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Roles of serum and biliary CEA, CA19-9, VEGFR3, and TAC in differentiating between malignant and benign biliary obstructions

BILIARY

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Original Article

ABSTRACT

Background/Aims: Despite the presence of many diagnostic methods, the differential diagnosis between benign and malignant biliary obstructions is still not easy. We aimed to evaluate the role of serum/biliary carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9), vascular endothelial growth factor receptor-3(VEGFR-3), and total antioxidant capacity (TAC) tests in this differential diagnosis.

Materials and Methods: Patients (n:225; 110♂, 115♀) with diagnosis of malignant (n:96) or benign (n:129) biliary obstruction were included in this cross-sectional study. Serum and biliary CEA, CA 19-9, VEGFR-3, and TAC tests were analyzed, statistics were obtained, and significance was defined as $p<0.05$.

Results: Mean age was 54.9±16.4 for the benign and 54.2±19.6 for the malignant group ($p=0.89$). Head of pancreas cancer (18.2%), cholangiocarcinoma (11.4%) and choledochal stone (48%) were the most common etiologies. The area under the curve (AUC)s by ROC analysis of serum/biliary CA 19-9, VEGFR-3, and TAC and serum CEA were 0.701/0.616, 0.622/0.663, 0.602/0.581, and 0713, respectively. Serum TAC had higher sensitivity (61.1%) and CEA had lower sensitivity (42.7%), whereas CEA had higher specificity (89.9%) and TAC had lower specificity (60.5%). In biliary tumor markers, CA 19-9 had higher sensitivity (74%) and VEGFR-3 had lower sensitivity (56.2%); however, VEGFR-3 had higher specificity (79.1%) and CA 19-9 had lower specificity (34.1%). Additionally, combination of serum CEA ($p<0.001$), CA 19-9 ($p<0.001$), VEGFR-3 ($p<0.001$), and biliary CA 19-9 ($p=0.028$) markers achieved 95% estimation probability, and the sensitivity, specificity, and accuracy were 88.5%, 45.7%, and 64%, respectively.

Conclusion: Serum and biliary CEA, CA 19-9, VEGFR-3, and TAC tests would not be useful in the differentiation between malignant and benign biliary obstructions.

Keywords: CA 19-9, CEA, biliary obstruction, vascular endothelial growth factor receptor-3, total antioxidant capacity

INTRODUCTION

Bile duct obstructions may occur at any level within the biliary tree due to either benign or malignant causes (1). The most frequent causes of benign obstructions are strictures caused by previous stone passage, Mirizzi's syndrome, and chronic pancreatitis, while those of malignant obstruction are cholangiocarcinoma (CCA), adenocarcinoma of the head of the pancreas, and adenocarcinoma of the ampulla of Vater.

Cholangiocarcinoma accounts for 3% of all gastrointestinal cancers, and its worldwide incidence is increasing consistently across different populations, including those in North America, Europe, Asia, and Australia (2,3). According to a report of the National Cancer Institute, the annual incidence and mortality rate of liver and intrahepatic bile duct cancers are increasing, presently being 7.8 per 100,000 (4).

Several tumor markers have been used to identify the presence of malignancy; some of these, such as carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA), play an important role in diagnosis, while others, such as total antioxidant capacity (TAC) and vas-

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cular endothelial growth factor receptor-3 (VEGFR3), are useful for monitoring carcinogenesis and prognosis, respectively. Although CA19-9 and CEA are the most commonly used markers in the clinical setting, they lack high sensitivity and specificity (5). Tests for CEA and CA19-9 have been evaluated for their efficacy in differential diagnoses for malignant and benign biliary obstructive lesions. Although CA19-9 levels have been shown to increase in benign and malignant obstructive biliary lesions (especially in malignancies), CEA levels only increase in malignancies. Further, the measurement of CEA and CA19-9 in bile has not been found to be clinically useful (6). In a study, the measurement of biliary/serum CEA levels has been proven useful for differentiating between benign and malignant hepato-biliary disorders; moreover, higher CEA values suggest the presence of malignancy in addition to residual choledocholithiasis-typically, cancer of the head of the pancreas or of the duodenal ampulla (7). CA19-9 has also been evaluated in another study examining its ability to differentiate between patients with pancreatobiliary disorders for malignant [n=106: pancreas cancer (n=73), CCA (n=19), ampullary cancer (n=7), neuroendocrine tumors (n=4), and duodenal cancer (n=3)] and benign disease [n=142: chronic pancreatitis (n=115) and biliary calculous disease (n=27)] (8). This study concluded that differentiation between malignant and benign disease was significantly improved when a cutoff value for CA19-9 (>70.5 U/mL) was used, along with radiology findings (8). Reports regarding the sensitivity and specificity of CEA and CA19-9 have varied considerably. Patel et al. (9) observed that for diagnosing CCA, the sensitivity of CA19-9 levels >100 U/mL was 53%. Nichols et al. (10) found the sensitivity and specificity of CA19-9 to be 89% and 86%, respectively. Qin et al. (11) demonstrated that the sensitivity and specificity of serum CEA for diagnosing CCA were 68.6% and 81.5%, respectively. Chen et al. (12) found the sensitivity of biliary CA19-9 to be less than 70%, while the specificity was 60%.

Cholangiocarcinoma cells stimulate the development of a rich vascular network (13). Correlations between lymphangiogenesis, lymph node metastases, and prognosis have been shown in CCA. The activation of VEGFR3 induces lymphatic endothelial cell proliferation *in vitro* and new lymphatic vessel formation *in vivo* (14-17). Lymphatic invasion and node metastasis are important prognostic factors for intrahepatic CCA (18,19). Although the original location of VEGFR3 is the cell membrane, it can also be present in serum. The role of serum VEGFR3 levels in the diagnosis and prognosis of cancers has been demonstrated in ovarian (20) and gastric cancers (21), as well as in melanoma (22). Although VEGFs have been studied in pancreatobiliary cancers, there are very few reports concerning VEGFR3, as shown by a PubMed search. Elevated VEGF-A levels have been shown in blood monocytes of CCA patients (23). Expression of VEGFR1 and VEGFR2 is also observed in human CCA biopsies by immunohistochemistry (24). Higher VEGFR2 levels have been found in patients with pancreatic ductal adenocarcinoma as compared to those in patients with intraductal papillary mucinous neoplasms (IPMN) (24,25). VEGF-A and VEGFR2 are necessary for the invasion and migration of pancreatic cancer cells, and VEGF/VEGFR inhibitors decrease the motility of pancreatic cancer cells (26).

Oxidative stress caused by increased free radical generation and decreased antioxidant levels in target cells and tissues may play an important role in carcinogenesis. TAC measurement in biological samples was developed during the beginning of the 1990s (27). The role of oxidative damage in cancer has been established, being caused by the generation of free radical DNA damage and mutations, cell death by necrosis or apoptosis, and cellular neoplastic transformation (28-31,8). TAC has been reported to be decreased in cancer patients (32); TAC is thus considered to be a significant marker for diagnosing cancers of the lung (33), breast (34,35), stomach (36), colon (37), and Barrett's esophagus (38). Although reduced antioxidant capacity has been noted in pancreatic cancer, this has not been observed in ampullary cancer, IPMN, or CCA (39).

Although clinical and laboratory findings, endoscopy, and radiology may help clinicians in determining the differential diagnosis for benign and malignant biliary obstructions, this is often difficult (40). The easiest and most frequently preferred method for differential diagnosis is by examining serological and/or biliary biomarkers. To date, no studies have examined the role of TAC or VEGFR3 in differentiating between benign and malignant biliary obstructive tumors. Therefore, in this study, we analyzed the role of biliary and serum levels of CEA, CA19-9, VEGFR3, and TAC as biomarkers for differentiating between benign and malignant biliary obstructions.

MATERIALS AND METHODS

This cross-sectional study was conducted between December 2011 and December 2012 in the gastroenterology clinic of the School of Medicine, Bezmialem Vakif University, Turkey. It was approved by the institutional review board (B.30.2.BAV.0.05.05/217-18.01.2012).

Patients

Patients with biliary obstruction, referred to our endoscopic ultrasound/endoscopic retrograde cholangiopancreatography (EUS/ERCP) unit, were included in the study after obtaining informed consent. Benign and malignant biliary obstructions were diagnosed based on imaging techniques (computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET)) and laboratory tests (CEA and CA19-9 levels). The benign and malignant etiologies diagnosed in our patients are shown in Table 1. Choledocholithiasis and other benign events, such as Mirizzi's syndrome, were diagnosed by laboratory tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transpeptidase (GGT), bilirubins, amylase, and lipase) and radiological methods (magnetic resonance cholangiopancreatography (MRCP), upper abdominal MRI,

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Table 1. Demographic and etiologic characteristics of patients

		Study Group		p
		Benign (n=129)	Malignant (n=96)	
Age		54.9±16.4	54.2±19.6	0.89
Gender	Male	56 (43.4%)	54 (56.3%)	0.057
	Female	73 (56.6%)	42 (43.7%)	
Etiology				
Ampullary Tumor		-	10 (4.4%)	
Head of Pancreas Tumor		-	41 (18.2%)	
Papillary Tumor		-	11 (4.9%)	
IPMN		-	5 (2.2%)	
Biliary Cancer		-	25 (11.4%)	
Other Malignancies		-	4 (1.8%)	
(Behaving as Biliary Cancer)				
Other Benign Events		3 (1.3%)	-	
Choledocholithiasis		108 (48%)	-	
Chronic Pancreatitis		5 (2.2%)	-	
Dysfunction of the Sphincter of Oddi		13 (5.8%)	-	

IPMN: intraductal papillary mucinous neoplasm

ERCP, and EUS). Chronic pancreatitis was diagnosed using EUS examination based on the Rosemont criteria and exclusion of malignancy. Diagnosis of sphincter of Oddi dysfunction was established by exclusion of other etiologies with the help of abdominal CT, transabdominal ultrasonography, gastroscopy, liver function tests (LFTs), amylase, and lipase tests. Diagnosis of papilla Vateri carcinoma was confirmed with biopsy. Diagnosis of CCA was established with the help of tumor marker levels, MRI, MRCP, CT, cholangiography, brush cytology, and biopsy. Histological confirmation of CCA was possible only in 30% of cases, as reported previously. The diagnosis of pancreas cancer was established using EUS, CT, and MRI. Tumor markers (CEA and CA19-9), as well as the pathological evaluation of tissue samples, were used in the diagnosis of malignant conditions.

All of the study patients were over 18 years of age. Patients in poor general condition and those suffering from cholangitis; sepsis; or severe heart, lung, kidney, and/or liver problems were excluded from the study. Patients considered unsuitable for ERCP (e.g., bleeding tendency) were also excluded.

Laboratory tests

Serum samples were obtained before ERCP, and bile samples were obtained during ERCP using a catheter and EUS syringe before contrast dye injection; samples were immediately transferred to 2-mL Eppendorf cups. All samples were stored at -80°C. Serum and biliary CEA, CA19-9, EGF3, and TAC test analyses were completed in the Biochemistry Department of İstanbul Cerrahpaşa Medicine Faculty.

CEA

Serum and biliary CEA levels were analyzed by chemiluminescence immunoassay (CEA Reagent Kit, Abbott Diagnostics).

CA19-9

Serum and biliary CA19-9 tests were measured in the laboratory using routine automated methods with the ADVIA Centaur CA19-9 Assay, an *in vitro* immunoassay for the quantitative measurement of CA19-9 tumor-associated antigens. The ADVIA Centaur® CA19-9 assay is a two-step sandwich immunoassay using direct chemiluminometric technology.

VEGFR3

Serum and biliary VEGFR3 levels were analyzed with enzyme-linked immunosorbent assay kits (R&D Systems, MN, USA). A 96-well microplate was coated with diluted capture antibody and incubated overnight. After washing, the plate was blocked by adding diluent reagent, thus finishing the plate preparation. Samples or standards were added; then, the plates were washed, the detection antibody was added, and washing was repeated. Streptavidin-horseradish peroxidase was added to each well. After washing again, substrate solution was added to each well. Finally, stop solution was added to each well. The plate was tapped gently. The optical densities of each well were quantified within 30 min at dual wavelengths of 450 nm corrected to 540 nm using a micro-plate reader.

Table 2. Mean values for tests in patients with benign and malignant biliary obstructions

	Tests	Benign (n=129)	Malignant (n=96)	t	p
Carcinoembryonic Antigen (ng/mL)	Serum *Mean S.D.*	1.58±4.71	8.83±21.42	-3.26	0.002
	Bile *Mean S.D.*	37.91±8.62	37.44±7.99	0.41	0.677
Carbohydrate Antigen 19-9 (U/mL)	Serum *Mean S.D.*	44.03±106.08	226.51±311.03	-5.51	0.001
	Bile *Mean S.D.*	22.15±6.12	25.26±8.05	-3.16	0.002
Vascular Endothelial Growth Factor Receptor-3 (ng/mL)	Serum *Mean S.D.*	39.15±10.55	44.83±14.77	-3.19	0.002
	Bile *Mean S.D.*	39.92±13.1	48.59±16.55	-4.23	0.001
Total Antioxidant Capacity (mmol/L)	Serum *Mean S.D.*	1.84±0.66	2.11±0.81	-2.7	0.007
	Bile *Mean S.D.*	1.81±0.4	2.15±2.2	-1.7	0.09

SD: standard deviation

Total antioxidant capacity (TAC)

Serum and biliary TAC were measured on an autoanalyzer using a kit supplied by Randox Laboratories Ltd. (Cat. No. NX2332). The sample volume was 5 µL (serum or biliary sample) in a total assay volume of 305 µL. The assay is based on the reduction of free radicals (ABTS •+- 2.2'-azinobis (3-ethylbenzothiazoline-6-sulfonate), measured as a decrease in absorbance at 600 nm for 3 min by antioxidants.

Statistical analysis

After performing descriptive analyses, the chi-square test was used for the comparison of 2-group categorical variables. Student's *t*-test was used for comparison of 2-group quantitative variables. Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and accuracy values were determined. Receiver operating characteristic (ROC) analysis was used for determination of cutoff values for the various parameters. Binary logistic regression analysis was used in order to determine which parameters were significant. Data analysis was performed using SPSS version 20.0 (IBM Corporation, Armonk, New York). Statistical significance was defined as $p<0.05$.

RESULTS

Demographic characteristics and etiologies

Overall, 225 patients (110 men and 115 women) were included in the study (Table 1). They were grouped based on the etiology; i.e., benign (n=129) or malignant (n=96) biliary obstruction. The mean age of the benign group was 54.9±16.4 years, while that of the malignant group was 54.2±19.6 years (p=0.89). Among the patients enrolled in this study, benign etiologies included choledocholithiasis (n=108; 48%), chronic pancreatitis (n=5; 2.2%), sphincter of Oddi dysfunction (n=13; 5.8%), and other benign events, such as Mirizzi's syndrome (n=3; 1.3%); malignant etiologies included ampullary tumor (n=10; 4.4%), IPMN (n=5; 2.2%), head of pancreas tumor (n=41; 18.2%), papillary tumor (n=11; 4.9%), biliary cancer (n=25; 11.4%), and other malignancies behaving as biliary cancers (n=4; 1.8%).

Serum markers

The mean serum levels of the tumor markers have been summarized in Table 2. Serum CEA, CA19-9, VEGFR3, and TAC levels were significantly higher in CCA patients as compared to those in the benign biliary obstructed group ($p<0.05$).

ROC curve analysis for serum markers

ROC curve analysis was performed for identifying cutoff values for serum markers in order to differentiate between malignant and benign biliary obstructions; the results are summarized in Table 2 and Figure 1. The area under the curve (AUC) for serum CEA (cutoff >2.39 ng/mL) was 0.713 (0.649-0.771); for CA19-9 (cutoff >47.8 U/mL), 0.701 (0.637-0.760); for VEGFR3 (cutoff >46.9 ng/mL), 0.622 (0.555-0.685); and for TAC (cutoff >1.95 mmol/L), 0.602 (0.534-0.666). The AUCs of the ROC were statistically significant for serum CEA ($p<0.0001$), CA19-9 ($p<0.0001$), VEGFR3 ($p<0.001$), and TAC ($p=0.008$).

Biliary markers

Table 2 summarizes the mean values for the biliary tests. No significant difference was observed in the levels of biliary CEA or TAC in the benign or malignant groups. However, the levels of biliary CA19-9 and VEGFR3 were significantly higher in CCA patients as compared to those in the benign group of patients ($p<0.05$).

ROC curve analysis for biliary markers

The results of ROC curve analysis are summarized in Table 3 and Figure 1. The AUC for biliary CEA (cutoff <40 ng/mL) was 0.516

Table 3. Tests for the prediction of malignant biliary obstruction

Test/Cutoff Values		ROC Curve Analysis [95% Confidence Interval (%)]							
		Sens	Speci	PPV	NPV	FPR	FNR	Accu	p value
Serum	CEA (>2.39)	42.7	89.9	76	68	24	68	70	<0.0001
	CA19-9 (>47.8)	49	84.5	57	68	43	68	64	<0.0001
	VEGFR3 (>46.9)	48.4	82.9	68	69	32	69	68	0.0014
	TAC (>1.95)	61.1	60.5	54	68	46	68	61	0.0084
Bile	CEA (≤40)	57.3	68.2	46	64	54	64	52	0.6873
	CA19-9 (>21)	74	34.1	70	69	30	69	69	0.0023
	VEGFR3 (>48)	56.2	79.1	67	71	33	71	69	<0.0001
	TAC (>1.8)	65.6	50.4	50	66	50	66	57	0.0363

Sens: sensitivity; Speci: specificity; Accu: accuracy; ROC: receiver operator characteristic; PPV: positive predictive value; NPV: negative predictive value; AC: accuracy; FPR: false-positive rate; FNR: false-negative rate; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9; VEGFR3: vascular endothelial growth factor receptor-3; TAC: total antioxidant capacity

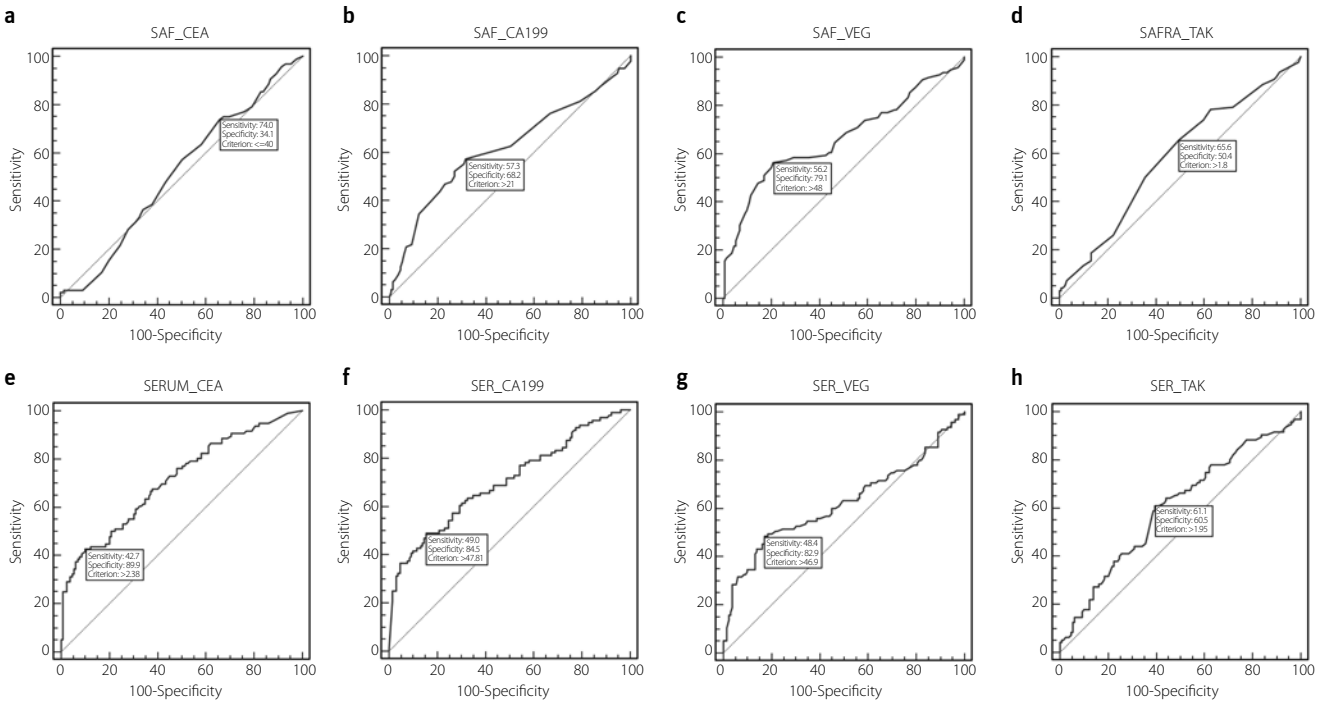

Figure 1. a-h. The area under the curve (AUC) of the ROC for biliary CEA (cutoff <40 ng/mL) was 0.516 (0.448-0.583) **(a),** for biliary CA19-9 (p=0.0023) is significantly higher than a chance value (0.05) **(b),** for biliary VEGFR-3 (p<0.0001) is significantly higher than a chance value (0.05) **(c),** for biliary TAC (p=0.03) is significantly higher than a chance value (0.05) **(d),** for serum CEA (p<0.0001) is significantly higher than a chance value (0.05) **(e),** for serum CA 19-9 (p<0.0001) is significantly higher than a chance value (0.05) **(f),** for serum VEGFR-3 (p<0.001) is significantly higher than a chance value (0.05) **(g),** for serum TAC (p=0.008) is significantly higher than a chance value (0.05) **(h).**

(0.448-0.583); for CA19-9 (cutoff >21 U/mL), 0.616 (0.549-0.68); for VEGFR3 (cutoff >48 ng/mL), 0.663 (0.597-0.724); and for TAC (cutoff >1.8 mmol/L), 0.581 (0.514-0.646). The AUCs of the ROC were statistically significant for CA19-9 (p=0.002), VEGFR3 (p<0.0001), and TAC (p=0.036).

Binary logistic regression analysis

Binary logistic regression analyses were used to determine which of the tumor markers tested in the present study provided maximum specificity and sensitivity. The results demonstrated that a combination of serum CEA, CA19-9, VEGFR3, and biliary CA19-9 tests achieved a 95% predictive probability. The sensitivity, specificity, PPV, NPV, accuracy, false positive values, and false negative values for this 4-test combination were 88.5%, 45.7%, 54.8%, 84.3%, 64%, 31.1%, and 4.9%, respectively.

DISCUSSION

CA19-9 levels are known to be upregulated in malignant disorders, such as pancreatic, biliary, gastric, colorectal, hepatocellular, and ovarian cancers, as well as in benign disorders associated with

jaundice, such as choledocholithiasis, pancreatitis, and cholangitis (41-43). In the presence of cholangitis, CA19-9 and CEA levels are known to increase. Therefore, patients with cholangitis were excluded from our study. Elevated serum CA19-9 concentrations have been previously observed in CCA patients, and it was concluded that CA19-9 could aid in the differentiation of pancreatobiliary disease using a cutoff value of 70.5 U/mL value in combination with routine radiological examinations. The sensitivity, specificity, PPV, and NPV of CA19-9 were 84.9%, 69.7%, 67.7%, and 86.1%, respectively, and it has been shown that CA19-9 was directly correlated with serum bilirubin levels in benign diseases but not in malignant diseases (44). Further, serum CA19-9 levels were higher in pancreatic carcinoma and CCA than in other malignancies ($p<0.001$); it was thus deduced that CA19-9 has a clear relationship with tumor location, stage, and resectability (8). In our study, the optimal cutoff value for serum CA19-9 levels was detected as >47.81 U/mL by ROC analysis (sensitivity: 49%, specificity: 84.5%, PPV: 57%, NPV: 68%, accuracy: 64%, $p<0.0001$). For biliary CA19-9, the optimal cutoff value was detected as >21 U/mL by ROC analysis (sensitivity: 74%, specificity: 34%, PPV: 70%, NPV: 69%, accuracy: 69%, $p=0.0023$) (Table 3). In a different study, the sensitivity of biliary CA19-9 was shown to be less than 70%, with a specificity of 60% (12). Although the mean values of serum and biliary CA19-9 were significantly higher in the malignant group than in the benign group ($p=0.001$ vs. $p=0.002$), the sensitivity of serum CA19-9 and the specificity of biliary CA19-9 were low in the present study. This corresponds to the low values for the true positives (CCA) of serum CA19-9 and true negatives of biliary CA19-9. It may be noted that the differences in the sensitivity and specificity were closely related to the cutoff values of the tests; i.e., if higher cutoff values were used, the sensitivities were also higher. Similarly, the sensitivity of serum CA19-9 was low in our study. In general, the sensitivities were low and specificities were high for CEA and CA19-9 in the studies mentioned above, similar to our results (5,9,6).

Carcinoembryonic antigen has also been used for the differentiation of benign and malignant biliary obstructions. Significantly higher serum [>22 µg/L (-1); sensitivity: 68.6%, specificity: 81.6%] (12) and biliary CEA levels (50.2 ± 5.8 ng/mL) have been detected in CCA patients (45). Patients with highly elevated preoperative CA19-9 and CEA serum levels have a lower chance of survival, and the frequency of nonresectability is significantly higher in these patients as compared to patients with lower corresponding values (46). While CA19-9 is increased in both malignant and benign diseases, CEA increases only in malignant diseases. Akdoğan et al. (6) concluded that the measurement of these markers in the bile is of little or no value. Chen et al. (12) found that the sensitivity and specificity of biliary CEA was less than 70% and 33.3%, although the sensitivity was higher in the presence of cholangitis. In our study, the sensitivity, specificity, and accuracy of serum CEA (cutoff value >2.39 ng/mL) were 43%, 90%, and 70%, respectively ($p<0.0001$), and the sensitivity, specificity, and accuracy of biliary CEA (cutoff ≤40 ng/mL value) were 57%, 68%, and 52%, respectively ($p=0.687$). Despite the presence of higher mean values for serum CEA, the biliary CEA mean was not higher in the malignant patients as compared to the benign group of patients. While the sensitivity of serum CEA was low (43%) and the specificity was high (90%), the sensitivity and specificity of biliary CEA were 57% and 68%, respectively. This outcome was in agreement with the majority of the previously mentioned studies (6,11). Increased levels of CEA have been reported in benign and malignant epithelium of the gall bladder, human bile (47), and in benign and malignant pancreatic duct lesions (48). The lack of difference in biliary CEA levels between the malignant and benign groups could be explained by the presence of CEA-related glycoproteins.

Higher VEGFR3 levels have been demonstrated in many solid cancers. VEGF-C and VEGFR3 expression are detected more frequently in gastric cancer tissues than in normal gastric tissues (54.90% and 35.29%) (49). The median survival time in gastric cancer patients with low serum VEGFR3 levels was found to be significantly greater than in those with higher VEGFR3 levels (15.4 months vs. 7.7 months, $p<0.001$) (21). VEGFR3 immunoreactivity was detected in colon cancer cells; positivity >25% was correlated with significantly poorer overall survival ($p<0.05$) (50). As compared to healthy donors, the median level of pre-treatment serum VEGFR3 in melanoma patients was significantly higher ($p=0.00001$) (22). Thus far, our study appears to be the first to examine VEGFR3 for the differential diagnosis of malignant and benign biliary obstructions. The mean values of both serum and biliary VEGFR3 were significantly higher in the malignant group than in the benign group ($p=0.002$; serum, $p=0.001$; bile). The sensitivity, specificity, and accuracy of serum VEGFR3 (cutoff >46.9 ng/mL) were 48%, 83%, and 68%, respectively ($p=0.014$), while those for biliary VEGFR3 (cutoff >48 ng/mL) were 56%, 79%, and 69%, respectively ($p<0.0001$). In our study, both serum and biliary VEGFR3 sensitivities were low and specificities were high, indicating that while these tests can detect malignant strictures with low sensitivity (48% and 56%), they can accurately predict that the patient does not have a malignant stricture with rates of 83% and 79% because of their higher specificities.

Total antioxidant capacity is a potential focus of research for differentiation between benign and malignant biliary obstructions, since TAC levels are known to decrease in many cancers. However, TAC levels can also be affected by other health conditions. Therefore, patients with poor general condition; cholangitis; sepsis; and severe heart, lung, kidney, or liver problems and patients considered unsuitable for ERCP (e.g., bleeding tendency) were excluded from the present study. Moreover, smoking may also affect TAC levels. If the rate of smokers to nonsmokers is assumed to be constant in a population at a defined time, this rate will also be constant (or will be at an equal rate) in both our study groups; i.e., benign and malignant disease. By assuming that the rate of smokers and nonsmokers was identical in our study groups, the statistical results may be considered not to change significantly. Although the role of TAC has been investigated for many cancers, no study has been published thus far (as shown by a search

in PubMed) concerning the relationship between TAC levels and malignant biliary obstructions. The mean serum antioxidant levels, such as selenium, zinc, manganese, vitamin E, and vitamin C, were found to be significantly lower in gallbladder cancer patients as compared to patients with gallstones as well as healthy subjects ($p<0.001$) (51). Moreover, the roles of chronic inflammation and oxidative stress have been shown in CCA (52). Jaiswal demonstrated that increased oxidative damage of DNA by inducing inducible nitric oxide synthase (iNOS) with nitric oxide (NO) generation during chronic inflammation is a risk factor for the development of CCA (53). It has also been shown that N-acetylcysteine maintains antioxidant defenses in biliary obstructed rats (54). In our study, the sensitivity, specificity, and accuracy of serum TAC (cutoff >1.95 mmol/L) were 61%, 60%, and 61% ($p=0.008$), respectively. Even if the mean biliary TAC level was not significantly different in the malignant and benign group of patients (cutoff >1.8 mmol/L) ($p=0.09$), the sensitivity, specificity, and accuracy of the biliary TAC test were significant at 66%, 50%, and 57%, respectively, in our study ($p=0.036$). Finally, serum ($p=0.008$) and biliary ($p=0.036$) TAC levels were found to be statistically significant for differentiating between benign and malignant biliary obstructions with medium sensitivity and specificity.

Interestingly, binary logistic regression analysis for a combination of serum CEA, CA19-9, VEGFR3, and biliary CA19-9 achieved 95% predictive probability for estimating the presence of malignancy. However, a low degree of specificity (45.7%) indicated that this 4-test combination would not be useful in differentiating malignant biliary obstructions from benign ones. In conclusion, based on the results of the study, a combination of serum and biliary CEA, CA19-9, VEGFR3, and biliary CA19-9 may not be useful in differentiating between malignant and benign biliary obstructions; however, they may be useful for screening of patients suspected to have a malignant etiology.

The limitations of the study are as follows. 1) The sample size of the study was comparatively small. 2) VEGFR3 and TAC tests are not as routinely available as CEA and CA19-9 tests.

The strengths of the study are as follows: 1) Thus far, this study appears to be the first study to evaluate the role of VEGFR3 and TAC tests in the diagnosis of CCA. 2) VEGFR3 and TAC tests not only can be used for prognosis of and monitoring carcinogenesis but also may be useful for the differential diagnosis of malignant and benign biliary obstructions.

Ethics Committee Approval: Ethics committee approval was received for this study from Bezmialem Vakif University Institutional Review Board.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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