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Postnatal Histogenesis of Islets of Langerhans in Rat

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Abstract.- The current study was carried out to analyze the process of growth, differentiation and development of alpha and beta cells of pancreatic islet during early post-natal period. Pups born 22-23 days postcoitum were divided into two groups A & B, having ten pups each. Group A pups were sacrificed on day 2 postnatal and group B on day 7 postnatal to obtain pancreatic tissue. Adult animals (10-12 weeks) were taken as group CIn both postnatal groups Islets of Langerhans were observed as groups of light staining cells in well-developed acinar parenchyma however, group B pancreatic tissue was comparable to adult tissue. Both groups showed strong association of pancreatic tissue with developing ducts whereas, group A showed mesenchymal tissue present in the vicinity of developing islets. Quantitative variables were applied and compared using one way ANOVA. The mean of 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 ($p<0.05$). The mean of the diameter of an islet was 112±1for 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. To conclude, all the parameters which were studied increased gradually postnatally. Number of islets though decreased but the diameter of islets increased gradually. Both postnatal groups showed ductal epithelium as well as mesenchymal tissue association with histogenesis of islets.

Key Words: Islets of Langerhans, histogenesis, postnatal developmentα, ß cells.

INTRODUCTION

The pancreas is a mixed gland composed of two different cell populations, exocrine and endocrine. The exocrine component makes up the majority of pancreas and includes acinar and ductal cells that secrete and transport digestive enzymes respectively into small intestine. Theendocrine cells are segregated in groups and are called 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 g (Langerhans, 1869).

Although the mature pancreas is a single organ, it is initially derived from separate dorsal and ventral buds. During embryogenesis these two pancreatic buds arise differently, the dorsal pancreas arises from just ventral to the notochord and caudal to the region of stomach, while the ventral pancreatic bud develops from the endodermal hepatic diverticulum. Recent work has shown that signals released from both the notochord and endothelial cells are important for proper regulation of the dorsal pancreas, while signals released from the cardiac mesoderm affect the development of ventral pancreas (Jarikji *et al*., 2009).

Immunocytochemical methods have shown the islets to be composed of α cells producing glucagon (15-20%), β cells producing insulin and amylin (65-80%), δ cells producing somatostatin (3-10%), PP cells producing pancreatic polypeptide (3-5%), and epsilon cells producing ghrelin, an antisatiety hormone (<1%) (Elayat *et al*., 1995; Andraloic *et al*., 2009).

Islets have a central core of insulin-secreting β cells and a surrounding mantle of α, δ and PP cells. The beta cells are differnt in pattern amongst species but they are always clumped together with an adjacent mix of non-beta cells. When rat islets are dispersed into single cells and allowed to reaggregate in tissue culture, the beta cells form a central core with non beta cells around it, thus re-establishing the natural pattern (Halban *et al*., 1987).

During mouse embryogenesis, beta cells are generated from a population of pancreatic progenitor cells. The beta cells are post- mitotic and differentiated from progenitor cells. During postnatal development, however, replication of differentiated beta cells can lead to additional new beta cells. High rates of beta cell replication during the neonatal period results in a massive increase in

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the cell mass (Georgia and Bhushan, 2004).

However, the controversy about the postnatal origin of pancreatic beta cells remained unresolved. It had been reported that pre-existing beta cells, rather than pluripotent stem cells, were the major source of new beta cells after pancreatectomy in mice and during adult life, suggesting that terminally differentiated beta cells retained a significant proliferative capacity in vivo thereby casting doubt on the idea that adult stem cells play a significant role in beta cell replacement (Dor *et al*., 2004).

Pancreatic stem cells have been demonstrated to generate cells of exocrine and endocrine parts of pancreas as well as cells of hepatic phenotype thus favoring the process of trans-differentiation (Zulewski *et al*., 2001).

In rats, perinatal malnutrition impaired neogenesis and the capacity of beta cell regeneration but preserved beta cell proliferation which remained the only choice to increase beta cell mass. This observation implied an impaired capacity of malnourished animals to neogenesis of beta cell; its mass improved with aging or pregnancy on account of proliferation of the cells which aggravated glucose intolerance (Garofano *et al*., 2000).

In humans, pancreatic acinar cells can serve as progenitor for pancreatic islets; a concept with substantial implications for therapeutic efforts to increase insulin producing beta cell mass in diabetic patients (Gao *et al*., 2003). In contrast, a substantial role for replication of preexisting acinar cells had been reported in the regeneration of new acinar cells after partial pancreatectomy. These results indicated that mature acinar cells harbor a facultative acinar but not endocrine progenitor capacity (Desai *et al*., 2007).

Postnatal expansion of the pancreatic beta-cell mass was reported to be 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 (Jiang *et al*., 2008).The current study was undertaken to evaluate the process of growth and development of islets of Langerhans during early postnatal period using rat as an experimental model.

MATERIALS AND METHODS

Adult non-diabetic albino rats (15 female, 5 male) were procured from National Institute of Health, Islamabad and kept under standard conditions of temperature, humidity and dark and light cycle. After acclimatization mating was allowed by keeping female and male adult rats in the same cage in the ratio of 3:1. Observation of vaginal plug was taken as day 0 of pregnancy. After 22-23 days of gestation pregnant rats delivered pups which were divided into two groups A and B. Group A pups were sacrificed on day 2 postnatal and group C pups were sacrificed on day 7 postnatal to procure the pancreatic tissue, while the adult male rats (10-12 weeks) were taken as adult controls which constituted group C. Ten animals were included in each group. Four sections were taken randomly for each animal .

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. The paraffin blocks were prepared and 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 islets of Langerhans.

Measurement of islets

Micrometry was used for measuring the diameter of the islets by using eyepiece reticule and a stage micrometer. Two diameters were taken for each islet at right angle to each other and then a profile diameter was calculated by taking the average of the two (Fig. 1A) (Morini *et al.*, 2006). At least four islets were selected at random. Measurement of each section was done and then their average was taken as diameter of islet of that particular section. Four sections of each block were used accordingly.

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 of the cells.

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 taken from each tissue and four islets were counted on each section randomly and the average was taken. Later on, ratio of beta and alpha cells was calculated.

Statistical analysis was done using one way ANOVA. Multiple comparisons were made using Post Hoc Tuckey test and qualitative variables were compared by applying the Pearson Chi Square test.

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 after H&E staining. Cell counting was done after Chrome alum Hematoxylin-Phloxin staining.

In group A, islets were seen in abundance, scattered among exocrine acini which were differentiating and branching to form lobes and lobules (Fig. 1B). Islet tissue could be recognized because of staining reaction and specific cellular arrangement but there was nothing to separate islet from exocrine pancreas. However, there was an intercalated duct presents almost in close proximity to islet and abundant mesenchymal tissue. Light microscopy of group B tissue showed well differentiated lobes and lobules; islets were well circumscribed with clear demarcation from the surrounding acinar tissue (Fig. 1C). In group C, light microscopy showed pancreatic tissue clearly demarcated into lobular structure with well defined inter lobular septa and intercalated and interlobular ducts (Fig. 1G).

By applying one way ANOVA with P,0.05 significant difference in the number of islets per section was observed among the groups. Post Hoc Tucky test was applied for multiple comparisons and significant difference was observed between groups A&B ($P<0.05$), A&C ($P<0.05$), and groups B&C ($p<0.05$) (Table I).

A significant difference was observed in mean diameter of islets among the groups with a p value of < 0.05. Multiple comparison showed significant difference in the mean diameter of islets between groups A&C ($P=0.05$), but no significant difference was observed in the mean diameter between groups A&B and B&C.

Table I.- Showing different parameters (mean±SD) compared among the groups (n=10)

Parameters	Group A	Group B	Group C	P-value
Islets per field	6.3±1	7.8±1	3.1±0	0.001*
Diameter of islet	112±18	136±21	171±55	0.001*
Cells per islet	126±29	119±56	136±41	0.58
Beta cells/section	95± 21	76±42	102±34	0.001*
Alpha cells/section	31±16	44±39	33±15	0.07
Beta: Alpha ratio	3.3±1.8	2.8±1.8	3.6±18	0.75

* p value < 0.05 is clinically significant.

In group A 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 fine granular cytoplasm. Islets were observed to have numerous capillaries interspersed among the cells (Fig. 1E).

In group B, 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 (Fig. 1F).

In group C the islet cells were observed in parallel cords with intervening capillaries. Beta cells were present more toward center of islet, mostly arranged in cord like fashion around rows of capillaries. Beta cells were round to oval in shape having granular cytoplasm each with prominent round nucleus and high nuclear cytoplasmic ratio. Alpha cells on the other hand, were flat or disc shaped with compressed nuclei and rather homogenous cytoplasm (Fig. 1G).

Total number of cells per islet section were counted for each group; four random islets per section were taken and four of the sections in each tissue of each group were used. Islets were randomly selected in each section. No significant difference was observed regarding number of total islet cells per section.

Chrome Alum Hematoxylin-Phloxin stain was used to differentiate between beta and alpha

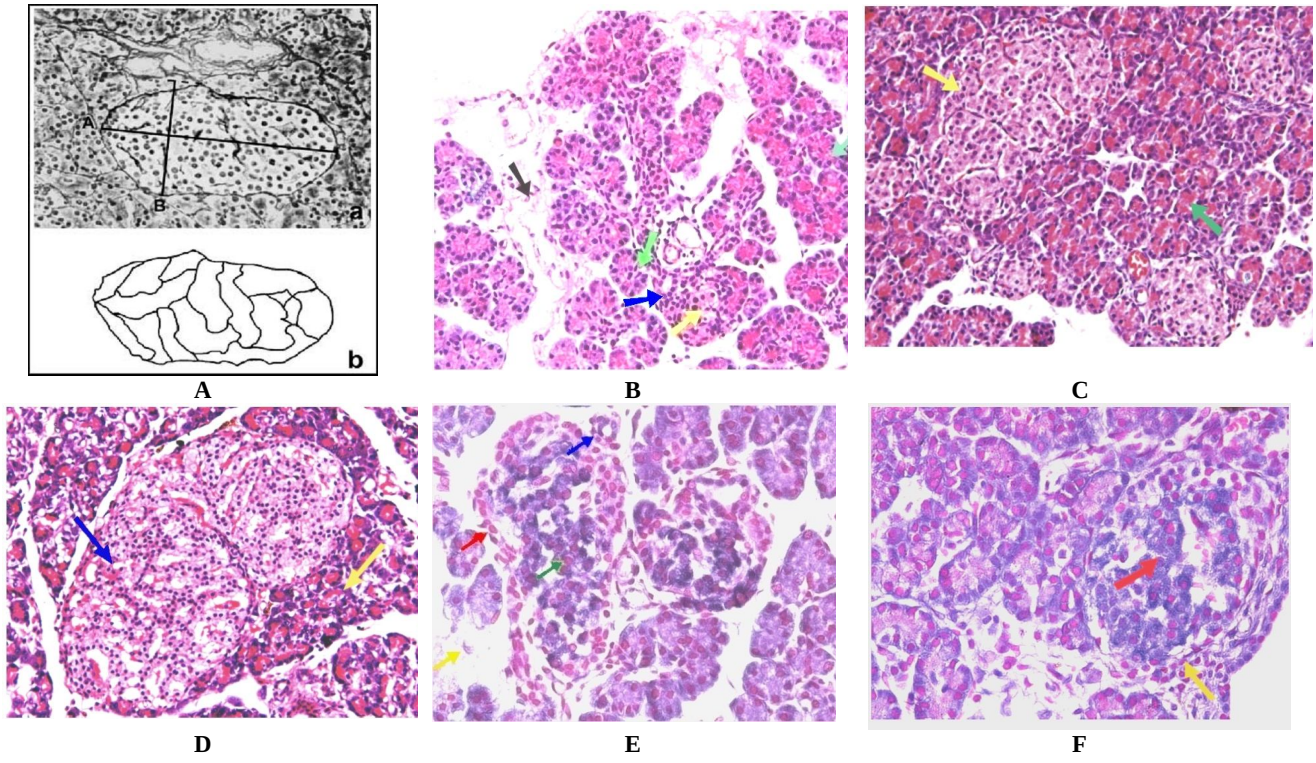

Fig. 1. Histological structure of rat islets of Langerhans; A, showing the method of taking two diameters of an islet; B, group A showing developing islets (yellow arrow) intercalated duct (blue arrow), mesenchymal tissue (black arrow), acinar tissue (green arrow); C, group B, showing pancreatic tissue with well defined acinar lobules (green arrow), well-demarcated islets (yellow arrow); D, group C pancreatic tissue, showing well-defined encapsulated islets (Blue arrow), interspersed with well organized exocrine tissue (yellow arrow); E, group A with CAH showing an islet with bluish cytoplasmic stain for beta cells (green arrow), and pinkish staining alpha cells (red arrow) an intercalated duct (blue arrow), and mesenchyme (yellow arrow); F, group B showing beta cells in the center staining bluish cytoplasm (red arrow) and pinkish staining cytoplasm of alpha cells (yellow arrow); G, group C showing bluish staining central cords of beta cells (yellow arrow), and pink staining peripherally arranged alpha cells (red arrow) and well organized acinar parenchyma (green arrow).
Stain: A, B, C, D, H & E; E, CAH-chrome alum hematoxylin-phloxin stain; F, G, CAH.
Magnification: A, B, C, D, 200x; E, F, G, 400x.

cells. Beta cell cytoplasm stained bluish while alpha cell cytoplasm stained pinkish andthe 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 islets, consisting of bluish staining beta cells in the central core and pink staining alpha cells forming a mantle around it (Fig. 1E). Groups B and C showed almost similar arrangement of centrally placed beta cells in cordlike arrangement around capillaries and peripherally placed alpha cells (Figs. 1F, G).

Statistically, significant difference was observed in mean number of beta cells per islet section among the different groups. Multiple comparison showed statistically significant difference between groups B& C ($P<0.05$) but no significant difference between groups A&B and A&C (Table I).

No significant difference was observed in

mean number of alpha cells per section among the different groups. Multiple comparisons were statistically non significant (Table I).

No significant difference was observed in the ratio of beta cells to alpha cells per islet field amongst the different groups with a p value of >0.05 (Table I).

Mesenchymal tissue was present in all 10 specimens of group A while it was absent in all twenty specimens of groups B&C. Chi square test showed significant association of mesenchymal tissue with age ($p< 0.05$). Intercalated ducts were present close to developing islet tissue in the twenty specimens of groups A&B and not present in group C. Duct association can be considered a feature of developing islet tissue.

DISCUSSION

In this study the structure and quantitative parameters of islets of Langerhans were observed in two early post natal groups and compared with that from the adult rat of group C. We found strong association of ductal and mesenchymal tissue with developing phases of islets in early postnatal period. Previous studies support both ductal and mesenchymal origins of islets cells (Badawoud, 2003).

It was also observed that the developing islets were found in close relation to an intercalated duct in early postnatal stages that surely indicates the ductal origin of islets, perhaps out of proportion of exocrine tissue which suggests that from the pancreatic bud there is formation of ductal and acinar element in the first place and then these ductal epithelium differentiates into islet cells. Also there was quite a large amount of mesenchymal tissue in the area of developing islets, suggesting a role of mesenchymal tissue in the differentiation of islet cells. Previous studies support the concept of direct differentiation of mesenchymal tissue to islet endocrine cells. Lucini *et al*. (1998) studied bovine fetuses for pancreatic development and observed that pancreas of 60 day old bovine fetuses was made up of cell clusters and ductules whose cell buds were surrounded by an abundant mesenchymal mass.

Transition of epithelial to mesenchymal tissue had been suggested to generate proliferative human islet precursor cells. This study showed that fibroblast like cells derived from human islets, proliferated readily in vitro. These mesenchymal type cells then could be induced to differentiate into hormone expressing islet like cell aggregates, which reestablished the epithelial character typical of islet cells (Gershengorn *et al*., 2004).These findings suggest that early postnatal period is the period of maximum differentiation and growth, perhaps postnatal stress of maintaining glycaemic level within normal limits is the stimulus for accelerated rate of growth and development, whether it occurs through ductal differentiation or from mesenchyme is not clear.

On the contrary another study suggested that during postnatal development, replication of differentiated beta cells can lead to addition of new beta cells, so high rates of beta cell replication during the neonatal period results in a massive increase in the cell mass (Georgia and Bhushan, 2004).

In this study the morphometric parameters measured the total number of islets per section, mean diameter of islet, total cell populating the islets, differential alpha and beta cells count and their relative ratio. Total number of islets was highest in group B that was more than double of that in adult. The mean diameter of islets showed gradual increase towards adult value which was $2/3^{rd}$ of adult in one week old pups. Mean number of cells per islet were slightly higher on the 2nd postnatal day with slight fall in next few days, probably remodeling by apoptosis or attrition of ill formed cells as suggested in an earlier study (Scalgia *et al*., 2008).

During postnatal period beta cells were the main cells populating islet on 2^{nd} 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 number of alpha cells to be more than beta cells in late fetal period; Probably alpha cells are the first of the endocrine cells to differentiate followed by beta cells or the alpha cells are an early stage of differentiation of beta cells (McEvoy, 1981).

The relative ratio of beta and alpha cells was seen to be closer to the adult ratio than in early postnatal days.

Immunohistochemical and morphometric study of the development of newborn rat pancreatic islets found that all the morphometric parameters for 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 newborn islets, while the other islet cells were present in the periphery of the islets (Badawoud, 2003).

Similar observations were noticed regarding the location of different types of islet cells, as the beta cells formed the central core with alpha and other cell types forming the mantle around beta cells. The particular organization of the islets may reflect the mutual 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 beta cell core to the peripheral endocrine cell mantle (Bonner-Weir and Orci, 1982; Samols and Stagner, 1988). The direction of this microcirculation permits the beta cell core to act on mantle cells (Samols and Stagner, 1991).

The distribution of endocrine cells in the pancreatic islets may differ between mammalian species. In the buffalo pancreas, the small islets sometimes showed cords of alpha cell crossing the centre of the islets (Lucini *et al*., 1998). Similar findings have been described for primates (Bonner Weir, 1991). In the horse and Japanese serow alpha cells were found located in the centre of the islet (Atoji *et al*., 1990). This variability in the organization of islet endocrine 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 endothelial cells during the first week after birth which raised the possibility of functional interaction between endothelial and islet cells, contributing to their postnatal maturity (Johansson *et al*., 2006).

CONCLUSIONS

Development of islet tissue showed a strong association with mesenchymal tissue as well as with ductal epithelium in early postnatal period, suggesting a strong role of ductal epithelium and mesenchymal tissue in the genesis of islet tissue. Islet tissue continued to increase gradually during early postnatal period. Further, the number of islet tissue and its diameter showed a steady increase to 7^{th} day postnatal. The islet tissue preparation from the adult rat was comparable, as far as the pattern of distribution of alpha and beta cells, to that seen in the early postnatal period. The number of beta cells increased rapidly in early postnatal period and outnumbered the alpha cells which got oriented at the peripheral region of the islet tissue. To elaborate the sequence of genesis of the islet of Langerhans, it is suggested to extend the study through the entire period of organogenesis to adulthood in albino rat, using hormone labeling antibodies.

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