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Research Article

Endoscopic Ultrasound (EUS) Guided Fine Needle Aspiration (FNA) Biopsy of Solid Pancreatic Lesions: A Review of 111 Cases and Comparative Study of Diff-Quik/PAP/Thin Prep Staining Techniques

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- EUS guided FNA
- Pancreatic malignancy
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- PAP

Abstract

Objective: Endoscopic Ultrasound (EUS) guided Fine Needle Aspiration (FNA) biopsy of the pancreas is a standard practice for diagnosis and staging of pancreatic malignancy. The material obtained by EUS guided FNA forms the basis of therapeutic decisions.

Methods: 111 cases of EUS guided FNA biopsy of the pancreas performed during the year January 2008 to December 2009 having solid pancreatic mass/lesion on the USG/CT and suspicious for the malignancy or malignant on clinical and radiological investigations were reviewed in August 2011 at the Department of Pathology and Laboratory Medicine, Hospital of University of Pennsylvania, Philadelphia U.S.A

Results: There was an 83.9% correlation between Diff-Quick diagnosis and the final cytological diagnosis. The overall diagnostic accuracy for the malignancy was 89.7%, sensitivity 90.6%, and specificity 100% of the cases where the cytological diagnosis was correlated with histological diagnosis and the other investigations. The positive predictive value for the malignancy was 100%. The false negative diagnosis was encountered in 10.3% cases. 51% of the cases showed intranuclear vacuoles, in the malignant cases on Diff-Quik Stain.

Conclusions: EUS FNA of the pancreas is a safe and reliable technique with high diagnostic accuracy, sensitivity, and specificity. The Diff-Quik stain smear is a useful technique for the rapid on-site cytological evaluation for the detection of malignancy of the pancreas. Thin Prep was found superior to the PAP / Diff-Quick stain for the diagnosis of the malignancy. The EUS FNA samples processed by multiple staining techniques help to improve the diagnostic accuracy and sensitivity.

ABBREVIATIONS

EUS: Endoscopic Ultrasound; FNA: Fine Needle Aspiration; PAP: Papanicolau

INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer-associated deaths in the United States and ranks ninth in its incidence [1]. EUS Guided FNA cytology has emerged as an important diagnostic tool since Vilman and Grimm (1992) made the first reports of endoscopic guided fine needle aspiration. In the last two decades, EUS-guided FNA has become popular in the clinical field, especially Western Countries [2]. Currently, EUS- guided FNA biopsy of the pancreas is a standard practice for diagnosis and staging of pancreatic malignancy [3]. Various published series on EUS guided FNA of Pancreas report diagnostic accuracy between 79-97%, sensitivity 77-95% and specificity 96-100%. False negative diagnosis range from 3.5-15% and false positive diagnosis range from 0-1% [4-13].

The present study was conducted for a short term research project of the Union International for Cancer control. The present study was carried out with the following objectives.

1. To find out the diagnostic accuracy, sensitivity, and specificity of EUS- guided FNA of the pancreatic malignancy by correlating cytological diagnosis with histological diagnosis and other investigations.

2. To know the diagnostic accuracy of air-dried smears using Diff-Quik Stain.

3. To compare the diagnosis obtained by Diff-Quik, conventional PAP Stain smears/ Cytospin Preparation and Thin Prep (Liquid Based Media).

Inclusion criteria: The cases with solid or solid cystic lesions suspicious or malignant on clinical and radiological investigations were selected for this study.

Exclusion criteria: Cystic lesions not suspicious or malignant on clinical and radiological investigations or pancreatitis were excluded from the study.

MATERIALS AND METHODS

111 cases of EUS guided FNA of Pancreas performed for the year January 2008 to December 2009 having a solid mass/lesion on Ultrasound/CT and suspicious for malignancy or malignant on clinical and radiological investigations were selected for the review in this study. The material for cytological examination was obtained from patients under conscious sedation by EUS guided FNA using 22 gauge needles by an experienced endosonographer in the endoscopic suite. An average 2 to 5 passes were used to obtain material for the cytological examination. The material obtained was processed for the Diff-Quik, PAP, Cytospin, Thin Prep and Cellblock as per institutional standard operating procedures. Direct smears were prepared on the glass slides for the Diff-Quik and the Pap staining from the samples obtained by EUS guided FNA. Remaining sample was poured into a normal saline container for Cytospin (49 cases) and Liquid-based media container for Thin Prep preparation. Diff-Quik stain slides were used for rapid on-site evaluation. PAP staining technique was used for the Cytospin preparation. The core biopsy and pancreatectomy specimen for histopathological examination were stained with Hematoxyline and Eosin stain. There were no major complications encountered. The cytology smears were evaluated by two pathologists independently with one having experience in EUS FNA for about two decades. The Cytological diagnosis was correlated with histological diagnosis and other investigations (Lymph node Aspirate, Liver Aspirate and Ascitic Fluid Cytology) to obtain the diagnostic accuracy, sensitivity, and specificity of the cytological diagnosis. EUS FNA samples were reported as

1. Unsatisfactory

2. Benign/Reactive

3. A typical

4. Suspicious

5. Malignant

RESULTS

Adequate cellularity was obtained in 111 cases for definitive final cytological diagnosis using a combination of multiple staining techniques Diff-Quik, PAP/Cytospin and Thin Prep. There were two unsatisfactory smears on the Diff-Quik stain and seven unsatisfactory smears on Pap/Cytospin and one on Thin Prep preparation. However, use of multiple staining techniques avoided unsatisfactory results on cytology.

Site within Pancreas

Head of the Pancreas was the commonest site affected, followed by the tail (Figure 1).

Sex Distribution

Males were more commonly affected than the females. Male: Female ratio was 1.13:1 (Figure 2)

Age Distribution

Pancreatic carcinoma was common in older age groups. Maximum age incidence was seen in the 7th decade (Figure 3) (Table 1).

Malignancy was the commonest diagnosis encountered followed by the suspicious for malignant and benign/reactive diagnosis. The diagnosis of malignancy was obtained in the 76.8% cases on Diff-Quik stain smears, 70.6% cases in the Thin Prep smears and 56.4% cases in the Pap/Cytospin stain smears (Figure 4,5) (Table 2).

The commonest diagnosis was adenocarcinoma in 36 (76.6%) out of 47 cases. Additional material was obtained from cell block and processed for immune histochemistry markers Thyroid Transcription Factor 1 to confirm metastases from the lung carcinoma (Table 3).

The final cytological diagnosis was malignancy in 83 cases (74.8%) followed by reactive/benign in 16 cases (14.4%) (Table 4).

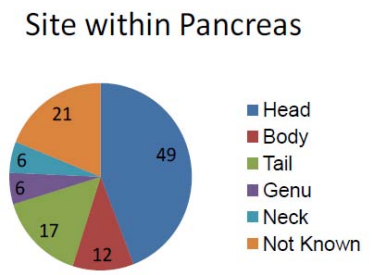

Figure 1 This pie diagram shows the distribution of cases at various sites within the pancreas.

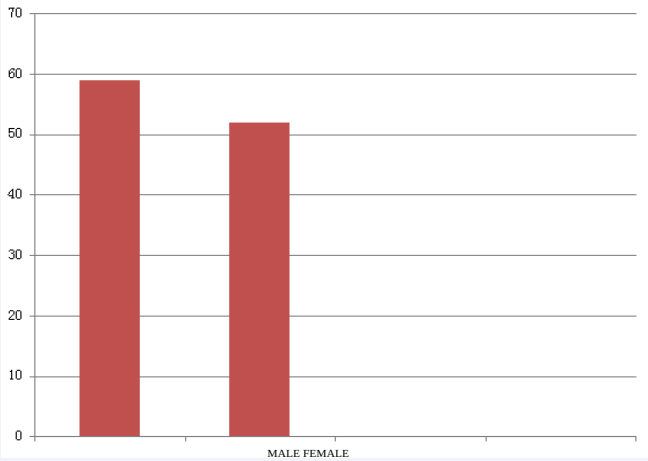

Figure 2 This bar diagram shows the gender distribution of cases.

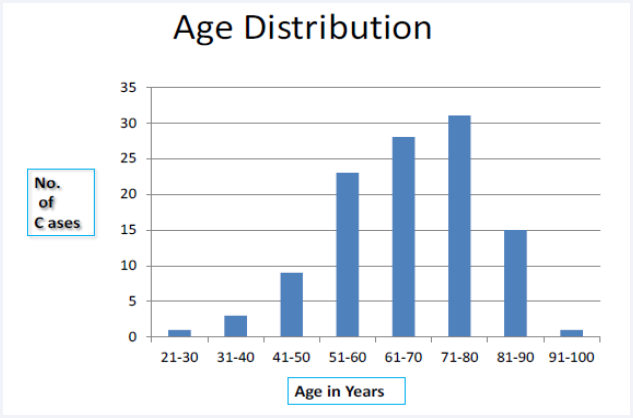

Figure 3 This bar diagram shows the age distribution of cases.

Table 1: Distribution of cases on Diff-Quik, Pap/Cytospin and Thin Prep.

Diagnosis	Diff-Quik No. of cases	Pap/Cytospin No. of cases	Thin Prep No. of cases
Benign/Reactive	04	17	10
Atypical	01	07	02
Suspicious for malignancy	06	17	12
Malignancy	43	62	72
Unsatisfactory	02	07	06
Total no. of cases	56	110	102

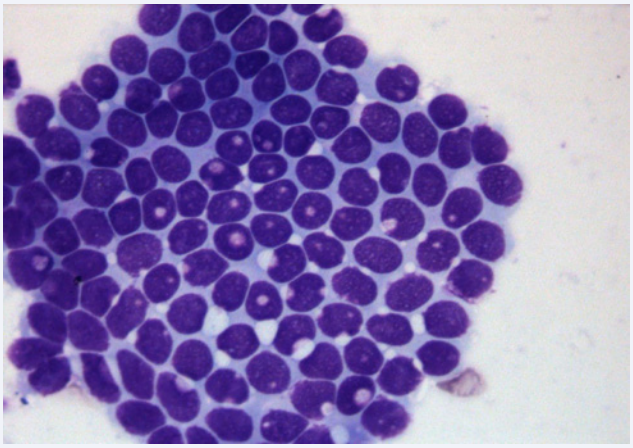

Figure 4 EUS-FNA smear from a case of Pancreatic Carcinoma shows malignant cells in the loosely cohesive cluster with intranuclear vacuoles. Diff-Quik Stain.400X.

There was 83.92% (47 out of 56 cases) correlation between the diagnosis of Diff-Quik and the final cytological diagnosis. If we include suspicious cases and malignant cases as malignant the correlation between the diagnoses improved further to 92.85% (52 out of 56 cases). The false negative diagnosis was encountered in 3.5% (2 out of 56 cases). 5 out of 6 cases in the suspicious group on the Diff-Quik stain smears were reported as malignant on the Pap /Thin Prep stain smears. This shows the superiority of the Pap/Cytospin and the Thin Prep smear staining over the Diff-Quik Stain (Table 5).

There was a 100 % correlation between the malignancy if malignant and suspicious of the malignancy cases were considered as malignant. In 3 out of 17 cases of the benign group, 2 out of 7 cases in A typical group and 4 out of 17 in a suspicious group on Pap/Cytospin smears were confidently reported as malignant on Thin prep smears. This shows the superiority of Thin Prep over Pap/Cytospin stain (Table 6).

In 39 patients where Cytological diagnosis was correlated with histopathology and other diagnostic investigations (Ascitic Fluid Cytology, Lymph node, and Liver FNA), the diagnostic accuracy of cytology were 89.75%, sensitivity 90.69%, and specificity 100% if suspicious for the malignancy were included as malignant. In the malignant group, there was a 100 % correlation and all the suspicious cases on cytology were found to be malignant on histopathology or other investigations. In the benign group on

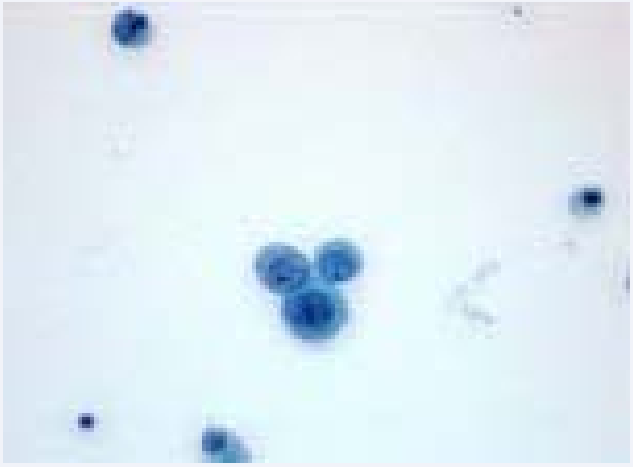

Figure 5 EUS- FNA smear from a case of Pancreatic Carcinoma shows malignant cells having enlarged vesicular nuclei with high N: C ratio and prominent nucleoli. Thin Prep.400X.

Table 2: Distribution of cases on cellblock.

Diagnosis	No. of cases
Benign/Reactive	04
Atypical	01
Suspicious	01
Adenocarcinoma	36
Metastatic Adenocarcinoma (Lung)	01
Insufficient	04
Total	47

Table 3: Final cytological diagnosis.

Diagnosis	No. of cases	Percentage (%)
Benign/Reactive	16	14.4
Atypical	03	02.7
Suspicious for malignancy	09	08.1
Malignant	83	74.8
Total no. of cases	111	100

Table 4: Comparison of Diff-Quik diagnosis with Pap and Thin Prep.

Diff-Quik	No. of cases	Pap and Thin Prep			
		Benign	Atypical	Suspicious	Malignant
Benign	04	03	0	0	01
Atypical	01	0	01	0	0
Suspicious	06	0	0	01	05
Malignant	43	0	0	01	42
Unsatisfactory	02	01	0	0	01
Total	56	04	01	02	49

Table 5: Comparison of Pap/Cytospin diagnosis and Thin Prep.

Diagnosis Pap/Cytospin	No. of cases	Thin Prep					
		Unsatisfactory	Benign	Atypical	Suspicious	Malignant	No material
Benign	17	01	09	0	0	03	04
Atypical	07	02	0	02	01	02	0
Suspicious	17	02	0	0	10	04	01
Malignant	62	0	0	0	02	58	02
Unsatisfactory	07	01	01	0	0	05	0
Total no. of cases	110						

Table 6: Correlation of cytological diagnosis with histopathology and other investigations.

Cytology diagnosis	No. of cases (%)	Histopathology diagnosis	No. of cases (%)	Other investigations No. of cases (%)
Malignant	28 (71.8%)	Malignant	21 (75%)	07 (25%)
Suspicious	07 (17.9%)	Malignant	06 (85.7%)	01 (12.3%)
Benign	04 (10.3%)	Malignant	02 (50%)	02 (50%)
Total	39 (100%)		29	10

Table 7: EUS FNA reported in the literature and present series.

Reference	Accuracy (%)	Sensitivity (%)	Specificity (%)	False negative (%)	False positive (%)
Chang et al,[7] 1994	87	91	100	14	0
Giovannini et al,[8] 1995	79	77	100	12	0
Gress et al,[9] 1997	87	89	100	NS	NS
Wiersema et al,[10] 1997	89	86	99	06	0
Bentz et al,[11] 1998	93	90	100	07	0
Williams et al,[4] 1999	86	84	96	15	0
Chhieng et al,[12] 2002	83 (97)*	74 (95)*	100	3.5	0
Shin et al,[13] 2002	80.3	81.7	100	13.2	0
Present series, 2011	89.7	90.6	100	10.3	0

EUS-FNA, Endoscopic Ultrasound-Guided Fine Needle Aspiration
NS: not specified
*Values in parentheses are those derived if atypical or "suspicious" diagnoses are considered diagnostic for malignancy.
Data in Table 7 reproduced from Jhala NC, et al. Am J Clin Pathol 2003:120:351-367

cytology, there were 4 (10.3%) false negative diagnosis and no false positive diagnosis was encountered.

DISCUSSION

Diff-Quik is a useful stain for preliminary on-site diagnosis as we reported malignancy in 43 (76.8%) out of 56 cases. The presence of the cytopathologist in endoscopic suit can avoid unsatisfactory aspirates by rapid on-site evaluation, obtain a preliminary diagnosis and collect additional samples for immunohistochemistry wherever needed [3]. The Cellblock was useful for confirming the cytological diagnosis and also confirmed metastases from the lung by additional immunohistochemical

staining for the Thyroid Transcription Factor 1. In the present series final cytological diagnosis of malignancy was obtained in the 74.8% cases. Williams et al (1999) [4] reported malignancy by EUS-FNA in 62% of patients with clinically suspicious lesions, Shah SM, et al (2008) [10]: reported malignancy by EUS- FNA in 87.8% of patients with a pancreatic mass.

We found that there is an 83.92% correlation between the diagnosis of Diff-Quik and the final cytological diagnosis. If we include suspicious cases and the malignant cases as malignant the correlation between the Diff-Quik and final cytological diagnosis further improved to 92.85%. We found intranuclear vacuoles in 51% cases (22 out of 43 cases) on Diff-Quik stain, but were rare on Pap stain (3 cases only). False negative diagnosis of 3.5% was encountered when we compared Diff-Quik and PAP/Thin Prep diagnosis. False negative on Diff-Quik was due to sampling error.

When we compared PAP/Cytospin with the Thin Prep Smear, there was a 100 % correlation between the malignancy if malignant and suspicious for malignancy were considered as malignant. 3 benign, 2 atypical and 4 suspicious cases on Pap/Cytospin smears, were confidently reported as malignant on Thin prep smears. Thin prep, therefore, improves diagnostic accuracy, sensitivity, and specificity due to better morphological features, improved cellularity, and clean background. This shows the superiority of Thin Prep preparation over Pap/Cytospin smears for the diagnosis of the malignancy. However, Lebalanc JK [11] et al in their prospective study found smear method more sensitive and accurate than Thin Prep in detecting malignancy from EUS FNA samples of the Pancreas. They reported sensitivity on Smear 98% and Thin Prep 62% and the diagnostic accuracy of the smear 98% and Thin Prep 64% (Table 7).

In 39 patients where the cytological diagnosis was correlated with the histological diagnosis and other investigations, the diagnostic accuracy of cytology was 89.7%, sensitivity 90.69%, specificity 100% and positive predictive value (PPV) 100% negative predictive value (NPV) 25% if suspicious for the malignancy is included as malignant. Our findings were compared with other reported series in the literature. The false negative diagnosis was encountered in 10.3% cases in the present series while it ranges between 3.5-15% in other reported series. The false negative diagnosis was encountered due to sampling error. No false positive diagnosis was encountered in the present series, which was comparable with other reported series.

A close interaction between endoscopist and the cytopathologist is essential to improve the diagnostic yield. The unsatisfactory sample is avoided if more than one pass is used for the cytological diagnosis and the material is obtained for the multiple staining techniques viz. Diff-Quik, Pap/Cytospin/Thin Prep and Cell Block [12,13]. The final diagnosis is always based on the clinical, EUS and the cytology features. Repeat EUS FNA is recommended in the cases where the diagnosis is suspicious for the malignancy or confirmed by the core biopsy.

CONCLUSION

EUS FNA of the pancreas is a safe and reliable technique with high diagnostic accuracy, sensitivity, and specificity. The Diff-Quik stain smear is a useful technique for the rapid on-site cytological evaluation for the detection of malignancy of the pancreas. We have reported intranuclear vacuoles as additional morphological criteria on the air-dried Diff-Quik smears for the diagnosis of malignancy which needs to be confirmed by the larger studies. Thin Prep was superior to the PAP/Diff-Quick stain and improved the diagnosis of the malignancy and also reduced the incidence of suspicious diagnosis in the malignant cases. We, therefore, recommend samples obtained by EUS FNA to be processed by multiple staining techniques to improve the diagnostic accuracy and sensitivity.

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CONFLICT OF INTEREST

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