Original Article

Indian Journal of Anatomy
Volume 4 Number 2, May - August 2015
https://dx.doi.org/10.21088/ija.2320.0022.4215.8

Morphological Study of the Developing Human Exocrine Pancreas at Various Gestational Ages

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Abstract

Background: The exocrine pancreas is of immense importance as regards carbohydrate, fat and protein metabolism. It comprises roughly 98% of the total volume of pancreas. The pancreas develops from two evaginations of the foregut – a dorsal and a ventral pancreatic bud. These buds fuse to form a single organ when the fetus is 12mm crown rump length. These buds give rise to tubules which differentiate into ducts. The primitive duct epithelium provides the stem cell population for all the secretory cells of the pancreas including the exocrine acini. The exact timing of appearance of various enzymes is not yet clear due to insufficient number of human studies. Research has revealed that neonatal pancreas does not show maturation as regards secretory capacity even though it acquired a mature morphological appearance. Also, any insult to the developing exocrine pancreas can result in a variety of exocrine insufficiencies. *Purpose of study:* It is a baseline study attempting to describe the sequential development of the parenchyma with particular focus on the cytogenesis of exocrine component. This knowledge will guide us in identifying any abnormalities during pre-natal development. *Results:* The exocrine acini developed from the lining epithelium of the tubules that arose as an evagination of the foregut endoderm. It was found that by 18 weeks of gestation the acinar cells showed evidence of zymogenic granules in the apical cytoplasm. By the 5th month of gestation, a mature morphological appearance was acquired by the fetal pancreas. *Conclusion:* Any factor that causes deviation from the normal development during this period will result in improper development. *Potential implications:* This knowledge will help in exploring any factors that result in abnormal development and hence, exocrine pancreatic insufficiency.

Keywords: Acini; Developing; Exocrine; Morphological; Pancreas.

Introduction

The name pancreas has been derived from the Greek words 'pan' meaning 'all' and 'creas' meaning 'flesh'. The pancreas is a lobulated gland located behind the stomach. It has an exocrine portion which makes up the major portion of the gland. The endocrine portion makes up 2% of the total volume and secretes hormones into the bloodstream. The exocrine pancreas is a lobulated, branched, acinar gland covered by a thin capsule [1]. The zymogenic acinar cells are pyramidal in shape and have an intensely basophilic cytoplasm due to presence of a network of rough endoplasmic reticulum. The zymogen granules in the apical part of the cells are acidophilic. There is a basal spherical nucleus [2]. Their secretions are important for the digestion of carbohydrates, proteins and fats. These enzymes are in an inactive state while they are inside the acinar cells. They are released by exocytosis and are carried to the duodenum by the pancreatic ducts. They become active after mixing with duodenal contents [3]. It has been observed that the newborns have a transient exocrine insufficiency. Adult levels of pancreatic function are attained only by the age of two years. The first enzyme detected in fetuses is lipase at 16 weeks. But the level is as low as 0.02% of the adult level [4]. There are many developmental disorders that affect the exocrine pancreas. The most common and well understood is cystic fibrosis. There are many other conditions such as Schwachman Diamond syndrome, Johanson Blizzard syndrome

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and isolated enzyme deficiencies. In these disorders, environmental insult to the developing fetal pancreas is the proposed etiology. A case of exocrine pancreas aplasia has also been reported [5]. Thus it is evident that exocrine pancreas plays an important role in the metabolism and any defective development can lead to myriads of conditions. Hence, we conducted a study to observe the development of fetal exocrine pancreas.

Materials and Methods

Aborted fetuses of gestational age 10-36 weeks were procured from the Department of Obstetrics and Gynaecology, Lok Nayak Hospital, New Delhi, after obtaining institutional ethical clearance and informed consent of parents. The fetuses of gestational age up to 20 weeks were obtained from medical termination of pregnancy in accordance with the Medical Termination of Pregnancy act. The foetuses above this age were obtained from still births. A detailed maternal history was recorded and diabetic mothers were excluded from the study. An initial assessment of the fetus was done to rule out any gross abnormality. Only normal foetuses were included in the study. The gestational ages of the procured fetuses was determined by measuring-Crown-rump length, Crown-heel length, Biparietal diameter and Foot length.

Incision was given longitudinally on the anterior abdominal wall in the median plane for better penetration of the fixative into the abdomen. The fetus was then immersed in 10% paraformaldehyde. After 1 week, the pancreas was dissected out and preserved in fresh fixative for 1-2 weeks. The specimens were labelled and processed for paraffin embedding. 7 micrometer thick serial sections were generated on a rotary microtome with the long axis of pancreas as the cutting surface. Serial sections were stained with haematoxylin and eosin stain to see the morphology of the developing pancreas and; Masson's trichrome stain to differentiate the connective tissue.

Results

The human fetal pancreas was studied from 10-36 weeks of gestation age. Emphasis was laid on the development of exocrine part of the pancreas.

At 10 weeks of gestation, the dorsal and ventral pancreatic buds had already fused to form one single mass. The anlage of pancreas was very small. A thin connective tissue capsule was seen around the gland. The parenchyma consisted of large amounts of mesenchymal tissue with scattered elongated nuclei of fibroblasts. In the parenchyma were embedded many branching tubules lined by columnar epithelium (Fig.1a). The basement membrane of these tubules was visible on the luminal side. The cells had a lightly eosinophilic cytoplasm and oval vesicular nuclei present towards the base. The nuclei had a prominent nucleolus (Fig.1b). At places, the tubules showed stratified lining epithelium. Cells were budding out from these stratified areas in the form of cords and small clusters to give rise to acini and islets. Some of these cords were solid and some had a common lumen with the tubules indicating that they are in the process of branching out. At this stage, it was difficult to distinguish between the cells in the terminal tubules and the primitive acinar and endocrine cells.

By the 12th week, connective tissue in the capsule around the gland was better defined. The parenchyma of the gland still had abundant loose connective tissue with fine collagen fibres and fibroblasts and small blood vessels. In the parenchyma, many branching tubules with a wide lumen were seen (Fig. 2a). The acini were seen budding out from the tubules. The cells in the acini had a darker staining cytoplasm compared to the ducts. Very small islets could be seen at this stage, in the form of few cords of cells budding out from the tubules. The parenchymal tissue had begun organising into ill-defined lobes. Mesenchymal tissue is beginning to surround the budding out acini (Fig. 2b).

At 14 weeks of gestation, the general features of capsule and parenchyma were same as at 12 weeks (Fig. 3a). The relative amount of mesenchymal tissue in the parenchyma had decreased due to the rapid proliferation of the acini. The primitive tubules were fewer in number (Fig. 3b). Many acini were seen branching out from the tubules and ducts and organising into lobes. The cells showed a faint apical acidophilia and basal basophilia. Small islets are also seen in close association with the tubules. They had few and loosely packed cells which were pale staining compared to the acinar cells. They were beginning to enclose capillaries within them.

At 18 weeks, the anlage of the pancreas was gradually increasing in size. The connective tissue capsule was well defined. It was sending septa into the parenchyma dividing it into lobes. These interlobar connective tissue septa were well defined with many collagen fibres, connective tissue cells and blood vessels (Fig. 4a). The intralobular connective

tissue was sparse and delicate (Fig. 4b). The exocrine component of the gland was expanding and showed well developed acini. The acinar cells now appeared pyramidal in shape and had a prominent nucleus. The cells showed marked basal basophilia and apical acidophilia. They were surrounded by delicate connective tissue capsules. Some fibroblasts were also seen in the connective tissue around the acini. Many islets of variable size were seen. The larger islets were well vascularised and encapsulated.

At 22 weeks, the anlage of pancreas had increased in both length and breadth. The features of the capsule and parenchyma were same as in the previous age. However, due to growth and proliferation, the size of the lobes appeared larger. The acini were proliferating and their cells were closely packed (Fig. 5a). The parenchyma now had very few tubules, possibly, because they had differentiated to form ducts. The larger ducts were lined by low columnar epithelium. The smaller ducts were lined by cuboidal epithelium. The ducts were surrounded by many layers of loose connective tissue with many fibroblasts and some blood vessels (Fig. 5b). Many large islets were visible especially in the tail region of pancreas. They were pale staining compared to the surrounding acini.

At 28 weeks, the size of pancreas was bigger. The capsule and connective tissue septa had features similar to those seen earlier. The parenchyma showed a distinctly pronounced lobular structure with larger lobes and lobules. The intralobular connective tissue had become denser. Ducts of varying sizes are seen. The main pancreatic duct was seen lined by columnar epithelium. The cells had a faintly stained cytoplasm and well defined elongated nuclei. It was surrounded by large amount of connective tissue. In some places, duct still showed stratification and ducts were seen to be budding out from it. These were connected by cords of cells to the main duct from which they were budding out. The smaller ducts were lined by cuboidal epithelium. The acini were larger in size and more in number. The islets had become larger in size. Many islets were still seen close to the ducts.

By the 36 weeks, the anlage of pancreas was seen to increase in size in all dimensions. The capsule was thicker. The architecture of the lobes and lobules was highly well developed in the parenchyma (Fig. 6a). In the parenchyma, the branching tubules were almost absent. The duct system was well developed. The smaller ducts were lined by columnar epithelium with very few goblet cells seen mainly towards the termination of these ducts (Fig. 6b). The features of acini were same as the features at earlier age. The main pancreatic duct could be seen entering the connective tissue around the duodenum along with the common bile duct. The duct was lined by simple to stratified columnar epithelium with many goblet cells. The goblet cells had an empty cytoplasm with a basal well defined nucleus. The ducts were surrounded by large amount of connective tissue containing abundant collagen fibres, fibroblasts and some blood vessels (Fig. 6c). The islets were easily recognisable. Most of the islets were very large. Their cytoarchitecture was well developed with a capsule of delicate loose connective tissue. The cells in them were loosely packed compared to the acini and were pale staining. The islets were well vascularised.

Fig.1a: Haematoxylin and eosin staining (H&E) at 10 weeks showing the pancreas with a large amount of primitive mesenchyme (M) with branching tubules (T) embedded in it and lined by columnar epithelium. (Scale Bar- 1cm = 15µm.)

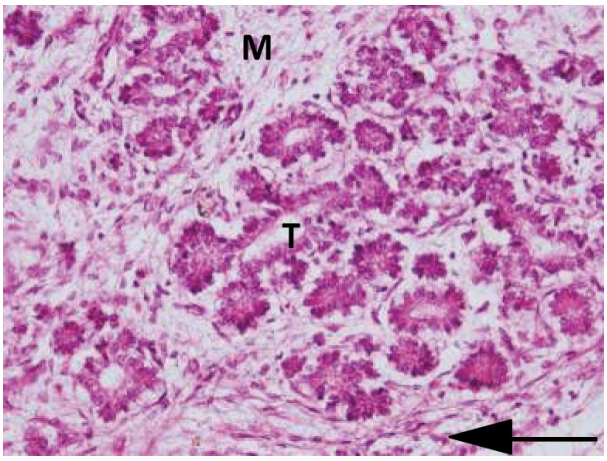


Fig. 1b: Masson's Trichrome staining at 10 weeks showing the branching tubules with their basement membrane visible on the luminal side (arrow). Scattered flat and elongated nuclei of fibroblasts were seen (arrowhead). (Scale Bar- 1cm = 15µm.)

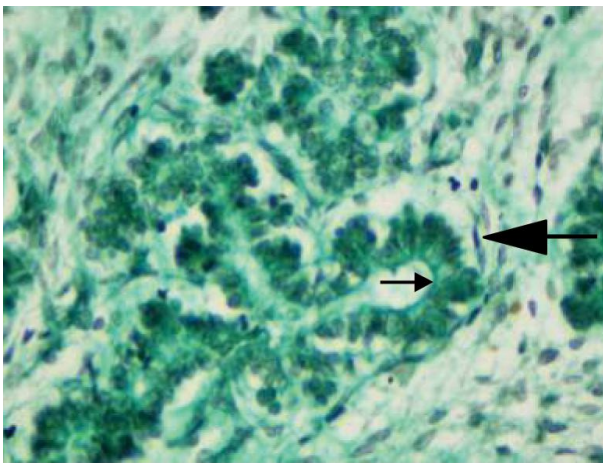


Fig 2a: H&E staining at 12 weeks showing tubules lined by columnar epithelium. The cells show a light eosinophilic cytoplasm, well defined, elongated nuclei present towards the base (arrow). At places, the tubules show stratified lining epithelium (arrowhead). Cells are seen budding out from the tubules especially from these areas in the form of cords and small clusters. (Scale Bar- 1cm = 15µm.)

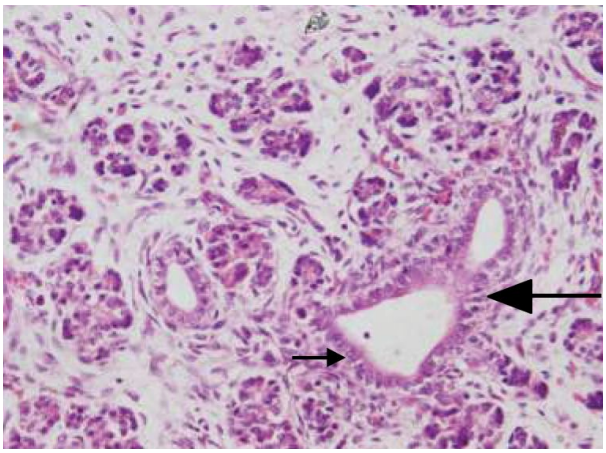


Fig. 2b: Masson's Trichrome staining at 12 weeks depicts the abundant mesenchymal between the glandular components. The glandular components show an ill-defined organisation into lobes (arrow). (Scale Bar- 1cm = 15µm.)

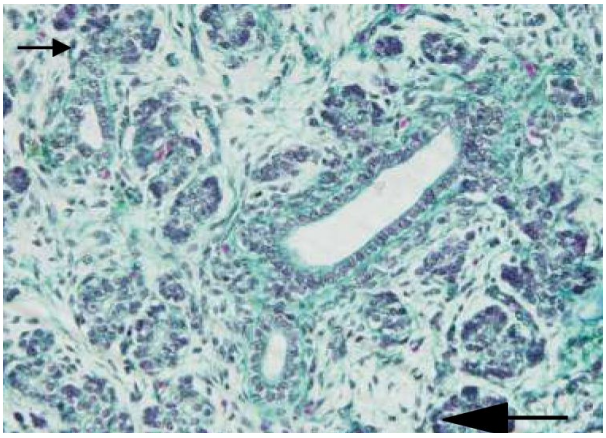


Fig. 3a: H&E staining at 14 weeks showing an oblique section of the main pancreatic duct (D) surrounded by abundant connective tissue (arrowhead). Some small islets with few pale staining cells were seen in close proximity to the tubules (arrows). (Scale Bar- 1cm = 15µm.)

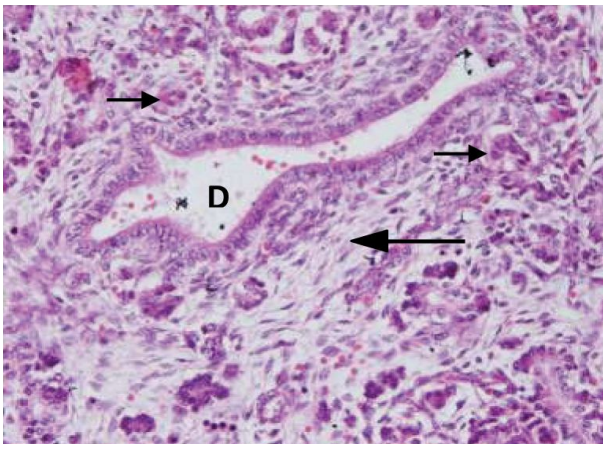


Fig. 3b: Masson's trichrome at 14 weeks showing proliferating acini (A) and branching tubules (T) in mesenchymal tissue (M). (Scale Bar- 1cm = 15µm.)

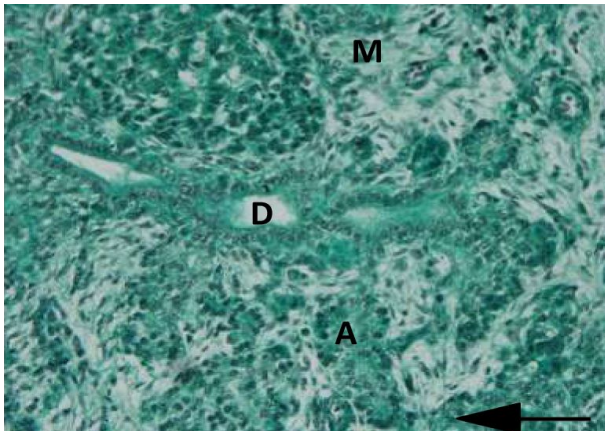


Fig. 4a: H&E staining at 18 weeks depicting the lobular architecture of the gland. Islet (arrowhead) with well-developed capillaries (arrow) are seen. (Scale Bar- 1cm = 15µm.)

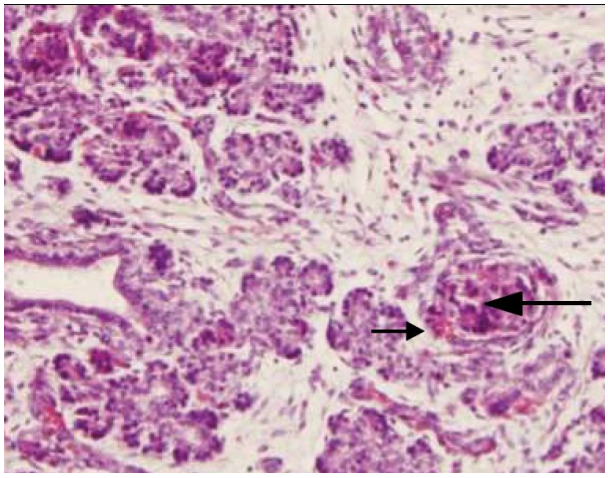


Fig. 4b: Masson's Trichrome stain at 18 weeks showing well developed interlobar connective tissue septa (arrow), whereas, the intralobular connective tissue was sparse and delicate (arrowhead). (Scale Bar- 1cm = 15µm.)

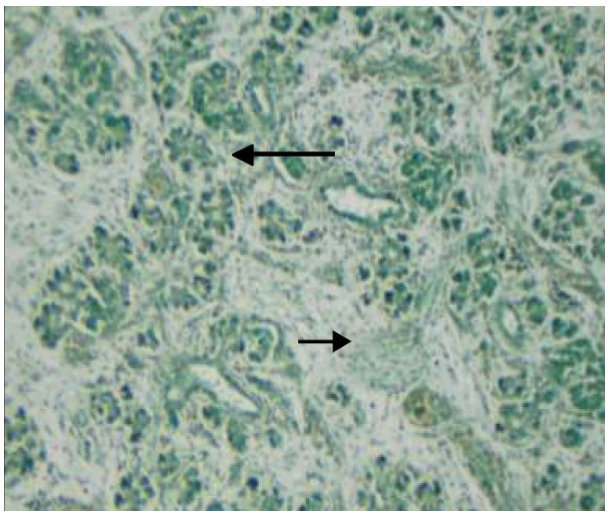


Fig. 5a: H&E staining at 22 weeks showing differentiation of the pancreas into lobes. The acinar cells showed a basal basophilia and apical acidophilia (Arrow). (Scale Bar- 1cm = 100µm.)

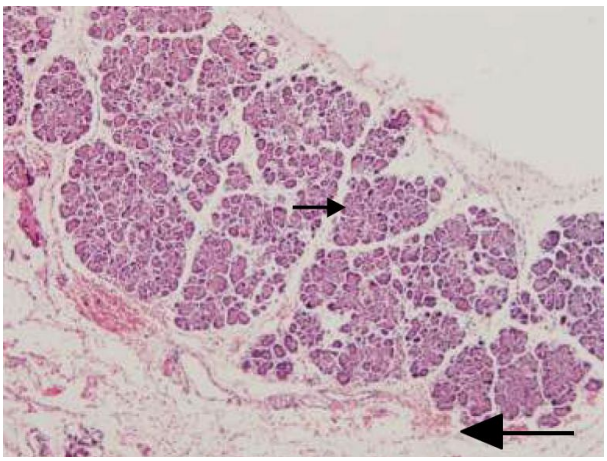


Fig. 5b: Masson's Trichrome staining at 22 weeks with prominent interlobular septa (arrowhead) and delicate intralobar septa (arrow). (Scale Bar- 1cm = 100µm.)

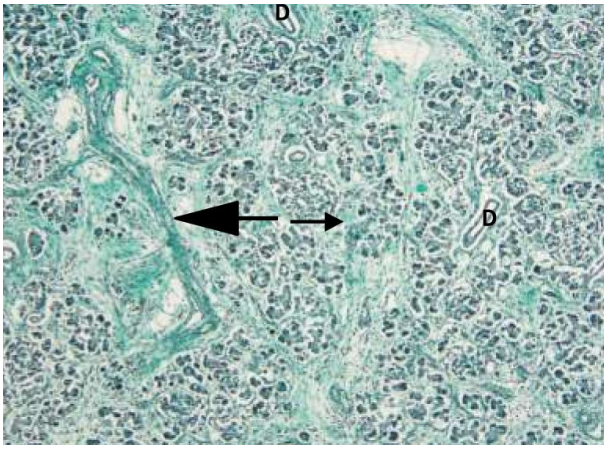


Fig. 6a: H&E staining at 36 weeks showing a distinct lobular architecture. There is well defined basal basophilia and apical acidophilia in the pyramidal acinar cells. The duct is surrounded by abundant connective tissue. Many large islets surrounded with a delicate connective tissue capsule are seen (arrows). (Scale Bar- 1cm = 100µm.)

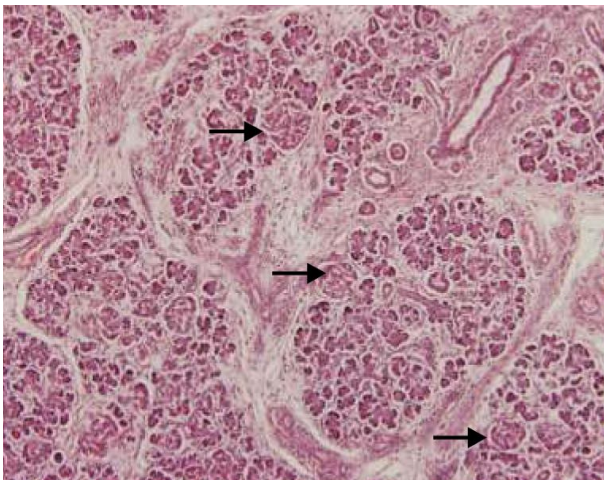


Fig. 6b: Masson's Trichrome stain at 36 weeks showing distinct lobular architecture in the gland. (Scale Bar- 1cm = 100µm.)

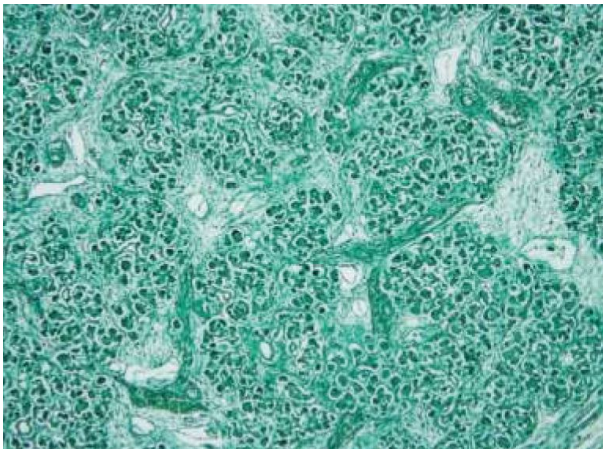
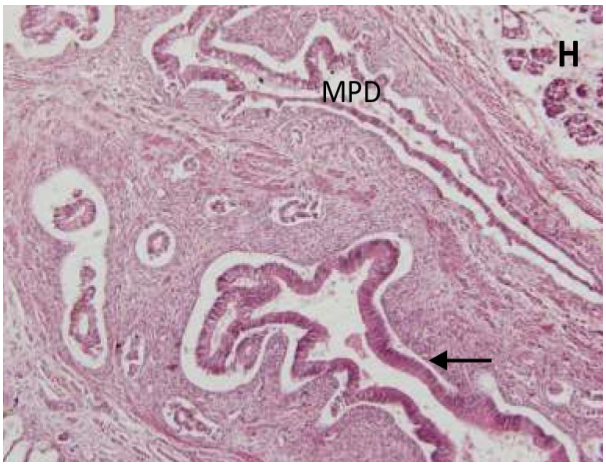


Fig. 6c: H&E staining at 36 weeks showing the main pancreatic duct (MPD) coming from the head of pancreas (H) and containing many goblet cells. The common bile duct is also seen (arrow). (Scale Bar- 1cm = 100µm.)



Discussion

Our study was directed towards understanding the morphological maturation of human fetal exocrine pancreas through the different gestational ages. The pancreas develops from two evaginations of the foregut endoderm – a dorsal and a ventral pancreatic bud, which later fuse to form a single organ [6]. Thus, the inferior part of the head of pancreas and the uncinnate process are derived from the ventral bud while the rest of the head, neck, body and tail are derived from the dorsal bud [7].

The youngest fetus in our study belonged to the 10th week of gestation. By then, the dorsal and ventral pancreatic buds had already fused to form one single mass. Falin LI has noted that from 9-11 weeks of fetal life, the gland had abundant primitive mesenchymal tissue in which were embedded branching tubules lined by low columnar epithelium. The cells in the

tubules had oval nuclei towards the base [8]. Our findings were similar to this study and also another study by Meier JJ et al [9]. The pancreas was easily differentiated as a glandular structure developing in the connective tissue around the duodenum. Literature reveals that epithelia-mesenchymal interactions are very important in pancreatic organogenesis [10, 11]. The tubules were branching to form primitive acini especially where they were lined by stratified epithelium similar to the findings other studies [12]. This suggests that the epithelium lining of the tubules serves as the precursor for acinar and islet cells.

In the 12 weeks old fetus, the parenchyma had less of primitive mesenchyme. Branching tubules with a wide lumen were present. The vasculature was developing and small blood vessels could be seen. Our findings are in corroboration with the work of Conklin JA [13]. Clark A and Grant AM have described that by 10 weeks of gestational age, lobular architecture became apparent and thereafter, up till week 20, there was rapid expansion of the glandular component of pancreas [14].

Gupta V et al have reported that from 18 week onwards, a mature lobular architecture was visible in the pancreas [15]. The findings in our study are comparable to the findings by these authors. Multiple blood capillaries are now associated with the islets and acini signifying that functional maturation of the gland is associated with coordinated vascular development. The importance of vascular supply for pancreatic development, especially the islets, has been highlighted by a series of studies. It has been demonstrated that vascular endothelial cells provides signals for embryonic pancreatic development [16, 17].

Falin LI has described that by 17-18 weeks month of gestation, the glandular acini were tightly packed in a well-developed connective tissue stroma. The acinar cells showed a distinct apical acidophilia at 5th month of gestation. This is due to formation of zymogenic granules in apical cytoplasm and implies functional maturity [8]. Our findings corroborate his study. As the gestation age advanced, we found that the lobular architecture became more evolved. The parenchyma had fewer tubules. This is because they had differentiated into ducts. The previous works show that by the age group of 28 weeks, the parenchyma had sharply defined lobe and lobules with dense connective tissue septa. These septa had interlobular ducts and large blood vessels. The acini were tightly packed. Our findings are in agreement to these studies [12, 13]. In the 36 weeks old fetus, the pancreas had acquired a mature appearance.

Conclusion

The exocrine pancreas begins to differentiate early in fetal life. The acini aggregate and undergo a phase of rapid growth and maturation. Their cell shape and staining characteristic of cells undergo transformation at around 18 weeks. The formation of new acini from the ducts continues throughout fetal life. There is a simultaneous increase in the growth of exocrine and the endocrine tissue. It is evident that by 28 weeks of age, the pancreas attains considerable morphological maturation in order to meet the increasing demands of the developing fetus. The acini and the exocrine part, both are functional in fetal life. Islet cells have a role in glucose metabolism of the fetus. This is proven by studies that show that as a result of hyperglycaemia in foetuses of diabetic mothers, islets undergo hypertrophy and hyperplasia [18].

Studies indicate that despite the morphological maturation of pancreas during the pre-natal period, adult functional capacity of the acini is not yet achieved. The neonates show some pancreatic insufficiency which subsides by the end of first year of life. Also, any insult to the developing fetal pancreas results in exocrine and endocrine insufficiency. Further studies are required to understand the mechanism of these injuries and hence prevent them.

Conflict of Interests

None.

Authors' Contributions

All the authors have made substantive intellectual contributions to this study as regards: (1) conception and design, or acquisition of data, or analysis and interpretation of data, and; (2) drafting the manuscript or revising it critically for important intellectual content. All the authors have given final approval of the version to be published. Each author has participated sufficiently in the work to take public responsibility for appropriate portions of the content.

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