Levothyroxin Drug Ameliorates the Pancreatic Changes Occurred After Induced Hypothyroidism in Male Albino Rats

Samar A. Mustafa, Zienab A. Gouda*, Mohamed E. Ali Khalifa, Aisha A. Alkhodary
Histology and Cell Biology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

*Corresponding author
Dr. Zienab Abdullah Gouda
Histology and Cell Biology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
Tel.: 002 01065498038
E. mail: dr_zienab@hotmail.com
Mail address: Histology and Cell Biology Department, Faculty of Medicine, Zagazig University44159, Zagazig, Egypt

Abstract
Hypothyroidism is one of the common thyroid disorders in humans. Although there is a recognized association between thyroid disease and Diabetes Mellitus, there is no definite answer about pancreatic affections occurred in patients with hypothyroidism. Levothyroxin (L-T4) is a synthetic thyroxin hormone (T4). To study antidotal impact of the L-T4 on the pancreas of induced hypothyroidism, three albino rat groups; control, hypothyroidism induced (HIG) & hypothyroidism induced with L-T4 replacement (HIG+LT4) were used. Serum T3, T4, TSH, blood glucose levels, plasma malondialdehyde MDA and superoxide dismutase SOD levels were determined. Pancreatic tissues were visualized by light microscope. In addition, area % of collagen fibers, area % of pancreatic acini and diameters of acini and islets of Langerhans were estimated. Statistically, HIG revealed a highly significant decrease in T3 and a significant decrease in T4 levels. While, TSH and blood glucose levels showed significant increase. Histologically, many septal affections as; fibrosis, cellular infiltration, fat cells deposition and precipitation of pale acidophilic fine fibriller material were recorded. Deformed acinar cells with less apical eosinophilia and more basal basophilia were observed. The positive reaction (detected by immunostaining of insulin protein) was intense centrally while, peripherally, some cells expressed weak positive reaction. Morphometrically, a highly significant increase of area % of collagen fibers was revealed. A highly significant decrease of area % of acini and a significant decrease of the acinar diameters were noted while, the islets diameters showed a significant increase. HIG+L-T4 treated group revealed normal shaped acini and normal islets of Langerhans. Moreover, thyroid hormones, blood glucose, MDA, SOD levels appeared closer to values of control group.

Keywords: Levothyroxin, pancreas, rats, hypothyroidism

Introduction
Nowadays, there is a considerable interest of hypothyroidism, as in most cases, it is accompanied with other endocrine insufficiency. In humans, hypothyroidism may be congenital, acquired or occur as a complication during treatment of hyperthyroidism. A
high prevalence of iodine deficiency in some rural areas \(^2\) was documented. Hypothyroidism and Diabetes Mellitus account the most two common endocrinopathies encountered in clinical practice \(^3\).

Interestingly, in insulin physiology, thyroid related hormones play a pivotal role e.g. Thyrotropin-releasing hormone (TRH) has been shown to be synthesized in the pancreatic β cells \(^4\). Fasting blood glucose levels of the TRH knockout mice were markedly higher than those of the normal mice \(^5\). In rodents, triiodothyronine (T3) is important to ensure normal development and function of exocrine portion of pancreas as it is a powerful inducer of pancreatic acinar cell proliferation \(^6\). Moreover, it is important for insulin secretion and sensitivity as it decreases insulin resistance via expression and stimulation of proteins e.g. G proteins, CAMP, GLUT-2 and GLUT-4 of cellular membranes \(^7\). Finally, both T3 and insulin hormones stimulate the expression of glycogen synthase which is responsible for uptake and disposal of glucose as glycogen. So, by previous mechanisms, T3 help insulin to keep normal blood glucose level \(^8\).

Levothyroxin (L-T4) is a synthetic thyroxin hormone (T4) that is biochemically and physiologically indistinguishable from the natural human hormone. The endocrinologists prefer use of T4 alone for treatment of hypothyroidism. As a combination of T3 and T4 hormones in treatment of hypothyroid patients showed a little improvement in comparison with T4 only \(^9\).

Carbimazole is a widely used drug in treatment of thyrotoxic cases. Hypothyroidism is one of carbimazole complications during treatment \(^10\). Any thyroid disturbance will affect all body organs including pancreas. Correction of hypothyroidism by Levothyroxin in many disorders e.g. retinopathy of diabetic patients \(^11\) was the purpose of several deep investigations. However, a little information was available about treating hypothyroidism on pancreatic associated changes. So, the aim of this study was to analyze histological and immunohistochemical pancreatic affections after induced hypothyroidism by carbimazole in adult male albino rats and after correction by Levothyroxin.

**Materials and Methods**

**Animals**

Forty two adult male albino rats (2-3months) weighing about 180-230 gm were utilized in this study. The animals obtained from the breeding animal house, Faculty of Medicine, Zagazig University, Egypt. They were housed in cages under strict care and cleaning measures. All ethical protocols for animal food and treatment were according to the guidelines of Faculty of Veterinary, Zagazig University, Egypt. Following acclimatization 25 ± 2°C; 12:12 hours light/dark for one week. Animals were divided into three groups.

- **Group I (control group- 14 rats):** given distilled water only.
- **Group II (hypothyroid induced group (HIG)-14 rats):** given daily oral dose of Carbimazole\((0.05 \text{ mg/kg of body weight})\) for one month \(^12\).
- **Group III (hypothyroid group treated with Levothyroxin(HIG+ T-L4)-14 rats):** given previous Carbimazole dose for one month. Then, given daily oral dose of Levothyroxin sodium\((10 \text{ mcg/100gm of body weight})\) for another month \(^13\).
Chemicals
Carbimazole 50mg and Levothyroxin 10 mcg tablets were purchased from pharmacy. One Carbimazole tablet was dissolved in 100ml and one Levothyroxin sodium tablet was dissolved in 5 ml distilled water.

General observations
During the experimental period, the activity, food consumption and mortalities of the rats were followed.

Biochemical study
At the end of the experiment, rats were sacrificed. Blood samples (6 ml / each rat) were collected in a capillary tube by retro-orbital puncture. The samples were centrifuged at 3000 rpm.

Determination of serum levels of T3, T4, and TSH
Serum levels of T3, T4, and TSH were determined as described by (using commercially available Chemiluminescence Immunoassay (CLIA, catalogue no. ABIN504750; ABIN, Canoga Park, C.A. USA) following the manufacturer’s instructions.

Determination of Blood glucose level
Blood glucose level was tested with glucose tester (Bionime. GmbH, Switzerland).

Determination of plasma oxidative marker
The superoxide dismutase (SOD) was assessed colorimetrically (absorbance 450 nm) using a commercially available kit (catalogue no. K335-100; Biovision, San Francisco, USA) following the manufacturer’s instructions.

The lipid peroxidation marker malondialdehyde (MDA) was assessed colorimetrically (absorbance 532 nm) using a commercially available kit (catalogue no. K739-100; Biovision, San Francisco, USA) following the manufacturer’s instructions.

Histological study
The pancreatic specimens were fixed in Bouin's solution, dehydrated and embedded in paraffin. Sections of 5 µm were cut and stained with H&E and MT (14) and immunohistochemical staining for insulin protein (15). For Toluidine blue (TB) stained sections, small specimens were taken from the pancreata, fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide, dehydrated and embedded in epoxy resin. Thick sections of 1 µm, were mounted on glass slides and stained with TB (16).

Image analysis and morphometric study
The image analyzer computer system Leica Qwin 500 (Leica Imaging system, Ltd, Cambridge, UK) was used to evaluate the diameter and area percentage of pancreatic acini, area percentage of collagen fibers and the diameter of islets of Langerhans in (Pathology Department, Faculty of Dentistry, Cairo University, Egypt). Ten non-overlapping high-power fields from each slide of all animals of each group were used.

Statistical analysis
The data obtained were subjected to statistical analysis by Analysis of Variance (ANOVA) using statistical analysis program SPSS version 16. The data obtained were expressed as

© 2015 British Journals ISSN 2047-3745
mean values ± SD. Differences were considered to be significant when $P$ value < 0.05 against the control group.

**Results**

**Biochemical results**

In HIG, a highly significant decrease in the levels of T3 and a significant decrease of T4 were observed. While, TSH and blood glucose levels showed a significant increase (Table 1). There was a highly significant increase in MDA level whereas, the SOD showed a significant decrease (Table 2). In group III, serum T3 level was significantly decreased while T4 and TSH showed nearly normal levels (Table 1). MDA and SOD levels showed non significant differences (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>T3(ng mL)</th>
<th>T4(ng mL)</th>
<th>TSH(ng mL)</th>
<th>blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>112 ±13</td>
<td>4.6 ±1</td>
<td>0.5 ±0.1</td>
<td>88 ±10</td>
</tr>
<tr>
<td>HIG</td>
<td>59.5 ±10***</td>
<td>2.5 ±0.9*</td>
<td>8 ±2*</td>
<td>108 ±11*</td>
</tr>
<tr>
<td>HIG+L-4</td>
<td>75 ±20*</td>
<td>5 ±2</td>
<td>1 ±0.4</td>
<td>95 ±13</td>
</tr>
</tbody>
</table>

Table (1): Statistical analysis of T3, T4, TSH and blood glucose levels in all studied groups
Means ± SD; *$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001 vs. control animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA(nmol/mL)</th>
<th>SOD(U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>9.28±1.30</td>
<td>177.46 ± 11.54</td>
</tr>
<tr>
<td>HIG</td>
<td>18. 98 ± 1.04 ***</td>
<td>134.24 ± 20.72*</td>
</tr>
<tr>
<td>HIG+L-thyroxin</td>
<td>10.10 ± 0.11</td>
<td>160.96 ± 17.94</td>
</tr>
</tbody>
</table>

Means ± SD; *$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001 vs. control animals.

**Histological Results:**

**Control Group (I)**

Light microscopic examination of H & E stained sections revealed normal architecture of the pancreas in the form of thin capsule and thin septa dividing it into multiple lobules (Figure 1.A). Each lobule was formed of closely packed acini (49.4-63.81µm in diameter) and multiple different sized islets of Langerhans (91.66-250.73 µm in diameter). The acinar cells cytoplasm displayed apical acidophilia and basal basophilia and had basal rounded vesicular nuclei (Figure 2.A).

Three different locations of islets were noted: interlobular, intralobular and scattered simple islets cells (few numbers). They appeared either as irregular branching cords of cells or clumps of indistinct bordered islet cells separated by extensive network of blood capillaries (Figures 3.A&B). The interlobular islets were small closely related to ducts (Figures 1.A&3.A). The intralobular islets were larger and showed crowded small cells formed the mantle at the periphery while, their center contained a network of blood vessels whose penetrated and branched inside the islets. The β cells were intermingled with non β cells (Figure 3.B).
Mallory's trichrome stained sections showed few collagen fibers in the inter-lobular septa and around the blood vessels (Figure 1.B). Immunostaining of insulin protein detected central location of β cells (Figure 3.C). Toluidine blue stained sections showed pyramidal shaped acinar cells studded with apical zymogen granules. Blood vessels, some interstitial cells and pancreatic stellate cells were seen in between the acini (Figure 2.D). A high vascularity at the center of the islet (Figure 3.D) was noted.

Figure (1): Photomicrographs of control pancreas sections. (A): Indicating thin capsule (c), thin septa(arrow), lobules(lo) and an interlobular islet of Langerhans(i)(H&E,X100;Bars,100µm). (B): Indicating a thin layer of collagen fibers in septum (arrowhead) and around the blood vessels (arrows) (Toluidine blue, X 100; Bar, 100µm).

Figure (2): Photomicrographs of control pancreas sections. (A): Indicating multiple pancreatic acini. Their cells are characterized by apical acidophilia(*),basal basophilia(arrows) and rounded vesicular basal nuclei (arrowheads) (H&E,X1000; Bar, 20 µm). (B): Indicating pyramidal shaped acinar cells studded with apical granules (g). Blood vessels (bv), some interstitial cells (arrows) and a stellate cell (arrowhead) appear in between the acini(Toluidine blue,X 1000; Bar, 20µm).
Figure (3): Photomicrographs of control pancreas sections. (A): Indicating interlobular small islet of Langerhans (i) nearby a duct (d) (H&E, X 400; Bar, 100µm) (B): Indicating an intralobular large islet of Langerhans characterized by high vascularity (circles) (H&E, X 1000; Bar, 20µm). (C): Indicating central position of β-cells of the islet with positive reaction (*) and peripheral position of non-β-cells with negative reaction (arrow) (Immunostaining, X 400; Bar, 100µm). (D): Indicating clumps of indistinct bordered islet cells with high vascularity at the center of the islet (circles) (Toluidine blue, X 1000; Bar, 20µm).

HIG Group (II)

This group revealed thickening of the capsule and the septa (Figures 4.A&B). Many septal affections were noted in the form of cellular infiltration as lymphocytes, neutrophils and vacuolated cells (Figures 5.A&B). Sporadic fat cells or clusters of fat cells (Figures 5.A&C) also infiltrated the septa. Precipitation of pale acidophilic fine fibriller material (Figure 5.D) was observed. Deformed acini lined by cells with less apical eosinophilia and more basal basophilia were recorded (Figures 6.A&B). Some lobules were infiltrated with large number of irregular shaped cells and the nearby acinar cells contained many vacuoles (Figures 7.A&B). Both pale vacuolated and dark stained acinar cells (Figure 7.C) were noted. Some islets cells showed dark basophilic nuclei and the others contained pale basophilic nuclei (Figure 7.D). Other islets of Langerhans appeared with normal small mantle cells but separated from the acini (Figure 8.A). Some peripheral cells (forming the mantle) characterized by deep acidophilic cytoplasm and pale basophilic nuclei were encountered. The others were vacuolated (Figure 8.B). The positive reaction (detected by immunostaining of insulin protein) was intense in centrally located cells while, was weak positive reaction in peripherally located cells (Figures 8.C&D).
Figure (4): Photomicrographs of HIG pancreas sections. (A) & (B): Indicating thick capsule (c) and thick septa (arrows) (A&B: H&E, X100; Bars, 100µm).

Figure (5): Photomicrographs of HIG sections indicating different septal affections. (A): Inflammatory cells (arrow head) and sporadic fat cells infiltration (f) (H&E, X 400; Bar, 100µm). (B): Lymphocytes (arrowheads), neutrophils (arrows) and vacuolated cells (circles) infiltration (H&E, X 1000; Bar, 20µm). (C): A cluster of fat cells (f) infiltrate the septum (MT, X 400; Bar, 100µm). (D): Precipitation of pale acidophilic fine fibriller material (m) with thickening layer of collagen fiber around blood vessels (arrow) (MT, X 400; Bar, 100µm).
Figure (6): Photomicrographs of HIG sections indicating acinar affections. (A): Less apical acidophilia(*) and more basal basophilia(arrows) of acinar cells (H&E,X 1000; Bar, 20µm). (B): Deformed shaped acini and diminished size (a). Note some acinar cells are devoid of granules(e) (Toluidine blue,X 1000; Bar, 20µm).

Figure (7): Photomicrographs of HIG sections. (A): Indicating intralobular cellular infiltration (if) (Toluidine blue,X 400; Bar, 100µm). (B): A higher magnification of (A) indicating a large number of irregular shaped cells (if) and vacuolated acinar cells (v) (Toluidine blue,X 1000; Bar, 20µm). (C): Indicating pale (p) vacuolated (v) and dense (d) stained acinar cells (Toluidine blue,X 1000; Bar, 20µm). (D): Indicating some islets cells with deep basophilic nuclei (arrowheads) and the others with pale basophilic nuclei (arrow). Note decreased vascularity (Toluidine blue,X 1000; Bar, 20µm).
Figure (8): Photomicrographs of HIG pancreas sections. (A): Indicating space between acini and islets(s). The peripheral islet's cell appeared normal (arrowhead) (H&E, X 400; Bar, 100µm). (B): Indicating peripheral located large islet cells (arrows) characterized by deep acidophilic cytoplasm and pale basophilic nuclei and vacuoles (v)(H&E, X 1000; Bar, 20µm). (C): Indicating an intense staining for insulin protein in central located cells(Immunostaining, X 400; Bar, 100µm). (D): A higher magnification of (C) indicating an intense positive reaction for insulin protein in centrally located cells while, peripherally, some cells express a weak positive reaction(Immunostaining, X 1000; Bar, 20µm).

HIG+L-T4 replacement group (III)

This group showed unnoticed thin capsule, thin septa and pale areas (islets of Langerhans)(Figures 9.A&B). Some acinar cells appeared with less basal basophilia, granular apical acidophilia and contained dark basophilic nuclei (Figure 10.A). While the other pancreatic acinar cells were devoid of granules (Figure 10.B). The islets peripheral cells forming the mantle appeared normal. The vascularity of the islets appeared more in the center of them (Figure 11.A). Immunohistochemical reaction for insulin protein showed an intense positive reaction in central located cells and a negative reaction in the peripheral cells (Figure 11.B).
Figure (9): Photomicrographs of HIG+L-T4 replacement pancreas sections. (A): Indicating unnoticed thin capsule (c), thin septa (arrow) and multiple pale areas (islets of langerhans) (i) (H&E, x100; bar, 100 µm). (B): Indicating thin layer of collagen fibers in between the lobules (arrows) and around the blood vessels (arrowhead) (MT, x100; bar, 100 µm).

Figure (10): Photomicrographs of HIG+L-T4 replacement pancreas sections. (A): Indicating acinar cells with less basal basophilia (arrows), apical acidophilia (*) and dark basal basophilic nuclei (arrow heads) (H&E, X 1000; Bar, 20µm). (B): Indicating nearly normal shaped acinar cells with apical granules (g). Some acinar cells are devoid of granules (arrowheads) (Toluidine blue, X 1000; Bar, 20µm).
Figure (11): Photomicrographs of HIG+L-T4 replacement pancreas sections. (A): Indicating a highly vascular normal islet of Langerhans (circles) (H&E, X 400; Bar, 100µm). (B): Indicating an intense positive reaction of β-cells that occupying most of the islet and a negative reaction of some centrally located non β-cells and peripheral located cells (Immunostaining, X 400; Bar, 100µm).

Morphometric study
Statistical analysis of HIG revealed a highly significant increase area % of the collagen fibers, a highly significant decrease in the area % of the acini and a significant decrease in the acinar diameters. In contrast, the islets diameters showed a highly significant increase. The HIG+L-T4 replacement revealed a highly significant increase area % of the collagen, a significant decrease in acinar area % and non significant differences in acinar and islets diameters when compared with control group (Table 3).
Table (3): Statistical analysis of the area % of the collagen fibers, area % of the acini, acinar and islets diameters in different studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>area % of the collagen fibers</th>
<th>area % of the acini</th>
<th>acinar diameter</th>
<th>islets diameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>2.12±0.02</td>
<td>91.1 ±4.2</td>
<td>55.9±5.4</td>
<td>98.2 ±9</td>
</tr>
<tr>
<td>HIG</td>
<td>13.8±1.2**</td>
<td>63. 3 ±7 **</td>
<td>44.7±10.8</td>
<td>133.8 ±20**</td>
</tr>
<tr>
<td>HIG+L-thyroxin</td>
<td>7.5±1.12**</td>
<td>87.3 ± 2.7*</td>
<td>54.8±7.7</td>
<td>107. 6 ± 30.6</td>
</tr>
</tbody>
</table>

Means ± SD; *P < 0.05; **P < 0.01; ***P < 0.001 vs. control animals.

Discussion

The pancreas has both exocrine and endocrine functions. The exocrine secretions are important for digestion of food into components that are then available for absorption by intestinal epithelium. Without exocrine pancreas, malabsorption and malnutrition may result. The endocrine portion of the pancreas achieves normoglycemia via controlling carbohydrates metabolism. The successful relationship between the thyroid gland and the endocrine portion of the pancreas achieves this aim. This study aimed to clarify the histological changes occurred in pancreas as a result of deficiency in the thyroid hormones and the role of L-thyroxin replacement in elimination of these changes.

In this study, the rat was chosen as an experimental model because the cellular populations and distribution of rat islets is so similar to human islets.

Hypothyroidism is two types: peripheral and central. In the peripheral (primary) hypothyroidism, the thyroid is stimulated properly but it is unable to produce enough thyroid hormones for the body to function properly. The central hypothyroidism is characterized by insufficient stimulation of the thyroid gland as result of lesion in the pituitary gland (secondary) or in the hypothalamus (tertiary). It is rarely isolated; it occurs more commonly in conjugation with other pituitary hormones deficiency. So, the primary hypothyroidism type was chosen in this study to avoid deficiency in other pituitary hormones which may interact with the effect of the hypothyroidism on the pancreas.

In the current study, antithyroid drug was used to induce hypothyroidism in the rats because the misuse of antithyroid drugs is one of the common causes of primary hypothyroidism. Carbimazole is the first choice as many physicians avoid the use of propylthiouracil because of its hepatotoxicity. Also, pancreatitis is a very rare complication of carbimazole. So, the pancreatic changes resulting of its use can be excluded. Moreover, it is cheaper than other regimes overall and is widely used in treatment of thyrotoxicosis. In the current work, L-T4 was used to correct induced hypothyroidism. Although it is man-made, it is exactly the same as natural T4 hormone. So, guidelines from all professional societies, including the American Thyroid Association and the American Association of Clinical Endocrinologists recommend L-T4 mono-therapy as a treatment of choice for all hypothyroid patients. In the present study, serum levels of T3, T4 and TSH were measured as they are the reliable indicators of the thyroid function in humans and experimental animals. The HIG revealed a highly significant decreased T3 and T4 and a
significant increased TSH levels. Decreased T3 and T4 levels occur due to inhibition of iodination of tyrosyl residues in thyroglobulin caused by carbimazole\(^\text{25}\). While, increased TSH level is due to stimulation of the pituitary gland as a negative feedback mechanism to lowered T3 and T4 levels\(^\text{26}\).

The blood glucose level in HIG was significantly increased (108±11) in comparison to the control group (88±10). Marked hyperglycemia accompanied impaired insulin secretion in TRH deficient mice (tertiary hypothyroidism) was recorded\(^\text{27}\). They explained hyperglycemia to decreased β-cells sensitivity to blood glucose level due to low T3 and T4 level and consequently, decreased insulin secretion. Also, hypothyroidism leads to increase insulin resistant state and consequently, impairment of glucose utilization in peripheral tissues\(^\text{28}\).

In the present work, a highly significant increase in MDA levels whereas; a significant decrease in SOD levels was recorded in HIG. Under hyperglycemic conditions, glycation of phospholipids in the cell membrane or the organelles occur. These glycated lipids are the causative agents of oxidative stress (lipid peroxidation) in organs\(^\text{29}\). Moreover, one of the factors of hypofunctions of human body is protein denaturation which primarily results from glycation as well as oxidation. Improvement of the blood glucose level in the HIG+L-T4 replacement group (95±13) was in agreement with\(^\text{3}\) who revealed that treatment of thyroid dysfunction in diabetic patients will benefit glycaemic control and improve general well-being. In addition, the thyroid hormones potentiate insulin signaling and attenuate hyperglycemia and insulin resistance in a mouse model of type 2 diabetes\(^\text{30}\).

The HIG showed profound changes in pancreatic septa in the form of fibrosis, fatty infiltration and precipitation of pale acidophilic fine fibrillar material. Fat cells deposition was noted either singly or in clusters in pancreatic septa. Wide spread lipoid deposits throughout the exocrine tissue was observed in diabetic animals\(^\text{31}\). This alteration is due to elaborated free radicals from impairment of glucose utilization\(^\text{31}\). However, the process of fatty infiltration is often related to atrophy of the involved site as there is a propensity for adipocytes to fill a vacuum, in a sense, left by atrophic processes\(^\text{32}\). The highly significant decrease in the acinar area % recorded in HIG confirms this explanation.

Lymphocytes, neutrophils and vacuolated cells recruitment the larger septa were noted in HIG. Increased immune cell infiltration of the exocrine pancreas may contribute in the pathogenesis of Diabetes Mellitus type I, even without insulitis\(^\text{33}\).

In the HIG of the present work, immunohistological studies of HIG group showed a tense positive reaction in the islets of langerhans. This result is in agreement with\(^\text{7}\). They referred this tense reaction to decreased insulin secretion and consequently its accumulation inside granules as the thyroid hormones are important for insulin secretion. The detected large acidophilic, peripheral cells which expressed in immunostained sections a weak positive reaction for insulin protein were described\(^\text{34}\). They detected the capacity of α cell to co-express insulin and glucagon. The similarity between α-cell and β-cell transcriptomes in rodent and humans might be the cause\(^\text{35}\). As they discovered that hormone gene promoters in different islet cell types present similar methylation patterns. However, other study revealed that the adult functional β cells can be generated under need from adult pancreatic exocrine tissue by trans-differentiation\(^\text{36}\).

In this study, we had registered signs of improved morphological structure of the pancreas in HIG+LT4 replacement. Improvement of skin manifestations after treatment of hypothyroidism was reported\(^\text{37}\).
Conclusions
Hypothyroidism could seriously affect the pancreatic function with subsequent disturbance in the blood glucose level. These changes may carry the risk of Diabetes Mellitus development. On the other hand, correction of hypothyroidism by levothyroxine showed a little improvement of pancreatic affections. Prevention of hypothyroidism via adding iodine to water or salt especially in iodine deficiency rural areas and early detection of cases via routine analysis of thyroid function tests for rapid and good control are recommended to avoid its harmful effects on the pancreas. Further investigations on using a long-acting, slow-release form of T3 will be required.

Conflict of interest: None
Funding: This work was personal supported

Authors’ contributions

<table>
<thead>
<tr>
<th>Authors’ contributions</th>
<th>SAH</th>
<th>ZA G</th>
<th>ME Kh</th>
<th>AA A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research concept and design</td>
<td>--</td>
<td>✓</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Collection and/or assembly of data</td>
<td>✓</td>
<td>✓</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Data analysis and interpretation</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Writing the article</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>--</td>
<td>--</td>
<td>✓</td>
<td>--</td>
</tr>
<tr>
<td>Design of figures</td>
<td>--</td>
<td>✓</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Critical revision of the article</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Final approval of article</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Acknowledgements
The authors would like to thank Dr. Eman El Shahate for her aid in statistical analysis and Dr. Mohamed Shahin for his aid in morphometrical analysis.

References


© 2015 British Journals ISSN 2047-3745


Authors

Samar Abd Aziz Mustafa, Demonstrator of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt E.mail: ommar.abdallah@yahoo.com

Zienab Abdulla Gouda, Lecturer of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt E.mail: dr_zienab@hotmail.com.

Mohamed El Sayed Ali Khalifa, Associate professor of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt. E.mail: khalkhalifa@yahoo.com

Aisha Abd Elmoneum Alkhodary Professor of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt. E.mail: r_i_P0_18@hotmail.com

© 2015 British Journals ISSN 2047-3745