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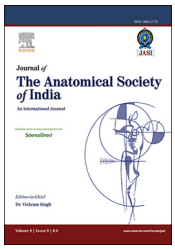

Original Article

Age-related changes in the ductular system and stellate cells of human pancreas

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ABSTRACT

Introduction: Age associated progressive fibrosis may be a major causative factor that leads to pathogenesis of many diseases. Activated pancreatic stellate cells (α-SMA positive) play a major role in fibrogenesis that affects the cytoarchitecture and functioning of pancreas. This study dealt with age-related fibrotic changes in the ductular system of the tail and body of pancreas and the morphology of pancreatic stellate cells.

Methods: Pancreata ($n = 36$) from cadavers aged 30–80 years were obtained after due clearances and processed for Masson's trichome staining. Fibrosis was quantified using Adobe Photoshop (CS2) and Image-J software. Hierarchical cluster analysis was done on the luminal area and total ductal area that were measured by the nucleator probe of StereoInvestigator software (MBF, Vermont, USA). Pancreatic stellate cells (α-SMA positive cells) were identified by immunohistochemistry and quantified stereologically around periacinar, periductular, perivascular, and peri-Islet areas.

Results: An increased fibrosis was noted in body and tail regions of the pancreas with increasing age. Three duct populations were identified in clustering. Their area and corresponding lumen showed a significant increase with progressive decades ($p < 0.001$). α-SMA positive cells increased significantly from 4th to 7th decades ($p = 0.002$, 0.004 and 0.002, respectively).

Discussion: Pancreatic stellate cells may be important contributors to increased fibrosis in pancreas. The classification of pancreatic ducts into three clusters may serve to be a useful tool.

1. Introduction

Progressive fibrosis is a characteristic of aging in organs such as liver, kidney,[1] lung,[2] and pancreas.[3] Fibrosis is the excessive accumulation of extracellular matrix (ECM) proteins as a result of imbalance between the deposition and degradation of ECM.[4] The accumulating ECM affects tissue function that leads to the progression of fibrosis, often as a vicious cycle.

In the pancreas, fibrosis may contribute to the initiation of disease by deposition of ECM, production of cytokines, and

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restriction of blood flow. These changes destroy the tissue structure and cause degeneration of the gland,[5] which affects the functioning of its exocrine and endocrine components.[6] Fibrosis is also an important component of chronic pancreatitis,[23] pancreatic cancer[5] and type 2 Diabetes Mellitus.[7] Many workers have observed progressive fibrosis occurring in the pancreas with age.[8,9,26] In addition, some researchers have found pancreatographic changes in the ductal system of the pancreas.[22,25] In the European population, fibrotic changes start at a relatively late age around 60 years[12] than what is seen in Indian people.[10] The quantitative study of[11] using colorimetric method found a significant correlation between collagen and degree of fibrosis in pancreas. The presence of myofibroblast-like cells, in association with the fibrotic foci, indicates an ongoing fibrogenic process.[12] It has been reported that these myofibroblast-like cells called pancreatic stellate cells (PSC) play a major role in pancreatic fibrogenesis.[4]

The similarities of PSCs with hepatic stellate cells and their association with fibrotic areas suggest that PSCs participate in the development of pancreatic fibrosis.[13] Activated PSCs are found in areas of extensive necrosis and inflammation that are associated with cytokines, growth factors, and reactive oxygen species.[14] Animal models of experimental pancreatitis[15,16] indicate that parenchymal necrosis, and inflammation takes place before the activation of PSCs. These activated PSCs proliferate, migrate, and deposit ECM proteins.[5] It would be interesting to determine, whether the increasing fibrosis observed in the aging pancreas has a similar pathogenesis as in other diseases causing fibrosis of the pancreatic tissue. One of the methods of assessment of the extent of fibrosis in the tissues is by specifically staining for fibrous tissue in histological sections. A simple histological staining technique such as Masson's Trichrome can differentiate the fibrous component in the tissues. Further, immunohistochemistry for the identification of activated stellate cells that express α-smooth muscle actin (α-SMA), glial fibril associated protein (GFAP), etc.,[14] would determine, if these cells are located at the sites of fibrosis.[17]

To the best of our knowledge, there are no studies from India that have recorded age-related fibrotic changes in the human pancreas. The results could provide useful insights upon the progression of various diseases that seem to appear as a part of the aging process, particularly type 2 Diabetes Mellitus.

In the present study, we have determined the relationship between age, fibrosis, and alterations in the morphology of the various components of the pancreas. We have also morphometrically classified the pancreatic ducts into three clusters according to their luminal and ductal areas. We also attempted to determine a fibrogenic mechanism by quantifying PSC around periacinar, periductular, periacinar and periislar areas.

2. Materials and methods

Thirty-six adult human pancreata were collected from the mortuary at the Department of Forensic Medicine, All India Institute of Medical Science, New Delhi (AIIMS), in accordance with the protocol approved by the Institutional Human Ethics Committee. The pancreatic tissue samples from age group of 30–80 were taken and divided into four groups (4th, 5th, 6th, and 7th decades). Samples of known alcoholic, chronic smoker, pancreatic, metabolic, and hepatobiliary disease, traumatic injury and unclaimed bodies were excluded. The body and tail parts of the pancreas were excised from the cadavers, washed with normal saline and fixed in 4% buffered paraformaldehyde (pH 7.4) at 4 °C. After initial fixation, the body and tail of the pancreas were sectioned in the sagittal plane. One of the numerous parasagittal sections was randomly selected and further sectioned in the coronal plane into three equidistant segments. These segments were further divided into equal portions depending on the original length of the segments (Supplementary Fig. 1). These blocks were processed for paraffin embedding. Thereafter, 5 μm sections of these tissue blocks were cut on a rotary microtome and stained with Masson's trichrome.

Supplementary Fig. 1 related to this article can be found, in the online version, at doi:10.1016/j.jasi.2015.10.011.

2.1. *Microscopy and Image analysis for quantification of fibrosis.*

Masson's trichrome stained slides were viewed under, 10× and 40× objectives of a BX61 Olympus microscope for qualitative and semi-quantitative assessment of fibrosis. From each slide, 6–8 high power fields were systematically and randomly selected and images were clicked with a CX9000 digital camera attached to the microscope. The digital images were processed using the Adobe Photoshop software (v7) with the purpose of mapping the fibrotic areas by selecting the pseudocolor of the fibrotic areas, stained green (collagen) with Masson's trichrome, with a color-picker tool (Supplementary Fig. 2). This picked up all the areas in a similar shade of green, wherein the selectivity of the color was kept at 80% accuracy in order to pick up all areas of fibrosis and the variability that arises from performing the staining in different batches. The color of the fibrotic areas was made more distinct by adjusting the fuzziness. Each image was then imported to Image J Basics software, version 1.38 (http://rsb.info.nih.gov/ij/). The image was then converted to gray scale, which converts the image to 256 (8 bit) shades of gray. Thresholding of the image was done by converting to binary image (black/white) by defining gray scale cut off point. In this scale 0 (zero) is pure black and 255 is pure white. Gray scale values below the cut off become black and those above become white. By this procedure all the fibrotic areas in the field appeared black (Supplementary Fig. 2c). These black areas were the stained areas of fibrosis and were measured in this program. The percentage of fibrotic areas in each field was computed as follows:

$$\text{Percentage of fibrotic area} = \text{Total Fibrotic area(Black)}/\text{Total Image area(White)} \times 100.$$

Supplementary Fig. 2 related to this article can be found, in the online version, at doi:10.1016/j.jasi.2015.10.011.