Insulin-Like Growth Factor-1 and Vascular Endothelial Growth Factor in Malignant and Benign Biliary Obstructions

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ABSTRACT

Background: Despite the presence of various diagnostic tools, the differential diagnosis between malignant and benign biliary obstructions is so difficult. This study aimed to evaluate the role of serum and biliary insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) in this differential diagnosis.

Materials and Methods: Patients ($n = 109$, 61 men and 48 women) with diagnosis of benign ($n = 62$) or malignant ($n = 47$) biliary obstruction were included. Serum and biliary IGF-1 and VEGF markers were analyzed by the chemiluminescent immunometric method.

Results: Mean age was 62.7 ± 8.1 years for the malignant group and 58.5 ± 15.4 years for the benign group ($P = 0.092$). Choledocholithiasis (79%), cancer head of the pancreas (53.2%) and cholangiocarcinoma (38.3%) were the most common etiologies. No statistical difference was detected regarding serum IGF-1 and VEGF levels between 2 groups. At a cutoff value of 308.55 and 0.5 ng/mL, biliary IGF-1 and VEGF had (91.4% and 90.3%) sensitivity and (89.5% and 84.9%) specificity differential diagnosis between malignant and benign biliary obstructions (area under the curve: 0.943, 0.915), respectively.

Conclusions: Biliary levels of IGF-1 and VEGF significantly increase in malignant than benign obstructive lesions. Measurement of these markers in the bile of these patients may aid in the detection of biliary tumors.

Key Indexing Terms: Insulin-like growth factor-1; Vascular endothelial growth factor; Biliary obstruction. **[Am J Med Sci 2016;351(3):259–264.]**

INTRODUCTION

Biliary tract carcinomas are relatively rare, demonstrating less than 1% of cancers.[1] Although, the elevation of their incidence and fatality rate in the industrialized countries like the United States of America and Japan has been observed.[2]

Generally, chronic inflammation is thought to enhance carcinogenesis by modifying proto-oncogenes, DNA mismatch repair genes/proteins, tumor suppressor genes and by increasing growth factors and local cytokines capable of hastening the cell cycle, to favor an accumulation of somatic mutations.[3] Among the growth factors and cytokines involved in the pathogenesis of cholangiocarcinoma (CCA) are insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF) and interleukin 6.[4]

IGF-1 is a 70-amino-acid protein produced mostly by the *liver* as an *endocrine* hormone as well as in target tissues in an autocrine or paracrine fashion.[5] Previous studies proved that it has an important role in tumor development as it inhibits apoptosis, stimulates mitotic cell division and promotes cancer cell proliferation.[6] CCA, which is an estrogen-sensitive neoplastic cell, is induced for metastasis and proliferation by IGF-1.[7]

VEGF is a highly characteristic mitogen for vascular endothelial cells. *In vivo* plays a noteworthy role in the order of vasculogenesis. When VEGF is overexpressed, it may be in the disease progression and even cancer. It may also be interpreted as an early step in the process of metastasis.[8] Many studies reported its significant use in tumor angiogenesis, correlating its serum levels with tumor metastasis and invasion. In addition, raised levels of VEGF are associated with poor prognosis in different cancers, including pancreatic cancer.[9] The elevated VEGF expression is reported in CCA.[7]

This study aimed to investigate the roles of serum and biliary levels of IGF-1 and VEGF in differentiating between malignant and benign biliary obstructions.

PATIENTS AND METHODS

In this cross-sectional study, we recruited 136 patients with obstructive jaundice who were referred to the endoscopic retrograde cholangiopancreatography (ERCP) unit, Gastroenterology Center-Mansoura University, in Egypt from March 2013-June 2014.

Exclusion criteria included age less than 18 years, postsurgical biliary and anatomy stricture after liver

transplantation. Patients suffering from cholangitis, sepsis, or kidney, lung, severe heart or liver problems (especially the bleeding tendency) were omitted from the study. The proper diagnosis of CCA and pancreatic cancer was based on tissue diagnosis either in surgery, or on fine needle aspiration on subsequent follow-up. The primary sclerosing cholangitis diagnosis was established on the ground of either magnetic resonance cholangiopancreatography or ERCP. Among the 47 patients with malignant biliary obstruction, histopathological evaluation was confirmed in 42 patients with either fine needle aspiration at the time of surgery or during follow-up visits.

After careful history taking and clinical examination, all cases were subjected to laboratory assessment of the following: (1) complete blood count; (2) liver function tests; (3) serum gamma-glutamyl transferase (γ-GT) and alkaline phosphatase; (4) serum C-reactive protein (CRP); (5) serum tumor markers, carcinoembryonic antigen (CEA) and CA19-9, with normal values 0-10 ng/mL and 0-33 U/mL respectively; (6) serum and biliary levels of IGF-1 and VEGF; (7) imaging modalities, abdominal ultrasound,[10] spiral computed tomography, magnetic resonance cholangiopancreatography or ERCP and upper abdominal MRI as reliable noninvasive imaging in diagnosing and locating biliary carcinomas[11] and (8) histopathology by endoscopic brush cytology. In some cases, operative data and postoperative biopsies were assessed.

Blood Sampling

Fasting 8 mL venous blood samples were picked up on the day of ERCP. One mL on ethylenediaminetetraacetic acid for complete blood count and the remaining 7 mL blood was centrifuged; the serum was immediately stored in small aliquots at −70°C until analysis could be performed.

Biliary Fluid Sampling Procedure

During ERCP, after cannulation of the common bile duct and before contrast injection, approximately 5-10 mL of bile was aspirated through the sphincterotome and into a sterile syringe. The iced bile samples were immediately sent to the laboratory, centrifuged for 10-15 minutes at 4000 RPM and immediately stored in small aliquots at –70°C until analysis could be performed.

Analysis

Complete blood count was measured on CELL-DYN Emerald cell counter (Abbott, Wiesbaden, Germany), liver function tests were evaluated on the Dimension Xpand plus chemistry analyzer using its kits (both were supplied by Siemens Technology, Ramsey, MN), γ-GT and alkaline phosphatase were measured using kinetic kit supplied by EliTech (Zone Industrielle, 61500-France). Serum CRP concentrations were measured on COBAS C111 using its commercial kits (Roche Diagnostics, Basel, Switzerland). Serum levels of tumor markers CA19-9 and CEA were assessed by chemiluminescent immunometric technique on ELECSYS 2010 (Roche Hitachi, Carnation, WA) with serum normal values 0-33 U/mL and 0-10 ng/mL respectively. Serum and biliary levels of IGF-1 were measured by enzyme-linked immunosorbent assay (ELISA) kits supplied by BioVision (S. Milpitas Blvd., Milpitas, CA) with its detection range (0-30 ng/mL), and serum and biliary levels of VEGF were measured by ELISA kits supplied by Invitrogen Corporation (Flynn Road, Camarillo, CA) with its detection range (0-1.5 ng/mL).

RESULTS

Patients' Characteristics

Of 136 consecutive patients, only 109 patients with obstructive jaundice were included in this study. After approximately 2 weeks of admission of each case, the final diagnosis of obstructive jaundice was assessed. A total of 27 patients were excluded from this study, as follows: 7 patients with hepatocellular carcinoma, 3 patients with metastatic colorectal carcinoma, 5 patients with metastatic gastric carcinoma, 3 patients with lymphoma, 3 patients with biliary stenosis in patients who received a liver transplant, 2 patients of bile duct leak and 4 patients with unsuccessful intubation of the common bile duct.

According to the clinical, biochemical and radiological data, patients were divided into a benign group, including 62 patients (34 men and 28 women) and a malignant group of 47 patients (27 men and 20 women). The average age of the benign group was 58.5 ± 15.4 years whereas the malignant group was 62.7 ± 8.1years ($P = 0.092$). Among the patients enrolled in this study, benign etiologies included choledocholithiasis ($n = 49$, 79%), chronic pancreatitis ($n = 2$, 3.2%), sphincter of Oddi dysfunction ($n = 4$, 6.5%) and primary sclerosing cholangitis ($n = 7$, 11.3%); malignant etiologies included cancer head of pancreas ($n = 25$, 53.2%), CCA ($n = 18$, 38.3%), ampullary tumor ($n = 3$, 6.4%) and papillary tumor ($n = 1$, 2.1%). Hemoglobin concentration, platelet count, serum bilirubin, alkaline phosphatase, ALT and γ-GT levels were significantly different between the benign and malignant groups ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.025$ and $P < 0.001$, respectively). On the contrary, WBCs, serum albumin, AST and CRP levels show no difference between the benign and malignant groups ($P = 0.552$, $P = 0.154$, $P = 0.06$ and $P = 0.301$, respectively) (Table 1).

Serum Markers and Their Receiver Operating Characteristic Curve Analysis

The mean serum levels of IGF-1 and VEGF have been reported in Table 2. No statistical difference was detected regarding serum IGF-1 and VEGF levels between 2 studied groups. ROC curve analysis was done to identify serum marker cutoff values differentiating between malignant and benign biliary obstructions; the results are summarized in Table 3 and the Figure.

TABLE 1. Demographic and etiologic characteristics of patients.

Parameters	Studied groups		P Value
	Benign ($n = 62$)	Malignant ($n = 47$)	
Age	58.5 ± 15.4	62.7 ± 8.1	0.092
Sex (M:F)	34/28	27/20	0.81
Causes			
Malignant			
Head of pancreas tumor	–	25 (53.2%)	–
Cholangiocarcinoma	–	18 (38.3%)	–
Ampullary tumor	–	3 (6.4%)	–
Papillary tumor	–	1 (2.1%)	–
Benign			
Choledocholithiasis	49 (79%)	–	–
Chronic pancreatitis	2 (3.2%)	–	–
Primary sclerosing cholangitis	7 (11.3%)	–	–
Sphincter of Oddi dysfunction	4 (6.5%)	–	–
Hemoglobin (gm/dL)	13.2 ± 1.5	11.3 ± 1.2	<0.001
WBCs ($\times 10^3$/cmm)	7.7 ± 3.9	8.1 ± 2.8	0.552
Platelets ($\times 10^3$/cmm)	299 ± 96	191 ± 99	<0.001
Albumin (g/dL)	3.8 ± 0.8	3.6 ± 0.6	0.154
Bilirubin (mg/dL)	2.6 ± 2.1	8.2 ± 4.1	<0.001
Alkaline phosphatase (U/L)	125.7 ± 73.6	388.6 ± 155.1	<0.001
AST (U/L)	77.6 ± 93	110 ± 81.3	0.06
ALT(U/L)	85.4 ± 76	121 ± 87	0.025
γ-GT (U/L)	231 ± 271	612 ± 351	<0.001
CRP (mg/L)	43.9 ± 26.8	49.7 ± 31.4	0.301

AST, aspartate aminotransferase; ALT, alanine aminotransferase; WBC, white blood cell.

Biliary Markers and Their ROC Curve Analysis

The mean biliary levels of IGF-1 and VEGF have been summarized in Table 2. Contrary to results of serum markers, biliary IGF-1 and VEGF levels were significantly higher in the malignant group as compared to those in the benign biliary obstructed group ($P < 0.05$). Results of ROC analysis are summarized in Table 3 and the Figure.

Biliary IGF-1, at a cutoff value of 308.55 ng/mL, had 91.4% sensitivity and 89.5% specificity for differentiation between malignant and benign biliary obstructions (area under the curve [AUC] = 0.943 with PPV and NPV for biliary IGF-1 92% and 91%, respectively). Similarly, biliary VEGF, at a cutoff value of 0.5 ng/mL had 90.3% sensitivity and 84.9% specificity for differentiation between malignant and benign biliary obstructions (AUC = 0.915 with PPV and NPV for biliary VEGF 91% and 88%, respectively).

It is well known that pancreatic cancer mainly causes cholestasis by external compression and a CCA grows within the choledochal lumen, so we evaluated these markers in the malignant group to differentiate CCA and pancreatic cancer.

In malignant group, the biliary IGF-I and biliary VEGF concentrations in the CCA patients were statistically significantly increased compared with the pancreatic cancer patients ($P < 0.001$). Besides, biliary IGF-1, at a cutoff value of 385.12 ng/mL, had 97.6% sensitivity and 95.5% specificity for differentiation between CCA patients and pancreatic cancer patients (AUC = 0.952

TABLE 2. Mean values for different markers in patients with benign and malignant biliary obstructions.

Markers	Serum/bile	Benign ($n = 62$)	Malignant ($n = 47$)	P Value
IGF-1 (ng/mL)	Serum	201.23 ± 32.52	219.15 ± 65.42	0.064
	Bile	41.60 ± 9.86	541.25 ± 75.66	<0.001
VEGF (ng/mL)	Serum	0.48 ± 0.27	0.56 ± 0.24	0.112
	Bile	0.5 ± 0.4	1.8 ± 1.2	<0.001
CEA	Serum	11.45 ± 7.87	36.77 ± 21.65	<0.001
CA 19-9	Serum	123.54 ± 67.85	418.06 ± 213.66	0.017

CA19-9, carbohydrate antigen 19-9.

TABLE 3. ROC Curve Analysis of different markers in patients with benign and malignant biliary obstructions.

Markers/cutoff values	AUC	Sensitivity	Specificity	PPV	NPV
Serum					
IGF-1 (195.65 ng/mL)	0.605	62	51	76	64
VEGF (0.42 ng/mL)	0.544	58.3	57.3	56	65
Bile					
IGF-1 (308.55 ng/mL)	0.943	91.4	89.5	92	91
VEGF (0.5 ng/mL)	0.915	90.3	84.9	91	88

with PPV and NPV for biliary IGF-1 93% and 92%, respectively). Similarly, biliary VEGF, at a cutoff value of 0.98 ng/mL, had 95.4% sensitivity and 93.8% specificity for differentiation between CCA patients and pancreatic cancer patients (AUC = 0.938 with PPV and NPV for biliary VEGF 91.8% and 90%, respectively).

Correlation Between Serum and Biliary Levels of IGF-1 and VEGF With Routine Laboratory Data in the Malignant Group

Serum IGF-1 concentrations were positively correlated with bilirubin, serum VEGF levels and biliary IGF-1 (Table 4). Serum VEGF was positively correlated with serum bilirubin, ALP, AST, ALT, γ-GT, platelets, serum IGF-1 levels, biliary IGF-1 and biliary VEGF. Biliary IGF-1 was positively correlated with bilirubin, CRP and biliary VEGF levels. Biliary VEGF was positively correlated with bilirubin, CRP, serum IGF-1 and biliary IGF-1. The serum and biliary levels of IGF-1 and CRP were inversely correlated with hemoglobin.

Biliary levels of IGF-1 and VEGF were positively correlated with serum CEA and CA 19-9 in malignant group. However, the serum levels of IGF-1 and VEGF did not show any correlation with tumor markers, as shown in Table 4.

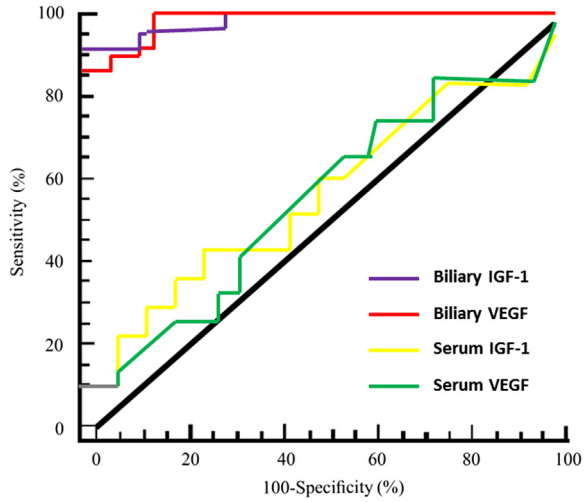

FIGURE. Receiver operating characteristic curves of serum and biliary IGF-1 and VEGF in differentiation between malignant and benign biliary obstructions.

DISCUSSION

Biliary tract tumors are characterized by delayed growth but because of the late presentation of their symptoms, they are usually diagnosed in late stages when the majority of therapeutic options are palliative.[12] Therefore, there is an increasing demand for new markers to provide a superior diagnosis and therapeutic approach.

The IGF-1 axis has been demonstrated to play characters in the suppression of cell destruction and the promotion of cell proliferation.[13] Higher serum concentrations of IGF-1 and its receptor in association with pancreatic, colorectal, prostate, breast and lung cancers have been reported in previous studies.[14]

In this study, serum IGF-1 levels were similar in 2 groups ($P = 0.064$), whereas biliary levels of IGF-1 were significantly increased in the malignant group than the benign group ($P < 0.001$). This finding is consistent with Alvaro et al[15] who reported the same results. Besides, this study noted that mean biliary IGF-1 in the malignant group (541.25 ± 75.66 ng/mL) was ~13-fold higher than those with the benign biliary lesions (41.60 ± 9.86 ng/cc), this latter determination is in agreement with Elsadek and Hassaneen,[16] who found the same relation as biliary IGF-1 was significantly raised in the extrahepatic malignant patients in comparison to other groups ($P < 0.001$).

Alvaro et al[7] reported that in the normal cholangiocytes, at least immunohistochemically, IGF-1 is not expressed; however, it is substantially expressed in biopsies of human intrahepatic CCA. Moreover, IGF-1 was secreted in the supernatant of cholangiocytes exposed to agents that enhance cell reproduction.

In this work, biliary IGF-1 was correlated with serum bilirubin, CRP and biliary VEGF. This result is not supported by Elsadek and Hassaneen,[16] who reported that the elevation of biliary IGF-1 was not correlated to the degree of the cholestasis.

In this study, at a cutoff value of 308.55 ng/mL, biliary IGF-1 had the highest sensitivity and specificity for differentiation between malignant and benign biliary obstructions. These interesting results were in agreement with Hassan et al,[17] who reported that at a cutoff value of 314.92 ng/mL, biliary IGF-1 had 100% sensitivity and 100% specificity for differentiation between malignant and benign biliary obstructions with an AUC of 1.

As regard to VEGF, there was no difference in serum levels of VEGF between 2 groups ($P = 0.112$), while there was a statistically significant increase in the biliary level of

TABLE 4. Spearman's coefficients showing correlations between serum IGF-1, serum VEGF, biliary IGF-1 and biliary VEGF with routine laboratory data in the malignant group.

	Serum IGF-1		Serum VEGF		Biliary IGF-1		Biliary VEGF	
Parameters	***rho***	***P* Value**	***rho***	***P* Value**	***rho***	***P***	***rho***	***P* Value**
Hg	−0.288	0.318	−0.020	0.946	−0.552	0.041	−0.631	0.016
WBCs	0.477	0.085	0.125	0.670	0.154	0.599	0.423	0.135
Albumin	0.373	0.189	0.516	0.059	0.044	0.881	0.475	0.097
Bilirubin (total)	0.676	0.008	0.744	0.002	0.547	0.043	0.745	0.002
ALP	0.009	0.976	0.618	0.019	0.162	0.581	0.487	0.074
AST	0.435	0.120	0.590	0.026	0.581	0.077	0.253	0.383
ALT	0.364	0.178	0.547	0.043	0.360	0.420	0.025	0.930
γ-GT	0.259	0.372	0.556	0.039	0.569	0.053	0.212	0.466
Platelets	0.209	0.474	0.600	0.023	0.466	0.078	0.210	0.486
CRP	0.379	0.182	0.433	0.122	0.685	0.006	0.655	0.011
Serum IGF-1	–	–	0.565	0.038	0.139	0.669	0.756	0.001
Serum VEGF	0.638	0.014	–	–	0.275	0.361	0.220	0.461
Biliary IGF-1	0.642	0.017	0.678	0.008	–	–	0.622	0.019
Biliary VEGF	0.266	0.371	0.665	0.005	0.756	0.001	–	–
Serum CEA	0.244	0.498	0.061	0.962	0.574	0.042	0.711	0.002
Serum CA 19-9	0.055	0.231	0.395	0.456	0.712	0.003	0.654	0.012

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CA19-9, carbohydrate antigen 19-9; Hg, hemoglobin; WBC, white blood cell.

VEGF in the malignant group than the benign one ($P < 0.001$) in this study. The latter finding is in agreement with Navaneethan et al,[18] who reported the same findings. On the contrary, a previous Italian study by Alvaro et al[15] noted that the biliary VEGF concentration was similar in both benign and malignant groups, whereas serum VEGF levels were increased in the pancreatic tumors and CCA compared with patients with benign biliary abnormalities.

This study reported a correlation between biliary VEGF levels and bilirubin, CRP, serum IGF-1 and biliary IGF-1. These previous results were not supported by Navaneethan et al,[18] who reported that there is no correlation between biliary VEGF levels and serum markers of the cholestasis including alkaline phosphatase and bilirubin indicating that biliary VEGF levels are independent of the degree of biliary obstruction.

Expression of VEGF in the pancreatic tumor has been shown to be positively correlated with the invasion and growth of pancreatic malignancies.[19] In addition, CCA can express VEGF that appears secreted into the basolateral side of the biliary epithelium. A previous study has found elevated serum levels of VEGF in CCA patients.[15] In this study, at a cutoff value of 0.5 ng/mL, biliary VEGF had the highest sensitivity and specificity for differentiation between malignant and benign biliary obstructions.

There are many limitations to this study that merit consideration. First, we included all patients with malignant obstructive causes, irrespective of the etiology. It may be that our observations cannot be generalized to all patients with the malignant cause of obstructive jaundice. Second, the measurement of serial serum and bile levels after relief of obstruction may be of advantage in order to study the influence of cholestasis on the IGF-1 and VEGF levels. Third, our sample size was comparatively small and larger studies are needed to evaluate these markers in various clinical settings and to establish a reliable cutoff for serum and biliary levels of IGF-1 and VEGF in patients with obstructive jaundice to identify malignant and benign causes of cholestasis in these patients. Fourth, IGF-1 and VEGF tests are not as routinely available.

CONCLUSIONS

Biliary IGF-1 and VEGF levels are significantly increased in malignant more than benign obstructive biliary diseases. Assessment of these markers in the bile, not in the serum, of obstructive lesions may help in the detection of biliary tumors. Unfortunately, no tumor markers are accurate enough to provide reliable information about tumor diagnosis in such conditions.

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