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Review Article

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# Mucinous Cystic Lesions of the Pancreas an EUS-FNA-based Study with Clinico-Pathological Follow Up

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## Abstract

***Background:*** *Mucinous cystic lesions of pancreas harbor a pre-malignant potential thus necessitating their distinction from the non-mucinous ones. To make this distinction, EUS-FNA cytology along with cyst fluid CEA and amylase levels are utilized in addition to endoscopic and radiological findings. Evaluation of K-ras mutations has emerged as a useful adjunct for the evaluation of mucinous cystic neoplasms of the pancreas.*

***Aim:*** *We aimed to study mucinous cystic lesions of the pancreas diagnosed on EUS-FNA cytology, in conjunction with cyst fluid CEA and amylase levels and the frequency of K-ras mutation in a cohort of patients seen at the largest cancer hospital in our country.*

***Materials and Methods:*** *After approval from the institutional review board, all the cases of mucinous cystic lesions of pancreas evaluated between July 2005 and August 2019 were reviewed. Patient data, including age, gender, endoscopic and radiological findings, cytological and/or histological diagnosis, cyst fluid CEA, and amylase levels were collected.*

***Results:*** *Twenty-three patients enrolled in the study demonstrated an equal gender distribution with a mean age of 67.4 years. The sensitivity of EUS-FNA for mucinous cystic lesions of the pancreas was 84.6%. Cyst fluid CEA levels were elevated in some MCNs but not IPMNs resulting in a sensitivity of 37.5%. The specificity of cyst fluid amylase was 90%. K-ras mutation was found to have a sensitivity and specificity of 50% and 100% respectively, for mucinous lesions of the pancreas.*

***Conclusion:*** *EUS-FNA is a useful technique for evaluation of pancreatic cystic lesions, especially since cytological diagnosis can be augmented by cyst fluid CEA and amylase levels. K-ras analysis can add further to the diagnostic utility of EUS-FNA*

**Keywords:** Mucinous cystic neoplasm, EUS-FNA, K-ras, CEA

## Introduction

Cystic lesions of the pancreas are classified as pseudo-cysts, simple retention cysts and cystic neoplasms. Rarely, pancreatic cystic lesions are associated with systemic and hereditary diseases, such as cystic fibrosis and von Hippel-Lindau disease. Distinction between various types of pancreatic cystic lesions is important since this has therapeutic and prognostic implications. Pancreatic cystic neoplasms are further sub-divided into benign cysts (cysts/ cyst adenomas), borderline tumors (mucinous cystic neoplasm (MCN) and intra-ductal papillary mucinous neoplasm (IPMN)) and invasive carcinomas [1]. It is important to distinguish mucinous from non-mucinous cysts as the former are pre-malignant [2].

The evolution of endoscopic ultrasound (EUS) has drastically improved the evaluation of gastrointestinal and pancreatic tumors. It not only provides high resolution images but also has the advan-

tage of allowing the performance of fine needle aspiration (FNA) during the same procedure. Over time, EUS-FNA has been found to be a more effective and superior technique as compared to computed tomography (CT)-guided or ultrasound-guided per cutaneous aspiration, particularly in the evaluation of smaller lesions [3]. EUS-FNA is now considered the standard procedure to obtain a cytological diagnosis of pancreatic masses, with a sensitivity of 85-89% and specificity of 96-99%, as reported by three meta-analyses and by Mehmood et al [4-7].

When distinguishing mucinous from non-mucinous cystic lesions, quantitative analysis of various tumor markers in the cyst fluid, including CA19-9, CA72-4, CA125, amylase and CEA, has also been studied. Of these, carcinoembryonic antigen (CEA) has been shown to be the most reliable in helping with this characterization. The agreed upon cut-off value for cyst fluid CEA is 192 ng/ml, as suggested by Brugge et al [8]. In addition, cyst fluid amylase level is often used to evaluate communication of pancreatic cysts with the main pancreatic duct and is helpful in excluding pancreatic pseudo-cysts. Although there is no universally accepted cut-off value, cyst fluid amylase levels less than 250 IU/L can virtually exclude a pseudo-cyst [8].

Evaluation of K-ras mutations by polymerase chain reaction (PCR) has emerged as a useful adjunct for the evaluation of mucinous cystic neoplasms of the pancreas. Both MCN and IPMN harbor somatic K-ras mutations with a frequency ranging from 30% to 80%, increasing with the grade of the lesion [9].

Currently, the recommendation is to evaluate pancreatic cystic lesions using a multidisciplinary approach involving clinical, radiological and endoscopic ultrasound findings, combined with the results of cytology obtained by fine needle aspiration, together with analysis of cyst fluid for CEA and amylase levels. Despite the use of these modalities, distinguishing mucinous cystic neoplasms (MCN) and intra-ductal papillary mucinous neoplasms (IPMN) from non-mucinous cysts may still pose a challenge [10]. In accordance with best-practice guidelines, it is a routine practice in our hospital to assess pancreatic lesions with a combination of radiological, endoscopic and cytological findings, along with cyst fluid analysis.

We aimed to study mucinous cystic lesions of the pancreas diagnosed on EUS-FNA cytopathology, cyst fluid CEA and amylase levels and frequency of K-ras mutation in a cohort of patients seen at the largest cancer hospital in our country.

## Material and methods

Approval from the institutional review board was taken prior to the study. In this retrospective study, a data search was performed to identify mucinous cystic lesions of the pancreas i.e. mucinous cystic neoplasms and intra-ductal papillary mucinous neoplasms, diagnosed on EUS-FNA cytology in combination with endoscopic and radiologic findings. Cases evaluated between July 2005 and August 2019 were reviewed. Patient data, including age, gender, endoscopic and radiological findings, cytological and/or histological diagnosis, cyst fluid CEA and amylase levels were collected. EUS-FNA was conducted in the endoscopy suite, utilizing linear array echo-endoscope, after obtaining informed consent in all cases. Procedures were carried out under sedation, using intravenous nalbuphine and midazolam. FNA was performed using a 22-gauge needle. Rapid on-site evaluation for adequacy was carried out in all cases by a consultant cytopathologist. Cyst fluid aspirated was also subjected to analysis for CEA and amylase levels. PCR was performed on paraffin embedded blocks to assess for K-ras mutation at codon 12, 13, 59, 61, 117 and 46. Patients were followed up clinically and for subsequent histopathology, where available. Cases with limited material were rejected. Data was analyzed using SPSS 20 software. Qualitative and quantitative variables were analyzed by calculating mean, frequency and percentages. Sensitivity, specificity and positive and negative predictive values were calculated using 2 x 2 tables. Histological diagnosis and clinical follow up was used to identify true positive and false positive cases.

## Results

During the study period twenty-three patients with mucinous cystic lesions of the pancreas were diagnosed using cytology on EUS-FNA, in combination with cyst fluid CEA levels, endoscopic and radiological findings. There was an almost equal gender distribution, with a mean age of 67.4 years. In 6 (26%) cases the pancreatic lesions were detected as an incidental finding while investigating for other health conditions. Lesions were found to be equally distributed in the head and body (43.5 %), while only 3 cases (13.6%) were located in the tail of pancreas (table 1). Of the 23 cases evaluated, 65.2% cases were cystic, 26.1% were both solid and cystic while 8.7% were completely solid. Lesions ranged in size from 1.5 to 14.9 cm with a mean size of 4.5 cm. Radiologically, most patients (69.6%) were assessed by CT scan alone. Trans-abdominal ultrasonography was performed in addition to CT scan in 4 patients (17.4%). One (4.3%) patient was evaluated using CT scan and MRI both while no prior radiological investigation was carried out in 2 (8.7%) patients. Cytological diagnosis of mucinous cystic neoplasm (shown in fig. 1 and 2) was made in 16 cases (69.6%), intra-ductal papillary mucinous neoplasm was reported in 5 cases (21.7%) and a differential of MCN or IPMN was given in 2 cases (8.7%). Histological resection followed EUS-FNA in 6 (26%) cases, all of which were reported as MCN on cytology. Histological diagnosis was concordant with the cytological opinion in 4 cases. One of the reported MCN's turned out to be a serous cyst adenoma on evaluation of the resected specimen while another was reported as mucinous adenocarcinoma. Clinical follow up was available in 13 (56.5%) cases. One patient died due to another health condition (endometrioid adenocarcinoma) while 9 patients were lost to follow up. The sensitivity of EUS-FNA in diagnosing these lesions was 84.6% and 2 cases were false positive. Cyst fluid analysis for CEA levels was carried out in 16 patients and was found to be elevated in only 6 (37.5%) cases.

**Table 1: Demographics of the enrolled patients and characteristics of pancreatic cysts**

| | All | MCN | IPMN | MUCINOUS NEOPLASM |
|---|---|---|---|---|
| Total patients | 23 | 16 | 5 | 2 |
| Age (years) mean ± SD | 67 ± 13 | 66 ± 14 | 73 ± 9 | 72 |
| Sex M:F | 11:12 | 6:10 | 4:1 | 1:1 |
| Cyst Location H:B:T | 10:10:3 | 4:9:3 | 5:0:0 | 1:1:0 |
| Mean size, cm (range) | 4.5 (1.5-14.9) | 5.3 (1.7-14.9) | 2.7 (1.5-3.5) | 2.8 (1.7-4) |
| Histological diagnosis | 6 | 5 | 0 | 1 |
| Cyst fluid amylase, U/L Median (min-max) | 64 (39-2176) | 74 (49-2176) | 54 (39-94) | NA |
| CEA, ng/mL Median (min-max) | 21 (0.30-22191) | 7189 (0.30-22191) | 6.51 (5.09-35.70) | 7.38 |
| KRAS positive: negative | 4:5 | 4:4 | NA | 0:1 |

M: Male, F: Female, H: Head, B: Body, T: Tail

All the lesions with increased cyst fluid CEA levels were reported as MCN's. None of those diagnosed as being an IPMN had increased cyst fluid CEA levels. The range of CEA results in this group was 0.301 to 27,717 ng/mL with a mean of 4,434.6 ng/mL. The sensitivity of cyst fluid CEA levels was 37.5 % with a positive predictive value of 100%. Cyst fluid amylase levels were assessed in 10 cases and were increased in only one case (10%), taking 250U/L as a reference value [7]. However, the single case with an increased cyst fluid amylase level had a cytological and histological diagnosis of mucinous cystic neoplasm (shown in fig. 3 and 4). The specificity of cyst fluid amylase was 90% with a negative predictive value of 100%. PCR for K-ras mutation was performed in 9 cases including 7 MCN's, 1 serous cyst adenoma and 1 mucinous adenocarcinoma. K-ras mutation at codon 12 was detected in 3 out of 7 cases of MCN. The patient with a serous cyst adenoma did not harbor a K-ras mutation, while this mutation was detected in the patient with mucinous adenocarcinoma. None of the reported IPMN's was tested for K-ras mutation.

## Discussion

EUS FNA is of great importance in patient management because of its diagnostic accuracy. While benign conditions may not require intensive treatment and can be followed up clinically, malignant neoplasms usually require aggressive treatment, with either surgical resection (when possible), chemotherapy, with or without additional radiation treatment, or appropriate palliative care. Diagnostic accuracy varies according to the organ/site of aspiration. In pancreatic lesions, the most common lesions assessed by EUS-FNA, the diagnostic accuracy is 92.04% [11]. In a meta-analysis performed by Thosani et al, EUS-FNA cytology had a pooled sensitivity and specificity of 0.63 and 0.88 respectively [12].

Our study emphasizes the diagnostic utility of EUS-FNA, with a sensitivity of 84.6%. In a study conducted by Levy et al, the specificity, sensitivity, positive & negative predictive values of EUS-FNA for the diagnosis of pancreatic adenocarcinoma were 80.3%, 78.4%, 92.3%, 94.2%, and 75.0%, respectively [13]. False positive results rarely occur with EUS-FNA specimens. Gleeson et al reported a false positivity arte with EUS-FNA cytology of 5.3%, which they attributed to the occurrence of dysplasia or an autoimmune process [14]. We report 2 (8.7%) false positive cytological diagnoses, both of which were reported as MCN. One of these was subsequently diagnosed as mucinous adenocarcinoma while the other was a serous cyst adenoma on final histology. It is a well-established fact that mucinous adenocarcinoma can undergo cystic degeneration and create diagnostic difficulty, since it typically yields mucin and bland appearing cells on cytology.

In a study conducted by Stefano Crippa et al, mucinous cystic neoplasm of the pancreas showed a predilection for females (95%) and was mostly encountered in the tail region [15]. In another study, Abdullah Al-Rashdan et al [16] reported that cystic lesions of the pancreas were more prevalent in females, accounting for 73% of the study population and were mostly encountered in the head and neck region (77%). Our study showed an almost equal gender distribution and a mean age of 67.4 years. Lesions were equally distributed in the head and the body of the pancreas with only three cases located in the pancreatic tail.

Kucera et al proposed that even though CEA concentration in cyst fluid is valuable in the classification of cystic lesions of the pancreas, clinical decisions should not be based on CEA levels alone[17]. Brugge et al established that the optimal cut-off value of cyst fluid CEA for distinguishing mucinous from non-mucinous cysts is 192ng/ml. In the same study, cyst fluid CEA had a sensitivity of 75.0% for diagnosing a mucinous cyst with a specificity of 83.6% [8]. In our study a different pattern of cyst fluid CEA levels was noted. Cyst fluid CEA levels were elevated in 6 out of 11 (54.5%) cases of MCN while none of the IPMN's had any increase in cyst fluid CEA levels. However, the difference between the two was not statistically significant (p value = 0.446). The sensitivity of cyst fluid CEA levels was 37.5% with a positive predictive value of 100%. Specificity, however, could not be calculated because of the absence of true negative cases. Unfortunately, cyst fluid CEA levels were not assessed in patients with serous cyst adenoma. If performed, this would have added value to the utility of cyst fluid

CEA levels. Correa-Gallego et al suggested that CEA elevation in cyst fluid is not a predictor of malignant transformation within IPMN [18].

A pooled analysis of twelve studies carried out by van der Waaij et al concluded that cyst fluid amylase level of < 250U/L virtually excludes the possibility of a pseudocyst [19]. In our study, cyst fluid amylase levels were assessed in 10 cases and were found to be elevated in only one case, with a histologic and cytological diagnosis of MCN. Taking 250U/L as a cut-off, the specificity of cyst fluid amylase is 90%, with a negative predictive value of 100%. Jimenez et al demonstrated that sequential accumulation of mutations is involved in the tumorigenesis of MCN. K-ras mutations appear early in the disease process and increase with increasing dysplasia [20]. The PANDA study, carried out by Khalid et al, concluded that detection of the K-ras mutation in cyst fluid helps in diagnosis of mucinous cysts, with a specificity of 96%. The presence of high amplitude K-ras mutation and allelic loss has a specificity of 96% for malignancy and DNA analysis should be carried out when cytological examination negates the presence of a malignant process [21]. In our study, the K-ras mutation was found to have a sensitivity and specificity of 50% and 100% respectively for mucinous lesions of the pancreas. However, it cannot differentiate between mucinous cystic neoplasm and mucinous adenocarcinoma.

When used in combination with CEA levels, K-ras testing can increase the diagnostic yield of fine needle aspiration of cystic lesions. In surgically resected cysts of the pancreas, K-ras mutations have been found to have 100% specificity for mucinous differentiation, with a sensitivity of 54%. In a study conducted by Nikiforova et al, only 14 % of MCN exhibited the K-ras mutation, while in IPMN, KRAS prevalence was 67% [10].

In conclusion, EUS-FNA is a useful technique for evaluation of pancreatic cystic lesions, especially since cytological diagnosis can be augmented by cyst fluid CEA and amylase levels. K-ras analysis can add further to the diagnostic utility of EUS-FNA.

The current study is limited by the fact that it is a retrospective, single cancer centre study; with limited clinical and histological follow up. Additionally, all patients were selected with a disease, adding selection bias. Clearly, no comparison group was available. Of note, our results of EUS-FNA and cyto-histologic correlation are in concordance with previously reported studies but CEA levels were not always increased in mucinous lesions of the pancreas in our study population, which is rather unusual and warrants further study with a larger cohort [12-14].

**Statement of ethics:** Approval from institutional review board was taken prior to the research.
Conflict of interest: Authors have no conflict of interests to declare.

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**Author's contribution:**

- Concept and design: Asif Loya and Muhammed Aasim Yusuf
- Data collection and analysis: Sehar Bashir
- Preparation of initial draft/manuscript: Sehar Bashir
- Editing of manuscript: Asif Loya, Muhammed Aasim Yusuf, Umer Nisar Sheikh
- All authors approved the final manuscript.

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