Effect of Induced Hyperthyroidism on Pancreas of Adult Female Albino Rats

Zeinab M. Alazouny, Osama Y. Ibrahim, Mariam A. Abd Elmaksoud*, Karima F. Abdelfadeel
Medical Histology Department, Faculty of Medicine, Zagazig University, Egypt.
***Corresponding author:** Mariam A. Abd Elmaksoud, **Email:** maryam_ahmed17@yahoo.com

ABSTRACT

Introduction: A condition known as hyperthyroidism can lead to a wide range of health issues, including osteoporosis, oxidative liver damage, diabetes mellitus, as well as cardiovascular disease.

Objective: To examine the detrimental effects of hyperthyroidism on the pancreatic structure of adult female albino rats.

Materials and Methods: twenty-one adult virgin female albino rats were assigned to 2 groups; control and induction of hyperthyroidism. Serological analysis to assess thyroid functions, oxidative stress and lipid peroxidation analysis was carried out. Pancreatic samples were processed for light microscopic examination.

Results: Hyperthyroidism caused both biochemical and histological changes on pancreas of adult female albino rats. The biochemical changes in the form of significant decreased in the thyroid stimulating hormone serum level, significant reduction in level of serum superoxide dismutase and the malondialdehyde level was significantly increased in the hyperthyroid rats. The histological changes were loss of its general architecture. The pancreatic acini appeared irregular, vacuolated and had dark nuclei. Some islets of Langerhans appeared shrunken and the acini did not have the normal smooth demarcation from the surface.

Conclusion: Hyperthyroidism seriously affected the histological structure of the pancreas with subsequent disturbance in the biochemical markers.

Keywords: Hyperthyroidism, Pancreas, Adult Female Albino Rats

INTRODUCTION

Hyperthyroidism (HT) is one of hyper-metabolic problems that result from increased free T4 and/or free T3 serum levels [(1)]. T4 is the thyroid gland's most important hormone. Administration of L-T4 drug produces effect similar to that of thyroid hormone in vivo. It is converted to T3 in kidneys and liver and then binds to thyroid binding hormones in the blood to be transferred to various tissues in order to exert its functions. It is given as a replacement therapy in the hypothyroid patients. At high doses, it can develop the same symptoms and distress of hyperthyroidism [(2)].

Hyperthyroidism is a serious disorder as it can lead to a wide range of health issues, including osteoporosis, oxidative liver damage, diabetes mellitus, as well as cardiovascular disease [(3)].

The thyroid gland is linked to the endocrine portion of pancreas through regulation of carbohydrate metabolism. Excess thyroid hormones (THs) produce diabetogenic effect through stimulation of intestinal absorption of glucose, decrease in the ability of the liver to store glucose as glycogen, impairment of pancreatic insulin release, increase in peripheral insulin resistance (IR), decrease in plasma insulin half-life and increase in the expression of catecholamine receptors[(4)].

Physiologically as well as anatomically, the pancreas' endocrine and exocrine systems are interconnected. The pancreas' endocrine function can be compromised due to pathological abnormalities in the exocrine tissue, and conversely insulin and glucagon hormones govern exocrine tissue (e.g., glucagon and somatostatin), so a deficiency of these hormones causes enzyme production to be out of whack and exocrine dysfunction to ensue[(5, 6)].

The aim of the present study was to examine the detrimental effects of hyperthyroidism on the pancreatic structure of adult female albino rats.

MATERIALS AND METHODOLOGY

Substances:

L-Thyroxine (L-T4): To make a solution containing 1 ml of 100 µg g of L-thyroxine, one tablet (100µg) was dissolving in 1 ml of water. It was purchased from GlaxoSmithKline, Cairo, Egypt.

Animals used in research:

We used a total of twenty-one adult, virgin albino female rats for this study, ten to 18 weeks old. They weighed between 180 and 200 grams. At Zagazig University, they were taken from the Breading Animal House. A room temperature and regular light/dark cycles were maintained for the animals with ad libitum access to food and water. Before beginning the experiment, the animals were maintained for at least two weeks to get used to their new surroundings.

The rats were divided into two major groups:

Group I (control): a total of 14 rats divided into two groups of equal size.

Subgroup Ia (negative control): were left without intervention for two weeks.

Subgroup Ib (vehicle L-T4): which received 1 ml of distilled water by oral gavage for two weeks.

Group II (induction of hyperthyroidism): in which 7 rats were hyperthyroid by daily oral gavage of L-T4

Received: 27/8/2021
Accepted: 13/10/2021

(600 µg/kg/ b.wt.) dissolved in distilled water for 2 weeks [7].

General observations:

The general appearance and daily food consumption of the rats were observed during the period of the experiment; rats' mortalities and weight were also documented.

Serological analysis:

We performed serological analysis on each experimental group at the end of the study. Rats were allowed free access to water while fasting for the duration of the experiment. The retro-orbital plexuses of sedated rats were punctured with a capillary glass tube to obtain blood samples. In order to perform the analysis later, the serum samples were centrifuged for ten minutes at 3000 r.p.m. and then stored at -20 Celsius degrees.

Thyroid Function Analysis:

Rat TSH ELISA kit was used to assess TSH levels in the blood. (LS-F5124, LSBio, North America).

Oxidative stress and lipid Peroxidation Analysis:

Malondialdehyde (MDA) has been identified as the product of lipid peroxidation. Thiobarbituric acid reactive compounds were used to measure MDA (TBARS) [8].

Assessment of Antioxidant Status

Superoxide dismutase (SOD) activity was assessed colorimetrically at 610 nm as described by **Sinha** [9].

Histopathological investigation:

An inspection with a light microscope:

After obtaining blood samples, all animals were euthanized by pentobarbiturate anesthesia (50 mg/kg/rat) then cutting of the aorta was done after cervical dislocation [10]. We performed an incision in the middle of the abdomen and dissection of pancreas was carried out. Fixation of pancreatic samples was done in 10% neutral buffered formalin for 48 hours then procession to get 5 µm-thick paraffin sections [11]**.** The following stains were used in this study:

Hematoxylin and Eosin (H and E) stain [11]**:** as a routine method for studying the general architecture of the pancreas.

Mallory's trichrome stain [11]: collagen fibres can be identified using this method.

Morphometric study:

For stained sections with H and E and Mallory's trichrome morphometry and image analysis were done by Fiji image J (1.51n, NIH, USA) program in unit of the image analysis at Department of Pathology, Faculty of Dentistry, Cairo University. Camera, display, and IBM hard disc were all connected to the microscope and operated by Leica Qwin 500 software, which allowed the user to evaluate images. For starters, the image analyzer's measurement units (pixels) were calibrated automatically to obtain the actual micrometer units.

In Mallory trichrome stained slices, the diameter of the Langerhans islets and the pancreatic acini, as well as the percentage of collagen fibers, were assessed.

Ethical approval:

National Guide for the Care and Use of Laboratory Animals was used to ensure that all rats were cared for in accordance (NIH Publications No. 8023, revised 1978). University IACUC, Zagazig, Egypt, has given its approval to the experiment's design.

Statistical analysis:

The data collected as body weight, food consumption, analyses of biochemical and morphometrical data were represented as mean (standard deviation) and compared using SPSS version 20.0 software. Independent t-test was used for comparison. P less than 0.05 was deemed statistically significant.

RESULTS

Generally: After doing a series of experiments, the hyperthyroid group revealed signs of hyperthyroidism including increased appetite, mild diarrhea, irritability and increased body temperature. In the control groups no signs were detected. Also, three rats died from the hyperthyroid group only. In the hyperthyroid group, the end body weight was significantly lower than that of the control groups. Compared to the control groups, the hyperthyroid group showed a significant increase in food consumption as a result of their decreased body weight **(Table 1).**

Table (1): Body weight and food consumption among the studied groups

Variable	Control groups (N=14)	Hyperthyroid (N=4)
Body weight (gm): Mean ± SD	225 ± 12.5	160.3 ± 10.8*
Food consumption (mg/day/rat): Mean ± SD	13.2 ± 0.9	16.7 ± 1.5*

Data are shown as Mean ± SD. Significant difference at $p<0.05$ (*).

Biochemical results: After administration of LT4 (600 µg/kg/day) for 2 weeks, the blood TSH levels of hyperthyroid rats were considerably lower than those of control groups, showing that the model worked **(Table 2).**

Serum SOD levels decreased significantly in the hyperthyroid group. When compared to the control groups, the MDA level in the hyperthyroid group was considerably elevated **(Table 2).**

Table (2): Biochemical data among the studied groups:

Variable	Control groups (N=14)	Hyperthyroid (N=4)
TSH (µIU/ml): Mean ± SD	0.08 ± 0.01	0.019 ± 0.002*
Serum MDA (mmol/l): Mean ± SD	7.9 ± 0.6	15.2 ± 1.6*
Serum SOD (U/l): Mean ± SD	8.9 ± 0.8	4.5 ± 0.7*

Significant difference at p<0.05 (*)

The morphological findings:

Mallory's trichrome stained sections were statistically analyzed and presented in the results of H and E stained sections **(Table 3, 4).** In comparison of the hyperthyroid group to the control, the mean value of the diameter of the islets and the acini in random sections indicated significant decrease in the hyperthyroid group **(Table 3).**

Table (3): Diameter of islet and acini data among the studied groups

Variable	Control groups (N=14)	Hyperthyroid (N=4)
Diameter of islet: Mean ± SD	110.5 ±23.6	28.7 ± 3.5*
Diameter of acini: Mean ± SD	44.6 ± 4.7	19 ± 1.4*

Significant difference at p<0.05 (*)

The mean value of the area % of the collagen fibers in random sections showed a substantial rise in the hyperthyroid group as compared to the control group **(Table 4).**

Table 4: Collagen area percent data among the studied groups

Variable	Control groups (N=14)	Hyperthyroid (N=4)
Collagen area (%): Mean ± SD	4.8 ±1.2	15.9 ± 2.3*

Significant difference at p<0.05 (*)

Histopathological results: The control sub groups (a, b) were examined by the light microscopic and closely related results were found. As a result, only results from subgroup Ia are presented in this paper.

Stain results from H and E: Sections stained with H and E showed an extremely well-defined pancreatic structure, with the endocrine (islets of Langerhans) as well as the exocrine (pancreatic ducts) components clearly defined. The septa dividing the gland into lobules were variable in size and were formed of acini (serous acini). In the connective tissue septa, ducts and blood arteries were found (**Fig. 1a**).

The exocrine component was formed of closely packed serous acini. It was discovered that the nuclei of these cells had round or oval shapes and the cytoplasm was granular, apical acidophilic and basal basophilic. Despite the fact that the acinar margins were clearly defined, the lumen was difficult to discern. The lobules also contained pale areas (islets of Langerhans). The islets of Langerhans were seen interspersed between the acinar cells. The cells were arranged in a densely packed cords that had pale rounded nuclei and acidophilic cytoplasm with blood capillaries in between appeared more in the center **(Fig. 1b)**.

Pancreas of the hyperthyroid group revealed loss of its general architecture. The septa were thick and infiltrated with inflammatory cells. Many blood vessels were congested and the ducts were dilated.

The acini were deeply basophilic **(Fig. 1c)**. The pancreatic acini appeared irregular, their cells in some lobules were vacuolated and had dark nuclei. Flat cells with flat nuclei lined the other acini. Some interlobular ducts were dilated and lined with flattened epithelium. Some islets of Langerhans appeared shrunken and the acini did not have a smooth demarcation. They had dark nuclei and vacuolated cytoplasm **(Fig. 1d).**

Results from Mallory's trichrome: In stained sections with Mallory's trichrome there were few collagen fibers in the narrow septa, around the pancreatic acini, the blood vessels, and the duct system **(Fig. 1e).** Some pancreatic ducts and blood vessels as well as the septa in the hyperthyroid group showed a significant buildup of collagen fibers **(Fig 1f)**.

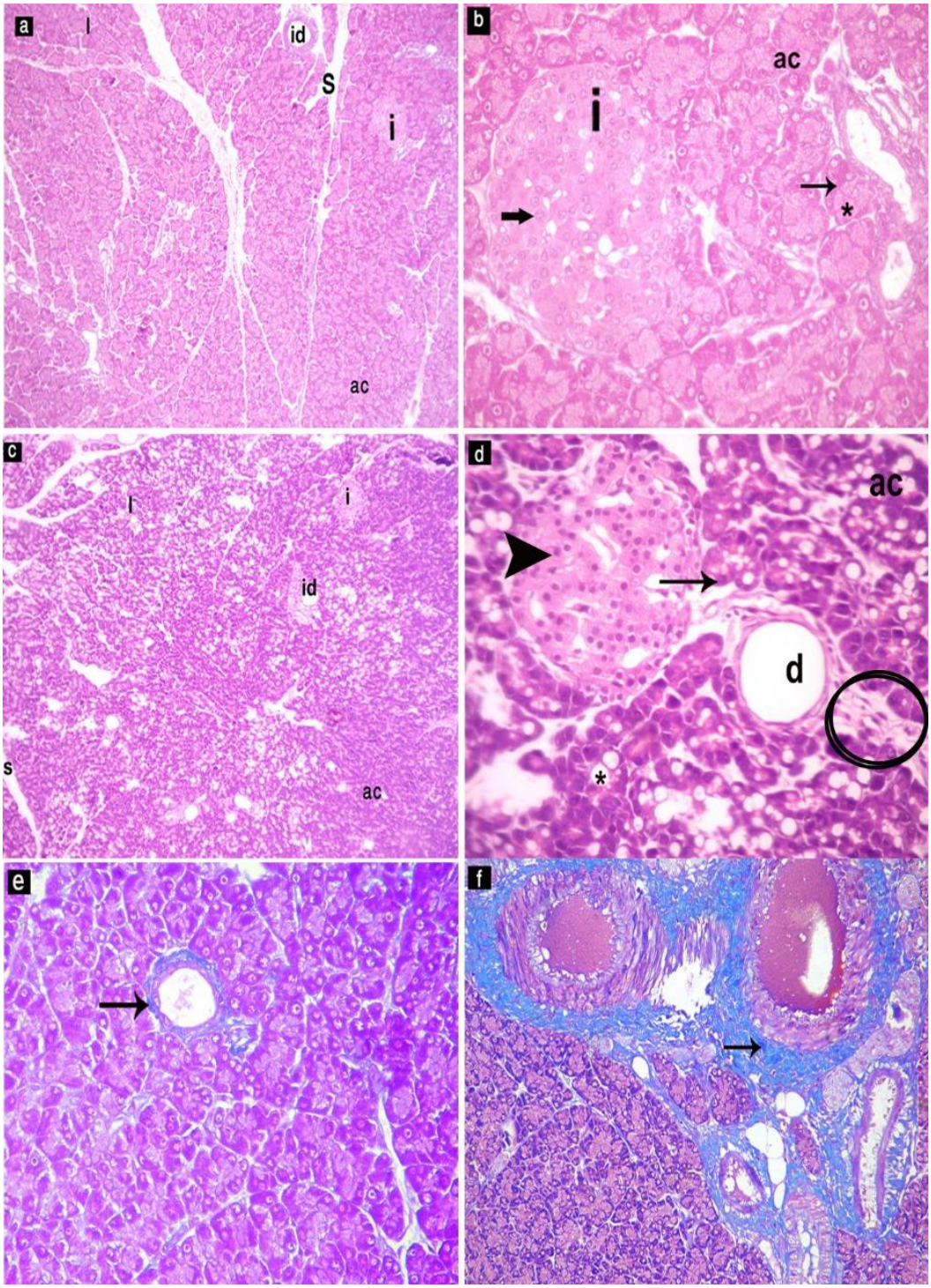

Fig. (1): **(a, b)** H and E-stained slices of pancreas from albino rats of the control groups. **(a)** Low magnification showing its general architecture. The exocrine and endocrine systems are two different sections of the system (islets of Langerhans). Multiple lobules of the pancreas are divided into lobules by thin septa (S) (l). Each lobule is formed of closely packed serous acini (ac). Intra lobular ducts (id) are seen within the lobules. The islets appear lightly stained than the surrounding acinar cells (i). (x100). **(b)** Higher magnification showing the serous acini (ac) lined by pyramidal cells with acidic granules (*) at the top and basal basophilic cytoplasm (arrow) that contain rounded vesicular nuclei. The cells of the islet (i) appear closely packed irregular cords of cells. They have pale nuclei (thick arrow) and acidophilic cytoplasm (arrow head) (x400). **(c, d)** Pancreas sections stained with H and E from albino rats with hyperthyroidism. **(c)** Low magnification showing loss of its general architecture. Thick connective tissue septa (s) with interlobular ducts (d) are present within it. The pancreatic acini are irregular in shape (ac) (x100). **(d)** Distorted acini (ac) that are lined by cells with dark nuclei (arrow) and vacuolated cytoplasm (*). The acinar borders are irregular and hardly seen. The cells of islet have dark nuclei (arrow head) (x400). **(e, f)** The pancreas of albino rats from the research groups were stained with Mallory's trichrome. (**e)** Shows delicate collagen fibers around the pancreatic duct (arrow) in the control groups. (x400). **(f)** Shows prominent deposition of collagen fibers (arrow) around some pancreatic ducts and blood vessels (x100)**.**

DISCUSSION

In the present study, the development of hyperthyroidism was confirmed biochemically and statistically as the level of TSH decreased significantly as compared to the control groups. These changes were in agreement **Hashem and Saad** [12]. That meant that hyperthyroidism was well established experimentally. **Croker *et al.*** [13] explained induction of hyperthyroidism by L-T4 administration by hypothalamic-pituitary-thyroid (HPT) axis activation. Hyperthyroidism was induced in our experimental model by using L-thyroxine which is a synthetic T4 hormone. It is considered a replacement therapy of choice for patients with hypothyroidism. It is also used as suppressive therapy in patients with thyroid nodules, diffuse goiters, or thyroid cancer, through suppression of TSH production [14].

The pancreas of adult female albino rats was studied in this study. As hyperthyroidism and thyroid disorders are related to autoimmunity, which more commonly occurs in females than males. This may be due to genetic, environmental and lifestyle factors [15].

The mortality from hyperthyroidism through the experiment might be due to cardiovascular or cerebrovascular complication according to **Chakera *et al.*** [16] who said that hyperthyroidism leads to sympathetic excitation, furthermore, certain disorders particularly in the elderly as severe angina or myocardial infarction in persons with asymptomatic ischemic heart disease maybe the cause of mortality.

The body weights showed a significant decrease in spite of more food consumption of the hyperthyroid group compared to the control. This observation is in agreement with **Messarah *et al.*** [17]**. Ajayi *et al.*** [18] explained that by increase in metabolic rate, energy expenditure and decrease in adipose tissue.

SOD and MDA levels decreased significantly in hyperthyroidism compared to the control groups, according to the findings of this study. **Joshi *et al.*** [19] showed that the overall antioxidant capacity decreased in hyperthyroidism may be related to a decrease in the extracellular free radical scavenging mechanism. As a result of hyperthyroidism, researchers observed that MDA levels were elevated in hyperthyroid rats because thyroid hormones influence fat content in the rat tissues, promoting reactive oxygen species (ROS) production and nitric oxide production, and increasing free radical formation and lipid peroxidation levels, as a result, MDA is a good indicator of the presence of these activities. **Babu *et al.*** [20] mentioned that hyperthyroidism is a hypermetabolic condition that resulted in increased production of ROS but SOD acts as a first line of defence.

H and E-stained slices of the pancreas of control rats revealed the following findings in the current study: normal architecture this was in agreement with **Arafa *et al.*** [21] who described that it was formed of serous acini in which the cells were pyramidal in shape with round nuclei at the base of the cell. The cytoplasm appeared basal basophilic and apical acidophilic. The acini were separated by thin connective tissue septa dividing it into lobules. Ducts and blood vessels were observed in the septa. Between the acinar cells, the islets of Langerhans were seen scattered. They were formed of irregular cords of cells closely packed and had pale nuclei and acidophilic cytoplasm. The blood capillaries were present in between.

Examination of the H and E stained sections of the pancreas of the hyperthyroid group revealed the folowing: loss of the general architecture. The septa weye thick with cellular infilterations. The pancreatic acini were irregular in shape and the cells had dark pyknotic nuclei and vacuolated cytoplasm. The acinar borders were irregular and hardly seen. Some interlobular ducts were dilated and their linig were flat. Many blood vessels were dilated and congested. Many shrunken islets of Langerhans were present. Many cells of the islets had irregular outlining, dark pyknotic nuclei and vacuolated cytoplasm. Inflammatory cells were seen in the septa. This was in agreement of **Hassan and Zahran** [22] as they examined the tongue mucosa of hyperthyroid rats and noticed signs of vacuolar degeneration and nuclear changes of the altered spinous cells.

Hashem and Saad [12] observed several vacuoles in the acini of the parotid in hyperthyroid rats and they explained this by lipid peroxidation and oxidative stress that led to damage of cell membrane, increase in water content and hydropic degeneration.

Previous studies, explained presence of inflammatory cells in connective tissue septa that inflammatory conditions such as hyperthyroidism caused movement of fluids and leukocytes from the blood into the extravascular tissue as a reaction of microcirculation. Also, high levels of prostaglandins synthesis might induce smooth muscle relaxation with subsequent sinusoidal dilatation, while loss of fluid from the blood made the vessels engorged with RBCs [23].

Examination of Mallory's trichrome stained sections revealed that deposition of collagen fibers significantly increased in the connective tissue septa around the pancreatic acini, ducts and blood vessels of the hyperthyroid group. This was in accordance with **Meligy *et al.*** [24] who examined the liver of hyperthyroid rats and observed significant increase in the collagen deposition and **Hegazy *et al.*** [25] who found that hyperthyroidism accompanied with marked thickness in the capsule of the testes indicating that the collagen fibers deposition increased in the capsule as well as around the blood vessels. **Safadi and Friedman** [26] explained the increase of liver fibrosis that it might resulted from activation of stellate cells by ROS and subsequent increase in collagen synthesis and fibrogenesis.

CONCLUSIONS

In conclusion, from this study it was demonstrated that hyperthyroidism could seriously affect histological structure of the pancreas with subsequent disturbance in the biochemical markers.

Financial support and sponsorship: Nil.
Conflict of Interest: Nil.

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