Physiological and Histological Changes in Pancreatic Gland Associated with Ageing in Local Rabbits in Iraq

Rasheed, Kh¹*, Thamer, I², Hussine, F. A², Ibraheem, A²

1. Tikrit University, College of Science, Department of Biology, Iraq
2. Tikrit University, College of Veterinary Medicine, Department of Anatomy and Histology, Iraq

Corresponding Author: najiiirasheed@yahoo.com

Abstract

Pancreas is a flat organ resembling the letter L or pear shaped, almost yellowish to pink, is of medical significance as the target in two life-threatening diseases of diabetes mellitus and pancreatic cancer. The study was conducted on male rabbits which are distributed into 3 groups by age (6 months, 1 year, 3 years). Physiological and histological changes of pancreas were studied according to age groups adopted. Physiological aspect was done to analysis level of pancreatic gland hormones, the histological structure of pancreas was also studied during the same periods in which hormonal changes were analyzed. Results showed significant differences in the concentration of pancreatic gland hormones, where insulin was in the second group more than in the first and third groups, while blood sugar (glucose) was the highest concentration in third group compared to the first and second. Changes were also show in the tissue structure of pancreas during age, although the basic structure is similar to all samples. As age increase (1-3) years, Langerhans islets increased in size, contained alpha and beta cells that were surrounded by a loose connective tissue in the third stage, and there was no significant difference in diameters of cells which produce enzymes at all stages of life. Physiological and histological changes indicated to the role of age in function and structure of pancreas gland during different stages of life. In addition, this study indicated that the hormonal variability of pancreas is closely related to the histological composition of gland components, therefore more detailed studies such as sex, different breeds or environmental conditions are worthwhile and may give more information on factors that may affect on effectiveness and activity of gland.

Keywords: Pancreas and Age Progress, Rabbit's Pancreatic Gland, Langerhans Islets Diameters

1. Introduction
Rabbit is a small mammal from Leporidae family, adapted to live in different environments of the world, feeds on plants (1) and has many characterizes that make it desirable for breeding, such as a beautiful shape, good taste, its usefulness, rapidly of reproduction and the quality of its furs (2, 3), most of which are 25-29 cm in length, its colors range from white, black, gray and spotted, and are about four to seven years old (4). Rabbits are laboratory animal favored by many of researchers for scientific experiments for several reasons, including: short life, as well as, many of which are rapidly reproductive, allowing scientists an opportunity to monitor the effect of treatment regimens on several generations of their in a relatively short period of time (3). One of the characteristics of rabbits that they are temperate and fast-adapting organisms to any environment, and do not affected by that, as behavior of many living organisms are affected of the changing environment and require an environment similar to their original in order to survive and reproduce, so experiments on their are true to a large extent and are not affected by any behavioral or genetic change that affected the animal as a result of its presence in a different environment to its natural environment (5).

Pancreas is a flat organ resembling the letter L or pear shaped, almost yellowish to pink, is of medical significance as the target in two life-threatening diseases of diabetes mellitus and pancreatic cancer (6), located horizontally behind the stomach deep in the upper left posterior of the abdomen against the spine at the level of lumbar spines first and second. It is a mixed gland, exocrine and endocrine, that contain two types of cells: 1) Acinar cells, which constitute more than 95% of the pancreas mass, are relatively large cells and less dense than the second type that surrounds it. Acinar cells secrete enzymes that help digest proteins, fats and carbohydrates such as amylase, lipase and protease. Protease are excreted as inactive zymogens such as trypsinogen, which is activated by duodenum to trypsin, which in turn activates other proteases such as chymotrypsinogen to chymotrypsin. These enzymes are carried through a special channel that flows into the duodenum. 2) The second type of pancreas cells are named Langerhans islets, which constitute (1–2)% of pancreas mass, are lumps of elliptical or spherical forms of endocrine secretion that embedded within the acinar cells, produce several hormones directly into the blood, consisting of several types of cells, most important are:- Alpha cells (α) which are located peripherally, secrete glucagon that increase level of glucose in blood throughout glycogenolysis stored in the liver.- The most centrally located beta cells (β) are insulin releasing, which lower level of blood glucose by promoting it entry into muscles and tissues of body as a source of energy, as well as, storing it in form of glycogen in the liver to be used when needed. Delta cells (δ) which are scattered and least number, secreted somatostatin that inhibits secretion of growth hormone (GH) and thyroid stimulating hormone (TSH) from anterior lobe of the
pituitary, in addition, inhibit secretion of HCL from gastric parietal cells. Secondary cells are secreted pancreatic polypeptide, which activates chief cells in the stomach, inhibits secretion of bile and bicarbonate, as well as, inhibits intestinal motility (7-9). Pancreas is equipped with blood from the inferior intestinal pancreatic artery with its anterior and posterior branches of the superior mesenteric artery, as well as, superior intestinal pancreatic artery in the anterior and posterior branches of the gastrointestinal tract branch of the common hepatic artery of the celiac stem, in addition to the branches of splenic artery branching out of celiac trunk, where three vessels (back pancreatic artery, large pancreatic artery, caudal pancreatic artery) perpendicular to the longitudinal axis of the body and tail of pancreas which reaches between splenic artery and inferior pancreatic artery. Veins drain is similar to arterial perfusion, as it passes through the tributaries to both portal vein and inferior, superior mesenteric vein and splenic vein (10).

The aim of the current study was to investigate physiological and histological changes of pancreas associated with ageing.

2. Materials and Methods

2.1. Animals

The research was carried out on male rabbits (n=9) which distributed into 3 age groups. Group 1 consisted of three 6-month-old male rabbits; group 2 consisted of three 1-year-old and group 3 had has three 3-year-old male rabbits. All the three groups used to study the physiological and histological changes in pancreatic gland at different age groups. All instruments were calibrated and maintained in accordance with routine quality control procedures overseen by the Quality Assurance Department of the Tikrit University, College of Science, Department of Biology, Iraq. Local breed rabbits were housed under controlled environmental conditions (20±2°C, 14:10h light:dark cycle) and allowed ad libitum access to food and water.

2.2. Blood sampling and hormonal assays

As for investigating the physiological aspect, a hormonal analysis was performed using ELISA device through blood samples. The blood samples were collected from the ear vein in anticoagulant Venoject (BD
Life Sciences, Cockeysville, Md, USA), the concentration of somatostatin and insulin were measured with Rabbit Somatostatin ELISA Kit (ARP, Catalog #: E04S0192, USA), Rabbit Insulin ELISA Kit (Crystal Chem, Catalog # 90186, USA), respectively. Also the blood sugar was measured for all the animals.

2.3. Tissue processing and histological examination

While for the tissue processing technique, pancreas was quickly eradication and the samples were prepare after cutting at a sagittal level of 5×5×10 mm per part (head, body and tail) according to routine tissue technique (11). Briefly the routine tissue techniques included of the following steps: fixation, washing, dehydration, clearing, infiltration, embedding, sectioning, mounting and staining. After the mentioned steps the histological examination and microscopic photography with 40× magnification was done to study the morphological changes of both exocrine and endocrine cells, as well as, counting the pancreatic acini and the islets of Langerhans in the microscopic field by calculating these structures in twenty microscopic fields for each sample by the objective lens (10×) (12).

2.4. Statistical analyze

Results were statistically analyzed by using correlation analysis and ANOVA test to assess the importance of variance between different age groups, the relationship between age and different variables, as well as, the explain differences between mean values of age groups using analysis of variance and standard deviations (SD), as these statistical tests were conducted by the SPSS and Microsoft Excel XP system, the differences were considered significant at the probability level (P≤0.01).

3. Results and Discussion

The results showed statistical differences in concentration of insulin, somatostatin and blood sugar in blood samples, as well as, clear changes or differences in pancreas tissue structure during the age progress, as following:

3.1. Hormonal changes

Statistical analysis showed significant difference of insulin and somatostatin in second group (1 year), which were higher compared to first (6 months) and third group (3 years) at probability level (P≤0.01). While blood sugar was statistically higher in the third group than the first and second groups at the same probability (P≤0.01), as it shown in table 1. About diameters of acinar cells, there was no statistical
significance at (P≤0.01) according to the three age groups (Table 2). As for diameters of Langerhans islets, table 3 showed statistical significance for the second group compared to the first and third group at (P≤0.01).

3.2. Histological changes

Histological examination of the early life stage (6 months) showed that pancreas was found in small lobules separated or spaced from each other, and external secretory units consisting of a number of pyramidal cells with intercalated ducts which were very small inside the lobules, as well as, interlobular ducts (Figures 1 and 2), in addition to internal secretory units represented by Langerhans islets were a number of hormone-secreting cells of small sizes, shapes and diameters, which were adjacent to the blood vessels (Figure 3). When pancreas was examined at middle age, which is puberty, pancreatic cells were found to be interconnected with each other, as well as, external secretory units, where their cells have a purple cytoplasm, dark-blue nuclei, and large multicellular Langerhans islets with abundant capillaries (Figures 4 and 5), and large-size pancreatic ducts lined with simple cuboidal cells surrounded by a medium-sized blood vessel (Figure 6). As for Pancreatic specimens for the advanced age stage or perennial rabbits (3 years), the results showed that pancreas also contained lobules with external secretion, which comprise of pyramidal cells do not differ in size than in the previous two stages (6 months and 1 year), but the intercalated ducts were difficult to show inside the lobules (Figures 7 and 8). It was observed at this stage that there is adipose tissue between lobules in the parenchyma of pancreas as large adipocytes, which also appeared between external secretory units (Figures 9 and 10).

As known that pancreas is a mixed gland (exocrine and endocrine secretion), surrounded by a capsule of very thin connective tissue that invaginates into the gland to form septa, further, these septa divide pancreas into lobes which contain many distinctive lobules (5). Pancreas is a highly affected sensitive tissue for any metabolic disorder in the body, which can cause an imbalance of essential hormones secretion that released from cells of Langerhans islets (endocrine secretion), which are located within the structure surrounded by acinar cells (exocrine secretion) (1). Through the results obtained, it was found that the first stages of life, hormonal secretion had a role in metabolism of glucose, as normal results were recorded with normal histological structure, as well as, age progress showed better effectiveness of pancreatic hormonal activity, with greater extent in size of Langerhans islets, which indicates that the average age of animal had a vital
role in making the enzymatic and hormonal activities at a better level, and this is consistent with the mentioned at other studies (9, 13).

In conclusion results showed that the middle age of the animal is consider as the best period in the life time of the animal for vital activities of the body, but when the animal goes to the later steps of its life time, the stage of aging, a significant reduction in the Langerhans islets diameters, with an increase in fibrous and adipose tissue, that reduces the ability of cells to release hormones and enzymes, as it means a decline in capacity of pancreas to perform its function of metabolism, which refers that the histological structure has a role in that, and this is consistent with mentioned in another research conducted by Seymour, Bennett (6), since age has a role in path of the histological and functional structure of the animal, as well as, corresponds with Tadokoro, Takase (14).

![Figure 1. (6 months): Lobules of pancreas are small in size and separated from each other (A), fibrous septa (B), duct between lobules (C). (H&E. 40X).](image-url)
Figure 2. (6 months): Pancreatic secretory units (A), duct between lobules (B), blood vessels (C). (H&E. 40X).

Figure 3. (6 months): Capillaries between pancreatic lobules (A), islet of Langerhans with endocrine cells (B). (H&E. 40X).
Figure 4. (12 months): Crowding of external pancreatic units (A), large size and diameter of Langerhans islets with abundance of their endocrine cells (B), capillaries in islets of Langerhans (C). (H&E. 40X).

Figure 5. (12 months): Large duct between lobules (A), blood vessel congestion (B). (H&E. 40X).
Figure 6. (12 months): Large Langerhans islet with its endocrine cells (A), external excretory units (B). (H&E. 40X).

Figure 7. (36 months): Pancreatic lobule including the external secretory units (A), Langerhans islets (B). (H&E. 40X).
Figure 8. (36 months): Langerhans islets are irregular shape (A), fibrous septa between pancreatic lobules (B). (H&E. 40X).

Figure 9. (36 months): Langerhans islets with some endocrine cells (A), fatty tissue between the lobules (B). (H&E. 40X).
Figure 10. (36 months): Pancreatic duct between the lobes (A), adipocytes between external secretory units (B). (H&E. 40X).

Table 1. Assessment number of pancreatic parameters according to the age groups(1,2 and 3)

<table>
<thead>
<tr>
<th>Group</th>
<th>Insulin</th>
<th>Somatostatin</th>
<th>Blood sugar</th>
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<tbody>
<tr>
<td>1</td>
<td>0.2 ± 0.011</td>
<td>0.03 ± 0.012</td>
<td>96 ± 3.469</td>
</tr>
<tr>
<td>2</td>
<td>0.89 ± 0.052</td>
<td>0.013 ± 0.017</td>
<td>89 ± 1.155</td>
</tr>
<tr>
<td>3</td>
<td>0.56 ± 0.035</td>
<td>0.03 ± 0.005</td>
<td>112 ± 1.149</td>
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Table 2. Average diameters of acinar cells at groups (1,2 and 3), (micrometer/ 40X magnification)

<table>
<thead>
<tr>
<th></th>
<th>First group</th>
<th>Second group</th>
<th>Third group</th>
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<tr>
<td></td>
<td>5.91 ± 0.50</td>
<td>5.08 ± 0.36</td>
<td>5 ± 0.37</td>
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</table>

Table 3. Average diameters of Langerhans islets at groups (1,2 and 3),(micrometer/ 40Xmagnification)

<table>
<thead>
<tr>
<th></th>
<th>First group</th>
<th>Second group</th>
<th>Third group</th>
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<tr>
<td></td>
<td>19.90 ± 0.75</td>
<td>39.66 ± 3.99</td>
<td>26.87 ± 3.29</td>
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References