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Biliary and Serum Insulin-Like Growth Factor-I: Are They Reliable Diagnostic Markers in Cholangiocarcinoma?

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Abstract: Cholangiocarcinoma (CCA), the commonest biliary malignancy, is difficult to diagnose owing to its anatomic location, growth patterns and lack of definite diagnostic criteria. CCA cells express and secrete insulin-like growth factor I (IGF-I) which participate in the modulation of enhancing cell growth and reducing apoptosis. Measuring IGF-I in bile and serum of patients with cholangiocarcinoma and to evaluate them as diagnostic markers. This was a cross sectional study that included 60 patients who had endoscopic retrograde cholangio-pancreatography (ERCP), including 20 patients with cholangiocarcinoma, 20 patients with pancreatic cancer and 20 patients with benign biliary abnormalities. Mean biliary IGF-I level, in patients with cholangiocarcinoma, was 14 fold higher than patients with pancreatic cancer and 19 fold higher than in those with benign biliary abnormalities with high statistical significance (each recorded $p< 0.001$). Biliary, not serum, IGF-1 proved to be a significant discriminator between the studied groups (AUC=1), moreover, IGF-1 assay was independent from degree of obstruction. Biliary IGF-I is a reliable marker in differentiating CCA from other causes of extrahepatic biliary obstruction.

Key words: Chonagiocarcinoma (CCA) · Insulin-like growth factor-I(IGF-I) · Endoscopic retrograde cholangiopancreatography (ERCP)

INTRODUCTION

Cholangiocarcinomas (CCA), involving the union of the hepatic ducts, represent < 2% of all cancers [1]. Recently, cancers of the biliary tract and the pancreas have increased. However, they are difficult to be diagnosed early and their clinical signs, such as jaundice and abdominal mass indicate advanced stage [2]. There are various tumor markers used for diagnosis of pancreatic and biliary tract cancer including carbohydrate antigens 19-9 (CA19-9) and carcinoembryonic antigen (CEA), their sensitivity and specificity are low in the early stage [3]. Their combination improves their sensitivity and specificity [4].

Insulin like growth factor-I (IGF-1) stimulates mitotic cell division, inhibits apoptosis and promotes cancer cell proliferation [5]. CCA, which is an estrogen-sensitive neoplastic cells, is induced for proliferation and spread by IGF-I [6]. Biliary IGF-I, retrieved by ERCP for biliary obstruction, may differentiate extrahepatic CCA from either benign lesions or pancreatic cancer [7].

So this study was conducted to evaluate IGF-I as diagnostic marker in bile and serum of patients with cholangiocarcinoma.

MATERIALS AND METHODS

In this study, 147 patients with biliary obstruction, as demonstrated by abdominal ultrasonography [8], were referred to ERCP unit for drainage and management, Kasr El-Ainy hospitals, Cairo University, from November 2011 till November 2012. Sixty patients were included in the study. The following cases were excluded:

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Other malignancies (like hepatocellular carcinoma, colorectal carcinoma and gastric carcinoma), cases with unsuccessful intubation of the common bile duct, bile duct leak, biliary stenosis in patients who received a liver transplant, or rare diseases of the biliary tree (for example, Caroli disease).

After thorough history taking and clinical examination, all cases were subjected to: (a) Liver biochemical profile: bilirubin, ALP,AST and ALT, Prothrombin concentration, (b) Tumor markers; CA19-9 and CEA, with normal values 0-37U/ml and 0-10 ng/ml respectively, (c) Imaging modalities; abdominal ultrasound [8] and spiral CT as reliable non invasive imaging in diagnosing and locating biliary carcinomas [9], (d) Histopathology by endoscopic brush cytology and in some cases, operative data and post-operative biopsies, (e) Serum and Bile IGF-I Analyses: Blood samples were obtained just before ERCP, while biliary fluid was aspirated immediately after endoscopic selective intubation of the common bile duct. Both samples were collected in glass tubes. Bile samples were immediately stored in small aliquots at-20°C. Blood samples were centrifuged and the serum was immediately stored in small aliquots at -20°C. IGF-I in bile and serum was measured by commercial enzyme-linked immunosorbent assay (ELISA) kit (DRG instruments GmbH, Germany).

This study was approved by the institutional ethical committee and all patients provided informed consent in accordance with The Code of Ethics of the World Medical Association.

Patients were divided into 3 groups; Group A: patients with extra hepatic CCA, Group B: patients with pancreatic cancerand Group C: patients with benign biliary lesions. The ERCP endoscopist was aware of the definitive, presumptive, or undefined diagnosis.

Statistical Analysis: Done by applying Statistical Package for Scientific Studies (SPSS 17) for Windows. Comparison between quantitative variables was carried out by student T-test of two independent samples. While Analysis of Variance (ANOVA) test was used when comparing between more than two groups of independent variables. Comparison between qualitative variables was carried out by Chi-Square test (X2). A Receiver operating characteristic (ROC) curve was graphed to determine an appropriate IGF-I level in detecting CCA that gives optimal sensitivity and specificity among different study groups. Parameter is considered significant and reliable discriminator when area under the curve (AUC) is $\geq$ 0.9 and suggestive for discrimination when 0.7-0.89. Results were expressed in the form of P-value and were considered non-significant when P-value > 0.05, significant when $\leq$ 0.05 and highly significant when $\leq$ 0.01.

RESULTS

This study included sixty patients with biliary obstruction who were referred to ERCP unit, Kasr El-Ainy hospital, Cairo University, from August 2012 till August 2013. Patients were divided into 3 groups, each constituted of 20 patients. Group A: patients with CCA.Group B: patients with pancreatic head cancer. Group C: patients with benign biliary lesions; 12 of them (60%) had biliary stones, 6 (30%) with benign biliary stricture and 2 (10%) had primary sclerosing cholangitis. Sixteen patients (80 %) from group A were males and 4 (20%) were females, while 13 (65%) of group B were males and 7 (35%) were females and 10 (50%) of group C were males and 10 (50%) were females. The mean age ± SD of group A was 70.0 ± 3.68, while in group B was 68.0 ± 4.8 and in group C was 55.0± 7.47 P value < 0.001.

Laboratory tests are shown in Table (1).

Diagnostic approach of the studied patients via variable imaging modalities and histopathology are shown in tables (2-a) and (2-b) respectively. Imaging was done to

Table 1: Laboratory results of the studied groups

Laboratory parameter (mean ±SD)		Group A	Group B	Group C	P value A/B	A/C	B/C	A/B/C
Bilirubin (mg/dl)	Total	12.65± 2.73	5.54± 1.81	7.24± 4.64	< 0.001	<0.001	0.547	0.547
	Direct	8.61±2.14	3.93±1.38	1.38± 3.12	< 0.001	<0.001	0.678	0.678
ALP (U/ml)		562.65 ± 219.38	258.5 ± 91.66	345.0 ± 140.28	< 0.001	<0.001	0.026	0.026
Trans-aminases (U/ml)	AST	50.2 ± 25.93	37.35 ± 7.58	63.75 ± 58.8	0.033	0.242	0.64	0.640
	ALT	62.6 ± 35.27	44.35±9.50	85.0 ± 84.51	0.006	0.289	0.718	0.718
PC (%)	91± 8	98± 4	96± 11	0.005	0.108	0.758	0.758	
Tumour markers	CA19-9 ug/ml	469.65±152.80	252.6 ± 63.29	17.65 ± 7.34	< 0.001	<0.001	<0.001	<0.001
	CEA ng/ml	73.60±16.85	76.9 ± 39.99	3.09 ± 0.89	0.383	<0.001	<0.001	<0.001

Table 2-a: Diagnostic approach via variable imaging modalities in all studied patients

	Imaging modality n (%)		
Diagnosis	US	CT	ERCP
No diagnosis	22 (36.6%)	14(23.3%)	0
Cholangiocarcinoma	10 (16.6%)	12 (20%)	20(33.3%)
Cancer pancreas	20 (33.3%)	20 (33.3%)	--
Choledocholithiasis	8 (13.3%)	11(16.6%)	12(20%)
Biliary stricture	0	3(5%)	26 (43.3%)
Primary sclerosing cholangitis	0	0	2 (3.3%)

Table 2-b: Diagnostic approach via histopathology

	Histopathological sampling	
Diagnosis	Endoscopic Brush cytology (n=46)	Operative (n=26)
No diagnosis	39 (84.7%)	0
Cholangiocarcinoma	7(15.2%)	14(53.8%)
Cancer pancreas	0	12 (46.2%)
Benign biliary stricture	0	--

Table 3: Bilairy and Serum IGF-I in the studied groups

				P value			
IGF-I (ng/ml) Mean ± SD	Group A	Group B	Group C	A/B	A/C	B/C	A/B/C
Biliary	639.14 ± 86.77	44.93 ± 20.55	33.60 ± 8.75	< 0.001	<0.001	0.13	0.030
Serum	223.06 ± 76.53	247.09 ± 54.84	198.34 ± 38.74	0.183	0.192	0.004	0.004

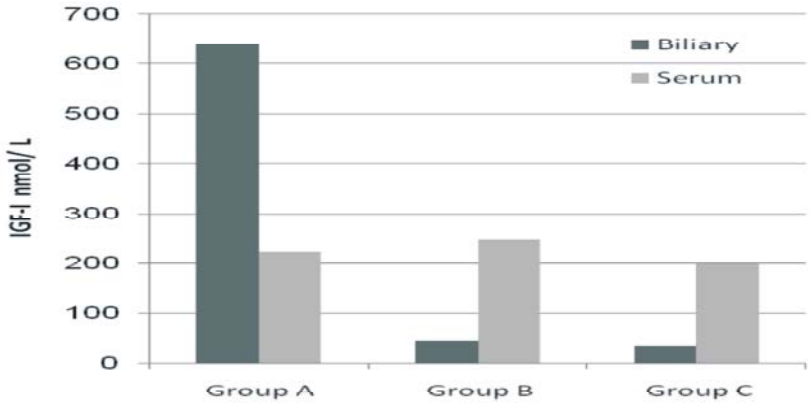

Fig 1: Bilairy and srum IGF-1 in the studied groups

all studied patients. The diagnostic yield of US was 63%, CT was 76% and ERCP was 100%.All CCA cases were extrahepatic; 13 of them (65%) located at bifurcation of CBD i.e Klatskin, while the rest were located at the distal CBD. Brush cytology was done for all patients with benign and malignant biliary stricture (n=46), it was positive in 7 patients with CCA. Operative histopathologywas done in 26 malignant patients; 14 with CCA and 12 with pancreatic cancer while the rest of the malignant patients were inoperable.

Biliary and serum in the studied groups are shown in table (3) and figure(1).

IGF-I proved to significantly differentiate extrahepatic CCA from other biliary lesions. ROC curves in figures (2-a), (2-b) and (2-c). On the contrary, serum IGF-I was not a reliable differentiating marker.

ROC curve of IGF-I in CCA versus other 2 groups in figure (2-a) showed that the AUC of biliary IGF-I was 1, p<0.001(CI =95%;1-1). Sensitivity and specificity were 100% at cut-off level of 314.92 ng/ml. While AUC for serum IGF-I was 0.499, p=0.987 (CI= 95%; 0.336-0.662). Sensitivity and specificity were 60% and 50% respectively at cut-off level of 214.05 ng/ml and 45% and 60% respectively at cut-off level of 231.1 ng/mL.

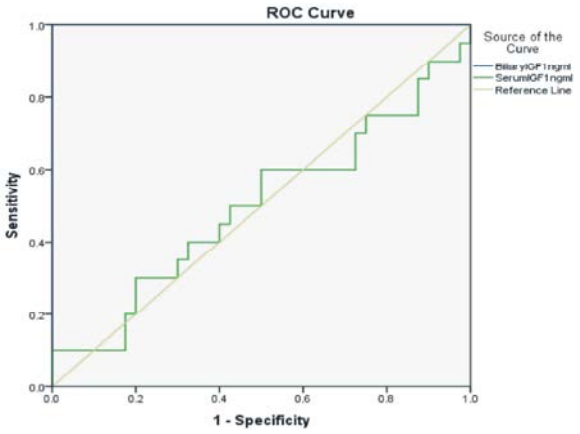

Fig 2-a: ROC curve of IGF-I level in cholangiocarcinoma

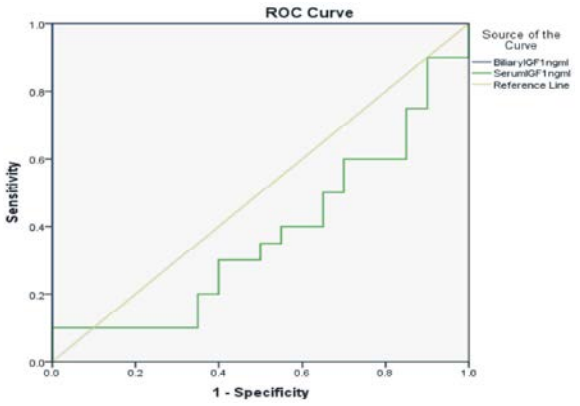

Fig 2-b: ROC curve of IGF-I in cholangiocarcinoma versus pancreatic carcinoma

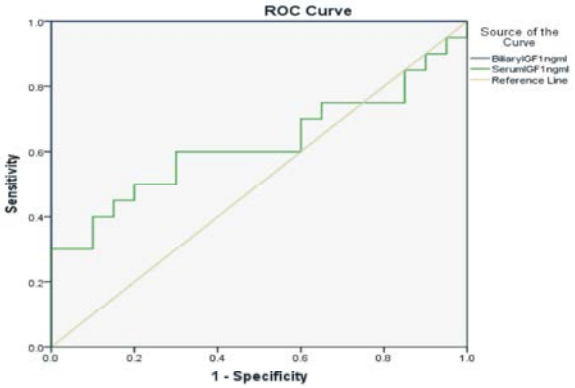

Fig 2-c: ROC curve of IGF-I in cholangiocarcinoma versus benign biliary lesions

Table 4: Correlation between IGF-1 in the studied groups and patients' age, bilirubin and alkaline phosphatase

	Age	IGF-I (ng/ml)			
	Total/ direct bilirubin	Biliary		Serum	
The studied Group	Alkaline phosphatase	r	P-value	r	P-value
Group I Cholangiocarcinoma		-0.096	0.688	-0.096	0.688
		0.282/ 0.262	0.228/ 0.265	0.282/ 0.262	0.228/ 0.265
		0.283	0.227	0.283	0.227
Group II Cancer pancreas		0.265	0.258	0.265	0.258
		0.306/0.306	0.189/0.189	0.306/ 0.306	0.189/ 0.189
		0.307	0.188	0.306	0.189
Group III Benign biliary lesions		-0.103	0.665	-0.103	0.665
		0.2331/0.2327	0.323/0.323	0.2331/0.2327	0.323/0.323
		0.2331	0.322	0.2331	0.322

Among the Studied Groups: ROC curve of IGF-I in CCA versuspancreatic cancer in figure (2-b) revealed that AUC of biliary IGF-I was 1, p?0.001 (CI=95%; 0-1).Sensitivity and specificity were 100% at cut-off level of 314.92 ng/ml. While that of serum IGF-I was 0.375, p=0.09 (CI= 95%; 0.176-0.199). Sensitivity and specificity were 60% and 30% respectively at cut-off level of 209.34ng/ml and 30% and 60% respectively at cut-off level of 276.43 ng/mL.

ROC of IGF-I in CCA versus benign biliary lesions in figure (2-c)showed that the AUC of biliary IGF-I was 1, p=0.0 (CI=95%; 1-1). Sensitivity and specificity were 100%

at cut-off level of 294.15ng/ml. While that of serum IGF-I was 0.623, p= 0.092 (CI=95%; 0.442-0.803). Sensitivity and specificity were 65% and 40% respectively at cut-off level of 186.63ng/ml and 50% and 80% respectively at cut-off level of 227.41ng/mL.

Correlation between IGF-1 in the studied groups and patients' age, bilirubin and alkaline phoaphatse is shown in table (5). No positive correlation between IGF-1 and any of the studied parameters confirming its independent assay from age or degree of obstruction.

DISCUSSION

The incidence of CCA has increased recentlyin developed countries for unknown trend [10]. Two thirds of CCAs are extrahepatic while the remaining third is intrahepatic [11] Tumor markers, CA19-9 and CEA, are successful markers to differentiate malignant from non malignant causes in cases of jaundice [12].

In our study, males were commoner than females (39 and 21 patients respectively), however, males were commoner in malignant groups (A and B) and equal in non malignant group © with no statistical difference (p= 0.138). In CCA group, the ratio of male: female was 4: 1. This was higher than that reported beforee.g 1.5: 1 [13, 14] and ranged 0.6-1.8: 1 in *Parkin et al* series [15]. While in pancreatic cancer, it was 1.85: 1, also higher than that reported before which was 0.86:1 [14].

Meanwhile, all patients were rather old (mean age was 64.33 years), however, malignant groups were significantly older than non malignant one (69 versus 55 years respectively, p<0.001). This agrees with previous studies like *Shaib and El-Serag, 2004* [13] who stated that CCA rarely occurs at age younger than 40 and that the 7th decade is the typical age at presentation. Mean ages recorded in this study were slightly higher than that recorded by *Alsadek and Hassaneen, 2013* [14] in their similar study with three groups; 62.4, 61.1 and 49.4 years respectively with statistical difference among the studied groups (p<0.001).

Laboratory tests suggestive of obstructive jaundice i.e bilirubin and ALP, in our study, were significantly higher in malignant groups than in non malignant group (all recorded p<0.001). Also, *Lazaridis and Gores, 2005* [11] and *Alsadek and Hassaneen, 2013* [14] reported the same findings.

In our study, CA 19-9 was significantly different among the 3 studied groups (p<0.001). In CCA group, it was 1.88 fold higher than group B (mean 252.6 ug/ml). Serum CA 19-9 value>100 U/mL has a sensitivity and specificity for CCA of approximately 75% and 80%, respectively [16]. While CEA was comparable in malignant groups (A and B) (mean =75.25 ng/ml) and significantly higher than benign group (p<0.001).

Our recorded values were higher than that recorded by *Qin et al., 2004* [17] who reported serum CA19-9 and CEA, which were significantly elevated (P<0.001 and P<0.05 respectively) in patients with CCA (290.31KU/L and 36.46 mg/L respectively) compared with patients with benign biliary diseases (13.38KU/L and 13.84mg/L respectively). They also found that the accuracy of CA19-9 and CEA were 82.68% and 77.95%, their sensitivity were 77.14% and 68.57% respectively and their false positive rates were 15.22% and 18.48%, respectively.The AUC for both CEA and CA19-9 was 0.76.

CA19-9 may be expressed non-specifically in several benign and malignant diseases, false negative results in Lewis negative genotype were found and increased false positive results in presence of obstructive jaundice were seen [18, 19]. As for CEA, many cancers do not produce an increased CEA level and conversely, other conditions, such as hepatitis, pancreatitis, inflammatory bowel disease and obstructive pulmonary disease, may cause an elevated CEA [20].

In our study, the mean biliary IGF-I level in patients with extrahepatic CCA (639.14ng/ml), was 14 fold higher than patients with pancreatic cancer (44.9 ng/ml) and 19 fold higher than those with benign biliary abnormalities (33.6ng/ml) with high statistical significance (each recorded p< 0.001), while the mean biliary IGF-I level was comparable between patients with pancreatic cancer and benign biliary abnormalities (p=0.13). On studying its discriminatory role, AUC of its ROC curve was always 1 when compared among the 3 studied groups and when compared individually to the other 2 groups. Our figures were higher but in concordance with another similar study by *Alvaro et al. 2007* [7] who found that mean biliary IGF-I level in patients with extrahepatic CCA (84.6 nmol/L), was 15 fold higher than patients with pancreatic cancer (5.8nmol/L) and 20 fold higher than those with benign biliary lesions (4.1nmol/L) with high statistical significance (each recorded p< 0.001) and AUC was 1.

Also, *Alsadek and Hassaneen, 2013* [14] found the same relation as biliary IGF-1 was significantly elevated in extrahepatic CCA patients in comparison to other groups (P<0.001).

On the other hand, serum IGF-I, in our study, did not show a statisticaldifference between the studied groups, meanwhile its AUC of its ROC never recorded a

significant discriminator level (0.499, 0.375 and 0.623 respectively). It only showed a significant difference between patients with pancreatic cancer and benign biliary abnormalities (p=0.004). Similarly, *Alsadek and Hassaneen, 2013* [14] found no significant difference in serum level of IGF-1 in their different groups, despite being higher in CCA and cancer head of pancreas than others.

In conclusion, biliary IGF-I is a useful marker in differentiating extrahepatic CCA from other causes of extrahepatic biliary obstruction.

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