Serum Transforming Growth Factor Beta 1 as a Diagnostic Marker for Cholangiocarcinoma

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Background and study aim: Cholangiocarcinoma (CCA) is a rare epithelial malignancy of the biliary system. Delayed diagnosis is the primary factor contributing to the elevated mortality rate, often occurring when the disease has progressed significantly, resulting in a poor prognosis. Studies demonstrated that the TGF-β1 signaling pathway is involved in tumor initiation and progression. Elevated levels of TGF-β1 in the bloodstream have been observed in patients with various cancer types, often correlating with disease prognosis. We aimed to evaluate TGFβ1 as a diagnostic marker for CCA by analyzing its correlation with clinical and laboratory data.

Subjects and Methods: Serum levels of TGF-β were assessed via the sandwich ELISA method across four patient groups: Group 1 (30 CCA patients), Group 2 (20 HCC patients), Group 3 (20 patients with pancreatic cancer), and Group 4 (30 controls).

Results: TGF-β1 levels showed a significant difference across groups, with the highest concentrations observed in pancreatic cancer patients. TGF-β1 can differentiate between healthy controls and CCA patients at a cut-off ≥ 36.22 ng/mL, demonstrating 90.0% sensitivity and 66.7% specificity. TGF-β1 effectively distinguished pancreatic cancer patients from healthy controls at a cut-off of ≥ 53.77 ng/ml, demonstrating 85% sensitivity and 83.3% specificity. TGF-β1 at a cut-off of ≤ 79.55 ng/ml effectively distinguishes between pancreatic cancer and CCA patients, demonstrating 70% sensitivity and 70% specificity. The ROC curve analysis revealed that TGF-β1 was unable to differentiate between the groups of CCA and HCC. With a cutoff of ≥ 70.75 pg/mL, sensitivity was 70.0% and specificity was 60.0%.

Conclusion: Although serum TGF-β1 demonstrated limited diagnostic and prognostic value for CCA, it showed a superior discriminative capability between CCA and pancreatic cancer when compared to CA19.9.

INTRODUCTION

Cholangiocarcinomas (CCAs) represent a diverse group of hepatobiliary malignancies that may arise at any location within the biliary tree. It is the second most common hepatic malignant tumor following hepatocellular carcinoma (HCC). Despite being rare, CCA is increasingly observed globally [1]. CCA is categorized based on its anatomic location intodistal (dCCA), perihilar (pCCA), or intrahepatic (iCCA) [2].

CCA is regarded as the most aggressive biliary system malignant tumor, characterized by a poor prognosis, elevated mortality rates, and limited treatment options [3]. It is often identified at an advanced stage, resulting in restricted treatment options, and is generally asymptomatic during its early stages. It is primarily identified when jaundice or metastasis-related symptoms arise when tumor compression affects the extrahepatic bile duct [4].

Although in over 65% of cases, due to factors such as tumor size, metastasis, and lymph node invasion, it remains the most effective treatment option for CCA. Metastases and recurrence, whether local or distant, frequently occur, even in patients who have undergone potentially curative surgical resection [5]. The advancement of targeted therapeutics and the identification of reliable biomarkers for early detection rely on studies to deepen the understanding of the pathogenic mechanisms underlying CCA [2].

Common risk factors for CCA encompass infection, inflammatory conditions, and cholestatic liver diseases [6, 7, 8]. Most risk factors lead to cholestasis or chronic inflammation of the biliary epithelium, irrespective of the underlying cause. Cholangiocytes exhibit increased exposure to inflammatory mediators due to chronic inflammation [9, 10].

In CCA, the protein induced by transforming growth factor-β (TGF-β) is one of the elevated mediators. Fibroblasts, chondrocytes, and smooth muscle cells release this extracellular matrix (ECM) protein, which binds to different kinds of collagens, such as fibronectin and laminin. The TGF-β family of released cytokines comprises three distinct isoforms: TGF-β1, TGF-β2, and TGF-β3 [11]. Each isoform exhibits a distinct expression pattern and function. The formation of the tumor microenvironment (TME) and the malignancy of cancer are significantly influenced by the TGF-β1 isoform. Numerous studies indicate that TGF-β regulates extracellular matrix (ECM) production and immune infiltration, acting as a tumor promoter in carcinoma cells while serving as a tumor suppressor in premalignant cells [12].

TGF-β acts as a tumor suppressor by inhibiting growth and inducing apoptosis during the early stages of carcinogenesis. Cancer and fibrosis exhibit a significant association with the dysregulation of TGF-β signaling. The mechanism is complex; tissue lesions are directly promoted by TGF-β overexpression under pathological conditions [13]. Numerous studies present conflicting evidence regarding the correlation between TGF β1 expression and clinical outcomes, suggesting both positive and negative associations. Research indicates that TGF β1 may serve as a diagnostic biomarker for gastrointestinal and colorectal malignancies [14].

The progression of CCA is significantly affected by the activity of TGFβ1. Numerous studies have demonstrated that TGFβ1 plays a crucial role in promoting EMT within CCA cell lines[15].

Currently, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are frequently utilized as tumor markers for the detection of CCA. These markers, however, demonstrate limited accuracy owing to their low sensitivity and specificity. Due to the frequent absence of early symptoms and the lack of reliable diagnostic tools, the median survival time for most individuals diagnosed with CCA is less than one year [16]. The role of TGF-β1 in cancer development, specifically in CCA, remains undetermined. This study investigates the role of TGFβ as a diagnostic marker for CCA and its ability to differentiate between individuals with pancreatic cancer, HCC, and CCA.

SUBJECTS AND METHODS

The study population:

One hundred participants were divided into 4 groups for this case-control study: 30 patients with CCA, 20 with HCC, twenty with pancreatic cancer, and thirty healthy controls. Diagnosis of intrahepatic cholangiocarcinoma (iCCA) was established by contrast enhancement patterns in triphasic computed tomography (CT), magnetic resonance imaging (MRI), MRCP, and/or histopathology. Imaging features included mass-forming lesions in the hepatic parenchyma with upstream biliary ductal dilatation and characteristic delayed enhancement patterns. CCA cases were also classified according to tumor location using imaging modalities (ultrasound, CT, and MRCP) into intrahepatic (iCCA), hilar (perihilar, pCCA), or distal (dCCA) types. HCC diagnosis was based on the European Association for the Study of the Liver (EASL) guidelines, utilizing dynamic MRI, triphasic CT, and abdominal ultrasonography. Ultrasonography was used to identify cirrhosis in patients by measuring the size of the liver and spleen, as well as the existence of focal lesions that focused on the portal vein diameter (PVD) and periportal fibrosis, ascites, or cirrhosis.

The control group consisted of 30 healthy subjects matched for age and sex. The trial excluded patients with acute hepatitis, sepsis, primary biliary cirrhosis, primary sclerosing

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cholangitis, medical jaundice, and a history of hepatic resection .

During the 12-month study, all subjects were recruited from the inpatient and outpatient units of the hepatology and gastroenterology department at Menoufia University's National Liver Institute (NLI). Each patient underwent a comprehensive assessment of their medical history, which included age, sex, smoking status, comorbidities (such as diabetes and hypertension), surgical history, thorough abdominal and general examinations, radiographic evaluations, and pelviabdominal ultrasonography. To diagnose and evaluate the tumor's characteristics, such as location, size, and number, while also assessing the biliary system for classification, site, and degree of dilation, narrowing, or obstruction; to identify vascular involvement; and to detect any metastases beyond the liver, triphasic CT and/or MRI imaging of the abdomen and pelvis were performed in conjunction with MRCP.

Laboratory investigations :

Each subject underwent the following laboratory tests: The complete blood count (CBC) was conducted using the automated hematology analyzer (XP-300, Sysmex Cooperation, Japan). The electrochemilumine scence immunoassay (ECLIA) technique was employed on the fully automated immunoassay module (e601 module) of the Cobas C6000 analyzer (HITACHI Corporation, Japan) to assess kidney functions, specifically creatinine, urea, and C-reactive protein. Liver function tests were conducted to measure levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total and direct bilirubin, and albumin. Prothrombin time (PT) and international normalized ratio (INR) were evaluated utilizing an automated coagulation analyzer (CS-1600, Sysmex Corporation, Japan).

Serum TGF-β1 Level Measurement

Serum Collection and Processing

A 3 ml blood sample was collected from each participant and transferred into a plain tube. The sample was permitted to clot at room temperature for two hours prior to centrifugation at approximately 1000 xg for 20 minutes. The serum was subsequentlystored at -20 °C until further analysis.

Principles of the test

TGF-β1 levels were measured using the DEVELOP ELISA Kit (DL Sci & Tech Development Co., Ltd, Cat. No. DL-TGFb1-Hu). Latent TGF-β1 was activated using 1N HCl, followed by neutralization with 1.2N NaOH in 0.5M HEPES. The assay was conducted according to the sandwich ELISA protocol outlined in the manufacturer's instructions. Wells pre-coated with anti-TGF-β1 antibodies were loaded with standards, controls, and activated samples, incubated for 2 hours at room temperature, then washed. Biotin-conjugated anti-TGF-β1 antibodies were added, followed by incubation with HRP-conjugated avidin. Following the removal of excess unbound material, the substrate solution was introduced to each well, incubated at room temperature for 30 min, and subsequently protected from light exposure. Hydrochloric acid was employed to terminate the reaction, after which the plates were assessed for optical density (OD) at 450 nm utilizing a Thermo-Fisher ELISA reader. The standard curve was employed to compute the results. The results were expressed in nanograms per milliliter.

Statistical analysis:

For all analyses, SPSS 22.0 was utilized. Parametric data were summarized using mean ± SD and range, while non-parametric data were summarized using median and IQR. Categorical variables were represented using frequencies and percentages. The Student's t-test was employed for two groups, ANOVA for multiple groups with normal data, and the Kruskal-Wallis test for non-normal data to assess group differences. Chi-square analysis was employed to examine categorical variables. Spearman's correlation assessed the relationships between skewed numerical variables. The significance threshold was set at $p < 0.05$. The diagnostic accuracy of TGF-β1 was assessed using the ROC curve and AUC (95% CI).

RESULTS

Demographic data, laboratory investigations, and radiological findings across all groups

The study involved 30 CCA patients, including 20 males and 10 females, with a mean age of 60.67 ± 7.17 years. Additionally, it included 20 HCC patients, consisting of 15 males and five females, with a mean age of 59.85 ± 6.13 years.

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Twenty patients with pancreatic cancer (PC) were studied, comprising 14 males and six females, with a mean age of 58.85 ± 6.43 years. A control group of 30 healthy individuals included 18 males and 12 females, with a mean age of 56.37 ± 5.62 years. Table 1 presented the demographic characteristics, laboratory investigations, and radiological findings. No statistically significant differences were observed between the study groups in terms of age, sex, smoking status, hypertension, and diabetes mellitus. A significant difference in serum levels of TGF-β1 was observed among the groups, with the highest values in PC patients, followed by HCC patients, and then CCA patients. The median values were 110.96, 86.88, and 63.47 ng/ml, respectively. Laboratory results indicated significant differences between the studied groups regarding liver function tests, tumor markers, INR, and complete blood count. However, creatinine levels did not differ significantly. All patients with HCC tested positive for HCV antibodies, while 43.3% of CCA patients and 45% of PC patients were HCV-positive. The Child-Pugh score was used to evaluate the severity of liver disease in cirrhotic individuals. In CCA cases, 58.3% were classified as Child B, compared to 55% of HCC patients. Among the 30 patients with CCA, 4 (13.3%) had intrahepatic CCA, 16 (53.3%) had a hilar CCA, and 10 (33.3%) had a distal CCA, as determined by imaging findings.

Relationship between serum TGF-β1 levels, clinicopathological information, and lab findings across several groups were presented in (Table 2)

The analysis revealed that in patients with CCA, higher serum TGF-β1 levels were significantly associated with elevated ALP and bilirubin (P = 0.027), along with higher CA 19.9 levels (P = 0.001). TGF-β1 levels exhibited an inverse correlation with both age and albumin concentration. No significant associations were identified between TGF-β1 and hemoglobin levels, WBC count, Child-Pugh classification, spleen size, or portal vein diameter. A direct positive correlation was observed between TGF-β1 levels and the size of the focal hepatic lesion.

There is no correlation between TGF-β1 levels and various parameters in HCC patients. Conversely, a positive correlation exists between CA19.9, CRP, ALP, and Bilirubin levels with TGF-β1 levels in patients with pancreatic cancer. The size of pancreatic tumors exhibits a positive correlation with TGF-β1 level. Pancreatic tumor size positively correlated with TGF-β1level.

Relation between serum levels of TGF-β1 and demographic and clinical parameters in the CCA group (Table3)

No significant relation was found between serum levels of TGF-β1 and different parameters in the CCA group of patients, except for lymph node involvement. Furthermore, higher values of serum TGF-β1 were found in patients with lymph node involvement .

Diagnostic performance of TGF-β1 to differentiate different groups against healthy controls: (Table4)

Serum TGF-β1 had high diagnostic utility in differentiating CCA, HCC, and PC from healthy controls with high AUC values of 0.829, 0.890, and 0.920, respectively, all with high significance ($p < 0.001$). TGF-β1 was best for PC (84.2%), while HCC was highly specific (93.3%) but moderately sensitive (70%), and CCA was highly sensitive (90%) but less specific (66.7%). These results indicate that TGF-β1 is effective in distinguishing cancer patients from healthy individuals, but its diagnostic efficiency varies slightly according to the tumor type, as illustrated in Table 4.

The diagnostic efficacy of serum TGF-β1, CA19.9, and their combined assessment in differentiating between CCA and PC:

A ROC curve was developed to differentiate between patients with CCA and those with pancreatic cancer. A cut-off value of 19.9 was ineffective in distinguishing between the two groups, while a TGF-β1 cut-off of ≤ 79.55 ng/ml successfully differentiated PC from CCA patients, demonstrating 70% sensitivity and 70% specificity. Furthermore, the integration of TGF-β1 and CA 19.9 demonstrated an enhancement in specificity, reaching 75%, while sensitivity remained unchanged at 70%, as illustrated in Table 5 and Fig. 1.

The diagnostic efficacy of serum TGF-β1, AFP, and their combined assessment in differentiating between CCA and HCC:

Table 6 shows the diagnostic potential of serum TGF-β1, AFP, and their combination in differentiating between HCC and CCA. At a cut-off value of ≤ 70.75 ng/mL, TGF-β1 yielded an

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AUC of 0.623 with a non-significant p-value (p = 0.146), 70.0% sensitivity, 60.0% specificity, 53.8% positive predictive value (PPV), 75.0% negative predictive value (NPV), and 65.0% overall accuracy. AFP at a cutoff level of ≤ 22.00 ng/mL had a modestly better performance with an AUC of 0.703 (p = 0.027), 76.7% sensitivity, 66.7% specificity, 64.3% PPV, 78.6% NPV, and 71.0% accuracy. When both TGF-β1 and AFP (≥ 0.76) were combined, the diagnostic accuracy was improved considerably with an AUC of 0.763 with a highly significant p-value (p = 0.002), 86.7% sensitivity, 73.3% specificity, 72.2% PPV, 87.5% NPV, and the highest overall accuracy of 80.0%., as shown in Table 6 and Fig. 2.

Table 1: Demographic data, laboratory, radiological findings among the different groups

Parameters	CCA (n=30)	HCC (n=20)	PC (n=20)	Healthy controls (n=30)	P-value
Age (year), Mean ± SD*	60.67 ± 7.17	59.85 ± 6.13	58.85 ± 6.43	56.37 ± 5.62	0.065 NS, 1
Sex (male/ female), n (%)*	20 (66.7) / 10 (33.3)	15 (75.0) / 5 (25.0)	14 (70.0) / 6 (30.0)	18 (60.0) / 12 (40.0)	0.723 NS, 2
Smoking (no/ yes), n (%)*	18 (60.0) / 12 (40.0)	11 (55.0) / 9 (45.0)	12 (60.0) / 8 (40.0)	24 (80.0) / 6 (20.0)	0.222 NS, 2
DM (No/ Yes), n (%)*	19 (63.3) / 11 (36.7)	12 (60.0) / 8 (40.0)	13 (65.0) / 7 (35.0)	24 (80.0) / 6 (20.0)	0.398 NS, 2
HTN (No/ Yes), n (%)*	21 (70.0) / 9 (30.0)	13 (65.0) / 7 (35.0)	14 (70.0) / 6 (30.0)	26 (86.7) / 4 (13.3)	0.289 NS, 2
TGF-β1 (ng/mL)	63.47 (48.17) a	86.88 (82.75) a	110.96 (102.28) a	25.29 (35.05) b	**<0.001** HS,3
CA19.9 (IU/mL)	1121.00 (2861.00) a	122.00 (199.50) b	721.50 (1525.75) a	16.50 (21.10) c	**<0.001** HS,3
AFP (ng/mL)	3.75 (11.44) a	870.00 (1841.00) b	3.15 (4.08) a,c	2.59 (2.85) c	**<0.001** HS,3
CRP (mg/L)	52.25 (60.90) a,b	75.00 (73.50) a	18.00 (33.25) b	3.74 (2.58) c	**<0.001** HS,3
ALT (U/L)	66.00 (40.75) a	71.00 (66.75) a	64.00 (73.50) a	25.00 (12.50) b	**<0.001** HS,3
AST (U/L)	74.00 (55.00) a	104.00 (110.50) a	72.50 (80.00) a	21.50 (15.25) b	**<0.001** HS,3
ALP (U/L)	323.50 (306.75) a	276.00 (237.25) a	390.00 (518.25) a	65.00 (37.75) b	**<0.001** HS,3
GGT (U/L)	242.00 (229.25) a,b	198.50 (288.75) a	374.00 (594.00) b	29.50 (14.50) c	**<0.001** HS,3
Total bilirubin (mg/dL)	12.45 (11.68) a	8.48 (7.94) b	10.09 (9.54) a,b	0.72 (0.17) c	**<0.001** HS,3
Direct bilirubin (mg/dL)	9.66 (8.91) a	4.95 (4.73) b	7.75 (8.36) a,b	0.19 (0.04) c	**<0.001** HS,3
Albumin (g/dL)	3.00 (1.05) a,b	2.70 (0.85) a	3.30 (0.50) b	4.06 (0.29) c	**<0.001** HS,3
Total protein (g/dL)	6.45 (1.13) a	6.60 (1.42) a	6.75 (0.98) a	7.45 (0.65) b	**<0.001** HS,3
INR	1.32 (0.28) a	1.50 (0.61) a	1.20 (0.14) b	1.05 (0.09) c	**<0.001** HS,3
Urea (mg/dL)	33.00 (41.50) a	48.50 (37.50) a	36.00 (33.25) a	21.50 (14.00) b	**<0.001** HS,3
Creatinine (mg/dL)	0.85 (0.35)	0.99 (0.84)	0.79 (0.47)	0.87 (0.23)	0.147 NS,3
Hemoglobin (g/dL)	11.30 (2.80) a	10.40 (2.50) a	10.75 (2.42) a	12.75 (1.45) b	**<0.001** HS,3
WBCs (10^3 cell/µL)	8.20 (4.43) a	8.50 (6.43) a	8.15 (3.65) a	5.65 (1.72) b	**<0.001** HS,3
Platelets ($10^{\wedge 3}$ cell/µL)	206.50 (120.25) a	103.50 (58.75) b	229.50 (134.75) a,c	240.50 (48.75) a	**<0.001** HS,3
HCV-Ab (Negative/ Positive), n (%)*	17 (56.7) / 13 (43.3) a	0 (0.0) / 20 (100.0) b	11 (55.0) / 9 (45.0) a		**<0.001** HS,2
Child score, Mean ± SD*, ¥	9.08 ± 1.31	9.20 ± 1.54	8.57 ± 1.27		0.608 NS,1
Child-Pugh Score (B/ C), n (%)*	7 (58.3) / 5 (41.7)	11 (55.0) / 9 (45.0)	6 (85.7) / 1 (14.3)		0.343 NS, 4
CT Findings:					
Liver cirrhosis (No/ Yes), n (%)*	18 (60.0) / 12 (40.0) a	0 (0.0) / 20 (100.0) b	13 (65.0) / 7 (35.0) a		**<0.001** HS,2
Ascites, n (%)*					0.083 NS, 4
Absent	20 (66.7)	5 (25.0)	14 (70.0)		
Minimal perihepatic	5 (16.7)	7 (35.0)	3 (15.0)		
Mild	3 (10.0)	6 (30.0)	2 (10.0)		
Moderate	2 (6.7)	2 (10.0)	1 (5.0)		
Spleen (cm)	11.00 (2.25) a	13.50 (6.00) a	10.50 (2.75) a		**0.024** S,3

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Largest FLs (cm), Mean ± SD*	7.52 ± 1.95	4.78 ± 2.47		**<0.001** HS,5
HCC FLs multiplicity (Single/ Multiple), n (%)*		14 (70.0) / 6 (30.0)		-
Pancreas tumor size (cm), Mean ± SD*			5.23 ± 0.87	-
Lymph nodes (Negative/ Positive), n (%)*	9 (30.0) / 21 (70.0)a	15 (75.0) / 5 (25.0)b	12 (60.0) / 8 (40.0)a, b	**0.005** HS,2
IHBRD, n (%)*	a	b	a	**<0.001** HS,4
Minimal bilobar	0 (0.0)	10 (50.0)	2 (10.0)	
Mild bilobar	10 (33.3)	6 (30.0)	8 (40.0)	
Moderate bilobar	8 (26.7)	4 (20.0)	6 (30.0)	
Marked bilobar	12 (40.0)	0 (0.0)	4 (20.0)	
MRCP Findings:				
CBD (mm)	11.00 (8.00) a	8.00 (2.75) b	19.50 (5.00) c	**<0.001** HS,3
Focal Lesions site,n (%)*				-
Distal	10 (33.3)			
Hilar	16 (53.3)			
Intrahepatic	4 (13.3)			
Gall Bladder, n (%)*	a	a	b	**0.001** HS,4
Contracted	22 (73.3)	17 (85.0)	6 (30.0)	
Distended	8 (26.7)	2 (10.0)	14 (70.0)	
Calicular	0 (0.0)	1 (5.0)	0 (0.0)	

1: ANOVA 2: Pearson chi-sqaure test 3: Kruskal-Wallis test 4: Fisher's Exact test 5: Student t-test

*: Values are expressed as median (interquartile range) unless otherwise indicated

¥: No of cases in CCA= 12 and in PC =7

The values for the same parameter not sharing the same subscript letter are significantly different after adjustment for multiple comparisons by post hoc test at the level of 0.05

NS: Non significant at P-value ≥ 0.05 S: Significant at P-value < 0.05 HS : Highly significant at P-value < 0.01

INR: International Normalized Ratio **WBCs:** White Blood Corpuscles **ALT:** Alanine Aminotransferase **AST:** Aspartate Aminotransferase **ALP:** Alkaline Phosphatase **GGT:** Gamma-Glutamyl Transpeptidase **AFP:** Alpha-Fetoprotein **CBD:** Common Bile Duct **CRP:** C Reactive Protein **DM:** Diabetes Mellitus **HTN:** Hypertension **IHBRD:** Intra Hepatic Biliary Radicle Dilatation

Table 2: Correlation between TGF-β1 level in serum with clinicopathological data and laboratory results in different groups

Correlated parameters	TGF-β1 (ng/mL)					
	CCA		HCC		PC	
	r_s	P-value	r_s	P-value	r_s	P-value
Age (year)	-0.10	0.609 NS	-0.08	0.733 NS	-0.03	0.899 NS
CA19.9 (IU/mL)	0.57	**0.001** HS	0.14	0.569 NS	0.52	**0.019** S
AFP (ng/mL)	0.31	0.100 NS	0.43	0.057 NS	0.24	0.306 NS
CRP (mg/L)	0.30	0.107 NS	0.32	0.175 NS	0.46	**0.042** S
ALT (U/L)	0.26	0.160 NS	0.36	0.118 NS	0.11	0.636 NS
AST (U/L)	0.18	0.331 NS	0.28	0.241 NS	0.25	0.297 NS
ALP (U/L)	0.40	**0.027** S	0.38	0.101 NS	0.55	**0.013** S
GGT (U/L)	0.35	0.058 NS	0.44	0.052 NS	0.25	0.286 NS
Total bilirubin (mg/dL)	0.40	**0.027** S	0.28	0.227 NS	0.46	**0.040** S
Direct bilirubin (mg/dL)	0.48	**0.007** HS	0.4	0.081 NS	0.46	**0.041** S
Albumin (g/dL)	-0.37	**0.045** S	-0.11	0.646 NS	-0.32	0.168 NS
Total protein (g/dL)	0.11	0.567 NS	0.01	0.982 NS	0.31	0.192 NS

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INR	0.34	0.071 NS	0.17	0.474 NS	0.26	0.266 NS
Urea (mg/dL)	0.23	0.219 NS	0.26	0.269 NS	0.04	0.855 NS
Creatinine (mg/dL)	0.32	0.089 NS	0.36	0.120 NS	0.21	0.365 NS
Hemoglobin (g/dL)	0.02	0.922 NS	0.06	0.801 NS	0.13	0.591 NS
WBCs (10^3 cell/µL)	0.05	0.778 NS	0.44	0.052 NS	0.02	0.922 NS
Platelets (10^3 cell/µL)	0.17	0.371 NS	0.37	0.107 NS	0.16	0.506 NS
Child Pugh Score	0.52	0.084 NS	0.35	0.127 NS	0.67	0.097 NS
Spleen(cm)	0.22	0.239 NS	0.22	0.354 NS	0.12	0.610 NS
CBD(mm)	0.23	0.217 NS	0.25	0.292 NS	0.04	0.857 NS
Largest Fls (cm)	0.66	**<0.001** HS	0.36	0.117 NS		
Pancreas tumor size(cm)					0.56	**0.011** S

rs: Spearman correlation coefficient
NS: Non-significant at P-value ≥ 0.05 S: Significant at P-value < 0.05 HS: Highly significant at P-value < 0.01
INR: International Normalized Ratio **WBCs:** White Blood Corpuscles **ALT:** Alanine Aminotransferase
AST: Aspartate Aminotransferase **ALP:** Alkaline Phosphatase **GGT:** Gamma-Glutamyl Transpeptidase
AFP: Alpha-Fetoprotein **CBD:** Common Bile Duct **CRP:** C Reactive Protein

Table 3: Relation between serum levels of TGF-β1 and demographic and clinical parameters in cholangiocarcinoma group

Parameters		**TGF-β1 (ng/mL)**			**P-value**
		Cases [n (%)]	**Median** (IQR)	**Range**(min-max)	
Gender	Male	20 (66.7)	62.45 (30.50)	26.89 - 205.00	0.725 NS, a
	Female	10 (33.3)	72.07 (76.58)	18.60 - 155.00	
Smoking	No	18 (60.0)	63.47 (60.76)	18.60 - 179.00	0.933 NS, a
	Yes	12 (40.0)	64.77 (28.31)	30.36 - 205.00	
Child-Pugh Score	B	7 (58.3)	67.43 (79.44)	36.60 - 155.00	0.223 NS, a
	C	5 (41.7)	130.00 (116.90)	45.63 - 205.00	
Liver cirrhosis	No	18 (60.0)	59.14 (33.85)	18.60 - 140.00	0.162 NS, a
	Yes	12 (40.0)	86.22 (96.19)	36.60 - 205.00	
Ascites	Absent	20 (66.7)	59.14 (35.04)	18.60 - 140.00	0.245 NS, b
	Minimal perihepatic	5 (16.7)	105.00 (73.84)	41.38 - 155.00	
	Mild	3 (10.0)	130.00 (116.90)	62.10 - 179.00	
	Moderate	2 (6.7)	125.32 (159.37)	45.63 - 205.00	
Lymph nodes	Negative	9 (30.0)	49.61 (18.87)	18.60 - 89.60	**0.032** S, a
	Positive	21 (70.0)	70.00 (66.83)	26.89 - 205.00	
IHBRD	Mild bilobar	10 (33.3)	59.81 (30.39)	30.36 - 155.00	0.918 NS, b
	Moderate bilobar	8 (26.7)	65.12 (38.21)	26.89 - 105.00	
	Marked bilobar	12 (40.0)	66.05 (86.90)	18.60 - 205.00	
Focal lesions site	Distal	10 (33.3)	72.73 (45.36)	18.60 - 179.00	0.680 NS, b
	Hilar	16 (53.3)	58.39 (35.08)	26.89 - 205.00	
	Intrahepatic	4 (13.3)	91.46 (70.87)	46.98 - 130.00	
Gall Bladder	Contracted	22 (73.3)	65.79 (43.97)	18.60 - 205.00	0.778 NS, a
	Distended	8 (26.7)	58.79 (51.77)	26.89 - 140.00	
HCV-Ab	Negative	17 (56.7)	55.47 (33.85)	18.60 - 140.00	0.161 NS, a
	Positive	13 (43.3)	67.43 (83.02)	36.60 - 205.00	
Diabetes Mellitus	No	19 (63.3)	62.10 (34.37)	26.89 - 205.00	0.747 NS, a
	Yes	11 (36.7)	64.14 (76.58)	18.60 - 155.00	
Hypertension	No	21 (70.0)	62.80 (35.26)	18.60 - 205.00	0.354 NS, a
	Yes	9 (30.0)	78.09 (71.21)	41.38 - 155.00	

a: Mann-Whitney U testb: Kruskal Wallis test **IHBRD:** Intra Hepatic Biliary Radicle Dilatation
NS: Non significant at P-value ≥ 0.05 S: Significant at P-value < 0.05

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Table 4: Diagnostic performance of serum TGF-β1between cholangiocarcinoma and healthy controls, HCC and healthy controls and pancreatic cancer patients against healthy controls

Test characteristics of TGF-β1 (ng/mL)					
Cutoff value	**AUC**	**P-value**	**Sensitivity %**	**Specificity %**	**PPV %**
≥ 36.22	0.829	**<0.001** HS	90.0	66.7	73.0
≥ 70.75	0.890	**<0.001** HS	70.0	93.3	87.4
≥ 53.77	0.92	**<0.001** HS	85.0	83.3	77.2

AUC: Area under the curve
PPV: Positive predictive value NPV: Negative predictive value
NS: Non-significant at P-value ≥ 0.05 S: Significant at P-value < 0.05 HS: Highly significant at P-value < 0.01

Table 5: Diagnostic performance of serum TGF-β1, CA19.9, and their combined measurement for discrimination between cholangiocarcinoma and pancreatic cancer

Biomarkers	**Test characteristics**							
	Best cutoff value	**AUC**	**P-value**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**	**Accuracy %**
TGF-β1 (ng/mL)	≤ 79.55	0.691	**0.023** S	70.0	70.0	77.8	60.9	70.0
CA19.9 (IU/mL)	≥ 920.00	0.613	0.178 NS	60.0	70.0	75.0	53.8	65.0
Combined TGF-β1 and CA19.9	≥ 0.63	0.772	**0.001** HS	70.0	75.0	80.8	62.5	72.5

AUC: Area under the curve
PPV: Positive predictive value NPV: Negative predictive value
NS: Non-significant at P-value ≥ 0.05 S: Significant at P-value < 0.05 HS: Highly significant at P-value < 0.01

Table 6: Diagnostic performance of serum TGF-β1, AFP, and their combined measurement for discrimination between cholangiocarcinomaand HCC.

Biomarkers	**Test characteristics**							
	Best cutoff value	**AUC**	**P-value**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**	**Accuracy %**
TGF-β1 (ng/mL)	≤ 70.75	0.623	0.146 NS	60.0	70.0	75.0	53.8	65.0
AFP (ng/mL)	≤ 22.00	0.927	**<0.001** HS	93.3	85.0	90.3	89.4	89.2
Combined TGF-β1 and AFP	≥ 0.76	0.928	**<0.001** HS	93.3	85.0	90.3	89.4	89.2
HCC vs. CCA	≥ 70.75	0.623	**0.146** NS	70.0	60.0	53.8	75.0	65.0

AUC: Area under the curve
PPV: Positive predictive valueNPV: Negative predictive value
NS: Non-significant at P-value ≥ 0.05 HS: Highly significant at P-value < 0.01

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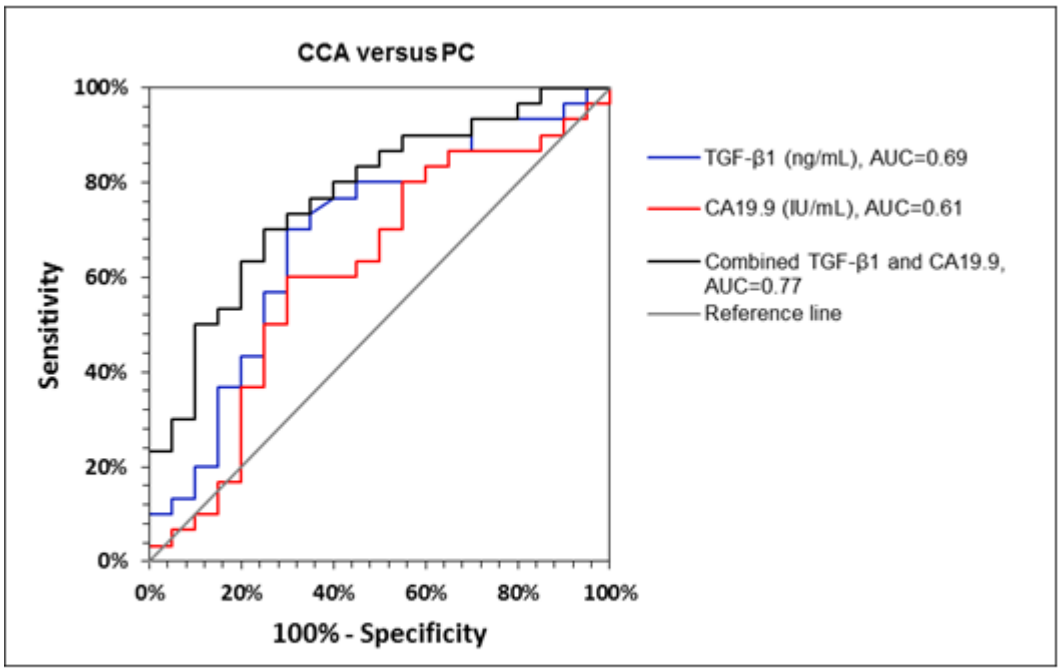

Figure 1: ROC curves of TGF -β, CA19.9, and combined measurement for pairwise discrimination between CCA and PC

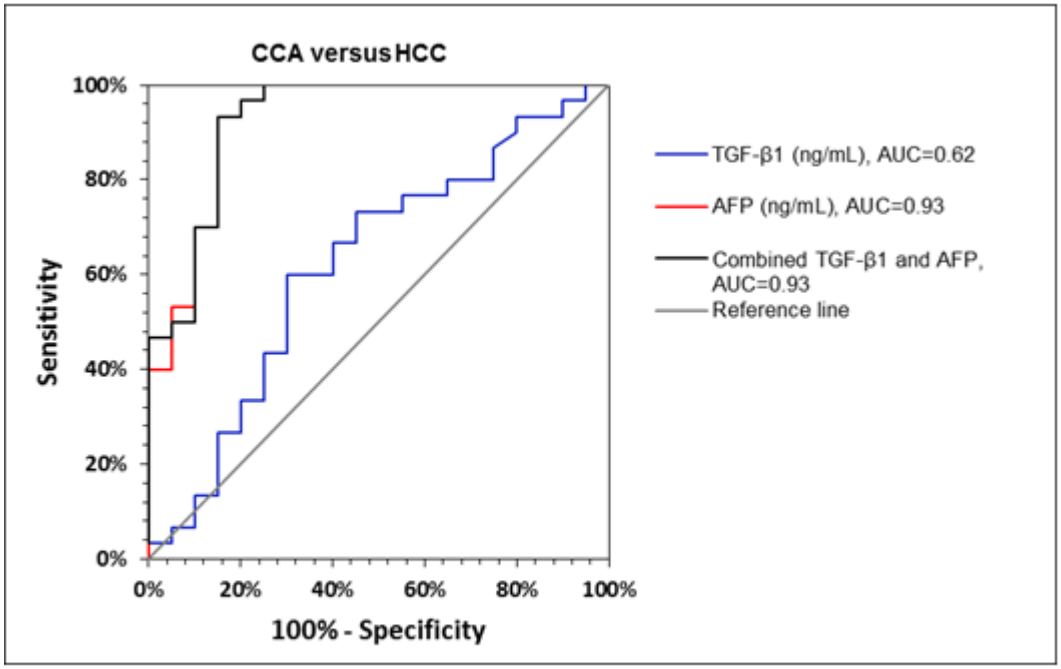

Figure 2: ROC curves of TGF -β1, AFP, and combined measurement for pairwise discrimination between CCA and HCC

DISCUSSION

The early detection of CCA, an aggressive malignancy characterized by a poor prognosis and often delayed diagnosis, presents significant challenges. A multidisciplinary approach is essential to improve screening and reduce mortality associated with late presentation and inadequate diagnostic accuracy. Tumor markers such as CA 19-9, CEA, and CA-125 are frequently utilized; however, their sensitivity and

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specificity are limited as they can be elevated in various benign and malignant diseases [17].

This study investigated the ability of TGF β1 to differentiate CCA from HCC and PC, as well as its potential utility as a diagnostic and prognostic marker for CCA. Our findings indicate that patients with pancreatic, HCC, and CCA exhibit significantly elevated levels of TGF-β1 compared to healthy individuals. Patients with PC exhibited the highest serum concentrations of TGF-β1, followed by those with HCC and CCA. A recent study indicates that TGF-β1 contributes to oncogenesis by inducing epithelial-mesenchymal transition (EMT) and promoting tumor invasion and metastasis, as evidenced by elevated serum levels in patients with various cancer types [18].

Our findings arecorroborated by Zhao et al.'s study [19], which showed a statistically significant difference in TGF-β1 blood levels between the control group and patients with pancreatic ductal adenocarcinoma (PDAC). PDAC patients had serum TGF-β1 levels of 237.6±45.3 ng/mL, whereas healthy control subjects had serum TGF-β1 levels of 57.6±23.2 ng/mL.

Serum TGF-β1 levels did not significantly correlate with any of the criteria in the CCA group of patients, except for lymph node involvement, where patients exhibiting lymph node involvement had elevated serum TGF-β1 levels. A positive correlation between TGF-β1 levels and focal lesion size was identified. Increased serum TGF-β1 levels were significantly associated with bilirubin and ALP levels in patients with CCA. Additionally, a positive correlation was observed between TGF-β1 levels and CA 19.9 values, consistent with findings by Kimawaha et al. [20], which indicated that serum TGF-β1 levels increase alongside rising serum CA19-9 levels. Furthermore, the study indicated a strong association between TGF-β1 and the progression of CCA, as well as a poorer prognosis, with significant expression levels observed in CCA tissues. The metastatic status of CCA patients correlates with their serum TGF-β1 level. According to Chen et al. [21], patients with ICC exhibiting TGF-β1 overexpression demonstrate significantly higher incidence of distant metastases, lymphatic metastases, lymphovascular invasion, and tumor recurrence compared to those with TGF-β1-negative tumors .

There was no correlation between TGF-β1 levels and other indicators in patients with HCC. Lee et al. [11] reported that elevated plasma levels of TGF-β1 were linked to accelerated disease progression, which contrasts with our results. Individuals with extrahepatic metastases or portal vein thrombosis exhibited significantly greater values compared to those without metastases. A positive correlation exists between the size of HCC focal lesions and plasma TGF-β1 levels. Additionally, a negative correlation was observed between patient survival and plasma TGF-β1 levels. This is evidenced by the dual function of TGF-β1 in cancer progression. In the initial phases, TGF-β inhibits tumor development by inducing programmed cell death [21]. In advanced stages, TGF-β contributes to tumor progression via three primary mechanisms: promoting epithelial-to-mesenchymal transition, inhibiting the immune response, and increasing angiogenesis [23].

Furthermore, elevated blood TGF-β1 levels may not accurately reflect the activation of the TGF-β1 pathway within the tumor microenvironment, which accounts for its limited utility as an independent prognostic factor [24].

Furthermore, in pancreatic cancer patients, a positive correlation was found between CA19.9, CRP values, ALP, and Bilirubin values with TGF-β1 level. Pancreatic tumor size positively correlated with TGF-β1 level. Furthermore, assessed the diagnostic efficacy of TGF-β1 as a potential biomarker for CCA and its ability to differentiate CCA from other research cohorts. The TGF-β1 level can differentiate healthy controls from CCA patients at a cut-off of ≥ 36.22 ng/mL, demonstrating 90.0% sensitivity and 66.7% specificity, as indicated by ROC curve analysis. The findings align with those of Kimawaha et al. [20], who reported that the TGF-β1 level exhibited a sensitivity of 71.1% and a specificity of 68.9% at a cut-off of 38.54 ng/mL, effectively differentiating between the CCA and normal groups .

Currently, CA 19-9 is the most reliable blood biomarker for the identification of pancreatic adenocarcinoma and CCA. This study assessed the capacity of TGF-β1 to differentiate between CCA and pancreatic carcinoma. ROC curve analysis indicated that CA19.9 was ineffective in

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differentiating between patients with CCA and those with pancreatic cancer. TGF-β1 at a cut-off of ≤ 79.55 ng/ml effectively distinguished between CCA patients and those with pancreatic cancer, demonstrating 70% sensitivity and 70% specificity. The combination of TGF-β1 and CA 19.9 demonstrated an enhancement in specificity, reaching 75%, while sensitivity remained unchanged at 70% .

Although our study evaluated TGF-β1 and CA19.9 levels in distinguishing CCA from pancreatic cancer, we did not specifically analyze the differentiation between dCCA infiltrating the pancreatic head and pancreatic adenocarcinoma. This limitation was primarily due to the relatively small sample size of dCCA cases (n = 10). Considering that this distinction has significant clinical implications for diagnosis, treatment planning, and surgical approach, future studies with larger studies focusing specifically on distal CCA are warranted to clarify these diagnostic challenges.

ROC curve analysis indicated that TGF-β1 was unable to differentiate between HCC and CCA. AFP demonstrated superior performance compared to TGF-β1 at a cut-off value of ≤ 22.0 ng/ml, effectively distinguishing between the two groups with a sensitivity of 93.3% and a specificity of 85.0%. Combined measurements of AFP demonstrated no alteration in sensitivity or specificity.

Although AFP is a well-established, highly sensitive marker of hepatocellular carcinoma, its finding within this study was employed more as a control against which to gauge relative diagnostic utility of TGF-β1 across the spectrum of hepatobiliary cancers than as an equivalent specificity marker. This control was employed to cast emphasis upon the relatively narrow discriminatory capability of TGF-β1 employed in contrast to an established HCC-specific marker such as AFP.

Overall, the findings suggest that while TGF-β1 alone is not sufficient as a standalone diagnostic biomarker for CCA, its comparative performance against CA19.9 highlights potential value in combined marker assessment, particularly for distinguishing CCA from pancreatic cancer. Although serum TGF-β1 levels were actually significantly elevated in all cancers and suggested a shared role in tumor formation, this is also a reflection of its relatively low specificity for CCA. Since our study population had not been stratified by TNM classification or stage of disease, the prognostic significance of TGF-β1 could not be evaluated fully. Further studies including tumor staging and long-term follow-up would be necessary to assess whether levels of TGF-β1 increase with disease burden and to define its true prognostic value.

CONCLUSION

TGF-β is a poor diagnostic and prognostic marker for CCA; however, it exhibits superior capability in differentiating between CCA and HCC, and AFP demonstrates better diagnostic performance. Additionally, while TGF-β1 is not a useful prognostic and diagnostic marker for CCA, its elevation in other tumor types points toward its general role in carcinogenesis. Further studies including disease staging must be conducted to ascertain its prognostic value. Furthermore, due to limited sample size, our study did not separately evaluate distal CCA infiltrating the pancreatic head versus pancreatic adenocarcinoma; future investigations focusing on this subgroup are needed to establish clinically relevant diagnostic distinctions.

Ethics approval and consent to participate

Well informed written consent was obtained from all individuals included in the study. The Ethics Committee of the National Liver Institute at Menoufia University approved the study protocol; with IRB number (00696/2025).

Consent for publication

- All authors gave consent with manuscript content.
- On behalf of all authors, I have the pleasure for communication with your Journal. I wish that our research work is under your kind care and observation.

Availability of data and material

- All data is available upon request.

Competing interests

- No conflict of interest regarding the publication of this paper.
- No fund was received.

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Authors' contributions

- All authors shared in: design of the work, conceptualization, resources detection, formal analysis, data curation, interpretation of data, creation of new software used in the work, validation and methodology plus revision.

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List of abbreviations: OFTGF-β: Transforming growth factor β; CCA: cholangiocarcinoma; TME: tumor microenvironment; HCC: Hepatocellular carcinoma; EMT: epithelial-mesenchymal transition

HIGHLIGHTS

- TGF-β1 levels showed a significant difference across groups, with the highest concentrations observed in pancreatic cancer patients. TGF-β1 can differentiate between healthy controls and CCA patients at a cut-off ≥ 36.22 ng/mL, demonstrating 90.0% sensitivity and 66.7% specificity.
- TGF-β1 effectively distinguished pancreatic cancer patients from healthy controls at a cut-off of ≥ 53.77 ng/ml, demonstrating 85% sensitivity and 83.3% specificity.
- TGF-β1 at a cut-off of ≤ 79.55 ng/ml effectively distinguishes between pancreatic cancer and CCA patients, demonstrating 70% sensitivity and 70% specificity.
- TGF-β is a poor diagnostic and prognostic marker for CCA; however, it demonstrates superior capability in distinguishing between CCA and pancreatic cancer compared to CA19.9.

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