

Is Insulin-Like Growth Factor-I (IGF-I) efficient as a Diagnostic Biomarker in Differentiating Cholangiocarcinoma from Benign Biliary Obstruction?

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phy (ERCP)

Background and study aim: Cholangiocarcinoma (CCA) is a rarely curable cancer and is infrequently diagnosed early. There is a growing interest in evaluating CCA biomarkers in serum and bile. CCA cells express insulin-like growth factor-I (IGF-I) which modulates cell growth and reduces apoptosis. The aim of this study is to evaluate biliary and serum IGF-I as diagnostic biomarkers in patients with extrahepatic cholangio-carcinoma vs. benign biliary obstruction.

Patients and Method: We conducted a prospective cross-sectional study on 60 patients with extrahepatic biliary obstruction divided into: CCA group (n=30) and benign obstruction (n=30). All patients had diagnostic and therapeutic ERCP with IGF-I assessment in serum and bile.

Results: The CCA group mean age was significantly higher than the benign obstruction group (69.0±4.68 vs. 56.0±6.47years, p<0.001). The etiologies of benign biliary obstruction were bile

duct stones (n=18), benign stricture (n=11) and cholangitis (n=2). The mean biliary IGF-I in CCA patients was significantly higher (19-20 folds) than the benign biliary obstruction group (639.14±86.77 vs. 33.60±8.75, p<0.001). The mean serum IGF-I in the CAA group was higher than the benign group, however, this was non-significant (223.06±76.53 vs. 198.34±38.74, p=0.192). Biliary IGF-I cutoff of 292.24ng/ml in CCA vs. benign group showed 100% sensitivity and specificity (AUC=1, p<0.001). While a serum IGF-I cutoff of 236.32ng/mL in CCA vs. benign group showed a sensitivity/specificity of 50%/80% respectively (AUC=0.614, p=0.081).

Conclusion: Biliary but not serum IGF-I was an excellent marker in differentiating extrahepatic CCA from extrahepatic benign biliary obstruction with a 100% sensitivity and specificity. The diagnostic utility of serum IGF-I in biliary malignancies needs further study .

INTRODUCTION

Cholangiocarcinoma (CCA), whether extra or intrahepatic, represent <1% of all cancers. However, CCA is infrequently diagnosed early and clinical signs indicate advanced stage. Histopathologic diagnosis for CCA is difficult to obtain as only a few patients are eligible for operation and endoscopic tissue sampling by brush cytology or biopsy forceps have limited diagnostic sensitivity, generally not exceeding 70-60% [1,2].

Widely used serum markers, such as carbonic anhydrase 19-9 (CA19-9) and carcino-embryonic antigen

(CEA), are not specific for CCA and have unsatisfactory diagnostic sensitivity in early stages of cancer. However, their combination improves their sensitivity and specificity [3].

Because bile fluid can be easily aspirated during Endoscopic retrograde cholangiopancreatography (ERCP) aimed to decompress biliary ducts in extrahepatic biliary obstruction, there is a growing interest in evaluating cancer biomarkers in the bile fluid [4, 5].

Insulin-like growth factor-I (IGF-1) IGF-1 is a 70-amino-acid protein produced mostly by the liver as an

endocrine hormone. IGF-1 plays an important role in tumorigenesis by promoting mitotic cell division, inhibiting apoptosis and stimulating cancer cell proliferation [6]. Neoplastic cells of CCA are estrogen-sensitive, so IGF-I induces their proliferation and spread [7]. Alvaro et al. (2007) found that biliary IGF-I, retrieved by ERCP through cannulation of biliary ducts, may differentiate extrahepatic CCA from either benign lesions or pancreatic tumor [4].

As far as we know there are very few studies concerning the role of biliary IGF-I in the evaluation of bile ducts lesions. So, in this study, we aimed to evaluate biliary and serum IGF-I as diagnostic biomarkers in patients with extrahepatic cholangiocarcinoma in comparison to benign biliary obstruction lesions.

SUBJECTS AND METHODS

Study population:

We had conducted a prospective cross-sectional study on 60 patients with extrahepatic biliary obstruction enrolled from the Endoscopy Unit (Endoscopic retrograde cholangiopancreatography; ERCP unit) of Faculty of Medicine, Suez Canal University from May 2015 till April 2017. The extrahepatic biliary obstruction was diagnosed by abdominal ultrasound (US) with dilatation of extrahepatic and intrahepatic biliary system with/without triphasic CT or MRCP to diagnose and locate biliary lesions/carcinomas.

These patients were divided into two groups (each had 30 patients); **Group (I)**: patients with extrahepatic cholangiocarcinoma (CCA), **Group (II)**: patients with benign biliary strictures/lesions. All ERCP examinations were planned as therapeutic procedures (to stent or dilate biliary duct strictures) and diagnostic procedures (Brush cytology and bile aspiration sample). Brush cytology (during ERCP) was done for all cases to obtain a pathologic diagnosis, also, in some cases, operative records and postoperative biopsies were evaluated. The ERCP team was informed, before the procedure, of the initial diagnosis whether definitive, presumptive or undefined.

The diagnosis of cholangiocarcinoma patients was based on the guidelines criteria for the diagnosis of cholangiocarcinoma [8].

Study protocol

We excluded patients with (1) Age less than 18 years (2) Intrahepatic CCA for possible difficult or misleading diagnosis (3) Unsuccessful or difficult cannulation of the common bile duct e.g. post-laparoscopic cholecystectomy (4) Congenital biliary anomalies (5) Post liver transplant stenosis (6) Malignancy other than biliary (HCC, pancreatic,...) (7) Patients suffering from hemobilia and (8) Patients suffering from cholangitis, sepsis, or kidney, lung, severe heart or liver problems.

All our patients were subjected to: (a) Labs assessment: CBC, liver biochemical profile: bilirubin, AST, ALT and ALP, INR, creatinine, tumor markers (CA19-9, CEA) (b) Imaging methods: US, \pm triphasic CT \pm MRCP (c) Histopathology by endoscopic brush cytology and post-operative biopsies in some cases (d) Biliary and serum IGF-I analyses.

Serum and biliary Insulin-like growth factor-I (IGF-I) analyses:

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

Blood samples were obtained in the same ERCP setting and were collected in glass tubes. Blood samples were centrifuged, and the serum was immediately stored in small aliquots at -20°C . IGF-I in bile fluid and serum was measured by commercial enzyme-linked immunosorbent assay (ELISA) kit (DRG Instruments GmbH, Germany).

Statistical Analysis:

Data were analyzed by Statistical Package for Scientific Studies (SPSS 17) for Windows. Comparing the quantitative variables was done by student T-test of two independent samples. Comparing the qualitative variables was done by Chi-Square test (X²). Results were expressed in the form of P-value and were considered significant when ≤ 0.05 , and highly significant when ≤ 0.01 .

A Receiver operating characteristic (ROC) curve was created to determine an optimum IGF-I level, with the best sensitivity and specificity, for diagnosing CCA between the two groups. The parameter was considered significant and reliable discriminator when area under the curve (AUC) is ≥ 0.9 , and suggestive for discrimination when 0.7-0.89.

RESULTS

Basic patients' features:

This was a prospective cross-sectional study on 60 patients with extrahepatic biliary obstruction divided into 2 equal groups (CCA group and benign obstruction group). The etiology of obstruction in the benign group consisted of bile duct stones (n=18), benign biliary stricture (n=11) and cholangitis (n=2) (Table 1).

The diagnosis of biliary cancer was based on the results of ERCP criteria in 30(100%) patients and radiologic diagnosis (US and CT/MRCP) in 24 (80%). Afterwards, a histopathologic diagnosis of CCA was found in 27/30 (90%) patients [by endoscopic cytology through ERCP in 15(50%) cases, operative biopsy in 9(30%)]. During follow-up visits, additional 3(10%) patients were proved by histopathology through surgical biopsy].

In group I (CCA); the mean age, serum bilirubin (total and conjugated), ALP, CA19-9, and CEA were significantly higher than in group II (Benign lesions) ($p < 0.001$ for all) (Table 1).

Biliary and serum IGF-I as markers of Cholangiocarcinoma (CCA):

In differentiating extrahepatic CCA from other biliary obstruction lesions, biliary IGF-I levels were significantly elevated in CCA group vs. benign group ($p < 0.001$) (Table 2). However, IGF-I levels in serum were not significantly different between the two groups ($p = 0.192$).

For reliable discrimination, a cutoff level of 292.24 ng/ml for biliary IGF-I was found to differentiate between CCA and benign biliary lesions with a 100% sensitivity and specificity (AUC= 1, 95% CI= 1-1, $p < 0.001$) (Figure 1).

However, a cutoff level of 236.32 ng/ml for serum IGF-I could differentiate between CCA and benign biliary lesions with only 50% sensitivity and 80% specificity (AUC=0.614, 95% CI= 0.442–0.803, $p = 0.081$) (Figure 1).

CA19-9 and CEA in the diagnosis of cholangiocarcinoma:

We had assessed the two tumor markers (CA19-9 and CEA) in differentiating CCA from benign biliary lesions. At a cutoff level of 130.50U/mL for CA19-9, the AUC was 1 (95% CI: 1 to 1) with a 100% sensitivity and specificity ($p < 0.001$). Also, at a cutoff level of 24.80ng/ml for CEA, the AUC was 1 (95% CI: 1 to 1) with a 100% sensitivity and specificity ($p < 0.001$) (Figure 2). However, with lower cutoffs for CA19-9 and CEA, the sensitivity and specificity decreased significantly, thus decreasing their diagnostic value in CCA.

Table (1): Demographic and lab characteristics of the two studied groups.

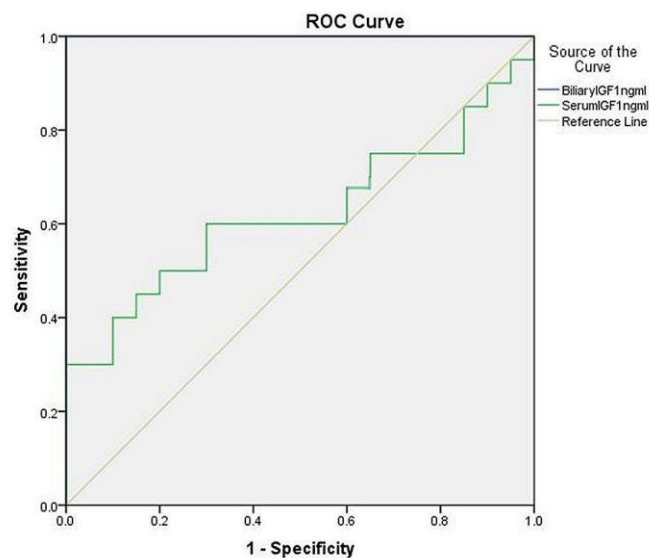
Demographic feature		Group I(CCA)	Group II (Benign)	P value
Males		21(70%)	16(53.3%)	0.248
Age (years)		69.0±4.68	56.0±6.47	<0.001
Etiology	Bile duct stones		18	-----
	Benign stricture	----	11	
	Cholangitis		2	
Laboratory parameters (mean ±SD)				
Bilirubin	Total	13.65±3.73	8.24±5.64	<0.001
	Direct	9.61±3.14	2.38±4.12	<0.001
ALT		73.6±34.27	91.0±90.51	0.188
AST		51.2±27.93	65.75±57.8	0.235
ALP (U/ml)		571.65±228.38	356.0±153.28	<0.001
Albumin (g/dL)		3.7±0.62	3.7±0.54	0.154
PC (%)		91±8	96±11	0.217
CA19-9 ug/ml		470.65±149.80	18.65±8.34	<0.001
CEA ng/ml		83.60±17.85	4.09±0.97	<0.001

ALT: Alanine transferase, AST: Aspartate transferase, ALP: alkaline phosphatase; PC: prothrombin concentration; CA19-9: carbonic anhydrase 19-9; CEA: carcino-embryonic antigen; SD: standard deviation.

Table (2): Biliary and serum IGF-I levels in the two groups.

IGF-I (ng/ml)	Group I (CCA)	Group II (Benign)	P value
Biliary (mean±SD)	639.14 ± 86.77	33.60 ± 8.75	<0.001
Serum (mean±SD)	223.06 ± 76.53	198.34 ± 38.74	0.192

IGF-I: Insulin-Like Growth Factor-I; CCA: Cholangiocarcinoma; SD: standard deviation

**Figure (1):** IGF-I levels in cholangiocarcinoma group versus benign biliary lesions group.

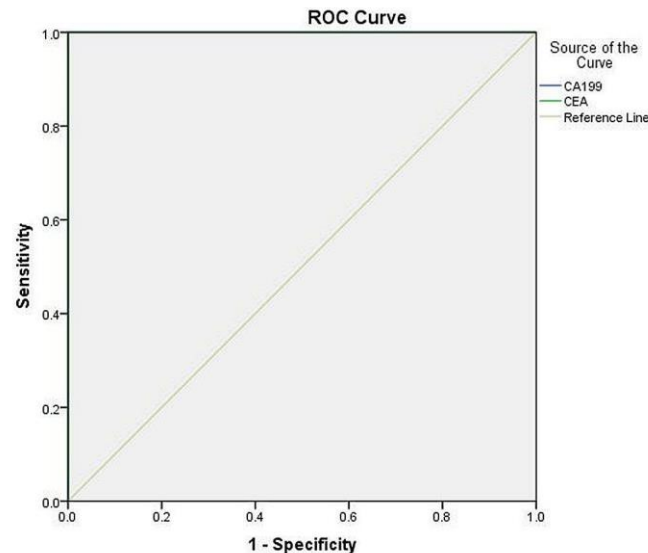


Figure (2): Serum CA19-9 and CEA in differentiating cholangiocarcinoma patients from benign biliary lesions patients

DISCUSSION

The incidence of cholangiocarcinoma (CCA) had, for an unfamiliar reason, increased recently in developed countries. Anatomically, CCA is categorized as intrahepatic (5-10%) or extrahepatic (90-95%), the latter is additionally divided into proximal (or perihilar) known as Klatskin tumor (60-70%) and distal (30-40%). CCA is highly fatal with 1- and 2-year survival rates of 25% and 13% respectively [8].

Cholangiocarcinoma is usually diagnosed in late stages, due to delayed growth, when the majority of therapeutic options are palliative. CEA and CA19-9 are frequently used serum markers of biliary tumors. However, both are expressed non-specifically in many benign and malignant diseases. Therefore, increasing demand for new markers is emerging for a better and earlier diagnosis. Multiple studies published over the past decades had suggested few diverse serum and bile markers that may help in prompt diagnosis and staging of CCA.

The IGF-1axis has been demonstrated to play a role in cell destruction suppression and cell proliferation promotion. Higher serum concentrations of IGF-1and its receptor in association with pancreatic, colorectal, prostate, breast and lung cancers have been reported in previous studies [9].

In our study, male sex was predominant in the CCA group, which is consistent with the finding

of Elsadek & Hassaneen [10] who studied serum and biliary IGF-1 in CCA and benign extrahepatic obstruction and found that male: female ratio was higher in the malignant group (M:F 27:18), compared to the benign group (M:F 18:27). However, in Abdel-Razik *et al* [11] study; they found that males were dominant in both the benign (M:F 34:28) and malignant (M:F 27:20) groups.

In our study, there was a significant (19-20 folds) increase in the mean biliary IGF-I level in patients with extrahepatic CCA in comparison to patients with benign biliary stasis. Also, by studying its discriminatory role, the biliary IGF-1 AUC was (1) when compared between the CCA and benign groups.

Our result was in concordance with another study by Alvaro *et al.* [4] on patients with extrahepatic CCA (n=29), and benign biliary abnormalities (n=25), they found that the mean biliary IGF-I level in extrahepatic CCA patients (mean 84.6nmol/L) was 20 folds higher than benign biliary lesions patients (4.1nmol/L) with high statistical significance ($P < 0.001$), also, they found AUC value of 1 when biliary IGF- in the extrahepatic CCA were related with benign biliary stasis.

Also, Alsadek and Hassaneen [10] found similar conclusions, as biliary IGF-1 was significantly raised in extrahepatic CCA patients (n=45, 83.4 ± 21.3 , range 10-118nmol/l) in comparison to other groups (non CCA n=37, 11.2 ± 9.0 , range

3-50nmol/l), and benign causes (n=45, 7.0±2.5, range 3-12nmol/l). They reported an AUROC of 0.992 on comparing the mean biliary IGF-1 in the extrahepatic CCA versus the other malignant or benign causes of extrahepatic biliary obstruction (sensitivity 95.56% and specificity 98.78%).

Similarly, in Abdel-Razik *et al.* [11] study; the biliary IGF-1 levels were significantly higher in the malignant compared to the benign biliary obstruction groups (541.25±75.66 vs. 41.60±9.86, P <0.001). At a cutoff value of 308.55, biliary IGF-1 had 91.4% sensitivity and 89.5% specificity in differentiating between malignant and benign biliary obstructions (AUC: 0.943, PPV/NPV 92%/91% respectively).

On the contrary, as far as we know, only one study had contradictory results, as Budzynska *et al* [12] prospectively studied IGF-1 in patients with CCA (n=15), pancreatic cancer (n=7), and benign strictures (n=18). Biliary IGF-1 levels were significantly increased in pancreatic cancer as compared to CCA and benign biliary strictures groups (966 vs. 137ng/mL, p=0.03 and 966 vs. 90.6ng/mL, p=0.01, respectively). While the biliary IGF-1 level was not significantly different between the CCA and the benign groups (p: 0.14).

Serum IGF-I level may be influenced by different factors including age, diet, menopause, and other pathological conditions. It should also be noted that in some patients with benign biliary stricture, cholangitis may be present and an influence of bacterial infection on IGF-I cannot be excluded.

The serum IGF-I, in our study, did not reach a significant difference between the CCA and the benign groups, also, the serum IGF-I AUC has never reported a significant discriminator level (AUC 0.614) with modest sensitivity and specificity. Similarly, Alsadek and Hassaneen [10] found no significant difference in serum level of IGF-I in their different groups (p=0.07), despite being higher in the CCA (28.8±9.7 [range 12-57]) and the cancer head of pancreas (30.4±10.4 [range 12-59]) groups compared with the benign group (25.9±6.7[range 14-43]).

Also, Abdel-Razik *et al.* [11] found a marginal statistical difference in serum IGF-I levels between extrahepatic CCA and benign biliary stricture groups (219.15±65.42 vs. 201.23±32.52, p=0.064). They reported that a cutoff of

195.65ng/mL had a 62% sensitivity and 51% specificity (AUC=0.605).

However, Alvaro *et al.* [4] reported that serum IGF-I levels were not significantly different between patients with pancreatic cancer, CCA and inflammatory biliary disease, but, interestingly, when the two malignant groups were combined, their serum IGF-I was significantly higher than the inflammatory group.

On the other hand, Budzynska *et al.* [12] surprisingly reported that serum IGF-I in malignant biliary occlusions patients was significantly lower than benign biliary stricture patients (74.4 vs. 117.0ng/mL, p=0.03). But CA19-9 (5689 vs. 38.9 U/mL, p<0.001) and CEA (27.5 vs. 1.9ng/mL, p<0.0001) differed significantly between patients with malignant and benign biliary strictures. Moreover, the AUC-ROC for serum IGF-I was 0.336, which was worse than that of CA19-9 (0.855) and CEA (0.794).

Taking into account the inconclusive results of different studies, the diagnostic utility of serum IGF-I in diagnosing biliary malignancies seems to be limited.

CA19-9 and CEA are commonly used biomarkers of pancreato-biliary cancers. However, serum CA19-9 may be expressed non-specifically in many benign and malignant diseases, patients with the Lewis negative genotype and patients having lower levels than our reported cutoffs had resulted in many false negative results. Also, increased false positive results in the presence of obstructive jaundice were found [13,14]. As for CEA, many malignancies do not increase CEA level, and conversely, benign conditions, such as hepatitis, pancreatitis, and inflammatory bowel disease may cause an elevated CEA [15]. So, there is a growing need for a reliable discriminator between CCA and benign obstruction.

In our study, serum CA19-9 and CEA levels were significantly higher in the malignant group than the benign group. However, with lower cutoffs, the sensitivity and specificity of both of them decreased significantly, thus decreasing their diagnostic value in CCA. Our results were similar to that reported by Qin *et al.* [16] who recorded that serum CA19-9 and CEA were significantly elevated (P<0.001 and P<0.05 respectively) in CCA patients (290.31KU/L and 36.46mg/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. Also, other studies showed that serum CA19-9 value >100U/mL has a sensitivity and specificity for CCA of approximately 75% and 80% respectively [17].

Also, Budzynska *et al.* [12] showed that CA19-9 and CEA had acceptable sensitivity and specificity in detecting malignant bile duct strictures. Both markers differentiated CCA and pancreatic cancer from benign diseases. CA19-9 at a cutoff 16.4U/mL had a sensitivity 95.5% and specificity 55.6% (AUC=0.855, accuracy 77.5%), while CEA at a cutoff 2.62ng/mL had a sensitivity 72.7% and specificity 77.8% (AUC=0.794, accuracy 75.0). They also think that although biliary cancer is of intestinal type, CEA seems to better reflect poor prognosis in pancreatic cancer patients than CCA.

CONCLUSION

In conclusion, biliary IGF-I was an excellent marker in differentiating extrahepatic CCA from benign causes of extrahepatic biliary obstruction with good sensitivity and specificity. However, the diagnostic utility of serum IGF-I in differentiating biliary malignancies vs. benign lesions seems to be limited.

There were some reported limitations this study: (1) small sample size (2) histopathological assessment of CCA was not through ERCP (3) single-center study

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Ethical considerations: All the included patients provided informed consent. This study was approved by the institutional ethics committee and was performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Conflict of interest: All authors disclose that there are no any potential conflicts (financial, professional, or personal) that are relevant to the manuscript.

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