Assessment of Tumor Markers in Bile in Patients With Pancreaticobiliary Malignancies: ERCP- Based Study

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Received :12 /1 /2012
Accepted after revision: 15 /2 /2012

Key words:
Carcinoembryonic antigen, carbohydrate antigen, CA19-9, hepatopancreatobiliary disease, bile.

Background and study aim: The value of serum tumor markers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) in the differential diagnosis of obstructive biliary disease is a matter of debate. We aimed to define their role prospectively.

Patients and methods: Thirty five cholestatic patients , 14 malignant group, their age ranged from 47-72 years (mean: 58.43 ± 9.08 years), 14 benign group, their age ranged from 46-72 years (mean: 60.21 ± 10.45 years) and 7 calcular, their age ranged from 48-71 years (mean: 60.14 ± 7.78 years) who were referred for endoscopic retrograde cholangiopancreatography examination for obstructive jaundice were included. Bile was obtained through cannulation of ERCP. Serum samples were taken from all patients at the time of acquisition of bile. Serum and bile samples were stored at -80 °C until they were tested. CEA and CA19-9 levels were measured with enzyme immunoassay methods in serum and bile samples by using Immunospec CA 19-9 and Immunospec CEA kits, respectively (Canoga Park,CA, 91303).

Results: In 14 patients with malignant disease, serum CEA levels were 36.77 (23.33-124.92) ng/ml and CA19-9 were 418.07 (1.23-483.47) U/ml, while in 14 patients with benign disease the serum CEA levels were 15.43 (0.38-30.80) ng/ml and CA19-9 were 144.6 (3.99-471.15) U/ml. The difference for both values was significant ($p<0.05$). In malignant disease bile CEA and CA19-9 levels were 5.05 (0-124.84) ng/ml, 455.61 (0.07-483.80) U/ml respectively, while in benign disease the corresponding levels were 3.22 (0-121.81) ng/ml for CEA and 421.45 (0-485.06) U/ml for CA19-9. The differences were not significant in this case ($p>0.05$).

Conclusion: It was concluded that serum CEA and CA19-9 levels are increased both in malignant and benign obstructive biliary diseases. However, levels of serum CEA are markedly increased and mostly restricted to malignant diseases. Measurement of these markers in bile was of no clinical significance.

INTRODUCTION

Tumor markers are antigens and bioactive substances produced by tumor cells because of the abnormal expression of correlated genes. They are either not produced or only minimally produced, in normal tissues, and can be detected in tissues, body fluids and excreta of patients with cancer [1]. Both carbohydrate antigen 19-9 (CA19-9) and serum carcinoembryonic antigen (CEA) are produced by epithelia of the pancreas, stomach, colon, liver, and biliary tract [2]. Small tumors produce detectable levels of the CEA or CA19-9 antigen only in the body fluids such as bile, and this was suggested as a useful tool in the patient with otherwise occult liver metastasis [3] or with primary tumors [4]. CEA and CA19-9 have long been used as tumor markers in gastrointestinal malignancies to help detect the primary tumor and determine tumor stage and prognosis [5]. They also allow

monitoring of therapeutic efficacy and tumor recurrence. Although CEA and CA 19-9 have been studied extensively in pancreatic head cancer [6]. Their roles in other nonpancreatic periampullary cancer have not been clearly established [7]. The present study has aimed to determine the role of both serum and biliary CA19-9 and CEA levels in the differential diagnosis of benign and malignant pancreaticobiliary diseases.

PATIENTS AND METHODS

Between July 2009 and September 2011, 35 patients (18 female, 17 male, median age: 54 yr, range: 19-81) with diagnosis of obstructive jaundice were studied. (The diagnosis was based on ultrasonography (US) and/or computerized tomography (CT) findings). Endoscopic retrograde cholangio-pancreatography (ERCP) was performed. Bile was obtained through cannulation of ERCP. Serum samples were taken from all patients for CEA and CA19-9 at the time of acquisition of bile. Serum and bile samples were stored at -80 °C until they were tested. Bile samples were mixed with 0.1 M. acetic acid at 70 °C for 15 minutes followed by centrifugation for 10 minutes at 3000 g for the elimination of bile pigments and other proteins before the measurements [8]. All CEA and CA19-9 bile samples were tested after dilution at titers of 1/10, 1/20, 1/40 and 1/80. The distinction between malignant and benign was based on clinical or radiological findings (US and/or CT, ERCP). The diagnosis of primary sclerosing cholangitis (PSC) was based on compatible cholangiographic features, biochemical and clinical findings. Histopathology was available in three-fourth of the patients as well. Alkaline phosphates, ALT, AST, total bilirubin levels and white blood cell (WBC) count were studied in all patients. CA19-9 and CEA levels were measured with enzyme immunoassay methods in serum and bile samples by using Immunospec CA 19-9 and Immunospec CEA kits, respectively (Canoga Park, CA, 91303). Serum upper limit of normal for CA19-9 was 37 ng/ml and for CEA was 5 ng/dl. Normal levels are undefined for bile samples .

Those subjects were selected from patients attending the outpatient clinic and inpatient unit of Tropical Medicine and Endoscopy Unit, Cairo University, and informed consents were obtained from each subject before blood samples were collected and pathological specimen were taken.

STATISTICAL ANALYSIS

Statistical analysis was performed using the SPSS 17(Statistical package for social science) . The quantitative data were presented in the form of mean, standard deviation, and range. The qualitative data were presented in the form of number and percentage. Pearson Moment Correlation tests were used to study the relation between variables. ROC curve was constructed for CA 19-9 and CEA. The sensitivity and specificity values of CA 19-9 and CEA were calculated with one cut-off value level. Significance was considered when P value less than 0.05.

RESULTS

Thirty five cholestatic patients, 14 malignant group [8 males and 6 females, their age ranged from 47-72 years (mean: 58.43 ± 9.08 years)], 14 benign group [6 males and 8 females, their age ranged from 46-72 years (mean: 60.21 ± 10.45 years)] and 7 calcular [3 males and 4 females, their age ranged from 48-71 years (mean: 60.14 ± 7.78 years)] who were referred for ERCP examination for obstructive jaundice were included. The etiology of malignant disease was cholangiocarcinoma in 2, papillary carcinoma in 2 and pancreatic head carcinoma in 10. Mean serum CEA level in the malignant group was 36.77 (23.33-124.92) ng/ml while bile CEA level was 5.05 (0-124.84) ng/ml, the area under ROC curve for serum CEA in malignant group was 0.964 (P<0.001) with cutoff value 26.72 ng/ml, the sensitivity and specificity was 92.9% and 85.7% respectively, while the area under ROC curve for biliary CEA level in malignant group was 0.561 (P=0.581) with cutoff value 3.99 ng/ml ,the sensitivity and specificity was 64.3% and 50% respectively. The levels of serum and bile CA19-9 levels in malignant group was 418.07 (1.23-843.47) U/ml and 455.61 (0.07-483.80)U/ml, respectively, the area under ROC curve for serum CA19-9 in malignant group was 0.0.765 (P=0.017) with cutoff value 290.005 U/ml the sensitivity and specificity was 85.7% and 71.4% respectively, while the area under ROC curve for biliary CA19-9 level in malignant group was 0.566 (P=0.55) with cutoff value 215.965 U/ml, the sensitivity and specificity was 71.4% and 42.9% respectively. The benign diseases were hydatid disease related biliary stricture in 1, post surgical biliary stricture in 7, primary sclerosing cholangitis in 4, cholangitis due to *Fasciola hepatica* in 1 and stricture

followed liver transplantation in 1. Serum CEA value in benign disease was 15.43 (0.38-30.80) ng/ml, while the area under ROC curve for serum CEA level in benign group was 0.036 (P<0.001) with cutoff value 28.675 ng/ml ,the sensitivity and specificity was 14.3% and 21.4% respectively, while CA19-9 was 144.6 (3.99-471.15) U/ml with area under ROC curve for serum CA 19-9 level in benign group was 0.235 (P=0.017) with cutoff value 346.73 U/ml ,the sensitivity and specificity was 28.6% and 35.7% respectively. The serum CEA for the malignant group (36.77 [23.33-124.92] ng/ml) was significantly higher than for the benign group (15.43 [0.38-30.80] ng/ml) (P<0.001). Serum CA19-9 concentration was significantly higher in patients with malignant disease (418.07 [1.23-483.47] U/ml) in comparison to patients with benign diseases (144.6 [3.99-471.15] U/ml) (p=0.017).

In benign group, mean bile CEA and CA19-9 levels were 3.22 (0-121.81) ng/ml, 412.45 (0-485.06) U/ml, respectively with area under ROC curve for biliary CEA level was 0. 439 (P=0.0.581) with cutoff value 12.74 ng/ml the sensitivity and specificity was 42.9% and 42.9% respectively and area under ROC curve for biliary CA 19-9 level was 0.434 (P=0.550) with cutoff value 312.2 U/ml ,the sensitivity and specificity was 57.1% and 35.7% respectively. Biliary CA19-9 levels in patients with malignant diseases were not significantly different from those in the patients with benign disease (p=0.550).

In malignant group, there was no correlation in malignant group between Age, Hb%, WBCs, platelet count, serum levels of ALT, AST and serum CEA (P=0.647; P=0.318; P=0.085; P=0.976; P=0.164 and P=0.191) respectively, but there is significant correlation also in malignant group between serum bilirubin, alkaline phosphate, serum CA 19-9 and serum CEA (P=0.007; P=0.002 and P= 0.023). Also, there is inversely correlation between serum albumin and serum CEA in malignant group (P=0.002). There was no correlation in malignant group between Age, Hb%, WBCs, platelet count, serum levels of albumin, ALT, AST and serum CA 19-9 (P=0.788; P=0.946; P=0.670; P=0.464; P=0.308; P=0.064 and P=0.318) respectively, but there is significant correlation also in malignant group between serum bilirubin, alkaline phosphate and serum CA 19-9 (P=0.030 and P= 0.035).

In benign group, There was no correlation in benign group between Age, Hb%, WBCs, platelet count, serum levels of albumin, ALT, AST and serum CEA (P=0.664; P=0.177; P=0.599; P=0.474; P=0.581;P=0.122 and P=0.182) respectively, but there is significant correlation also in benign group between serum bilirubin, alkaline phosphate, serum CA 19-9 and serum CEA (P=0.001; P=0.010 and P= 0.019). There was no correlation in benign group between Age, Hb%, WBCs, platelet count, serum levels of albumin, ALT, AST and serum CA 19-9 (P=0.358; P=0.823; P=0.714; P=0.418; P=0.881;P=0.064 and P=0.084) respectively, but there is significant correlation also in benign group between serum bilirubin, alkaline phosphate and serum CA 19-9 (P=0.033 and P= 0.037).

Table (1): Serum tumor marker in different groups

	Malignant group (n = 14)	Benign group (n = 14)	Control group (n = 7)	P value
CEA (ng/ml)	36.77 (23.33 – 124.92)	15.43 (0.38 – 30.80)	1.20 (0.29 – 5.28)	P1 < 0.001 P2 < 0.001 P3 = 0.037
CA19-9 (U/ml)	418.07 (1.23 – 483.47)	144.6 (3.99 – 471.15)	8.40 (3.99 – 104.78)	P1 = 0.017 P2 = 0.004 P3 = 0.021

P1: Malignant versus Benign, P2: P1: Malignant versus Control, P3: Benign versus Control

Table (2): Biliary levels of tumor marker in different groups

	Malignant group (n = 14)	Benign group (n = 14)	Control group (n = 7)	P value
CEA (ng/ml)	5.05 (0 – 124.84)	3.22 (0 – 121.81)	0.38 (0 – 1.14)	P1 = 0.581 P2 = 0.020 P3 = 0.044
CA19-9 (U/ml)	455.61 (0.07 – 483.80)	421.45 (0 – 485.06)	9.13 (0 – 189.58)	P1 = 0.550 P2 = 0.006 P3 = 0.012

P1: Malignant versus Benign, P2: P1: Malignant versus Control, P3: Benign versus Control

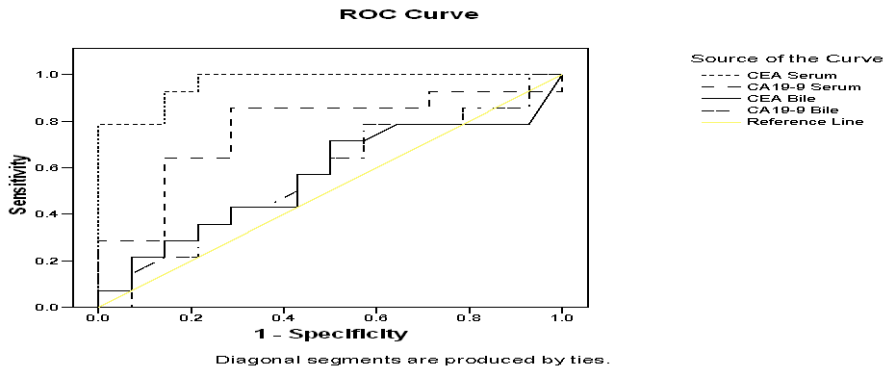

Figure (1): ROC curve analysis of serum and biliary tumor markers in malignant group

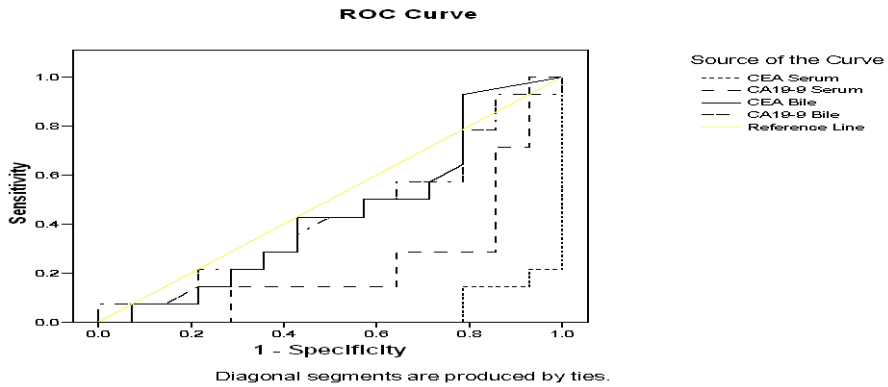

Figure (2) :ROC curve analysis of serum and biliary tumor markers in benign group

Table (3): Correlation between serum and biliary tumor markers with other variables in malignant group

	Serum				Biliary			
	CEA		CA19-9		CEA		CA19-9	
	r	p	r	R	r	p	r	p
Age (years)	0.134	0.647	0.079	0.788	0.334	0.244	0.145	0.620
Hb% (gm/dL)	0.288	0.318	-0.020	0.946	0.227	0.434	0.064	0.829
WBCs(10^3/µL)	-0.477	0.085	-0.125	0.670	-0.143	0.625	-0.196	0.503
Platelet (10^3/µL)	0.009	0.976	-0.213	0.464	0.117	0.690	-0.381	0.179
Bilirubin (mg/dL)	0.684	0.007*	0.579	0.030*	0.676	0.008*	0.590	0.026*
Albumin (gm/dL)	-0.645	0.013*	-0.294	0.308	-0.435	0.120	-0.259	0.372
alkaline phosphatase (IU/L)	0.745	0.002*	0.565	0.035*	0.744	0.002*	0.547	0.043*
ALT (IU/L)	0.393	0.164	0.508	0.064	0.550	0.042*	0.556	0.039*
AST (IU/L)	0.371	0.191	0.288	0.318	0.373	0.189	0.516	0.059
CA19-9 (U/mL)	0.209	0.474	-	-	0.600	0.023*	-	-

Table (4): Correlation between serum and biliary tumor markers with other variables in benign group

	Serum				Biliary			
	CEA		CA19-9		CEA		CA19-9	
	r	p	r	R	r	p	r	p
Age (years)	-0.128	0.664	-0.266	0.358	-0.112	0.703	0.117	0.691
Hb% (gm/dL)	-0.383	0.177	0.066	0.823	-0.180	0.537	0.403	0.153
WBCs	0.154	0.599	0.108	0.714	0.253	0.383	0.198	0.497
Platelet (10^3/µL)	0.209	0.474	-0.235	0.418	0.433	0.122	0.042	0.887
Bilirubin (mg/dL)	0.770	<0.001**	0.572	0.033*	0.638	0.014*	0.631	0.016*
Albumin (gm/dL)	-0.162	0.581	0.044	0.881	0.212	0.466	-0.217	0.457
alkaline phosphatase (IU/L)	0.662	0.010*	0.560	0.037*	0.552	0.041*	0.846	<0.001**
ALT (IU/L)	-0.433	0.122	0.508	0.064	0.099	0.737	0.420	0.135
AST (IU/L)	-0.379	0.182	0.478	0.084	-0.020	0.946	0.333	0.245
CA19-9 (U/mL)	0.618	0.019*	-	-	0.209	0.474	-	-

DISCUSSION

Carbohydrate antigen (CA19-9) and carcinoembryonic antigen (CEA) are tumor markers for the diagnosis of gastrointestinal cancers [9]. Carbohydrate antigens are a saccharide complex derived from certain tumor tissues or tumor cell lines. More than ten types of such antigens have been discovered to increase in the serum of cancer patients. They can be used in diagnosis and treatment of tumors as biological markers if they are highly sensitive and specific. A large number of potential tumor markers have been evaluated in pancreatic cancer, but none has been sufficiently sensitive or specific in detecting pancreatic cancer [10]. CA19-9 has been studied intensively in diagnosis of pancreas cancer for many years [11,12].

In this work, serum CA19-9 levels were found to be elevated in patients with malignant biliary group (P=0.004). This is agree with Safi et al [13] ; Goonetilleke and Siriwardena [14] who approved that elevations in serum CA 19-9 appear to be useful in the diagnosis of adenocarcinoma of the upper gastrointestinal tract and in monitoring of colonic carcinoma, its greatest sensitivity is in the detection of pancreatic adenocarcinoma. Also this is in accordance with Kau et al.,[15] who approved that CA 19-9 is superior in the diagnosis of pancreatic cancer and is often considered the standard marker for pancreas cancer.

In the present study, serum CA19-9 levels were found to be elevated in patients with benign biliary diseases (P= 0.021). This is consistent with Ong et al.[16] and Morris-Stiff et al.[17] who reported that serum level of Ca19-9 have

been found elevated in some benign diseases, such as pancreatitis, cholangitis, hepatitis and cirrhosis. This is due to the presence of jaundice itself leading to up regulation of CA19-9 in benign diseases.

As regard, CA 19-9 antigen is synthesized both by the epithelial cells of the normal biliary tract and by the tumor cells and excreted within the bile [9]. It is suggested that the CA 19-9 antigen, which is high in concentration in the bile of the patients with benign and malignant obstructive jaundice, refluxes into the bloodstream due to the increase in the permeability between bile and blood, secondary to the bile stasis; moreover, it is stated that there can be an inability to degrade the antigen in the liver due to a hepatic dysfunction[18]. Therefore, remeasurement of CA 19-9 after the jaundice subsides can be useful in differential diagnosis of some CA 19-9 positive patients with obstructive jaundice, and if the concentration is still high, then the malignancy potential is high[19]. Moreover, Marrelli et al.,[20] reported that in the presence of successfully drained obstructive jaundice, CA19-9 serum levels that remain unchanged or measure more than 90 U/mL are strongly indicative of a malignant cause of obstruction. However, the real clinical utility of this marker remains controversial.

In this work, elevation of serum Ca 19-9 was higher in malignant group than in benign one (P=0.017). This is in line with McLaughlin et al.,[21]; Morris-Stiff et al.,[17] who approved that CA19-9 levels were significantly lower in patients with benign pathology than those with malignant pathology.

In addition, ROC analysis is a graphic method to determine the optimal threshold for evaluation of sensitivity and specificity profiles of serum tumor markers[22]. In our study, ROC curve analysis of serum CA 19-9 in malignant group (table 19) shows the area under the ROC curve was 0.765 with (p=0.017). For these patients, serum CA19-9 proved to be useful. At a cutoff value of 290.005 U/ml, sensitivity and specificity were 85.7% and 71.4%, respectively for diagnosis of biliary malignancies. This is accordance with Bedi et al.,[23] who reported that ROC analysis has shown that at a threshold level of 300 U/mL, CA 19-9 has 100% specificity in diagnosing pancreatic carcinoma in patients with idiopathic chronic pancreatitis. Similar cut-off level has been proposed by Nouts et al.,[24] in a study comparing de novo pancreatic cancer and chronic pancreatitis.

Moreover, Morris-Stiff et al.,[17] founded that the CA19-9 levels were significantly greater for malignant than for benign disease, A ROC analysis provided an area under the curve for CA19-9 of 0.871 (0.820-0.922), giving an optimal CA19-9 of 70.5 U/ml for differentiating benign from malignant pathology. Using this cut-off, the sensitivity was 82.1%, while specificity improved to 85.9%. When standard radiology was included (US/ CT/MRCP) in the decision process, the results improved to 97.2% and 88.7% respectively.

In the present study, combined correlation between alkaline phosphatase and serum bilirubin with serum CA 19-9 in malignant group (r=0.565; P=0.035) and (r=0.579; P=0.030) respectively and also (r=0.560; P=0.037) and (r=0.572; P=0.033) in benign group respectively. This is in accordance with McLaughlin et al.,[21] who founded that there was a significant correlation between serum CA19-9 levels and alkaline phosphatase, ALT, AST, bilirubin, in obstructive jaundiced patients. Also, Ni et al.,[25] showed that there was a significantly positive correlation between serum CA19-9 and bilirubin, which suggested that obstructive jaundice, might result in increasing of CA19-9 levels and provided some fault positive results. In contrast, Haglund et al.,[26] and Bedi et al,.[23] who found that there is no correlation between serum CA 19-9 and bilirubin or alkaline phosphatase levels were detected. In addition, Morris-Stiff et al.,[17] found that for benign disease, the CA19-9 correlated directly with the serum bilirubin, but for malignant disease, CA19-9 levels were elevated independent of the bilirubin level. On other hand, there is no correlation between ALT and AST with serum CA 19-9 in malignant (r=0.508; p=0.064) and (r=0.288; P=0.318) respectively and also in benign groups (r=0.508; p=0.064) and (r=0.478; P=0.084) respectively in our study.

In our work, biliary level of CA19-9 levels were found to be elevated in patients with malignant biliary diseases (P=0.006) (table 12) and also elevated in benign group (P=0.44). This is agree with Akdoğan et al.,[27] and Duraker et al.,[19] who approved that the CA 19-9 antigen, which is high in concentration in the bile of the patients with benign and malignant obstructive jaundice.

CEA is a member of the immunoglobulin superfamily which was originally identified in human fetal colon and colorectal cancer. It is widely used as a tumor marker. However, little is known about its function except that it acts as a homotypic adhesion molecule that is implicated in cell aggregation. It is over-expressed in numerous human cancers where it is present on the surface of cancer cells[28].

CEA is mainly secreted by digestive glandular cancers and their metastases. It is also found in other types of cancer such as breast, lung, ovary, thyroid, etc[29,30].

In this work, serum CEA levels were found to be elevated in patients with malignant biliary diseases (P<0.001). This is in accordance with Ni et al., [25] who reported that levels of serum CEA and CA19-9 in patients with pancreatic cancer were higher than that of other malignant diseases and benign pancreatic diseases.

In the present study, serum CEA levels were found to be elevated in patients with benign biliary diseases (P= 0.037). This is consistent with Duraker et al.,[19] who reported that CEA increase in the serums of patients with benign biliary obstruction as follows: CEA-like substances are normally produced endogenously in small amounts; the degradation and excretion of these substances by the liver may be impaired in biliary obstruction. However, in malignant biliary obstruction, since CEA is produced in large amounts by the tumor, usually it will not return to the normal levels after the recovery of jaundice.

As regard to previous observations, ROC curve for serum CEA in malignant group shows area under the curve was 0.964 (p<0.001) with cutoff value 26.72, the sensitivity and the specificity were 92.9% and 85.7% respectively, while ROC curve for serum CEA in benign group shows area under the curve was 0.036 (p<0.001) with cutoff value 28.675, the sensitivity and the specificity were 14.3% and 21.4% respectively . This corresponds with the view of Groblewska et al.,[31]; Liao et al.,[32]; Mroczko et a.l,[33] who reported that carcinoembryonic antigen was the first marker used for clinical diagnostics in the seventies and eighties. Throughout the past 20 years, CEA has been replaced by markers with higher diagnostic performance such as CA 19-9. Nevertheless, there are many studies on CEA which can be detected at low levels in fetal and normal adult tissue while high serum levels indicate presence of pancreatic cancer. The main problem of this biomarker is its low sensitivity of 25–56% at a high specificity of 82–100% for discriminating carcinoma from controls.

In this work, elevation of serum CEA was higher in malignant group than in benign one (P<0.001). This is consistent with Akdoğan et al.,[34] who reported that serum CEA level, in particular, can be elevated in some benign conditions such as cholangitis and biliary obstruction, which can cause confusion when it is used as a diagnostic test for gastrointestinal malignancy. Akdoğan et al.,[27] observed that some patients with benign disease had an elevated level of CEA; however, these levels in the malignant group were markedly higher.

In the present study, combined correlation between alkaline phosphatase and serum bilirubin with serum CEA in malignant group (r=0.745; P=0.002) and (r=0.684; P=0.007) respectively and also (r=0.662; P=0.010) and (r=0.770; P=0.001) in benign group respectively. This is in accordance with McLaughlin et al.,[21] who founded that there was a significant correlation between serum CEA levels and alkaline phosphatase and bilirubin in cholestatic patients. In contrast, Ni et al.,[25] serum CEA and had no significant correlations with serum bilirubin in cholestatic studied groups.

In this work, biliary level of CEA levels were found to be elevated in patients with malignant biliary diseases (P=0.020) and also elevated in benign group (P=0.044). This is agree with Duraker et al.,[19] who approved that the level of CEA in the bile of patients, both with and without malignancy, were high and widely distributed.

As regard, in this study, there is no correlation between biliary CEA and CA 19-9 levels in patients with malignant diseases (r=0.338; P=0.238) and also no correlation between biliary CEA and CA 19-9 levels in patients with benign diseases (r=0.0.209; P=0.474). This is consistent with Akdoğan et al.,[27] who reported that the levels of CEA or CA19-9 in the bile of patients, both with and without malignancy, were high and widely distributed. Biliary CEA levels in patients with malignant diseases tended to be higher when compared to benign group; however, both markers' bile levels failed to discriminate between benign and malignant disease as there is no correlation. Furthermore,

bile levels have a poor discriminatory value in comparison with serum levels.

In addition, in this work, there is correlation between serum CEA and CA 19-9 levels in patients with malignant diseases (r=0.600; P=0.023) and also between serum CEA and CA 19-9 levels in patients with benign diseases (r=0.618; P=0.019). This is consistent with McLaughlin et al.,[21] who founded that there was a significant correlation between serum CA19-9 levels and CEA in cholestatic studied groups.

One limitation of our study can be only single determination of these markers. The measurement of serial serum and bile levels after relief of obstruction may be of advantage in order to decrease the influence of cholestasis on the CEA and CA19- 9 levels[35]. Nevertheless it has been reported that removal of the obstruction of the biliary tract in patients with carcinoma did not result in a marked decrease of the marker bile levels. The authors suggested that CEA or CA19-9 levels in the bile were more influenced by marker production by the cancer than by the hepatobiliary factors [36]. In our study, no difference was found in the levels of these tumor markers in the bile between patients with malignant and benign disease. It has also been reported that the measurement of these antigens in bile seemed to be of little diagnostic value in the differentiation between malignant and benign diseases[37].

In conclusion, serum CA19-9 levels and CEA are increased both in malignant and benign obstructive biliary diseases. However, an increase in serum CEA is mostly restricted to malignant diseases. Measurement of these markers in the serum of obstructive jaundice may help in the detection of early tumor and determination of tumor stage, prognosis and recurrence. Unfortunately, no tumor markers are accurate enough to provide reliable information about tumor diagnosis and prognosis. In addition, measurement of these markers in the bile appears to be of no value in differentiation between benign and malignant biliary disease.

Funding: Non .

Conflicts of interest: Non .

Ethical approval: The protocol of the study was approved by the ethical committee of Faculty of Medicine, Mansoura University. Informed consents were obtained from all patients.

REFERENCES

1. Zhenhua Ma, Qingyong Ma , Zheng W. An evaluation of the diagnostic value of CA19-9 and CEA levels in patients with pancreatic cancer. *JNMU* 2009; 23(3):199-202.
2. Yeatman TJ, Bland Ki , Copeland EM. Relationship between colorectal liver metastases and CEA levels in gallbladder bile. *Ann Surg* 1989; 210: 505-12.
3. Montgomery RC, Hoffman JP , Ross E A. Biliary CA19-9 values correlate with the risk of hepatic metastases in patients with adenocarcinoma of the pancreas. *J Gastrointest Surg* 1998; 2: 28-35.
4. Nakeeb A, Lipsett PA , Lillemoe KD. Biliary carcinoembryonic antigen levels are a marker for cholangiocarcinoma. *Am J Surg* 1996; 171: 147-53.
5. Lundin J, Roberts PJ, Kuusela P , Haglund C. The prognostic value of preoperative serum levels of CA 19-9 and CEA in patients with pancreatic cancer. *Br J Surg* 1994;69:515–519.
6. Haglund C, Lundin J, Kuusela P , Roberts PJ. CA 242, a new tumor marker for pancreatic cancer: a comparison with CA 19-9, CA 50 and CEA. *Br J Surg* 1994;70:487–492.
7. Yamaguchi K, Enjoji M , Tsuneyoshi M. Pancreatoduodenal carcinoma: A clinicopathologic study of 304 patients and immunohistochemical observation for CEA and CA 19-9. *J Surg Oncol* 1991;47:148–154.
8. Maiolini R, Bagrel A , Chavance C. Study of an enzyme immunoassay kit for carcinoembryonic antigen. *Clin Chem* 1980 ; 26: 1718-22.
9. Mann DV, Edwards R, Ho S, Lau WY , Glazer G . Elevated tumour marker CA19-9: clinical interpretation and influence of obstructive jaundice. *Eur J Surg Oncol* 2000; 26: 474–479.
10. Hayashi H., Nakamori S., Okami J., Nagano H., Dono K. , Umeshita K. Association between expression levels of CA19-9 and N-acetylglucosamine-beta;1,3-galactosyltransferase 5 gene in human pancreatic cancer tissue. *Pathobiology* 2004; 71:26-34.
11. Saad E.D., Machado M.C., Wajsbrot D., Abramoff R., Hoff P.M. , Tabacof J. Pretreatment CA19-9 level as a prognostic factor in patients with advanced pancreatic cancer treated with gemcitabine. *Int J Gastrointest Cancer* 2002; 32:35-41.
12. Micke O., Bruns F., Kurowski R., Horst E., de Vries A.F. , Hausler F.W. Predictive value of carbohydrate antigen 19-9 in pancreatic cancer treated with radiochemotherapy. *Int J Radiat Oncol Biol Phys* 2003 ;57:90-97.
13. Safi F., Beger H.G., Bittner R., Büchler M. , Krautzberger W. CA 19-9 and pancreatic adenocarcima. *Cancer* 1986; 57: 779-83.
14. Goonetilleke K.S. , Siriwardena A.K. Systematic review of carbohydrate antigen (CA 19-9) as a

biochemical marker in the diagnosis of pancreatic cancer. *EJSO* 2007; 33: 266-270.
15. Kau S.Y., Shyr Y.M., Su C.H., Wu C.W. , Lui W.Y. Diagnostic and prognostic values of CA 19-9 and CEA in periampullary cancers. *J Am Coll Surg.* 1999;188(4):415-20.
16. Ong S.L., Sachdeva A., Garcea G., Gravante G., Metcalfe M.S., Lloyd D.M. et al., Elevation of carbohydrate antigen 19.9 in benign hepatobiliary conditions and its correlation with serum bilirubin concentration. *Digest Dis Sci* 2008;53:3213–7.
17. Morris-Stiff G., Teli M., Jardine N. , Puntis M.C. CA19-9 antigen levels can distinguish between benign and malignant pancreaticobiliary disease. *Hepatobiliary Pancreat Dis Int*. 2009;8(6):620-6.
18. Ohshio G., Manabe T., Watanabe Y., Endo K., Kudo H., Suzuki T. , Tobe T. Comparative studies of DU-PAN-2, carcinoembryonic antigen, and CA19-9 in the serum and bile of patients with pancreatic and biliary tract diseases: Evaluation of the influence of obstructive jaundice. *Am J Gastroenterol* 1990;85:1370–1376.
19. Duraker N., Hot S., Polat Y., Höbek A., Gençler N. , Urhan N. CEA, CA 19-9, and CA 125 in the differential diagnosis of benign and malignant pancreatic diseases with or without jaundice. *J Surg Oncol.* 2007;95(2):142-7.
20. Marrelli D., Caruso S., Pedrazzani C., Neri A., Fernandes E., Marini M., et al., CA19-9 serum levels in obstructive jaundice: clinical value in benign and malignant conditions. *Am J Surg.* 2009; 198(3):333-9 .
21. McLaughlin R., O'Hanlon D., Kerin M., Kenny P., Grimes H. , Given H.F. Are elevated levels of the tumour marker CA19-9 of any clinical significance?--an evaluation. *Ir J Med Sci.* 1999; 168(2):124-6.
22. Zweig MH , Campell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;39:561–77.
23. Bedi M.M., Gandhi M.D., Jacob G., Lekha V., Venugopal A. , Ramesh H. CA 19-9 to differentiate benign and malignant masses in chronic pancreatitis: is there any benefit? *Indian J Gastroenterol.* 2009; 28(1):24-7.
24. Nouts A., Levy P., Voitot H. , Bernades P. Diagnostic value of serum CA 19-9 antigen in chronic pancreatitis and pancreatic adenocarcinoma. *Gastroenterol Clin Biol* 1998;22:152–9.
25. Ni X.G., Bai X.F., Mao Y.L., Shao Y.F., Wu J.X., Shan Y., et al., The clinical value of serum CEA, CA19-9, and CA242 in the diagnosis and prognosis of pancreatic cancer. *Eur J Surg Oncol.* 2005;31(2):164-9.
26. Haglund C., Roberts P.J., Kuusela P., Scheinin T.M., Mäkelä O., Jalanko H. Evaluation of CA 19-9 as a serum tumour marker in pancreatic cancer. *Br J Cancer* 1986; 53(2): 197–202.
27. Akdoğan M., Parlak E., Kayhan B., Balk M., Saydam G. , Sahin B. Are serum and biliary carcinoembryonic antigen and carbohydrate antigen19-9 determinations reliable for differentiation between benign and malignant biliary disease? *Turk J Gastroenterol.* 2003;14(3):181-4.
28. Al-Shuneigat JM, Mahgoub SS , Huq F. Colorectal carcinoma: nucleosomes, carcinoembryonic antigen and ca 19-9 as apoptotic markers; a comparative study. *J Biomed Sci.* 2011;18(1):50.
29. Hammarström S., Shively J.E. , Paxton R.J. Antigenic sites in carcinoembryonic antigen. *Cancer Res* 1989;49:4852–4858.
30. Bünger S , Laubert T, Roblick UJ , Habermann JK. Serum biomarkers for improved diagnostic of pancreatic cancer: a current overview. *J Cancer Res Clin Oncol* 2011;137:375–389.
31. Groblewska M., Mroczko B., Wereszczynska-Siemiatkowska U., Mysliwiec P., Kedra B. , Szmitkowski M. Serum levels of granulocyte colony-stimulating factor (G-CSF) and macrophage colony-stimulating factor (M-CSF) in pancreatic cancer patients. *Clin Chem Lab Med* 2007; 45:30–34.
32. Liao W.C., Wu M.S., Wang H.P., Tien Y.W. , Lin J.T. Serum heat shock protein 27 is increased in chronic pancreatitis and pancreatic carcinoma. *Pancreas* 2009; 38:422–426.
33. Mroczko B., Lukaszewicz-Zajac M., Wereszczynska-Siemiatkowska U., Groblewska M., Gryko M., Kedra B. et al., Clinical significance of the measurements of serum matrix metalloproteinase-9 and its inhibitor (tissue inhibitor of metalloproteinase-1) in patients with pancreatic cancer: metalloproteinase-9 as an independent prognostic factor. *Pancreas* 2009; 38:613–618.
34. Akdoğan M, Sasmaz N , Kayhan B. Extraordinarily elevated CA19-9 in benign conditions: a case report and review of the literature. *Tumori* 2011; 87: 337-9.
35. Mann D.V., Edwards R., Ho S., Lau W.Y. , Glazer G. Elevated tumour marker CA19-9: clinical interpretation and influence of obstructive jaundice. *Eur J Surg Oncol* 2000; 26: 474–479.
36. Tatsuta M, Yamamura H , Yamamoto R. Carcinoembryonic antigen in the bile in patients with pancreatic and biliary cancer. *Cancer* 1982; 50: 2903-9.
37. Buffet C, Fourre C , Altman C. Bile levels of carcinoembryonic antigen in patients with hepatopancreatobiliary disease. *Eur J Gastroenterol Hepatol* 1996; 8: 131-4.