A Promising Diagnostic Role of Immunohistochemical Expression of Insulin-Like Growth Factor II mRNA Binding Protein3 (IMP3) in Pancreatic Lesions Using Endoscopic Ultrasound –Guided –Fine Needle Aspiration (EUS-FNA) Cytology

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Abstract

Background: Poor prognosis and short survival of patients harboring pancreatic cancer emerge how advanced disease it is. In a trial to achieve the earliest and most accurate diagnosis to manage this progressive disease, we proposed that using endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) with an adjuvant diagnostic immunohistochemical marker would give better diagnostic results. IMP3 has gained recently wide attention, as many studies found that IMP3 has not only diagnostic but also prognostic role in different types of malignancies.

A**im of the study**: this prospective work is to assess the diagnostic role of EUS-FNA combined with the immunohistochemical expression of IMP3 on different benign and malignant pancreatic lesions.

Material and Method: the included pancreatic lesions (n=140) were obtained by EUS-FNA technique and stained for IMP3 immunohistochemically. Paraffin blocks from patients who underwent excision (n=92) or core biopsies (n=48) were performed for confirming diagnosis.

Results: the combined method for diagnosis showed that IMP3 was positive in 78.7%, 91.7%, 100% PAC, Mucinous neoplasm with high grade dysplasia, IPMN with high grade dysplasia, respectively, while almost all benign lesions showed negative IMP3. Also, this method showed sensitivity (78.26%), specificity (95.83%), and accuracy (84.3%).

Conclusion: EUS-FNA cytology with IMP3 could be a reliable diagnostic tool especially for assessment of malignant pancreatic lesions.

I. Introduction

Pancreatic cancer (PC) is considered one of the most lethal cancers with high mortality and morbidity. In 2021, an estimated 60,430 new cases of PC will be diagnosed in the US and 48,220 people will die from the disease. The death rate for PC has increased slightly (by 0.3% per year) since around 2000 (1). The 5-year relative survival of PC decreases with advanced stages at time of diagnosis, as the survival in localized disease is 39.4%, while apparently declines with regional (13.3%) and distant disease (2.9%) (2).

Patients with PC are usually presented with an advanced disease, so they are inoperable at time of diagnosis. An early and feasible diagnostic tool for diagnosis is needed. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is considered a safe and effective procedure for diagnosis of pancreatic lesions which is very important because of the risk for simultaneous or later malignancy development associated with benign lesions (3).

Insulin-like growth factor II messenger ribonucleic acid (mRNA) binding protein 3 (IMP3), a member of IMP family which involves IMP1, IMP2 and IMP3, is an oncofetal protein that contributes to different organs development including intestine, thymus, pancreas, and kidneys during embryogenesis (4, 5).

The evaluation of immunohistochemical markers like IMP3 protein to reach an appropriate discrimination between malignant and benign pancreatic lesions would essentially help in early diagnosis of pancreatic cancer and optimum patient management as several studies concluded that IMP3 is a promising marker for various malignancies (6–9). Overexpression was frequently seen in different tumor types and typically related to progressive tumor features. However, IMP3 was not expressed in various normal tissues, e.g., heart, striated muscle, uterus, esophagus, stomach, colon, kidney, urinary bladder, ovary, fat, skin, oral cavity, ectocervix, gallbladder, liver, pancreas, bone marrow, prostate, lung, breast, thyroid gland, cerebellum and cerebrum (10).

The aim of this work was to assess the diagnostic value of EUS-FNA procedure with the immunohistochemical expression of IMP3 in pancreatic lesions then comparing results with surgical resected specimens or core biopsies of unresectable specimens to reach an early and accurate diagnosis.

Ii. Material And Method

Patients' selection

This prospective study was held up in Pathology, Tropical and Surgical Departments, Faculty of Medicine, Zagazig University, Egypt, including 140 patients with pancreatic lesions. Samples were obtained by EUS-FNA procedure, and cytology specimens and/or cell blocks were prepared. Specimens from patients who underwent surgical resection or core biopsies were histopathologically evaluated for reaching the gold standard diagnosis, however cases who did not undergo either procedure were excluded. Approval has been obtained from the Institutional Review Board (IRB), Faculty of Medicine, Zagazig University. (approval number: 6130, May 30th, 2020). Patients' data was assessed including age, sex, site of the lesion, duct communication.

EUS-FNA specimens

EUS-FNA was performed with a 19, 22, or 25 gauge needle and an average of 3.1 ± 1.0 passes per session. The aspirate material was smeared onto a glass slide by air pressure and fixed with 95% ethanol for cytological evaluation. Cytology/ cell blocks specimens were fixed in formalin then embedded in paraffin. Staining with hematoxylin and eosin was done for histopathological diagnosis and with IMP3 marker for evaluation of protein expression. The diagnosis was considered as benign and malignant. Cystic fluid aspirates were investigated for levels of amylase enzyme and tumor markers CEA and CA19-9, IgG4.

IMP3 Immunohistochemical staining

Formalin-fixed paraffin-embedded cell blocks were serially sectioned into 3-5 μm and deparaffinized in xylene, then rehydrated in descending series of alcohols. For antigen retrieval processing, 10 mM citrate buffer (pH 6.0) at the microwave for nearly 20 min was used. Blocking of endogenous peroxidase was done by using 3% hydrogen peroxide for 10 min. Repeated washing in PBS was performed, then the

slides were incubated with primary antibody for mouse monoclonal antibody IMP3 (IGF2BP3 (E-2): sc-365640, 1:100 dilution, Santa Cruz Biotechnology). The polymer detection system; Dako EnVisionTM kit (Dako, Copenhagen, Denmark) was used. Finally, the tissue sections were counterstained with Meyer's hematoxylin.

IMP3 cytoplasmic expression was assessed semi-quantitatively according to both the extent of positively stained cancer cells and the intensity of stain at x100 then x400 HPF. The Intensity of staining was none, weak, moderate, and strong considered as 0, 1, 2, 3, respectively. The percentage of positive cells was calculated as <5 %; ≥5-<25 %; ≥25-<50 %; and ≥50 % and evaluated as 0, 1, 2, 3, respectively. A score was set by adding the two previous scores. A final score was considered as negative if <2, and positive if ≥2 (11).

Gold standard diagnosis (Surgically resected specimens or core biopsies)

The pancreatic specimens from patients who underwent surgical excision (n=92), and core biopsies (n=48) were collected, fixed in formalin, embedded in paraffin then stained with hematoxylin and eosin for setting the gold standard diagnosis relying on the clinical presentation and radiological findings. Then, the diagnosis was considered as benign and malignant to compare results with cytologic diagnosis and combined method.

STATISTICAL ANALYSIS

All data were collected, tabulated and statistically analyzed using SPSS 22.0 for windows (IBM Inc., Chicago, IL, USA) and MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium). Diagnostic performance of cytology, IMP3 IHC and their combination in diagnosis of pancreatic lesions was calculated depending on sample 2x2 contingency tables generation using the Tru-cut needle biopsy/surgical specimen pathology as the reference (Gold) standard. Sensitivity, specificity, positive predictive value, negative predicitive value, positive likelihood ratio, negative likelihood ratio and accuracy were calculated to compare between them. All tests were two sided. p-value < 0.05 was considered statistically significant (S), p-value < 0.001 was considered highly statistically significant (HS), and p-value ≥ 0.05 was considered statistically non-significant (NS).

Iii. Results

Patients' characteristics

This study included 140 specimens of different pancreatic lesions. The incidence rate of pancreatic lesions is higher in males (53.6%). The malignant lesions were higher in age group 55 years with slightly higher incidence in males. Diagnosis of these lesions comprises malignant lesions (n=92): pancreatic adenocarcinoma carcinoma (PAC) (n=47), intraductal papillary mucinous carcinoma (IPMN) with low (n=6) and high grade dysplasia (n=12), Mucinous cystic neoplasm (MCN) with low (n=6) and high grade dysplasia (n=12), solid pseudopapillary neoplasm (SPN) (n=9), and benign lesions (n=48): Serous

Microcystic Adenoma (SMA) (n=9), Pseudocyst (n=19), autoimmune pancreatitis (n=12) and chronic pancreatitis (n=8). The most common site for malignant lesions was the head followed by the body-tail (50%, 28.6%). Grossly, the pancreatic lesions were; mass nature (50%) are slightly higher than cystic lesions (45.7%) while the least common form is the mixed pattern (4.3%). Most of lesions present without duct communication (86.4%) **(Table 1, 2, 3, 4)**.

Results of the combined method of diagnosis (EUS-FNA with IMP3 immunohistochemistry)

The results of cytologic sampling obtained by EUS-FNA demonstrated that from 47 PAC, 72.3% were malignant by cytology compared by 78.7% by combined method (EUS-FNA with IMP3 immunohistochemistry). Regarding high grade MCN and IPMN, 1 case of MCN and 3 cases were detected by IMP3 immunohistochemistry over cytologic diagnosis. Two cases of low grade MCN, 2 of low grade IPMN and 2 of solid-pseudopapillary neoplasms were mistakenly considered benign by cytology, and correctly diagnosed as malignant depending on IMP3 expression. As regard the benign lesion, including serous microcystic adenoma, pseudocyst, chronic pancreatitis, and autoimmune pancreatitis were accurately benign by cytology, but only 1/9 of serous microcystic adenoma and 1/8 of autoimmune pancreatitis were positive for IMP3 and considered malignant **(Table 5, 6)**

Considering benign and malignant diagnosis only by the three methods, the results of combined method showed higher efficacy in diagnosing the malignant pancreatic lesions than depending on cytology only, and that was proved by reaching 78.2% of malignant lesions diagnosis compared with 64.1% by cytology only **(Table 6)** Fig. 1, 2, 3.

Assessment of sensitivity, specificity, and accuracy

Regarding using cytology (EUS-FNA) only for diagnosis of pancreatic lesions, the sensitivity was 64.13%; the specificity was (100%), and the accuracy was 89%. But the combination of cytological diagnosis using EUS-FNA with the expression of IMP3 in diagnosis showed sensitivity (78.26%), specificity (95.83%), and accuracy (84.3%). So, IMP3 with cytology has higher sensitivity, while cytology has more specificity and accuracy **(Table7).**

Vi. Discussion

Pancreatic cancer (PC) is characterized by poor prognosis, decreased survival and inoperable advanced stages. Presenting at advanced stages is considered a crucial point in patients' survival. Therefore, an early and accurate diagnosis is needed for those patients, hoping for early management that could improve prognosis and prolong survival. A study done by **Pasiliao et al 2015** observed a decrease in motility, invasion, and matrix adhesion following IMP3 knockdown suggesting that IMP3 promotes PAC progression by enhancing the pro-metastatic behavior of tumor cells (12). Moreover, the immunohistochemical IMP3 expression was considered a poor prognostic predictor in different malignancies as mucoepidermoid carcinoma of salivary glands, duodenal papillary carcinoma and pilocytic and pilomyxoid astrocytomas (13–15).

Also confirming the diagnostic importance of IMP3, using quantitative real-time RT-PCR by **Yantiss et al 2005** showed that mRNA IMP3 was highly expressed in pancreatic carcinomas (79%) (16). Also, **Wang et al 2015** demonstrated that increased mRNA IMP3 expression was associated with poor overall survival and considered as an independent risk factor and they suggested that combination with microdissection techniques to evaluate IMP3 mRNA expression in frozen pancreatic lesions can be worthy for diagnosis of PC (17).

Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) combined with evaluation of IMP3 immunohistochemical expression is a promising tool for achieving this goal. A meta-analysis study showed that the results of IMP3 immunostaining may be a useful diagnostic tool for confirming PAC but should be evaluated in parallel with the gold standard of histopathological morphology and clinical findings. Also IMP3 was suggested to be diagnostic tool for duodenal papillary carcinoma and endometrial cancers and their premalignant lesions (14, 18–19).

EUS approach is not only minimally invasive technique with low complications but also it provides us with high resolution images for different pancreatic lesions and may determine tumor stage. Many studies studied the combined diagnostic tool of (EUS-FNA) with IMP3 expression for discriminating malignant from benign lesions. As **Yantiss et al 2008** demonstrated that EUS-FNA for PAC expressed IMP3 in 92% of the cases, while all cases of chronic pancreatitis were IMP3 negative (20). **Zhao et al 2007** found that all benign cases expressed negative IMP3; IMP3 was expressed in 88% of PAC; suspicious cases were positive for IMP3; Cytology results combined with IMP3 expression revealed 95% positivity in PAC (21). IMP3 staining was positive in 97%, 79% of PAC, high grade dysplasia, respectively, while in mild to moderate dysplasia, IMP3 showed negative staining. Generally, IMP3 showed strong to moderate intensity (16). These results were agreeing with ours, as we found that high IMP3 was expressed in 77.8%, 78.7%, 91.7%, and 100% of SPN, PAC, mucinous neoplasm with high grade dysplasia, and IPMN with high grade dysplasia, respectively.

In low grade dysplasia, 2/6 cases of mucinous neoplasm and 3/6 cases of IPMN were positive for IMP3 staining. However, benign lesions showed negative staining in all cases of benign lesions except one case of serous microcystic adenoma and a case of autoimmune pancreatitis which were diagnosed by cytology as benign. Also, A study analyzed the expression of 26 immunohistochemical markers confirmed the diagnosis of PAC in both surgical and fine-needle aspiration specimens using the best diagnostic panel of immunomarkers including pVHL, maspin, S100P, and IMP3. IMP3 was 90% positive (intermediate to weak intensity) in PAC in surgical specimens and normal pancreatic ducts were usually negative for IMP-3, while in FNA specimens, IMP3 was positive in 93% of PAC compared with 77% and 10% for suspicious and benign cases, respectively (22).

The incidence of pancreatic cystic lesions is rising due to increase use of imaging techniques as part of routine clinical practice. Some cystic lesions have the potential for malignant neoplastic transformation and are considered malignant precursor for pancreatic ductal adenocarcinoma such as intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) whereas serous cystic

neoplasms (SCNs) are considered benign lesions. **Ezzat et al 2016 and Senoo et al 2018** demonstrated sensitivity, and specificity of IMP3 on cytology specimens for PAC were 91.2%, 86.7%, and 87.9%, 100%, respectively, with total accuracy 90.3% and 90.8%, respectively (23–24). Our findings concluded that using of combined IMP3 immunohistochemical staining with cytology diagnosis of malignant and benign pancreatic lesions has sensitivity, and specificity of 78.2% and 95.8% with total accuracy 84.3% compared with 64.1%, 100%, and 89% sensitivity, specificity, and total accuracy of cytology diagnosis only, respectively.

Limitations of the study:

Many problematic issues may arise due to limited skills of the endoscopy operator in terms of insufficient tissue yield and targeting-error, misinterpretation and misdiagnosis by pathologists and absence of on-site cytopathologists for adequacy assessment.

In conclusion, for diagnosing malignant pancreatic lesions, IMP3 expression based on EUS-FNA sampling could be very valuable. However, benign lesions rely more accurately on cytologic findings by EUS-FNA than IMP3 expression. Further research is recommended on largest samples of malignant and benign pancreatic lesions.

Declarations

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Tables

Table (1): Diagnosis of the studied patients (N=140).

Diagnosis	The studied patients (N=140)	
	Number	Percent
Cytologic diagnosis		
Malignant	59	42.1%
Benign	81	57.9%
Cytologic + IMP3 diagnosis		
Malignant	74	52.9%
Benign	66	47.1%
Gold standard diagnosis		
Malignant	92	65.7%
Benign	48	34.3%

Table (2): Gold standard diagnosis of the studied patients (N=140).

Gold standard diagnosis	The studied patients (N=140)	
	Number	Percent
Pancreatic Adenocarcinoma	47	33.6%
Mucinous neoplasm with high grade dysplasia	12	8.6%
Mucinous neoplasm with low grade dysplasia	6	4.3%
IPMN with low grade dysplasia	6	4.3%
IPMN with high grade dysplasia	12	8.6%
SPN	9	6.4%
Serous microcystic Adenoma	9	6.4%
Pseudocyst	19	13.6%
Pancreatitis	12	8.6%
Autoimmune pancreatitis	8	5.7%
Total	140	100%

Table (3): Comparison between benign and malignant pancreatic lesions regarding demographic data

Demographic data	All Studied patients (N=140)		Gold standard diagnosis				Test	p-value (Sig.)
			Malignant (N=92)		Benign (N=48)			
	No.	%	No.	%	No.	%		
Age (years)								
<55 years	56	40%	27	29.3%	29	60.4%	12.686[a]	<0.001 (HS)
=>55 years	84	60%	65	70.7%	19	39.6%		
Mean ± SD	56±14.5749		58.3261±15.56561		53.2917±11.92166		-2.956 [b]	<0.003 (HS)
Median (Range)	58 (12 – 85)		62.5 (12 – 85)		52 (30 – 77)			
Sex								
Male	75	53.6%	52	56.5%	23	47.9%	0.939[a]	0.333 (NS)
Female	65	46.4%	40	43.5%	25	52.1%		

a: Chi-square test; b: Mann Whitney U test; p-value< 0.05 is significant; Sig.: Significance.

Table (4): Comparison between benign and malignant pancreatic lesions regarding imaging findings

Imaging findings	All Studied patients (N=140)		Gold standard diagnosis				Test[a]	p-value (Sig.)
			Malignant (N=92)		Benign (N=48)			
	No.	%	No.	%	No.	%		
Site of lesion			-					
Head	70	50%	59	64.1%	11	22.9%	52.156[a]	<0.001 (HS)
Body-tail	40	28.6%	29	31.5%	11	22.9%		
Entire pancreas	11	7.9%	4	4.3%	7	14.6%		
Extra-pancreatic	19	13.6%	0	0%	19	39.6%		
Nature			-					
Mass	70	50%	54	58.7%	16	33.3%	9.395[a]	0.009 (S)
Cyst	64	45.7%	36	39.1%	28	58.3%		
Mixed	6	4.3%	2	2.2%	4	8.3%		
Duct communication			-					
No	121	86.4%	73	79.3%	48	100%	11.470[a]	0.001 (S)
Yes	19	13.6%	19	20.7%	0	0%		

a: Chi-square test; p-value< 0.05 is significant; Sig.: Significance.

Table (5): Comparison between cytological diagnosis and gold standard diagnosis

Cytologic diagnosis		Gold standard diagnosis		Total
		Malignant	Benign	
Malignant	No.	59	0	59
	%	42.1%	0%	42.1%
Benign	No.	33	48	81
	%	23.6%	34.3%	57.9%
Total	No.	92	48	140
	%	65.7%	34.3%	100%
Test[c]		31.030		
p-value (Sig.)		<0.001 (HS)		

c: McNemar’s test; p-value< 0.05 is significant; Sig.: Significance.

Table (6): Comparison between Cytologic+IMP3 and gold standard diagnosis

Cytologic+IMP3 diagnosis		Gold standard diagnosis		Total
		Malignant	Benign	
Malignant	No.	72	2	74
	%	51.4%	1.4%	52.9%
Benign	No.	20	46	66
	%	14.3%	32.9%	47.1%
Total	No.	92	48	140
	%	65.7%	34.3%	100%
Test[c]		13.136		
p-value (Sig.)		<0.001 (HS)		

c: McNemar’s test; p-value< 0.05 is significant; Sig.: Significance.

Table (7): Diagnostic performance of cytology and cytology+IMP3 IHC for diagnosis of malignant pancreatic lesions

	Cytology	Cytology+ IMP3 IHC
TP	59	72
FP	0	2
TN	48	46
FN	33	20
SN (95%CI)	64.13% (53.457 – 73.867)	78.26% (68.44 – 86.187)
SP (95%CI)	100% (92.06 – 100)	95.83% (85.746 – 99.49)
PPV (95%CI)	100%	97.297% (90.225 – 99.293)
NPV (95%CI)	59.259% (52.534 – 65.654)	69.69% (60.843 – 77.296)
Positive LR (95%CI)		18.78 (4.871 – 73.255)
Negative LR (95%CI)	0.359 (0.273 – 0.471)	0.227 (0.153 – 0.336)
Accuracy (95%CI)	89% (52.2 – 97.6)	84.3% (74.4 – 90. 7)
AUC (95%CI)	0.821 (0.747 – 0.88)	0.870 (0.803 – 0.921)

TP: True positive; FP: False positive; TN: True negative; FN: False negative; SN: Sensitivity; SP: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; LR: Likelihood Ratio; AUC: Area Under Curve; CI: Confidence Interval.

Figures

Figure 1

A- Cytology showed pleomorphic malignant cells with hyperchromatic nuclei of pancreatic adenocarcinoma (H&E x400) B- IMP3 immunohistochemical expression was positive in cell block of PAC (IHC x400).

Figure 2

A- MCN high grade showed dysplastic mucinous cells with high grade dysplasia in cell block (H&E x400) B- MCN high grade showed high expression of IMP3 in cell block (IMP3 IHC x400)

Figure 3

Core biopsy showed well differentiated pancreatic duct adenocarcinoma (H&E x400)