Evaluation of mesenchymal stem cell therapy on diabetic rats' thyroid function (Histological and Biochemical study)

Shaimaa M. Hassan[1], Maha M. Anani[2], Ahmed S. Salman[4,5], Abdullah A. Almilaibary[6], Seham A. Abdel Aziz[3],

[1] Histology and Cell Biology, Faculty of Medicine, Menoufia University, Menoufia, Egypt

[2] Department of Clinical Pathology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

[3] Histology and Cell Biology, Faculty of Medicine, Menoufia University, Menoufia, Egypt

[4] Department of Anatomy, Faculty of Medicine, Menoufia University, Egypt

[5] Department of Anatomy and Histology, Faculty of Medicine, The University of Jordan, Amman, Jordan

[6] Department of Family and Community medicine , Faculty of Medicine ,Albaha University , Saudi Arabia

SUMMARY

Thyroid hormones play a crucial role in the body's metabolism, and in patients with diabetes thyroid dysfunction diseases are common. Type 1 diabetes mellitus can be treated with daily insulin injections, but this treatment is often accompanied by multiple complications.

Studies have shown, however, that mesenchymal stem cells (MSCs), having produced remarkable improvement in diabetic rat models, can differentiate into insulin-producing cells that could be used for treatment of diabetes mellitus. To evaluate effects of bone-marrow-derived stem cell transplantation on rats with induced diabetes mellitus by assessing their thyroid hormones, thyroid autoantibodies, and structural changes in thyroid gland sections before and after MSC transplantation, this study used 40 adult male albino rats: 10 for MSC isolation; 30 randomly divided into control, diabetic, and MSC-treated groups. In 20, diabetes mellitus was induced by streptozotocin injection. Of the diabetic group, 10 were treated with bone-marrow-derived stem cells. Histological studies (light, electron microscopy) of thyroid sections were observed; thyroid hormones, thyroid peroxidase, and thyroglobulin antibodies were measured in the serum.

Diabetic rats' thyroid glands showed distorted histological structure, a drop in thyroid stimulating hormone (TSH), and elevation of thyroid hormone level. Microscopically, the thyroid gland of bone-marrow-derived stem cell-treated rats yielded significantly ameliorated histological appearance and significantly increased TSH levels, along with thyroid hormones decreased toward normal levels. Diabetes induces thyroid dysfunction and thyroid tissue injury. Bone-marrow-derived stem-cell therapy protects against thyroid gland diabetes-induced tissue injury.

Key words: Diabetes mellitus – Autoimmune thyroiditis – Mesenchymal stem cell – Histopathology – Streptozotocin

Corresponding author:
Dr. Ahmed Salman. Faculty of Medicine, The Jordan University Queen Rania Street, Amman, Jordan. Phone: +962790627433. E-mail: Ahmedsalman1971@gmail.com

Submitted: February 1, 2021. **Accepted:** May 23, 2021

INTRODUCTION

In clinical practice, diabetes mellitus (DM) and thyroid dysfunction are the most prevalently encountered endocrine diseases. In fact, incidence of thyroid disorders in diabetic patients is greater than in the normal population and is affected by autoimmune diseases, age, and gender (Kadiyala et al., 2010). Thyroid hormones participate in controlling functions of pancreases and carbohydrate metabolism. Moreover, diabetes influences thyroid function laboratory results (Leong et al., 1999). Patients with hypothyroid and type 1 diabetes mellitus (T1 DM) experience more hypoglycemic attacks than diabetic patients with normal thyroid function, and studies have reported hypothyroidism common in diabetic patients with insulin resistance (Maratou et al., 2009). Disturbance of thyroid hormones' levels has been reported in diabetic patients, particularly in those with non-controlled diabetes, who reveal depressed nocturnal thyroid stimulating hormone (TSH) peak and reduction of response to thyrotropin-releasing hormone (TRH) (Nilgün Gürsoy, 1999).

In addition, untreated thyroid dysfunction disease may augment existing cardiovascular disease and deteriorate diabetic patients' metabolic control (Mohn et al., 2002). Furthermore, autoimmune thyroid disease (ATD) includes Hashimoto's thyroiditis, Graves' disease, and postpartum thyroiditis. ATD and T1 DM have common predisposition genes in that both diseases can be associated with other autoimmune syndromes such as Addison's and coeliac diseases (Barker, 2006; Pilia et al., 2011). Besides that, ATD and T1 DM share in polyglandular autoimmune syndromes (Dittmar and Kahaly, 2003). ATD shows elevation of serum level thyroid peroxidase (TPO Ab) and thyroglobulin (TG Ab) antibodies (Weetman and McGregor, 1994), which are also detected in T1 DM patients (Hanukoglu et al., 2003); the existence of these antibodies predicts incidence of thyroid diseases (González et al., 2007).

Autoimmune demolition of pancreatic beta cells results in T1 DM with expression of insulin autoantibodies. TI insulin-dependent DM is a metabolic disease that causes beta cell autoimmune destruction requiring insulin replacement (Dave et al., 2015). In rat-model DM, streptozotocin is used to induce T1 DM by destruction of pancreatic beta cells, in turn leading to reduction of insulin and, consequently, significant hyperglycemia. Long-lasting hyperglycemia results in irreversible tissue damage and dysfunction of various organs (El Barky et al., 2017).

The main treatment for T1 DM is insulin injection, but, unfortunately, many complications occur in DM, even with long-term insulin therapy (Chen et al., 2004). Therefore, a new approach for treating DM and its complications has become a priority. Extracted from bone marrow and used in body-tissue repair and rebuilding, mesenchymal stem cells (MSCs) offer advanced DM therapy (Boháčová et al., 2018) because MSCs can differentiate into cells of all three germ layers; they also have high proliferative potential ex vivo, so they are considered for use in tissue engineering and cell transplantation (Das et al., 2013). In addition, the two endocrine disorders of DM and thyroid dysfunction are greatly associated in animals and humans (Duntas et al., 2011). Indeed, in animal models of diabetes, MSC transplantation has shown increased metabolism and is considered a new therapeutic choice for insulin-dependent DM (Dave et al., 2015).

Many studies have been conducted on MSC treatment's effect on various organs affected by diabetes, but few have examined its efficacy in the thyroid gland. Therefore, this study evaluated MSC's transplantation effects on diabetic rats' thyroid glands by assessing alterations of their thyroid hormone level, thyroid autoantibodies, and structural and ultrastructural changes.

MATERIALS AND METHODS

Animals

After the Medical Research Ethical Committee, Faculty of Medicine at Suez Canal University approved this study's protocol, forty 180-200 g adult male albino rats were allowed a laboratory-rat chow diet and water ad libitum. Care and hygiene were undertaken to maintain a constantly healthy location and atmosphere for the rats, housed under standard conditions of temperature and lighting (12-h light/dark cycles). All rats received care in accordance with the rules and regulations of the Medical Research Ethical

Committee, Faculty of Medicine, Suez Canal University.

Mesenchymal stem cells isolation

Ten rats were sacrificed to obtain MSCs from their bone marrow, which was harvested by flushing the tibiae and femurs of 2-4 week-old white rats with DMEM supplemented with 10% FBS. Nucleated cells were isolated with a density gradient (Ficoll) and resuspended in medium supplemented with 1% penicillin-streptomycin (Anani et al., 2014). Cells were plated and incubated at 37° C in 5% humidified CO_2 within a medium changed every 3-4 days. Typically, cells were maintained for 12-14 days as primary culture or upon formation of big colonies. Cultures were washed twice with phosphate buffer saline (PBS). Cells were then trypsinized (0.25%) in 1-mM EDTA for 5 min at 37° C. After centrifugation, cells were suspended with a serum-supplemented medium and incubated in a 50 cm^2 culture flask (Falcon). First-passage MSC cultures were characterized by their stickiness and fusiform shape (Rochefort et al., 2005), and 6 x 10^6 of cells were injected and adjusted for dosing in a 1-ml PBS solution (Alhadlaq and Mao, 2004). Streptavidin-biotin immunoperoxidase was used to detect CD29 (integrin beta-1) and CD44 (receptor for hyaluronate and osteopontin) (purchased from Labvision, New York, USA) as a marker of MSCs (Abdel Aziz et al., 2007).

Experimental groups

Thirty rats were divided into three experimental groups of 10: Group I (Control); Group II, diabetic group (DM); and Group III, diabetic group treated with mesenchymal stem cells (DM+MSC).

Group I (Control): Healthy rats received no medications during the experiments. Their fasting blood glucose was approximately 105 mg/dl.

Group II (DM) and Group III (DM+MSC): Diabetes mellitus was induced in these rats by a single intraperitoneal injection of streptozotocin (STZ) (Sigma-Aldrich, St. Louis, MO), 45 mg/kg body weight, freshly dissolved in 50 mM of sodium citrate buffer (pH 4.5). Two days after STZ administration, rats with 12-hour fasting blood glucose values exceeding 250 mg/dl were considered diabetic (Santos et al., 2013). Blood glucose was tested using a glucose monitor (Accu-Chek Active, Roche, Mannheim, Germany), and blood samples were taken from the rats' tail veins.

Four weeks after diabetes induction, diabetic rats were randomly divided. Half received mesenchymal cells (1×10^5 cells/rat), injected via tail veins (Antunes et al., 2014), while the other groups received the same amount of the vehicle. Thyroid stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4) levels, free T4, T3, and thyroid autoantibodies (TPO Ab, TG Ab) were measured for all groups; venous blood samples were collected from retro-orbital plexuses. Serum TSH, T3, T4, and free T4 and T3 concentrations were measured by Enzyme Linked Fluorescent Assay (ELFA) (Biomerieux Mini VIDAS Automated Immunoassay System, France). TPO Ab and TG Ab were measured by immunoradiometric assays (Abbott Laboratories, Abbott Park, IL, USA).

After 6 weeks of mesenchymal cell injection, all rats were sacrificed. Neck skin was incised, and the trachea was exposed and dissected. The thyroid gland was removed, one lobe then used for light microscope examination and the other for electron microscope examination.

Light microscopic (LM) study

After thyroid removal, portions of the thyroid gland were fixed in 10% buffered formaldehyde for 2 days and processed for paraffin sections. Sections 5 µm thick were cut and stained with hematoxylin and eosin (H & E) (Kiernan, 2015).

Electron microscopy (EM) study

Gluatraldehyde 3% buffer at pH 7.4 was used to fix thyroid gland specimens. Tissues were removed and fixed again in 1.3% osmium tetroxide in phosphate buffer (pH 7.4) for 1h, then inserted in an epoxy resin mixture and processed and embedded in Epon capsules. Semi-thin sections, i.e., one µm of Epon capsule, were cut using LKB ultra-microtome and then stained with toluidine blue. Ultrathin sections (70-90 nm) were prepared and stained with uranyl acetate and lead citrate (Bozzola and Russell, 1999). Stained grids were then examined by a (JEOL) EM 1010 at the Egyptian National Cancer Institute of Cairo.

Morphometric study

Hematoxylin and eosin slides were examined under a standard microscope. With an X40 lens, five fields of each slide were photographed using a Nikon E400 digital microphotography system (N150, Nikon Co., Tokyo, Japan). Follicular epithelial height was measured using Digimizer software program version 4.6.1.

Quantitative assessment and statistics

All data were analyzed statistically using Graph Pad prism version 4. Data were expressed as mean ± SD and analyzed using one-way analysis of variance (ANOVA) and the Kruskall-Wallis test, followed by Dunn's multiple comparison post-hoc test for comparison among all groups. Differences were significant at p-values of <0.05.

RESULTS

Light Microscopic results

Haematoxylin and eosin (H & E) staining:

Group I (control): Thyroid sections' histological examination revealed many thyroid follicles of variable size surrounded by a connective tissue capsule, and their cavities contained an acidophilic colloid. Each follicle was lined by a single layer of cuboidal follicular cells with round vesicular nuclei and parafollicular cells with flattened nuclei. Interfollicular cells and minute blood capillaries were found between follicles (Figs. 1a, 2a).

Group II (DM): In the diabetic group, the thyroid gland H & E-stained sections showed variable morphological changes: Thin capsule (Fig. 1b) and the parenchyma showed congested blood vessels (Figs. 1c, d, e, 2d). The tyroid follicles' size varied, some large and dilated (Figs. 1c, d), others shrunken and atrophied (Figs. 1d, 2d, e), and some follicles were fused (Fig. 1e).

Follicles were lined by flattened epithelium (Figs. 2b, e), with some lined by multilayers of follicular cells (Figs. 1b, 2b, c), while others had lost their epithelial lining (Fig. 2b), showing hyperemia between follicular cells (Figs. 1b, 2b, c). Several lining epithelial cells showed dark stained pyknotic nuclei (Fig. 2e). The follicles' lumen contained a vacuolated colloid or were completely devoid of colloid (Figs. 1b, c, d, 2d).

Group III (DM + MSC): For diabetic rats, thyroid gland H & E-stained sections treated with mesenchymal stem cells revealed marked improvement and restored normal thyroid histological architecture. Follicles were lined with one layer of cuboidal cells with rounded euchromatic nuclei. Interfollicular cells were present between follicles. Most follicles' lumen was filled with colloid, but some showed vacuolated colloid (Figs. 1f, 2f).

Electron microscopic results

Group I (Control): Electron microscopic examination of the control group's thyroid glands revealed that follicular cells contained euchromatic nuclei with prominent nucleoli and well- defined nuclear membranes. Their cytoplasm had rough endoplasmic reticulum, normal mitochondria, and, occasionally, small dense granules (lysosomes). Their apical surfaces showed apical abundant microvilli protruding into follicular lumen filled with colloid (Figs. 3a, 4a).

Group II (DM): In the diabetic group, electron microscopic sections of the thyroid glands showed follicular cells with irregular electron-dense nuclei with clumped peripheral heterochromatin. Their cytoplasm showed dilated rough endoplasmic reticulum (Figs. 3b, 4c, d), swollen degenerated mitochondria, few electron-dense granules (lysosomes), and many collagen fibers (Figs. 3b, e, 4b); other cells had marked shrunken nuclei and complete loss of cytoplasmic organelles (Fig. 3d). Moreover, some cells had flattened nuclei (Fig. 3e) or fragmented nuclei (Fig. 4b). Some follicles were lined by more than one layer of follicular cells with irregular shrunken dark nuclei with vacuolated cytoplasm (Figs. 5a, b, c). Follicular cells showed dome-shaped apical borders, and microvilli protruded into their follicular lumen (Figs. 5b, c). Dilated blood vessels were observed between follicular cells (Fig. 4e). Follicular cell division in the form of prophase, metaphase, and anaphase was also observed (Figs. 5d, e, f).

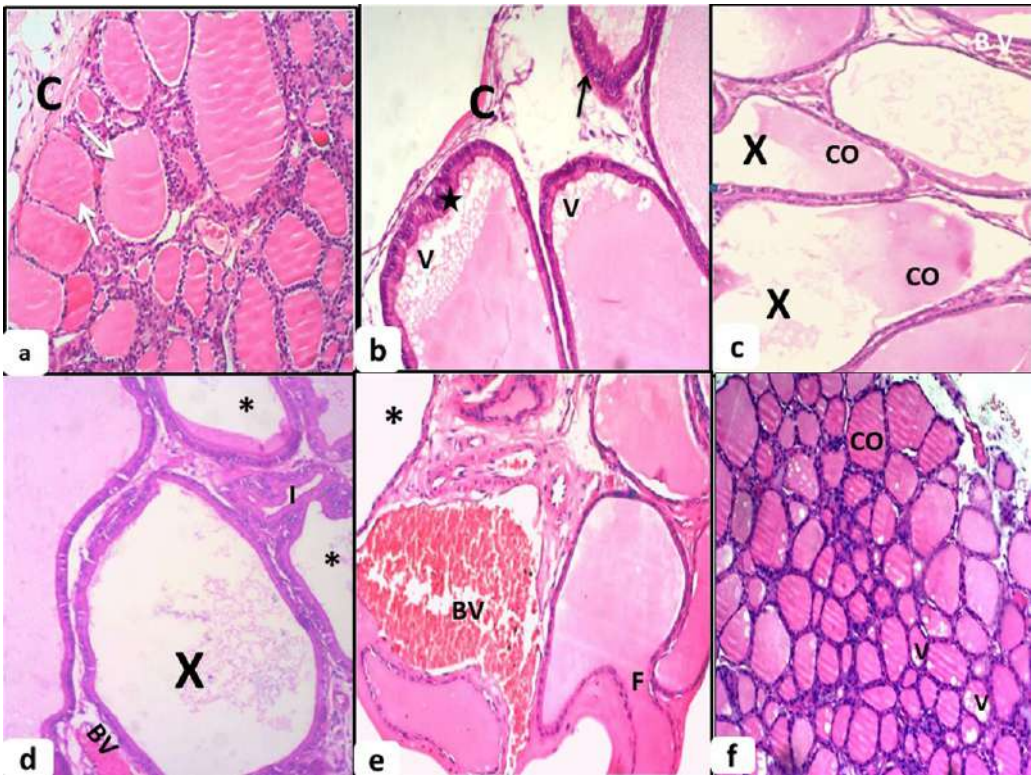

Fig. 1.- Photomicrographs of thyroid gland sections. **(a)** Control group (I) showing a thyroid gland surrounded by a connective tissue capsule (C). The parenchyma is composed of multiple relatively moderate-size rounded follicles (white arrows), and some larger follicles appear at the periphery. **(b-e)** Diabetic group (II) showing thin capsule (C). Some thyroid follicles are lined with multiple layers of follicular cells (stars), others show hyperemia between follicular epithelium (black arrows), and yet others have large dilated thyroid follicles (X) with decreased colloid (Co) and vacuolated colloid (V). Note: There are fused thyroid follicles (F), empty follicles (*), involuted follicles (I), and dilated congested blood vessels (BV). **(f)** The MSC-treated group (III) showing nearly normal thyroid follicles with colloid in their lumina (Co) but still with colloidal vacuoles (V). (H&E, x100).

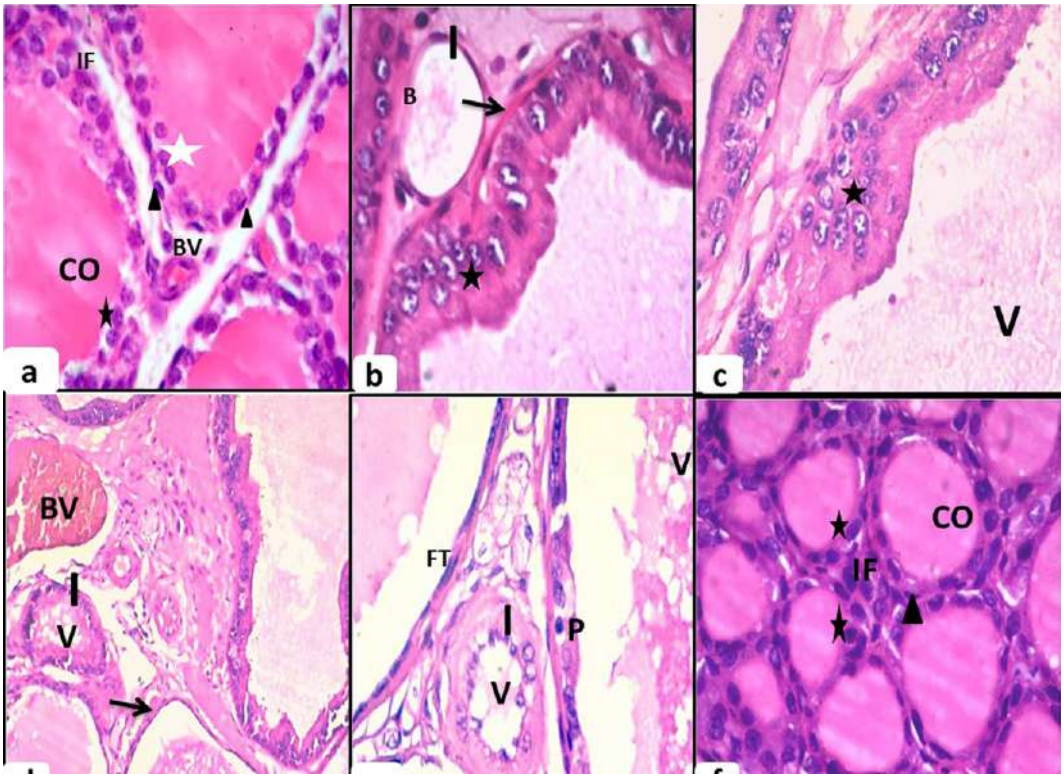

Fig. 2.- Photomicrographs of thyroid gland sections. **(a)** Control group (I) showing normal thyroid architecture with variable follicles lined with cuboidal follicular cells with rounded nuclei (white stars) and parafollicular cells with flattened nuclei (arrow heads). Follicular lumen is filled with homogenous acidophilic colloid (Co). Interfollicular cells (IF) and blood capillaries (BV) appear in connective tissue between follicles. **(b-e)** Diabetic group (II) showing part of thyroid follicles lined with multiple layers of follicular cells (black stars) and hyperemia between follicular epithelium lining (arrow). Additionally, microfollicles or involuted follicles (I) lined with flattened epithelium (FT) with areas of lost epithelium (B) and others with dark pyknotic nuclei (P) containing scanty vacuolated colloid (V). **(f)** MSC-treated group (III) showing improvement of thyroid gland's normal architecture, cuboidal follicular cells (stars) and parafollicular cells (arrow head); homogenous acidophilic colloid **(CO)** filling lumen and interfollicular cells (IF) are noted (H&E, x400).

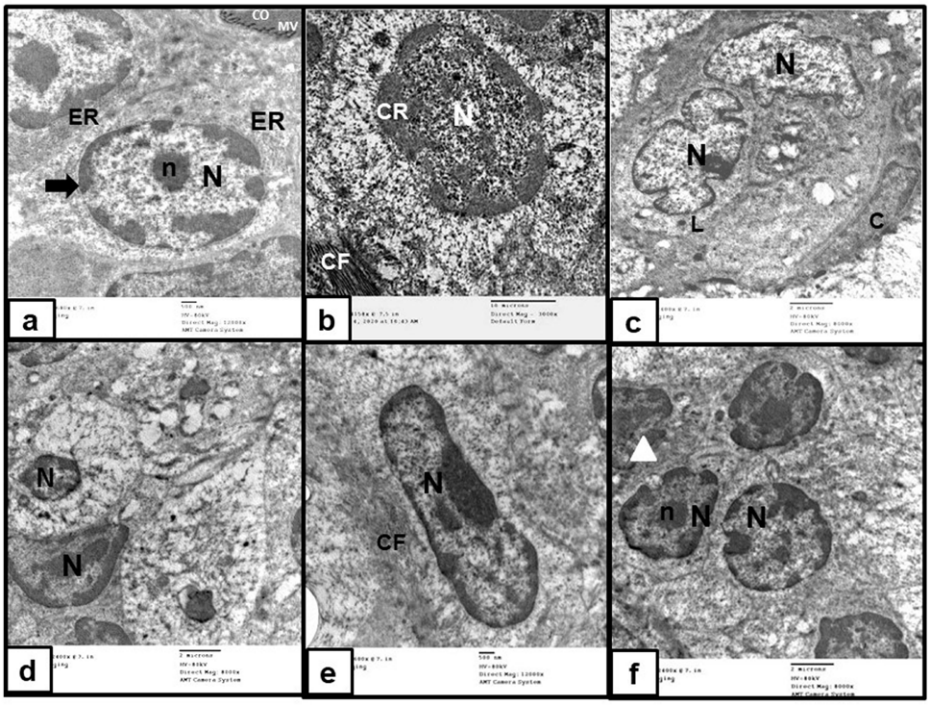

Fig. 3.- Electron micrograph of thyroid follicles of group: **(a)** Control group (I) showing cuboidal follicular cells with rounded euchromatic nuclei (N), prominent nucleoli (n), and well-defined nuclear membranes (white arrow). Their cytoplasm shows cisternae of rough endoplasmic reticulum (ER). Apical surfaces show apical abundant microvilli (MV) protruding into follicular lumen that contains colloid (CO) (x12000). **(b)** Diabetic group (II) showing follicular cell with irregular nucleus (N) with clumped peripheral heterochromatin (CR); many collagen fibers (CF) occupy its cytoplasm (x3000). **(c)** Others with irregular indented nuclei (N), cytoplasm containing lysosomes (L), surrounded by parafollicular C cell (C) (x8000). **(d)** Others with marked shrunken nuclei (N) and complete loss of cytoplasmic organelles (x8000). **(e)** Other cells with flattened nuclei (N) surrounded by many collagen fibers (CF) (x12000). **(f)** Stem cell treated group (III) showing marked amelioration of ultrastructure compared to Group II. Most follicular cells have nearly normal euchromatic nuclei (N) and prominent nucleoli (n). Their cytoplasm has nearly normal organelles, but some cells still show small irregular electron-dense nuclei (arrow heads) (x8000).

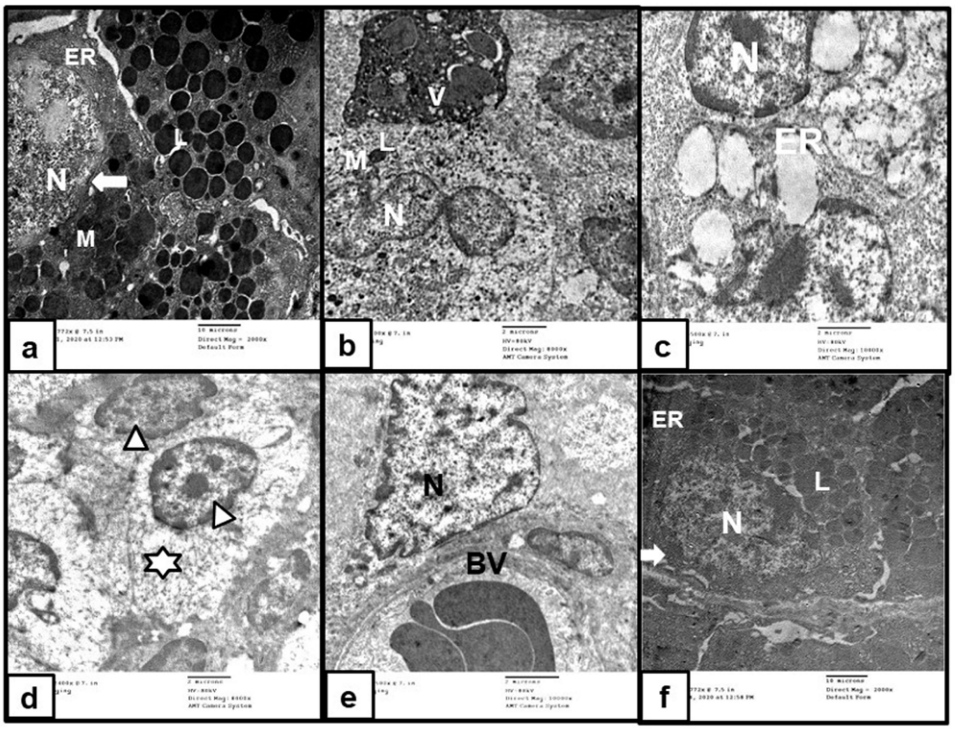

Fig. 4.- Electron micrograph of thyroid follicles: **(a)** Control group (I) showing cuboidal cells with euchromatic nuclei (N) and well-defined nuclear membranes (arrow). Their cytoplasm contains cisternae of rough endoplasmic reticulum (ER), mitochondria (M), and many lysosomes (L) (x2000). **(b-e)** Diabetic group (II) (b) showing follicular cells with fragmented nuclei (N), their cytoplasm containing degenerated mitochondria (M) and lysosomes (L). Vacuolated colloid are also noted (V) (x8000). (c) Others with dark nuclei (N) and large cytoplasmic areas occupied by dilated profiles of rough endoplasmic reticulum (ER) (x10000). (d) Irregular shrunken dark nuclei (arrow heads) surrounded by empty zones devoid of organelles (star) (x8000). (e) Dilated congested blood vessel are also observed (BV). **(f)** MSC-treated group (III) showing follicular cell with oval-to-rounded euchromatic nuclei (N) and well-defined nuclear membranes (arrow). Their cytoplasm shows cisternae of rough endoplasmic reticulum (R) and multiple lysosomes (L) (x2000).

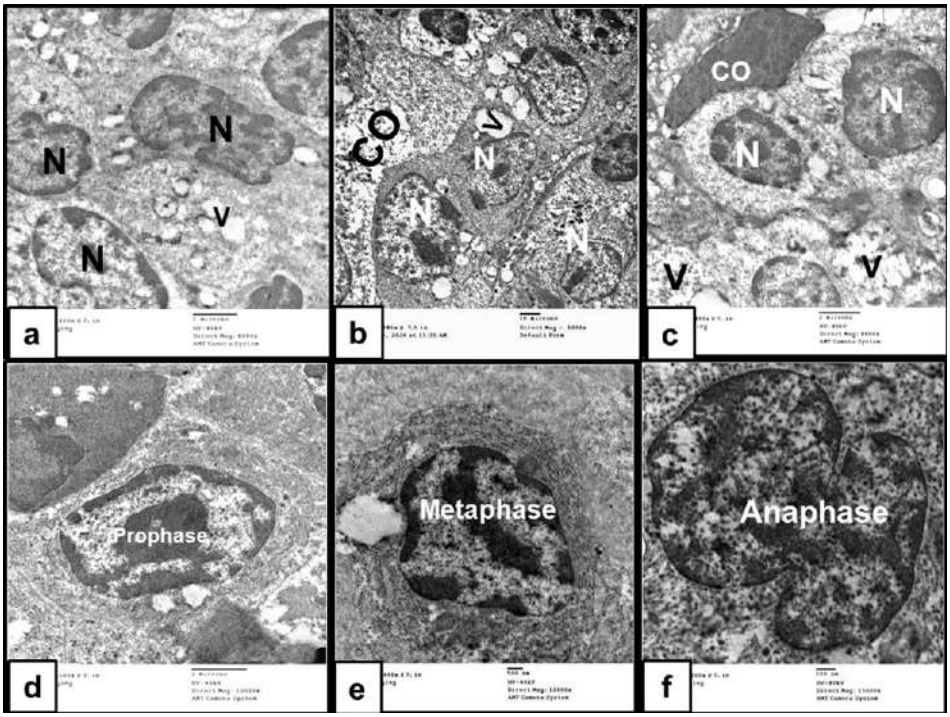

Fig. 5.- Electron micrograph of thyroid follicles of diabetic group (II): **(a-c)** Showing severely affected follicle lined by more than one layer of cells with irregular shrunken dark nuclei (N). The cells exhibit many empty zones devoid of organelles and multiple vacuoles (V). (b & c) Showing columnar follicular cells with dome-shaped apical border, with dark nuclei (N) and lost apical microvilli; the follicular lumen contains colloid (CO). **(d-f)** Showing follicular cell division in forms of prophase, metaphase, and anaphase. (a) (x8000); (b) (x1000); (c) (x8000); (d) (x10000); (e) (x12000); (f) (x15000).

Group III (DM + MSC): Electron microscopic sections of thyroid glands belonging to diabetic rats treated with mesenchymal stem cells revealed marked amelioration of ultrastructure compared to the diabetic group. Most follicular cells had nearly normal euchromatic nuclei with prominent nucleoli with well-defined nuclear membranes. Their cytoplasm had nearly normal organelles, that is, rough endoplasmic reticulum and lysosomes. However, some cells still showed small irregular electron-dense nuclei (Figs. 3f, 4f).

Biochemical results

Diabetic rats showed increased serum TSH concentration, along with reduced serum total T3, total T4, free T3, and free T4 concentrations (Table 1). In the MSC group, these thyroid hormones' serum concentrations were nearly the same as those of the control group (Table 1). However, compared with control rats, diabetic animals showed elevated sera TPO and TG antibodies that returned to normal in mesenchymal-treated rats (Table 1).

Morphometric results

Compared to the control group, the diabetic group had significantly higher follicular cell height. In the MSC-treated group, on the other hand, follicular height decreased markedly (Table 1).

DISCUSSION

Globally, DM is a remarkably common endocrine disease affecting various organs, but it is especially associated with thyroid dysfunction (Chen et al., 2004). In this study, however, serum TSH was elevated, whereas in diabetic rats, T3 and T4 levels decreased so that these biochemical markers improved toward normal in mesenchymal cell-treated rats. These results correspond to those of Da Silva et al. (2018), who reported reduction of T3 and T4 levels with an elevated TSH level in diabetic rats' thyroid function. Indeed, in many experimental studies of diabetic rats, researchers have reported that long-lasting diabetes affects thyroid function because significant hyperglycemia leads to depression of the hypothalamus pituitary-thyroid hormonal axis, in turn leading to reduction of thyrotropin-

releasing hormone (TRH) (Rondeel et al., 1992) and to decline of TSH secretion. Moreover, the thyroid response to TSH is diminished in diabetic rats (Bagchi et al., 1981). Furthermore, T3 and T4 synthesis and thyroperoxidase activity decrease (Moura et al., 1986), and deiodination of T4 to T3 in tissues diminishes (Schröder et al., 1992).

Decline in TSH could be due to augmentation of thyroid DUOX activity, crucial for thyroperoxidase and thyroid hormone synthesis (Fortunato et al., 2010), and otherwise to increased NOX4 activity in diabetic rats' thyroid glands, which participates in raising reactive oxygen species (ROS) synthesis. Accumulated ROS causes thyroid oxidative damage, thus exposing diabetic patients to thyroid hormones' disruption (Santos et al., 2013).

Increased serum TPO and TG antibodies in diabetic animals in our study has also been reported by López Medina et al., (2004), Ridha and Zubaidi (2019) and Sharifi et al. (2008). Type 1 diabetes is greatly associated with thyroid antibodies: thyroid peroxidase and thyroglobulin antibodies are predominant in type 1 DM patients and in their first-degree kin (Hanukoglu et al., 2003). These antibodies are common in uncontrolled diabetic patients and may cause elevated TSH and thus hypothyroidism by segregate thyroid hormone (Ridha and Al Zubaidi, 2019).

In this study, diabetic rats showed distorted thyroid histology with hyperemia and cellular infiltration. This finding accorded with that of Yetim et al. (2015), who reported that diabetic rats' thyroids showed large thyroid follicles with flat squamous or cuboidal lining epithelium and wide lumen. Follicles were distorted, diminishing colloid contents (Yetim et al., 2015). In another study of diabetes' effect on the thyroid, researchers found inflammation of thyroid tissue and its follicles with lymphocyte infiltration (Wright et al., 1983). This could be explained as hyperglycemia resulting from insulin deficiency causing Advanced Glycation End-Products (AGE). As reported by Hasegawa et al. (2011), these products connect receptors with cytokine release, causing inflammation, increased endothelial permeability, fibroblast proliferation, and increased extracellular matrix.

Table 1. Serum thyroid stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4) levels, free T4, T3, thyroid peroxidase antibodies (TPO Ab), thyroglobulin antibodies (TG Ab) and height of follicular cells (µm) in the studied groups. Data presented as Mean ± SD.

	Normal reference ranges	Group I (control) Mean ± SD (n=10)	Group II (DM) Mean ± SD (n=10)	Group III (DM + MSC) Mean ± SD (n=10)
TSH	0.4-4.5 uUl/ml	1.63 ± 0.74	5.8 ±0.85 ***	1.62 ± 0.98 ###
T3	65.0-205 ng/ dl	114.1 ± 41.66	48.6 ± 7.73 ***	114.5 ± 40.09 ###
T4	3.9-14 ug/dl	9.2 ± 3.259	2.63 ± 0.61 ***	9.20 ± 3.55 ###
Free T3	2-4.4 pmol/l	2.7 ±0.48	1.65 ± 0.29 ***	2.84 ± 0.64 ###
Free T4	0.9-1.7 ng/dL	1.3 ± 0.22	0.95 ± 0.26 *	1.3 ± 0.22 #
TPO Ab	0.0-0.6 IU/ml	1.28 ± 0.22	11.5 ± 3.89 ***	1.37 ± 0.23 ##
TG Ab	0.0-60 IU/ml	3.7 ± 1.7	59.6 ± 21.94 ***	26.5 ± 12.48 *
Height of follicular cells (µm)		10.4 ± 2.98	20.24 ± 7.57 *	7.9 ± 3.47 ###

*, ** or *** Denotes significant difference between DM or DM +MSC vs control, $p<0.05$, $p<0.01$ or $p<0.001$, respectively. #, ## or ### Denotes significant difference between DM and DM + MSC groups, $p<0.05$, $p<0.01$ or $p<0.001$, respectively.

Our study revealed diabetic rats' increased follicular cell height: TSH synthesized and secreted from pituitary thyrotrophs positively regulates thyroid gland activity, which is controlled by the hypothalamic TSH-releasing hormone. TSH acts on specific membrane receptors of follicular cells and stimulates activity of the sodium-iodine symporter of intracellular enzymes involved in thyroid hormones' synthesis. Therefore, when the level of serum thyroid hormones decreases, TSH feedback inhibition is attenuated, and more TSH is secreted; this supports hyperplasia and hypertrophy of thyroid cells and disturbs the thyroid gland's function (Boelaert et al., 2009; Chiamolera and Wondisford, 2009).

The present study's electron microscopic examination of diabetic rats' thyroid follicular cells showed marked dilatation of rough endoplasmic reticulum; swollen degenerated mitochondria; some, or complete, loss of cytoplasmic organelles; and irregular, shrunken, or fragmented nuclei—as also reported by (Bestetti et al., 1987). Denham et al. (1997) confirmed that mitochondrial damage was due to increased proinflammatory cytokines such as TNF-α.

Dilatation of rough endoplasmic reticulum was attributed to formation of oxygen-derived free radicals that induce lipid peroxidation and cause damage to mitochondria and cytoplasmic organelle membrane structures. Accordingly, membranes' stability and integrity are disrupted, leading to osmolality changes and hydropic cell degeneration (Guo et al., 2013). Lipid peroxidation triggers endonuclease enzymes, thus leading to nuclear degeneration (Zhang et al., 2012).

In our work, mesenchymal stem cells (MSC) improved thyroid hormone levels and ameliorated thyroid structure and ultrastructure. This result accords with an examination of MSCs' influence on thyroid function and ROS generation in type I diabetes; previous research found that MSC therapy controlled thyroid ROS generation, thyroid hormones levels remained low, and serum TSH concentrations increased. TPO activity decreased in diabetes, and MSC treatment did not normalize TPO. In our study, MSC treatment decreased serum TSH levels compared to the diabetic group, but TPO levels were normalized in the MSC-treated group, contrary to this study (da Silva et al., 2018).

Many experiments have tested MSC's efficacy for treatment of type 1 DM. Transplantation of 1×10^6 bone marrow MSCs with pancreatic islet cells to type 1 diabetic rats effectively reduced blood glucose levels to under 200 mg/dl after 15 days (Figliuzzi et al., 2009). In many additional experiments, MSC treatment has reduced blood glucose levels in the rat model of diabetes (Lin et al., 2009; Si et al., 2012). In clinical trials, MSC plays an effective role in controlling type 1 diabetes (Carlsson et al., 2015; Jiang et al., 2011). In in vivo studies, MSC is trans-differentiated into insulin-producing cells. Moreover, streptozotocin-induced diabetic rats have shown noticeable elevation of blood insulin levels (Lin et al., 2009).

MSCs' role in controlling blood glucose level can be explained by its secretion of a variety of cytokines and growth factors that help protect damaged cells from apoptosis and enable proliferation of intrinsic pancreatic progenitor cells. This paracrine effect correlates with angiogenesis, anti-inflammatory activities, cell protection, apoptosis resistance, and promotion of mitosis (El Barky et al., 2018). Moreover, MSCs can trans-differentiate into insulin-producing cells and prolong islet cells' viability and function with subsequent formation of pancreatic tissue having the capability of maintaining beta cell function. This leads to decreased blood glucose levels and increased serum insulin in both humans and animals (Dave et al., 2015; Timper et al., 2006). Thus, stem cells can replace the need for islet cell transplantation with semi- analogous effects. However, MSC transplantation is restricted due to the need for much equipment and cell viability (Luo, 2012). More studies are necessary for better understanding of MSCs' mode of action derived from bone marrow and its special effects on damaged tissues.

Further research is also required to understand more about the amount of transported MSCs and transplantation's proper timing so as to employ cells efficiently in regenerative medical applications.

CONCLUSION

Diabetes induces thyroid dysfunction and thyroid tissue injury, but MSCs can help protect against diabetes-induced tissue injury of the thyroid gland. Future studies are required to fully appraise the leverage of stem cell therapy in treatment of DM and its complications.

REFERENCES

ABDEL AZIZ MT, ATTA HM, MAHFOUZ S, FOUAD HH, ROSHDY NK, AHMED HH, RASHED LA, SABRY D, HASSOUNA AA, HASAN NM (2007) Therapeutic potential of bone marrow-derived mesenchymal stem cells on experimental liver fibrosis. *Clin Biochem*, 40(12): 893-899.

ALHADLAQ A, MAO JJ (2004) Mesenchymal stem cells: isolation and therapeutics. *Stem Cells Develop*, 13(4): 436-448.

ANANI M, NOBUHISA I, OSAWA M, IWAMA A, HARADA K, SAITO K, TAGA T (2014) Sox17 as a candidate regulator of myeloid restricted differentiation potential. *Dev Growth Differ*, 56(6): 469-479.

ANTUNES MA, ABREU SC, CRUZ FF, TEIXEIRA AC, LOPES-PACHECO M, BANDEIRA E, OLSEN PC, DIAZ BL, TAKYIA CM, FREITAS IPRG, ROCHA NN, CAPELOZZI VL, XISTO DG, WEISS DJ, MORALES MM, ROCCO PRM (2014) Effects of different mesenchymal stromal cell sources and delivery routes in experimental emphysema. *Respir Res*, 15(1): 118-118.

BAGCHI N, BROWN TR, SHIVERS B, LUCAS S, MACK RE (1981) Decreased thyroidal response to thyrotropin in diabetic mice. *Endocrinology*, 109(5): 1428- 1432.

BARKER JM (2006) Type 1 diabetes-associated autoimmunity: natural history, genetic associations, and screening. *J Clin Endocrinol Metab*, 91(4): 1210-1217.

BESTETTI GE, REYMOND MJ, PERRIN IV, KNIEL PC, LEMARCHAND-BÉRAUD T, ROSSI GL (1987) Thyroid and pituitary secretory disorders in streptozotocin-diabetic rats are associated with severe structural changes of these glands. *Virchows Archiv B*, 53(1): 69.

BOELAERT K, SYED AA, MANJI N, SHEPPARD MC, HOLDER RL, GOUGH SC, FRANKLYN JA (2009) Prediction of cure and risk of hypothyroidism in patients receiving 131I for hyperthyroidism. *Clin Endocrinol (Oxf)*, 70(1): 129-138.

BOHÁČOVÁ P, FAU-HOLÁŇ V, HOLÁŇ V (2018) Mesenchymal stem cells and type 1 diabetes treatment. [Mezenchymální kmenové buňky a léčba diabetu 1. typu.]. *Vnitr Lek*, 64(7-8): 725-728.

BOZZOLA JJ, RUSSELL LD (1999) Electron Microscopy: Principles and Techniques for Biologists (2nd ed.). London: Jones and Bartlett.

CARLSSON P-O, SCHWARCZ E, KORSGREN O, LE BLANC K (2015) Preserved □-cell function in type 1 diabetes by mesenchymal stromal cells. *Diabetes*, 64(2): 587-592.

CHEN L-B, JIANG X-B, YANG L (2004) Differentiation of rat marrow mesenchymal stem cells into pancreatic islet beta-cells. *World J Gastroenterol*, 10: 3016-3020.

CHIAMOLERA MI, WONDISFORD FE (2009) Thyrotropin-releasing hormone and the thyroid hormone feedback mechanism. *Endocrinology*, 150(3): 1091-1096.

DA SILVA DLSG, DE FREITAS ML, CAHIL GM, DE SÃO JOSÉ VS, NETO FM, CARDOSO RC, DA COSTA VMC, FORTUNATO RS, DE CARVALHO DP, MEDEI EH, FERREIRA ACF (2018) Influence of stem cell therapy on thyroid function and reactive oxygen species production in diabetic rats. *Horm Metab Res*, 50(04): 331-339.

DAS M, SUNDELL IB, KOKA PS (2013) Adult mesenchymal stem cells and their potency in the cell-based therapy. *J Stem Cells*, 8(1): 1-16.

DAVE SD, VANIKAR AV, TRIVEDI HL, THAKKAR UG, GOPAL SC, CHANDRA T (2015) Novel therapy for insulin-dependent diabetes mellitus: infusion of in vitro-generated insulin-secreting cells. *Clin Exp Med*, 15(1): 41-45.

DITTMAR M, KAHALY GJ (2003) Polyglandular autoimmune syndromes: immunogenetics and long-term follow-up. *J Clin Endocrinol Metab*, 88(7): 2983-2992.

DUNTAS LH, ORGIAZZI J, BRABANT G (2011) The interface between thyroid and diabetes mellitus. *Clin Endocrinol (Oxf)*, 75(1): 1-9.

EL BARKY A, HUSSEIN S, ALM-ELDEEN A-E, HAFEZ A, MOHAMED TM (2017) Saponins and their potential role in diabetes mellitus. *Diabetes Manag*, 7: 148-158.

EL BARKY AR, EZZ AAH, ALM-ELDEEN A-E, HUSSEIN SA, HAFEZ YA, MOHAMED TM (2018) Can stem cells ameliorate the pancreatic damage induced by streptozotocin in rats? *Canad J Diabetes*, 42(1): 61-70.

FIGLIUZZI M, CORNOLTI R, PERICO N, ROTA C, MORIGI M, REMUZZI G, REMUZZI A, BENIGNI A (2009) Bone marrow–derived mesenchymal stem cells improve islet graft function in diabetic rats. *Transplant Proc*, 41(5): 1797-1800.

FORTUNATO RS, LIMA DE SOUZA EC, HASSANI RA-E, BOUFRAQECH M, WEYEMI U, TALBOT M, LAGENTE-CHEVALLIER O, DE CARVALHO DP, BIDART J-M, SCHLUMBERGER M, DUPUY C (2010) Functional consequences of dual oxidase-thyroperoxidase interaction at the plasma membrane. *J Clin Endocrinol Metab*, 95(12): 5403-5411.

GONZÁLEZ GC, CAPEL I, RODRÍGUEZ-ESPINOSA J, MAURICIO D, DE LEIVA A, PÉREZ A (2007) Thyroid autoimmunity at onset of type 1 diabetes as a predictor of thyroid dysfunction. *Diabetes Care*, 30(6): 1611-1612.

GUO C, SUN L, CHEN X, ZHANG D (2013) Oxidative stress, mitochondrial damage and neurodegenerative diseases. *Neural Regen Res*, 8(21): 2003-2014.

HANUKOGLU A, MIZRACHI A, DALAL I, ADMONI O, RAKOVER Y, BISTRITZER Z, LEVINE A, SOMEKH E, LEHMANN D, TUVAL M, BOAZ M, GOLANDER A (2003) Extrapancreatic autoimmune manifestations in type 1 diabetes patients and their first-degree relatives. *Diabetes Care*, 26(4): 1235-1240.

JIANG R, HAN Z, ZHUO G, QU X, LI X, WANG X, SHAO Y, YANG S, HAN ZC (2011) Transplantation of placenta-derived mesenchymal stem cells in type 2 diabetes: a pilot study. *Front Med*, 5(1): 94-100.

KADIYALA R, PETER R, OKOSIEME OE (2010) Thyroid dysfunction in patients with diabetes: clinical implications and screening strategies. *Int J Clin Pract*, 64(8): 1130-1139.

KIERNAN JA (2015) Histological and Histochemical Methods: Theory and Practice (5th ed.). Scion Publishing Ltd., London.

LEONG KS, WALLYMAHMED M, WILDING J, MACFARLANE I (1999) Clinical presentation of thyroid dysfunction and Addison's disease in young adults with type 1 diabetes. *Postgrad Med J*, 75(886): 467-470.

LIN P, CHEN L, YANG N, SUN Y, XU YX (2009) Evaluation of stem cell differentiation in diabetic rats transplanted with bone marrow mesenchymal stem cells. *Transplant Proc*, 41(5): 1891-1893.

LÓPEZ MEDINA JA, LÓPEZ-JURADO ROMERO DE LA CRUZ R, DELGADO GARCÍA A, ESPIGARES MARTÍN R, BARRIONUEVO PORRAS JL, ORTEGA MARTOS L (2004) Beta-cell, thyroid and celiac autoimmunity in children with type 1 diabetes. [Autoinmunidad pancreática, tiroidea y relacionada con la celiaquía en niños con diabetes mellitus tipo 1]. *An Pediatr (Barc)*, 61(4): 320-325.

LUO L-G (2012) Islet transplantation challenge–human islet longevity: A potential solution from bone marrow cells. *J Bioanal Biomed*, 4(4): 1-5.

MARATOU E, HADJIDAKIS DJ, KOLLIAS A, TSEGKA K, PEPPA M, ALEVIZAKI M, MITROU P, LAMBADIARI V, BOUTATI E, NIKZAS D, TOUNTAS N, ECONOMOPOULOS T, RAPTIS SA, DIMITRIADIS G (2009) Studies of insulin resistance in patients with clinical and subclinical hypothyroidism. *Eur J Endocrinol*, 160(5): 785-790.

MOHN A, DI MICHELE S, DI LUZIO R, TUMINI S, CHIARELLI F (2002) The effect of subclinical hypothyroidism on metabolic control in children and adolescents with Type 1 diabetes mellitus. *Diabet Med*, 19(1): 70-73.

MOURA EG, PAZOS CC, ROSENTHAL D (1986) Insulin deficiency impairs thyroid peroxidase activity: a study in experimental diabetes mellitus. In: Medeiros-Neto G, Gaitan E (eds.). *Frontiers in Thyroidology*, Vol 1 (pp 627-630). Springer US, Boston, MA.

NILGÜN GÜRSOY ET, ERDINÇ E, ŞAZI İ, AYHAN ARıNıK (1999) The relationship between the glycemic control and the hypothalamus-pituitary-thyroid axis in diabetic patients. *Turkish J Endocrinol Metab*, 4: 163-168.

PILIA S, CASINI MR, CAMBULI VM, IBBAA, CIVOLANI P, ZAVATTARI P, INCANI M, MOSSA P, BARONI MG, MARIOTTI S, LOCHE S (2011) Prevalence of Type 1 diabetes autoantibodies (GAD and IA2) in Sardinian children and adolescents with autoimmune thyroiditis. *Diabet Med*, 28(8): 896-899.

RIDHA M, AL ZUBAIDI M (2019) Thyroid auto immune antibodies in children with Type-I Diabetes mellitus in relation to diabetes control. *Pak J Med Sci*, 35(4): 969-973.

ROCHEFORT GY, VAUDIN P, BONNET N, PAGES J-C, DOMENECH J, CHARBORD P, EDER V (2005) Influence of hypoxia on the domiciliation of mesenchymal stem cells after infusion into rats: possibilities of targeting pulmonary artery remodeling via cells therapies? *Respir Res*, 6(1): 125.

RONDEEL JM, DE GREEF WJ, HEIDE R, VISSER TJ (1992) Hypothalamo-hypophysial-thyroid axis in streptozotocin-induced diabetes. *Endocrinology*, 130(1): 216- 220.

SANTOS MCS, LOUZADA RAN, SOUZA ECL, FORTUNATO RS, VASCONCELOS AL, SOUZA KLA, CASTRO JPSW, CARVALHO DP, FERREIRA ACF (2013) Diabetes mellitus increases reactive oxygen species production in the thyroid of male rats. *Endocrinology*, 154(3): 1361-1372.

SCHRÖDER-VAN DER ELST JP, VAN DER HEIDE D (1992) Effects of streptozocin-induced diabetes and food restriction on quantities and source of T4 and T3 in rat tissues. *Diabetes*, 41(2): 147.

SHARIFI F, GHASEMI L, MOUSAVINASAB N (2008) Thyroid function and anti-thyroid antibodies in Iranian patients with type 1 diabetes mellitus: influences of age and sex. *Iran J Allergy Asthma Immunol*, 7(1): 31-36.

SI Y, ZHAO Y, HAO H, LIU J, GUO Y, MU Y. SHEN J, CHENG Y, FU X, HANW (2012) Infusion of mesenchymal stem cells ameliorates hyperglycemia in type 2 diabetic rats: identification of a novel role in improving insulin sensitivity. *Diabetes*, 61(6): 1616-1625.

TIMPER K, SEBOEK D, EBERHARDT M, LINSCHEID P, CHRIST-CRAIN M, KELLER U, MÜLLER B, ZULEWSKI H (2006) Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. *Biochem Biophys Res Commun*, 341(4): 1135-1140.

WEETMAN AP, MCGREGOR AM (1994) Autoimmune thyroid disease: further developments in our understanding. *Endocr Rev*, 15(6): 788-830.

WRIGHT JR, SENHAUSER DA, YATES AJ, SHARMA HM, THIBERT P (1983) Spontaneous thyroiditis in BB Wistar diabetic rats. *Vet Pathol*, 20(5): 522-530.

YETIM Z, UNAL D, KARAMESE S, MERCANTEPE T, SELLI J, POLAT E, BÜYÜK B (2015) Effects of menopause and diabetes on the rat thyroid gland: a histopathological and stereological examining. *J Interdiscip Histopathol*, 3.

ZHANG Y, CHEN Y, SUN L, LIANG J, GUO Z, XU L (2012) Protein phosphatases 2A as well as reactive oxygen species involved in tributyltin-induced apoptosis in mouse livers. *Environ Toxicol*, 29(2): 234-242.