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Keywords:

Fibrous stroma; Pancreatic ductal adenocarcinoma; Tumor tissue; Pathological features; Prognostic value

1. Abstract

1.1. Introduction:

The fibrous stroma (FS) in pancreatic ductal adenocarcinoma develops due to chronic injury during tumor invasion, yet emerging evidence indicates its crucial role in tumor invasion.

1.2. Aims:

This study aims to assess the fibrous stroma percentage (FSP) in pancreatic ductal adenocarcinoma (PDAC) tissue and its corelation with various factors including gender, tumor size, lymphatic and vascular invasion, distant metastases, tumor grade, disease stage and overall survival.

1.3. Materials and Methods:

We enrolled 62 patients categorized into two groups based on FSP percentage in tumor tissue: group with FSP ≤ 50% and group with FSP > 50%. FSP was determined using the Prika method, modified to fit two-tiered system of low and high degree of FS. Correlations between FSP and gender, tumor size, lymphatic and vascular invasion, distant metastases, stage, grade, and survival were assessed.

1.4. Results:

FSP in tumor tissue exceeding 50% was observed more in females compared to males. FSP more than 50% was prevalent in larger tumors, and in tumors infiltrating the celiac plexus. While FSP in stage III tumors was slightly higher, no statistically significant difference was found. Interestingly, FSP below 50% was more common in patients with distant metastases.

1.5. Conclusions:

Although higher FSP correlated with larger tumors, poorer differentiation, more advanced stage, and presence of lymphatic invasion, patients with FSP below and above 50% did not significantly differ in terms of survival time.

2. Introduction and Aims

Pancreatic cancer (PC) stands out as seventh leading cause of cancer-related death worldwide, due to its propensity for diagnosis at advanced stages and its resistance to therapy [1, 2]. The global incidence and mortality rates for PC are approximately 7/100,000 person/year respectively, with notably higher figures in the United States and reach the second leading cause of cancer-related death after lung cancer by 2040 [3, 4]. Prognosis of PC is extremely poor, with a less than 10 % 5-year survival rate [5, 6]. This type of malignant neoplasm usually is followed by a dense fibrous stroma (FS) comprised of mixture of rapidly proliferating myofibroblasts (pancreatic stellate cells), collagen type-1 deposition, hyaluronic acid, and various inflammatory cells including macrophages, lymphocytes, and plasma cells [7]. The presence of FS, or desmoplasia, characterized by excessive extracellular matrix (ECM) production and abundant activated pancreatic stellate cells (PSCs) is common in patients with chron-

ic pancreatitis and pancreatic ductal adenocarcinoma (PDAC) [8]. Growth factors produced by the stroma, such as fibroblastic growth factor, can directly promote tumor cell survival [9]. In the development of pancreatic adenocarcinoma, FS forms as a consequence of chronic injury during tumor invasion. However, recent evidence suggests that this fibrotic stromal reaction is indispensable for tumor invasion. A deeper understanding of the molecular mechanisms underlying pancreatic fibrosis has been facilitated by the identification, isolation, and characterization of pancreatic stellate cells [10, 11]. This desmoplastic replacement of normal parenchyma leads to impaired exocrine and endocrine function, a significant pathophysiological feature of PDAC. PDAC is renowned for its abundance of stroma compared to other solid tumors [12]. Desmoplasia poses challenges for radiotherapy due to difficulty in distinguishing between tumor growth and fibrous stroma, and it also contributes to increased drug resistance [13, 14].

TGF-β signaling pathway predominantly regulates extracellular matrix activators in the pancreas [15, 16]. Recent research has highlighted the direct involvement of TGF-β within the pancreatic stroma in cancer progression and metastasis [17]. The role of the TGF-β signaling pathway in PDAC is complex, with TGF-β acting as a tumor suppressor in the early stages by inducing apoptosis and halting the cell cycle in stage G1 [18]. However, in later stages, TGF-β promotes metastasis by activating pancreatic stellate cells, leading to excess matrix production. Fibrogenesis in the pancreas arises from various factors including external tissue damage, chronic pancreatitis, and cell apoptosis. This tissue damage triggers the release of inflammatory cytokines, chemokines, and growth factors, including TGF-β, VEGF, PDGF, and angiotensin, which activate pancreatic stellate cells, promoting the accumulation of myofibroblasts and excessive extracellular matrix deposition [19]. The tumor microenvironment also indirectly influences disease progression; for instance, pancreatic adenocarcinomas exhibit low microvascular density and limited perfusion, leading to intratumoral hypoxia [20, 21]. The high degree of fibrous stroma may exacerbate this reduced blood flow and high interstitial pressure, impairing drug sensitivity [22]. Fibroblasts play an important role in cancer, where they acquire phenotypic alterations and are referred to as cancer-associated fibroblasts (CAFs) [23, 24, 25].

Besides TGF-β, other signaling pathways like vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) also hold significant roles in triggering the desmoplastic response [26]. It is hoped that the increasing evidence regarding fibroblasts and stromal elements in the pancreas will prompt a shift in treatment approaches for pancreatic cancer. One implication of the widespread desmoplastic reaction in pancreatic cancer is intratumoral hypoxia, a key marker of resistance to pancreatic adenocarcinoma treatments [27]. Concurrently, stellate capillaries amplify the production of endostatin by pancreatic adenocarcinoma cells—an endogenous inhibitor of angiogenesis—surpassing the angiogenic potential of both pancreatic stellate cells (PSC) and pancreatic adenocarcinoma, thereby modulating microcirculation density. Consequently, the vasculature in pancreatic adenocarcinoma becomes highly disorganized, dysfunctional, and permeable [28].

Another significant aspect of the microenvironment of pancreatic adenocarcinoma is the suppression of the immune response and the induction of an inflammatory environment that fosters tumor genesis through paracrine interactions between tumor and immune cells [29]. These tumor microenvironment characteristics carry important implications for immuno-oncology therapy [30]. Additionally, the administration of agonist CD40 antibodies activates tumor immunity by promoting infiltration of pancreatic adenocarcinoma by tumor macrophages in both preclinical models and patients [31]. Furthermore, recent advancements include the use of vaccines targeting specific tumorigenic factors (e.g., mutant KRAS vaccine) as immunomodulatory therapy, showing promise in early-stage disease but limited efficacy in advanced stages in mouse models [32]. Overall, progress in tumor immunology holds potential for the development of novel and more efficacious strategies in combating pancreatic adenocarcinoma.

The aim of the study was to evaluate the FSP in pancreatic ductal adenocarcinoma tumor tissue and its relationship with various factors, such as gender, tumor size, lymphatic and vascular invasion, distant metastases, tumor grade and disease stage. Also, this marker may have prognostic value for survival.

Materials and Methods:

The prospective study involved 62 patients operated at the University Clinic for Digestive Surgery, Faculty of Medicine, Ss. Cyril and Methodius in Skopje, North Macedonia and histopathologically diagnosed with pancreatic ductal adenocarcinoma at the Institute of Pathology, Faculty of Medicine. Inclusion criteria encompassed resectable tumors with histopathologically confirmed diagnosis, while exclusion criteria included previous malignant diseases.

Histopathological analyses followed the criteria and recommendations of the Digestive System Tumors classification published by the World Health Organization for determining the type and grade of the tumor, according to the TNM classification of malignant tumors published by the Union for International Cancer Control (UICC) from 2016. Formalin-fixed paraffin-embedded tumor tissue slides were analysed under light microscope, and for each case, beside the pTNM categorisation, fibrous stroma percentage was additionally evaluated. The amount of fibrous stroma was assessed using a modified, two-tiered histological Pri-

ka method, involving examination of 10 visible fields at 200X magnification and semiquantitative evaluation following histochemical staining with Trichrome Azan. The tumor samples with low degree of fibrous stroma was sorted in Group I (≤50% of FS) and the tumor samples with high degree of fibrous stroma was sorted in Group II (> 50% of FS).

3. Results

The obtained results underwent statistical analysis using the SPSS for Windows 17.0 software. Normality in data distribution was assessed using the Kolmogorov-Smirnov test. Quantitative markers were presented using arithmetic mean and standard deviation for symmetric distributions, and median with quartile ranks for asymmetric distributions. Qualitative features were expressed through absolute and relative numbers.

Bivariate analysis was conducted to assess differences among variables using Chi-square and Fisher exact tests for qualitative features, and Mann-Whitney, Student t-test, and Analysis of variance for quantitative markers. Kaplan-Meier survival analysis determined the 6 and 12-month survival rates in patients with pancreatic adenocarcinoma, while the Log Rank (Mantel-Cox) test compared survival times among variables of interest. Univariate and Multiple Cox Regression analyses were performed to identify significant predictors of lethal outcomes, calculating Hazard ratios and a 95% confidence interval (CI).

Tabular and graphical representations were utilized for presenting the data, with significance considered for p-values <0.05.

Correlation Analysis of Fibrous Stroma Percentage with Clinicopathological Factors

A FSP lower than 50% was observed in 27 (43.55%) patients, while the remaining 35 (56.45%) exhibited values of FSP exceeding 50%. Notably, FSP exceeding 50% was less frequent in female patients compared to male patients—16 (72.7%) vs. 19 (47.5%).

FSP did not correlate with tumors size, for example FSP greater than 50% in varying frequencies is equal between tumor less than 2 cm in diameter or larger tumors than 4 cm. However, the observed differences in tumor size relative to FSP did not attain statistical significance (p = 0.075). In stage III tumors (pN2 11 (64.7%), FSP exceeding 50% was marginally more prevalent compared to stage II (N1 and N0)— 9 (50%), and 14 (53.85%), respectively (p = 0.65). Interestingly, in our study we have found that FSP was lower than 50% in tumor samples of patients with advanced Steg IV metastatic disease (6 - 66.7%).

The FSP more than 50% was noted in 24 (64.9%) in moderately-differentiated G2 tumors and 11 (47.8%) poorly-differentiated G3 tumors (p = 0.11).

FSP exceeding 50% was observed across various tumor stages, with no statistically significant association noted (p = 0.15). Notably, tumors with and without vascular invasion did not exhibit significant differences in FSP (p = 0.9).

Table 1: Percentage of tumor fibrous stroma in correlation with histological features.

Variable		Fibrous stroma percentage			p value
		N	≤50 n (%)	>50 n (%)	
Gender	Male	40	21 (52.5)	19 (47.5)	χ^2 =3.7
	Female	22	6 (27.27)	16 (72.73)	p=0.55ns
Tumor size	<2	7	6 (85.71)	1 (14.29)	Fisher exact
	2-4 cm	40	15 (37.5)	25 (62.5)	p=0.075 ns
	> 4 cm	14	6 (42.86)	8 (57.14)	
	in the celiac plexus	1	0	1 (100)	
Lymphatic invasion	N0	26	12 (46.15)	14 (53.85)	χ^2 =1.62
	N1	18	9 (50)	9 (50)	p=0.65 ns
	N2	17	6 (35.29)	11 (64.71)	
	Nx	1	0	1 (100)	
Distant metastases	M1	6	4 (66.67)	2 (33.33)	χ^2 =1.4
	Mx	56	23 (41.07)	33 (58.93)	p=0.23ns
Stage	IA	7	6 (85.71)	1 (14.29)	Fisher exact
	IB	8	2 (25)	6 (75)	p=0.15 ns
	IIA	13	6 (46.15)	7 (53.85)	
	IIB	13	4 (30.77)	9 (69.23)	
	III	21	9 (42.86)	12 (57.14)	
Grade	Well	2	2 (100)	0	χ^2 =4.35
	Moderate	37	13 (35.14)	24 (64.86)	p=0.11 ns
	Poor	23	12 (52.17)	11 (47.83)	
Vascular invasion	Present	27	12 (44.44)	15 (55.56)	χ^2 =0.016
	Absent	35	15 (42.86)	20 (57.14)	p=0.9 ns

X^2 (Pearson Chi-square).

4. Discussion and Conclusions

Numerous studies have explored the aggressiveness and prognosis of pancreatic cancer, aiming to elucidate various pathohistological tumor features for a deeper understanding of its biology. Among these studies is the investigation conducted by Chen P. et al., which focused on determining the presence of pancreatic fibrosis (PF) in pancreatic ductal adenocarcinomas. Their study examined the correlation between the FSP and various clinical-pathological characteristics in 143 patients who underwent operative treatment for pancreatic adenocarcioma located on the head of the pancreas [33]. This study marks the first to establish an association between FSP and overall poor survival following pancreatic adenocarcinoma resection, identifying FSP as an independent negative prognostic factor. Additionally, recent research has underscored the diagnostic significance of histologically present FSP in pancreatic adenocarcinoma, along with tumor necrosis, both of which have been linked to reduced survival rates. FSP has emerged as an independent prognostic indicator for post-resection outcomes [34].

In our study, we sought to correlate the FSP within tumors with diverse clinical parameters (gender, age) and histological tumor characteristics (size, differentiation, lymph node involvement, metastases, and disease stage). Patients were categorized into two groups based on the presence of fibrous stroma percentage, lower FSP group ≤50% and higher FSP group >50%.

However, tumors with a FSP exceeding 50% were less prevalent in female patients compared to male patients (72.7% vs. 47.5%). Furthermore, patients with tumor size between 2-4 cm more frequently exhibited FSP >50% (62.5%), as did those with tumors larger than 4 cm (57.14%). Notably, stage III tumors displayed a slightly higher FSP >50% compared to stage II and stage I (64.7%, 50%, and 53.85%, respectively). Moderately and poorly differentiated tumors exhibited a higher prevalence of FSP >50% compared to well-differentiated tumors (G1).

Analysis revealed no significant difference in FSP between tumors with and without vascular invasion ($p = 0.9$). Although higher FSP correlated with larger tumors, poorer differentiation, more advanced stage, and presence of lymphatic invasion, patients with FSP below and above 50% did not significantly differ in terms of survival time ($p = 0.64$). The 6-month survival rate was slightly higher in patients with FSP >50% (66.7%) compared to those with FSP ≤50% (40.7%). Similarly, the 12-month survival rate was slightly lower in patients with FSP ≤50% (40.7% vs. 42.9%). Cox's univariate analysis yielded a hazard ratio (Exp [B]) for FSP of 0.86395 with a 95% confidence interval (0.447 - 1.666). However, the role of FSP in pancreatic cancer survival did not achieve statistical significance in our study.

In summary, while certain tumor characteristics show trends in association with FSP, none of these associations reached statistical significance except for tumor grades. We found that the cases with Stage IV metastatic disease have FSP lower than 50%, which may mean that these patients initially devoloped tumors which comprised malignant cell clones with high metastic potential. This finding needs to be taken carefully mainly because of the small number of histopathologically proven Stage IV disease cases in this study (6). The findings suggest that FSP may be influenced by multiple factors and further research may be warranted to better understand its role in tumor biology and prognosis.

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