Morphology and Aging of the Human Adult Pancreas: An Electron Microscopic Study

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Abstract- Pancreas gets affected by fibrosis associated with aging. This study analyzed the age-related fibrotic changes in the exocrine as well as endocrine system of the pancreas. After obtaining necessary ethical clearances 30 post-natal and adult pancreases were collected and processed to obtain resin-embedded sections for transmission electron microscopy and paraffin-embedded sections for H and E staining and light microscopy. The sections were analyzed qualitatively and quantitatively. It was observed with ageing, the ductal epithelial cytoplasm contained many lipoid bodies. The basal lamina and the connective tissue around ducts increased. Periductal fibrosis appeared during fourth decade in Indian population whereas it appears sixth decade in Europeans. There was a direct correlation between area of the ducts and increasing age. Stellate cells and centroacinar cells increased with aging. The cytoplasmic processes of the centroacinar cells covered the acini and ductal epithelial cells, indicating their important function. The centroacinar cells have a regulatory role in secretory process during normal and pathological conditions. Increased fibrosis was noted in and around the islets of Langerhans. Epithelial hyperplasia, papillary projections, and periductal fibrosis around small and medium sized duct started very early in Indian populations indicating the vulnerability to pancreatic diseases in the Indian population in early ages.

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Introduction

Pancreas is the largest digestive gland that has both exocrine and endocrine functions. Both the exocrine and endocrine components are involved in various disease processes during one's lifetime. Acute pancreatitis (AP), an acute inflammatory condition affecting the exocrine pancreas, annually affects between 5 and 80 people per 100,000 (1). In chronic pancreatitis (CP), there are chronic inflammatory cells within the pancreas, progressive fibrosis, sclerosis, and parenchymal atrophy. Malfunction of the endocrine component in CP leads to diabetes mellitus (DM) (2). Diabetes, in all its forms, currently afflicts at least 200 million people in the world and this number is expected to double by the year 2025 (3). Hence, it becomes important to know various aspects related to aging with respect to acini, ducts and islets. In the present study, we investigated the detailed histology of pancreatic acinar, islets, and duct system during aging.

Materials and Methods

Thirty human pancreas from persons aged between the 40th post-natal day and 62 years were collected from the mortuary of the Department of Forensic Medicine, All India Institute of Medical Sciences; New Delhi in accordance with the protocol approved by the Institutional Ethics Committee. (Consent was deemed unnecessary by the Ethics Committee for the use of post-mortem samples). The pancreas was immersed in 4 % paraformaldehyde and preserved at 4° C to minimize post-mortem autolytic changes. The middle part of the pancreas was removed after fixation and a sagittal slice was processed for the study. In addition, a small portion of the pancreas was also immersed in modified Karnovsky's fixative as above.

After fixation middle part near the neck of pancreas tissue was processed for paraffin embedding haematoxylin and eosin staining and microscopy and suitable pancreas tissue were processed for electron microscopy.

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The stained sections of pancreas were examined under Zeiss Axiophot Research microscope (Oberkochen, Germany). The sections were assessed qualitatively and quantitatively. The ultrathin sections of the pancreas were stained with uranyl acetate and lead citrate and observed under the transmission electron microscope (Philips Morgagni 268D TEM, Field Emission Inc., Eindhoven, the Netherlands). Photomicrographs were obtained by a CCD camera, stored and analysed further.

The areas of the pancreatic ducts were estimated from the captured images using a video camera attached to the microscope. The nucleator probe of the Stereo Investigator software (MicroBrightfield Inc., USA) was used to measure the area of pancreatic ducts.

Statistical analysis

The data were expressed as mean ± standard deviation (S.D.). Ducts were divided into three types, according to the luminal size: the large duct (had luminal area more than 500 μ2), the medium sized duct (had luminal area between 150–499 μ2), and the smaller ducts, (the area was less than 150 μ2) (Table 1). The pancreases were divided into various groups according to the age of the deceased at the time of death. Group 1-immediately after birth up to 10 years; Group 2-up to 35 years of age; Group 3-above 35 years.

These data were analyzed using Kruskal-Wallis non-parametric test followed by post hoc test (Mann-Whitney-U test). To test the increase, the amount the connective around the duct after birth and up to 65 years, Pearson's correlation test was applied. Pearson's correlation test was also used for comparison of data for area of lumen of duct and total area of the duct, including its wall. The software SPSS (v12, IBM, USA) was utilized for the statistical analysis.

Results

Pancreas of Early Postnatal, Infantile Period and First Decade of Life.

The pancreas of this period of life contained differentiated acini. Many acini showed zymogen granules near their apices. Ducts were lined by simple columnar or cuboidal epithelium and had well-organized connective tissue around them. There were a few undifferentiated dark cells seen scattered in extra cellular matrix even up to 5 months after the birth. Ultra structurally, the majority of the cells of the acini showed well-formed zymogen granules with a diameter of 500–1000 nm and they were mostly found towards the apical region, establishing an apical to basal polarity to the cells. Centroacinar cells with electron-lucent cytoplasm and nucleus were noted on the apical part of the acinar cells. Some of the acinar cells had two nuclei. The larger ducts showed columnar epithelium with tight junctional complexes between the cells. Some of the ductal cells were observed to have monocilia on their apical surface.

Second decade

The exocrine component of pancreas consisted of closely packed secretory acini. Each acinus was made up of irregular cluster of pyramid shaped cells, the apices of which surrounded a minute central lumen. The apical membrane projected into the lumen as microvilli. The acinar cells were typical protei secreting cells. The nuclei were basally located and surrounded by basophilic cytoplasm with rough endoplasmic reticulum; the apices of the cells were packed with eosinophilic secretory granules (called zymogen granules). Some acinar cells contained greater amount of zymogen granules and some had lesser in amount at same time. The centre of the acini frequently contained one or more pale nuclei of centroacinar cells with sparse pale-stained cytoplasm; these represented the terminal lining cells of minute ducts. Adjacent acini were separated by inconspicuous supporting tissue containing numerous capillaries.

The larger ducts were mainly confined towards the central part of the pancreas, whereas the medium sized ducts were present in the intermediate area (between the periphery and the central part of the gland in a sagittal section of the gland). The smaller ducts were present in the periphery of the gland. In our qualitative study, it was noted that major changes in the duct system occurred in the fourth decade, therefore the quantitative data were analyzed and expressed in the following manner. Small ducts were seen to be lined by a single layer of cuboidal epithelium with centrally placed round or oval nucleus. Medium sized ducts were lined by low columnar to cuboidal cells. Both type of ducts had many dark cells in their basal regions. Large ducts were lined by a single layer of tall columnar cells, which had brush border on their luminal side. The large ducts had few dark cells in their basal regions. Some of these dark cells extended from basement membrane to lumen. Some of the ductal cells had dark granules in their apical cytoplasm.

Ultra structurally, it was noted that ductal cells possessed round or oval nuclei, rough endoplasmic reticulum, mitochondria and a well-developed Golgi apparatus. The epithelium had a large number of mucus-secreting cells that had many mucin granules in their

apical zone. There were also some endocrine cells that could be seen near the basal region of the epithelium. The centroacinar cells were seen close to the luminal aspect of the acinar cells (Figure 1a). Centroacinar cells closely resembled ductal cells. Ultra structurally, the centroacinar cells had fewer organelles and diffusely distributed polyribosomes. They had small mitochondria that were located towards the luminal surface, where the Golgi apparatus was also seen. The nucleus was round or oval with marginal indentation, usually having at least one nucleolus. Some centroacinar cells had complexly arranged, long cytoplasmic processes extending between the acinar cells.

The endocrine part of pancreas (islets of Langerhans) were scattered among the exocrine glandular tissue. They were spheroidal in shape, measuring 52–210 lm in diameter and the cells in the islets were organized as irregular cords. They were composed of groups of secretory cells, supported by a fine network of connective tissue, containing numerous fenestrated capillaries. The same connective tissue formed a delicate capsule that surrounded each islet. The endocrine cells were small with pale staining granular cytoplasm and these were in contrast to the large acinar cells of surrounding exocrine pancreas that stained strongly with H&E.

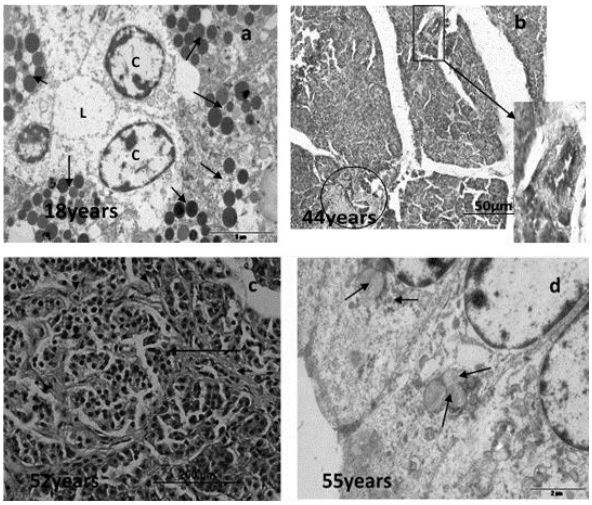

Figure 1. (a) Electron micrograph of the pancreas of 18 years showing the centroacinar cells (C) covering the apical part of the acinar cells (arrows). Note the lumen (L) of the acinus. The centroacinar cells had few organelles and diffusely distributed polyribosomes. They form the beginning of the smallest ducts. (b) Photomicrograph of 44 years pancreas showing fibrosis around small ducts along shown in the circular area. The ducts show papillary projections of their epithelium (rectangle, inset show magnified view). H & E stain. (c) Photomicrograph of 52 years pancreas showing increased fibrous tissue (arrowheads) within and around the disorganized islet of Langerhans (I). H & E stain. (d) Electron micrograph of 55 years pancreas showing lipid accumulation (arrows) in the apical region of large duct epithelium. Scale bar: a= 5 µm, b = 50 µm, c= 200 µm, d= 2 µm

Ultra structurally, these endocrine cells were filled with electron dense secretory vesicles that had a maximum diameter of 300 nm with no polarity to the cells. Apart from the clustered endocrine cells in the islet of Langerhans, some unitary endocrine cells were also seen between ductal cells.

The extra cellular matrix in the pancreas was distributed throughout the gland, but was more prominent around the large ducts and blood vessels. Small number of macrophages, neutrophils, mast cells, plasma cells, lymphocytes and fat cells were seen scattered in the ECM. Stellate cells were also seen in the ECM around the acini. These cells were spindle-shaped that also contained lipid vesicles and intermediate filaments in their cytosol.

Third decade

The pancreas in third decade of life did not show any major differences from the histological features observed in the second decade.

Quantitative observations-stereological estimates

The nucleator probe was used for the estimation of areas of pancreatic ducts. The measurements of the ducts were taken with respect to their lumen (A); lumen including epithelium (B) and duct lumen with the fibrous wall of the duct (C). The estimations of area are summarized in table 2. There was significant increase of connective tissue around the large sized ducts (H=10.38, *P*=0.016) and area of connective tissue around the small ducts (H=7.75, *P*=0.051) with aging, at all age groups studied. There was no statistically significant increase in

area of the connective tissue around the medium sized ducts with aging (H=2.44, *P*=0.485). A maximum increase in the connective tissue was noted in 3rd age category, up to 35 years old and onwards.

The Pearsons Correlations test showed significantly increase in the connective tissue organization around the small ducts (55%, *P*=0.005) and connective tissue around the medium sized ducts (42.4%, *P*=0.039) between 5 months' pancreas to 65 years' pancreas. But no significant changes were observed in the connective tissue around the large sized ducts (77%, *P*=0.72) (Table 3, Figure 2). Maximum increase in fibrosis occurred in the fourth decade.

Table 1. Age related changes in pancreas

Decade	Acini	Ducts	Islets	ECM (Extracellular Matrix)
4th		Fibrosis was confined to the periductal region of small and medium sized ducts		Increased
5th	-Disruption of the basement membrane of the acinar epithelium	-Fatty infiltration and fibrosis and epithelial hyperplasia -Papillary projections into the lumen of the duct (Figure 1b).	-Fibrous tissue increased in the islets of Langerhans -Normal architecture of islet of Langerhans was disrupted	Increased number of fibroblast cells
6th	-Tiny cytoplasmic processes of the centroacinar cells covering a small or longer portion of the luminal surface of the neighboring acinar cells	-Accumulation of lipid droplets in epithelial cells of large ducts (Figure 1d) -Distortions of the lumen of large ducts	-Fibrous tissue increased -Islets were scattered in small fragment (Figure 1c)	-Stellate cells increase

Table 2. Area of pancreatic ducts in adults in different decades showing the various components

Age category	Size of ducts	Luminal area (A) (in µm²)	Lumen + epithelium (B) (in µm²))	Lumen+ epithelium+fibrous wall (C) (in µm²)
Age category 1	Small ducts	94.36± 35.79	426.78± 150.80	1082.14± 239.68*
	Medium ducts	247.23± 22.84	974.91± 46.08	2370.86± 941.03
	Large ducts	963.19± 382.38	2455.15± 1024.93	4550.6± 1709.94**
Age category 2	Small ducts	89.72± 27.89	545.99± 223.22	1336.05± 430.89*
	Medium ducts	271.55± 70.11	1085.12±364.88	2817.89± 929.69
	Large ducts	2353.14± 4177.27	6821.94±13097.01	19291.84± 32862.58**
Age category 3	Small ducts	82.18± 22.99	488.25± 223.93	1898.23± 696.92*
	Medium ducts	318.05± 77.34	1264.39± 464.67	4084.37± 2248.55
	Large ducts	1724.56± 982.98	4910.52± 2389.81	14161.13± 9480.86**

H and *P* for fibrous wall around the small ducts were respectively 7.75, 0.051. H and *P* for fibrous wall around the medium sized ducts were respectively 2.45, 0.485. H and *P* value for fibrous wall around the large ducts were respectively 10.38, 0.016. * $P < 0.05$, ** $P < 0.01$

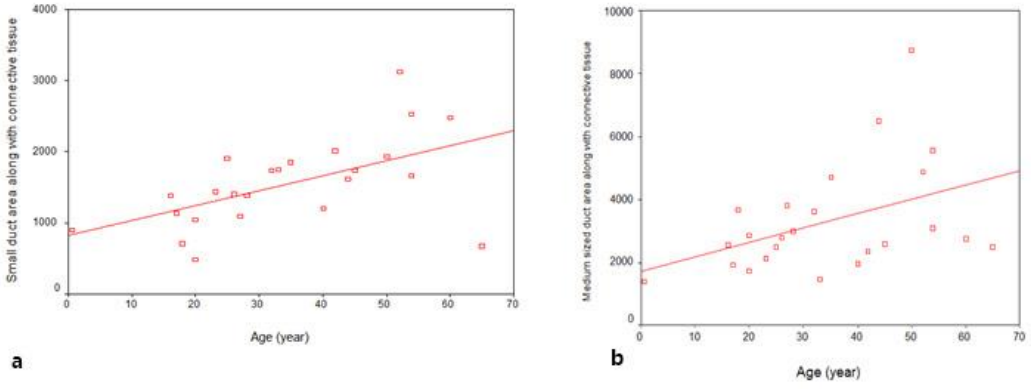

Figure 2. Showing relation between fibrosis around small and medium sized duct and age

Table 3. Pearsons correlation test

		AGECAT1	LOWLUMI	LOWEPI	LOWCONNE	MEDLUM	MEDEPI	MEDCONNE	HIGLUM	HIGEPI	HIGCONNE
	Pearson correlation	1	-.021	.174	.550*	.390	.367	.424*	-.077	-.120	-.077
AGECAT1	**Sig.(2-tailed)**	.	.922	.417	.005	.059	.078	.039	.719	.576	.721
	N	24	24	24	24	24	24	24	24	24	24
	Pearson correlation	-.021	1	.415*	.433*	-.024	.133	.143	-.481*	-.512*	-.470*
LOWLUMI	**Sig.(2-tailed)**	.922	.	.044	.034	.913	.536	.506	.017	.011	.020
	N	24	24	24	24	24	24	24	24	24	24
	Pearson correlation	.174	.415*	1	.593	.107	.355	0.66	-.212	-.275	-.258
LOWEPI	**Sig.(2-tailed)**	.417	.044	.	.002	.618	.089	.761	.319	.193	.223
	N	24	24	24	24	24	24	24	24	24	24
	Pearson correlation	.550*	.433*	.593*	1	.365	.441*	.336	-.325	-.376	-.320
LOWCONI	**Sig.(2-tailed)**	.005	.034	.002	.	.080	.031	.109	.121	.070	.128
	N	24	24	24	24	24	24	24	24	24	24
	Pearson correlation	.390	-.024	.107	.365	1	.791*	.612*	-.296	-.250	-.337
MEDLUM	**Sig.(2-tailed)**	.059	.913	.618	.080	.	.000	.001	.160	.239	.107
	N	24	24	24	24	24	24	24	24	24	24
	Pearson correlation	.367	.133	.355	.441*	.791*	1	.842*	-.198	-.157	-.174
MEDEPI	**Sig.(2-tailed)**	0.78	.536	.089	0.31	.000	.	.000	.353	.463	.417
	N	24	24	24	24	24	24	24	24	24	24
	Pearson correlation	.424*	.143	.066	.336	.612*	.842*	1	-.245	-.209	-.196
MEDCONI	**Sig.(2-tailed)**	.039	.506	.761	.109	.001	.000	.	.249	.327	.429
	N	24	24	24	24	24	24	24	24	24	24
	Pearson correlation	-.077	-.481*	-.212	-325	-.296	-.198	-.245	1	.948*	.980*
HIGLUM	**Sig.(2-tailed)**	.719	.017	.319	.121	.160	.353	.249	.	.000	.000
	N	24	24	24	24	24	24	24	24	24	24
	Pearson correlation	-.120	-.512*	-.275	-.376	-.250	-.157	-.209	.948*	1	.941*
HIGEPI	**Sig.(2-tailed)**	.576	.011	.193	.070	.239	.463	.327	.000	.	.000
	N	24	24	24	24	24	24	24	24	24	24
	Pearson correlation	-.077	-.470*	-.258	-.320	-.337	-.174	-.169	.980*	.941*	1
HIGCONN	**Sig.(2-tailed)**	.721	.020	.223	.128	.107	.417	.429	.000	.000	.
	N	24	24	24	24	24	24	24	24	24	24

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed)

Lowlumi – luminal area of small ducts, lowepi – area of small duct along with lining epithelium, lowconne – area of small duct along with connective tissue, medlum - luminal area of medium ducts, medepi - area of medium sized duct along with lining epithelium, medconne - area of medium sized duct along with connective tissue, higlum – luminal area of large ducts, higepi - area of large sized duct along with lining epithelium, higconne - area of large sized duct along with connective tissue

Discussion

This study provides for the first time, the detailed light and electron microscopic morphology of age related changes in the exocrine and endocrine part of the human pancreas in Indian population. We have used the unbiased stereological technique to measure the various ducts, its epithelium and the connective tissue organization around the ducts, which has not been reported earlier.

The pancreas grew gradually to the adult size during the 2nd decade. Connective tissue around the whole gland was very less up to the 3rd decade, after which it increased dramatically up to the 6th decade, the maximum age group available in the present study.

Pancreatic acini

Differential distribution of zymogen granules in a single acinus indicated that all the cells of an acinus were not active at a particular time. Some cells are active while other cells are in resting phase. The resting cells contained fewer zymogen granules. This observation needs further morphological and correlated physiological study. Occasional presence of double nucleus in cells has been reported in hepatocytes, which are highly metabolically active cells (4,5). In the present study binucleated acinar cells were noted during early postnatal period and in adult pancreas. Cells with double nucleus are present during mitosis in physiological conditions. Double nucleus may be present in cancerous cells (6). This indicates that pancreas is also a highly active organ like the liver. From extensive literature survey, we conclude that this feature has not been reported earlier.

Duct system

In the present study, microvilli and occasional cilia were noted in larger ducts, which regulate the flow of the pancreatic secretion, which are in agreement with studies conducted by Nagata and Monno (7).

Accumulation of lipid inside the epithelial cells of the larger ducts was noted frequently in the aged pancreas. Accumulation of membrane bound lipid inside the cell indicates fatty degeneration of these cells (8,9). A marked feature of the ducts in our study was periductal fibrosis of the larger ducts, which started during 4th decade and continued.

Ultra structurally, it was noted that there were thickening of the basement membrane along with fibrosis. In addition to fibrosis there were epithelial hyperplasia in the large ducts and papillary projections observed inside the lumen of the smaller ducts. The peripheral parts of the glands showed the formation of fibrotic foci that involved one or two lobules. Here, the acinar cells were supplanted by connective tissue. The pattern of fibrosis is mainly intralobular because the fibrotic changes look as if they start within the lobule and not in perilobular space. Epithelial hyperplasia, papillary projections and periductal fibrosis started very early in Indian populations whereas it started in 6th decade in German populations (10). No clinical study is available for comparison. Therefore, this observation needs an in depth study.

In the present study, the pancreatic ducts were divided into three types on the basis of their area. We also quantified the area of the lining epithelium, and fibrous wall beyond the epithelium, to observe the changes with aging. Quantitative observations of pancreatic duct revealed a significant increase amount of the connective tissue around the small and medium sized duct with aging. Louis and Brenda, observed a steady increase in duct calibre with age throughout the whole of the pancreas head, body, and tail and this increase was at the rate of 0–8% per year (11). Due to the small sample size in our study, it is too early to predict a similar trend for Idian population. Increase in the connective tissue component of the small and medium sized ducts in the present study indicate the vulnerability to pancreatic diseases in the Indian population in early ages. This needs further confirmation using a larger sample size.

Islets of Langerhans

In present study, we observed increased amount of fibrous tissue within the islets as well as around the islets in aged pancreas. Not only was the fibrous tissue increased in islets but also they were scattered in small fragments within the lobules of the pancreas. Earlier literature showed in aged rats, the population of small islets loses their ability to respond rapidly to glucose in vitro. This ability is retained in the large islets (12). Movassat et al. observed that when there was a reduction in the total beta-cell mass in adult GK rats, it was associated with an obvious change in the architecture of some islets (13).

Recent studies of the human pancreas seem to indicate that the reduced beta cell mass seen in early stages of diabetes are caused by accelerated apoptosis of the beta cells. One of the reasons that may be causing the death of islet cells may be the increasing fibrosis within the islets and the inadequate capacity of beta-cells to proliferate in diabetic patients. Pancreatic stellate cells (PSCs) are involved in the progression of fibrosis within pancreatic islet in patients with type 2 diabetes (14,15). The histo-morphology of islets from patients with type 2 diabetes showed the presence of an inflammatory process that was evidenced by the occurrence of cytokines, apoptotic cells, immune cell infiltration, amyloid deposits, and fibrosis (16). Although we have not estimated the inflammatory or apoptotic markers, the fibrotic changes in the islets of the aged pancreas alarms a need for in depth study of aging of islet cells in Indian population, which may contribute literature on the onset of type2 diabetes.

Pancreatic fibrosis

Kloppel and Maillet observed that there were variations in the ducts that drained fibrotic Lobules (2). These changes in the duct were defined as “ductal papillary hyperplasia” (17) and were recently recognized as a precursor lesion of pancreatic ductal adenocarcinoma (18). Shimizu et al. observed that the commonest changes seen in the ducts are epithelial hyperplasia (88 %) and periductal fibrosis (74 %) (19). These authors observed that the ducts showed cystic widening (62 %) and intraluminal protein precipitates (40 %), within the parenchyma as an intralobular fibrosis or total fibrosis of lobules (88 %). These fibrotic changes increased with aging, both in the number of the foci and their extent. In the present study, we noted papillary projections and epithelial hyperplasia beginning at the age of 44 years and it increased gradually up to 65 years, the maximum age studied. This signifies that in Indian population pancreatic ductal changes starts earlier than in Europeans (10). This needs further study including a larger population size.

Age changes in pancreas

Aging is defined as the increasing accumulation of changes with time that are either linked to or are responsible for perpetually increasing vulnerability to disease and death, which is the final event of age. Pitchumoni et al., Shimizu et al., and Stamm showed

that the pancreas has fibrotic changes that increase with the age of the person but they did not grade the fibrosis nor discussed its pathogenesis (19-21). One study quantified fibrosis in the normal pancreas and compared it to that found in CP. In the study, photometric evaluation was done to determine the collagen content in histological sections of the pancreas with CP; however, the authors did not provide any information regarding either the distribution of fibrotic foci or the association of fibrosis with age (22).

In our study, we observed an overall increase amount of fibrous tissue around the ducts with aging; more around the small and medium sized ducts in comparison with large sized ducts. Results from earlier literature report that there is decrease in weight of the pancreas after the seventh decade of life in humans, and it becomes harder and more atrophic (23,24).

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