Original Article

PERINATAL HISTOLOGY OF ENDOCRINE PANCREAS IN ALBINO RAT AN EXPERIMENTAL STUDY

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Objective: To analyze the process of growth, differentiation and development of pancreatic islet alpha and beta cells during late fetal and early post-natal period.

Methods: An observational experimental study was conducted in the animal research laboratories of University of Health Sciences, Lahore. Adult non-diabetic male and female albino rats were procured from National Institute of Health, Islamabad and kept under standard conditions in animal house. Mating was allowed by keeping female and male rats in the same cage with ratio of 3:1. Pregnancy was confirmed by the observation of vaginal plug. Pregnant rats were divided into 3 groups. First group rats were sacrificed on day 20 of gestation and their foetuses were dissected to procure the pancreatica of Study group A. Pups were born after 22-23 days postcoitum for rest of the pregnant rats. They were divided into two groups B &C, having ten pups each. Group B pups were sacrificed on day 2 postnatal and group C on day 7 postnatal to obtain pancreatic tissue. The pancreatica, so obtained were fixed, processed and sectioned at 4µm thickness. Sections were stained with H&E for light microscopy and with Chrome Alum Hematoxylin-Phloxin stain for differential counts. Observations were made regarding number of islets/section, mean diameter of islets, mean number of total cells /islet, mean number of α cell and β cell/ islet and the mean ratio of β: α cell.

Results: The pancreatic tissue on light microscopy showed both exocrine and endocrine elements; the former predominated later. Islets of Langerhans were observed as clumps of light staining cells in well-developed acinar parenchyma in both fetal and postnatal groups; Both postnatal groups showed strong association of pancreatic tissue with ducts whereas, group A showed mesenchymal tissue in close vicinity of developing islets. Quantitative variables were compared using one way ANOVA. Mean islets per section for group A was 6.3±1, for group B 7.8±1 and for group C 3.1 with significant difference among the groups ($p<0.05$). Mean diameter of an islet was 112±1 for group A, 136±2 for group B, and 171±5 for group C with statistically significant difference among the groups. Total number of cells per islet did not show statistically significant difference ($p>0.05$). Number of β cells per islet was 95±2 for group A, 76±4 for group B and 102±3 for group C, which was statistically significant ($p<0.05$). Number of α cell per islet and the ratio of β and α cell was not statistically significant among the groups.

Conclusion: All the parameters studied showed gradual increase postnatally. Number of islets though decreased but the diameter of islets increased gradually. Differential cell counts showed gradual increase in number with their relative proportion comparable to adult ratios in late postnatal group.

Keywords: Perinatal, Histology. Endocrine, pancreas.

Introduction

The pancreas is a mixed gland, comprising of two different cell populations, exocrine and endocrine. The exocrine component dominates the parenchyma of pancreas and consists of acinar and ductal cells that secrete and transport digestive enzymes respectively into small intestine. Its endocrine cells are segregated in discrete groups; islets of Langerhans, first described by Paul Langerhans. The Islet tissue comprises 1-2% mass of healthy adult human pancreas; there are about one million islets collectively weighing 1-2 grams.[1] Although the adult pancreas is a single organ, it is derived from two growth buds; dorsal and ventral buds. During embryogenesis, the dorsal pancreas arises from foregut tube just ventral to the notochord and caudal to the region of stomach, while the ventral pancreatic bud develops from the endodermal hepatic diverticulumSignals released from both the notochord and endothelial cells are shown to be mandatory for proper regulation of the dorsal pancreas,while signals from the cardiac mesoderm

are found to influence the development of ventral pancreas.[2] Endocrine pancreas comprises of ; alpha (α) cells producing glucagon (15-20%), beta (β) cells producing insulin & amylin (65-80%), delta (δ) cells producing somatostatin (3-10%), PP cells producing pancreatic polypeptide (3-5%), and epsilon cells producing ghrelin, an antisatiety hormone (<1%)[3,4].In adult human pancreas, Islets have a central mass of insulin-secreting β cells and a surrounding mantle of α, δ and PP cells. This relative pattern of cell arrangement may be different amongst species but the beta cells are always clumped together with an adjacent mix of non-beta cells. In experimental studies, rat islets dispersed into single cells, when allowed to re aggregate in tissue culture, the beta cells formed a central core with non-beta cells around it, thus re-establishing the natural pattern which may have a role in normal functioning of the islets.[5]Developmentally, beta cells are believed to be generated from a population of pancreatic progenitor cells. These progenitor cells divide and differentiate into beta cells. Once formed these beta cell are post mitotic. However, replication of differentiated beta cells can lead to addition of new beta cells. High rates of beta cell replication during the early post-natal period results in tremendous increase in the beta cell mass.[6] Both insulin and glucagon are detectable by 8th week of development in humans with insulin positive cells being more prevalent type in early fetal life. The relative delay in hormone biosynthesis and earlier expression of insulin rather than glucagon in human is suggestive of the relative difference of endocrine differentiation programme of human from that of rodents.[7]Perinatal malnutrition impairs neogenesis and beta cell regeneration with preserved beta cell proliferation, thereby, implying an impaired capacity of malnourished animals to neogenesis of beta cell.[8] Postnatal expansion of the pancreatic beta-cell mass is dependent on survivin, a gene critical for cell division and cell survival in cancer cells; it was also implied that survivin had a role in the maintenance of beta cell mass through both replication and anti-apoptotic mechanisms.[9] Perinatal is term used to describe the time around birth originating from two words "peri" means about or around, and "natus" means birth. It is different for different species depending on the length of gestation; in humans, it is considered to range between 28weeks of gestation to 4weeks after birth. In rat, however, it spans between seventeenth day of pregnancy to eleventh day postnatally.[10]

The current study was undertaken to evaluate the process of growth and development of islets of Langerhans during late fetal and early postnatal period using rat as an experimental model.

Methods

20Adult non-diabetic albino rats (15 female, 5 male) were obtained from National Institute of Health, Islamabad and kept under standardized conditions in animal laboratory of University of health sciences. After acclimatization animals were facilitated to mate by keeping female and male adult rats in the same cage in the ratio of 3:1. Pregnancy was confirmed by observation of vaginal plug. Pregnant rats were randomly divided into three groups, A, B and C, each containing five animals. Progression of Pregnancy was monitored by weighing the pregnant animals on alternate days. Group A pregnant rats were sacrificed on 20th day of gestation to extract the fetuses which were dissected to procure fetal pancreatic tissue, while groups B and C females were allowed to continue pregnancy. After 22-23 days of gestation pregnant rats delivered pups which were divided into two groups B and C. Group B pups were sacrificed on day 2 postnatal and group C pups were sacrificed on day 7 postnatal to procure the pancreatic tissue. The pancreatic tissue was dissected out and fixed in 10% formalin for 72 hours. Then it was cut into small pieces and treated in automatic processor, blocks so obtained were cut into 4 μm sections and stained with H&E for light microscopy and Chrome Alum Hematoxylin-Phloxin stain for differential counts of alpha and beta cells of pancreatic islets.

Micrometry

Micrometry was done for diameter of the islets by taking two diameters for each islet at right angle to each other and then a profile diameter was calculated by averaging the two[11]. At least four islets selected at random were so measured on each section and then their average was taken as diameter of islet of that particular section. Four sections of each block were so observed.

Micrometry for cell counts

Cell counting was done at 40X by using the grid on which the islet section was superimposed, cells were counted in all squares of grid superimposing the islet; leaving lower and left lines to avoid double counting..

The total number of cells per islet section and the beta and alpha cells were counted as they differentially stained with chrome alum Hematoxylin-phloxin. Four sections were observed for each tissue and four islets were counted on each section randomly and the average taken. Later on, ratio of beta and alpha cells

One way ANOVA was used for statistical analysis. Multiple comparisons were made using Post Hoc Tuckey test. A p-value of < 0.05 was considered as statistically significant.

Results

The pancreas was identified on dissection as pinkish lobulated mass clamped between duodenum and spleen. Light microscopy was done to observe exocrine and endocrine portions of the pancreas in all three groups on H&E staining. Both exocrine and endocrine tissue were observed in group A pancreatic tissue. Exocrine acini were well-formed but lobulation was less marked **Fig 1**. Most of pancreatic tissue comprised of exocrine parenchyma with rather scanty endocrine tissue; developing islet tissue looked like a bunch of pale staining cells.

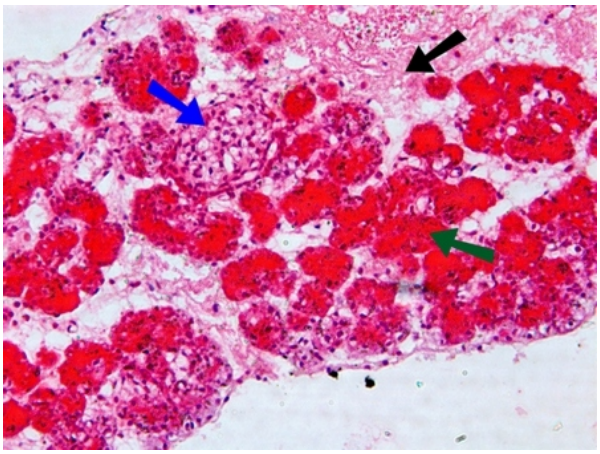

Fig-1: Photomicrograph from group A pancreatic tissue, showing poorly lobulated exocrine acini and islet tissue in scarce amount, with ill-defined capsule around it. Blue arrow showing islets, green arrow (acini) and black arrow (mesenchymal tissue). H&E 200X.

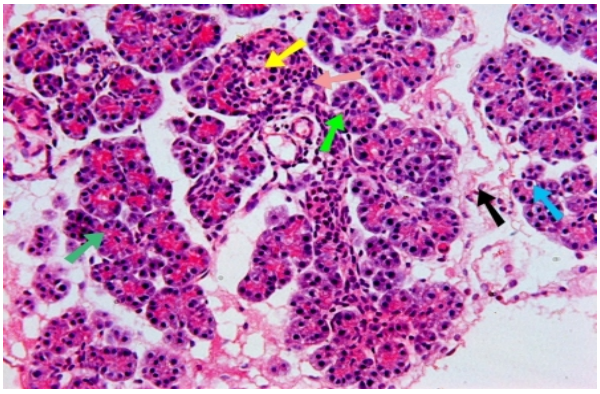

Fig-2: Photomicrograph from pancreatic tissue of group B, showing developing islets (yellow arrow), intercalated ducts (pink arrow), mesenchymal tissue (black arrow), apoptotic body (blue arrow) and exocrine tissue (green arrow). H&E 200X.

Light microscopy of group C tissue showed well differentiated lobes and lobules; islets were well circumscribed with clear demarcation from the surrounding acinar tissue **Fig3.**Islets were independent from ducts and mesenchymal tissue was absent

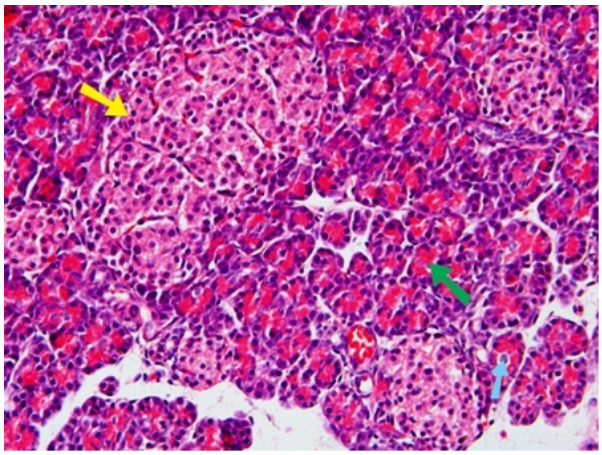

Fig-3: Photomicrograph of a pancreatic tissue preparation from group C, showing pancreatic tissue with well-defined acinar lobules (green arrow) and well-demarcated islets (yellow arrow) in abundance. H&E 200 X.

In group B on light microscopy, islets were in abundance, scattered among exocrine acini, differentiating and branching to form lobes and lobules **Fig -2.** Number of islets per unit field area was counted on random fields, four fields were taken randomly for each slide and then the average was taken. Four slides were studied for each tissue specimen. Mean number of islets per unit field was taken for all groups and then compared **Table1 and Fig 4**.

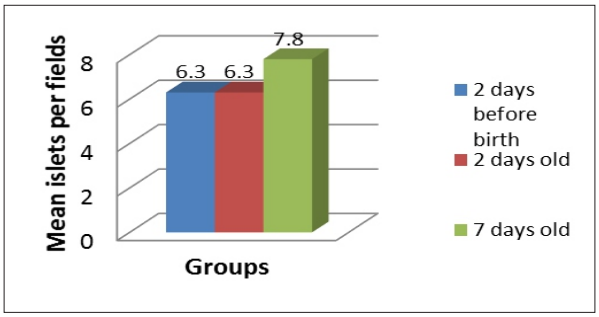

Fig-4: Bar chart showing the mean number of islets per unit field in various groups.

Significant difference was observed in the mean number of islets per field between groups B and C (P=0.05), and C and A (p=0.05) but no significant difference was observed in the mean number of islets per unit field between groups B and A (P>0.05). The mean diameter of islets was taken by measuring maximum and minimum profile dimensions of an islet and then taking average diameter as the islets were of different shapes like oval, round and elliptical. Diameters of four random islets were taken for each slide and four sections were randomly observed for each tissue of each group. Mean diameters were taken and then compared among the groups **Table1 and Fig 5**.

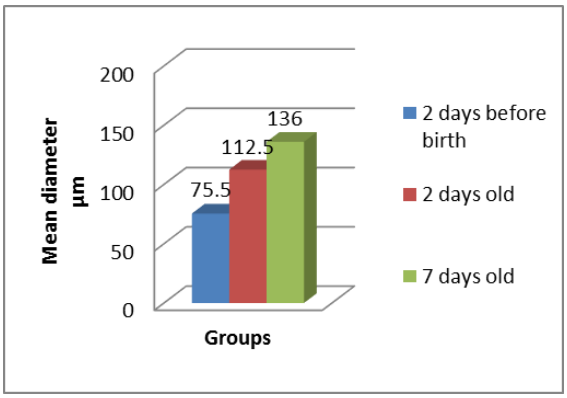

Fig-5: Bar Chart showing the mean diameter of islets in different groups.

Significant difference was observed in the mean diameter of islet between groups A and C (P=0.001). No significant difference was observed in the mean diameter of islet between groups B and A (p-value = 0.07) and groups B and C (P=0.401).

On light microscopy with H & E staining, group A specimens were observed to have two prominent type of cells; the more centrally placed cells were very pale, large and ovoid with basic staining, round nuclei containing prominent nucleoli. Cells were arranged like groups or clump not a particular pattern. In group B specimens the islet cells towards central portion were larger with big, round; prominent nuclei and granular cytoplasm. Cells towards periphery were rather flattened and smaller with small oval or irregular nuclei and finely granular cytoplasm. Islets were observed to have numerous capillaries interspersed among the cells. In group C, the islets were well circumscribed from the surrounding acinar tissue. Cells in the islets were more organized in arrangement than in the previous groups, being arranged in single or double cell cords around well-developed capillaries.Total numbers of cells per islet section were counted for each group; four random islets per observed per section for four of the sections in each tissue of each group. Islets were randomly selected in each section. Mean number of cells per islet were calculated and compared among the groups **Table 1 and Fig 6.**

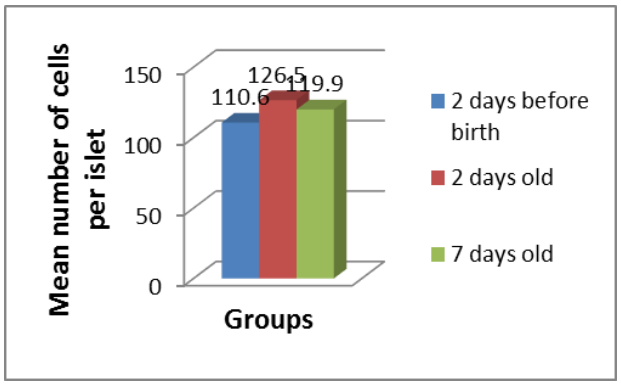

Fig-6: Bar chart showing the mean number of cells per islet in various groups.

No significant difference was observed in the mean number of total cells per islet section on multiple comparisons among the various groups (P > 0.05).

Chrome alum haematoxylin was used to differentiate beta and alpha cells. Beta cell cytoplasm stained bluish while alpha cell cytoplasm stained pinkish while the nuclei stained violet. All the groups showed central concentration of beta cells with an occasional presence of alpha cells in between beta cells. Group A showed at places only few beta cells clumped together **Fig 7.**

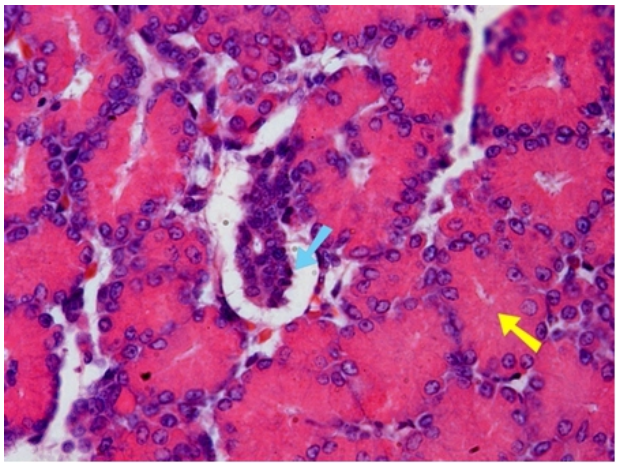

Fig-7: Photomicrograph from pancreatic tissue preparation of group A, showing beta cells stained blue (light blue arrow), these appear like a column of irregularly arranged cells budding out of a duct. Acinar formation of exocrine part of the organ is also being arranged (Yellow arrow). CAH 200 X.

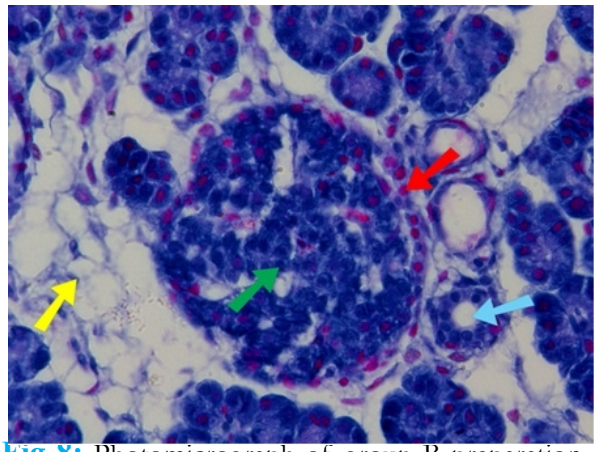

Fig-8: Photomicrograph of group B preparation showing an islet with blue cytoplasmic stain for beta cells (green arrow) , pink staining cytoplasmic granules of alpha cells (red arrow), an intercalated duct bordering islet (light blue arrow) with plenty of mesenchyme (yellow arrow) CAH.400 X.

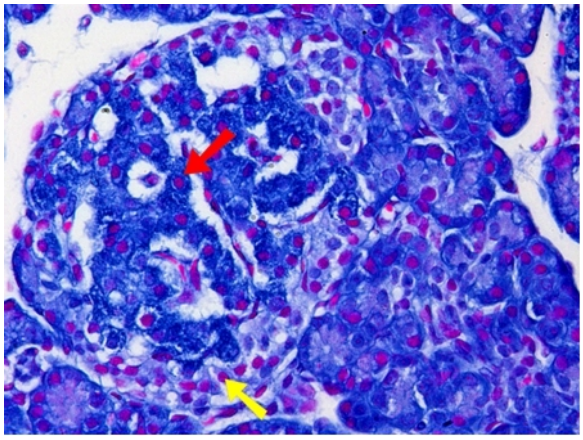

Fig-9: Photomicrograph of group C preparation, showing beta cells in the center staining bluish cytoplasmic granules (red arrow), and pink staining cytoplasmic granules of peripherally arranged alpha cells (Yellow arrow). CAH 400 X.

Group B showed few islets, consisting of only alpha cells, rest of them showed the same pattern (Fig 8). Groups C showed almost similar arrangement of centrally placed beta cells in cordlike arrangement around capillaries and peripherally placed alpha cells (Fig. 9). Mean number of beta cells were calculated for all four groups and compared among the groups **Table and Fig 10.** By multiple comparisons made amongst the different groups, Significant difference was observed in the mean number of beta cells per islet section between groups A and B (p=0.001), groups A and C (p=0.03). No significant difference was observed in the mean number of beta cells per islet section between groups B and C (p=o.497).

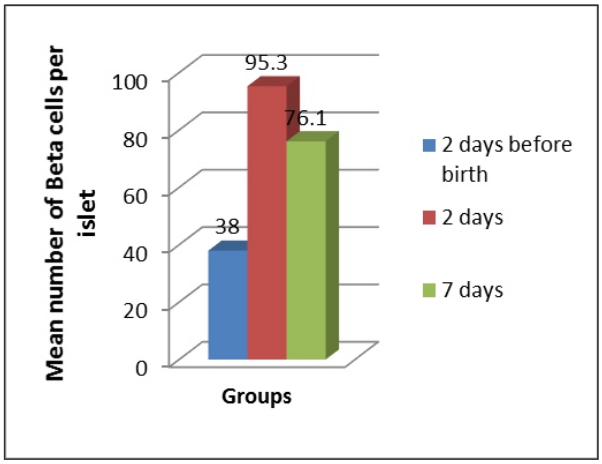

Fig-10: Bar chart showing the mean number of beta cells per islet.

Total numbers of alpha cells per islet section were counted for each experimental group on chrome alum hematoxilin staining. Four random islets were used from each slide and for each tissue of each group four random sections were taken. Mean value was calculated from the total values and then comparison was made between the groups **Table 10 and Fig 11.**

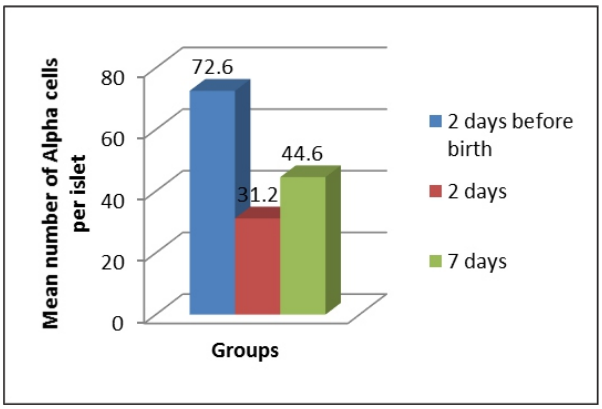

Fig-11: Bar charts showing the mean number of alpha cells per islet section.

ignificant difference was observed in number of alpha cells per islet field in groups with a p value of 0.007. On multiple comparisons significant difference was observed in the mean number of islets per unit section between groups A and B (p=0.009), while no significant difference was observed between groups A and C (p=0.12) and between groups B and C (p=0.69). From the mean number of beta cells per islet field and mean number of alpha cells per islet field the ratio between beta and alpha cells per islet field was calculated for each group and then compared among the groups. **Table and Fig- 12.**

.For group A the ratio ranged from 0.37 to 1.30 with a mean value of 0.58 ± 0.26.The same values were observed to range from 1.30 to 6.50 for group B with a mean of 3.38 ± 1.82 and for group C they were observed to vary in beta alpha ratio from 0.48 to 5.70 with a mean value of 2.81 ± 1.81.

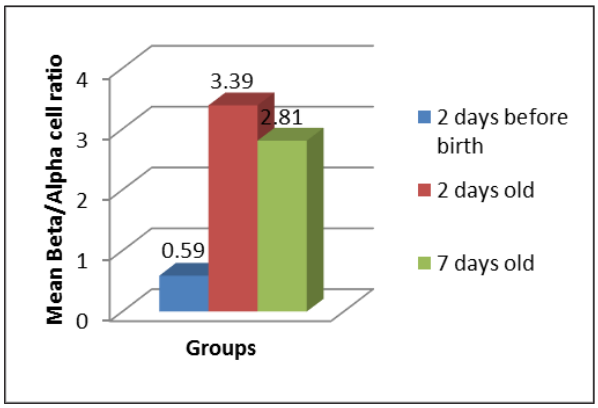

Fig-12: Bar chart showing the mean beta cell /alpha cell ratio in different groups.

Multiple comparisons were made in mean ratio between beta and alpha cells per islet field which showed significant difference in the mean beta cell/alpha cell ratio between groups B and A (p=0.002), between groups A and C (p=0.018).There was no statistically significant difference observed in the mean ratio of beta and alpha cells between groups B and C (p=0.85).

Discussion

Our study observed one fetal and two early post-natal groups and compared the histological structure and quantitative parameters of the endocrine portion of the pancreas among the groups.

Morphometric parameters measured in our study were a total number of islets per section, the mean diameter of islets, the total cell populating islets, differential beta and alpha cells and their relative ratio. The total number of islets was highest in the group C. The mean diameter of islets showed gradual increase towards one-week old pups. The mean number of cells per islet were slightly higher on the 2nd postnatal day with slight fall in next few days, suggesting remodelling by apoptosis or attrition of ill formed cells as observed in literature earlier.[12] During postnatal period beta cells were the main cells populating islet on 2nd postnatal day, followed by some fall in number in one-week old pups. Alpha cells, on the other hand, showed relatively low counts in early postnatal period and slight gain in one-week old age group. However, previous studies showed alpha cells to outnumber beta cells in the late fetal period; implying thereby, alpha cells are the first endocrine cells to differentiate followed by the beta cells or, the alpha cells represent an early stage in the differentiation of beta cells.[13] The relative ratio of beta and alpha cells was seen to be more close to adult ratio in early postnatal day.

Immunohistochemical and morphometric study of the development of new-born rat pancreatic islets found that all the morphometric parameters for the beta cells showed gradual increase during the first four days after birth. The beta cells were well stained and present in the central part of the new-born islets, while the other islet cells were present in the periphery of the islets as seen earlier.[14] We made similar observations regarding the location of different islet cell types, as the beta cells formed the central core with the alpha and other cell types forming the outer mantle around beta cells. This particular organization of the islets may reflect the functional relationships between different cell types. In the rat, the intra-islet microcirculation has been observed to run from the arteriolar entry point outwards, i.e. from the beta cell core to the peripheral endocrine cell mantle[15, 16]. The direction of this microcirculation permits the beta cell core to act on mantle cells.[17] However, the relative distribution of islet cells in the pancreatic may differ

Table-1: The comparative values of various study parameters amongst the three groups.

Parameters	Group A Mean ± SD n=10	Group B Mean ± SD n=10	Group C Mean ± SD n=10	P-value
Islets Per Field	6.3±1.61	6.3±1.41	7.8±1.03	<0.001*
Dia meter of Islet	75.5±22.7	112.5±18.59	136±21.44	<0.001*
Cells per islet	110.60±40.63	126.50±29.93	119.90±56.24	0.588
Number of alpha cells/islet field	72.60±30.45	31.20±16.61	44.60±39.50	0.007*
Beta cells per islet per unit field	38±11.87	95.3±21.99	76.10±42.91	<0.001*
Beta/Alpha ratio	0.58±0.26	3.38±1.82	2.81±1.81	<0.001*

Among various mammalian species. In the buffalo pancreas, the small islets showed alpha cell cords crossing the centre of the islets.[18] Similar findings also described for primates[19]. In the horse and Japanese serow, alpha cells were found located in the centre of the islet[20]. This variation in the organization of islet cells may reflect differences in the interactions between the cells of the islets and their metabolic role.The accelerated rate of growth and development of islet cells in the early postnatal period was also supported by a study in which perinatal development of islet vasculature in rat was investigated; it showed a
pronounced proliferation of the vascular endothelium during the first week after birth which raised the possibility of functional interaction between endothelial and islet cells, contributing to their postnatal maturity.[21]

Conclusion

The endocrine pancreas comprised a large percentage of the pancreatic mass in early rat fetuses. This percentage increased gradually during late embryonic and early postnatal development. All study parameters showed rise in late fetal and early postnatal stage. The proportion of alpha to beta cells was high in late fetal period but later it showed a reversal i.e. the beta cells outnumbered alpha cells in islet which got oriented at the peripheral region of the islet tissue. It is suggested that the whole process of organogenesis of pancreas be studied along with the genetic and epigenetic factors affecting growth and development of pancreas

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