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Observational Study

Role of Endoscopic ultrasound, and cyst fluid tumor markers in the diagnosis of cystic pancreatic lesions

Okasha HH *et al*. EUS and cyst fluid tumor markers

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Abstract

BACKGROUND

Pancreatic cystic lesions (PCLs) are common in clinical practice. The accurate classification and diagnosis of these lesions are crucial to avoid unnecessary treatment of benign lesions and missed opportunities for early treatment of potentially malignant lesions.

AIM

To evaluate the role of cyst fluid analysis of different tumor markers as cancer antigens (*e.g.*, cancer antigen (CA 19-9 and CA 72-4), carcinoembryonic antigen (CEA), the serine protease inhibitor Kazal-type 1 (SPINK1), interleukin 1 beta (IL1-β), vascular

endothelial growth factor A (VEGF-A), prostaglandin E2 (PGE2), amylase, and mucin stain in diagnosing pancreatic cysts and differentiating malignant from benign lesions.

METHODS

This study included 76 patients diagnosed with PCLs using different imaging modalities. All patients underwent endoscopic ultrasound (EUS) and EUS-fine needle aspiration (EUS-FNA) for characterization and sampling of different PCLs.

RESULTS

The mean age of studied patients was 47.4 years ± 11.4 SD, with a slight female predominance (59.2%). Mucin stain showed a high statistical significance in predicting malignancy with a sensitivity of 87.1% and specificity of 95.56%. It also showed a positive predictive value and negative predictive value of 93.1% and 91.49%, respectively (P value < 0.001). We found that positive mucin stain, cyst fluid glucose, SPINK1, amylase, and CEA levels have a high statistical significance with a P value of < 0.0001. In contrast, IL-1β, CA 72-4, VEGF-A, VEGFR2, and PGE2 did not show any statistical significance. Univariate regression analysis for prediction of malignancy in PCLs showed a statistically significant positive correlation between mural nodules, lymph nodes, cyst diameter, mucin stain, and cyst fluid CEA. Meanwhile, logistic multivariable regression analysis proved that mural nodules, mucin stain, and SPINK1 were independent predictors of malignancy in cystic pancreatic lesions.

CONCLUSION

EUS examination of cyst morphology with cytopathological analysis and cyst fluid analysis could improve the differentiation between malignant and benign pancreatic cysts. Also, CEA, glucose, and SPINK1 could be used as promising markers to predict malignant pancreatic cysts.

Core Tip: Nowadays, the awareness of pancreatic cystic lesions became an essential issue, especially with the increased incidence of asymptomatic pancreatic cysts in the general population. Therefore, the proper diagnosis, meticulous differentiation, and staging of these pancreatic cystic lesions are crucial for proper management and avoiding unnecessary treatment of benign lesions and missing early treatment of the malignant/pre-malignant lesions. Endoscopic ultrasound examination of cyst morphology with cytopathological and chemical analysis and cyst fluid could improve the diagnostic capability. Also, many developing markers are valuable for predicting a malignant pancreatic cyst.

INTRODUCTION

Pancreatic cystic lesions (PCLs) are not rare; they vary from a simple benign cyst to a highly malignant one[1]. Awareness of these lesions has been increased in recent years, especially with the increased incidence of asymptomatic pancreatic cysts in the general population primarily due to improved detection by the different advanced imaging modalities[2,3]. Therefore, the proper diagnosis, meticulous differentiation, and staging of these PCLs are crucial for proper management and avoiding unnecessary treatment

of benign lesions and missing early treatment of the malignant/pre-malignant lesions[4,5].

Endoscopic ultrasound (EUS) has become an indispensable tool for diagnosing many pancreatic lesions; it has a benefit for better evaluation of number, location, dimensions, wall thickness, and the content of pancreatic cysts. Also, it is crucial in distinguishing the internal septae and solid areas within the cysts[6].

The morphological features of the PCLs are not independent factors in differentiating malignant from nonmalignant lesions. The combination of both EUS-fine needle aspiration (EUS-FNA) findings with cystic fluid tumor markers analysis, along with clinical, radiologic, histologic, genetic, and molecular characteristics, enhances the diagnostic accuracy of PCLs and helps to construct a novel model in the era of PCLs diagnosis[4].

Currently, many tumor markers, both in the serum and in pancreatic cyst fluid, have been widely studied as a tool for distinguishing mucinous/malignant and non-mucinous pancreatic cystic lesions such as carcinoembryonic antigen (CEA), CA19-9, CA125, CA15-3, and CA72-4[7].

MATERIALS AND METHODS

Study design and aims

This single tertiary referral center prospective study, the samples were collected and stored then all markers were done in the same specimens in the same time. The study aimed primarily to evaluate the role of cyst fluid amylase and tumor markers as CA 19-9, CEA, serine protease inhibitor Kazal-type 1 (SPINK1), IL1-β, CA 72-4, vascular endothelial growth factor A (VEGF-A), and prostaglandin E2 (PGE2) in addition to mucin stain in diagnosing pancreatic cysts and differentiating malignant from benign lesions.

Patients and recruitment

This prospective study was conducted on 76 patients diagnosed with PCLs using different imaging modalities such as computed tomography (CT), EUS, abdominal ultrasound, or magnetic resonance imaging (MRI). The candidates were recruited over three years from the Gastroenterology, Endoscopy, and Hepatology Unit, Internal Medicine Department, Kasr Al-Ainy, Cairo University. Fluid analysis was performed for CA 19-9, CA 72-4, CEA, VEGF-1, SPINK-1, IL1-b, PGE2, amylase, mucin stain, and cytopathology. We compared these data with the final diagnosis based on histopathology after surgical resection, positive cytopathology (positive for malignancy), and a long period of follow-up of the patients for at least 18 mo.

All patients underwent EUS examination for cyst characterization and sampling of the cystic lesions. All included patients were above 18 years of age. Patients included in this study were diagnosed with large pancreatic cysts (larger than 3 cm), suspicious intraductal papillary mucinous neoplasm (IPMN), or pancreatic duct dilatation proved by magnetic resonance cholangiopancreatography. However, patients with small cysts (less than 1 cm), calculous cholecystitis, a potential risk for anesthesia, a bleeding tendency (international normalized ratio > 1.5, or severe thrombocytopenia, with platelet count < 50000/mm³) and patients who refused to participate were excluded from the study. Also, those who missed the follow-up were ruled out from the study. Our institution's Research Ethical Committee approved the study, and all patients gave their informed written consent before inclusion in the study, according to the ethical guidelines of the 1975 Declaration of Helsinki.

Examination procedure

All the patients, after thorough full history taking and clinical examination, were subjected to: (1) EUS examination using a linear Echoendoscope PENTAX EG3870UTK (HOYA Corporation, PENTAX Life Care Division, Showanomori Technology Center, Tokyo, Japan) connected to an ultrasound unit Hitachi AVIUS machine (Hitachi Medical Systems, Tokyo, Japan). All examinations were performed under deep sedation with IV propofol. For EUS-FNA, we used the Cook 19G and 22G needles (Echotip;

Wilson-Cook, Winston Salem, NC). Prophylactic ceftriaxone (1 gm) was administrated before the procedure; (2) Characterization of the PCLs. All the characteristics of the PCLs were documented, including localization, number, dimensions, wall thickness, presence of septations or mural nodules, calcification, lymph nodes, and cystic dilatation of the main pancreatic duct. The color, transparency, and viscosity of the cyst fluid were also recorded; and (3) Evacuation of the cystic fluid entirely with a single needle pass. Aspirated material inside the needle was spread over dry slides. Also, a proportion of the fluid sample (at least 2 mL) was sent for cytopathological examination, including mucin staining using alcian blue stain. At least 5 mL of cyst fluid were analyzed for CEA, SPINK1, IL1-β, CA 72-4, VEGF-A, PGE2, and CA-19-9 using two-site immunoassays (Beckman Coulter). Amylase was measured by the enzymatic colorimetric assay on a modular system (Roche).

Cysts were considered malignant when any of the following is present: (1) Cytopathological detection of malignancy; (2) Presence of metastasis in the absence of other concomitant malignancies; (3) Presence of mural nodules that progress in size within six months; and (4) Postoperative pathological diagnosis of malignancy if available. Cysts were considered benign when proved negative for malignancy by cytopathological examination and follow-up for 18 mo without increasing its size, the appearance of mural nodules or metastasis, or occurrence of obstructive jaundice.

The overall complication rate of EUS-FNA in the prospective series ranges from 0% to 2.5%[8]. Such complications include pain, infection, bleeding, acute pancreatitis, perforation of the esophagus or duodenum, bile peritonitis, and seeding of tumorous cells along the needle track[9]. Therefore, a prophylactic antibiotic in the form of 1 gm IM or slow IV third-generation cephalosporin was administered 6 h before the procedure. No major complications occurred in our series. However, self-limiting intracystic bleeding occurred in one patient, and mild pains occurred in 3 patients. All patients were discharged on the same day, and no hospital admission was needed.

Statistical analysis

Data management and analysis were performed using Statistical Package for Social Sciences v. 25. Numerical data were summarized using means and standard deviations, medians, or ranges, as appropriate. Categorical data were summarized as numbers and percentages. Estimates of the frequency were calculated using the numbers and percentages. Numerical data were explored for normality using the Kolmogorov-Smirnov test and the Shapiro-Wilk test. To the measure association between variables: (1) Chi-square or Fisher's tests were used to compare independent groups concerning categorical data; (2) Kappa statistics were computed to test the agreement between categorical variables. Their values ranged from zero to one; (3) The Mann-Whitney U test implemented comparisons between two groups for non-normally distributed numeric variables; and (4) P value ≤ 0.05 was considered significant.

RESULTS

This study included 76 patients [31 males (40.8%) and 45 females (59.2%)] with a mean age of 47.4 years ± 11.4 SD (Table 1).

EUS evaluation showed that most patients had a unilocular cyst (40 patients, 52.6%), while 36 patients (47.4%) had a multilocular cyst. Mural nodules were found in 24 patients (31.6%). In addition, most cysts had thin walls (77.6%) and clear contents (78.9%). Calcifications and lymph nodes were not found in 92.1% and 82.9% of patients, respectively. The pancreatic duct was dilated in 10 patients (13.2%) (Table 2).

Pancreatic cysts were diagnosed as malignant/potentially malignant or benign in 38.2% and 61.8% of patients, respectively. Malignant cysts included mucinous cystadenocarcinoma (14.5%) (Figure 1A) and pancreatic adenocarcinoma (5.3%). On the other hand, potentially malignant cysts included IPMN with low (7.9%) and high-grade dysplasia (13.2%) and mucinous cystadenoma. Benign cysts included serous and mucinous cystic neoplasms (17.1%), pseudocysts (39.5%) (Figure 1B), and cystic lymphangioma (1.3%) (Table 3).

Evaluating PCLs using mucin stain to differentiate between mucinous and non-mucinous pancreatic cystic lesions showed a sensitivity of 100%, specificity of 94%, and

an accuracy of 96.04% (Table 4). Also, we found that there was a high statistical significance for mucin stain in predicting malignancies with a sensitivity of 87.1%, specificity of 95.56%, positive predictive value (PPV) of 93.1%, and negative predictive value (NPV) of 91.49% (*P* value < 0.001) (Table 5).

The median cyst fluid (CF) CEA level was 90 (8.39- 2750) ng/mL. Also, the median CF SPINK1 Level was 0.56 (0.35-0.97) ng/mL, and the median CF glucose level was 50 mg/dL (Table 6). When we categorized the CF level of CEA above and below 192 ng/mL, the malignant/potentially malignant cysts were more likely to have CEA level above 192 ng/mL (*P* value = 0.001), as shown in Table 7.

As shown in Table 6, CF CEA level and CF amylase were significantly higher in malignant/potentially malignant cysts than benign cysts with a median of 15.8 *vs* 6.4 and 130.5 *vs* 3060 (*P* values = 0.004 and 0.034, respectively). Also, CF amylase and CF CEA showed statistical significance in predicting malignancy with *P* value 0.028 and < 0.001, respectively. Furthermore, the SPINK1 Level in cyst fluid was significantly higher in malignant/potentially malignant cysts compared to benign ones (0.91 *vs* 0.47, *P* value = 0.001). Meanwhile, glucose was markedly consumed in malignant/potentially malignant cysts that were significantly lower than the glucose level of benign cysts (68.5 *vs* 21.5, *P* value = 0.0001) (Table 7).

Comparing different CF markers in predicting malignant PCLs among the studied patients reveals that positive Mucin stain, CF Glucose, SPINK1, amylase, and CEA showed a high statistical significance with a *P* value of < 0.0001, 0.0001, 0.001, 0.034, and 0.004, respectively. However, IL1-β, CA 72-4, VEGF-A, VEGFR2, and PGE2 did not show any statistical significance (Table 8).

Univariate regression analysis showed a statistically significant association between malignancy in PCLs and mural nodules, lymph nodes, cyst diameter, mucin Stain, CF CEA, SPINK1, and CEA level > 192 ng/mL. In comparison, multivariable regression analysis proved that mural nodules, mucin Stain, SPINK1, and CEA level > 192 ng/mL are independent predictors of malignancy in cystic pancreatic lesions (Table 9).

Receiver operating characteristic (ROC) curves were constructed to assess the diagnostic accuracy of cyst fluid CEA, SPINK1, IL1-β, CA 72-4, VEGF-A, PGE2, and CA-19-9 in predicting malignant cysts. It revealed that the area under the curve was comparable for CEA, glucose, and SPINK1 (0.75, 0.76, and 0.72, respectively) (Figures 3, 2, and 5).

The sensitivity of EUS diagnosis in detecting malignant and premalignant pancreatic cyst was 66.7%, while 69.2% for the specificity, 60% PPV, 75% NPV with an overall accuracy of 68.2% (Table 10).

Out of 76 patients, two patients died. Both patients had pancreatic adenocarcinoma. Most of the patients showed a stationary course (40 patients, 52.6%), and only three patients (3.9%) ran a regressive course, as demonstrated in Table 11. Two patients with inflammatory pseudocyst underwent a percutaneous pig-tail insertion; one of them was complicated by abscess formation and proceeded to surgery. Most of the patients required no intervention (56 patients, 73.7%). However, some patients were referred to surgeries (17 patients, 22.4%), and only one patient underwent cystogastrostomy, as demonstrated in Table 12.

DISCUSSION

There are great challenges in diagnosing and managing PCLs that have become a common problem faced by many physicians and surgeons[10]. Some PCLs have a malignant potential with a significant risk of developing invasive cancer[11]. Therefore, the accurate classification and diagnosis of pancreatic cysts provide a potential for preventing and early detection of pancreatic cancer. On the other hand, misdiagnosis or unnecessary surgeries may lead to high cost and harm to the patients[10].

Unfortunately, imaging modalities such as CT and MRI have insufficient sensitivity and specificity to characterize PCLs and provide a suboptimal classification and diagnosis due to poor interobserver variability[12].

EUS is considered the most sensitive tool in delineating the pancreatic cyst characteristics with the capacity to identify the presence of mural nodules and solid

components[13]. Also, it has a benefit in enabling EUS-FNA for cytology[14]. Nonetheless, and cytology still has a limited diagnostic yield with a pooled sensitivity of 63% and specificity of 88%[15].

Owing to the limited diagnostic accuracy of different pancreatic cysts with the current diagnostic modalities, analysis of the pancreatic cyst fluid obtained *via* EUS-FNA could improve the diagnostic accuracy for pancreatic cysts and help determine the malignant potentiality. Therefore, there is still a growing research interest in discovering and validating novel cyst fluid biomarkers that may improve diagnostic accuracy. The present study was designed to determine the role of cyst fluid amylase and tumor markers as CA 19-9, CEA, SPINK1, IL1-β, CA 72-4, VEGF-A, and PGE2 in addition to mucin stain in diagnosing pancreatic cysts and differentiating malignant from benign lesions.

The presence of solid components inside the cyst on imaging could be a significant predictor of malignancy, as reported in many kinds of research[16-18]. Also, we found that the presence of mural nodules is highly predictive of malignancy in univariate and multivariate logistic regression analysis (*P* value = 0.0006 and 0.0172, respectively) along with cyst diameter (*P* value 0.0189 for shortest diameter and 0.0112 for longest diameter) and lymph node enlargement (*P* value = 0.0024).

In a study conducted by Okasha *et al*[19] analyzing the cyst fluid amylase of 44 patients, they concluded that pancreatic cyst fluid amylase level could differentiate between malignant/potentially malignant and benign cysts with a sensitivity of 58%, specificity of 75%, PPV of 73%, NPV of 60%, and accuracy of 66%.

In our study, CF CEA level and CF amylase were significantly higher in malignant/potentially malignant cysts than in benign cysts with *P* values of 0.004 and 0.034, respectively. This finding agrees with other studies stating that the pancreatic cyst fluid CEA offers the best diagnostic performance than any other single test, especially in differentiating mucinous and non-mucinous cysts[20].

A large multi-institutional study conducted on 1,861 patients reported that CEA > 192 ng/mL could differentiate mucinous from non-mucinous cysts with an accuracy of

77%[21]. Their findings are in concordance with our study that reported that the malignant/potentially malignant cysts had CEA levels above 192 ng/mL with P value = 0.001.

In CF, positive mucin stain was significantly more frequent in malignant cysts (87.1%) with a P value < 0.0001. Twenty-seven cysts were positive for mucin stain, with a sensitivity of 87.1% and specificity of 95.56% in differentiating benign from malignant PCLS. Also, mucin staining differentiates mucinous from non-mucinous cysts with a sensitivity and specificity of 100% and 94%, respectively. The results in the current study were more compatible with an Egyptian study by Okasha and his colleagues. They showed that pancreatic cyst fluid positive mucin stain is 85% sensitive and 95% specific in detecting mucinous or non-mucinous pancreatic cysts with 92% PPV, 91% NPV and accuracy of 91%. Also, positive mucin staining is 63% sensitive and 97% specific in differentiating malignant/potentially malignant from benign pancreatic cysts with PPV of 96%, NPV of 72%, and overall accuracy of 80%. This outcome is in concordance with a recent study by Okasha and his colleagues that showed that a cyst fluid positive mucin stain has a sensitivity of 85.5% and a specificity of 86.1% for detecting mucinous cystic neoplasm with 72.3% PPV, 93.3% NPV, and 85.9% accuracy[4]. Many studies also reported that the mucin staining could be complementary to cyst CEA levels and cytology and when 1 out of 3 was found to be positive, this increases sensitivity to 92% and specificity to 52%, as in a study conducted by Morris-Stiff et al[22].

In our study, cyst fluid glucose was markedly consumed in malignant/potentially malignant cysts than in benign cysts (21.5 *vs* 68.5, P value = 0.0001). Therefore, since it is a simple and cheap biomarker; it could be used as a marker for differentiation between benign and malignant pancreatic cysts with a relatively low cost[23-25].

In 2004, Raty et al[26] were the first to evaluate the role of cyst fluid SPINK1 in differentiating potentially malignant from benign cysts. They reported that the SPINK1 Level was higher in malignant/potentially malignant than benign cystic pancreatic lesions (1609 ± 418 *vs* 46 ± 21 ug/L; P value = 0.0001). These findings matched our study

that showed that SPINK1 Level was higher in malignant/potentially malignant cysts than benign ones (0.91 *vs* 0.47, *P* value = 0.001) with a sensitivity and specificity of 70.59% and 65.33%, respectively (Table 8).

In our study, mural nodules, cyst diameter, lymph node enlargement, mucin stain, CF CEA, SPINK1, and glucose measurements in cyst fluid were highly predictive of malignancy in univariate analysis. In comparison, only mural nodules, mucin stain, and SPINK1 were highly predictive of malignancy in multivariate analysis.

Of all these markers measured in cyst fluid, CEA, glucose, and SPINK1 were independent predictors of malignancy, suggesting that these markers could help differentiate potentially malignant cysts from benign cysts.

The analysis of recent markers -not investigated in this study- as cyst fluid DNA is recommended for future research because it might add more diagnostic value in differentiating benign from malignant cysts.

CONCLUSION

Conclusion

EUS examination of cyst morphology with cytopathological and chemical analysis and cyst fluid could improve the differentiation between malignant and benign pancreatic cysts. Also, CEA, glucose, and SPINK1 are valuable markers for predicting a malignant pancreatic cyst.

Recommendations

Further studies addressing new markers are recommended, which will provide a panel of laboratory data to recognize the malignant and potentially malignant lesions to establish a standard protocol for diagnosis and management. Also, cyst fluid DNA is considered a potential diagnostic agent with a particular possible use in differentiating between benign and malignant cysts. Further investigation regarding this biomarker is recommended.

ARTICLE HIGHLIGHTS

Research background

Nowadays, the awareness of pancreatic cystic lesions became an essential issue, especially with the increased incidence of asymptomatic pancreatic cysts in the general population. Therefore, the proper diagnosis, meticulous differentiation, and staging of these pancreatic cystic lesions (PCLs) are crucial for proper management and avoiding unnecessary treatment of benign lesions and missing early treatment of the malignant/pre-malignant lesions. Endoscopic ultrasound (EUS) examination of cyst morphology with cytopathological and chemical analysis and cyst fluid could improve the diagnostic capability. Also, many developing markers are valuable for predicting a malignant pancreatic cyst.

Research motivation

EUS examination of cyst morphology with cytopathological and chemical analysis and cyst fluid could improve the differentiation between malignant and benign pancreatic cysts. Also, carcinoembryonic antigen (CEA), glucose, and the serine protease inhibitor Kazal-type 1 (SPINK1) are valuable markers for predicting a malignant pancreatic cyst.

Research objectives

To evaluate the role of cyst fluid analysis of different tumor markers as cancer antigens (*e.g.,* CA 19-9 and CA 72-4), carcinoembryonic antigen (CEA), SPINK1, interleukin 1 beta (IL-1β), vascular endothelial growth factor A (VEGF-A), prostaglandin E2 (PGE2), amylase, and mucin stain in diagnosing pancreatic cysts and differentiating malignant from benign lesions.

Research methods

This study included 76 patients diagnosed with PCLs using different imaging modalities. All patients underwent EUS and EUS-FNA for characterization and sampling of different PCLs.

Research results

The mean age of studied patients was 47.4 years ± 11.4 SD, with a slight female predominance (59.2%). Mucin stain showed a high statistical significance in predicting malignancy with a sensitivity of 87.1% and specificity of 95.56%. It also showed a positive predictive value and negative predictive value of 93.1% and 91.49%, respectively (P value < 0.001). We found that positive mucin stain, cyst fluid (CF) glucose, SPINK1, amylase, and CEA levels have a high statistical significance with a P value of < 0.0001. In contrast, IL-1β, CA 72-4, VEGF-A, VEGFR2, and PGE2 did not show any statistical significance. Univariate regression analysis for prediction of malignancy in PCLs showed a statistically significant positive correlation between mural nodules, lymph nodes, cyst diameter, mucin stain, and cyst fluid CEA. Meanwhile, logistic multivariable regression analysis proved that mural nodules, mucin stain, and SPINK1 were independent predictors of malignancy in cystic pancreatic lesions.

Research conclusions

EUS examination of cyst morphology with cytopathological analysis and cyst fluid analysis could improve the differentiation between malignant and benign pancreatic cysts. Also, CEA, glucose, and SPINK1 could be used as promising markers to predict malignant pancreatic cysts.

Research perspectives

Further studies addressing new markers are recommended, which will provide a panel of laboratory data to recognize the malignant and potentially malignant lesions to establish a standard protocol for diagnosis and management. Also, cyst fluid DNA is considered a potential diagnostic agent with a particular possible use in differentiating between benign and malignant cysts. Further investigation regarding this biomarker is recommended.

5 ACKNOWLEDGEMENTS

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