Serum and Biliary Insulin-like Growth Factor I As A Diagnostic Marker for Cholangiocarcinoma

Thesis
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Abstract

Background: Cholangiocarcinoma (CCA) is the most frequent biliary malignancy. It is difficult to diagnose owing to its anatomic location, growth patterns and lack of definite diagnostic criteria. Its cells express and secrete Insulin-like growth factor I (IGF-I). **Objective:** Measurement of IGF-I in bile and serum of patients with extrahepatic CCA and its evaluation as a diagnostic marker. **Methods:** The study is a cross sectional study including 60 patients presented to endoscopic retrograde cholangiopancreatography (ERCP) unit for extrahepatic bilairy obstruction. Patients were grouped as follows: Group A including 20 patients with extrahepatic CCA, Group B 20 patients with pancreatic cancer and Group C 20 patients with benign biliary lesions. **Results:** Biliary IGF-I was significantly different among the studied groups ($p=0.030$, AUC =1, and at cut-off of 314.92 ng/ml, the sensitivity and the specificity was 100%). The mean biliary IGF-I level, in patients with extrahepatic CCA, was 14 fold higher than in those with pancreatic cancer ($p < 0.001$), and 19 fold higher than in those with benign biliary lesions ($p < 0.001$), while it was comparable among patients with pancreatic cancer and benign biliary lesions ($p = 0.13$). On the other hand, the mean serum IGF-I was comparable among the studied patients, only showing statistical difference between patients with pancreatic cancer and those with benign bilary lesions ($p=0.004$). **Conclusion:** Biliary IGF-I is a useful reliable diagnostic test in differentiating extrahepatic CCA from other causes of biliary obstruction.

Key words: Insulin-like growth factor I; Cholangiocarcinoma; Pancreatic cancer; Benign biliary lesions.

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LIST OF ABBREVIATIONS

AJCC	American Joint Committee on Cancer
ALP	Alkaline phosphatase
ALS	Acid Labile Subunit
ALT	Alanine aminotransferase
APS	Associate Protein Substrate
AST	Aspartate aminotransferase
BilIN	Biliary Intraepithelial Neoplasia
CA 19-9	Carbohydrate Antigen 19-9
CCA	Cholangiocarcinoma
CEA	Carcinoembryonic Antigen
CI	Confidence interval
CT	Computed Tomography
CYFRA21-1	Cytokeratin 19 Fragment Antigen 21-1
Da	Dalton (atomic mass unit)
DIA	Digitized Image Analysis
DNA	DeoxyriboNucleic Acid
ECM	Extracellular Matrix
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
ELISA	Enzyme-linked immunosorbent assay
ERCP	Endoscopic Retrograde Cholangiopancreatography
EUS	Endoscopic Ultrasound
FDG	Fluorodeoxyglucose
FISH	Fluorescence In Situ Hybridization
FNA	Fine Needle Aspiration
GH	Growth Hormone
GH-R	Growth Hormone Receptor
GRB2	Growth Factor Receptor Bound Protein-2
HBV	Hepatitis B Virus
HCC	Hepatocellular carcinoma

HCV	Hepatitis C Virus
HIV	Human immunodeficiency virus
ICD	International Classification of Disease
IDUS	Intraductal Ultrasound
IGF	Insulin-like Growth Factor
IGF-I	Insulin-like Growth Factor I
IGF-II	Insulin-like Growth Factor II
IGFBPs	IGF- Binding Proteins
IGFBP-1	Insulin-like Growth Factor Binding Protein 1
IGFBP-2	Insulin-like Growth Factor Binding Protein 2
IGFBP-3	Insulin-like Growth Factor Binding Protein 3
IGFBP-4	Insulin-like Growth Factor Binding Protein 4
IGFBP-5	Insulin-like Growth Factor Binding Protein 5
IGFBP-6	Insulin-like Growth Factor Binding Protein 6
IGF-IR	Insulin-like Growth Factor- I Receptors
IL-6	Interleukin- 6
InsR	Insulin Receptor
IPMN	Intraductal Papillary Mucinous Neoplasm
IRS	Insulin Receptor Substrates
kDa	kilodaltons
MAPK	Mitogen Activated Protein Kinase
Mcm5	Minichromosome Maintenance protein 5
MRCP	Magnetic Resonance Cholangiopancreaticography
MRI	Magnetic Resonance Imaging.
MUC1	Mucin 1
MUC5AC	Mucin-5AC
NO	Nitric Oxide
PC	Prothrombin Concentration
PET	Positron Emission Tomography
PDGF	Platelet-Derived Growth Factor
PI3K	Phosphatidyl Inositol 3-Kinase

PLR	Platelet–Lymphocyte Ratio
PSC	Primary Sclerosing Cholangitis
PTC	Percutaneous Transhepatic Cholangiography
R	Correlation coefficient
RTK	Receptor Tyrosine Kinase
SH2	Src Homology-2
SHC	SH2 Containing Protein
Shc	Src Homology Collagen
SOS	Son-Of-Sevenless
TNF	Tumor Necrosis Factor
US	Ultrasonography

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INTRODUCTION

Various tumors may result in biliary obstruction, such as cholangiocarcinomas (CCA), ampullary carcinomas, gallbladder carcinoma, pancreatic and metastatic tumors. Although endoscopic retrograde cholangiopancreatography (ERCP) and magnetic resonance cholangiopancreatography (MRCP) are extensively and efficiently used in the management algorithm for biliary obstruction, the differential diagnosis of malignant and benign conditions is not easy all the time ***(Goonetilleke and Siriwardena, 2007)***.

CCA is a malignant cancer arising from the neoplastic transformation of cholangiocytes, the epithelial cells lining the intrahepatic and extrahepatic bile ducts ***(Blechacz and Gores, 2008)***.

Globally, CCA is the second most common primary hepatic malignancy. Several epidemiological studies have shown that the incidence and the mortality rates are increasing worldwide. Its incidence rates vary markedly worldwide, presumably reflecting differences in local risk factors and genetics ***(Gatto et al., 2010)***.

The radiological modalities for evaluation of these patients include ultrasonography (US), contrast-enhanced computed tomography (CT) scan, magnetic resonance imaging (MRI) and MRCP. These non-invasive diagnostic methods provide useful information about the level of obstruction, extent of biliary dilatation and the presence of a mass or distant metastasis. ERCP and percutaneous transhepatic cholangiography (PTC) are more accurate imaging tests for bile duct evaluation and allow a tissue diagnosis through

brush cytology and endoscopic US (EUS) -guided fine-needle aspiration (FNA). However, these are associated with a significant risk of morbidity ***(Saluja et al., 2007)*.**

The clinical findings and laboratory values including tumour marker levels are not specific enough to determine the precise cause of a biliary stricture of the proximal bile duct. Markers for early diagnosis are lacking. The most frequently tested marker is serum Carbohydrate Antigen 19-9 (CA19-9), but its yield for early diagnosis of CCA is considered unsatisfactory ***(Singh and Patel, 2006)*.**

Both Carcinoembryonic Antigen (CEA) and CA 19-9 have been measured in bile from patients with benign and malignant diseases of the pancreaticobiliary tract, but results are contradictory and no consistent differences have been found ***(Nehls et al., 2004)*.**

Serum tumour markers are attractive because of the ease of obtaining samples and their relative low cost. Therefore, they have been the object of extensive investigation to aid CCA diagnosis but, unfortunately, none of them has reached adequate specificity ***(Yachimski and Pratt, 2008)*.**

Given the challenges associated with serum markers, bile was investigated as a logical alternative fluid for identifying CCA-specific biomarkers. Bile is, in fact, more proximal than serum to the tumour and its flow through the biliary tree should favour the enrichment of CCA-derived products. Unfortunately, collection of bile samples could only be performed by using invasive technique and therefore the use for screening purposes or surveillance of population at risk is limited. Different studies have

recently shown that human CCA tissues express insulin-like growth factor I (IGF-I), growth factor involved in cancerogenesis and the growth and spreading of different cancers ***(Mancino et al., 2009)*.**

IGF-I is a circulating peptide hormone and locally acting growth factor with endocrine, paracrine and autocrine functions. The liver is the main source of circulating IGF-I. The IGF-I produced by the liver is under the control of growth hormone (GH) which, by acting on specific receptors (GH-R), induces the synthesis and release of IGF-I ***(Alvaro et al., 2005)*.** Scientific interest in the relationship among IGF-I, IGF-I receptor, and cancer is increasing because high serum concentrations of IGF-I are associated with an increased risk for breast, prostate, colorectal, pancreatic, and lung cancer ***(Lin et al., 2004)***. It has a strong influence on cancer cell proliferation and is a potent inhibitor of apoptosis. The action of IGF-I is predominantly mediated through IGF-I receptor, which is involved in several oncogenic processes and overexpressed in many tumor cell lines and in some human tumors, where it seems to play a critical role in transformation, cancerogenesis, and metastasis**.** Especially for estrogen-sensitive cells, such as CCA, IGF-I has been considered a candidate growth factor for cross-talk with estrogens in the modulation of neoplastic cell proliferation and spreading. Its measurement in bile fluid collected during ERCP for biliary obstruction may be useful in differentiating extrahepatic CCA from other causes of biliary obstruction, thus helping in the clinical work-up of these abnormalities ***(Alvaro et al., 2007)*.**

Aim of the work

The aim of this work is to measure Insulin-like growth factor I in bile and serum of patients with extrahepatic cholangiocarcinoma and its evaluation as a diagnostic marker in differentiating it from other causes of extrahepatic biliary obstruction, whether malignant like pancreatic cancer, or benign like benign biliary lesions.

Chapter (1)

CHOLANGIOCARCINOMA (CCA)

INTRODUCTION — Biliary tract cancers were traditionally divided into cancers of the gallbladder, the extrahepatic ducts, and the ampulla of Vater, while intrahepatic tumors were classified as primary liver cancers. Recently, the term CCA has been used to refer to bile duct cancers arising in the intrahepatic, perihilar, or distal (extrahepatic) biliary tree, exclusive of the gallbladder or ampulla of Vater (figure I) ***(Robert et al., 2012)*.**

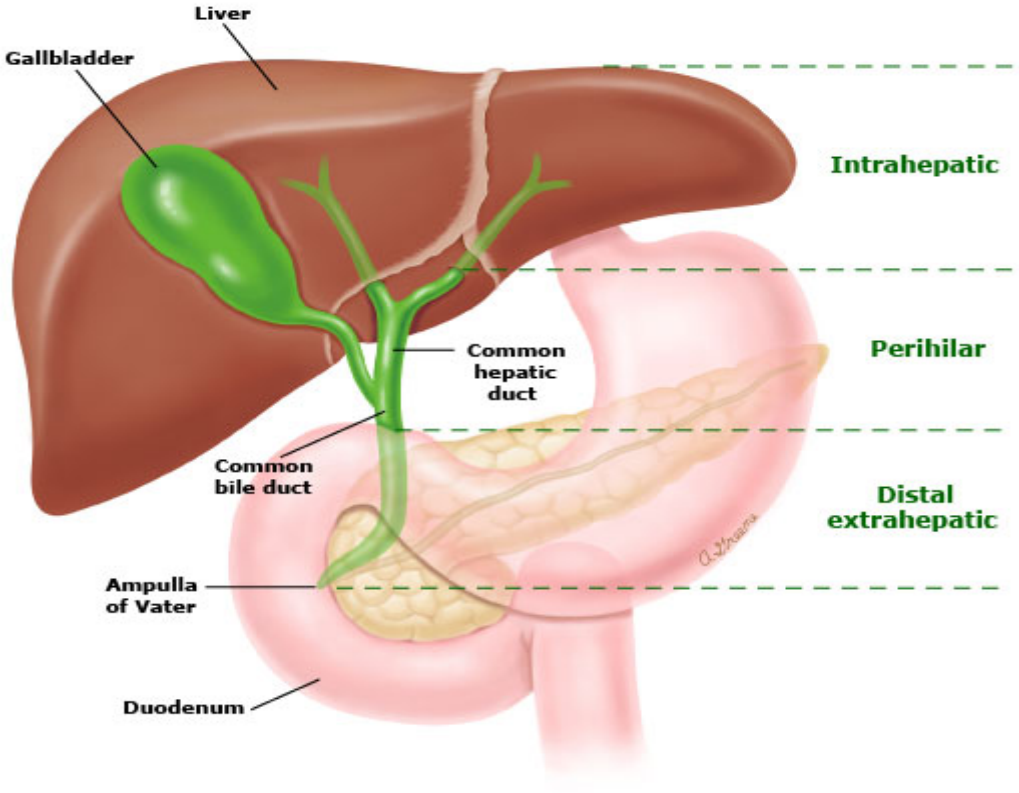

Figure (I): Anatomic classification of cancers of the human biliary tract.
(Modified from de Groen PC, Gores GJ, LaRusso NF, et al. N Engl J Med 1999; 341:1368)

EPIDEMIOLOGY:

CCA is a malignant neoplasm of the biliary duct system accounting for 3% of gastrointestinal tumors. It is the 2nd most common primary hepatic malignancy, representing 10–25% of primary hepatic malignancies worldwide ***(Gatto et al., 2010)*.**

CCA is considered a tumour with a worldwide low incidence, with the exception of Eastern countries in which endemic liver fluke is present. Nevertheless, during the last decade, the incidence of this cancer is increasing in many countries. If the worldwide trend is confirmed in the near future, this could possibly have an impact on public-health programs. An increased incidence of intrahepatic CCA versus extrahepatic CCA has been reported across many countries ***(Hammill and Wong, 2008)*.** The increase in intrahepatic CCAs may be attributable to new diagnostic methods for obstructive jaundice that identify biliary malignancies which previously might have gone undiagnosed ***(Jarnagin, 2000)*.** Other studies suggest that the increasing incidence of intrahepatic CCA may be related to a concomitant increase in certain risk factors such as cirrhosis, alcoholic liver disease, and hepatitis C virus (HCV) infection ***(Shaib et al., 2005)***, apparent differences may be due to changes in International Classification of Disease (ICD) ***(Khan et al., 2012)*.** On the other hand, the decreased incidence of extrahepatic CCAs is also unexplained. An epidemiologic study could not attribute this to time trends in known risk factors such as inflammatory bowel disease, diabetes, smoking, or exposure to thorotrast ***(Jepsen et al., 2007)*.**

The peak age for CCA is the 7th decade, with a slightly higher incidence in men ***(Shaib and E1-Serag, 2004)*.** It is more common