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Effect of thyroid dysfunction on apoptosis markers: Caspase-3 and Bcl-2 among Iraqi patients

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Abstract

Thyroid hormones are considered vital for cellular life history starting from its proliferation, differentiation, and ending up with its apoptosis. However, there are very limited human studies concerning the effect of thyroid dysfunction on the levels of apoptosis markers. Therefore, the aim of this cross-sectional study was to examine the effect of thyroid dysfunction (hyperthyroidism and hypothyroidism) on the levels of serum caspase-3, B-cell lymphoma (Bcl-2) and thyroid stimulating hormone (TSH) among patients in Babylon, Iraq. The study included 52 male patients (aged 25-50 years) with thyroid dysfunction, visited the Endocrinology center in Al-imam al-Sadiq hospital located in Babylon province, Iraq during the period from November 2023 to May 2024. Patients were split into two groups (patients with hypothyroidism, N=26; patients with hyperthyroidism, N=26), in addition to 26 subjects without thyroid dysfunction as a control group. Levels of serum caspase-3, Bcl-2, and TSH were measured in all study subjects. The results indicated that levels of serum caspase-3 were significantly increased in both patients' groups compared to the control group (p<0.001), while serum Bcl-2 was significantly increased in patients with hypothyroidism compared to other study groups (p<0.001). Levels of TSH were significantly greater among patients with hypothyroidism comparing to other groups (p<0.001). It can be concluded that patients with hyperthyroidism and hypothyroidism enhance apoptosis through activation of caspase-3 specifically.

Keywords: Apoptosis, B-cell lymphoma (Bcl-2), Caspase-3, Thyroid dysfunction, Iraqi patients, SDG3.

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Introduction

Disorders of the thyroid gland, a little gland shaped like a butterfly situated at the base of the neck, are medical illnesses that impair thyroid function. Thyroid disorders can be ranked into two features: hyperthyroidism which is recognized by gland over-activity and hypothyroidism that is recognized by a gland underactivity. Thyroid hormones are necessary for proper growth and energy metabolism since they practically affect all nucleated cells. Although thyroid dysfunction is frequently seen, easily recognized, and treatable, it can have serious negative repercussions if left undetected or mistreated. Cases of severe

thyroid dysfunction still happen occasionally, despite a greater knowledge of thyroid disease and the availability of sensitive laboratory assays for the assessment of thyroid hormones.[1] One of the most prevalent endocrine disorders is hyperthyroidism, which affects 3% of women and 0~3% of men. Despite being benign, hyperthyroidism can lead to major side effects like heart failure, osteoporosis, or atrial fibrillation and the most common complaints are heat intolerance, perspiration, palpitations, anxiety, and exhaustion.[2,3] Weight loss, tremors in the extremities, and tachycardia are the physical symptoms of thyrotoxicosis.[4]

Subclinical hypothyroidism is defined by biochemical means, i.e., high blood levels of thyroid stimulating hormone (TSH) in conjunction with normal serum levels of free thyroid hormones. Patients with subclinical hypothyroidism, in contrast to those with overt hypothyroidism, frequently go undiagnosed during normal medical exams and may not exhibit any clinical signs of hypothyroidism and determining this "subclinical" status is difficult.[5]

Thyroid hormones are considered vital for cellular life history starting from its proliferation, differentiation, and ending up with its apoptosis,[6] the latter is an outcome between pro- and anti-apoptotic molecules. [7,8] Apoptosis is essential in regulating different sorts of diseases. Schizophrenia, for example, is a neural disease that may be caused by an imbalance between pro- and anti-apoptotic factors.[9] Previous research also indicated that thyroid hormone could change the degree of apoptosis among the differentiating erythrocytic progenitor cells.[10]

B-cell lymphoma (Bcl-2) is the family of proteins that represents crucial regulators of cellular survival and apoptosis. This family involves Bcl-2, and Bcl-extra-large (xL) which serve as anti-apoptotic factors and Bax, Bcl-xS, Bad, and Bak serve as proapoptotic factors. [11-13]

Caspase-3 is one of the proteolytic enzymes that belong to the family of both amino acids (cysteine and aspartate). It is mainly involved in the cellular apoptotic pathway. [14-16] The activation of caspase-3 has usually occurred through two routes, the extrinsic route is firstly initiated by activation of cell death receptors like Fas, FasL, and tumor necrosis factor alpha (TNFα) while the intrinsic route is activated in response to cell damage by releasing different mitochondrial proteins like cytochrome c, activating factor-1 and caspase-9. [13,17,18,19]

To the best of our knowledge, we are not aware of a previous human study concerning the potential link between markers of apoptosis (caspase 3 and Bcl-2) and thyroid dysfunction in Iraq. Therefore, the current study aimed to examine the association between thyroid dysfunction (both hyperthyroidism and hypothyroidism) and serum markers of apoptosis (caspase 3 and Bcl-2) among patients in the Babylon province, Iraq.

Subjects and Methods

The current study included 52 male patients (aged 25-50 years) with thyroid dysfunction who visited the Endocrinology center in Al-imam al-Sadiq hospital located in Babylon province, Iraq. Based on the diagnosis of an endocrinologist, patients were split into two groups (patients with hypothyroidism, N=26; patients with hyperthyroidism, N=26). Another 26 normal people were randomly selected as a control group. Formal and written consent was taken from all participants in the current study.

A blood sample (5 ml) was collected from each study subject in plain tubes using a venipuncture method. The serum was separated by a centrifugation for 10 minutes at 1107 g. Then serum was then collected and kept frozen at -2^{o}C until used for markers analysis.

Thyroid Stimulating Hormone (TSH) level

TSH levels were measured by commercial kits (the mini VIDAS Kits, TSH-Biomerieux, France), according to the manufacturer's instructions.

Caspase-3 and Bcl-2 levels

Bcl-2 and caspase-3 were identified by the quantitative sandwich enzyme linked immunosorbent assay methods using commercial kits (Elabscience, China), according to the manufacturer's instructions.

Statistical Analysis

The Minitab software (Version 17) was used for data analysis. The normality of data was

examined and transformation applied for skewed data. The significant changes in the measured markers among groups were examined by applying a one-way ANOVA test. The correlation in the measured markers was tested using the Pearson correlation. The results were presented as a mean and standard error (SE). A *p*-value ≤0.05 was considered significant.

Results

The results indicated that the levels of serum caspase-3 were significantly increased in both patients with hypothyroidism and hyper-thyroidism compared to the levels observed in the control group (p<0.001) (Figure 1).

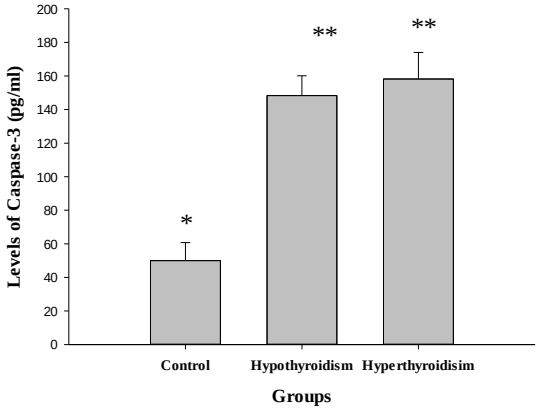

Figure 1. Levels of Caspase-3 (Mean± SE; pg/ml) in the patient and control groups. Groups that do not share stars are significantly different.

Levels of serum Bcl-2 were significantly increased in patients with hypothyroidism compared to the other groups (p<0.001). However, there was no difference between patients with hyperthyroidism and the control group (Figure 2).

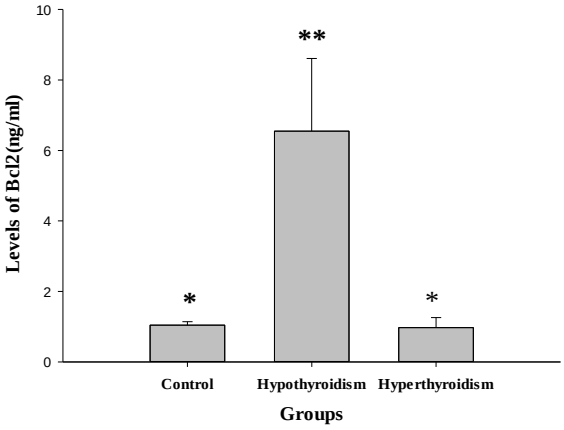

Figure 2. Levels of Bcl-2 (Mean± SE; ng/ml) in the control and patient groups. Groups that do not share stars are significantly different.

Levels of serum TSH were significantly greater among patients with hypothyroidism compared to the other groups. However, there was no difference in serum TSH levels between the other groups (Figure 3). No significant correlations were found in the measured markers among the groups studied.

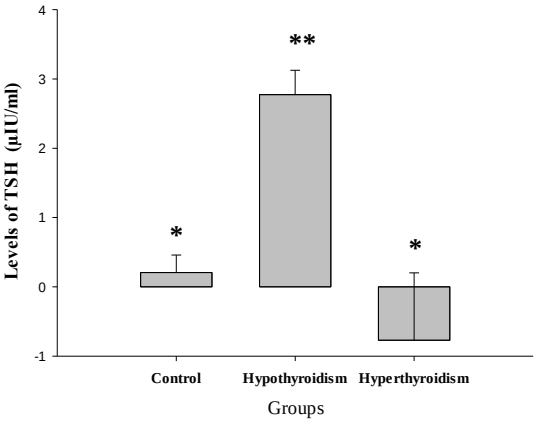

Figure 3. Levels of transformed TSH (Mean± SE; µIU/ml) in the control and patient groups. Groups that do not share stars are significantly different.

Discussion

The current study intended to measure the apoptosis markers (caspase-3 and Bcl-2) with regard to their association with thyroid dysfunction. Our results indicated that patients with thyroid dysfunction in both hyperthyroidism and hypothyroidism had significantly more apoptosis as measured by caspase-3 compared to the control group. This finding suggests that caspase-3 is involved in thyroid dysfunction induced apoptosis. Caspase-3 is one of the proteolytic enzymes that belong to the family of both amino acids (cysteine and aspartate). It is mainly involved in the cellular apoptotic pathway.[14-16] Caspase-3 and other types of caspases (including caspase 7, 8, 9, or 10) are ranked into pro-apoptotic molecules that mainly participated in the transduction of cellular death signaling.[19] Activation of caspase-3 can be achieved through two pathways. The intrinsic pathway, which is commonly known as mitochondrial pathway, occurs in the response of internal sources of stimulus like nuclear damage.[20] In this regard, several types of mitochondrial proteins are released from inner space of mitochondria of type 2 cells into cell cytoplasm.[21] In addition, Bcl-2 family members can also participate in this pathway via the permeabilization of mitochondrial outer membrane.[22] The extrinsic pathway, which known as a mitochondrial independent pathway, is adopted by type 1 cells in which the activation of caspase 3 caused by releasing high amounts of caspase 8.[19] The members of Bcl-2 family are not effective in this extrinsic pathway.[23] Although there is no previous human study to compare our results with, few experimental research were done on animal models, showed that the levels of caspase-3 were significantly elevated among rats with thyroid dysfunction. [13,24,25] It was concluded from these studies that both significantly higher and lower levels of thyroid hormones (hyperthyroidism and hypothyroidism, respectively) induced releasing different cellular proteins that activated caspase-3, and ultimately increased apoptosis.

Regarding the levels of Bcl-2, our results indicated the patients with hypothyroidism had significantly greater levels of Bcl-2 compared to other groups (hyperthyroidism and control), however, the difference was insignificant between hyperthyroidism and controls. In consistent with our results, levels of serum Bcl-2 among Iraqi patients with hyperthyroidism were not significantly different from those in the healthy control group.[26] Another observational study on human being indicated that the levels of mRNA Bcl-2 were significantly increased among Ukraine patients with hypothyroidism compared to the control group.[27] Data from experimental animals also showed that rats with hyperthyroidism did not significantly change the levels of liver Bcl-2 compared to the control group.[24] Contrary to the current findings, levels of Bcl-2 protein measured by immunohistochemistry in rat's spleen were significantly lower in rats with hyperthyroidism and hypothyroidism compared to the healthy control rats.[23] The results from another animal study also showed that the levels of Bcl-2 measured by western blot were significantly reduced in the cortex of cerebellum of developing hypothyroid pup rats.[13]

Despite that the exact reason for why Bcl-2 was not significantly decreased among patients in the current study is obscure, some of the potential explanations can be adopted. Unchanged levels of Bcl-2 among hyperthyroidism and control groups paralleled with significant increase in the levels of caspase-3 among groups with thyroid dysfunction could highlight the importance of selection-based apoptosis. It is well known that Bcl-2 is involved in the mitochondrial apoptosis pathway while the caspase-3 is involved in both dependent and independent mitochondria apoptosis pathways. This suggests that the considerable disturbances of thyroid hormones in both (hyperthyroidism and hypothyroidism) could directly act on the activation of caspase-3 specific rather than Bcl-2 and leading to apoptosis.[28] Another explanation could be due to the variations of Bcl-2 detection methods and the tissue being used for measuring of Bcl-2, as the previous studies and the current one have used different methods and tissues.

In conclusion, the current study has shed light on the apoptosis associated with human thyroid dysfunction. The outcomes of this study

revealed that patients with hyperthyroidism and hypothyroidism enhance apoptosis through activation of caspase-3 specifically.

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Author Contributions

AHA and ATA; designed the study. RKM and KHM; collected the samples and did the lab work. AHA; analyzed the results and wrote the manuscript. The manuscript was then approved by all authors.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Ethics Committee for Human Research of the College of Science, Babylon University (Project No, B551, dated September 2023).

Informed consent

A signed consent form was obtained from each study participant.

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