RESEARCH ARTICLE

Can Serum ICAM 1 distinguish pancreatic cancer from chronic pancreatitis?

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Abstract

   Background and aim: Pancreatic cancer is the fourth leading cause of cancer-related death worldwide, with an overall 5-year survival of <5% mainly due to presence of advanced disease at time of diagnosis. Therefore development of valid biomarkers to diagnose pancreatic cancer in early stages is an urgent need. This study concerned the sensitivity and specificity of serum ICAM 1 versus CA 19-9 in differentiation between pancreatic cancer and healthy subjects and acohort of patients with chronic pancreatitis with a focus on assessing validity in diagnosis of early stages of pancreatic cancer. **Methods:** A cohort of 50 patients with histologically diagnosed pancreatic tumors, 27 patients with chronic pancreatitis, and 35 healthy controls were enrolled. Serum samples for measurement of CA19-9 and I-CAM 1 were obtained from all groups and analyzed for significance regarding diagnosis and disease stage. **Results:** At a cut off value of (878.5 u/ml) I-CAM 1 had 82% and 82.26% sensitivity and specificity for differentiation between cancer and non-cancer cases, with higher sensitivity and specificity than CA19-9 at different cut offs (CA19-9 sensitivity and specificity ranged from 64-80% and 56.4 – 61.2% respectively). The AUC was 0.851 for I-CAM and 0.754 for CA19-9. Neither of the markers demonstrated significance for distinguishing between early and late cancer stages. **Conclusion:** ICAM 1 is a useful marker in differentiation between malignant and benign pancreatic conditions, and superior to CA19-9 in this regard. However, neither of the markers can be recommended for use in differentiation between early and late stage pancreatic cancers.

   Keywords: : Pancreatic cancer- I-CAM- CA19-9- chronic pancreatitis

Introduction

   Pancreatic cancer is considered the fourth leading cause of cancer-related deaths across the globe with poor prognosis (Yang et al., 2013). In United States, Pancreatic cancer accounts for about 3.0% of all cancers, and about 7% of cancer deaths (Fong et al., 2012).

   Epidemiological differences were noted in Egypt based on Mortality data that were obtained from the electronic national mortality records of the Ministry of Health from 2000 to 2004, which demonstrated higher rates in Egypt compared to the United States for subjects under age 20 years (relative risks (RR) of 7.7 and 4.2, for the age groups 0-15 and 15-20, respectively), and also that the highest mortality rates were observed in the Nile Delta compared to southern Egypt and the oasis (Soliman et al., 2006).

   Pancreatic ductal adenocarcinomas (PDAC) which constitute 85–90% of pancreatic cancer is known to be highly lethal with a median survival of 6.0 months and an overall 5-years survival of <5%. This is mainly attributed

to being asymptomatic till late stages and the presence of advanced disease at the time of diagnosis (Michaud, 2004). On the other hand; median survival following surgical resection for early pancreatic cancer is of the order of 11-20 months, with five-year survival ranges from 7-25% (Richter et al., 2003). Therefore, there is an urgent need to develop biomarkers with enough sensitivity and specificity to help diagnose pancreatic cancer in early stages (Alison et al., 2013).

   In 2002, CA 19-9 (carbohydrate antigen 19-9) radioimmunoassay for monitoring of pancreatic cancer patients received marketing clearance from the US Food and Drug Administration (FDA) (oncology, 2002). However, it has only a 79-81% sensitivity and 82-90% specificity for diagnosis (Ballehaninna et al, 2012) with false-positive results observed in benign pancreatico-biliary diseases such as pancreatitis, cholangitis and obstructive jaundice. Furthermore, CA19-9 is not expressed in 8-10% of the Caucasian population with the Lewis a-b- genotype (Lamerz, 1999). Despite this,

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CA19-9 has proved useful for disease management, where increased post-therapy levels indicate poor prognosis and poor therapy response (Ziske et al., 2003).

There has been a recent explosion in the pancreatic cancer biomarker field with more than 2000 biomarker studies implicating thousands of informative genes as candidate biomarkers. Among these biomarkers is the expression of ICAM (Intercellular adhesion molecule molecule 1), ICAM-1 is a glycoprotein that functions in cell–cell and cell–extracellular matrix adhesion and has a physiological role in tight adhesion of leukocytes, and can act as a chemoattractant for macrophages. Attracted macrophages release matrix degrading enzymes including matrix metalloproteinase 9 (MMP9), as well as cytokines such as TNF that synergize with Kras mutations to drive acinar cell metaplasia (Roland et al., 2010, Liou et al., 2015). It was found also that Increased ICAM-1 expression correlates with poor prognosis in pancreatic cancer (Roland et al., 2010).

The current study aims at assessing the sensitivity and specificity of ICAM 1 expression versus CA 19-9 in differentiation between pancreatic cancer and healthy subjects &cohort of patients with chronic pancreatitis. It also aims at assessing the validity of using CA19-9 and ICAM-1 in diagnosis of early stages of pancreatic cancer (Stages 1 and 2 tumor of American Joint Committee on Cancer (AJCC) staging system (Greene et al., 2002)

Material and Methods

Our cross sectional study enrolled 112 subjects, 50 patients with histologically diagnosed pancreatic tumors, 27 patients with chronic pancreatitis, and 35 healthy controls (without evidence of pancreatic disease (either medical history, clinically, laboratory or imaging).

Study subjects were subjected to:

-Informed consent was obtained from each subject and the study was carried out in accordance with the Helsinki Declaration.

-History taking regarding risk factors for acquiring chronic pancreatitis or pancreatic cancer as smoking habits, alcohol intake, history of gall stones, dietary history and family history of pancreatic illness.

-History taking regarding current pancreatic illness in chronic pancreatitis and cancer groups, including onset and course of illness, presence of pain, jaundice, fever, significant weight loss, or perception of body masses.

-None of the patients with chronic pancreatitis or the healthy controls had a history of any malignancies

-Clinical examination including BMI, vital signs, full general and local examination with emphasis on presence of lymphadenopathy, abdominal tenderness, masses, presence of ascites or localized fluid collections.

-The diagnosis of the patients with chronic pancreatitis was clinical and guided by standard radiological imaging tests (ultrasound and contrast enhanced CT studies).

-The diagnosis of the patients with pancreatic cancer was clinical and guided by standard radiological imaging tests (ultrasound and contrast enhanced CT studies), and confirmed with tissue biopsy from the pancreatic lesion that was either obtained by ultrasound guidance or endosonographically in inaccessible cases.

-Staging of pancreatic cancer was done according to American Joint Committee on Cancer (AJCC) staging system (Greene et al., 2002).

-The healthy controls were recruited from the same geographical location and were confirmed they had normal results of all physical, blood and imaging examinations.

-Samples for CA19-9 and ICAM assessment were obtained from all patient groups prior to any treatments and were analyzed regarding diagnosis and disease stage.

Samples collection and biochemical analysis

-Ten ml of venous blood were withdrawn from each patient in dry sterile vacutainers. After centrifugation, portions of blood were allowed to clot and then centrifuged at 3500g for 10 min to separate the serum designed for assessment of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltranspeptidase (GGT) activities, and the content of total bilirubin and direct bilirubin.

-Serum aliquots were stored at -80 °C until assayed and thawed on ice before measurement of ICAM 1 blood levels.

-For other parameters such as AST, ALT, GGT, total bilirubin, direct bilirubin, albumin were assayed using Beckman CX4 chemistry analyzer (NY, USA).

-Serum level of ICAM 1was determined by a commercially available Assay Max Human ELISA kit (Cat# BE 59011, Germany).

-Serum CA19-9 level was determined using an enzyme-linked binding protein assay kit (CanAg Cat#120-10, FUJIREBIO Diagnostics CO, Sweden).

-Levels of ICAM 1and CA19-9 were calculated by interpolation from a reference curve generated in the same assay with reference standards of known concentrations. All assays were performed in duplicate according to the manufacturer's instructions.

Statistical analysis

Continuous variables were summarized as mean ± standard deviation (SD). Categorical and ordinal data were presented as frequencies and percentages. Differences between means of continuous variables were assessed using Student´s t-test. The association of cancer pancreas with potential risk factors was assessed using the Chi square and Fisher´s exact tests where appropriate. A ROC curve was drawn and the area under the curve was calculated to evaluate whether ICAM-1 and CA19-9 were capable to predict the development of cancer. p values of < 0.05 were considered statistically significant. Analysis were performed using SPSS version 15.

Results

Demographic data of the studied population revealed predominance of male gender in all groups and higher mean age for pancreatic cancer group (Table 1).

Liver biochemical profile (apart from albumin level), Blood glucose level, serum cholesterol and Alpha

Table 1. Demographic and Laboratory Data of the Study Groups

| | | Cancer (n=50) | Non- cancer (n=62) | | P value between non-cancer and cancer groups |
			Pancreatitis (n=27)	Control (n=35)	
Sex*	Male	33 (66%)	18 (66.7%)	19 (54.3%)	0.48
	Female	17(34%)	9 (33.3%)	16 (45.7%)	
Age (years)†		62.± 7.8	57.6± 9.1	55.1± 13.2	0.002 ‡
AST (u/l) †		153.7 ± 65.1	161.5±77.9	43.9±44.0	0.000
ALT (u/l) †		78.3 ± 64.6	61.6±18.7	45.9±30.2	0.000
GGT (u/l) †		444.3 ± 505.9	155.7±73.9	61.1±63.2	0.000
ALP (u/l) †		579.1 ± 936.3	290.0±130.8	79.7±19.8	0.000
T_BIL (g/dl) †		7.1 ± 6.2	2.7±0.8	0.8±0.2	0.000
D_BIL (g/dl) †		3.2 ± 3.2	0.7±0.3	0.1±.06	0.000
ALB (g/dl) †		3.5 ± 0.6	2.7±0.5	3.7±0.5	0.057
INR†		1.3 ± 0.2	1.3±0.2	1.0±0.1	0.000
Glu (mg/dl) †		303.7 ±.499.1	221.4±158.4	115.2±45.9	0.001
TG†		212.2 ± 90.9	249.1±134.8	175.3±60.9	0.08
chol†		236.9 ± 127.2	218.6±85.7	179.6±85.6	0.001
AFP†		248.3 ± 235.5	±	17.2±20.6	0.000

AST(Aspartate transaminase); ALT (Alanine transaminase);, GGT(Gamma glutamyltransferase); ALP (Alkaline phosphatase); T BIL (total Bilirubin); D BIL (Direct Bilirubin); ALB (Albumin); INR (Interntional normalized ratio); GLU (Glucose); TG(Triglycerides); Chol. (Cholesterol); AFP (Alpha feto protein);*Data are given in number of cases (%); † Data are given in mean ± SD

Table 2. Risk Factors for Development of Pancreatitis or Cancer in the Study Group

| | Cancer (n=50) | Non- cancer (n=62.0) | | P value between non-cancer and cancer groups | Odds Ratio between non-cancer and cancer groups. | 95% Confidence Interval |
		Pancreatitis (n=27)	Control (n=35)			
Smoking *	25 (50%)	11.0 (40.0%)	7.0 (20.0%)	0.023†	2.4	(1.1 - 5.3)
Obesity*	26 (52%)	13.0 (48.1%)	14.0 (40.0%)	0.373	1.4	(0.7 - 2.9)
Coffee consumption*	27 (54%)	16.0(59.3%)	9.0 (26.5%)	0.172	1.7	(0.8 - 3.6)
High meat diet*	32 (64%)	17.0 (63.0%)	0.0 (0.0%)	0.928	1.0	(0.4 - 2.8)
Alcohol intake*	6 (12%)	7.0 (9.1%)	1.0 (2.9%)	0.227	3.5	(0.4 - 31.1)
Family history of the disease*	13 (26%)	6.0 (22.2%)	0.0 (0.0%)	0.714	1.2	(0.4 - 3.7)
Urban residence*	17(34%)	15.0(55.6%)	25.0 (73.5%)	0.001†	3.7	(1.7 - 8.1)

*Data are given in number of cases (%); †Statistically significant

Table 3. I-CAM 1and CA19-9 Values of Cancer and Non- Cancer (Pancreatitis and Control) Groups*

| | Cancer (n=50) | Non- cancer (n=62) | | P value between non-cancer and cancer groups |
		Pancreatitis (n=27)	Control (n=35)	
I-CAM 1	3,259.6±2,400.7	1,005.9±1,511.2	641.8±414.4	0.000
CA19-9	260.3±248.9	223.8±228.0	30.46±30.454	0.000

*Data are given in mean ± SD

fetoprotein levels were significantly different between cancer and non -cancer groups as shown in (Table 1).

Smoking and urban residence were the only significant risk factors between cancer and non-cancer groups (Table 2).

Of the pancreatic cancer group, 30.0 patients (60%) had histological early stage tumor (stage 1 and 2), while 20 patients (40%) had late stage tumor (stage 3 and 4).

I-CAM1 and CA19-9 levels were significantly higher in cancer than non-cancer group (Table 3), with I-CAM 1 sensitivity of 82% and specificity of 82.3% at cut off value 878.5, which is higher than CA19-9 at different cut off values (sensitivity range 64-80% and specificity range 56.4 – 61.2%),) (Table 4).Moreover; AUC for I-CAM 1 (0.851) was higher compared to CA19-9 (0.8) (Figure 1). However, both markers failed to demonstrate
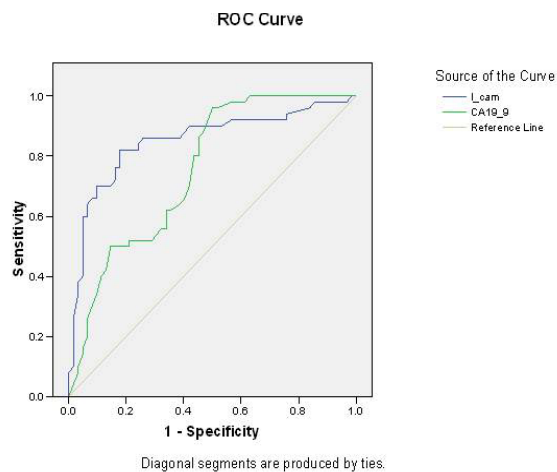
Figure 1. AUC of I-CAM 1 (0.851) and CA19-9 (0.754) between Cancer and Non-Cancer Groups

Table 4. Cut off Values of I-CAM1 and CA19-9 between Cancer and Non-Cancer Groups

	Cut off value	Sensitivity*	Specificity*
I-CAM 1	665.0	86.0	74.2
	674.0	84.0	75.8
	878.5	82.0	82.3
CA19-9	55.5	80.0	56.4
	60.5	70.0	58.1
	71.5	66.0	59.7
	79.0	64.0	61.3

*Data are given in %

significance difference when compared between early and late cancer stages.

Discussion

In the current study we evaluated the possible role of ICAM 1 in diagnosis of pancreatic cancer (with specificity and sensitivity higher than CA 19-9) versus healthy subjects and patients with chronic pancreatitis that commonly could be misdiagnosed as malignant.

ICAM 1 level identified in our analysis were highly discriminatory for pancreatic cancer versus non cancer (Healthy subjects and chronic pancreatitis)demonstrating sensitivity 82% and specificity 82.3% at cut off value 878.5, these sensitivity and specificity were higher than CA19-9 at different cut off values (sensitivity range 64-80% and specificity range 56.4 – 61.2%), even at lower I-CAM 1 cut off values (665 and 674) there is higher sensitivity and specificity than CA 19-9 (84-86/74-75.8 Vs 64-80/56.4-61 respectively). Also I-CAM 1 demonstrated significant improvement over CA19-9 by using ROC analysis (0.851 vs 0.754 respectively).

Some other reports have matching results to ours, Brand and colleagues analyzed 83.0 circulating proteins in sera of patients with PDAC (n=333.0), compared with benign pancreatic conditions (n=144.0) and healthy controls (n=227.0), and found that the panel of CA19-9, ICAM-1 and osteoprotegerin was best able to discriminate PDAC patients from healthy controls with a sensitivity and specificity of 78% and 94% (Brand et al., 2011).

Another study analyzed the values of ICAM-1, VCAM-1, and ELAM-1 between 20 pancreatic cancer specimens and 20 normal pancreatic tissues, and found 5.4-fold increase of ICAM-1 (P<0.01) and a 3.7-fold increase in VCAM-1 (P<0.01) mRNA expression in cancer samples in comparison with normal controls (Caliera et al, 2002). Among more recent reports matching our results, ICAM-1 expression was found in regions of acinar to ductal metaplasia, but not in adjacent "normal" tissue or pancreatic intraepithelial neoplastic lesions (Liou et al, 2014).

On contrary to our results regarding poor significance of CA19-9 measurement in detection of early stage pancreatic cancer, O'Brien and colleague studied levels of serum CA19-9 one and two years prior to clinical presentation of pancreatic cancer on 154.0 cases and 304.0 matched controls, and found 95% specificity for CA19-9 level (>37.0 U/mL), and encouraging sensitivity of 68.0% up to 1 year, and 53.0% up to 2.0 years before diagnosis (O'Brien et al., 2015).

The lower cut off values for CA19_9 (60.5 U/mL) in our study matches with that found by Kim and colleagues who assessed CA 19-9 serum levels in 70940 asymptomatic individuals and identified only 4 patients with pancreatic cancer among 1063 patients with elevated CA 19-9 serum levels (>37.0 U/mL, mean values 50.5±16.8 U/mL), with a sensitivity and specificity of 100.0 and 98.5% respectively, however poor positive predictive value (PPV) of only 0.9% (Kim et al., 2004).

As regards the demographic data of our study population, the mean age of cancer group was 62 years, and most of them were males (66% versus 34% for females). In literature, it is mentioned that most of cases of pancreatic cancer occur by the age older than 55 years, and only 10% below this age, and that men have higher incidence rates than women (Yadav et al., 2013).

In our study, smoking and urban residence were the only significant risk factors between cancer and non-cancer groups. Smoking was present in 50% of cancer patients, with odds ratio of 2.4, and 95% confidence interval of (1.1 - 5.3), urban residence was present in 34% of cancer patients of our study, with odds ratio of 3.7, and 95% confidence interval of (1.7 - 8.1). These results support the hypotheses regarding the role of specialized pathways related to smoking and other factors related to urbanization as dietary habits in the precipitation of pancreatic cancer. The pathophysiologic role of cigarette smoking in influencing pancreatic carcinogenesis is thought to be through the interference with physiological pathways (altering secretion, increasing proliferation and reducing apoptosis) and the interaction with DNA (DNA damage as well as DNA independent alterations) (Wittel et al., 2012), and it is known that high consumption of fats, sugars and red meet with low consumption of healthy food has a negative impact on pancreatic cancer risk (La Vecchia, 2009).In this aspect our results matches with Bosetti et al, 2012 and Zou et al., 2014 who mentioned that pancreatic cancer risk is 2.2 times higher in current smokers compared with those who never-smoke, and that the risk increases with the number of cigarettes smoked

per day, and duration of smoking (Bosetti et al, 2012, Zou et al, 2014).

Based on our data, we can conclude that ICAM 1 is a useful marker in the differentiation between malignant and benign pancreatic conditions (pancreatitis and healthy controls) with superiority to the well-known CA19-9 marker in terms of specificity and sensitivity. However, the use of both markers in the differentiation between early and late stages of pancreatic tumors cannot be recommended.

Acknowledgements

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