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.