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FULL PAPER

The diagnostic utility of fibroblast activating protein (FAP) alpha in relation to transforming growth factor (TGF) beta and beta catenin immunohistochemical markers in pancreatic adenocarcinoma versus pancreatitis

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Pancreatic adenocarcinoma and chronic pancreatitis may present complex challenges in diagnosis due to overlapping clinical and histopathological features. Pancreatic adenocarcinoma is a particularly lethal neoplasm with a grim 5% overall 5-year survival rate. Recent research underscores the pivotal role of the tumor microenvironment in neoplastic initiation and progression, with stromal components supporting tumor growth, invasion, and metastasis. Fibroblast Activation Protein alpha (FAPα) and TGF-beta have been identified as significant players in this context. This study collected paraffin blocks from 32 patients with pancreatic adenocarcinoma and 11 patients with chronic pancreatitis to investigate the expression and localization of FAPα in relation to TGF-beta, and B-Catenin expressions. Both patient groups revealed a higher prevalence of males, but no statistically significant gender difference. Notably, age emerged as a crucial distinguishing factor, with pancreatic adenocarcinoma patients being notably older. Biomarker analysis showed that B-Catenin was the superior diagnostic marker, detecting 68.6% of cases, particularly in late stages, and exhibiting a positive relationship with tumor size and spread. FAP α plays a critical role in early detection, identifying 40.6% of cases and showing a weak negative relationship with tumor size and spread. Combining B-Catenin and FAP α proved to be the most effective approach, detecting all cases with variable intensity. These findings have significant implications for improving early and accurate diagnosis, which can lead to more tailored management, however further research is still needed to validate and refine diagnostic approaches as our understanding of these diseases evolves.

KEYWORDS
Pancreatic carcinoma; immunohistochemistry; fibroblast activating protein; transforming growth factor; tumor makers.

Introduction

Pancreatitis and pancreatic carcinoma are two significant diseases that affect the pancreas, and their exact pathogenesis is not fully understood. Pancreatic carcinoma is one of the most fatal neoplasms as it is the 7th primary cause of cancer mortality, and it is estimated to be the 3rd primary cause of

cancer mortality by 2025 and the second cause by 2030 (1,2). It has enormously bad prognostic criteria with an overall 5-year survival is 5%. The mainstream of pancreatic carcinoma patients is detected with advanced stages either with local invasion or metastases, making the neoplasm to be un-respectable by surgery. Whereas chemo- and radio-therapy function as alternate treatment options, the results of these approaches continue to be disappointing (3,4).

Lately, light has been shed on the microenvironment role in the growth and development of malignant neoplasms. Previous related studies propose that the tumor microenvironment is interrelated with neoplastic angiogenesis, proliferation and spread, distant metastasis, immune evasion and treatment resistance (5).

In pancreatic carcinoma, cells of the stroma and stromal components in neoplastic microenvironment can sustain and stimulate neoplastic cell development, invasion and distant metastasis (6).

Furthermore, immune cells penetration and the pancreatic stroma structure are linked to the progression-free patient survival after resection of pancreatic carcinoma (7). The plentiful pancreatic carcinoma stroma is formed by cancer-associated fibroblasts or the pancreatic stellate cells (PSCs).

Closeness to neoplastic cell triggers PSCs and leads to amplified cell proliferation, movement, and manufacture of extracellular matrix (ECM). Simultaneously, activated PSCs help neoplastic proliferation, penetration of matrix, metastasis and cancer stem cell preservation via various growth factors and signals of immune modulation (8).

Fibroblast Activation Protein (FAPα), one of the prolyl-specific serin proteases family, contributes in tumor cell local invasion and distant metastasis. As a result of its marked expression in fibroblasts of neoplastic stroma, FAPα has been regarded as stimulated fibroblasts marker in various cancer types and was concerned in different functions of neoplastic development.

It has been suggested that FAPα+ PSCs in pancreatic carcinoma microenvironment play a significant role in the tumor evolution (9,10).

Transforming growth factor beta (TGF-beta) was basically designated as a hormonal polypeptide which can transform target cells malignant and applies critical effects on cell cycle growth and regulation, differentiation, synthesis of extracellular matrix (ECM), chemotaxis, blood cells synthesis, and the immune response (11).

Former investigations have established that malignant cells microenvironment obstructs the immune reaction by generating factors like TGF-β1, to escape T-cell immune response. Other researches have shown that TGF-β1 can lead to epithelial-mesenchymal transformation (EMT), causing upsurges in migration of malignant cells. It was previously concluded that TGF-beta is well thought-out as a core player in tumorigenesis as it applies numerous context-related impacts on all functioning neoplastic cells.

A distinct role was documented for TGF-beta-activated cancer associated fibroblasts which are rich in cancer stromal cells (12-14).

Further research is always needed to fully investigate these proteins expression in both inflammatory and carcinogenic cases and their correlation with clinical features of the diseases, where the FAP alpha expression has been studied and compared between cancer and normal pancreatic tissue and seen significantly higher in primary tumor tissues than the normal tissues, but still limited in the challenging pancreatitis cases.

Therefore, this study aimed to investigate the expression and localization of FAP, TGF-beta, and beta-catenin in pancreatic tissue in patients with pancreatitis and pancreatic carcinoma, and to correlate their expression and localization with clinical features of the diseases.

Material and methods

Clinico-pathological data

Following the research approval granted by the Ethical Committee of the Faculty of Medicine at our University, a total of 32 paraffin blocks from pancreatic carcinoma cases and 11 paraffin blocks from pancreatitis cases were obtained from the pathology departments' archives at the university hospitals in Cairo. The clinical data, which encompassed the patient's age, sex, tumor size, metastatic status, preoperative treatment status, and tumor recurrence, were gathered from the pathology records and patients' medical files. In cases where necessary, communication with the relevant physician was established to get the required information.

The inclusion criteria for this study encompassed individuals who had undergone a Whipple procedure performed by the surgical oncology team after getting general anesthesia at our institution and were diagnosed with primary adenocarcinoma of the pancreas or chronic pancreatitis .

The exclusion criteria encompassed patients who had insufficient clinical data, non-representative tumor samples, substantial or whole tumor necrosis, and recurring tumors without data of the initial. All instances were treated with anonymity and managed in accordance with established ethical protocols.

Radiological assessment of the tumors

All included cases' CT scans were revised by the radiologist author to record the findings and the maximum dimension of primary pancreatic tumors measured in any direction (on pre-operative CT). Three tumor dimensions were also reported by the same radiologist for each pancreatic tumor on almost all scans.

Histopathological evaluation

Histological sections stained with hematoxylin and eosin (H&E) were generated to re-validate the diagnosis, evaluate the grade of the tumor, determine the existence of tumor necrosis (defined as an area of necrotic tumor cells adjacent to viable tumor tissue), measure the depth of invasion, and identify any lymphovascular and perineural invasion. The histopathological grading of pancreatic carcinoma holds significant prognostic value. To ensure accuracy, this grading process involves the examination of all slides by a minimum of three histopathologists on-site. Remote histopathological consultation is sought when necessary, adhering to established the WHO criteria. These criteria encompass various factors such as the distinction between tubular structures and solid growth, the presence of mucin, nuclear polymorphism, and the count of mitoses. Poorly differentiated carcinomas exhibit a lesser degree of development or absence of the desmoplastic stromal response (15).

Immunohistochemical staining

The paraffin-embedded tissue blocks were sectioned into slices with a thickness of 5 µm. These slices were then subjected to deparaffinization using xylene, followed by rehydration using a series of ethanol solutions with decreasing concentrations. The antigen retrieval process involved incubation with a sodium citrate buffer at a pH of 6.0. The sections obtained from each case were subjected to overnight incubation at a temperature of 4 °C in the presence of the antibodies. Subsequently, the slides underwent counter-staining using hematoxylin. The stained slides, prepared using the immunohistochemical technique, were evaluated by two pathologists who were unaware of the clinicopathological characteristics of the patients.

Evaluation of immunohistochemical staining

The assessment of FAP α staining was conducted by three independent examiners, who evaluated the stained area and intensity as separate variables. In this study, a numerical value of 0 was designated to denote the presence of a stained area containing less than or equal to 10% of the tumor cells. Similarly, a value of 1 was assigned to indicate an area with tumor cells ranging from 11% to less than or equal to 25%. Moreover, a score of 2 was utilized to signify an area with tumor cells exceeding 26% up to 50%, while a score of 3 was employed to represent an area with tumor cells surpassing 51%. The staining intensity was evaluated using a scoring system. A value of 0 was given to samples with zero or weak staining, which served as the negative control. A score of 1 was provided to samples with weak staining that was noticeably stronger than the negative control. Samples with moderately intense staining were given a score of 2, while those with intense staining received a score of 3. The overall grade of the section was determined by combining the marks for stained area and intensity. This assessment of FAP α expression was conducted following the methodology suggested by Cao *et al.* (16).

The cytoplasmic staining of the β-catenin marker was assessed and categorized into four levels based on staining intensity: negative (0), faintly positive (1), moderately positive (2), or strongly positive (3). In addition, an assessment was conducted on the membrane staining of β-catenin. In instances when membrane staining was absent, the absence of cytoplasmic staining was also observed. White *et al.* (17), deemed the highest score from each sample as typical for analysis.

The staining strength of the TGF beta marker was assessed using a scoring system, where a score of 0 indicated a negative result, a score of 1 indicated weak staining, a score of 2 indicated intermediate staining, and a score of 3 indicated strong staining. The scoring of the percentage of cells exhibiting positive staining was as follows: 0 denoted the absence of staining, 1 indicated staining in 1-50% of cells. According to Yin *et al.* (18), the final staining score was determined by multiplying the intensity score with the percentage score.

Statistical analysis

The statistical analysis was performed using SPSS (version 20) software in Chicago, IL, USA. The median and standard deviation were computed for the age of the patients. The association between the expression of biomarkers and clinicopathological variables was assessed using the Chi-square (χ^2) test. A Spearman correlation coefficient (r) was utilized to evaluate the association between the expression patterns of RRBP1 and p53-immunophenotype. The statistical significance of the results was determined based on a p-value threshold of less than 0.05.

Results

The studied cases were divided into two groups, group I (carcinoma) and group II (chronic pancreatitis); Male predominance was detected in both groups with no significant difference in the studied groups. However, a significant difference is seen in the age groups where the pancreatic carcinoma affected older age groups (Table 1).

Regarding to the tumor size of the carcinoma, the majority of cases were between two to four cm in maximal diameter according to the radiological and pathological measurements giving a T2 stage (TNM staging system) and the most frequent anatomical site affected was the head of the pancreas rather than tail or the body of pancreas. The vascular permeation was seen in the majority of cases in our study where 75% of cases were involving the vessels according to the histopathological examination, in addition to

the perineural invasion that was seen in nearly 85% of the studied cases (Table 2).

Computed tomography (CT) scan findings in the group I was informative for malignant diagnosis (Figure 1) without clear evidence of the specific type (adenocarcinoma) which was provided on histopathology (Figure 2), however in group II (chronic pancreatitis) radiological examination revealed atrophy in 9% of cases, enlargement in 9% of cases, enlargement with calcification in 45% of cases, irregular area in 9% cases, irregular firm area in 9% of cases and mass lesions (irregular mass) in the rest 18 % of the studied chronic pancreatitis cases (Table 3).

FAP α expression (Figure 3) in pancreatic cancer is shown in Table 5 with no significant difference between the expression and the patients' age, sex, tumor size, site or invasion and Beta catenin expression is presented in Table 4 in the pancreatic cancer cases revealing no significant difference in relation to sex, age, tumor size, anatomical site or the vascular invasion. Table 6 lists the expression of TGF beta in the cancer cases, also no significant difference between the expression and all the previous parameters (Figure 4).

Expression of beta catenin shows a significant difference in both groups (carcinoma and pancreatitis) as well as the FAP α marker and the use of both markers may be helpful for diagnosis of the challenging cases. β-catenin was expressed (moderate and strong expression) in 24 out of 32 cases of pancreatic carcinoma (75%), but 25% of adenocarcinomas were negatively expressed β-catenin (Figure 5). However, TGF beta was only weak expressed in 25% of cancer cases and no expression in all pancreatitis cases with no significant difference and likely no diagnostic utility of this marker in differentiation.

TABLE 1 A comparison between the two studied groups according to demographic and immunohistochemical expression

	Pancreatic adeno carcinoma (n = 32)	**Chronic pancreatitis (n = 11)**	**Test of Sig.**	***P*-value**
Demographic data				
Sex				
Male	27 (84.4%)	8 (72.7%)	χ^2=	^{FE}p=
Female	5 (15.6%)	3 (27.3%)	0.733	0.401
Age (years)				
Mean ± SD.	60.7 ± 6.1	41.4 ± 9.1	t=	<0.001*
Median (Min. – Max.)	60 (48 – 72)	41.0 (28.0 – 55.0)	6.528*	
Immunohistochemical markers' expression				
B-Catenin				
Negative	0 (0%)	11 (100.0%)	χ^2=	^{FE}p
Weak	0 (0%)	0 (0%)	40.557*	<0.001*
Moderate	10 (31.3%)	0 (0%)		
Strong	22 (68.8%)	0 (0%)		
FAP				
Negative	0 (0%)	5 (45.5%)	χ^2=	^{FE}p
Weak	3 (9.4%)	6 (54.5%)	30.164	<0.001*
Moderate	16 (50%)	0 (0%)		
Strong	13 (40.6%)	0 (0%)		
TGF				
Negative	24 (75.0%)	11 (100.0%)	χ^2=	^{FE}p=
Weak	8 (25.0%)	0 (0%)	3.379	0.090

SD: **Standard deviation**, **t: Student t-test**, χ^2: **Chi square test**, FE**: Fisher Exact**, and *P*-value: *P*-value for comparing between the two studied groups.
*: Statistically significant at p ≤ 0.05.

TABLE 2 Distribution of the studied cases according to different parameters in Pancreatic carcinoma group (n = 32

	No. (%)
Perineural	27 (84.4%)
Vascular	24 (75.0%)
Grade II	32 (100%)
Tumor size (cm)	
<2cm(T1)	2 (6.3%)
≥2 - <4cm (T2)	22 (68.8%)
≥4cm (T3)	8 (25.0%)
Mean ± SD.	3.3 ± 1.3
Median (Min. – Max.)	3 (1.8 – 7.5)
Radiological CT - Findings	
Body Mass	2 (6.3%)
Head Mass	22 (68.8%)
Periampullary	8 (25.0%)

TABLE 3 Distribution of the studied cases according to Radiological CT-Findings in chronic pancreatitis group (n = 11)

	No. (%)
Radiological CT - Findings	
Atrophy	1 (9.1%)
Enlargement	1 (9.1%)
Enlargement with calcification	5 (45.5%)
Irregular area	1 (9.1%)
Irregular firm area	1 (9.1%)
Irregular mass	2 (18.2%)

TABLE 4 Relation between B-Catenin and different parameters in Pancreatic adeno carcinoma (n = 32)

	B-Catenin		Test of Sig.	P-value
	Moderate (n= 10)	Strong (n= 22)		
Sex				
Male	7 (70%)	20 (90.9%)	χ^2=2.280	FEp= 0.293
Female	3 (30%)	2 (9.1%)		
Age (years)				
Mean ± SD.	58.5 ± 6.9	61.6 ± 5.6	t=1.366	0.182
Median (Min. – Max.)	58 (48 – 70)	61.5 (49 – 72)		
Perineural				
Negative	1 (10%)	4 (18.2%)	χ^2=0.349	FEp= 1.000
Positive	9 (90%)	18 (81.8%)		
Vascular				
Negative	2 (20%)	6 (27.3%)	χ^2=0.194	FEp=

Positive	8 (80%)	16 (72.7%)		1.000
Tumor size (cm)				
<2cm	1 (10%)	1 (4.5%)	χ^2=0.823	MCp= 1.000
≥2 - <4cm	7 (70%)	15 (68.2%)		
≥4cm	2 (20%)	6 (27.3%)		
Mean ± SD.	2.8 ± 1	3.5 ± 1.4	U=65.0	0.070
Median (Min. – Max.)	2.5 (1.8 – 5)	3 (1.8 – 7.5)		
Radiological CT - Findings				
Body Mass	1 (10%)	1 (4.5%)	χ^2=1.090	MCp= 0.559
Head Mass	6 (60%)	16 (72.7%)		
Periampullary	3 (30%)	5 (22.7%)		

χ^2: **Chi square test**, MC: **Monte Carlo**, FE**: Fisher Exact**, **t: Student t-test**, **U: Mann Whitney test**, and *P*-value: *P*-value for comparison between the studied categories.
*: Statistically significant at p ≤ 0.05.

TABLE 5 Relation between FAP α and different parameters in Pancreatic carcinoma (n = 32)

	FAP			Test of Sig.	*P*-value
	Weak (n= 3)	**Moderate (n= 16)**	**Strong (n= 13)**		
Sex					
Male	3 (100%)	12 (75%)	12 (92.3%)	χ^2=1.689	MCp= 0.470
Female	0 (0%)	4 (25%)	1 (7.7%)		
Age (years)					
Mean ± SD.	55.7 ± 4	61.2 ± 6.3	61.2 ± 6.1	F=0.013	0.909
Median (Min. – Max.)	55 (52 – 60)	59.5 (49 – 72)	62 (48 – 71)		
Perineural					
Negative	0 (0%)	4 (25%)	1 (7.7%)	χ^2=1.689	MCp= 0.042
Positive	3 (100%)	12 (75%)	12 (92.3%)		
Vascular					
Negative	2 (66.7%)	3 (18.8%)	3 (23.1%)	χ^2=2.874	MCp= 0.265
Positive	1 (33.3%)	13 (81.3%)	10 (76.9%)		
Tumor size (cm)					
<2cm	0 (0%)	1 (6.3%)	1 (7.7%)	χ^2=1.255	MCp= 0.010
≥2 - <4cm	2 (66.7%)	11 (68.8%)	9 (69.2%)		
≥4cm	1 (33.3%)	4 (25%)	3 (23.1%)		
Mean ± SD.	3.1 ± 0.8	3.5 ± 1.6	3 ± 0.9	H=0.256	0.613
Median (Min. – Max.)	3 (2.4 – 4)	3 (1.8 – 7.5)	2.8 (1.8 – 5)		
Radiological CT – Findings					
Body Mass	0 (0%)	2 (12.5%)	0 (0%)	χ^2=3.262	MCp= 0.589
Head Mass	3 (100%)	9 (56.3%)	10 (76.9%)		
Periampullary	0 (0%)	5 (31.3%)	3 (23.1%)		

χ^2: **Chi square test**, MC: **Monte Carlo**, **F**: **F for One way ANOVA test**, H: H for **Kruskal Wallis test**, and *P*-value: *P*-value for comparison between the studied categories.
*: Statistically significant at p ≤ 0.05.

TABLE 6 Relation between TGF and different parameters in Pancreatic carcinoma (n = 32)

	TGF		Test of Sig.	*P*-value
	Negative (n= 24)	**Weak (n= 8)**		
Sex				
Male	19 (79.2%)	8 (100%)	χ^2=1.975	^{FE}p= 0.296
Female	5 (20.8%)	0 (0%)		
Age (years)				
Mean ± SD.	60.6 ± 6.2	60.9 ± 6.3	t=0.115	0.909
Median (Min. – Max.)	60.0 (48.0 – 72.0)	61.0 (49.0 – 71.0)		
Perineural				
Negative	5 (20.8%)	0 (0%)	χ^2=1.975	^{FE}p=0.296
Positive	19 (79.2%)	8 (100%)		
Vascular				
Negative	6 (25%)	2 (25%)	χ^2=0.0	^{FE}p=1.000
Positive	18 (75%)	6 (75%)		
Tumor size (cm)				
<2cm	1 (4.2%)	1 (12.5%)	χ^2=1.204	^{MC}p= 0.804
≥2 - <4cm	17 (70.8%)	5 (62.5%)		
≥4cm	6 (25%)	2 (25%)		
Mean ± SD.	3.3 ± 1.4	3.0 ± 1.1	U=84.50	0.623
Median (Min. – Max.)	3.0 (1.8 – 7.5)	3.0 (1.8 – 5.0)		
Radiological CT - Findings				
Body Mass	2 (8.3%)	0 (0%)	χ^2=1.204	^{MC}p= 0.804
Head Mass	17 (70.8%)	5 (62.5%)		
Periam pullary	5 (20.8%)	3 (37.5%)		

χ^2: **Chi square test**, MC: **Monte Carlo**, FE**: Fisher Exact**, **t: Student t-test**, **U: Mann Whitney test**, *P*-value: *P*-value for comparison between the studied categories

*: Statistically significant at p ≤ 0.05.

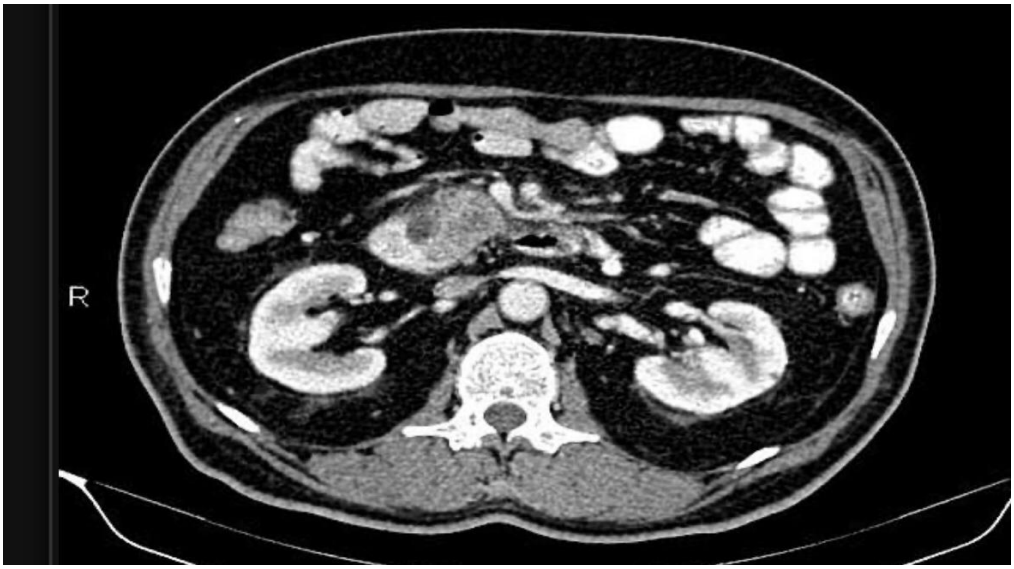

FIGURE 1 A CT picture showing a heterogeneous mass in the pancreatic head, infiltrate the adjacent duodenal wall along with multiple regional lymph nodes. Parenchymal atrophic changes with main pancreatic duct dilatation distal to the mass also seen

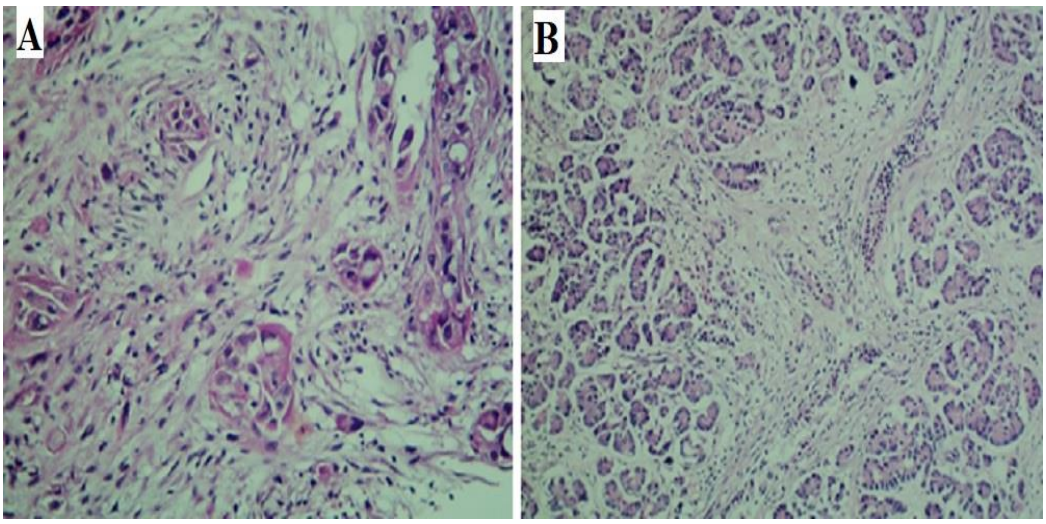

FIGURE 2 H and E stained pancreatic tissue of 2 different cases; focus of malignant glands surrounded by dense fibrotic stroma (x400) (A) and a section of chronic pancreatitis showed prominent stromal fibrosis mimicking cancer (x200) (B)

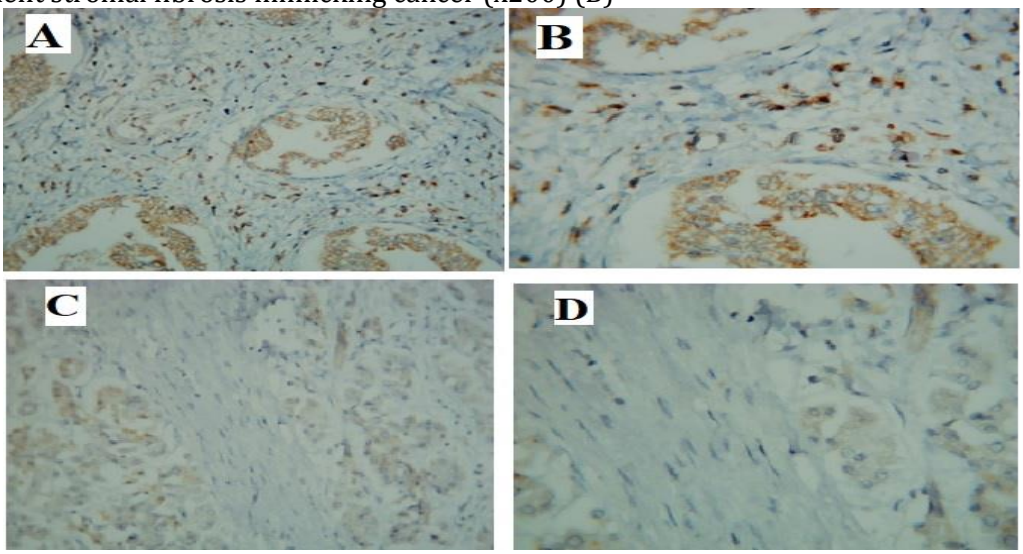

FIGURE 3 FAP expression: strong positive cytoplasmic staining of glandular epithelium and stromal fibroblasts (A) (x200) & (B) (x400). Negative FAP expression in stroma of chronic pancreatitis (C) (x200) & (d) (x400)

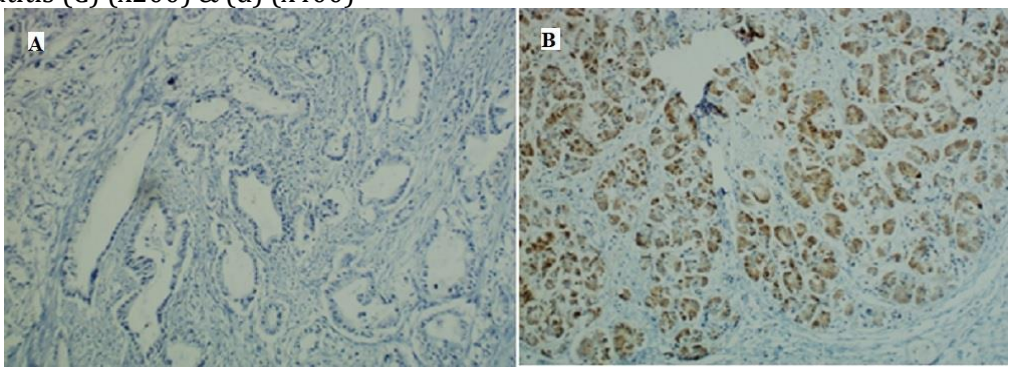

FIGURE 4 TGF beta 1 expression: negative staining of stromal fibroblasts in pancreatic adenocarcinoma(x200), (A). Negative expression in stroma of chronic pancreatitis while positive cytoplasmic staining in acinar cells (x200), (B)

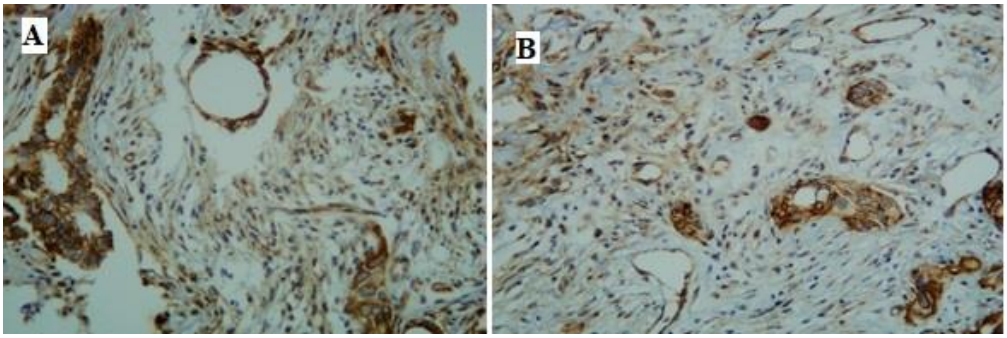

FIGURE 5 A and B: B catenin expression: strong positive membranous and cytoplasmic staining of glandular epithelium and stromal fibroblasts (x200)

Discussion

Since the five-year survival rate of pancreatic ductal adenocarcinoma is less than 5%, it has become and remains the 4th cause of cancer-related death worldwide. Although a repeatedly researched topic in recent years, pancreatic adenocarcinoma is still predicted to become the second leading cause of death due to cancer by 2030 (19), this awfully poor prognosis could result from rapid progression, delayed symptom manifestations, and a lack of early and accurate specific diagnostic methods (20).

Immunohistochemistry plays a crucial role in the precise identification and classification of neoplastic disorders affecting the gastrointestinal tract, liver, biliary tract, and pancreas. There exists a range of immunomarkers that prove to be valuable in discerning malignant neoplasms from benign situations, identifying the sources of organs, and further classifying neoplasms that exhibit morphological and biological heterogeneity. In the realm of personalized medicine, the utilization of certain immunomarkers plays a crucial role in both guiding patient therapy and evaluating the severity of the disease. These immunomarkers work as essential tools in ensuring the effectiveness of tailored medical approaches. The pathologists involvement in the identification, verification, and implementation of novel biomarkers will remain pivotal, leading to enhanced healthcare outcomes for patients (21,22).

Despite the rapid progress and ongoing research in molecular and genetic testing, immunohistochemistry remains a crucial ancillary tool for histopathologists to make critical contributions to patient care in almost all countries. This is particularly true when markers can be used to aid the diagnostic efforts to distinguish between challenging malignant and non-malignant conditions at early stages with proper imaging and surgical technologies. Immunomarkers can further help predict the biologic behavior of diseases, guiding treatment and surveillance decisions (23).

A number of immunohistochemical markers have been tested in pancreatic conditions, and β-catenin is one of the most commonly studied and widely used markers. In various solid tumors, Wnt/β-catenin signaling activation is involved in invasion and metastasis. In colorectal cancer, it promoted cancer cell scattering and metastasis by inhibiting the PI3K-Akt signaling pathway, and β-catenin expression was detected in more than 80% of colorectal cancers (24,25).

Nan *et al.* (2019) concluded that the Wnt/β-catenin signaling pathway may be involved in the invasion of pancreatic cancer (26). To the best of our knowledge, a few articles studied the expression of β-catenin in chronic pancreatitis. However, most of them studied its expression in carcinoma. We found a highly statistically significant difference in the expression of β-catenin in

chronic pancreatitis versus carcinoma. Three-fourths of the malignant cases (adenocarcinoma) in this study expressed β-catenin, while the rest of the cases (25%) were negative, however all cases of pancreatitis were negative.

TGF-beta inhibits the growth of many types of epithelial cells; however, in other types of epithelial neoplastic conditions, it loses its inhibitory effect, and p21 (WAF1/CIP1) is considered one of the cyclin-dependent kinase inhibitors induced by TGF-beta1, which is a downstream effector of this growth inhibitory function of TGF-beta1 (27).

In non-malignant epithelial cells of the pancreas, p21 is a tumor suppressor gene established to be required for TGFβ-induced cell cycle arrest, which can oppose acinar to ductal metaplasia and subsequently early pancreatic carcinogenesis in vivo (28). This is consistent with the clinical observations by many researchers that p21 positively associates with both TGFβ1 and SMAD4 expressions and that cancer patients with robust p21 expression in pancreatic tissue have an improved prognosis (29). In this study, we found that all cancer cases were negative or weakly expressed TGF-beta and the chronic pancreatitis cases were all negative, revealing a lack of clinical utility in diagnosis or differentiating the challenging cases of chronic pancreatitis and adenocarcinoma on a biopsy.

Fibroblast activation protein (FAP) is classified as a type II transmembrane cell surface proteinase. FAP α is a member of the prolyl dipeptidyl aminopeptidase family, responsible for enzymatically removing amino-terminal dipeptides from polypeptides that contain alanine or proline in the penultimate position (P1Pro or P1Ala). FAP α also has endopeptidase activity, displaying a preference for cleavage after the Gly-Pro sequence motif (30). The phenomenon of FAP α overexpression has been observed in both malignant neoplastic cells and stromal fibroblasts. Previous research has provided evidence indicating that the activity of FAP α protease plays a crucial role in the processing of cytokines, chemokines, and extracellular matrix components. Furthermore, it has been observed that FAP α protease activity influences the secretome and proteome of cancer-associated fibroblasts (CAFs), as indicated by many studies (31). Moreover, it has been observed in recent research that, despite its limited cytoplasmic tail, FAP α is capable of modulating signal transduction pathways and influencing the behavior of malignant tumors in a cell-autonomous manner (32).

In primary pancreatic adenocarcinomas, the deposition of extracellular matrix elements (hyaluronan and collagen) was revealed to negatively correlate with survival. Moreover, both primary and metastatic ductal tumors of the pancreas exhibit stromal fibrosis (33). These findings indicate that the desmoplastic reaction may affect the progression of the primary malignant tumor as well as the distant metastasis. Interestingly, FAP-alpha protease-mediated remodeling of tumor stroma was proven to promote tumor cell migration (34). We found a diagnostic significant role of FAP α expression in adenocarcinoma versus chronic pancreatitis however no significant difference between the expression and the patients' age, sex, tumor size, site or invasion. Our results come in agreement with Shi *et al.* (35) who reported expression of FAP in the pancreatic cancer cells (76%) as well as the fibroblasts (73 %). In contrast to our finding, Cohen *et al.* (36) reported that FAP α expression was seen mainly in fibroblasts adjacent to tumor tissues but was very rare in the cancer cells. Recently published article by Cheng *et al.* (37) described the functional role of FAP α overexpressing stromal cells, in tumor microenvironments, and summarized the mechanism underlying this contribution of overexpressing CAFs in pancreatic carcinoma progression and treatment resistance. Expression of FAP α in non-neoplastic

pancreatic tissue was very limited; Wen *et al.* (10) compared the expression of FAP α in tumoral lesions with the adjacent tissues, revealing that the stromal tissues in pancreatic cancer showed high FAPα expression. We recommend further studies on the distribution of FAP expression in different pancreatic lesions.

This study has some limitations that should be considered in future studies: genetic testing, higher number of studied cases, including the control group from normal pancreatic tissue, and the correlation with more clinical features of the included patients.

Conclusion

FAPα plays a critical role in early detection, showing a weak negative relationship with tumor size and spread. Combining B-Catenin and FAPα may be the most effective approach, detecting all cases with variable intensity. Histopathological and radiological findings provided additional insights into both diseases, and the study emphasized the potential utility of combining biomarkers to enhance diagnostic accuracy. The findings have significant implications for improving early diagnosis, which can lead to more tailored treatment strategies, offering promise for patients with pancreatic adenocarcinoma. Further research with larger and diverse patient cohorts is needed to validate these findings and refine diagnostic approaches as our understanding of these diseases evolves.

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Authors' contributions

Conceptualization: WG, TMMA, AH, HAS, AMS, SMMB. Methodology: WG, TMMA, AH, MSA, MH, AS, ZFZR, MT, NRA, AMS, HAS, MEAE, SMMB. Investigation: All authors. Data Collection: WG, NRA, MF, HAS, YME, SMMB. Writing drafts and Revision: All authors. Supervision: WG.

Conflicts of Interest

The authors declare that they have no competing interests.

Ethical Approval

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