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Research Article

The histogenesis of developing human fetal pancreas – an electron microscopic study

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Abstract

Background: For the prospect of successful replacement therapies in treatment of Diabetes mallitus it is necessary to know events occurring during normal human pancreas development. Literature of human pancreas development are few in number as well as mainly related to first trimester because of ethical and technical difficulties. So the study was conducted on 12 fetuses from 12 gestational weeks (GW) to 5 months of infant to know normal development of exocrine and endocrine part of human pancreas.

Material and Methods: Human fetal pancreases were screened by haematoxyline and eosin staining and done electron microscopy for suitable specimens to know ultra structural detail of fetal pancreas.

Results: It was observed arborized tubules, the cells budding out from these tubules differentiated into primitive acini and islets in 12thGW. At 14 weeks scanty granules were observed in the endocrine cells which coincided with the capillary invasion of the islets. The ducts and acini were surrounded by well-organized connective tissue. The acinihad elongated cells, small amount of cytoplasm and large open face euchromatic nuclei with single nucleolus. The mature form of islets of Langerhans was observed close to the acini and duct in 20 GW fetus. Connective tissue around the duct was well organized.No significant developmental change was observed early postnatal infant.

Conclusion: The development of both component exocrine as well as endocrine part of human fetal pancreas was studied by light and electron microscopy. Observations suggested that the fetal pancreas contained mainly ducts, few acini, many centroacinar cells, and large undifferentiated tissue.

Keywords: Gestational weeks (GW), acini, islets of Langerhans, ducts

1. Introduction

Pancreas performs both endocrine and exocrine functions. The major part of the gland is exocrine, secreting a range of enzymes, which are involved in the digestion of lipids, carbohydrates, and proteins. The endocrine function of the pancreas is confined to Islets of Langerhans, which are small islands scattered throughout the substance of gland but they are more in density in the tail of pancreas[1]. 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[2]. The incidence of diabetes mellitus (DM) is increasing exponentially.

The pancreas is of considerable interest in medicine and biology. Understanding pancreatic epithelial differentiation may fundamentally modify the approach to treating its major afflictions. A major thrust of research in DM is the search for a renewable source of islet tissue for use as cell replacement therapy.

The embryonic events of pancreatic development can be conceptually resolved into three phases. First, a restricted portion of multipotentialforgut endodermal epithelium is specified to become pancreatic anlagen. Second, the cell fates of these multipotential epithelium cells are determined in a regulated manner. Third, proliferation and organization of these pancreatic precursors ultimately leads to specialized islets of Langerhans and the extensively arborized epithelial tree of the adult pancreas[3]. In human the process of islet differentiation is divided into two phases. Phase 1, characterized by proliferation of polyhormonal cells, occure from weeks 9-15. Phase 2, characterized by differentiation of monohormonal cells, are seen from weeks 16 onwards. The β cells, differentiate first, followed by α-cells. The dorsal bud give rise mostly to α cells, and the ventral bud to most of the pancreatic polypeptide producing cells. The β cells develop from duct epithelium throughout development and into the neonatal period. Later in weeks of 10-15, some of the primitive ducts differentiate into acinar cells in which zymogen granules or acinar cell markers can be detected at 12-16 weeks[4].

By reviewing all these fact knowledge regarding normal pancreatic growth and differentiation during development will inform ongoing studies of pancreatic regeneration following surgical pancreatectomy. Therefore research into the development of the pancreas has great implications in day-to-day clinical practice and treatment protocol. But studies of human pancreas development are few in number as well as mainly related to first trimester because of ethical and technical difficulties. Apart from that some immunological studies regarding development of pancreas has done but not detailed electron microscopic study in human fetus has been done. So we describe the development of exocrine and endocrine part of human pancreas in 12 fetuses from 12 GW to 5 months of infant by ultramicroscopy.

2. Material and method

12 fetuses were collected during the period of 2007 to 2010 from the labor room of Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi within 8 hrs of delivery and were preserved at 4°C to minimize the postmortem changes. The fetuses less than 20 weeks of gestation (GW) were obtained from cases where medical termination of pregnancy was performed for family planning (legalized in India under MTP Act, 1971) while those more than 20GW were stillbirths. Prior to use of abortuses and stillborn fetuses a written consent was obtained from mothers or legal representatives of the demised fetuses. The consent to participate in the study was entirely voluntary and dissociated from the abortion decision. None of the mothers suffered from any medical illness during pregnancy and the fetus used in study had no congenital anomalies. However, the causes of death of the stillbirths were undetermined. The fetuses were weighed and measured for crown - rump length (CRL), foot length (FL)[5] and bi-parietal diameter (BPD)[6].

Together with above parameters and the clinical history, the fetal age was determined (table 1). After making a paramedian incision in the abdomen the fetuses were immersed in 4 % paraformaldehyde for proper fixation. The pancreas of the fetuses was removed after fixation.

Table 1: Morphometric parameters of fetuses used in the study

Fetus ID	Age	CRL (In cm.)	BPD (In cm.)	*FL* (In cm.)	Body wt. (In gm.)	Use
Aim -9	10 GW	6.4	2.4	1.0	50	Pancreas seen to be autolysed
Aim -1	12 GW	8.0	2.6	1.6	100	Used for electron microscopy
Aim -6	13 GW	9.8	3.0	1.8	75	Pancreas seen to be autolysed
Aim -5	14 GW	10.0	3.6	1.9	120	Used for light microscopy
Aim -11	15 GW	11.1	4.0	2.2	130	Used for light microscopy
Aim -2	16 GW	11.8	4.7	2.9	160	Used for light microscopy
Aim -8	20 GW	17.2	5.4	3.2	400	Used for light microscopy
Aim -3	20 GW	17.0	5.2	3.4	390	Suitable for electronmicroscopy
Aim -12	22 GW	18.1	5.5	4.5	475	Used for light microscopy
Aim -10	2 days PN	38	35∗	9.0	2500	Used for light microscopy
Aim -7	40 days PN	50.0#	36∗	9.2	3000	Suitable for electronmicroscopy
Aim -4	5 month PN	55.0#	42∗	11	5000	Suitable for electronmicroscopy

∗HC (head circumference) was measured in these fetuses; # Full length was measured in these fetuses.
BPD – biparietal diameter; CRL – crown rump length; FL – foot length; GW - gestational weeks ,PN-Postnatal.

After fixation of the whole pancreas, it was dissected from the surrounding connective tissue and a small sagittal slice from middle part near the neck of the organ was placed in fresh fixative at 4ºC. After proper fixation the slice was processed for paraffin embedding and haematoxyline and eosin staining. The hematoxyline& eosin stained section of pancreas at various gestational ages were examined under Zeiss (Oberkochen, Germany) Axiophot Research microscope.

A small piece (1-2mm³) of pancreas slice was excised and the specimen was placed in Karnovsky′s fixative (4% Para formaldehyde, 1% glutaraldehyde in 0.1M phosphate buffer, pH 7.4) for 48 hrs at 4°C. The pancreas of preserved human fetus aged 14, 20 GW, 40 postnatal day (PN), 5 month were suitable for electron microscopy.

2.1 Ethical Considerations

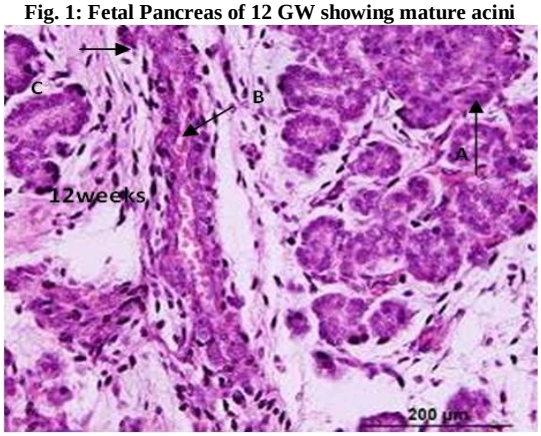
Informed consent was taken from the patients prior to operation and for the inclusion to the study.

3. Results

The pancreas in the fetus was fleshy and multilobulated. Fibrous and connective tissue capsule was thin in the fetal period. The neonatal pancreas had all of the features of an adult pancreas and its various subdivisions could be recognized. The head was proportionately large in newborn and it was continuous with the body and tail. The inferior border of the head of pancreas was in contact with the 'C' shaped duodenum.

The pancreas of 12th gestational weeks (GW) contained numerous ducts and few acini. In between the ducts and acini there was abundant connective tissue containing blood vessels. Simple columnar or cuboidal cells formed the lining epithelium of the ducts. Acini had small lumens and were lined by columnar to cuboidal cells with diffuse connective tissue around them. Extra cellular matrix was much disorganized and undifferentiated dark cells were seen in it. Blood vessels contained RBCs and were lined by single layer of flattened epithelium (Fig 1). Endocrine component i.e. the islets, were small and mainly spherical well defined constituents of the pancreatic parenchyma. The cells of the islets were aggregated in the center in clusters. The mesenchymal connective tissue formed an ill-defined capsule around the islet. The cytoplastmic stain (haematoxylin and eosin) showed the presence of more numbers of 'α' cells than 'β' cells, the former had eosinophillic and the later had basophillic cytoplasm. The characteristic granules of 'α' and, 'β'cells were not seen at this stage. Few mitotic figures were also seen. Capillaries were not seen within the islets but were present in the surrounding mesenchyme.

Fig. 1: Fetal Pancreas of 12 GW showing mature acini

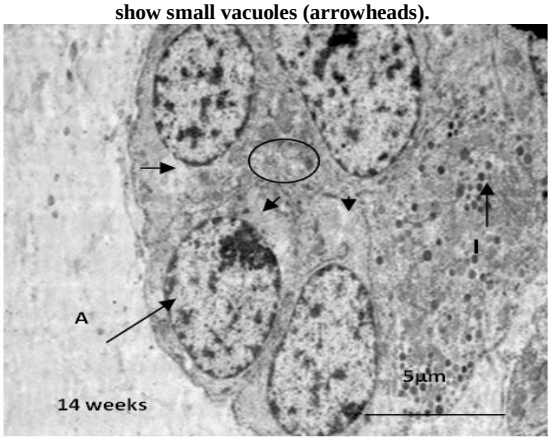


(A) along with well-organized connective tissue (C) and blood vessel (B) lined by single layer of flattened cells containing RBC, in H&E stain. Scale bar-200µm.

The acini were small and their lumens were visible in 14th GW fetal pancreas. Ducts were lined by simple epithelium. The ducts and acini were surrounded by well-organized connective tissue. The extra-cellular matrix was disorganized.The size of the islets had increased having a distinct capsule and showed vascularisation. The cells were more evenly distributed. The 'α' and 'β' cells granules were seen in the 14 weeks embryo and stained eosinophilic and basophillic with haemotoxylin and eosin. The capillaries were seen to be forming a network within the varied size islets in the 14 weeks embryo.

Ultrastucturally, the acini of the pancreas at 14 gestational weeks had elongated cells with small amount of cytoplasm and large open face euchromatic nuclei with single nucleolus. The apical parts of these cells were in close apposition and showed small vacuoles. There were small spaces between these cells indicating attempts of acini formation. There were many ducts and in some areas the ductular cells contained endocrine cells among the ductal cell. These cells contained multiple dark granules in the cytoplasm. The close proximity of the acini and islets in the fetal pancreas were also observed (Fig 2).

Fig. 2: Ultrastucture of the pancreas at14 gestational week showing elongated cells (arrow) the apical parts of these cells are in close apposition and show small vacuoles (arrowheads).



There are small spaces between these cells (inside the circle) indicating attempts of acini formation. Note the presence of multiple dark granules in the cytoplasm of the cell (I) on the right side of the acinar cells. Scale bar: 5µm

Large acini with lumen were clearly visible in the parenchyma of the pancreas in 20th GW and ducts were lined by single layered columnar epithelium. Some of the ducts had compound epithelium. Connective tissue around the duct was well organized. Extra cellular matrix (ECM) was organized and less compact in comparison with the postnatal pancreas.The islets were larger and well encapsulated and the cells were closely packed. The capillaries appeared to be more compressed. The 'α' and 'β' cells had increased in number as well as in size. The granules appeared well established and densely arranged within the cytoplasm at this stage. The 'α' cells were relatively more in number than the 'β' cells but after 20 weeks increase in the proportion of 'β' cells was observed.

Under the electron microscope the cells of the acini showed small number of zymogen granules towards the apical part. Undifferentiated cells were scattered in the ECM especially outside the basement membrane of the acini and the duct (Fig 4). The mature form of islets of Langerhans was observed close to the acini and duct (Fig 3 and 4).

Fig. 3: Electron micrograph of the acinus of 20 gestational weeks showing its clear lumen (L) with few cells containing zymogen granules (z). In close proximity to the acini, the mature form of the islets (I) can be seen. Scale bar: 5µm

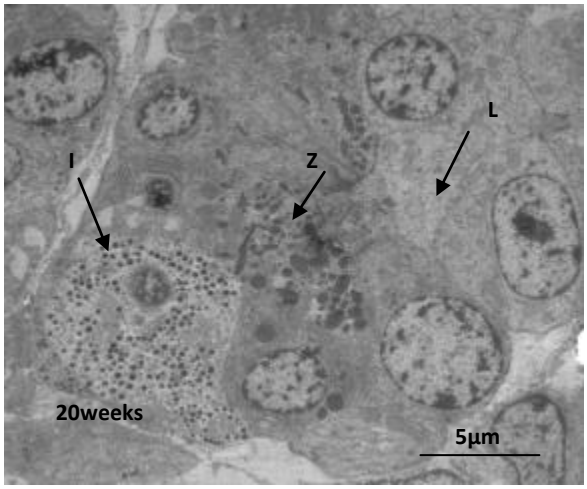
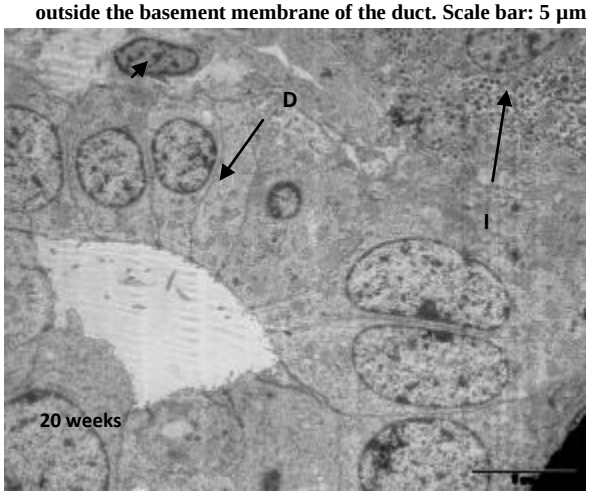


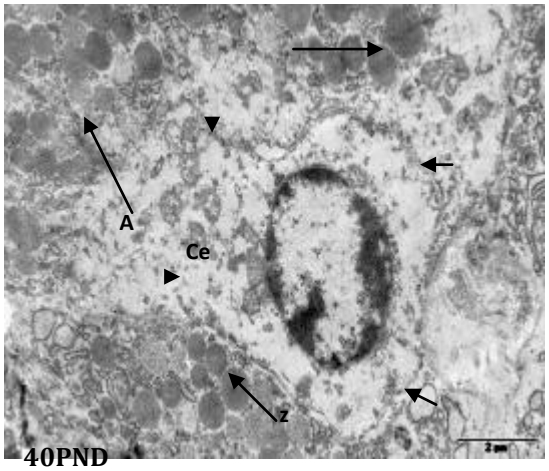
Fig: 4Ultrastructrually The mature form of islets (I) was observed close to the duct (D). Undifferentiated cell (arrowhead) was observed in ECM outside the basement membrane of the duct. Scale bar: 5 µm



The pancreas of the early postnatal, infant had similar appearance as has been observed in the fetal period. The pancreas contained acini that were mostly differentiated. Some acini showed zymogen granules in the apices. Ducts were lined by simple columnar or cuboidal epithelium with well-organized connective tissue around them. The islets were markedly increased in number and widely distributed. The 'β' cells were more in number and were larger than 'α' cells. The density of granules in 'α' and "β' cells appeared similar to that seen in laterfetuses of previous group and localized closer to the capillaries.Some undifferentiated dark cells were scattered in extra cellular matrix even five months after the birth.

Ultrastructurally, the majority of the cells of the acini showed well-formed zymogen granules towards the apical region. Centroacinar cells with electrolucent cytoplasm and nucleus were noted on the apical part of the acinar cells (fig 5).

Fig.5: Electron micrograph of the centroacinar cell (Ce) with electronlucent cytoplasm with processes (arrowhead) extending between the apical parts of the acinar cells (A) containing zymogen granules (z) at 40 postnatal days.



Some of the acini showed double nuclei without any plasma membrane dividing the acinar cell. Close to acinar cell, centroacinar cells were also visible. The larger ducts showed columnar epithelium with tight junctional complex between the cells. Occasional cilia were noted emerging from the apical cytoplasm and projecting into the lumen of the duct.

4. Discussion

The selection of an appropriate developmental stage of fetal pancreas is of paramount importance for the successful transplant of pancreas in patients of insulin dependent diabetes mellitus. The earliest fetus procured in the study was of 12 weeks gestation. It's parenchyma appeared as collection of branched tubules lined by cuboidal cells. Groups of cells from these proliferated to form primitive acini, islets and ducts.

The pancreas plays a key role in pathogenesisof diabetes mellitus (DM). For the prospect of successful replacement therapies in treatment of DM it is necessary to know events occurring during normal human pancreas development. Many studies in pancreatic islets of experimental animals (mainly rats and mice) were performed but morphological organization of islets of Langerhans and exocrine part in human differ from rodents. β- cells of rodents occupy central position , α- cells are localized at periphery in pancreatic islets but mosaic distribution of β- and α- cells is observed in human pancreatic islets[7,8]. The proportion of β- cells were 55% and α- cells were 38% observed in human islets[7]. The first endocrine cells appear in the center area of body of the pancreas, and single endocrine cells and small clusters mainly in the periphery are found out in further increase of the mass of the pancreas[9-11]. Same type of result observed in our study that initially islets appear in the center of mass and α- cells predominant but after 20 weeks of gestation β- cells gear up and form larger constitute of islets.

The pancreatic exocrine cells differentiate from the endodermal ventral and dorsal pancreatic buds respectively. The fusion of these two buds of the pancreas occursat the end of the embryonic period (the 56th day of development) in human embryo[10,11]. Initially, the buds are solid, surrounded by undifferentiated mesenchyme. Later these buds proliferate several times forming smaller terminal buds. Canaliculi appear in between the cells of the solid buds thereby forming the acini. The endocrine part of pancreas form in the similar manner from the solid buds. The terminal buds separate from the main bud to form isolated group of cells during 8- 10 embryonic weeks[12]. Initially, these endocrine cells are located in the duct walls or in buds arising from them; later they accumulate as pancreatic islets. Later, in weeks 10-15, some of the primitive ducts differentiate into acinar cells[13]. Are cells of the early pancreas multipotent, capable of contributing to both the endocrine and exocrine compartments, or do they arise already committed to one or the other lineage? The only direct evidence bearing on this question is a single report, based on retroviral 'tagging' in vitro, which showed that single cells in theE11.5 dorsal bud can give rise to both acinar and islet descendents[14]. Cultured without mesenchyme, pancreatic epithelium shows little proliferation and fails to produce acinar cells[15]; more-recent studies have shown that endocrine differentiation is actually enhanced in the absence of mesenchyme, as thoughmultipotent progenitors choose islet fates by default[16].

The origin of pancreatic endocrine cells in the islet however is controversial. It has been suggested that they arise from neural crest cells[17], and from epithelial cells of pancreatic ducts[18-21], from cells in the islets[17], or from cells in bone marrow[22].Collin 1995 observed that the endocrine secretions start 8- 10 gestational weeks[4] on other hand it is reported that insulin secretion starts approximately in the fifth month of intrauterine life[23]. We observed electron dense granules in the endocrine cells at the 14th gestational weeks.In our study, ultrastructural investigation revealed that the duct cells and endocrine cells were in close apposition, may alarm the possibility of similar endodermal origin of exocrine as well as endocrine part of pancreas. A close proximity of the acini and the isolated endocrine cells were observed during 14 gestational week open a question of similar source of origin but due to lack of funding we were unable to perform immunohistochemistry so this fact needs further investigation to conform source origin of cells.

In the present study, it was noted that the pancreatic acini were well formed by 12th week of gestation. The acini showed small lumen and active secretory cells containing zymogen granules scattered among the ducts. Previous authors have shown that the zymogen granules and acinar cell markers could be detected at 12-16 gestational weeks[13], confirming our observations. However, the proteolytic pancreatic enzymes are produced by the acini from the fifth month onwards[12]. In our study, ultrastructural investigation revealed that the duct cells and endocrine cells were in close apposition, indicating that there may be similar endodermal origin of exocrine as well as endocrine part of pancreas. A close proximity of the acini and the isolated endocrine cells were observed during 14 gestational week suggested that the exocrine and endocrine part arise from a common source in the embryonic life.

The development of both component exocrine as well as endocrine part of human fetal pancreas was studied by light and electron microscopy. Previous studies either based on exocrine part or endocrine part. First time we were try to formulate normal different developmental stages of human fetal pancreas from 12 GW to 5 months of extra uterine life. The fetal pancreas contained mainly ducts, few acini, many centroacinar cells, occasional stellate cells and large undifferentiated tissue. A close association between acini and endocrine cells needs further investigation. Presence of binucleated cells indicated high activity of the fetalacini.

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