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Full Length Research Paper

Value of serum and bile levels of IGF-1 in discriminating cholangiocarcinoma from other causes of extrahepatic biliary obstruction

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Cholangiocarcinoma (CCA) is a highly fatal malignant tumor with increasing incidence rates worldwide. Many biomarkers were suggested for diagnosis of CCA. Insulin-like growth factor 1 (IGF-1) stimulates mitosis, inhibits apoptosis and plays an important role in molecular pathogenesis of CCA. The aim of this study was to evaluate the performance of IGF-1 level in serum and bile as a diagnostic marker for extrahepatic CCA. The study was conducted on 127 patients with extrahepatic obstructive jaundice diagnosed as extrahepatic CCA (group 1, 45 cases), other malignant causes of extrahepatic obstructive jaundice (group 2, 37 cases) and benign causes of extrahepatic biliary obstruction (group 3, 45 cases). Level of IGF-1 was measured for all patients in collected serum samples as well as in bile samples collected during endoscopic retrograde cholangio-pancreatography (ERCP) or through percutaneous transhepatic cholangio-drainage (PTCD). Results: Bile level of IGF-1 was significantly elevated in extrahepatic CCA patients in comparison to other groups *(P<0.001).* On the other hand, no significant difference in serum level of IGF-1 was noticed among different groups. Conclusion: Measurement of IGF-1 level in bile (but not in serum) can distinguish CCA from other causes of extrahepatic biliary obstruction.

Key words: IGF-1, Cholangiocarcinoma, extrahepatic, obstructive jaundice.

INTRODUCTION

Cholangiocarcinoma (CCA) is a devastating cancer arising from biliary epithelium. It is characterized by a progressive increase in incidence and prevalence worldwide (Gatto and Alvaro, 2010). CCA accounts for more than 40% of biliary tract carcinomas (representing around 1% of cancers) and 3% of all gastrointestinal cancers (Malaguarnera et al., 2011). From the anatomical point of view, CCA is classified as intrahepatic (5-10%) or extrahepatic (90-95%), the latter is further divided into proximal (or perihilar) that is also known as Klatskin tumor (60-70%) and distal (30-40%) (Nakeeb et al., 1996 and Lim & Park, 2004). As a result of early invasion of vascular structures, most cases of CCA are inoperable on first discovery and liver transplantation is the only curative therapy. CCA is highly fatal with 1- and 2-year survival rates of 25% and 13% respectively (Malaguarnera et al., 2011 and Hashim et al., 2011).

Attention has been paid to the origin of CCA from the neoplastic transformation of resident hepatic stem cells (Sell & Dunsford, 1989 and Nomoto et al., 2006). The recognized risk factors for CCA share, as a common basis, a condition of chronic inflammation of the biliary epithelium together with a partial biliary obstruction e.g. primary sclerosing cholangitis (PSC) (Hashim et al., 2011). In general, chronic inflammation is thought to promote carcinogenesis by altering DNA mismatch repair genes/proteins, proto-oncogenes, and tumor suppressor genes and, by increasing local cytokines and growth factors capable to accelerate the cell cycle, to favor accumulation of somatic mutations (Jaiswal et al., 2000 and Wise et al., 2008).

Among the cytokines and growth factors involved in pathogenesis of CCA are interleukin 6 (IL-6), Insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF) (Gatto and Alvaro, 2010).

Multiple trials published in the last two decades had proposed some serum and bile biomarkers that could help in early diagnosing as well as in staging and

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predicting prognosis of CCA e.g. CA19-9, carcinoembryonic antigen (CEA), CA125, IL-6, cytokeratin 19 fragment (CYFRA 21-1), mucin-5AC (MUC5AC), IGF-1 and pancreatic elastase/amylase ratio table 4) (Nichols et al., 1993, Qin et al., 2004, Chaube et al., 2006, Cheon et al., 2007, Uenishi et al., 2008, Silsirivanit et al, 2011, Chen et al., 2002, Alvaro, 2007 and Chen et al., 2008. However, further confirmation is needed to support their applicability in the clinical setting. IGF-1 seems very promising in this field, especially in cases which are equivocal and present diagnostic dilemma (Gatto and Alvaro, 2010 and Alvaro, 2009).

IGF-1 is a circulating peptide hormone and a locally acting growth factor (Jones and Clemmons, 1995). IGF-1 stimulates mitosis, strongly inhibits apoptosis and hence accelerates cancer cell proliferation. The action of IGF-1 is predominantly mediated through IGF-1 receptor (Renehan et al., 2004). Previous studies showed high serum concentrations of IGF-1 and IGF-1 receptor in association with prostate, pancreatic, colorectal, breast, and lung cancers (Stattin et al., 2000, Kaaks et al., 2000, Lin et al., 2004, Endogenous Hormones and Breast Cancer Collaborative Group et al., 2010 and Shersher et al., 2011).

Alvaro et al. (2006) stated that IGF-1 stimulates cancer cell proliferation and spreading in estrogen-sensitive cancer cells, such as CCA. They also reported that IGF-1 is strongly expressed in biopsies of human intrahepatic CCA.

Being expressed by CCA cells, IGF-1 is suggested as a promising more sensitive biomarker for diagnosis of CCA than other known biomarkers. The aim of the current study is to evaluate the performance of IGF-1 level in serum and bile as a diagnostic marker for CCA.

METHODS

This cross sectional comparative study was conducted on 127 patients with extrahepatic obstructive jaundice in 2 big medical centers; Royal Commission Medical Center, Yanbu, Saudi Arabia (83 cases) and Zagazig University Hospital, Egypt (44 cases), since May 2010 till November 2012. Diagnosis of extrahepatic obstructive jaundice was made depending on clinical, laboratory and imaging criteria. Imaging procedures included ultrasonography for all patients, computed tomography (CT) and/or magnetic resonance cholangio-pancreatography (MRCP) for some selected cases. Endoscopic retrograde cholangio-pancreatography (ERCP) was attempted for all patients with intention of both diagnosis and drainage of retained bile (therapeutic or palliative), it was completed with successful biliary drainage in 110 cases. Percutaneous transhepatic cholangio-drainage (PTCD) was done for the 17 cases of failed ERCP drainage. Patients were assigned into 3 groups according to the cause of extrahepatic obstructive jaundice; ***group 1*** included 45 patients with unresected extrahepatic CCA; ***group 2*** included 37 patients with non-cholangiocarcinoma malignancy, these were 28 cases of cancer head of pancreas, 4 cases of hepatocellular carcinoma (HCC), 3 cases of colorectal carcinoma and 2 cases of gastric carcinoma and lastly ***group 3*** included 45 patients with benign biliary obstruction in the form of 43 cases of choledocholithiasis and 2 cases of primary sclerosing cholangitis (PSC). The diagnosis of either CCA or cancer head of pancreas was confirmed by a combination of CT and/or MRCP, ERCP, elevated serum levels of tumor markers (CA19-9 and/or CA-125 in cases of cancer head of pancreas) as well as positive cytology and/or histopathology (done in 17 cases of CCA and in 11 cases of cancer head of pancreas). Diagnosis of HCC was made by elevated serum alfa fetoprotien and abdominal triphasic CT which revealed an enlarged lymph node at portahepatis in 3 cases and an extrahepatic intraductal tumor thrombus in the forth case. Diagnosis of gastric and colorectal carcinomas was made by pelviabdominal CT, elevated serum CEA (in colorectal cancer patients), endoscopy and histopathology (done in all 4 patients). In all cases of colorectal carcinoma and one case of gastric carcinoma a metastasis at lymph node of portahepatis was noticed, while in the other case of gastric carcinoma a duodenal wall invasion was found. Diagnosis of suspected choledocholithiasis was confirmed by ERCP (preceded in some cases by MRCP). The two patients of PSC had associated inflammatory bowel disease, both cases had high serum level of ANA, one of them showed positive pANCA and both of them had a histopathologic confirmation of diagnosis.

Exclusion criteria included cholangitis at the time of bile collection, as evidenced by clinical assessment and negative cultures, diabetes mellitus, obesity (BMI ≥ 30) and malignancy if not the cause of obstructive jaundice e.g. cancer breast, prostate and lung.

Bile and Serum Analysis

Approximately 4 ml of bile were obtained during ERCP, after cannulation of the common bile duct and before contrast injection or by PTCD. Blood samples were centrifuged, and 4 ml serum samples were obtained. Bile and serum samples were collected and stored at −20°C. IGF-1 was measured in bile and serum using enzyme-linked immunosorbent assay kit depending on extraction and separation of IGF-1 from binding proteins followed by immunoassay in accordance with the manufacturer's instructions (DSL-10-5600, Diagnostic System Laboratories).

Written consent was obtained from each patient before the sample collection. The statistical analysis was performed using SPSS version 19 (SPSS, Chicago, IL, USA). Chi square, one way ANOVA, correlation tests were used and statistical significance was set at P values less than 0.05.

Table 1. Demographic features of all patients.

Group (n , %)	Sex		Age *in years*	BMI
	Male (n, %)	Female (n, %)	Mean ±SD (range)	Mean ±SD (range)
Group 1 CCA (n=45, 35.4%)	27 , 60	18 , 40	62.4 ± 8.1 (44-79)	20.5 ± 3.6 (14-29)
Group 2 non CCA malignancy (n=37, 29.1%)	18 , 48.6	19 , 51.4	60.8 ± 7.6 (47-76)	19.4 ± 3.2 (15-28)
− Ca head of pancreas(28)	13 , 46,4	15 , 53.6	61.1 ± 8.0	19.9 ± 3.4
− HCC(4)	2 , 50.0	2 , 50.0	60.0 ± 5.2	18.0 ± 2.2
− Colorectal CA(3)	2 , 66.7	1 , 33,3	58.7 ± 11.6	18.0 ± 2.6
− Gastric CA(2)	1 , 50.0	1 , 50,0	60.5 ± 2.1	17.0 ± 1.4
Group 3 benign biliary obstruction (n=45, 35.4%)	18 , 40	27 , 60	49.4 ± 7.4 (36-65)	26.1 ± 2.7 (16-29)
− Choledocholithiasis(43)	17 , 39.5	26 , 60.5	49.9 ± 7.1	26.5 ± 2.1
− PSC(2)	1 , 50.0	1 , 50.0	38.5 ± 3.5	19.0 ± 4.2
P value	0.164 (NS)		< 0.001	< 0.001
TOTAL	63 , 49.6	64 , 50.4	57.3 , 9,7 (36-79)	22.2 ± 4.3 (14-29)

RESULTS

Table 1 shows sex distribution, mean age, and mean BMI among all groups. Table 2 shows significant increases of cholestatic parameters (serum total and direct bilirubin as well as CBD diameter) in malignant groups (1 and 2) than in group 3.

As shown in table 3, there was no significant difference in serum IGF-1 level among all groups (figure 1). On the other hand, mean biliary level of IGF-1 was significantly higher in CCA group (83.4 ± 21.3, range 10-118 nmol/l) compared to other groups, being 11.2 ± 9.0 (range 3-50 nmol/l) in group 2 and 7.0 ± 2.5 (range 3-12 nmol/l) in group 3 *(P < 0.001)* (figure 2).

The area under the receiver-operating characteristic (ROC) curve was 0.992 on comparing mean value of biliary IGF-1 in the extrahepatic CCA patients (group 1) with its mean values in patients with other malignant or benign causes of extrahepatic biliary obstruction (groups 2 and 3). Sensitivity of the test was 95.56% with confidence interval: 84.82 % to 99.33% and specificity was 98.78% with confidence interval: 93.37% to 99.80%.

DISSCUSION

CCA is a highly fatal tumor, with increasing incidence rates worldwide. Current diagnostic techniques of CCA are sometimes unsatisfactory. Some emerging biomarkers are promising for its diagnosis (Malaguarnera et al., 2011 and Hashim et al., 2011). With this background, we evaluated the usefulness of measurement of IGF-1 in bile and serum for discrimination of extrahepatic CCA from other causes of extrahepatic bile duct obstruction. Based on the fact that IGF-1 level may be altered by obesity and diabetes mellitus, we excluded obese and diabetic patients from our study to confirm absence of factors affecting IGF-1 level other than the disease underlying the biliary obstruction. Moreover, malignancies (if not the cause of obstructive jaundice) were also excluded from my study because these may increase serum IGF-1 level (Juul, 2003). Regarding serum IGF-1 level in current study, there was no significant differences among all groups. However, it was higher in some groups and subgroups like cases of CCA, cancer head of pancreas and colorectal carcinoma than others. This goes with the results of previous studies that showed some elevation of serum IGF-1 in multiple types of cancers as colorectal, pancreatic cancers, without significant sensitivity or specificity (Kaaks et al., 2000 and Endogenous Hormones & Breast Cancer Collaborative Group et al., 2010). In this study, it was found that biliary level of IGF-1 was significantly higher in CCA group than in other cases of malignant extrahepatic biliary obstruction including pancreatic head cancer and metastatic tumors (HCC, colorectal carcinoma and gastric carcinoma) or In benign

Table 2. Some diagnostic features of obstructive jaundice in all patients.

Group (n , %)	Total Bilirubin in mmol/l Mean ±SD (range)	Direct Bilirubin in mmol/l Mean ±SD (range)	CBD *in mm* Mean ±SD (range)
Group 1 CCA (n=45, 35.4%)	89.5 ± 28.3 (39-148)	65.9 ± 25.1 (30-124)	9.7 ± 1.8 (7.7-14)
Group 2 non CCA malignancy (n=37, 29.1%)	89.9 ± 25.8 (48-148)	66.4 ± 22.0 (33-124)	10.1 ± 1.8 (7.5-14)
▪ Ca head of pancreas(28)	88.8 ± 25.6	64.7 ± 22.7	9.8 ± 1.8
▪ HCC(4)	85.2 ± 17.5	66.8 ± 18.2	10.8 ± 1.2
▪ Colorectal CA(3)	76.7 ± 12.2	61.0 ± 14.7	9.8 ± 1.1
▪ Gastric CA(2)	135.0 ± 18.4	97.5 ± 2.1	13.0 ± 1.4
Group 3 benign biliary obstruction (n=45, 35.4%)	56.0 ± 11.1 (38-80)	40.3 ± 9.7 (22-60)	8.6 ± 0.9 (7-11)
▪ Choledocholithiasis (43)	56.2 ± 11.3	40.4 ± 9.9	8.6 ± 0.9
▪ PSC(2)	51.0 ± 4.2	37.5 ± 3.5	8.4 ± 0.3
P value	< 0.001	< 0.001	0.001

Table 3: Biliary and serum level of IGF-1 in all groups.

Group (n , %)	Serum IGF-1 *nmol/l* Mean ± SD (range)	Biliary IGF-1 *nmol/l* Mean ± SD (range)
Group 1 CCA (n=45, 35.4%)	28.8 ± 9.7 (12-57)	83.4 ± 21.3 (10-118)
Group 2 non CCA malignancy (n=37, 29.1%)	30.4 ± 10.4 (12-59)	11.2 ± 9.0 (3-50)
▪ Ca head of pancreas(28)	32.2 ± 10.1 21.3 ± 5.1 25.3 ± 4.2	12.1 ± 10.0 7.8 ± 3.1
▪ HCC(4)	31.5 ± 2.1	7.3 ± 4.2
▪ Colorectal CA(3)		10.5 ± 3.5
▪ Gastric CA(2)		
Group 3 benign biliary obstruction (n=45, 35.4%)	25.9 ± 6.7 (14-43)	7.0 ± 2.5 (3-12)
▪ Choledocholithiasis(43)	24.3 ± 7.0	7.0 ± 2.6
▪ PSC(2)	21.0 ± 7.1	7.0 ± 1.4
P value*	0.07 (NS)	< 0.001

* Significantly lower than group 1 ($p < 0.05$)

extrahepatic biliary obstruction including choledocholithiasis and PSC *(P<0.001).* Moreover, in current study biliary, IGF-1 level was higher than serum IGF-1 level in group 1, however, in groups 2 and 3, serum

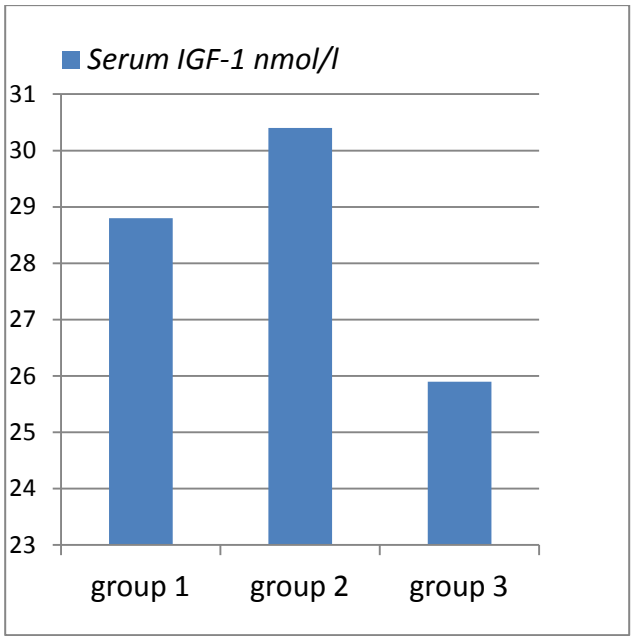

Figure 1. Pattern of serum IGF-1 level among all groups (P = 0.08).

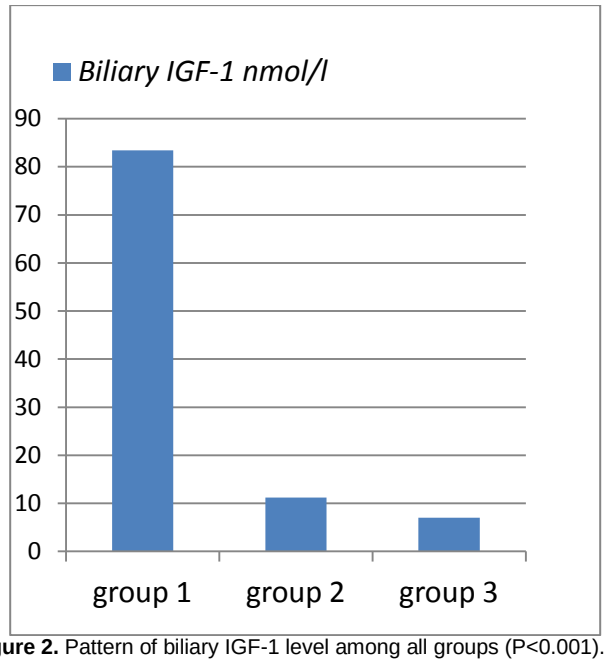

Figure 2. Pattern of biliary IGF-1 level among all groups (P<0.001).

IGF-1 level was higher than biliary IGF-1 level. The above results were supported by the findings of Alvaro et al. study (2006) who reported that IGF-1 is not expressed, at least immunohistochemically, in normal cholangiocytes; however, it is strongly expressed in biopsies of human intrahepatic CCA, they also found that IGF-1 was secreted in the supernatant of cholangiocytes exposed to agents that promote cell proliferation.

Although a significant difference of bilirubin level was noticed among different groups of current study, but this difference was due to elevation of bilirubin in both malignant groups 1 and 2, a pattern that was not correlated with biliary levels of IGF-1 among different groups, indicating that the elevation of biliary IGF-1 was not related to the degree of cholestasis. Similarly, the significant elder age and lower BMI noticed in this study in favor of both groups 1 and 2 than group 3, could not had produced or added to the elevation of biliary IGF-1 in group 1, because it is well known that IGF-1 is expected to get lower in these conditions (Juul, 2003). The present study showed a 95.56% sensitivity and a 98.78% specificity of biliary IGF-1 testing in discriminating extrahepatic CCA from other malignant or benign causes of extrahepatic biliary obstruction. Despite, extraction of IGF-1 from binding proteins was done before measurement but it seems that a remaining attached portion of these proteins led to the few false results (positive or negative) found in the study. However, a statistical cut off value of biliary IGF-1 of 55 nmol/l was found, above which CCA was always diagnosed. The above results were in agreement with Alvaro et al. (2007) who reported a sensitivity of 100% and a specificity of 100% for biliary IGF-1 measurement in discriminating CCA from other causes of obstructive jaundice. However, unlike the present study, Alvaro et al. (2007) conducted their study on a relatively small number of patients with extrahepatic obstructive jaundice (73 cases), they did not include malignant cases other than CCA and cancer pancreas and they did not exclude obese or diabetic patients.

On comparing the sensitivity and specificity of biliary IGF-1 in current study with the sensitivity and specificity of multiple biomarkers used for discrimination of CCA in other studies, a great superiority was seen for biliary IGF-1 testing over other biomarkers including serum biomarkers as CA19-9, CEA, CA125, IL-6, CYFRA 21-1 and MUC5AC and biliary biomarkers as CA19-9, CEA and pancreatic elastase/amylase ratio, as shown in table (4).

Although the sensitivity and specificity of serum CA 19-9 were relatively high (89% and 86% respectively) in the study of Nichols et al. (1993), but, this study was done on selected cases of CCA superimposed on PSC which might affected the results. Another more informative study of Qin et al, (2004) included non selected cases of CCA and compared the sensitivity of serum CA 19-9 and serum CEA that were as low as 77.14% and 68.57%, respectively.

The idea of cytologic examination of aspirated bile samples in cases of obstructive jaundice to help in diagnosis of CCA had been in use for a long time and is still being performed in some institutions (Abdelghani et

Table 4: Proposed serum and bile biomarkers for the diagnosis of CCA

Study	Biomarker	Sensitivity (%)	Specificity (%)	Comment
	SERUM			
Nichols et al., 1993	**CA19-9**	89	86	Increases in biliary cancer, pancreatic cancer and benign biliary and pancreatic conditions as PSC. Marked elevation of serum CA 19-9 is associated with advanced and unresectable biliary cancers.
Qin et al., 2004	**CEA**	68	81	Found in patients with malignant tumors of the digestive system such stomach, colon, biliary tract and pancreas cancer.
Chaube et al., 2006	**CA125**	40-50	> 86	Increases in other gastrointestinal or gynecological malignancies, in several cholangiopathies and in patients with ascites.
Cheon et al., 2007	**IL-6**	73	92	It is also elevated in many patients with hepatocellular carcinoma, benign biliary disease and metastatic lesions.
Uenishi et al., 2008	**CYFRA 21-1**	74	92	A useful marker for non-small-cell lung cancer and a prognostic factor for cervical, breast, esophageal, gastric and biliary cancers.
Silsirivanit et al, 2011	**MUC5AC**	71	90	It is an aberrant secreted mucin expressed in biliary tissues. High expression is correlated with tumor size, metastases and poor outcome of CCA.
	BILE			
Chen et al., 2002	**CA19-9**	46-61	60-70	Poor sensitivity and specificity.
Chen et al., 2002	**CEA**	67-84	33-80	Poor sensitivity and specificity.
Alvaro, 2007	**IGF-1**	100	100	Biliary IGF-1 levels in patients undergoing ERCP for biliary obstruction may differentiate extrahepatic CCA from either pancreatic cancer or benign biliary abnormalities.
Chen et al., 2008(18)	**Pancreatic elastase/amylase ratio**	82	89	This ratio distinguished malignant from benign causes of biliary obstruction. Biliary amylase activity was used to correct for pancreaticobiliary reflux.

al., 2012). Although the specificity of cytology is very high (usually 100%) but the low sensitivity (75% or less) and the need for frequent sampling (up to 6 times) as shown in multiple studies (Xing et al., 2005, Geraci et al., 2008 and Abdelghani et al., 2012) make cytology insufficient and less convenient method to confirm diagnosis of CCA than biliary IGF-1 testing used in this study.

CONCLUSION AND RECOMMENDATION

Measurement of biliary IGF-1 level in patients with extrahepatic biliary obstruction can distinguish cholangiocarcinoma from other malignant or benign causes of extrahepatic biliary obstruction. I recommend its use in cases with still uncertain diagnosis after the use of different imaging modalities. Further studies may

be needed to assess biliary IGF-1 before and after surgical resection of CCA and to correlate its level to the stage of the disease. Future studies are needed to help impleme-ntation of IGF-1 targeted therapy for treatment of CCA.

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