Age-related pancreatic tissue postnatal histogenesis in the albino rat (*Rattus norvegicus*): A morphological, morphometrical, and immunohistochemical study with emphasis on alpha and beta cells

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**Abstract**

Age is an essential factor that causes histological changes in the body's cells, tissues, and organs, affecting the functioning of all body systems. This study aimed to assess the histological changes of the pancreas during the stages of its lifetime. Seventy-two male rats of different ages were used in the present investigation to study the histological changes of the pancreas during age progress from birth until puberty with an impact on alpha and beta cells. The samples were categorized into six groups according to the postnatal ages; 1, 7, 14, 21, 30, and 70 days. The results showed that the pancreas did not appear clearly in rats of one day and 7 days old, while the shape and lobes of the pancreas were clearly observed in rats of 14 days old and the subsequent ages. Histologically, the serous acini, duct system, and islet of Langerhans were ill-developed in the first two ages and became well-developed at 21 days old. Statistical analysis showed that the number of alpha and beta cells in histological sections of the pancreas of the islets of Langerhans shows a decrease in the alpha cells with age progress. At the same time, it was observed that the number of beta cells increased gradually with age advancement; at the same time, serum insulin level increased, and glucagon decreased with age advancement. The current study revealed that pancreatic tissue histogenesis continues in postnatal life, at least through the first 2 weeks in rats where dramatic changes in pancreatic tissue occurred, including the endocrine portion.

**Keywords**

Rat, Pancreas, Alpha cell, Beta cell

**Introduction**

The pancreas is considered an essential organ from a medical point of view, as it is an organ and a target for two common diseases globally, namely pancreatic cancer and diabetes. It is necessary to understand and study the development of the pancreas, which is closely linked with the pathological changes associated with these diseases (1-3). It is important to identify hormone-secreting cell types of the islets of Langerhans in the pancreas and understand their function in glucose homeostasis and the pathophysiology of diabetes. The main theme of this account focuses on the cells that secrete the recognized islet hormones insulin and glucagon (4,5). The growth pattern of the pancreas was examined in numerous types. The results assumed that the pancreas was not completely developed at birth, so the full differentiation of its exocrine and endocrine parts occurs throughout postnatal revival (6). Therefore, the present work was piloted to show histological changes of the pancreas during the growth period from the first week after birth until puberty with an impact on beta and alpha endocrine cells number.
Materials and methods

Ethical approve

This work was achieved under ethical approve No. 390 from College of Education for Pure Sciences, University of Mosul in 7/2/2021.

Animals

The current study was conducted on male white rats, *Rattus norvegicus*, which were obtained from the animal house of the College of Veterinary Medicine / University of Mosul for the period from 15/3/2021 to 4/15/2022 under ethical approvement No. 930 of College of Education for pure sciences in 7/2/2021. Female white laboratory rats aged 2-3 were used in this study and placed with males for reproduction in plastic cages. Pregnant females were isolated in special cages and left until birth in standard laboratory conditions, feed, ad libitum (7,8). The date of birth was recorded, and animals (males only) were selected according to the ages specified in the design of the experimental groups.

Experimental design

Experimental animals were selected from newly born 72 white rats of different ages and divided equally into six groups as follows: The first group of one-day-old (newly born rats), the second group of 7-day-old rats (Rats fed their mother’s milk-only), third group of 14 days old (Rats fed mother’s milk and herbs for their nourishment), fourth group of 21 days old (freshly weaned rats), Fifth group of 30 days old (rats depend on solid diet) and sixth group of 70 days old (adult rats).

Blood sampling

Blood sampling from the animals of the first and second groups was done through a puncture in the heart directly using a syringe to obtain the largest possible amount of blood. In contrast, blood samples were taken from other groups through the orbital sinus. The blood was placed in test tubes free of anticoagulant. Serum was separated via Centrifuge at 3000 rpm for 15 minutes. Keep the serum at 4°C until serum insulin and glucagon levels are made (9).

Euthanization of animals

The animals of the first group (1 day old) and the second group (7 days old) were euthanized by decapitation or cervical dislocation method. In contrast, the other animals were euthanized by carbon dioxide inhalation (10).

Histological samples

The pancreas samples were taken from all experimental animals, placed directly in 10% neutral formalin solution, kept in the solution for 72 hours, then processed with routine histological processing method to get histological sections about 5-6µm thick. Hematoxylin and Eosin, Masson's Trichrome, and Gomori stain achieved the study's goals (11). Histological sections were photographed by tube microscope camera 18MP (Omax, China) that was provided with photo processing software (Toupview) (12).

Immunohistochemistry approach

A special marker Ki-67 was used to detect cell proliferation within the pancreatic tissue at different ages. The assay was performed according to the instructions provided by the manufacturer (Abcam) Immunohistochemistry protocols for Anti-Ki67 antibody ab16667 (13).

Estimation of the level of glucagon and insulin in the blood serum

The Competitive ELISA technique was used to estimate the glucagon level in the blood serum. Estimation kit manufactured by Elabscience® Rat GC (Glucagon) ELISA Kit No. E-EL-R0425. While the sandwich ELISA method was used to estimate the insulin level in the blood serum. Estimation kit manufactured by Elabscience® Rat INS (Insulin) ELISA kit catalog no: E-EL-R3034. The examination was carried out according to the instructions attached by the manufacturer by using the competitive ELISA test.

Statistical analysis

A computerized platform of Sigma stat -V13.0/ SYSTAT software) was used to achieve the statistical analysis. The data presented as means ± standard error was evaluated by (ANOVA), and the significance was set at P≤0.05. The differences among the study groups were determined using Duncan’s multiple-range test.

Results

A macroscopic study of the pancreas in albino rats showed its presence in the form of a diffuse gland in the mesentery associated with the duodenum, as it did not appear clearly in the first groups at the age of one day and the second at the age of 7 days, while the shape of the pancreas was clearly observed in rats. The third group of age (14 days), where it was observed after fixation in a 10% formalin solution in the form of a white to pink gland suspended in the mesentery extending between the stomach and the duodenum and the ascending colon (Figure 1). The histological sections of the pancreas of rats showed, in general, that it was composed of several lobules and surrounded by a capsule of connective tissue and that the type of glands was tubular-acinar complex glands. The exocrine part consists of the secretory acini and its various ducts. The pancreatic capsule was observed in the rats of the first two groups at as few scattered, not well-formed fibers surrounding some pancreatic lobes. The connective tissue
between the lobes was poorly formed. In contrast, the histological sections of the third group at 14 days exhibited an increment in connective tissue density formed between the lobes (Figure 2).

Figure 1: Macroscopic photo Showing the development of the pancreas (arrow) in rats at different postnatal days. Stomach (a), spleen(b), duodenum (c).

Figure 2: Histological section of the pancreas of 7 days aged rats shows immature fibrous tissue (a) and serous acini (b). Masson's trichrome stain, 400x.

The histological sections also showed, at this age, the stability of blood vessel differentiation significantly in the connective tissue among the lobules. Several elongated, dark-colored cells were observed surrounding the blood vessels, believed to be the Pericytes, which are believed to be the source of the emergence of many blood vessels. The histological sections of the pancreas of the fourth group at the age of 21 days and later ages showed a clear development of the connective tissue among the pancreatic lobules, while there was no clear difference in the thickness of the pancreatic capsule, as the results showed the presence of bundles of well-formed connective tissue fibers, they were of mature-type. Histological examinations also showed the presence of ganglionic neurons in the form of clusters as oval-shaped cells with very light nuclei in the connective tissue among the lobes, an indication of the development of the ganglion nerve part of the pancreas at this age (Figure 3).

Figure 3: Histological section of the pancreas of 30 days aged rats shows mature fibrous tissue (a) and serous acini (b), islet of Langerhans (c), and well-developed blood vessels (d). Masson's trichrome stain, 400x.

The serous secretory units of one-day-old and 7 days old rats appeared clearly at this age, but they were scattered. They were not organized into well-defined lobules, and they were composed of pyramidal-shaped cells of large size. These units had narrow cavities, and some had no cavities. Centro-acinar cells were not evident in one-day-old rats, although they were observed in some secretory units. The cells were distinguished by containing an acidic dye in the apical part of the cells lining the secretory units, the zymogen granules. Mesenchymal cells also appeared clearly and large in one-day-old rats in spindle-shaped cells with clear cytoplasmic processes (Figure 4).

The histological sections of the pancreas of 14-day-old rats showed complete structure regarding the density of the serous secretory units and the high percentage of Langerhans islets in the tissue section. An increase or development in the connective tissue was noted. However, the ductal system was not differentiated accurately in terms of composition. It was observed in some Histological sections of continued differentiation of secretory units from undifferentiated mesenchymal cell mass. The histological composition of the gastric lobe and the duodenal lobe at 14 days old were more developed than the splenic lobe, where the density of
secretory units and their size or diameter and the size of the cells lining these units were more developed. The cells lining the secretory units were characterized by their pyramidal shape, which contains one spherical nucleus in the cell's basal third. Two types of cells that line the secretory units were observed: the stable and active. The active cells were characterized by having a light-colored spherical nucleus located in the lower third of the cell or in the middle of the cell. In contrast, the stable cells were characterized by having a dark-colored nucleus close to the basement membrane. The results showed the presence of the Centro-acinar cells in most of the secretory units of rats' pancreas at 14 days. Large numbers of secretory units were observed in the differentiation phase, which was adjacent to the mass of mesenchymal cells, as they were distinguished by their irregular shape and did not contain a cavity (Figure 5).

The results of groups at the age of 21, 30 and 70 days showed well-developed serous secretory units within the lobules of the pancreas, where the cells lining these secretory units appeared as pyramidal cells with an acidic cytoplasm from the apical part of the cell and basophilic in the lower part of the cell. These cells possess pale spherical nuclei with clear nuclei located in the lower third of the cell, indicating that most of the cells lining the secretory cells were of the active type. The Centro acinar cells appeared clearly in large numbers of secretory units in the form of one or two nuclei in the center of the serous secretory units, indicating the completion of the formation of the beginning of the ductal nucleus. The histological study showed the presence of the ductal system in the pancreas of rats one and seven days of age. Still, it was not very developed, as it was noticed that the Centro acinar cells appeared in very few secretory units in the form of low cuboidal cells with round nuclei and were always observed in the form of one or two cells inside the lumen of the secretory units, the ducts were observed adjacent to the Langerhans islets, where it was observed in most of the histological sections as extensions of its cells in the form of a row or extending from the Langerhans islets towards the connective tissue close to it to form channels within the lobes (Figure 6).
Generally, it was observed that the differentiation and formation of ducts were still continuous at this age, as part of the mesenchymal cells are still continuous between Langerhans islets of newly formed ducts, especially those between the lobes. Histological examinations of the pancreas of rats of the third group at 14 days and the fourth at 21 days and later ages showed that the ducts between the lobes and ducts within the lobes continued to weave. It was observed that most of the ducts between the lobes were separated from the islets of Langerhans. The main pancreatic duct was clearly visible in the connective tissue as a large duct lined with two rows of cuboidal cells. Histological examinations of the pancreas of one-day-old rats showed few Langerhans islets, as they appeared in the form of a mass of light-colored cells. These masses of cells took different oval, spherical or irregular shapes adjacent to ducts. Most of the cells forming islets of Langerhans at this age had round, dark-colored nuclei and almost equal sizes, where alpha and beta cell differentiation did not occur clearly.

The results of examining tissue sections of the pancreas of 7-day-old rats showed a clear development in the composition of the islets of Langerhans, as these islets appeared larger in size and more numerous than in the previous group. The arrangement of cells within the islets also developed as the endocrine cells became visible in rows with one or two cells and noted the existence of capillary capillaries among these cells. Differentiation of types of endocrine cells was observed well at this age, as the presence of large cells with a basophilic cytoplasm (blue) with a large oval nucleus was observed in the peripheral part of the islets, which were alpha cells, while the presence of other smaller cells with eosinophilic cytoplasm (red) and small nuclei of dark color, which are concentrated in the central part of the islets, which were beta cells. It is noticeable at this age that the percentage of alpha cells was predominant in most of the islets of Langerhans, noting that the undifferentiated mesenchymal cells are still present in the peripheral part or around the islets of Langerhans, indicating the continuity of the process of developing the islets and that they did not acquire their final shape and function at this age.

The results of examining histological sections in the pancreas of rats at 14 days and 21 days showed the presence of well-developed Langerhans islets with large diameters. The endocrine cells within these islets were better organized than in the previous age. They were arranged in rows or cords with large blood vessels spread between them. The results revealed a substantial increment in the number of beta cells compared to alpha cells at this age; the differentiation continues in the later ages of 30 and 70 days in conjunction with the development of blood vessels and connective tissue and the appearance of ganglionic cells in the periphery of the islets (Figure 7).

Results of statistical investigation revealed that the numbers of alpha and beta cells in the histological section of the pancreas of the islets of Langerhans show a decrease in alpha cells with age progress. At the same time, it was observed that the number of beta cells increased gradually with age progress (Figure 8 and Table 1).

Table 1: Numbers of alpha and beta cells per islet in the histological sections of the pancreas at different postnatal ages of rats

<table>
<thead>
<tr>
<th>Cell Number</th>
<th>1 day</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>30 days</th>
<th>70 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>α cells</td>
<td>58.5±1.5 a</td>
<td>53.7±3.1 a</td>
<td>30±3.5 b</td>
<td>23.2±2.0 c</td>
<td>18.4±2.5 d</td>
<td>17.2±2.2 d</td>
</tr>
<tr>
<td>β cell</td>
<td>27.2±2.2 a</td>
<td>28.7±3.1 a</td>
<td>36.5±5.5 b</td>
<td>37.2±2.5 b</td>
<td>40.4±7.4 c</td>
<td>48±5.3 d</td>
</tr>
</tbody>
</table>
**Immunohistochemistry results**

The results of immunohistochemistry of Ki67 showed a strong activity for the proliferation of the cells of the endocrine and exocrine units as well as the ducal system at the age of 7 days and 14 days through the presence of a clear interaction of these proliferating cells that took a brown color and the cells were concentrated which showed a positive reaction in the mass of undifferentiated mesenchymal cells as well as in the external secretory units. It was observed that the percentage of the positive cells decreased to a large degree, except in the cells around the blood vessels and some cells of the serous secretory units. This reaction was moderately intense, while some islets of Langerhans showed a weak interaction with Ki67. In contrast, the positive reaction with the cells of the exocrine and endocrine units disappeared at 30 and 70 days. Generally, it can be said that the cellular proliferation activity of the pancreatic tissue was evident in the first and second weeks of the animal’s life. At the same time, it seemed to gradually decrease with age progress (Figure 9).

**ELISA assay for the hormone’s insulin and glucagon**

The results of the statistical analysis for measuring the insulin and glucagon hormones in the blood serum showed an important decline in the level of glucagon with age progress, as a non-significant difference was detected between rats of the first group at the age of one day and the rats of the second group at the age of 7 days, while a clear significant difference was observed between the other study groups. Results also presented a rise in the level of insulin hormone gradually with advancing age, with a significance between the study groups, except for the fifth and sixth groups (Table 2 and Figure 10).

**Discussion**

An exact manifestation of perinatal progenitor cells in the pancreas may emphasize the development of new therapy, such as stimulation of new β cell formation (14,15). A current study revealed the fact that pancreatic tissues histogenesis continues in postnatal life, at least through the first 2 weeks in rats where dramatic changes in pancreatic tissue occurred, including the endocrine portion; this fact comes consistent with that of Rasheed et al. (16), Tadokoro et al. (17) and Morini et al. (18). Actually, Finegood et al. (19) cited that during lifetime, the beta-cell population is dynamic with compensative changes to fulfill demands and replace the slow turnover of the cells, proposing either a capacity of beta cells to adjust their mass or the attendance of stem cells that can differentiate to beta cells by histogenesis.

This study showed an obvious relationship between exocrine ducal cells and islets of Langerhans where the newly formed ducts observed adjacent to the islets, especially in the newly born rats, anyway, Solar et al. (20), Rajathi and Muthukrishnan (21) provide convincing evidence that the epithelium of ducts does not make an important contribution to acinar or endocrine cells during

**Table 2: Insulin and glucagon hormones level in the blood serum at different postnatal ages of rat**

<table>
<thead>
<tr>
<th>picogram/ml</th>
<th>1 day</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>30 days</th>
<th>70 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucagon</td>
<td>210.2±30.2 a</td>
<td>202.9±19.5 a</td>
<td>177.1±10.3 b</td>
<td>132.4±6.2 c</td>
<td>102.6±8.3 d</td>
<td>83.3±7.3 e</td>
</tr>
<tr>
<td>Insulin</td>
<td>121.8±7.1 a</td>
<td>170.4±9.7 b</td>
<td>258.2±12.3 c</td>
<td>270.4±13c</td>
<td>361.8±22.4 d</td>
<td>398.5±26.5 d</td>
</tr>
</tbody>
</table>

Figure 9: Histological section of rat pancreas of different ages (a) at the age of one week (b) at the age of 14 days (c) at the age of 30 days (d) at the age of seventy days, where the intensity of the reaction of the Ki67-positive cells of the proliferating cells with brown color (arrows) is noted at the age of one week and fourteen days. The reaction is less in advanced ages, Ki67 formula, A, C, D 400x; B 10X.

Figure 10: Histogram show the concentration of serum insulin and glucagon level at different postnatal ages of rat.
neonatal growth. In fact, our study explains the development of endocrine cells through postnatal life by increasing beta cell mass with age progress, especially in the first 3 weeks of life, concurrently with the increase of serum insulin level. On the other hand, Benitez et al. (22), Bertelli et al. (23), and Bouwens et al. (24) found an association between ductal cells and the neogenesis of insulin-producing cells in rats.

Fibrous connective tissue is a very important source for angiogenesis and mesenchymal cell migration in the process of histogenesis; our study revealed well-developed connective tissue and blood vessels, especially in the second week of life, in fact, Connective tissue contain growth factor (CTGF) which is particle founded in the ducts, endothelium, as well as in the developing beta cells that formerly exhibited is required for proliferation of beta cell, its differentiation, as well as islet histogenesis throughout the development. A recent study inspected tissue interfaces through which the CTGF encourages the normal development of Langerhans islets (25-30).

Conclusion

The current study showed that the histogenesis of the pancreas in rats continued after birth, and the dramatic changes in its structure occurred from day 1 till day 14 after birth, as well as; the number of alpha cells and level of serum glucagon decreased gradually with age progress while beta cells and level of serum insulin increased with age progress. This study explains the processes of catabolism and anabolism-related histological structure of the islands of Langerhans during the stages of their lifetime.

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Conflict of interests

The authors declared they have no competing interests.

References


