Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2016; 4(7A):2332-2337 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2016.v04i07.005

Original Research Article

Cellular Differentiation of Developing Pancreas in Human Fetuses of Manipuri Origin

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Abstract: Cellular differentiation of the developing pancreas was studied in the pancreatic specimens obtained from the aborted fetuses and still births of Manipuri origin using hematoxylin-eosin and modified aldehyde fuchsin stains. Both the exocrine and endocrine components were identified in the 13-16 weeks of gestation, the youngest age group of the study. However, the glandular components were more characterized and differentiated as the gestational age advanced. The pancreas showed presence of an outer covering of loose connective tissue. The islets were devoid of proper capsule, but separated from the surrounding structures by thin layer of connective tissue. The islet cells i.e. alpha, beta and delta cells showed intermingling type of arrangement in all the gestational age groups in the study. The development of glandular components continued throughout the gestational period as evident by the presence of glandular buds at the terminals of branching tubules.
Keywords: Fetuses, Pancreas, Gestation, Acini, Islets

INTRODUCTION

The pancreas, the second largest gland associated with the gastrointestinal tract is a derivative of the caudal part of the primitive foregut. It is a mixed gland having both exocrine and endocrine components. The exocrine portion elaborates digestive juice and the endocrine portion, also called the islets of Langerhans, secretes hormones important for regulation of glucose, lipid and protein metabolism [1]. The islets constitute about 1-2 % of the volume of the pancreas [2]. Four major cell types are identified in the mammalian islets - alpha (A), beta (B), delta (D) and pancreatic polypeptide producing (PP) cells. The alpha cells secrete glucagon, beta cells insulin and delta cells somatostatin [3].

There are various studies on the cellular differentiation of the pancreas. However, regarding the time of appearance and differentiation of the glandular components especially of the endocrine pancreas, different opinions do appear. Broadly, the time of appearance of the islets during fetal development can be put into two categories: 10-11 weeks [4-6] and 12-14 weeks [7, 8]. Considering the differences in the opinions of the workers, we take up the study on cellular differentiation of the developing pancreas in human fetuses of Manipuri origin to enrich our understanding. An attempt has been made to throw light on the time of appearance and histological changes of the developing pancreas in the different gestational periods.

MATERIALS AND METHODS

The study was carried out in the Department of Anatomy, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur for a period of two years after getting clearance from the Institutional Ethics Committee.

Collection of specimen:

The human fetuses of Manipuri origin after legal abortions and still births of less than 4 hours duration were obtained from the Department of Obstetrics and Gynaecology, RIMS, Imphal with due permission from the parents and the authority concerned. The collected fetuses were fixed in 10% formalin as quickly as possible. Fifty five dead fetuses of either sex without any gross abnormality were included in the study. The fetuses were grouped into

different categories of gestational age (Table-1) based on the crown-rump length (CRL) [9].

Dissection of pancreas

Abdomen was opened through bilateral subcostal incision extending laterally and downwards to the anterior part of iliac bone on both sides. The pancreas was exposed by retracting the stomach upwards and transverse colon downwards by dividing the gastrocolic omentum. The body and tail of the pancreas was mobilized by incising peritoneum along its inferior border. The hepatic flexure of colon was mobilized and peritoneal attachments of the duodenum were incised. Further, mobilization was continued proximally and superiorly to visualize the common bile duct. The duct was cut near its duodenal attachment. After dissecting out the distal portion of the duodenum along with the pancreas, the latter was separated from the duodenum.

Table 1: Gestational age based on the crown-rump length

Crown-rump length (cm)	Gestational age (weeks)	Number of fetuses
5-8	9-12	0
9-14	13-16	5
15-19	17-20	14
20- 23	21-24	20
24- 27	25-28	8
28- 30	29- 32	4
31-34	33- 36	2
35 - 36	37-38	2

Preparation for histological examination

The standard histological techniques were followed in the study [10]. Dissected out pancreas specimens were fixed in 10% formal saline for 7-10 days, followed by secondary fixation in Bouin's fluid for 24 hours. Each gland was trimmed and divided into three portions - head, body and tail. The tissues were dehydrated by immersing in different grades of alcohol, and then processed for paraffin sections of 5 microns (µ) thickness. Using hot water bath, the sections were mounted on albuminated slides. For light microscopic studies, the sections were stained with hematoxylin-eosin (H&E) and modified aldehyde fuchsin. The islet cells were identified with modified aldehyde fuchsin which imparted A (alpha) cells greenish yellow, B (beta) cells deep purple violet and D cells green. Their cell nuclei were stained blue black. The stained slides were examined under different magnifications. Those slides showing maximum clarity and differentiation of the tissues were chosen and photographed.

RESULTS

In our study, 20 fetuses were in the 21-24 weeks of gestation followed by 14 and 8 fetuses in the 17-20 and 25-28 weeks of gestation respectively. Five fetuses were in the 13-16 weeks, 4 in the 29-32 weeks and 2 each in the 33-36 and 37-38 weeks of gestation (Table 1).

The stained slides showed that the pancreas possessed thin layer of loose connective tissue as its outer covering and the lobes were not distinctly demarcated as the septae arising from the connective tissue were often incomplete. The islets were devoid of proper capsule and most of them were separated from the surrounding structures by a thin layer of connective tissue. The endocrine component was seen more in the tail than the body and head portions of the gland.

13-16 weeks of gestation (9-14 cm CRL):

Plenty of mesenchymal connective tissues and proliferating tubules were seen. At places, the cells at the terminal ends of tubules enlarged and grouped around a central lumen. The cuboidal cells with eosinophilic cytoplasm and basophilic nuclei lined the tubules. The various parts of the duct system could be recognized. The lining epithelial cells of the intralobular duct were cuboidal or low columnar. The islets of varying sizes were present. Solid masses of undifferentiated cells were also seen separating from the tubules. Some of the islets were surrounded by thin layer of connective tissues, separating from the surrounding parenchyma. The islet cells were clustered in the centre with wide peripheral spaces (Figure 1&2). With modified aldehyde fuchsin stain, occasional cell types with greenish yellow cytoplasm with blue black nuclei could be identified among the tubular and ductal epithelial cells (Figure 3).

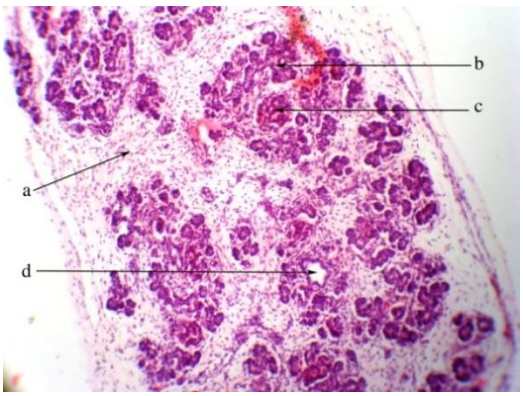

Fig-1: Pancreas (CRL-9.5 cm); a - connective tissue, b-branching tubule, c-islet, d-interlobular duct; Stain: H&E. 10x.

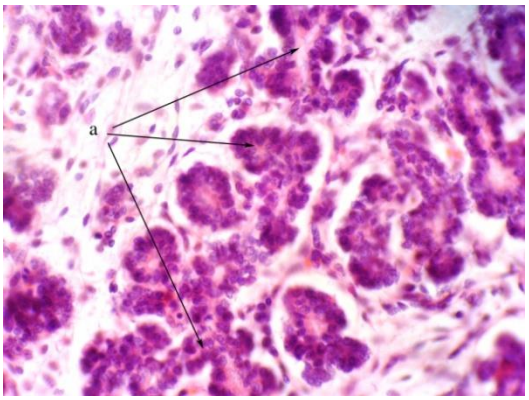

Fig-2: Pancreas (CRL-12 cm); a - branching tubules with terminal buds; Stain: H&E. 40x

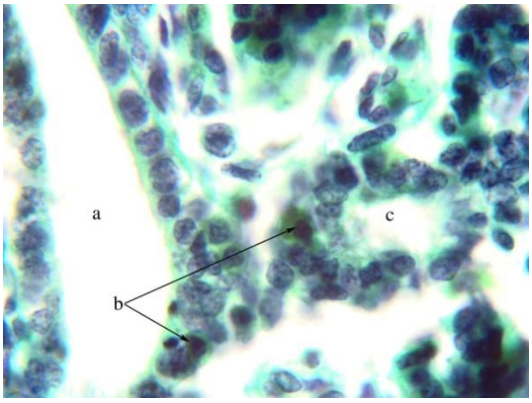

Fig-3: Pancreas (CRL-9.5 cm); a - tubule, b - A cells, c - intralobular duct; Stain: Modified aldehyde-fuchsin.100x

17-20 weeks of gestation (15-19 cm CRL)

The glandular tissues increased and more well-formed lobules and acini were seen. The acinar cells appeared more eosinophilic than before. Groups of undifferentiated or solid masses of cells were also present. The duct systems with branching tubules could be identified. Blood vessels were seen in the interlobular connective tissue (Figure 4). The columnar epithelial cells lined the interlobular ducts while the intralobular ducts had low columnar or cuboidal epithelium lining. The islets increased in size and vascularised as evident by increased RBCs. Some of the islets were well formed (Figure 5).

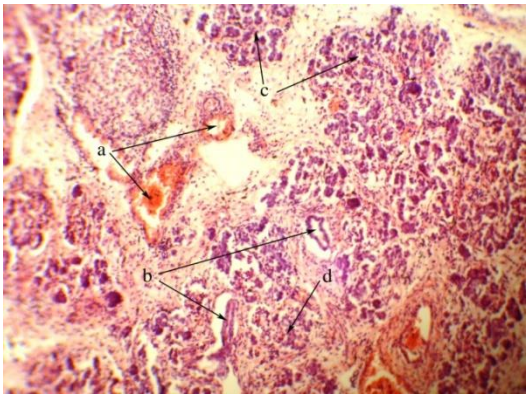

Fig-4: Pancreas (CRL-5.4 cm); a - blood vessels, b - Interlobular ducts, c-branching tubules, d-islet; Stain: H&E. 10x.

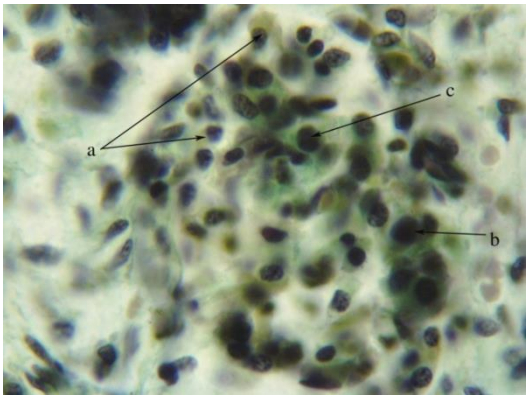

Fig-5: Islet (CRL-16 cm); a - A cells, b - B cells, c - D cells; Stain: Modified aldehyde- fuchsin. 100x

21-24 weeks of gestation (20-23 cm CRL)

The lobules and ducts were well formed. The branching tubules and interlobular connective tissues reduced than in the earlier weeks, but the acini were more numerous. Solid masses of undifferentiated cells were seen (Figure 6). The islets were present in varying sizes with the different cell types intermingling with each other (Figure 7).

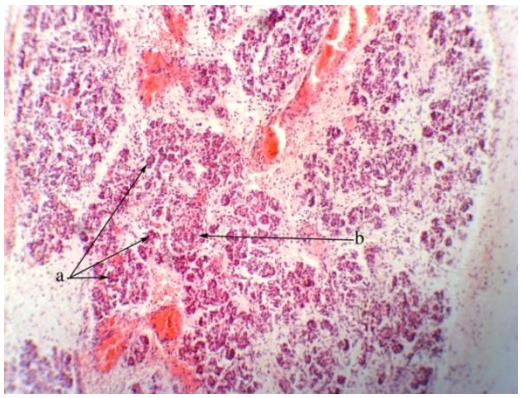

Fig-6: Pancreas (CRL – 22 cm); a - acini, b-islet; Stain: H&E. 10x

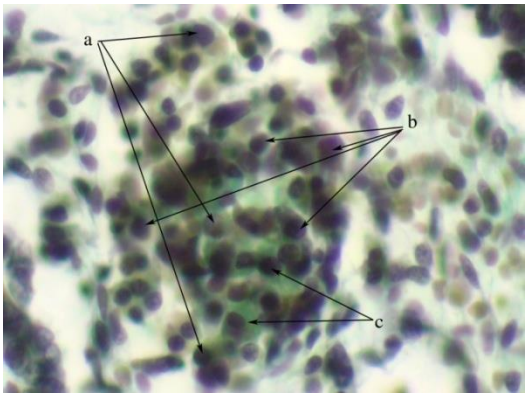

Fig-7: Islet (CRL-23 cm); a - A cells, b - B cells, c - D cells; Stain: Modified aldehyde-fuchsin. 40x. (enlarged)

25-28 weeks of gestation (24-27 cm CRL)

There was marked reduction in the interlobular connective tissue than before. The tubules showed less branching. Solid masses of undifferentiated cells were still present. The acini were well formed with markedly eosinophilic apical portions. The islets were found in groups of varying sizes and surrounded by the acini (Figure 8). The cellular arrangement of islets remained unchanged.

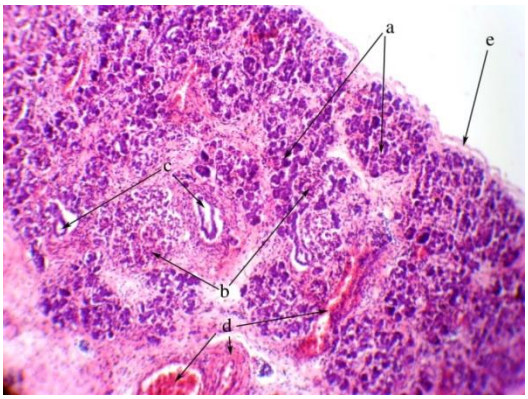

Fig-8: Pancreas (CRL – 25.7 cm); a - acini, b - islets, c-interlobular ducts,
Stain: H&E. 10x

29-32 weeks of gestation (28-30 cm CRL)

The lobules comprised of numerous acini separated by thin connective tissues. The acinar cells looked pyramidal in shape with more acidophilic apical and basophilic basal portions. Clusters of undifferentiated cells and cell buds were also seen. The islets increased in size (Figure 9).

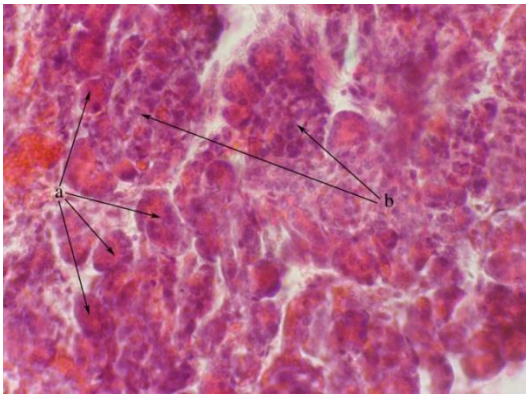

Fig-9: Pancreas (CRL-29.7 cm); a - acini, b-islets; Stain: H&E. 10x.

33–36 weeks of gestation (31-34 cm CRL)

The acini appeared larger. Occasional cell buds and undifferentiated clumps of cells could be identified. The islets increased in size and the different cell types showed intermingling arrangement (Figure 10).

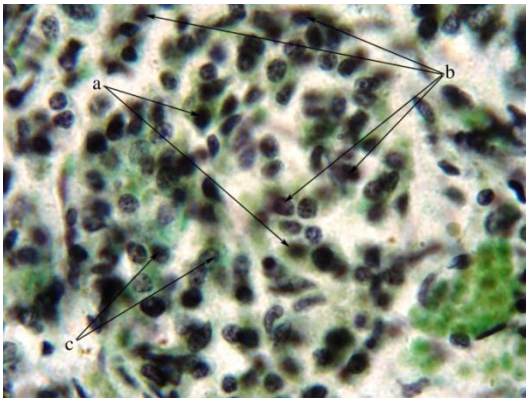

Fig-10: Islet (CRL-33 cm); a - A cells, b - B cells, c - D cells; Stain: Modified aldehyde-fuchsin. 100x

37-38 weeks of gestation (35-36 cm CRL)

There was no significant change in the cellular pattern except for increase in size of both the acini and islets. Occasional undifferentiated acinar and islet cell masses were still present (Figure 11).

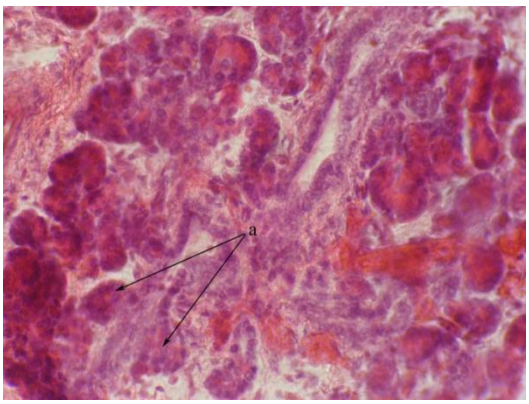

Fig-11: Pancreas (CRL-35.7 cm); a - glandular buds. Stain: H&E. 40x

DISCUSSION

The pancreatic specimens were obtained from fetuses fixed in 10% formalin as quickly as possible after legal abortions or still births. In tissues fixed within 2-3 hours after death the granulations took up stains as perfectly as in fresh specimens [11]. The gestational age was determined based on the crown rump length (CRL) of the fetuses as adopted by renowned authors [9, 12]. Hematoxylin-eosin (H&E) and modified aldehyde fuchsin stains were used for histological examination. H&E is the most widely used stain because of its simplicity and ability to demonstrate clearly a number of different tissue structures [13]. Modified aldehyde fuchsin is one of the special differential stains for the islet cells [10, 14].

The stained sections showed thin outer covering of loose connective tissue from which septae passed into the gland as reported by renowned workers [15, 16]. Mesenchymal connective tissues were seen in plenty in specimens of 12-13 weeks as observed by Gupta V *et al* [17]. The presence of lobules, branching tubules with buds, intralobular and interlobular ducts in the tissue sections of 13-16 weeks gestation was in agreement with the findings of some of the workers [18, 19]. Falin LI [6] reported the presence of acinar cells in various gestational age groups e.g. 17-20, 21-24, 25-28 weeks and similar pictures were observed in our study. The enlargement of acinar cells with well-defined eosinophilic apical and basophilic basal portions, reduction in the interlobular and intralobular connective tissues were the main histological observations in the exocrine pancreases of 29-32, 33-36 and 37-38 weeks of gestation. Gupta V *et al.* [17] reported that no branching pattern of the tubules was seen in fetuses above 34 weeks of gestation. However, in our study, clusters or solid masses of undifferentiated cells and cell buds from the tubules or ductules were observed in all the tissue sections from the different weeks of gestation.

The literature mentioned differentiation of the islets at different periods e.g. 6-10 weeks [7], 10 weeks [5], 9-10.5 weeks [20] and 10-12 weeks [6]. In our study, the youngest fetus available was in the 13-16 weeks (CRL-9.5cm) of gestation and therefore, we were unable to share our observations before 13 weeks of gestation. The observation of a few occasional cells presenting with similar staining characteristics of A cells among the tubular and ductal epithelium (Figure 3) could be an indication that the endocrine cells developed from the tubular or ductal epithelium.

Different workers observed different arrangement patterns of the islet cells e.g. mantle and intermingling type [5], bipolar or mantle type [6] and mantle type [18]. The patterns of cellular arrangement in our study were intermingling type in the different periods of gestation. Similar pattern of arrangement was also observed by Gupta V *et al* [17]. However, it was difficult to ascertain the predominant cell type on the whole as the cells particularly A and B cells showed varying predominance in different islets in the study. In older fetuses, the islets increased in size and vascularity as shown by the presence of numerous RBCs among the islets.

CONCLUSION

The pancreas possessed thin layer of loose connective tissue as its outer covering and the lobes were not distinctly demarcated as the septae arising from the connective tissue were often incomplete. The islets were devoid of proper capsule and their distribution was more in the tail portion of the gland. The presence of A cells among the tubular and ductal epithelial cells could be an indication that the endocrine component developed from the tubular and ductal epithelium.

Upto the 13^{th} week of gestation, both the exocrine and endocrine components did not developed fully. Both the components were characterised and differentiated with advancing weeks of gestation and the differentiation continued throughout the gestational period. There was reduction of the interlobular and intralobular connective tissues with the appearance of well-formed glandular components. The islet cells showed intermingling type of arrangement.

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