Bile carcinoembryonic cell adhesion molecule 6 as a potential diagnostic tool for malignant biliary stenotic lesions

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Received: 1 August 2019
Accepted: 20 August 2019
Published: 18 August 2020

The Egyptian Journal of Internal Medicine
2019, 31:544–549

Aim
Validate the biliary carcinoembryonic antigen-related cell adhesion molecule 6 in differentiating malignant from benign biliary lesions.

Background
The nature of biliary stenosis needs to be diagnosed early and accurately to give the patient the best chance of therapy. Imaging techniques still lack the high accuracy for this purpose. Different biomarkers were postulated to increase the diagnostic accuracy, and of them, carcinoembryonic cell adhesion molecule 6 (CEAM6) in bile was investigated in this study.

Patients and methods
Forty-four patients with biliary stenosis were enrolled in this prospective study in Assiut University Hospital from 2017 to 2019. CEAM6 concentration in bile and serum was measured using human carcinoembryonic antigen-related cell adhesion molecule 6 ELISA kit from SinoGeneClon Biotech Co. Ltd, and CA19-9 concentration in serum was assessed by the ELISA kit for CA19-9 from USCN Life Science Inc.

Results
The area under the curve, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of bile CEAM6 for diagnosis of stricture type among the study population were 0.841, 83, 74, 77, 82, and 78.5%, respectively, at a cut-off value of 6.15 ng/ml. Multivariate analysis showed age, CEAM6, CA19-9, and alkaline phosphatase as good predictors of malignancy.

Conclusion
CEAM6 in bile could be a good diagnostic tool to detect the nature of biliary stenosis.

Keywords:
biliary stenosis, carcinoembryonic antigen-related cell adhesion molecule 6, cholangiocarcinoma, pancreatic cancer

Egypt J Intern Med 31:544–549

1110-7782

Introduction

Many causes can lead to common bile duct stenosis, whether malignant causes such as pancreatic cancer, adenocarcinoma of ampulla of Vater, or cholangiocarcinoma, or benign ones such as primary sclerosing cholangitis or chronic pancreatitis [1,2].

The presence of such a wide range of causes and the need to differentiate between being malignant or benign was the main interest through the years. Early identification of malignancy allows early surgical intervention, which is the main treatment modality for such cases [3,4].

The development in the imaging techniques and the pathological examination of biliary samples aided somehow in the diagnosis of the nature of the stenosis. However, by the time these methods also showed some delay in the diagnosis, with decreased accuracy and negative predictive value of their diagnostic power [5,6].

One of the tumor biomarkers used extensively for detection of malignancies is carcinoembryonic antigen (CEA). It is related to a family of glycoproteins consisting of carcinoembryonic antigen-related cell adhesion molecule (CEACAM) and pregnancy-specific glycoprotein subgroup. The CAMs are involved in embryogenesis, neural tissue development, immune response, and inflammation besides hemostasis [7,8].

CEACAMs are involved in many processes such as angiogenesis, T-cell proliferation, insulin action regulation, neovascularization, and tumor-associated mechanisms [9,10].

CEACAM6 is related to cancer progression by many mechanisms such as antiapoptosis, resistance to treatment, and cell growth. Its overexpression is believed to promote invasion and metastasis in malignancy. Studies that were carried out *in vitro* postulated that antibodies for CEACAM6 on overexpressing cells can inhibit invasion and cell migration [11,12].

CEACAM6 is generally expressed in many tissues such as duct cells of pancreas, breast, myeloid cells of spleen and bone marrow and in bronchiole epithelium of the lungs [13].

It is believed that it is found in all diseases with adenoma or hyperplastic polyps, with bad prognosis in these cases [13–15].

Investigating CEACAM6 in bile is suggested to be helpful in differentiating between malignant and benign stenosis with high accuracy [16].

In this study, we aimed to validate the biliary CEACAM6 in differentiating malignant from benign biliary lesions.

Patients and methods

After approval of the ethical committee of the Faculty of Medicine at Assiut University, we conducted a prospective hospital-based study at Alrajhy Liver Hospital and Assiut University Hospital to evaluate the diagnostic accuracy of carcinoembryonic cell adhesion molecule 6 (CEAM6) in diagnosing the nature of biliary stricture in period spanning between March 2017 and March 2019.

Ethical Considerations

Before enrollment in our study, all participants signed a consent certificate. Before signing, they discussed in detail our study aim, and all possible complications (even the mildest and the rarest complications). Participants were clearly informed that refusing to participate in our study will not affect having full benefit from the available medical service and treatment.

Patients

Patients with bile duct stricture were enrolled and underwent endoscopic retrograde cholangiopancreatography (ERCP). Among 49 patients who were enrolled in the study, five patients were excluded because of loss of follow-up in three patients and insufficient sample in the other two patients. Thus, 44 patients were included in the data analysis.

Inclusion criteria

Patients presented with obstructive jaundice secondary to biliary stricture, as diagnosed by abdominal ultrasonography and confirmed by ERCP.

Exclusion criteria

Patients with one or more of the following conditions were excluded from the study:

(1) Extrahepatic obstruction secondary to cause (s) other than biliary stricture (e.g. choledocholithiasis, Mirizzi's syndrome).
(2) Strictures that would not permit passage of guide wire.
(3) Coagulopathy [international normalized ratio >1.5 and/or thrombocytopenia (platelets <50 000/ml)].
(4) Patients with a history of traumatic or iatrogenic bile duct injury (including biliary surgery within the last 6 months).

For each patient

(1) Full history and clinical examination.
(2) Laboratory investigations: liver function tests (total bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase, and prothrombin concentration), complete blood count, and kidney function tests (blood urea nitrogen and serum creatinine).
(3) Imaging: abdominal ultrasonography to confirm the obstruction and to exclude causes of obstruction other than stricture. Magnetic resonance pancreaticocholangiography was carried out to confirm the presence of stricture and determine its site and exclusion of other causes of obstruction.
(4) ERCP was carried out in the usual standard manner for all patients, and bile sample was taken.
(5) Bile and blood samples were collected, and CEAM6 concentration in bile and serum was measured.

Preparation before the procedure

(1) Preoperative fitness for ERCP and general anesthesia was assessed.
(2) Fasting for at least 8 h before the endoscopic procedure.

(3) Twenty milligrams N-butyl scopolamine bromide was administrated slowly intravenously (Buscopan, Boehringer Ingelheim US Ridgefieldand Danbury; 20 mg/1 ml) to relieve intestinal peristalsis during the procedure.

During the procedure

(1) ERCP was performed in the prone position.
(2) Continuous cardiorespiratory monitoring was carried out during the procedure.

Endoscopic retrograde cholangiopancreatography procedure and sample acquisition

ERCP was performed using side-viewing endoscope (Pentax ED-3440T/A01350, Tokyo, Japan) to diagnose and confirm biliary stricture and biliary drainage if indicated. Using the disposable dash-480 (Howell DASH direct access system; Cook) to cannulate the papilla of Vater in a conventional way, a 0.035 or 0.025-inch guide wire (Jag wire, 450 cm, Boston Scientific Corp., Indiana, USA) was introduced into the biliary passage. Bile was collected upstream to the bile duct stenosis by placing a catheter at the level of the stricture and aspirating the bile before contrast medium injection. All of the patients were previously uninstrumented.

After the procedure

(1) Oral intake was prohibited for 2 h.
(2) Follow-up was carried out for 24 h on the ward for the development of any complications.

Sample preparation

The bile sample was centrifuged for 10 min at 2000–3000 rpm at 2–8°C to remove any particulates, while the blood sample was centrifuged at a speed of 2000–3000 rpm for 20 min.

Enzyme-linked immunosorbent assay

CEAM6 concentrations in bile and serum were measured using human CEACAM6 ELISA kit from SinoGeneClon Biotech Co. Ltd (Hangzhou, P.R. China), and CA19-9 concentration in serum was assessed by the ELISA kit for CA19-9 from Uscn Life Science Inc. (Wuhan, P.R. China). The samples were diluted 1/5 (bile CEAM6) and 1/5 (serum CEAM6) and processed following the manufacturer's instructions. Each sample was analyzed in duplicate. A four-parameter logistics standard curve was used for data analysis.

Follow-up

The results of our study were compared with one of the following reference methods of diagnosis:

(1) Histopathological diagnosis by surgical excision of the lesion.
(2) Infiltration of adjacent organs proved by radiological examination or metastases by magnetic resonance pancreaticocholangiography and multislice computed tomography.

Statistical analysis

Data were verified, coded by the researcher, and analyzed using IBM-SPSS 21.0 (IBM-SPSS Inc., Chicago, Illinois, USA). Descriptive statistics: means, SDs, medians, ranges, and percentages were calculated. Tests of significance: χ^2 test was used to compare the difference in the distribution of frequencies among different groups. For continuous variables, the independent *t* test analysis was carried out to compare the means of normally distributed data, while Mann–Whitney *U* test was used to test the median differences of the data that do not follow normal distribution. The clinical and demographic factors with proven statistical significance from the univariate analyses were further included in the multivariate logistic regression models. Receiver operating characteristic curve depicted the diagnostic performance of biomarkers for diagnosis of thyroid function abnormalities, analyzed as area under the curve (AUC), SE, and 95% confidence interval. Validity statistics (sensitivity, specificity, positive predictive value and negative predictive value) were calculated. *P* value was considered to be significant when it was equal to or less than 0.05.

Results

Patients enrolled in this study were divided into 15 benign cases and 29 malignant ones with male predominance in malignant diagnosis. Death occurred in four cases. Pancreatic cancer was found to be the main diagnosis in the studied group and was recorded in 14 cases, followed by cholangiocarcinoma in 12 cases and periampullary carcinoma in two cases. The site of the stricture was mainly distal in both benign and malignant groups representing 86% in the first case and 65% in the second case. All the demographic and laboratory data of the two groups are shown in Table 1.

AUC was calculated in the three studied biomarkers. It was significant in bile CEAM6 and serum CA19-9, with *P* value less than 0.001 in both cases, but this was not the case for serum CEAM6, which did not show statistical significance (Table 2).

Different variables were tested to detect their ability to predict malignancy by regression analysis. Univariate

Table 1 demographic and basic data of patients divided into benign and malignant groups

Category	Benign (*N*=15)	Malignant (*N*=29)	*P* value
Age (years)	49.40±12.4	61.48±10.8	0.004
Sex			
Female	6 (40)	11 (37.9)	0.573
Male	9 (60)	18 (62.1)	
Outcome			
Death	0	4 (13.8)	0.001
Radiology	13 (86.7)	19 (65.5)	
Surgical and histopathological	2 (13.3)	6 (20.7)	
Final diagnosis			
Benign stricture	15 (100)	0	0.001
Pancreatic cancer	0	14 (48.3)	
Cholangiocarcinoma	0	12 (41.4)	
Periampullary carcinoma	0	3 (10.3)	
Stricture site			
Distal	13 (86.7)	19 (65.5)	0.024
Hilar	0	7 (24.1)	
Mid	0	2 (6.9)	
Long segment	2 (13.3)	1 (3.4)	
Hb level (mg/dl)	11.60±1.0	10.17±1.4	0.001*
Prothrombin concentration	77.67±12.2	73.36±16.1	0.329*
Serum bilirubin level (mg/dl)	6.27±3.9	10.50±4.4	0.003**
ALP (IU/l)	231.60 ±23.6	373.88±29.5	0.001*
ALT (IU/l)	42.33±5.5	53.48±6.6	0.201**
AST (IU/l)	46.87±5.6	56.07±7.2	0.320**
Bile CEAM6 Level (ng/ml)	4.71±3.23	10.50±4.1	<0.001**
Serum CEAM6 Level (ng/ml)	2.98±1.0	3.13±1.0	0.406**
CA19-9 Level (IU/l)	24.87±6.8	86.18±11.9	<0.001**

Data are presented as *n* (%) and mean±SD. ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; CEAM6, carcinoembryonic cell adhesion molecule 6; Hb, hemoglobin. *Independent *t*-test test was used to compare the mean difference between groups. **Mann Whitney test was used to compare the median difference between groups.

Table 2 Diagnostic performance of biomarkers for diagnosis of malignant biliary stenosis, analyzed as the area under the curve (95% confidence interval)

	AUC*	95% CI+	SE**	*P* value***
Bile CEAM6	0.841	0.724–0.959	0.060	<0.001
Serum CEAM6	0.575	0.393–0.756	0.092	0.421
CA19-9	0.859	0.741–0.976	0.060	<0.001

*AUC, area under the curve; CEAM6, carcinoembryonic cell adhesion molecule 6; +CI, confidence interval. **SE, Standard Error. ***Null hypothesis: true area = 0.5.

analysis showed that age, hemoglobin level, bilirubin, alkaline phosphatase, bile CEAM6, and serum CA19-9 have high statistical significance in predicting malignant stenosis. When using multivariate analysis, hemoglobin and bilirubin were not included as predictors (Table 3).

Table 3 Significant predictors of malignant biliary stenosis: logistic regression analysis

Factor	Odds ratio	95% CI*	*P* value
Univariate			
Age (years)	1.096	1.027–1.169	0.006
Hb (mg/dl)	0.393	0.204–0.760	0.003
Serum bilirubin (mg/dl)	1.271	1.067–1.514	0.007
ALP (IU/l)	1.009	1.003–1.016	0.005
Bile CEAM6 (ng/ml)	1.432	1.148–1.786	0.001
CA19-9 (IU/l)	1.047	1.014–1.081	0.005
Multivariate			
Age (years)	1.088	1.017–1.201	0.021
ALP (IU/l)	1.014	1.002–1.058	0.010
Bile CEAM6 (ng/ml)	1.653	1.218–2.205	0.001
CA19-9 (IU/l)	1.041	1.011–1.092	0.009

ALP, alkaline phosphatase; CEAM6, carcinoembryonic cell adhesion molecule 6; *CI, confidence interval; Hb, hemoglobin.

Table 4 Validity of biomarkers for diagnosis of malignant biliary stenosis

	Bile CEAM6 (ng/ml)	Serum CEAM6 (ng/ml)	CA19-9 (IU/l)
AUC	0.841	0.575	0.859
Cut-off	6.15	3.05	34.2
Accuracy (%)	78.5	53	80
Sensitivity (%)	83	76	86
Specificity (%)	74	40	74
PPV (%)	77	44	77
NPV (%)	82	62.5	84

AUC, area under the curve; CEAM6, carcinoembryonic cell adhesion molecule 6; NPV, negative predictive value; PPV, positive predictive value.

Validation of the investigated biomarkers showed that bile CEAM6 and CA19-9 have close sensitivity, negative predictive value and accuracy with equal specificity and positive predictive value. Serum CEAM6 showed the least accuracy (53%), sensitivity (76%), specificity (40%), negative predictive value (62.5%), and positive predictive value (44%) (Table 4).

Discussion

Early detection of malignant biliary stricture is the best way for total cure. Imaging techniques could not afford that unless the lesion was more than 2 cm. Endoscopic evaluation with brush cytology yields a sensitivity ranging only from 38 to 58% [17–19].

This encouraged the emergence of biomarkers that can give better results for early detection of malignant lesions. CA19-9 is a well-documented biomarker in serum for detecting malignancy, but with variability in sensitivity and specificity [16]. Bile examination was

introduced due to the proximity to the lesion, which carries a better result. CEAM6 is one of the investigated bile markers in recent years. It is a glycosylphosphatidylinositol-linked immunoglobulin superfamily member that is overexpressed in many gastrointestinal carcinomas [12]. CEAM6 is also related to metastasis and resistance to treatment [20,21].

Few studies have investigated its presence in bile in association with malignant biliary stenosis; its role in malignant biliary stenosis was not much investigated, except in few studies [22,23].

In this study, we decided to compare the conventional serum CA19-9 with CEACAM6 in serum and bile to diagnose malignant biliary stenosis. We examined patients with biliary obstruction who were divided into 15 with benign stenosis and 29 with malignant stenosis. The bile CEAM6 showed a high statistically significant *P* value of 0.001 in detecting malignant stenosis. It also showed high statistical significance with AUC of 0.841 and 95% confidence interval, 0.724–0.959. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of bile CEAM6 for diagnosis of stricture type among the study population were 83, 74, 77, 82, and 78.5%, respectively, at a cut-off value of 6.15 ng/ml.

It had close results to the conventional CA19-9, which had a high statistical significance of less than 0.001. It gave sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of serum CA19-9 for diagnosis of stricture type among the study population of 86, 74, 77, 84, and 80%, respectively, at a cut-off value of 34.2 IU/l. This was not the case in the study carried out by Farina *et al.*, as the bile CEAM6 had better accuracy than CA19-9 [16].

When we investigated serum CEACAM6 in diagnosing malignant biliary stenosis, it had a weak statistical significance. The AUC, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of serum CEAM6 for diagnosis of stricture type among the study population were 0.575, 76, 40, 44, 62.5, and 53%, respectively, at a cut-off value of 3.05 ng/ml. This variance in results between bile and serum CEAM6 may suggest that the biliary tumors excrete the marker directly into bile with no transport into blood. This finding was in concordance with Farina *et al.* [16], wherein no correlation was observed between bile and serum CEAM6 in detecting malignant biliary stenosis.

Age was increased by a decade when comparing between benign and malignant biliary stenosis. This was similar to the study carried out by Rose *et al.* [24], denoting the increased age with the malignancy.

Univariate analysis showed the relation between age, hemoglobin level, alkaline phosphatase, CA19-9, bilirubin, and bile CEACAM6 and malignancy. Investigating predictors of malignant biliary stenosis by multiple logistic regression analysis, bile CEACAM6, age, increased bilirubin, CA19-9, and alkaline phosphatase gave a high statistically significant value. This was in concordance with the study carried out by Rose *et al.* [24].

This can be explained by the obstructive effect of malignant biliary stenosis, which is more prolonged than the benign stenosis, with an associated greater increase in bilirubin, biomarkers and alkaline phosphatase.

A weak point in our study is that it needs to be carried out on a larger scale of patients for a better diagnostic performance. In contrast, the long duration of follow-up that approached 2 years in our patients increases the strength of the results.

Conclusion

Biliary CEAM6 is a good diagnostic tool for malignant biliary strictures. It should be investigated on a wider scale in patients with high-risk criteria for developing malignancy.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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