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Bile level of cytokeratin 7 as a diagnostic marker for cholangiocarcinoma: a case-control study in Egyptian patients

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Abstract

Background Cholangiocarcinoma (CCA) is an aggressive malignancy with a poor prognosis of less than 20% five-year survival rate. Early diagnosis is typically challenging due to asymptomatic characteristics at the earliest stages of the disease. This study aims to assess the potential utility of cytokeratin 7 (CK7) as a CCA diagnostic biomarker in bile. In total, 100 participants were included in this case-control study. Moreover, Group I had 30 CCA patients with malignant obstruction, and Group II had 20 patients with malignant biliary obstruction other than CCA formed. Group III included 20 patients with benign biliary obstruction, and 30 individuals undergoing cholecystectomy with no evidence of biliary obstruction made up the control group (Group IV). Bile samples were collected during endoscopic retrograde cholangiopancreatography or cholecystectomy for the control group. The CK7 levels in bile samples were measured using the enzyme-linked immunosorbent assay.

Results

The bile level of CK7 was significantly higher in cholangiocarcinoma patients (1555.4 ± 302.7 pg/mL) than those of the patients with malignancies other than CCA (581.9 ± 227.5 pg/mL), patients with benign obstruction (439.5 ± 255.7 pg/mL), and the control group (53 ± 26.4 pg/mL) (p value < 0.001). Furthermore, CK7 was significantly higher in CCA patients than in those with other malignancies (p value < 0.001). Patients with CCA with hilar lesions had the highest values compared to those with distal lesions. ROC curve analysis revealed that bile CK7 at a cut point of >1030 pg/mL yielded an area under a curve of 1 (95% CI: 1.000–1.000) in differentiating CCA from other groups.

Conclusion

The bile level of CK7 demonstrates outstanding performance that could help in diagnosing CCA.

Keywords Cholangiocarcinoma, Cytokeratin 7, Endoscopic retrograde cholangiopancreatography

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Background

Cholangiocarcinoma (CCA) constitutes a diverse group of malignancies emerging in the biliary tree [1]. CCA is considered the most common biliary tract malignancy and the second most common primary hepatic cancer, accounting for around 15% of cases and 3% of gastrointestinal tract (GIT) cancers. CCA is an uncommon GIT cancer [2].

Even though CCA is less common than other cancers, its prevalence rates remain on the increase globally. CCA, a type of cancer that mainly affects men, poses the most significant risk between the ages of 60 and 70. Only 30% of tumors are curative when they are first discovered, and the vast majority of CCA cases are identified at late stages. As a result, the prognosis is dismal, with a 7%–20% five-year survival rate [3].

Increased levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) tumor markers may be signs of bile duct cancer [4]. CA 19-9 and CEA provided wide ranges of sensitivity (47.2%–98.2%) and specificity (89.7%–100%) [5]. As no specific CCA radiology pattern exists, histopathological or cytological analysis is mandatory to confirm the diagnosis [6], which provided a sensitivity and specificity of 53% and 100%, respectively. Combining the histologic evaluation with the markers significantly increased the sensitivity [7].

Cytokeratins (CKs) belong to a group of approximately 20 cytoskeletal structural proteins present in epithelia and tumors derived from epithelia [8]. In epithelial cells, cytokeratins (CKs, keratins) are a category of intermediate filament proteins comprising those of type I (CK9–CK20) alongside type II (CK1–CK8) proteins in accordance with the traditional categorization [9]. They control the growth, proliferation, migration, apoptosis, immunity, and other aspects of cell metabolism, as well as provide structural support for epithelial cells [10]. CK expression is usually maintained by neoplastic cells; therefore, specific anti-CK antibodies are widely used in routine histopathology diagnostics to determine tumor origins, particularly in metastases [8].

CK7 is typical for ductal structures and can be observed in the pancreatic and bile duct and the gallbladder. Immunohistochemical staining indicates that CK7, an intermediate filament protein with a molecular weight of 54 kDa, is positively expressed in the cytoplasm of cells [11]. The immunostaining of CK7 and CK20 has been used to distinguish usual peripheral CCA and colorectal carcinoma metastasis (CRM) [12].

Contemporary medical research should be focused on identifying approaches to get beyond restrictions on diagnosis in CCA. Thus, this study's primary goal was to determine the potential role of CK7 in bile as a CCA diagnostic biomarker.

Methods

Study design and participants

This case-control study was performed at the National Liver Institute Department's of Hepatology and Gastroenterology and Clinical Pathology in collaboration with Tropical Medicine, Faculty of Medicine, Menoufia University. In this study, 100 participants were included. Over 18 months, between January 2022 to June 2023, they were recruited from the National Liver Institute's Hepatology and Gastroenterology Department. Participants were selected by the consecutive presentation and were divided into four groups: Group I, 30 patients with CCA and malignant obstruction; Group II, 20 patients with malignant obstructive jaundice other than CCA; Group III, 20 patients with nonmalignant obstructive jaundice; Group IV (control group), 30 individuals undergoing cholecystectomy by elective laparoscopy due to gallbladder stones with no evidence of biliary obstruction.

A comprehensive history, a clinical assessment, and laboratory tests such as C-reactive protein (CRP), liver function, renal function, and complete blood count were all performed. Tumor markers, including alpha-fetoprotein (AFP), CEA, and CA19-9, were measured using enzyme-linked immunosorbent assay (ELISA). Additionally, triphasic CT and or magnetic resonance imaging (MRI) on the abdomen and pelvis, as well as MRCP, were performed to confirm the diagnosis and to evaluate the tumor characteristics such as its site, size, and number, assessment of the biliary tree (site, type, and degree of dilatation, stricture, or obstruction), the vascular invasion, and the presence of extrahepatic metastasis. Subjects with medical jaundice, sepsis, primary sclerosing cholangitis or primary biliary cirrhosis, decompensated liver cirrhosis, and acute hepatitis were excluded.

Ethical consideration

After the research questions and goals were established, all participants were made aware of the purpose of the study and gave their written permission. The National Liver Institute, Menoufia University Ethics Committee, Egypt, approved the study (IRB: NLI 00003413) and the study followed the Helsinki Declaration.

Sample collection

Samples were collected during endoscopic retrograde cholangiopancreatography (ERCP) for patients presented with obstruction in Groups I, II, and III. Selective and deep cannulation was performed to help placement of more extensive accessories. An X-ray was done to visualize the biliary tree, and by using a syringe, 5 cm

bile samples were collected for analysis. For the control group, samples were collected during elective laparoscopic cholecystectomy for the calcular gall bladder.

Sample preparation

First, 2 mL of bile was allocated in a sterile container and then centrifuged for 20 min at the speed of 2000–3000 rpm. The supernatant is preserved at -20 till use.

Determination of Cytokeratin 7 (CK7) Bile Concentrations Using ELISA

CK7 was measured in bile using the Human Keratin 7 (KRT7) ELISA Kit. The kits were supplied by Shanghai Sunred Biological Technology Co. Catalogue (No.201-12-4092) [10]. The kit utilized a double-antibody sandwich ELISA to assay the level of KRT7 in samples. The assay was performed in accordance with the operating instructions for processing. Briefly, 40 µL of bile samples was introduced into wells that were pre-coated with KRT7 monoclonal antibody. This was followed by the addition of 10 µL of biotin-labeled KRT7 antibodies and 50 µL of streptavidin HRP. Finally, the wells were covered with a seal plate membrane. The samples were incubated for 60 min at 37°C. Subsequently, the wells were washed five times, and then 50 µL chromogen solution was added and allowed to react for 10 min at 37°C before washing again to remove the uncombined enzyme. Finally, we added 50 µL of Stop Solution to each well, and the sample's absorbance was detected at 450 nm wavelength. The chroma of color and the concentration of the KRT7 of the sample were positively correlated. The assay sensitivity was 22.682 pg/mL and the assay range was 25 pg/mL→7000pg/mL.

Statistical data analysis

The information was collected in the form of variables on a specially designed data collection questionnaire. Statistical analysis was performed using SPSS software version 20.0 [13]. Frequency and percentages were calculated for qualitative variables like gender, whereas quantitative variables such as age were presented in the form of mean and standard deviation. A chi-square test was performed to assess the significance of difference. The p value of ≤ 0.05 was considered to be significant. ROC curve was conducted by connecting the coordinate points using "1 – specificity (false positive rate)" as the x-axis and "sensitivity" as the y-axis for all cutoff values measured from the results.

Results

To distinguish CK7's role in CCA diagnosis, we used the ELISA technique to characterize 100 bile samples from 30 patients with CCA, 20 patients with malignant obstructive jaundice other than CCA, 20 patients with nonmalignant obstructive jaundice, and 30 participants with no obstruction. With a mean age of 57 (±13) years, 60% of the participants in this research were males. Additionally, 20 patients (20%) had hypertension, 32 patients (32%) had diabetes, and 53 patients (53%) were smokers.

Table 1 displays the demographic and laboratory characteristics of the 100 subjects that made up the clinical cohort used in this study. There were significant differences between the four groups regarding age and

Table 1 Comparison of the two studied groups according to demographic data and laboratory investigation

Variable	Group (I)	Group (II)	Group (III)	Group (IV)	*P*-value
Age (years)	66.00±9.418	62.35±8.536	48.60±12.059	48.63±12.444	*P*<0.001
Male gender, n (%)	22(73.3%)	16(80%)	8(40%)	14(46.7%)	*P*=0.011
Total bilirubin (mg/dl)	14.4±4.7	16.5±8.5	6.96±3.5	1±0.23	*P*<0.001
ALT (IU/L)	40.7±24.6	65.8±37.2	29.3±7.3	39.4±11.3	*P*<0.001
AST (IU/L)	74.8±47	89.4±40.4	28.8±7.8	43.1±10.1	*P*<0.001
GGT (IU/L)	385.7±157	366.6±224.3	169.6±87.1	30.8±9.9	*P*<0.001
ALP (IU/L)	545.2±165.96	508.95±170.9	311.8±102.2	104.97±29.4	*P*<0.001
Serum albumin (g/dl)	3.95±0.3	3.7±0.5	4.5±0.3	4.1±0.3	*P*<0.001
INR	1.3±0.14	1.51±0.3	1.16±0.13	1.16±0.09	*P*<0.001
HB (gm/dl)	12.10±1.39	13.14±1.3	12.65±0.89	12.2±1.5	*P*=0.037
WBCs x 10^9/L	12.1±5.8	13.78±5.55	10.48±5.23	4.73±1.7	*P*<0.001
CRP mg/L	121.2±46.27	133.9±65.1	61.34±47.85	3.6±2.5	*P*<0.001
Serum creatinine (mg/dl)	1.2±0.5	1.7±0.5	0.98±0.27	0.81±0.15	*P*<0.001

All values are represented in mean ± standard deviation (SD), *ALT* Alanine transaminase, *AST* Aspartate transaminase, *GGT* Gamma-glutamyl transferase, *ALP* Alkaline phosphatase, INR: international normalized ratio, *Hb* Hemoglobin concentration, *WBCs* White blood cells, *CRP* C-reactive protein. *p p* value for comparing the four studied groups. Statistically significant at $p < 0.05$

sex ($p < 0.001$ and $= 0.011$, resp.). Patients with malignant obstruction related to either CCA or another malignancy (Groups I and II) were older than those in other groups and were primarily males. In laboratory investigations of cases versus those of controls, there was highly significant variation in total bilirubin AST, ALT, GGT, serum albumin, and INR values with $p < 0.001$ for all.

Table 1 illustrates that patients in Groups I and II had significantly higher levels of total bilirubin, GGT, and ALP than those in the other groups. Group I (mean = 14.4 ± 4.7 mg/dl) and Group II (mean = 16.5 ± 8.5 mg/dl) had higher total bilirubin levels than the other groups, which was statistically significant (p value < 0.001). Additionally, CCA patients and those with malignant obstruction other than CCA had significantly higher mean values of GGT and ALP (385.7 ± 157 IU/L and 545.2 ± 166 IU/L; 366.6 ± 224.3 and 508.95 ± 170.9, resp.) than the other groups (p value < 0.001).

Patients with either benign or malignant biliary obstruction had higher WBC counts than those in the non-obstruction group (p value < 0.001). However, compared to other groups, patients with malignant obstruction, whether or not caused by cholangiocarcinoma, showed significantly higher CRP values (133.9 ± 65.1 mg/L and 121.2 ± 46.3 mg/L; p value < 0.001) than those with benign obstruction or control group as shown in Table 1.

Table 2 displays the patients' MRCP and ERCP results. According to MRCP, calcular GB was observed in 70% and 100% of Groups III and IV, respectively. There is

Table 2 Comparison between groups according to patient's radiological data

	Group (I)	Group (II)	Group (III)	Group (IV)	*P* Value
MRCP findings					
Gallbladder					
Calcular GB	0	0	14(70%)	30(100%)	*P*<0.001
Cholecystectomy	0	0	6(30%)	0	
Contracted GB	22(73.3%)	5(25%)	0	0	
Distended GB	8(26.7%)	15(75%)	0	0	
CBD (mm), mean ± S.D	9±2.15	19.5±6.1	14.70±3.4	3.87±0.8	*P*<0.001
CHD-dilatation, n (%)	16(53.3%)	20(100%)	20(100%)	0	*P*<0.001
Minimal IHBRD , n (%)	0	2(10%)	8(40%)	0	*P*<0.001
Mild IHBRD, n (%)	10(33.3%)	5(25%)	5(25%)	0	
Moderate IHBRD, n (%)	9(30%)	7(35%)	5(25%)	0	
Marked IHBRD, n (%)	11(36.7%)	6(30%)	2(10%)	0	
ERCP findings					
Minimal IHBRD , n (%)	0	2(10%)	4(20%)	-	*P*<0.001
Mild IHBRD, n (%)	10(33.3%)	5(25%)	9(45%)	-	
Moderate IHBRD, n (%)	7(23.3%)	7(35%)	5(25%)	-	
Marked IHBRD, n (%)	13(43.4%)	6(30%)	2(10%)	-	
CBD -dilatation , n (%)	11(36.7%)	20(100%)	20(100%)	-	*P*<0.001
Site of stricture					
Hilar , n (%)	17(56.7%)	1(5%)	0	-	*P*<0.001
Distal, n (%)	0	14(70%)	20(100%)	-	
Hilar and Intrahepatic, n (%)	6(20%)	0	0	-	
Hilar and distal, n (%)	7(23.3%)	3(15%)	0	-	
Distal and Intrahepatic, n (%)	0	1(5%)	0	-	
Distal, Hilar and Intrahepatic, n (%)	0	1(5%)	0		
Papilla enlargement, n(%)	7(23.3%)	8(40%)	0	-	*P*=0.001
Sphincterotomy, n(%)	18(60%)	12(60%)	13(65%)	-	*P*<0.001
Site of Focal lesions					
Right lobe, n (%)	21(70%)	4	0	-	*P*<0.001
Left lobe, n (%)	1(3.3%)	0	0	-	
Bilateral, n (%)	8(26.7%)	7(35%)	0	-	
Lymph node metastasis, n (%)	21(70%)	10(50%)	0	-	*P*<0.001

a statistically significant difference (p value < 0.001) between the studied groups regarding CBD diameter, with Groups II and III having the highest values, followed by Group I and Group IV. We noticed that out of all the patients in Groups II and III, all had CBD dilation with mean values of 19.5 ± 6.1 mm and 14.7 ± 3.4 mm, respectively, while only 11 patients (36.7%) of the CCA group had dilated CBD with a mean value of 9 ± 2.2 mm. Furthermore, all patients (100%) in Groups II and III exhibited common hepatic duct (CHD) dilatation with varying degrees of intrahepatic biliary radical dilatation (IHBRD). In contrast, 16 (53.3%) of the patients with CCA exhibited CHD dilatations. Additionally, near findings were noted during ERCP. In terms of IHBRD, ERCP revealed that 10 (33.3%) were mild, seven (23.3%) were moderate, and 13 (43.3%) were marked cases of CCA. None of the patients in Group IV had IHBRD. The degree of IHBRD varied statistically significantly between the groups (p value < 0.001).

As shown in Table 2, six patients (20.0%) in Group I and eight patients (40.0%) in Group II had enlarged papillae. Sphincterotomy was performed during ERCP on 18 patients (60.0%), 12 patients (60.0%), and 13 patients (65.0%) in Groups I, II, and III, respectively. All CCA lesions are hilar. Of the patients, 21 (70%) had masses found in the right lobe; eight (26.7%) had bilateral focal lesions; and only one (3.3%) had a focal lesion in the left lobe. Moreover, 12 patients (60.0%) in Group II had cancer head of the pancreas, and eight had hepatocellular carcinoma (HCC). Additionally, 21 patients (70%) in the CCA group and 10 patients (50%) in Group II had lymph node metastases.

In terms of diagnostic biomarkers, the mean of CA 19-9 for CCA was 3018.4 ± 2589 U/mL, whereas it was 559.8 ± 876.7 U/mL for other malignancies, Group II. The CA 19-9 level in patients with benign obstruction was 147.1 ± 102 U/mL, while it was 8.5 ± 3.4 U/mL in the control group (p value < 0.001). CEA values did not differ between the four studied groups (p value >0.05), with Groups I and II showing CEA values of 3.6 ± 4.8 ng/mL and 2.8 ± 4 ng/mL, respectively. For AFP, the highest levels were observed in Group II with a mean level of 1188 ± 1510.2 ng/mL, while in CCA, benign obstruction, and non-obstruction groups, its levels were 6.8 ± 6 ng/mL, 1.8 ± 1.1 ng/Ml, and 1.9 ± 1 ng/mL (p value <0.001). The bile level of CK7 was significantly higher in CCA patients (1555.4 ± 302.7 pg/mL) than patients with malignancies other than CCA (581.9 ± 227.5 pg/mL), those with benign obstruction (439.5±255.7 pg/mL), and those in the control group (53 ± 26.4 pg/mL) (p value <0.001) (Table 3).

Interestingly, we observed that compared to patients without strictures, patients with evidence of obstruction had significantly higher levels of CK7 and CA 19-9 (p < 0.001). Furthermore, CK7 and CA 19-9 were significantly higher in patients with malignant obstruction than those with benign obstruction (p value <0.001), as displayed in Fig. 1. Consequently, we can state that the presence of obstruction, either benign or malignant, significantly affects both CK7 and CA 19-9 levels compared to patients without obstruction Table 4.

Comparing CK7 levels throughout patients with various malignant obstruction aetiologies, we noticed that CK7 was significantly higher in CCA patients than in HCC or pancreatic cancer patients (p value <0.001). Similarly, CA19-9 was higher in CCA patients than in HCC or pancreatic cancer patients (p value <0.05), as demonstrated in Fig. 2.

Analysis of our data revealed that the CK7 levels in metastatic CCA did not differ significantly from non-metastatic CCA (p value = 0.669). Likewise, CA 19-9 was lower in metastatic CCA than in non-metastatic CCA, with a non-significant difference (p value = 0.773). The CK7 and CA 19-9 levels vary considerably depending on the location of the lesion. Patients with CCA who had hilar lesions had the highest values, followed by combined lesions, and the distal lesions had the lowest values, with p-values <0.001 for all (Fig. 3).

The ROC curve was applied to identify the sensitivity of each biomarker in differentiating between our groups. A bile CK7 cut point of >1030 pg /mL yielded area under curve (AUC) of 1 (95% CI: 1.000–1.000), 100% sensitivity, 100% specificity, while CA19-9's cutoff >1057 U/mL presented (AUC = 0.98) (95% CI: 0.961–1.000), 93.3% sensitivity, 95% specificity (p value<0.001), as shown in Table 3 and Fig. 4.

Table 3 Comparison between the studied groups as regards to patient's tumor markers

Variable	Group (I)	Group (II)	Group (III)	Group (IV)	*P* Value
Bile level of CK7 (pg/mL) mean± SD	1555.4±302.7	581.9±227.6	439.5±255.7	53±26.38	*P*<0.001
CA 19-9 (U/mL) mean± SD	3018.4±2589	559.8±876.7	147.1±102	8.5±3.4	*P*<0.001
AFP (ng/mL) mean± SD	6.8±5.98	1188±1510.2	1.8±1.1	1.9±1	*P*<0.001
CEA(U/mL) mean± SD	3.6±4.8	2.8±4	2.09±1.4	2.38±1.1	*P*=0.519

CK7 Cytokeratin 7, *CA 19-9* Carbohydrate antigen 19-9, *AFP* Alpha-fetoprotein, *CEA* carcinoembryonic antigen, *p p* value for comparing between the four studied groups. Statistically significant at $p < 0.05$

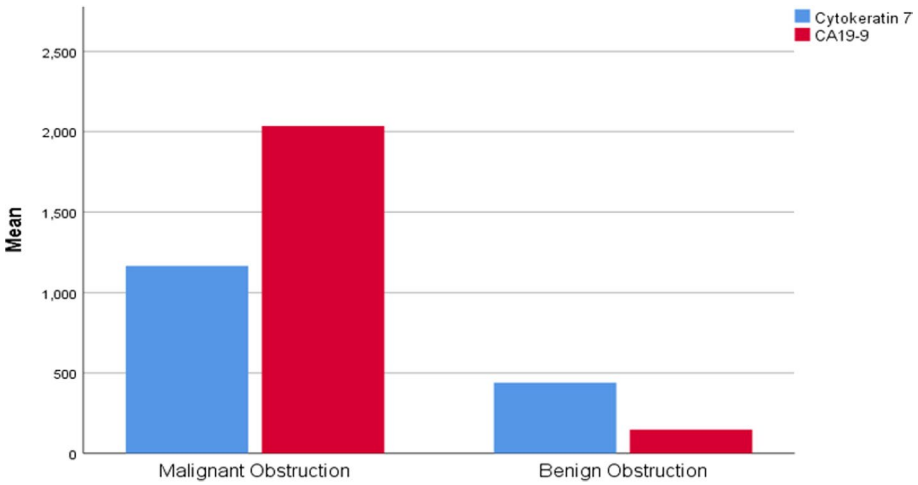

Fig. 1 Change in bile level of CK7 and serum CA 19-9 according to the type of obstruction: marked elevation was detected in patients with malignant compared to benign obstruction (p value < 0.001)

Table 4 Validity (AUC, sensitivity, specificity) of studied markers to discriminate CCA patients from between different groups

	Cut-off value	Sensitivity	Specificity	NPV	PPV	AUC	*P* value
Cytokeratin -7	>1030	100%	100%	100%	100%	1.000	<0.001
CA 19-9	>1057	93.3%	95%	90.5%	96.6%	0.984	<0.001

CK7 Cytokeratin 7, *CA19-9* Carbohydrate antigen 19-9, *AUC* Area under a curve, *p* value Probability value, *CI* Confidence intervals, *NPV* Negative predictive value, *PPV* Positive predictive value

*: Statistically significant at $p < 0.05$

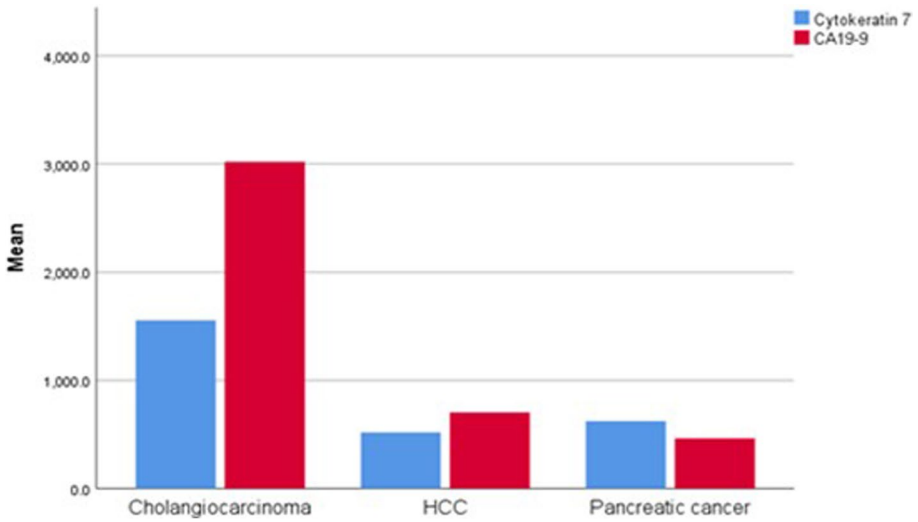

Fig. 2 Bile levels of CK7 and serum CA 19-9 were significantly elevated in cholangiocarcinoma compared to other malignancies (HCC and cancer head of the pancreas), benign obstruction, and in the control group

Our data evaluation demonstrated that CK7 bile level shows a positive significant correlation with CA 19-9 ($r = 0.584$ and $p < 0.001$), total bilirubin ($r = 0.584$ and $p < 0.001$), ALP ($r = 0.684$ and $p < 0.001$), and GGT ($r = 0.594$ and $P < 0.001$); however, no significant associated observed with CBD diameter (Table 5).

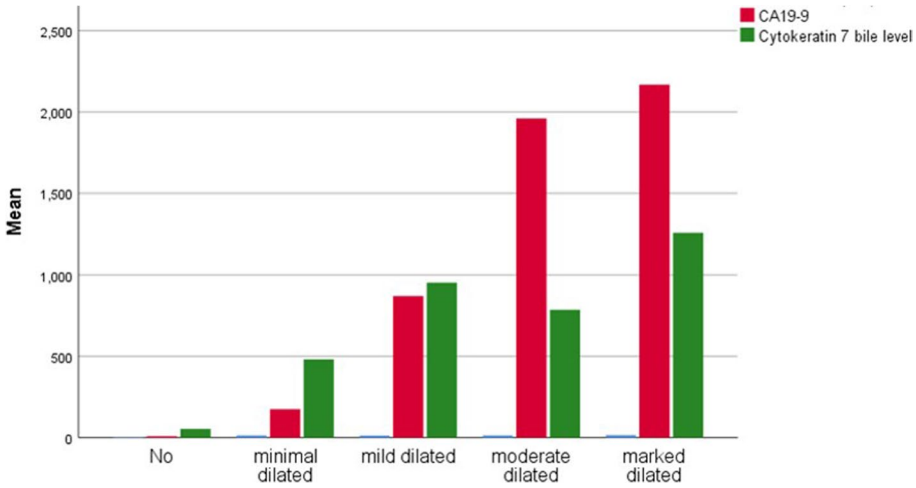

Fig. 3 Change in bile level of CK7 and serum CA 19-9 according to degree of IHBRD

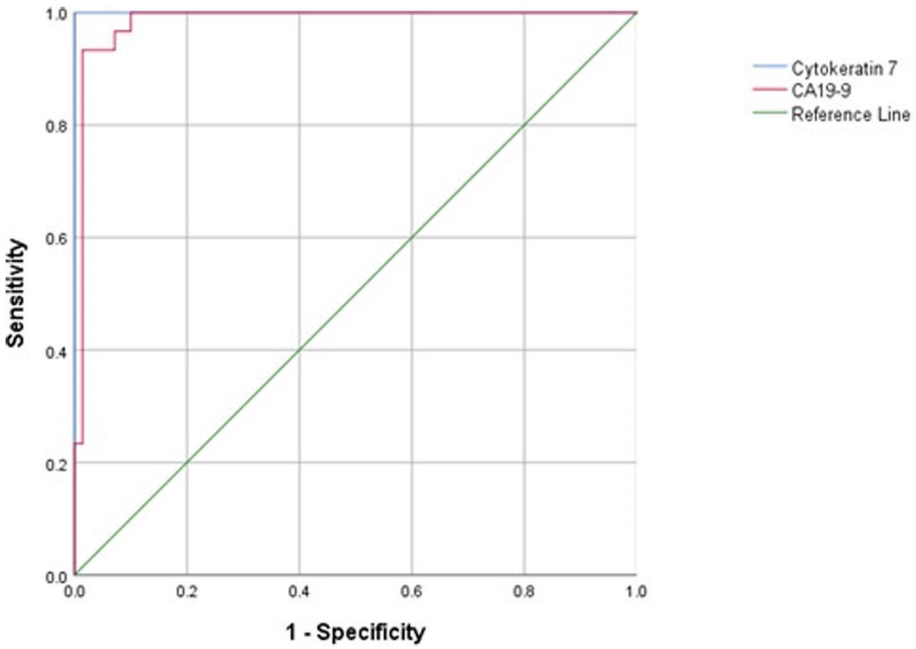

Fig. 4 Validity (AUC, sensitivity, and specificity) of studied markers to discriminate CCA patients from between different groups

For determining the impact of cholangitis on CA 19-9 levels and bile levels of CK7, we noticed that 37 patients presented with cholangitis had higher levels of bile CK7 and CA 19-9 than those without cholangitis (p value < 0.001). When CCA stratified by association with cholangitis, the performance of CK7 remains excellent: AUC of 0.905 (95% CI: 0.847–0.964) in noncholangitis-associated CCA (p value< 0.001). In contrast, CA19-9 had a twofold increase in response to infection (p value <0.05).

Discussion

Cholangiocarcinoma, a malignant tumor that arises from the bile duct epithelium, is the second most prevalent type of liver cancer, following HCC, and accounts for 10–20% of primary hepatic malignancies [6]. As no specific CCA radiology pattern exists, histopathological or cytological analysis is mandatory to confirm the diagnosis [9]. Cytological specimens can be classified as negative for malignancy, atypical, suspicious for malignancy, and diagnostic of cancer. Specimens obtained

Table 5 Correlation between cytokeratin 7 bile level and different parameters

	Cytokeratin 7 bile level	
	r	P
CA 19-9	0.584	<0.001*
CBD diameter	0.154	0.126
Total bilirubin	0.584	<0.001*
ALP	0.684	<0.001*
GGT	0.594	<0.001*

CA19-9 Carbohydrate antigen 19-9, *GGT* Gamma-glutamyl transferase, *ALP* Alkaline phosphatase

by brush cytology have a sensitivity of 15% when only diagnostic of cancer results are used for CCA diagnosis and 48% when diagnostic of cancer plus suspicious for malignancy cytology interpretations are combined for cancer diagnosis [14]. CK7 is a unique marker of bile duct epithelial cells, discerning cancers such as intrahepatic cholangiocarcinoma and HCC. Disparities in aberrant CK7 expression by hepatocytes can also sometimes be used to assess the prognosis of cancer patients [10]. Therefore, this investigation aims to determine whether CK7 in bile could serve as a diagnostic biomarker for cholangiocarcinoma.

In the current study, we observed that age and sex showed significant differences among the four groups. Patients in Groups I and II with evidence of malignant obstruction, either related to CCA (mean age = 66.00 ± 9.418) or another malignancy (mean age = 62.35±8.536), were predominantly older than those in the other groups and were primarily males. A systematic review and meta-analysis of eleven African studies found that the median age of CCA ranged from 52.5 to 61 years [15]. Additionally, studies from Egypt revealed that among CCA patients, the percentage of men was considerably higher than that of females [16].

In laboratory investigations of cases versus those of controls, CCA patients had higher levels of total bilirubin, GGT, and ALP than those in the other groups. According to Forner et al., total bilirubin generally exceeded 10 mg/dL and direct bilirubin was typically elevated in patients with extrahepatic CCA (usually increased 2- to 10-fold) [17]. In addition, Zhang et al. found that in ICC patients, ALP, total bilirubin, direct bilirubin, and GGT were independent predictors of poor outcomes [18].

Patients with either benign or malignant biliary obstruction (Groups I, II, and III) had higher WBC counts than the non-obstruction group, together with higher levels of LDH in these groups than the control group. It is also possible to hypothesize that strictures and the cholangitis that followed could be the etiology of the elevated WBC count CRP serum levels. Likewise, we observed that patients with malignant biliary obstruction had higher CRP levels than those with benign obstructions.

Being a member of the acute-phase protein family CRP level is influenced by a number of variables—infection, neoplasia, and injury all cause variations in its concentration. Cytokines, like tumor necrosis factor α (TNF-α), interleukin-8 (IL-8), and interleukin-6 (IL-6), are accountable for its upregulation [19]. Primarily, an inflammatory reaction to tumor invasion may also be the source of elevated CRP levels in malignant diseases [20]. Additionally, it was previously demonstrated that neoplastic tissue itself may express CRP in immunohistochemical investigations [21]. According to in vitro investigations, IL-6 is an autocrine growth factor that stimulates the expression of the antiapoptotic protein Mcl-1 in CCA cell lines [22]. Furthermore, patients with CCA had significantly elevated serum levels of IL-6, which sharply decreased following resection [23]. CRP also correlated with better OS (p = 0.002) and longer postoperative recurrence-free time (p = 0.032) [24].

Regarding the diagnostic biomarkers, the current study displayed that, CEA values did not differ between the four studied groups. In spite of being a well-known prognostic biomarker in patients with colorectal cancers, CEA utility seems to be restricted in patients with bile duct cancers. Kim et al. reported that only 9% of bile duct cancer patients had elevated serum CEA levels, low AUC, and low sensitivity. Besides, they also found that patients with benign diseases and cancer had similar serum CEA distributions. Due to its low sensitivity, CEA does not appear to be a helpful diagnostic biomarker for EBDC [25].

The mean of CA 19-9 for CCA was 3018.4 ± 2589 U/mL, whereas it was 559.8 ± 876.7 U/mL for other malignancies, Group II. Additionally, we noticed that the CA 19-9 level in patients with benign obstruction was significantly higher than that in the control group (p value < 0.001). When the ROC curve was applied to identify the sensitivity of CA 19-9 to discriminate CCA patients from other groups, we observed that CA19-9's cutoff >1057 U/mL presented 93.3% sensitivity and 95% specificity (AUC = 0.98; 95% CI: 0.961–1.000; p value < 0.001).

A systematic review and meta-analysis by Liang concluded that serum CA 19-9 is a useful noninvasive biomarker for CCA detection, which supports our findings [26]. Moreover, Younes et al. reported that CA 19-9 was higher in CCA patients than that in HCC patients (median was 155.7 and 67.4 U/mL, respectively) and that there was a statistically significant difference between the

two groups [27]. This finding is consistent with the current study.

Both malignant diseases and benign inflammatory conditions may cause an increase in the levels of CA 19-9 in patients [28–30]. According to Ahrendt et al., 13.8% of patients with benign biliary tract diseases showed a moderate increase in CA 19-9 concentration [31]. Both malignant and normal biliary ductal cells produced CA 19-9. In benign conditions like choledocholithiasis, if biliary obstruction prevents bile flow, epithelial cells become substantially hindered by inflammation and will proliferate simultaneously. Consequently, there is a likelihood that more CA 19-9 will be released into the bloodstream. When acute cholangitis is relieved or the common duct is appropriately decompressed, the raised CA 19-9 levels frequently come back to normal [32].

The only tumor-associated biomarker applied to pancreatic ductal adenocarcinoma (PDAC) and distal cholangiocarcinoma (dCCA) that the FDA has approved is serum carbohydrate antigen 19-9 (CA 19-9). However, because its levels could also be impacted by obstructive jaundice brought on by benign conditions or other pancreaticobiliary diseases, CA 19-9's accuracy in pinpointing the precise disease is low [25].

The intermediate filament-forming proteins, known as cytokeratins, were found to be unique to epithelial cells. The expression levels of cytokeratin in epithelial cells are controlled by differentiation- and context-dependent mechanisms in various types of epitheliums. Numerous cytokeratin proteins were extensively employed as biomarkers in diagnosis as well as prognosis forecasting due to the fact that they demonstrated characteristic patterns of expression in tumors [33].

Each kind of epithelial cell differs from the others as they have unique cytokeratin expression profiles. Although tumors retain most of their cytokeratin profiles during malignant transformation, there are variations in the cytokeratin expression levels. A poor clinical outcome is typically predicted by aberrant cytokeratin expression in malignant cells. For instance, high expression of CK20 in urothelial bladder cancers [34] and high expression of CK18 in gastric cancer [35] are linked with poor prognosis. Furthermore, El Raziky et al. found that in HCC, a combination of AFP and CK9 levels improves diagnostic accuracy and reported that in patients who have undergone interventional procedures, CK19 levels are a good indicator of ablation/recurrence [36]. As for CK7 and CK19, these are recognized as immunohistochemical markers of tissue derivation in ICC because they are constitutively expressed in healthy liver bile ducts but not in hepatocytes [10].

The current study demonstrates that the bile level of CK7 was significantly higher in CCA patients than that in patients with malignancies other than CCA, those with benign obstruction, and control groups. ROC curve analysis revealed that bile CK7 cut point of >1030 pg/mL yielded AUC of 1 (95% CI: 1.000–1.000), 100% sensitivity, and 100% specificity, in addition to significant positive correlation with CA 19-9, ALP, GGT, and total bilirubin. When compared to subjects with benign obstruction, the bile level of CK7 was higher in those with malignant obstruction. Besides, when compared to the absence of obstruction, the presence of either benign or malignant obstruction significantly showed higher CK7 levels.

Concentrating on cancers that originate in endoderm-derived (aerodigestive) organs, the prognosis for tumors that frequently express CK7, such as lung, pancreatic, gastric, and cholangiocellular carcinomas, is noticeably worse than that of colorectal cancer (CRC). A noteworthy finding is that in contrast to CRC, the lung and the pancreaticobiliary carcinomas frequently express CK7 and lack CK20 [12]. Moreover, the unique and varied expressions of CK7 and CK20 in carcinomas can help determine the site of origin of metastatic carcinoma of unknown primary site in a survey of 435 cases [37].

In a retrospective investigation scheduled to investigate the prognostic implications of CK7 and CK19 in ICC, Liu et al. encountered that CK7 expression in tissue specimens was significantly upregulated in CCA patients compared to their nontumor counterparts and positively correlated with aggressive tumor phenotypes, such as lymph node metastasis, larger tumor size, and CA 19-9 levels. Furthermore, high expression of CK7 predicted a significantly dismal postoperative survival rate [26]. Furthermore, Rahnemai-Azar demonstrated that in addition to other potential diagnostic markers circulating in serum or bile, CK7 and CK19 were reported as helpful immunohistochemical markers to distinguish ICC from HCC [38].

Among our study population, 37 patients with cholangitis had higher levels of CK7 compared to those without cholangitis. We noticed that the CK7 bile level was duplicated in the presence of cholangitis. Despite the aforementioned, the performance of CK-7 remains excellent: AUC of 0.905 (95% CI: 0.847–0.964) in non-cholangitis-associated CCA (p value < 0.001).

The bile level CK7 was significantly elevated in CCA patients, whether or not they had extrahepatic metastases or cholangitis. Additionally, CK7 was positively correlated with CA19-9. Thus, bile sampling during ERCP or other methods of biliary drainage with CK7 measurement could help diagnosis of cholangiocarcinoma, even in the presence of cholangitis.

Limitations

The current study was limited by a relatively small sample size and being a single-center study. Larger-scale research is still warranted to validate our findings regarding CK7 as a biomarker for CCA diagnosis.

Conclusion

The bile level of CK7 demonstrates outstanding performance that could help diagnose CCA.

Abbreviations

CCA	Cholangiocarcinoma
CK7	Cytokeratin 7
CRC	Colorectal cancer
HCC	Hepatocellular carcinoma
ICC	Intrahepatic cholangiocarcinoma
Hb	Hemoglobin concentration
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CEA	Carcinoembryonic Antigen
CA 19-9	Carbohydrate Antigen 19-9

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Authors' contributions

All authors made significant contributions to the work presented, whether in the areas of ideation, study design, implementation, data collection, analysis, and interpretation, or all of these. Additionally, they agreed to take responsibility for all aspects of the work, helped write, revise, or evaluate the article carefully, approved the final version to be published, and selected the journal to which it was submitted.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

After the research questions and goals were established, everyone who participated was made aware of the purpose of the study and requested to give their written permission before taking part. The National Liver Institute, Menoufia University Ethics Committee, Egypt, approved the study (IRB: NLI 00003413) and the study was carried out following the Helsinki Declaration.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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