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論　　文　　題　　名

（注：学位論文題名が英語の場合は和訳をつけること。）

Retrospective histopathological study of pancreatic fibrosis in cadaver samples

（Cadaver 標本を用いた膵臓線維症における回顧的組織病理学的研究）

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Content

Abstract

Background: Pancreatic fibrosis has also been found to occur in association with insulin insufficiency and pancreatic ductal adenocarcinoma. It is important to investigate the development, progression and causal factors of fibrosis.

Methods: We examined pancreatic fibrosis in 53 postmortem specimens from cadavers without any known pancreatic disease (mean age: 86.6 years; range, 58-104 years), as well as related types of fibrosis and other lesions found in these specimens. Fibrosis was classified as intralobular and interlobular fibrosis, each type of fibrosis was scaled as mild, moderate and marked.

Results: Intralobular fibrosis was seen in 51 of 53 (96.2%) cases (mild, 52.9%; moderate, 28.3%; marked, 15.1%). Interlobular fibrosis was seen in 22 of 53 (41.5%) cases (mild, 20.8%; moderate, 17.0%; marked, 3.8%]. Pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasm (IPMN), along with other lesions, islet cells loss, and inflammatory cell infiltration were also found in our study. A dependent relationship was found between intralobular fibrosis and interlobular fibrosis, and between intralobular fibrosis and PanIN, IPMN. A higher incidence of interlobular fibrosis was found at the body and tail region of the pancreas in comparison to the head region.

Conclusion: These findings suggested that pancreatic duct obstruction may be an important factor causing fibrosis in this study, and also suggest that some undocumented factors may underlie the formation of fibrosis. Thus, further studies may need to explore uncovered factors to elucidate the reasons the high prevalence of fibrosis among the elderly.

Keywords: Pancreatic fibrosis; Pancreatic intraepithelial neoplasia; Intraductal papillary mucinous neoplasm; Cadaver

Introduction

Chronic pancreatitis is a complex and irreversible disease of the pancreas that is associated with significant morbidity and mortality[1]. Studies reveal that pancreatic fibrosis plays fundamental roles in the pathogenesis of chronic pancreatitis[2]. Pancreatic fibrosis is a process that occurs during repeated necrosis, which involves the excessive deposition of extracellular matrix and collagen fibers to repair damaged pancreatic tissue[3]. Pancreatic fibrosis is also reported to be associated with pancreatic ductal adenocarcinoma[4].

It is therefore important to investigate the development, progression and causal factors of fibrosis. There have been many studies on these topics; however, the majority of these studies either relied on non-invasive measurement or used pancreas samples from patients with pancreatic disease[5, 6].

In order to study the natural prevalence and progression of pancreatic fibrosis, a histopathological analysis is the gold standard for the diagnosis and staging. In addition, samples from cadavers without any pancreatic related disease are superior than pancreas samples with pancreatic diseases and pancreas samples from surgeries, biopsies or autopsies. Firstly, pancreases from patients with pancreatic diseases are more likely to be affected by fibrosis; thus, the prevalence of fibrosis may be higher than that in the general population[7]. Secondly, in pancreas samples without any known pancreatic disease, the stages of fibrosis can be expected to be distributed naturally in accordance with the natural disease course of a given population within a specific age range. However, in the studies utilizing pancreas samples from patients with pancreatic disease, the fibrosis can be expected to progress to a more severe state before the onset of symptoms, causing the grades of fibrosis to become more severe[6], which would limit the analytical value. Finally, in many studies, the pancreas samples that were used were surgical and biopsy samples, or were obtained at autopsy. The volume of surgical

and biopsy samples was often limited and was not sufficient to analyze the pancreas as a whole; it is difficult to study the whole process of pancreatic fibrosis from such samples[6, 8]. Autopsy samples were often obtained for the purpose of performing a thorough medical checkup to investigate the cause of death, and not for a careful examination of the pancreas[9].

The aims of the present study were to examine the incidence of fibrosis in pancreas specimens from cadavers, to determine the subtypes and severity of fibrosis, and to explore the association of fibrosis with other lesions identified in the study, and to further explore the factors that contribute to pancreatic fibrosis.

Materials and methods

Tissue preparation

Fifty-three pancreas specimens were selected from 53 cadavers (male, n=21; female, n=32; mean age at the time of death, 86.6 years [range, 58–104 years]). The cadavers were used for anatomy research and teaching at the School of Life Dentistry at Tokyo, The Nippon Dental University Tokyo between 2014 and 2016. The 53 cadavers all had no record of pancreatic disease or history of abdominal surgery. The specimens in this study were identified as pancreatic head (including the uncinate process), pancreatic body and pancreatic tail. All pancreas specimens were divided into tissue blocks (thickness, approximately 5 mm) and embedded in paraffin blocks. Five-micrometer-thick sections were made and stained with hematoxylin and eosin (HE) and Masson's trichrome for a histological analysis.

All study procedures were approved by The Study Security Ethics Committee of Tokyo Metropolitan University (No.18051) and The Nippon Dental University Tokyo (NDU-T-2016-29), and this study was performed in accordance with institutional guidelines.

Masson's trichrome staining

Masson's trichrome staining was performed in order to better visualize fibrosis. Masson trichrome employs the combination of three dyes. Collagen, from which the fibrotic tissue is composed, is stained blue, rendering it distinguishable from acinar staining, which appears light red.

Histopathological grading

Pancreatic fibrosis was categorized into two classes: intra-lobular fibrosis and inter-lobular fibrosis. The severity of the fibrosis was scaled according to a study conducted by Suda et al.[10], as follows: mild, diffuse distribution of intra-lobular (Fig. 1A) and/or inter-lobular fibrosis, rarely accompanied by acinar atrophy (Fig. 1B); moderate, moderate distribution of intra-lobular and/or inter-lobular fibrosis, usually accompanied by acinar atrophy (Fig. 1C); marked, marked intra-lobular and/or inter-lobular fibrosis with quite evident acinar atrophy (Fig. 1D).

Statistical analyses

The χ^2 test or Fisher's exact test (SPSS, version 1.0.0.1406) were used as appropriate for the following comparisons: (1) frequency of interlobular fibrosis (higher than mild) between samples with and without intralobular fibrosis (moderate and marked); (2) frequency of PanIN (higher than 1A), IPMN, fatty degeneration, islet cell vacuolization and inflammatory cell infiltration between samples with and without intralobular fibrosis (moderate and marked); (3) frequency of PanIN (higher than 1A), IPMN, fatty degeneration, islet cell vacuolization and inflammatory cell infiltration between samples with and without interlobular fibrosis (moderate and marked); and (4) incidence of intralobular fibrosis and interlobular fibrosis in the head, body, and tail of the pancreas[11].

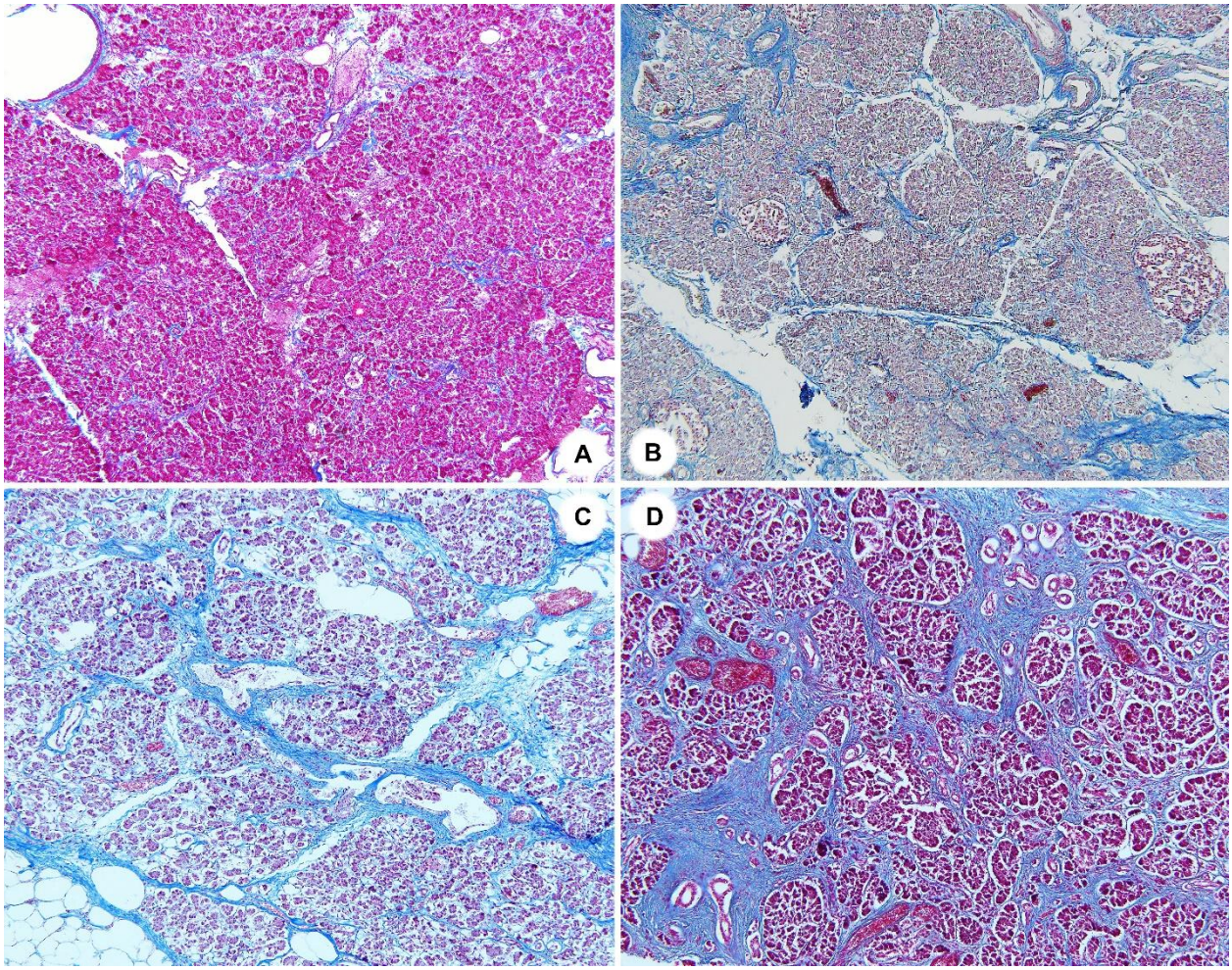

Figure 1. Representative example of pancreatic tissues stained with Masson's trichrome. The pancreatic fibrosis was categorized as intralobular and interlobular (perilobular) fibrosis. The extent and intensity of pancreatic fibrosis were as mild (A and B), moderate (C), or marked (D) (all 100×).

Results

Nearly all subjects were affected by intralobular fibrosis (51/53, 96.2%). A little over half (28/53, 52.9%) of the intralobular fibrosis was mild grade. In mild cases, the fibrotic bands were diffusely distributed. Nearly half (23/53, 43.4%) of the intralobular fibrosis cases fell into the moderate (15/53, 28.3%) and marked (8/53, 15.1%) grades (Table 1). In moderate cases, the fibrotic bands appeared to

be much wider than those in mild cases, and acinar atrophy was usually presented. In marked cases, a large area of acinar tissue was replaced by fibrotic tissue, acinar atrophy was quite evident (Fig. 2F). Less than half (22/53, 41.5%) of the cases showed changes of interlobular fibrosis, which was less common than Intralobular fibrosis. The grades of the cases were classified as follows: mild (n=11; 20.8%), moderate (n=9; 17.0%), and marked (n=2; 3.8%) (Table 1). The histological manifestations of the different grades of interlobular fibrosis resembled those of the intralobular fibrosis. A dependent relationship was identified between intralobular fibrosis (higher than mild) and interlobular fibrosis (higher than mild) (Table 2).

Some cases with intralobular fibrosis and/or interlobular fibrosis were affected by PanIN (Fig. 2A and B) and IPMN (Fig. 2C), which are major precursors of pancreatic cancer. Dependent relationships were found between intralobular fibrosis (higher than mild) and PanIN (higher than 1A) and IPMN (Table 3).

Table 1. Counts and the incidences of pancreatic fibrosis

Variable	n (%)
Intralobular fibrosis	51 (96.2%)
Mild	28 (52.9%)
Moderate	15 (28.3%)
Marked	8 (15.1%)
Interlobular fibrosis	22 (41.5%)
Mild	11 (20.8%)
Moderate	9 (17.0%)
Marked	2 (3.8%)

Table 2. Comparsion of cases with or without intralobular fibrosis (higher than mild) with interlobular fibrosis (higher than mild)

Variables	Without intralobular fibrosis (higher than mild) (n=30). n (%)	Intralobular fibrosis (higher than mild) (n=23). n (%)	P Value
Interlobular fibrosis (higher than mild)	2 (6.6%)	9 (39.1%)	0.003

Some other lesions, such as fatty degeneration (e.g., fat steatosis, fat infiltration, and fat replacement) (Tables 3 and 4) (Fig. 2B), islet cell loss (Fig. 2E) and inflammatory cell infiltration (Fig. 2D) also appeared in cases with intralobular fibrosis and/or interlobular fibrosis. However, it was found that intralobular or interlobular fibrosis were not related with any of these lesions (Tables 3 and 4).

Table 3. Comparsions of cases with or without intralobular fibrosis (higher than mild) with other pathological lesions of the pancreas

Variables	Without Intralobular fibrosis (higher than mild) (n=30). n (%)	Intralobular fibrosis (higher than mild) (n=23). n (%)	P Value
PanIN (higher than 1A)	6 (20%)	11 (47.8%)	0.031
IPMN	6 (20%)	11 (47.8%)	0.031
Fatty degeneration			
Fat steatosis	24 (80%)	18 (78.3%)	1
Fat infiltration	14 (46.7%)	12 (52.1%)	0.691
Fat replacement	15 (50%)	15 (65.2%)	0.268
Islet cells loss	14 (46.7%)	7 (30.4%)	0.231

Table 4. Comparsions of cases with or without interlobular fibrosis (higher than mild) with other pathological lesions of the pancreas

Variables	Without Interlobular fibrosis (higher than mild) (n=42). n (%)	Interlobular fibrosis (higher than mild) (n=11). n (%)	P Value
PanIN (higher than 1A)	12 (28.6%)	5 (45.5%)	0.301
IPMN	12 (28.6%)	5 (45.5%)	0.301
Fatty degeneration			

Fat steatosis	33 (78.6%)	9 (81.8%)	1
Fat infiltration	21 (50%)	5 (45.5%)	0.788
Fat replacement	22 (52.4%)	8 (72.7%)	0.313
Islet cells loss	18 (42.9%)	3 (27.3%)	0.494

Table 5. Comparsions of different parts of the pancreas with the incidences of intralobular fibrosis and interlobular fibrosis

Variables	Head*	Body*	Tail*	P Value	Head*	Body* and Tail* as a whole	P Value
Intralobular fibrosis	30 (93.8%)	30 (93.8%)	27 (84.4%)	0.496	30 (93.8%)	32 (100%)	0.492
Interlobular fibrosis	4 (12.5%)	7 (21.9%)	10 (31.3%)	0.193	4 (12.5%)	12 (37.5%)	0.021

The incidence rates of intralobular fibrosis and interlobular fibrosis were related to the locality of the pancreas. The body and tail of the pancreas were more frequently affected by interlobular fibrosis, however they (respectively) the incidence did not differ from that in the pancreatic head to a statistically significant extent (Table 5). When the pancreatic body and tail were assessed as a whole and the incidence of fibrosis in this region was compared to that of the pancreatic head, the difference

in the incidence rates was statistically significant (Table 5).

Table 6. Association between pancreatic fibrosis and age in References

Author (year)	Number of cases	Age (mean)	Dependent relationship with fibrosis
Pitchumoni et al. (1984)	101	Age range unspecified (62)	The prevalence of fibrosis was higher in 65 and older than in younger than 65
Shimizu et al. (1989)	76	42-94 (71.3)	The prevalence and degree of interstitial fibrosis was not statistically related with age
Stamm (1984)	112	18-89 (62)	Increasing degrees of fibrosis were seen with increasing age
Detlefsen et al. (2005)	89	20-86 (mean age unkonwn)	The prevalence of fibrosis was higher in age group 60 to 86 than in age group 20 to 59

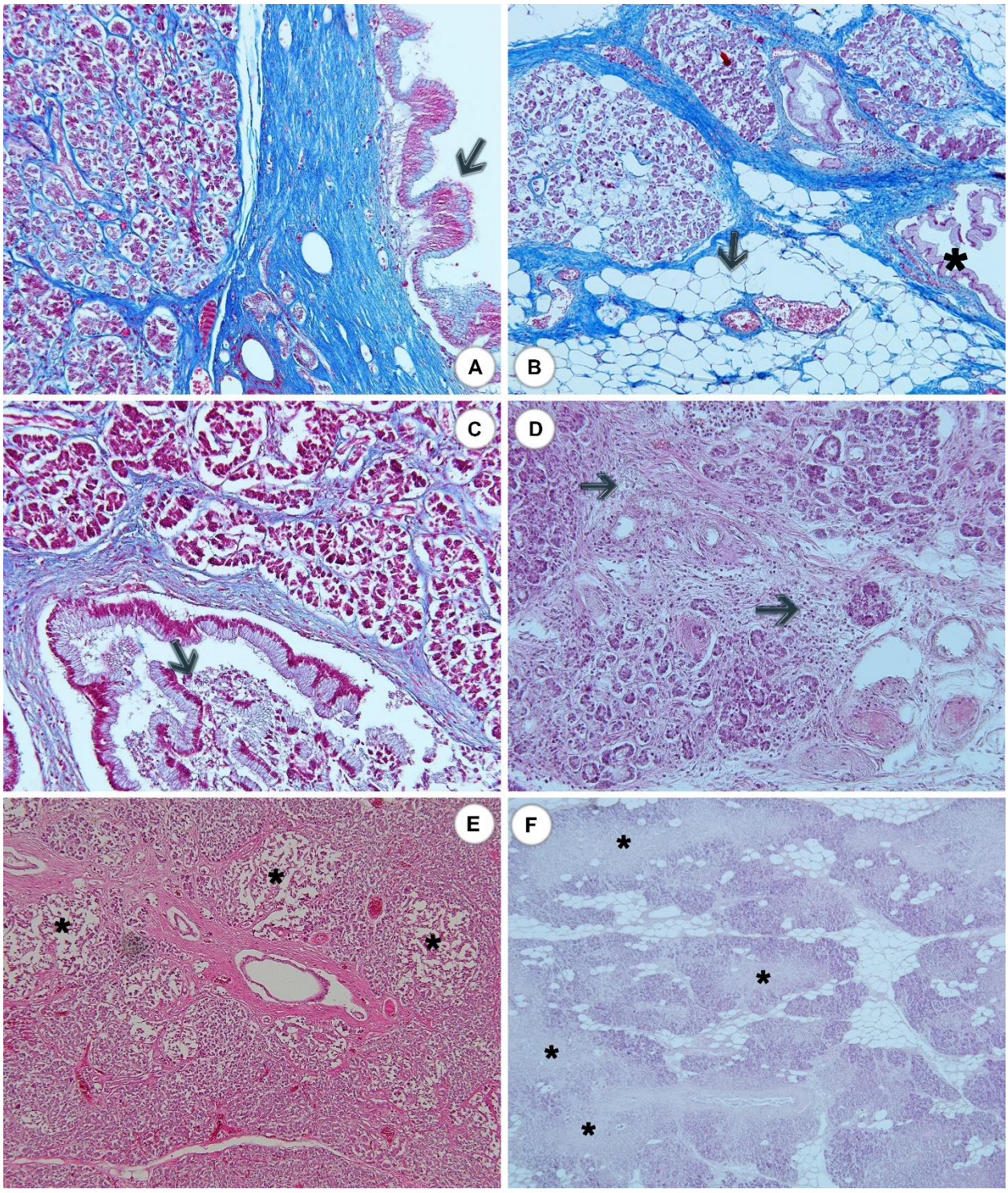

Figure 2. Pancreatic fibrosis accompanied by PanIN (A and B), fatty degeneration (B), IPMN (C), inflammatory cell infiltration (D), pancreatic islet cell defect (vacuolization) and lobulocentric atrophy. Magnification: 100× (B and E), 200× (A, C and D), and 40× (F).

Discussion

This study demonstrated a high prevalence of pancreatic fibrosis in cadavers (mean age, 86.6 years; range, 58-104 years) without any known diseases of the pancreas and surrounding organs. The incidence rates of other lesions, such as PanIN, IPMN, and fatty degeneration were also high in these cadavers.

Many studies have investigated the prevalence of pancreatic fibrosis; however, the number of histological studies utilizing pancreas samples from the elderly population without any known pancreatic disease is still limited. The prevalence of fibrosis in these populations were 92.1% (103/112; mean age, 62 years [range, 18-89 years])[12] and 64.0% (32/50; mean age, 72.4 years [range, 60-89 years])[13]. Due to the differences in era, age distribution, ethnic background, geographical influences, method used, and other factors, these incidence rates were not ideal for comparison with other populations. It is not appropriate to use these results to infer the current prevalence of fibrosis outside the countries in which these studies were conducted.

We investigated and analyzed the association between age and the incidence of pancreatic fibrosis. Previously, Pitchumoni et al.[14], Shimizu et al.[15], Stamm[12] and Detlefsen et al.[13] analyzed relationships between age and fibrosis (Table 6). Pitchumoni et al.[14] reported that the prevalence of fibrosis was higher among individuals of ≥65 years of age than among individuals of <65 years of age. Detlefsen et al.[13] found that prevalence of fibrosis among individuals of 60–86 years of age was higher than that among individuals of 20–59 years of age. Stamm[12] reported that increasing degrees of fibrosis were seen with increasing age (mean age 62 years [range, 18-89 years]). However, Shimizu et al.[15] reported that the prevalence and degree of interstitial fibrosis were not significantly associated with age. We did not find an association between age and fibrosis in our study. In our study, the majority of the cadavers were ≥65 years of age at the time of death; thus, a similar dependent

relationship was unlikely to exist due to the different age groups of our study population.

Fibrosis of the pancreas was also found to be related to other pancreatic lesions in this study. A dependent relationship was found between PanIN (1B and 2) and intralobular fibrosis (moderate and marked changes). PanIN is referred to as "pancreatic intraepithelial neoplasia" and have been recognized as precursor lesions of ductal adenocarcinoma, PanIN may present as flat, micropapillary, or papillary noninvasive intraductal lesions, which usually develop within small pancreatic ducts[16, 17]. These lesions can therefore lead to the narrowing of the pancreatic duct lumen. Formerly, tumors such as pancreatic ductal adenocarcinoma (PDAC) were thought to be able to cause fibrosis by obstructing the pancreatic duct. Animal experimental studies further validated this view by demonstrating the formation of fibrosis upstream of duct ligation, which resembled PDAC obstructing the pancreatic duct[18].

It is plausible that PanIN contributes to the formation of fibrosis through the same mechanism (i.e., by narrowing the pancreatic duct lumen) as occurred with PDAC and duct ligation. In PanIN 1A, the change only involved the metaplasia of epithelial cells, and the size of the lumen was well maintained[19]. Since PanIN 1A does not decrease the size of the lumen, it is unlikely to lead to the formation of fibrosis through narrowing of the pancreatic duct. When PanIN 1A progresses to a higher-grade lesion, it exhibits a papillary growth pattern, which leads to the downsizing of the lumen[19], the formation of fibrosis can therefore be induced. However, no dependent relationship was found between mild intralobular fibrosis and any-grade PanIN.

No association between interlobular fibrosis and PanIN was found. According to previous reports, multiple factors underlie the formation of fibrosis. Perhaps two of the most common factors are heavy alcohol use and increased pancreatic duct pressure caused by duct obstruction[20, 21]. Although increased pancreatic duct pressure tends to cause pancreatic fibrosis evenly across intralobular and

interlobular areas of the pancreas, alcoholic intake is more likely to lead to interlobular fibrosis than to intralobular fibrosis[5]. Since the intralobular fibrosis is more exclusively related to increased pancreatic duct pressure, interlobular fibrosis tends to be influenced by both pancreatic duct pressure and alcohol use; the intake of alcohol may interfere with the potential dependent relationship between interlobular fibrosis and PanIN. The potential relationship between PanIN and interlobular fibrosis could be influenced by the decreased proportion and the decreased consumption level of patients with interlobular fibrosis in comparison to patients without interlobular fibrosis.

Intraductal papillary mucinous neoplasm (IPMN), another PDAC precursor, was also found to be related to intralobular fibrosis (moderate and marked changes). IPMN, which is morphologically similar to PanIN[22], is defined as a grossly visible, predominantly papillary or rarely flat, noninvasive mucin-producing epithelial neoplasm arising in the main pancreatic duct or branch ducts[16, 21]. IPMN may give rise to the formation of intralobular fibrosis by the same mechanism through which PanIN causes the same type of lesion, as both IPMN and PanIN are able to cause the partial or complete obstruction of the pancreatic ducts.

In the present study, the incidence of interlobular fibrosis was higher in the pancreatic body and tail than in the pancreatic head, this phenomenon is unlikely to have occurred by chance. As previously discussed, alcohol consumption and elevated pancreatic duct pressure caused by duct obstruction—as two of the factors most commonly associated with the development of pancreatic fibrosis—duct obstruction that resulted from PanIN, IPMN or any other lesion, would only cause the pressure to increase upstream of the obstruction, inducing fibrosis upstream of the site of obstruction[18]. A very recent study even showed that pancreatic duct ligation at the tail region did not cause fibrosis of the head region[20]. Given that the duct obstruction in the head region would induce fibrosis of the body-tail region, but not visa versa, a higher incidence of fibrosis at the body-tail region is expected to

be observed. We did not come to a reasonable explanation of why the higher incidence at the body-tail region was associated with interlobular fibrosis but not intralobular fibrosis. Could it be possible that some undetermined aging-related factors, as mentioned above, caused intralobular fibrosis to form in almost all cases, blurring the association between the incidence of intralobular fibrosis and the site of the pancreas? Further research may need to explore undetermined factors causing intralobular fibrosis in aged populations.

To summarize the factors causing fibrosis in this study, pancreatic duct obstruction caused by PanIN and IPMN has been well documented. The formation of intralobular fibrosis surrounding the lumen in which lesions appeared was shown. Some cases showed inflammatory cell infiltration admixed with fibrotic tissue. This may be fibrosis caused by other factors, such as auto immune pancreatitis[23, 24]. However, this factor is not as common as PanIN and IPMN in our findings. Perhaps alcohol consumption was an important factor, although this factor was less manifested under the microscopic view. According to the national survey in 2003, in Japan, 84% of males and 64% of females were alcohol users[25], given the well established relationship between alcohol intake and pancreatic fibrosis[7, 14], we can also infer that alcohol intake was an important factor causing fibrosis in this study. However, as previously discussed, alcohol usually causes interlobular fibrosis; intralobular fibrosis likely resulted from increased pancreatic duct pressure caused by PanIN (1B, 2) or IPMN. It is noteworthy that over half of the cases (28/53) in this study did not show PanIN (1B, 2) and/or IPMN. Accordingly, it is difficult to conclude how intralobular fibrosis arises in these cases. It is clear that the pancreatic duct causes fibrosis by inducing elevated pancreatic duct pressure. In our study, we documented PanIN and IPMN as causal factors of elevated pancreatic duct pressure. Perhaps other potential factors were also able to cause elevated pancreatic duct pressure without actually obstructing the pancreatic ducts. Laugiers et al.[26] reported that the flow rate of pancreas

secretions decreased in their aged population. According to Bernoulli's principle[27], the intraductal pressure will increase with a decrease in the velocity of the flow. Although measurement of the pancreatic secretion flow rate cannot be applied in cadaver studies, it is logical to assume that—in many patients—the pancreatic secretion flow rate would decrease before their death as a process of aging. Factors may also cause fibrosis without the process of increasing pancreatic duct pressure. In Laugier's report[26], increasing calcium concentrations were also found in the pancreatic secretions of their aged population, and the increasing calcium concentration was directly linked to the deleterious effects on pancreatic cells, which could later cause fibrosis to arise in the pancreas[20, 28].

In conclusion, we found a high prevalence of pancreatic fibrosis in our cadavers of relatively advanced age. Moderate and marked intralobular fibrosis were associated with PanIN (1B and 2) and IPMN, possibly by PanIN and IPMN causing pancreatic duct obstruction leading to pressure-induced fibrosis. The incidence of interlobular fibrosis in the pancreatic body and tail is higher than that in the pancreatic head. This is possibly because the body and tail region is more likely to be affected by increased pancreatic duct pressure caused by obstruction in comparison to the tail region. In this study, the major causal factors of fibrosis were very likely to be pancreatic duct obstruction caused by PanIN and IPMN, and alcohol consumption. Other factors, such as inflammation, alternation of the pancreatic flow dynamics, and alternation of the pancreatic secretion composition may also contribute to the formation of fibrosis. However, the formation of fibrosis in the pancreas is a complex process, and whether all of the causal factors of fibrosis have been documented is unclear. Thus, further studies may needed to explore uncovered factors to elucidate the reasons the high prevalence of fibrosis among the elderly.

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Conflict of interest: None of the authors has any conflicts of interest to declare.

Ethics approval: All study procedures were approved by The Study Security Ethics Committee of Tokyo Metropolitan University (No.18051), and this study was performed in accordance with institutional guidelines.

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