmed colorectal carcinoma at Ankara Oncology Hospital from September 2001 through April 2002 were enrolled prospectively in the study after obtaining their informed consent. The diagnosis and clinical evaluation of colorectal carcinoma were based on all information including physical examination, endoscopic biopsy, routine biochemical investigations, and imaging studies such as chest X-ray, abdominal ultrasonography and

computerized tomography. A second population of healthy volunteers during the same period was included in the study as controls. Sera from patients (median age 55 years; range 29 to 80 years, and 17 male patients and 16 female patients) preoperatively and from controls (median age 54 years; range 27 to 75 years, and 6 males and 6 females) were obtained and evaluated for VEGF and CEA. The exclusion criteria of the study were as follows: previous neo-adjuvant chemotherapy or preoperative radiation therapy, and/or having recent trauma, surgery, pregnancy or diseases with possible increased VEGF activity (e.g. rheumatoid arthritis) for both populations. In the patient group there were 14 colon cancers and 19 rectum cancers. Pathological staging of the disease was performed according to the 2002 UICC/AJCC criteria [10] after tumor resections. Three patients had stage I disease, 11 stage II disease, 12 stage III disease and 7 had stage IV.

Assays. At the time of enrollment, 5 ml of whole blood were drawn from antecubital vein of each patient preoperatively after the final diagnosis was established and of each control after clinical evaluation. All samples were centrifuged immediately for 10 minutes at 1600 x g. Centrifuged serum was stored frozen at –20 ˚C until analyzed. Quantitative VEGF levels were determined by using the monoclonal human VEGF antibody (Biosource International, California, USA). The assessment of VEGF serum concentrations was done by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocols. All samples were run in duplicate and the mean values were calculated.

Serum CEA concentrations were measured by using an immunoluminometric assay (immulite 2000 kit, DPC Biermann GmbH, Nauheim, Germany) according to the manufacturer's recommendations.

Serum samples from patients and controls were assayed in parallel and at the same time.

Statistical analysis. Statistical analysis was performed using SPSS 9.05 for Windows statistical software (SPSS Inc, Chicago, IL, USA), with $\alpha<0.05$ considered to be statistically significant. Analysis included information regarding CEA and VEGF concentrations, age, and tumor size as a continuous and dichotomous variables; sex, tumor location, tumor differentiation, peritumoral vascular invasion, peritumoral neural invasion, peritumoral lymphatic invasion, lymph node status and distant metastasis as a dichotomous variables; depth of invasion, Astler-Coller and TNM stagings as multiple categorical variables. Categorical data were compared by the Wilcoxon's rank-sum test or the Kruskall-Wallis one-way analysis of variance. The Spearman's rank correlation, univariate linear and logistic regression models to predict relationships with CEA and VEGF levels, and with these markers and histopathological features were applied for independent variables, and proportion of variance explained (PVE) was calculated [8]. The analysis of residuals in evaluating the fit of the regression equation was used [13]. Serum levels of VEGF and CEA were considered as pathological when they exceed the mean plus two standard deviations of the control groups but the optimal cut-off points for each marker for discriminating between patients and controls were sought by constructing receiver operating characteristic (ROC) curves, which were generated by calculating the sensitivities and specifities of VEGF and CEA data at several predetermined cut-off points [24].

Results

The demographic and tumor characteristics of the 33 patients are summarized in Table 1. In all patients radical colectomy or abdominoperineal resection was performed related to tumor location. Metastasectomies were performed in seven patients additional to colorectal resection because of hepatic metastases. The median CEA levels in patients and the controls were 7 ng/ml (range, 0.2 to 63.3 ng/ml) and 1.3 ng/ml (range, 0.3 to 2.25 pg/ml), respectively ($p=0.004$), the median VEGF levels in these groups were 248 pg/ml (range, 5 to 1025 pg/ml) and 78.5 pg/ml (range, 5 to 276 pg/ml), respectively ($p=0.003$) (Fig. 1). In our series, 64% of patients had T_4 tumor and 19 (58%) had involved lymph nodes. Median tumor size was 6.0 cm (range, 1.5 to 11.0 cm). Although there was an increase in serum CEA and VEGF levels with advancing stages, this was not statistically significant ($p>0.05$) (Fig. 2). Both markers' levels in the patients who had great tumor and peritumoral vascular invasion were higher than those with smaller tumor and without peritumoral vascular invasion, and the differences were statistically significant. These results were confirmed by a significant correlation between the marker levels and the given pathological features (Tab. 2). The correlation of VEGF and tumor size was stronger than those with CEA. While 50% of the variation in the tumor size may be accounted for by knowing VEGF level or vice versa, this proportion was only 12% for CEA. There were significant but weak correlations between VEGF and peritumoral vascular invasion (PVI) ($p=0.004$, $r_s=0.49$) and, between VEGF and CEA ($p<0.0001$, $r_s=0.47$, Fig. 3A) as seen in Table 2. This regression model was rather satisfactory in residual analysis (Figure 3B). The logistic regression analysis showed that the serum levels of CEA and VEGF were able to determine peritumoral vascular invasion status ($p=0.01$ for both markers).

When serum cut-off levels were calculated as mean serum values of the controls plus 2SD (4 ng/ml for CEA and 269 pg/ml for VEGF), both markers did not demonstrate a great overlap. While the specificities were 100% for CEA and 92% for VEGF, their sensitivities were very low with these cut-off values (55% and 49%, respectively) (Tab. 3). The ROC curves for CEA and VEGF are presented in

Table 1. The demographic, tumor characteristics and preoperative CEA, VEGF levels of the patients

Features	n	CEA(ng/ml) median(range)	p-value*	VEGF(pg/ml) median(range)	p-value*
Sex					
Male	17	8.9(0.5–63.3)	NS	286(15–1024)	NS
Female	16	5.2(0.2–50.0)		165(5–584)	
Age					
≤55	17	6.5(0.2–56.0)	NS	176(5–676)	NS
>55	16	8.0(0.7–63.3)		283(60–1025)	
Tumor location					
Colon	14	2.3(0.7–63.3)	NS	286(92–1025)	NS
Rectum	19	10.3(0.2–56.0)		178(5–676)	
Tumor size					
≤5	23	0.9(0.2–22.2)	0.01	72(5–322)	0.001
>5	10	9.0(0.7–63.3)		348(88–1025)	
Tumor differentiation					
Well	10	1.5(0.2–21.7)	NS	176(5–462)	NS
Moderate-poor	23	9.0(0.5–63.3)		322(15–1024)	
Peritumoral vascular invasion					
Negative	27	2.7(0.2–34.3)	0.001	176(5–676)	0.006
Positive	6	30.0(11.5–63.3)		510(88–1025)	
Peritumoral neural invasion					
Negative	28	5.2(0.2–63.3)	NS	178(5–1025)	NS
Positive	5	24.4(0.7–56.0)		382(88–676)	
Peritumoral lymphatic invasion					
Negative	24	5.2(0.2–50.0)	NS	176(66–1025)	NS
Positive	9	9.0(0.8–63.3)		267(5–676)	
Lymph node status					
Negative	14	2.1(0.2–34.3)	NS	178(5–510)	NS
Positive	19	9.0(0.8–63.3)		322(60–1025)	
Distant metastasis					
No	26	5.2(0.2–56.0)	NS	178(5–1025)	NS
Yes	7	18.9(0.8–63.3)		410(92–888)	
Depth of invasion					
T2	3	1.2(1.0–1.8)	NS&	138(130–318)	NS&
T3	7	1.7(0.8–34.3)		248(92–462)	
T4	20	8.0(0.2–56.0)		261(5–1025)	
Astler-Coller staging					
B1	3	1.2(1.0–1.8)	NS&	138(130–318)	NS&
B2	11	2.3(0.2–34.3)		180(5–510)	
C2	12	14.5(0.9–56.0)		261(60–1025)	
D	7	18.9(0.8–63.3)		410(92–888)	
TNM staging					
I	3	1.2(1.0–1.8)	NS&	138(130–318)	NS&
II	11	2.3(0.2–34.3)		180(5–510)	
III	12	14.5(0.9–56.0)		261(60–1025)	
IV	7	18.9(0.8–63.3)		410(92–888)	

*Determined by Wilcoxon rank-sum test unless indicated, &determined by Kruskal-Wallis one-way ANOVA by ranks.

Figure 4. Area under the curves (AUC) of CEA and VEGF were 0.78 (p=0.004) and 0.80 (p=0.003), respectively. ROC analysis for the CEA data showed that the discriminating ability was limited and, decreasing the cut-off level increased its sensitivity from 55% to only 64%. Using the lower cut-off value for VEGF allowed decreasing the false negative results for colorectal carcinoma and the sensitivity and diagnostic accuracy for VEGF increased to 76% and 73%, respectively. Combining both markers with low cut-off levels, the sensitivity and accuracy were higher than those for each marker alone. Based on these cut-off points, patients with high level of both markers had risk of colorectal carcinoma that was 2.7 times higher than their counterparts.

Discussion

Angiogenesis, or the development of a vascularized stroma, is essential for tumors to grow beyond a minimal size and metastasize and is controlled by a variety of angiogenetic peptides and proteins, one of the most important being VEGF [5–7, 17, 22]. VEGF increases microvascular permeability and directly stimulates endothelial cell growth and angiogenesis. It has been reported to be synthesized and secreted by a variety of solid tumors [3, 9, 14, 20]. Recent studies have revealed a relationship between increased expression of VEGF and tumor growth, distant metastasis, and poor prognosis of patients with colorectal carcinoma [11, 16, 21]. In the presented study, patients with colorectal carcinoma had significantly higher serum concentrations of VEGF than the control group. Even the diagnostic values of VEGF and CEA have been evaluated in the literature [2, 16]. BROLL et al, who used the upper limit of 95% confidence interval for VEGF concentration in healthy controls as cut-off level, reported its sensitivity as 36% which is as low as the cut-off value for CEA [2]. When we applied the similar criterion, we observed that the sensitivities of VEGF and CEA were very low in our study. In contrast to Broll et al, KUMAR et al [16] indicated high

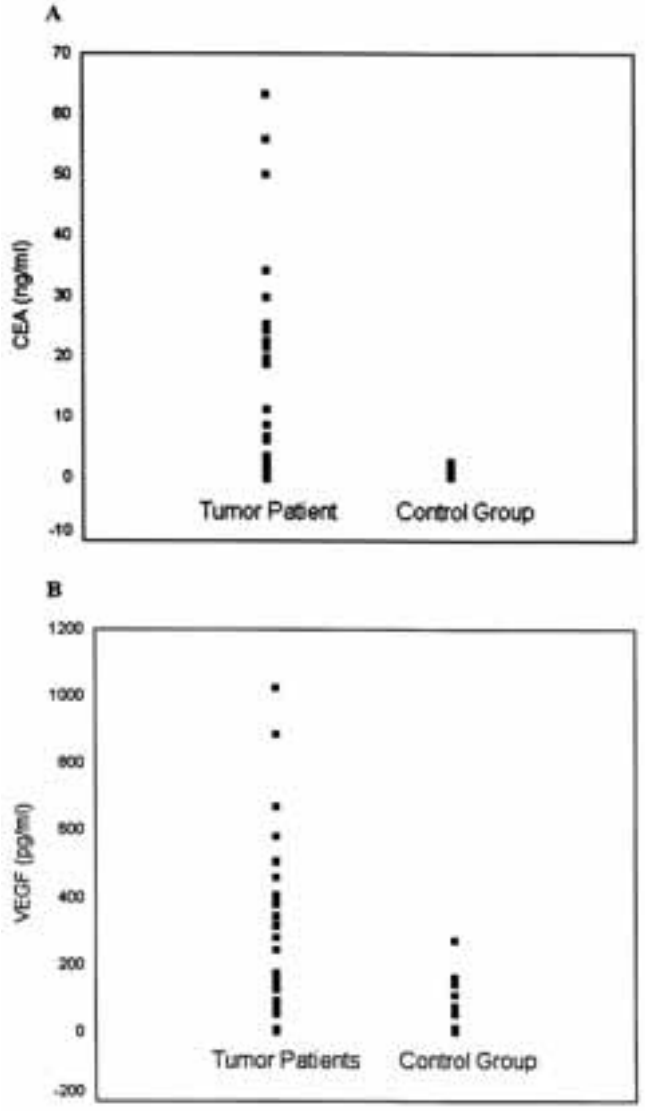

Figure 1. The serum CEA (A) and VEGF (B) levels of the tumor patients and the control group.

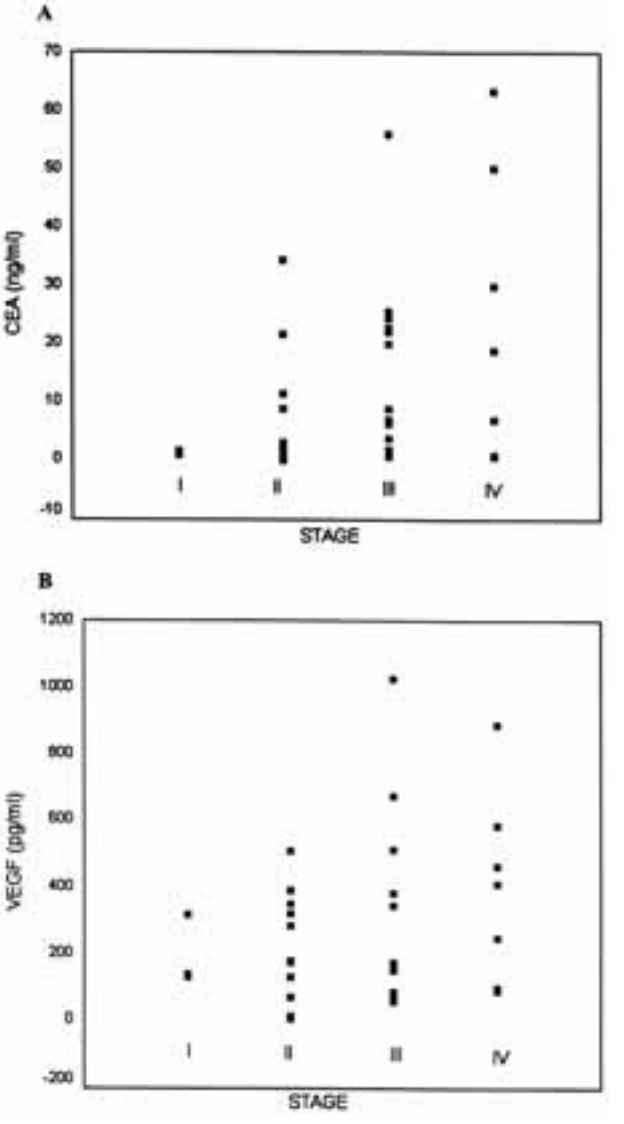

Figure 2. The serum CEA (A) and VEGF (B) levels according to TNM stages.

sensitivity (90.7%) of VEGF with lower cut-off level than those determined by the method mentioned above. In the current study, different cut-off values of both markers were considered by using ROC curves, their sensitivities at predetermined specificities were calculated. The ROC analysis revealed that using 122 pg/ml as the reference of VEGF and 2.5 ng/ml for CEA were optimal points and the sensitivities to predict colorectal carcinoma were 76% and 64%, respectively. Moreover, our study showed that the combination of both markers with these cut-off levels could increase the sensitivity to 88% which was higher than those reported by BROLL et al [2].

On the other hand, the presented study demonstrate a significant and strong correlation between VEGF and tumor size, and a significant but weak correlation between VEGF and peritumoral vascular invasion as poor histopathological feature. The correlations between CEA and the same parameters above were significant but weak. The same weakness was observed by correlation between VEGF and CEA. Thus, VEGF seemed to be more predictive factor especially for tumor than CEA, as mentioned in other studies [2].

In the study of KUMAR et al [16], they found a statistically significant correlation between increased levels of VEGF and all UICC and Dukes' stages of colorectal carcinoma except T_1 tumors. In addition they found a predictive correlation in serum VEGF levels and the incidence of liver metastasis and node-positivity. In our study although there was an increase in serum VEGF levels with advancing stages, this was not statistically significant. This may be due to the limited number of patients included in this study.

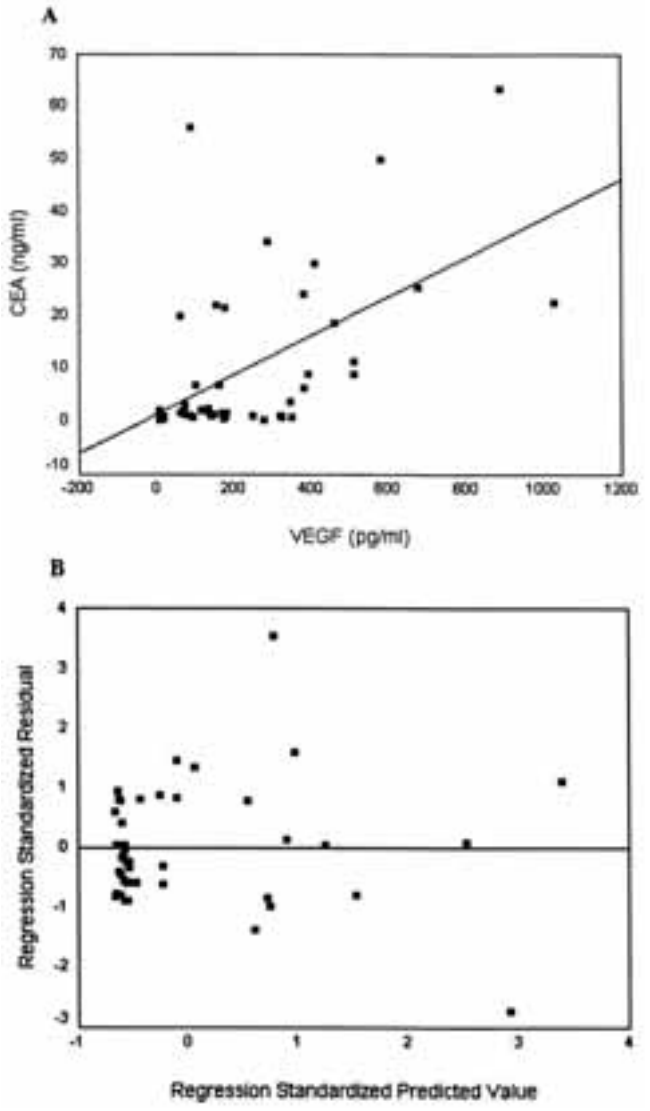

Figure 3. The correlation between VEGF and CEA in tumor patients (A) and its residual graphics (B).

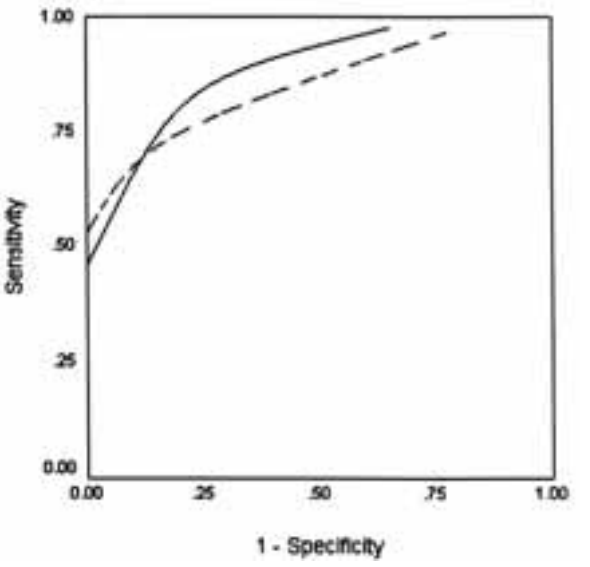

Figure 4. The ROC curves of CEA (dashed line) and VEGF (solid line).

Table 2. Correlation between pathological features and serum markers

Paired features	r_s*	p-value&	p-value#	PVE$
Tumor size-CEA	0.34	0.05	0.05	12%
PVI-CEA	0.58	<0.0001	<0.0001	47%
Tumor size-VEGF	0.80	<0.0001	<0.0001	50%
PVI-VEGF	0.49	0.004	<0.0001	34%
CEA-VEGF	0.47	0.001	<0.0001	30%

*Spearman's correlation coefficient, &two-tailed significans in Spearman's rho, #significance in univariate linear regression model, $proportion variance explained in linear regression model.

Microvessel invasion by tumor cells consist of two aspects: one is cancer cell invasion into the blood vessel and the second is invasion into the lymphatic vessels. As to the microvessel development around the tumor a close correlation between micro-blood vessel density and VEGF positivity was observed for some solid tumors [19, 23]. In the study of NAKATA et al, the VEGF mRNA expression level was significantly higher in cancer specimens with blood and lymphatic vessel invasion by cancer cells than in those without. Furthermore strong VEGF expression was detected in the cancer cells invading the blood and lymphatic vessels using immunohistochemistry [19]. In our study, higher levels of VEGF were seen in patients with vascular invasion and lymphatic invasion but this was statistically significant only for patients with vascular invasion.

VEGF-C, a fraction of VEGF, functions specially to induce lymphangiogenesis. AGAGI et al found that VEGF-C was highly expressed in the primary tumor of patients with both lymph nodes metastasis and lymphatic involvement, and VEGF-C expression increased in the metastatic tumor of lymph nodes, when compared with values in the primary tumor. They also noted the correlation between expression of VEGF-C and depth of invasion [1]. In our study, the deeper the invasion, the higher the VEGF levels were seen, but these were not statistically significant.

Many studies have shown that VEGF expression correlates with liver metastasis and hematogenous recurrence [4, 7, 12]. DAVIES et al pointed out a significant increase in plasma VEGF levels in colorectal liver metastasis compared with controls. There were significant correlation between plasma VEGF and tumor vessel count, tumor vessel volume and colorectal liver metastasis volume [7]. In the study of KANG et al, surgical specimens of 163 colorectal carcinomas were studied by immunohistochemical staining for p53 protein and VEGF. Positive p53 accumulation and VEGF ex-

Table 3. Evaluating diagnostic value of CEA and VEGF according to different cut-off levels

	CEA (4 ng/ml)*	CEA (2.5 ng/ml)&	VEGF (260 pg/ml)*	VEGF (122 pg/ml)&	CEA+VEGF (2.5 ng/ml+ 122 pg/ml)
Sensitivity	55%	64%	49%	76%	88%
Specificity	100%	100%	92%	67%	67%
Positive predictive value	100%	100%	94%	86%	88%
Negative predictive value	44%	50%	39%	50%	67%
Accuracy	67%	73%	60%	73%	82%
Relative risk of high level	1.8	2.0	1.5	1.8	2.7

*Determined by mean level of control group plus 2SD, &determined by ROC curve.

pression was found in 41.7% and 49.1% of tumors, respectively. The incidence of p53-negative or VEGF-positive tumors was significantly higher in patients with venous invasion and liver metastasis than in those without [12]. Similarly, in our series, patients with distant metastasis had higher VEGF levels than patients without distant metastasis but results were not statistically significant.

In conclusion, we evaluated the correlation between VEGF and clinicopathological features in colorectal carcinoma in comparison with CEA. Our study indicated that VEGF compared to CEA had a higher sensitivity for diagnosing of colorectal carcinoma and that combining both markers it seemed to reach higher sensitivity for discriminating colorectal carcinoma patients and healthy controls than those for each marker alone. On the other hand, VEGF may provide more additional information about tumor features and its level is more useful than CEA to reflect the tumor burden, but further studies must be carried out to confirm our results.

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