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Histogenesis of pancreas in human fetuses at different weeks of gestation with implications of cadaveric pancreatic transplants in insulin dependent diabetes mellitus patients

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Abstract
The pancreas is formed by two buds originating from the endodermal lining of the duodenum, the dorsal pancreatic bud is in the dorsal mesentery and the ventral pancreatic bud is close to the bile duct. The ventral bud forms the uncinate process and inferior part of the head of the pancreas whereas the remaining part of the gland is derived from the dorsal bud. The main pancreatic duct (of Wirsung) is formed by the distal part of the dorsal pancreatic duct and the entire ventral pancreatic duct. The proximal part of the dorsal pancreatic duct either is obliterated or persists as a small channel, the accessory pancreatic duct (of Santorini). In the third month of fetal life, pancreatic islets (of Langerhans) develop from the parenchymatous pancreatic tissue and scatter throughout the pancreas. Insulin secretion begins at approximately the fifth month. Glucagon- and somatostatin-secreting cells also develop from parenchymal cells. Splanchnic mesoderm surrounding the pancreatic buds forms the pancreatic connective tissue. Aborted human foetuses without obvious congenital anomaly of gestational age between 12 weeks and 36 weeks collected and processed for histological sections by H/E stain. This study was done to correlate the chronological pattern of pancreas development in this geographical eastern region of India, Odisha & compare the results from other researchers nationwide & worldwide.

Keywords: dorsal mesentery, pancreatic bud, pancreatic duct, pancreatic islets of Langerhans, splanchnic mesoderm

Introduction
The Pancreatic Islets Formation in Human Foetuses was 1st Reported by Pearce who described its development from Pancreatic Tubules in 54 mm Foetuses & Kardasewitch described two cell Duct System of a 45mm Foetus [1]. Most Recently Clinicians are making an attempt to treat the patients of Insulin Dependent Diabetes Mellitus by Transplantation of Human Foetal Pancreas (HFP) [2]. To date over 4000 Clinical Pancreas Transplants have been performed all over the World [3]. The World's 1st Transplantation of Cultured Islet Cells of HFP to a patient with Insulin Dependent Diabetes Mellitus (IDDM) Patient was performed in USSR in October 1979 [4]. It is still uncertain that which Stage (Weeks) of Human Fetal Pancreas is an Optimal Transplant Graft Material. The Use of Cultures Islets Cells from Fetal Human Pancreas has been successful even in the absence of Immunosuppression [5]. The parenchyma of the pancreas is derived from the endoderm of the pancreatic buds, which forms a network of tubules [6]. Early in the fetal period, pancreatic acini begin to develop from cell clusters around the ends of these tubules (primordial pancreatic ducts) [7]. The pancreatic islets develop from groups of cells that separate from the tubules and come to lie between the acini. Insulin secretion begins during the early fetal period (10 weeks) [8]. The glucagon and somatostatin containing cells develop before differentiation of the insulin secreting cells. Glucagon has been detected in fetal plasma at 15 weeks. The connective tissue sheath and interlobular septa of the pancreas develop from the surrounding splanchnic mesenchyma [9]. When there is maternal diabetes mellitus, the insulin secreting beta cells in the fetal pancreas are chronologically exposed to high levels of glucose. As a result, these cells undergo hypertrophy to increase the rate of insulin secretion [10]. As the endocrine cell lineage proliferates within the endodermal epithelium, these cells delaminate and subsequently aggregate into islets, where they continue to proliferate throughout the embryonic period [11]. Although several transcriptional factors and signalling molecules have been identified leading to the specification of exocrine and endocrine cell lineages, virtually nothing is known regarding the cellular mechanisms responsible for allocating endodermal cells to a ductal lineage [12]. Interactions with the mesoderm play an essential role in growth and differentiation of the pancreas, and the expression of several growth-promoting transcription factors expressed within the mesoderm are important in this. Isl1 (Insulin gene enhancer protein-1) is expressed by mesoderm surrounding the dorsal pancreatic bud [13]. If Isl1 is knocked out in mice, pancreatic mesenchyme is almost completely lost, and the expression of the pancreatic marker Pdx1 is greatly reduced [14]. Fgf10 is also expressed in pancreatic mesoderm and Fgf10 knockout mice have hypoplastic dorsal and ventral pancreatic buds [15]. Mice lacking Pbx1 (Pre-B-cell leukaemia transcription factor-1), another transcriptional marker for pancreatic endoderm, exhibit severe hypoplasia of dorsal pancreas and loss of acinar development. However, this defect can be rescued in culture by wild-type mesoderm [16].

Molecular Regulation of Histogenesis of Pancreas
With the expansion and branching of the pancreatic buds, the

pancreatic epithelium consists of convoluted sheets of epithelium that uniformly expresses Pdx1. From this epithelium arise exocrine cells and endocrine cells. As in hepatic cell specification, Notch signalling also plays an important role in mediating pancreatic cell specification, determining which cells activate the endocrine lineage within the pancreatic epithelium. One downstream target of Notch signaling in the pancreatic endoderm is Hes1 (Hairy and enhancer-of-split-like-1) [17]. This transcription factor downregulates the proendocrine bHLH transcription factor Neurogenin-3 (Ngn3), a member of Neurogenin/NeuroD family (Fig. 14-13). Ngn3 is transiently expressed within scattered cells of the pancreatic endoderm during the budding stage and later in early Glucagon-positive cells. Mice lacking Ngn3 not only fail to develop pancreatic endocrine cells but also lack intestinal enteroendocrine cells and gastric endocrine cells [18]. Moreover, Pdx1 promoter-driven Ngn3 expression in mice generates massive amounts of Glucagon-secreting endocrine cells in the gut. In the chick, ectopic Ngn3 expression induces the formation of Glucagon- and Insulin-secreting cells within endoderm outside the pancreatic endoderm. Finally, introducing the expression of Ngn3 in cultured human pancreatic ductal cells induces endocrine marker expression. Hence, Ngn3 is a proendocrine transcription factor that, in the absence of Notch signaling, is enough to initiate the endocrine pathway in pancreatic epithelium. Cells in which Ngn3 expression is repressed by Notch signaling (i.e., via expression of Hes1) become part of the exocrine pancreas [19]. Other members of the bHLH family, particularly the p48 subunit of the Ptf1a (Pancreas specific transcription factor-1a) complex, are thought to drive the differentiation of the exocrine pancreas. Mice lacking p48 do not develop acini or ductal epithelia, whereas pancreatic islets still form within the adjacent mesenchyme. What controls p48 expression in the pancreatic primordia is still unclear [20]. Glucagon-synthesizing cells are the first endocrine cells to form within the endoderm, appearing early in the pancreatic bud stage. For a long time, it was thought these cells were the precursors of both alpha cells (Glucagon producing) and beta cells (Insulin producing). However, recent experiments in mice using the cre-lox silencing elements for the Glucagon hormone allele and for the Insulin allele show that both alpha and beta cell lines develop independently [21]. Whereas Ngn3 seems to preferentially drive alpha cell specification of the endoderm, studies suggest Nkx2.2 and Nkx6.1 act downstream with Ngn3 to promote beta cell specification. Knockout mice for Nkx2.2 generate equal numbers of endocrine precursor cells as compared to their wild-type littermates but fail to activate the Insulin gene, suggesting they have a deficiency in beta-cell differentiation. Moreover, these endocrine precursors no longer express Nkx6.1 [22]. Knockout mice for Nkx6.1, a transcription factor specifically expressed in adult beta-islet cells, generate small numbers of Insulin-producing cells but fail to maintain or increase their numbers during subsequent development. Pax4 knockout mice have a complete lack of both beta and delta cell types and develop diabetes at birth, suggesting that Pax4 works downstream of Ngn3 but upstream of Nkx2.2 and Nkx6.1. However, recent evidence shows that activating Notch signalling in Pax4-positive progenitor cells can redirect early endocrine progenitors toward a ductal fate, suggesting that early pancreatic endocrine cells maintain a degree of developmental plasticity [23]. NeuroD1, a transcription fact or whose expression closely follows Ngn3 expression and can be activated by Ngn3, is expressed in all endocrine cells of pancreas after their specification and differentiation. NeuroD1 plays an important role in mediating the expression of differentiated endocrine products of the islet (e.g., Insulin) [24]. Mice lacking the NeuroD1 gene develop the normal complement of islet cell types, but the beta cell number is gradually reduced by apoptosis. Mutations in NEUROD1 in humans are associated with maturity-onset diabetes of the young, where the b-cells become insensitive to blood glucose levels and/or are unable to synthesize adequate amounts of Insulin. Mutations in NEUROD1 are also associated with human type II diabetes. Another transcription factor that may be involved in beta cell specification is MafA (v-Maf Musculoaponeurotic fibrosarcoma oncogene homolog A). MafA expression in the early pancreas is limited to beta cells, is a strong activator of the Insulin promoter, and seems to function downstream of Nkx6.1.

Aims and Objectives

The present study aims to study histogenesis and development of human pancreas in prenatal period to observe microscopic structure of liver at various gestational age groups and its future implications in cadaveric pancreas transplantations in Insulin Dependent Diabetes Mellitus patients.

Materials & Methods

This study was done to correlate the chronological pattern of pancreas development in this geographical eastern region of India, Odisha & compare the results from other researchers nationwide & worldwide. This is a hospital based, observational, cross sectional study conducted at Hi- Tech Medical Colleges & Hospital, Bhubaneswar, India by the Department of Anatomy in collaboration with Department of Obstetrics & Gynaecology from November 2011 to June 2013 on thirty two aborted human foetuses without obvious congenital anomaly of gestational age between 12 weeks and 36 weeks collected within 6 hours of delivery by spontaneous miscarriages & therapeutic legal abortions. Study samples were arbitrarily divided into groups of biweekly gestational age by duration of amenorrhoea from medical records & ultrasound fetometry after receipt of informed consent from mother and legal guardians.Fetuses were immediately fixed in 10% Formalin for 1-2 hrs. Pancreas was dissected by Dissecting Microscope, fixed in 10% Formalin for 48-72 hrs. After fixation by formalin, the tissues were transferred to 30%, 50%, 70%, 90% and Absolute alcohol each for 30 minutes. This ascending grading of the dehydrating fluid was done because when alcohol mixes with water, it produces diffusing current which can damage the tissues. Then the tissues were put in xylol for 24 hours to clear the residual alcohol. These tissues were processed for paraffin sections by tissue blocking (Paraffin Embedding). 3 pots of hard paraffin were taken; paraffin was melted in the incubator at 56 degrees, as hard paraffin is ideal for materials which are to be cut in thin sections about 12 mu. The tissue was put in the first pot containing equal parts of paraffin and xylol and then changed to second and third pots containing only fresh melted paraffin at 90 minutes interval. Then the tissues were mounted in fresh melted paraffin with L-Block. The L-Block was then trimmed to a rectangular shape. Then the L-Block was fixed with the block holder (choke) and the block holder was clamped in the rotary microtome. 5 mu sections were cut

in rotary microtome. The microtome was revolved at 40 per min and ribbon was formed. Then the ribbon was put in tissue flotation bath. Albuminised slide was then made by putting a drop of Mayor's albumin (equal parts of glycerine and egg white) and spreading it uniformly by rubbing with finger. The piece of ribbon was then taken on the slide and dried at room temperature. The slide was then put in the warming table. When the paraffin melted the slide was put into xylol for 2-3 minutes because xylol removes paraffin. Then the tissue was put in decreasing grades of alcohol (Absolute alcohol, 90%, 70%, 50% and 30%) then was put in the prepared Harris Alum Haematoxylin (nuclear) stain for 7 minutes and lastly washed with distilled water.2-3 drops of 1% acid alcohol (1cc Hcl in 75% alcohol) was added to remove the excess stain beyond the nucleus. The slide was then put in running tap water for 30 minutes to develop haematoxylin colour (bluish). Then the slides were again dipped in ascending grades of alcohol (30%, 50%, and 70%) and then put in eosin Y (cytoplasmic) stain for 30 seconds. Then the slide was washed with absolute alcohol for a few seconds so that excess of eosin was removed and lastly the slide was placed in xylol. The slide was then taken out from xylol and then put in 1-2 drops of DPX (Adhesive agent) and a cover slip was put on it and pressed slightly so that air bubbles were removed. Sections were then seen in light microscope under low power 10X followed by high power 45X magnification. Thereafter photomicrographs were taken by camera using microscope adapter [25].

Observations

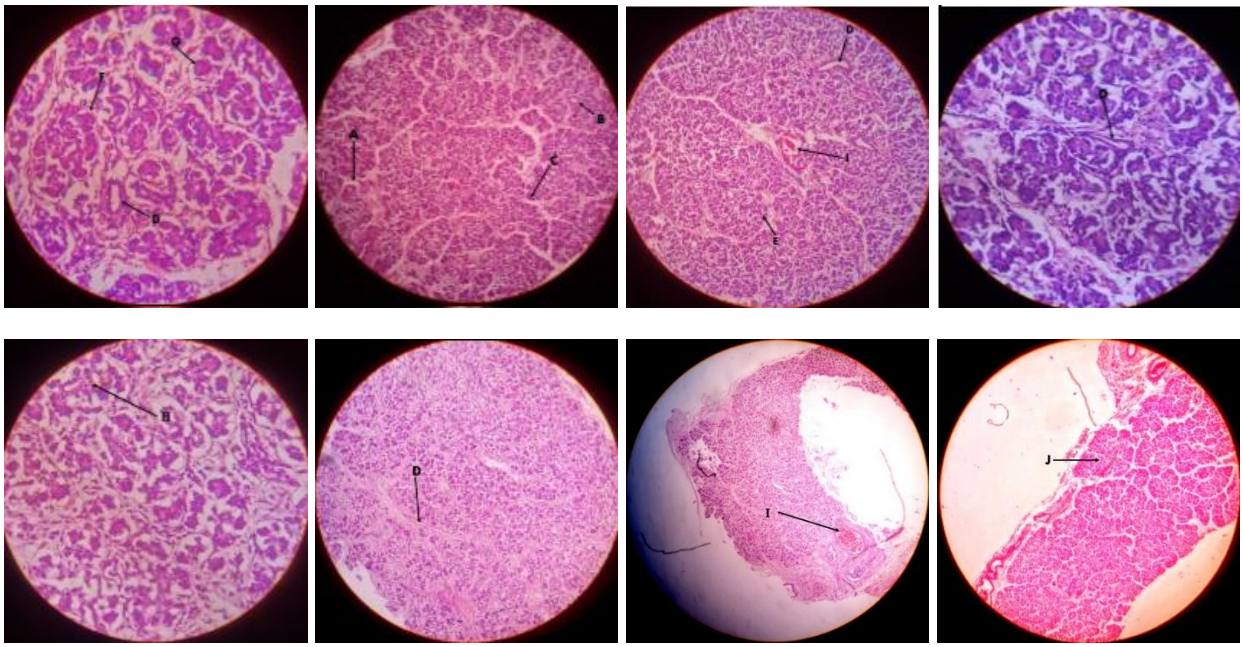

Fig 1

In earlier weeks of gestation Small Islets (A) with Primitive Ducts (B) and Undifferentiated Cells (C) which are mesenchymal tissues are seen as clusters of undifferentiated cells (J). The acini (G) and blood vessels (I) were starting to develop in earlier weeks but were not clearly demarcated. In later weeks of gestation these primitive ducts formed Intralobular ducts (D) lined by cuboidal epithelium separated by Interlobular septa and proliferation of acinar cells (E) were seen. In later weeks the Islets were seen surrounded by a thin capsule and blood (F) which later formed a large vascular Islet (H) as seen in adult pancreas.

The Presence of 2 types of Cells were seen within the Islets having Acidophilic & Basophilic Cytoplasm in 12 weeks Fetus but Granules were identified at 13-14 weeks.

The Argyrophilic Cell was present at the end of 2nd Month (30mm CR), followed by Alpha cells in middle of 3rd Month (50mm CR), Delta Cells slightly later & Beta Cells early in the 4th Month (85mm CR). The Islets begin to develop in the 2nd Month but Acinar Cells appear in 3rd Month. Mainly Beta Cells appears to have Secretory Activity in the Fetal Period from 4th Month Onwards whereas Alpha Cells appears to secrete in 5th Month. Beta cells (most numerous) are situated in the central regions of the islet & Alpha cells are generally arranged around the periphery. Delta cells and PP cells are much less numerous and do not display any obvious pattern of arrangement. Argyrophilic Cells are present within paratubular cell buds & Muddy Cells are not seen in the Parenchyma.

Discussion

No Stained Cells were found at 7 Weeks Alpha, Beta, Delta & PP Cells were found at 9 weeks in Primitive Islets or as Isolated Cells adjacent to Duct Cells [26]. Pancreatic Endocrine Tissue appears early in Foetal Development and is present at 10 Weeks Gestation in man [27]. A Higher density of Somatostatin containing D cells & PP Cells in lobules (PP rich) is found in neonate & in Mid-Term Foetal Pancreas than is seen in the Adult [28]. 70 mg of Fresh Tissue was needed to generate the Foetal Islets while only 30 mg was needed for Culture. Foetal Pancreatic Endocrine Cells grow & differentiate over time when Fresh Explants or Cultured Islet-Cell Clusters (ICCs) are Transplanted [29]. Total Insulin Content was found to be more in fresh tissue than ICCs done by Culturing the Pancreatic tissue the Collagenase containing preparation [30]. Appropriate Stage of Fetal Pancreas Development is crucial for optimal Transplant Material in Insulin Dependent Diabetes Mellitus [31]. Human Fetal Pancrease of 20-24 weeks may be a Suitable Donor

Transplant Material as Alpha & Beta cells start secreting from then onwards & capillaries present around the Islets Cells can grow well if transplanted [32].

Conclusion

Culture can eliminate the putative graft immunogenic Passenger Leukocyte, the cells responsible for initiating Graft Rejection so such immunogenic Cell-depleted Grafts can be transplanted across histocompatibility barriers without need for recipient immunosuppression. Connective Tissue Stroma is very well developed in the Cadaveric Pancreas of Adult Human Donors making it difficult to isolate Islet Cells [33]. Islets Cells comprise only 1-2% of total weight of the Adult Pancreas whereas it is 30% in Fetal Pancreas [34]. Enzymatic Activity of Exocrine Portion of the Pancreas in the postembryonic period is high compared to Fetal Pancreas where it is absent [35].

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