Eurasian Journal of Molecular and Biochemical Sciences

https://dergi.erzurum.edu.tr/ejmbs

Experimental hypothyroidism increases oxidative stress and apoptosis in ovary of rats

Nevra Aydemir CELEP[1,2*], Semin GEDİKLİ[1], Seçil Nazife PARLAK[3], Elvin ALİYEV[4]

[*1]*Atatürk University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Erzurum/Turkey*

[2]*Atatürk University, Faculty of Medicine, Department of Pharmacology, Erzurum/Turkey*

[3]*Ağrı İbrahim Çeçen University, Faculty Of Medıcıne, Department f Hıstology And Embryology, Ağrı/Turkey*

[4]*Lankaran State University, Department of Biology and Medical Knowledge, Lankaran/Azerbaycan*

Cite: *Celep A. N, Gedikli S,Parlak N.S, Aliyev E.Experimental hypothyroidism increases oxidative stress and apoptosis in ovary of rats. Eurasian Mol Biochem Sci 2023;2(2): 21-29.*

Received: 04 October 2023, Accepted: 25 October 2023

Abstract

This study aimed to investigate hypothyroidism's effects in experimental hypothyroidism-induced female rats. Materials and Methods: To induce hypothyroidism in rats, 0.05% 6-propyl-2-thiouracil (PTU) was given orally to the hypothyroid (HT) and L-thyroxine (LT) groups with their drinking water for eight weeks. In the LT group, 0.8 µg/100 g L-thyroxine was provided by subcutaneous for ten days of the experiment. Results: In the histopathological evaluation, hypothyroidism induced severe dilation in vessels atretic and irregular follicles and damaged the germinal epithelium. These pathologies were decreased in the LT group. In the biochemical analysis, SOD activity, GSH activity, and MDA level were lower in the HT group than Control and LT groups. In the relative protein analysis, Caspase-3, mTOR, P2X7R, NfkB, IL-1β, and TNF-α levels were lower in the Control and LT groups, but the relative protein expression of Caspase-3, mTOR, P2X7R, NfkB, IL-1β, and TNF-α was found as higher in the HT group. The protein expression of Bcl-2 level was higher in the Control and LT groups. Conclusion: The results showed that hypothyroidism causes ovarian tissue injuries —the histopathological and biochemical changes induced by Hypothyroidism in the experimental rat model were ameliorated with L-thyroxine treatment.

Keywords: Hypothyroidism, ovarian, apoptosis, oxidative stress, rat

Introduction

Thyroid hormone (TH) is an essential factor in regulati ng many biological processes, including growth, differe ntiation, metabolism, embryo development, and femal e reproduction (1-3). Altered TH levels are associated with impaired folliculogenesis, reduced fertilization rate, and reduced embryo quality in severe cases (4-7). Proper ovarian follicular development depends on the delicate balance between pituitary gonadotropic hormones and locally and peripherally produced factors. These factors include transforming growth factor beta, insulin-like growth factor, leptin, and adiponectin factors. (8-12). Active T3 (3,3', 5-

[*]**Correspondence**: Nevra Aydemir CELEP
Atatürk University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Erzurum/Turkey
E-mail: nevraaydemir@hotmail.com
Tel: +90 0541 471 8874

This article licensed under Creative Commons Attribution Non-Commercial 4.0 International (CC BY-NC 4.0)

triiodothyronine) functions as a peripheral factor by enhancing the effect of follicle-stimulating hormone (FSH), a gonadotropic hormone, on follicle growth (13). FSH and T3 exert their effects on follicular development by stimulating granulosa cell proliferation and inhibiting apoptosis through the activation of the PI3K/Akt pathway (14).

Hypothyroidism is a common health condition (15). Its prevalence increases with age and is also 5-10 times more common in women than in men (16). Although hyperthyroidism has a relatively high incidence in women, there are only a few studies that address the effects of elevated TH (thyroid hormone) concentrations in neonatal female reproductive system development, despite its overall prevalence being around 1% (17). For example, Soliman and Reineke have shown that mild thyroid stimulation in young female mice delayed the age of vaginal opening and accelerated the onset of estrus cycles compared to healthy thyroid control group. The ovaries of these hyperthyroid mice contain multiple growing follicles and corpus luteum (18).

The effects of thyroid hormone (TH) deficiency on ovarian follicular development are typically studied in animal models using propylthiouracil (PTU) as a goitrogen. However, long-term PTU treatment has been associated with various adverse effects, including agranulocytosis and hepatotoxicity (19). The administration of PTU during pregnancy often leads to preterm birth. As a result, treatment is typically initiated at the time of birth and discontinued before the offspring reach adolescence (20,21). These studies demonstrate that neonatal hypothyroidism in rats leads to a delay or even complete inhibition of vaginal opening and sexual maturation. It also results in smaller ovaries with fewer antral follicles and more atretic follicles (20).

Thyroid hormone can act as an oxidant and induce DNA damage (22). Reactive oxygen species (ROS) are continuously generated in cells as a result of both biochemical reactions and external factors (23). It is increasingly understood that ROS, when present in physiological amounts, play an important signaling role in cells (24). Indeed, regulated production of ROS is necessary for the initiation of the first meiotic division of the primary oocyte (25). This indicates that ROS serves as an important mediator during ovulation (24,26). Reducing ROS production is necessary during the secondary meiotic division, demonstrating the need for a balance between ROS production and cellular ROS defense. Disruption of cellular antioxidant defenses, especially with mitochondria playing a significant role as a source, can lead to oxidative stress and associated pathologies (27).

It is not clear that chronic hypothyroidism affects the expression of proteins associated with ovarian redox balance and cell survival. The reduced plasma TH concentration affects the ovarian follicular reserve and ovulation. We believe that there is a relationship between oxidative stress and ovarian reserve. To evaluate this hypothesis, the relative expression of Bcl-2, Caspase-3, NF-κB, mTOR, P2X7R, IL-1, TNF-alpha, mTOR, and oxidative parameters will be examined at the end of the experiment.

Materials and Methods

Animal Procedure: Ethical approval for the study was obtained from the Ataturk University Animal Experiments Local Ethics Committee. A total of 36 twelve-week-old Sprague-Dawley female rats, weighing between 200 and 250 grams, were procured from the Ataturk University Experimental Research and Application Center. Before commencing the experiments, the animals were acclimated to their environment and then divided into groups. Throughout the study, the rats were provided with water and pellet food ad libitum. The animals were kept in laboratory conditions with a 12-hour light/dark cycle, at a temperature of 21-23°C, and a relative humidity of 45-50% during the experiments.

Animal Grouping: For the experiment, three groups were formed, each consisting of 12 rats. The initial weight measurements of the animals were conducted on the day the experiment commenced, and subsequently, they were weighed at consistent weekly intervals throughout the study.

Experimental groups: No drug treatment was administered to the Control group. In the Hypothyroid (HT) and Treatment groups (LT), a 0.05% PTU (6-propyl-2-thiouracil) (Sigma, P3755) solution was freshly prepared daily and administered by mixing it into the drinking water for eight weeks to induce a hypothyroid model (28,29). Treatment Group (LT): The rats were administered 0.05% PTU, freshly prepared daily, by mixing it into their drinking water for a duration of eight weeks. Once the hypothyroidism model was successfully induced, the rats received a subcutaneous daily dose of 0.8µg/100g L-thyroxine (Santa Cruz, sc-207813A) for ten consecutive days (28).

Surgical Procedures: To conclude the experiment, the animals were intraperitoneally anesthetized with 100 mg/kg ketamine and 15 mg/kg xylazine. They were euthanized by decapitation, and ovarian tissues were then extracted. Tissues designated for histopathological examination were promptly immersed in a 10% formaldehyde solution.

Histopathological Analysis: The obtained ovarian tissues were fixed in 10% naturally buffered formaldehyde solution for 24 hours, then passed through a graded alcohol series, cleaned with xylene, and embedded in paraffin blocks. Blocks were cut to a thickness of 5 µm and stained with Crossman Modified Triple staining for histopathological evaluation. The slides were evaluated by photography with a Trinocular Microscope (Zeiss, Axio1, German).

Biochemical Analyzes in Ovarian Tissues: After the experiment was terminated, ovarian tissue samples taken from rats in each group (n: 6) were ground using the TissueLyser II grinding set (Qiagen, Hilden, Germany). 50 mg of each of the tissue samples ground into 2mL Eppendorf tubes will be taken and homogenized in 1 mL of phosphate buffer (100 mM, pH 7.0) using the TissueLyser II grinding set (Qiagen, Hilden, Germany). After the homogenization process, the samples will be centrifuged, and superoxide dismutase (SOD) activity, lipid peroxidation levels (LPO), and glutathione (GSH) levels will be measured from the supernatant sections.

Measurement of Superoxide Dismutase Enzyme Activity in Ovarian Tissues: Superoxide dismutase (SOD) activity in ovarian tissue tissues was determined by Sun et al (30). According to the protocol of this experiment, 50 mg of tissue was homogenized with 1 mL phosphate buffer (0.1 mM, pH 3) and then centrifuged at 18000xg for 60 min. After pipetting 500 µL supernatant and 2450 µL measurement mixture (0.3 mM xanthine, 0.6 mM EDTA, 150 µM NBT, 0.4 M Na_2CO_3, 1.2 g/L BSA) and 50 µl xanthine oxidase into the tubes prepared for each sample, from the supernatant obtained after centrifugation. Then it was incubated for 20 minutes. At the end of the incubation period, 100 µL $CuCl_2$ (0.8 mM) was pipetted to stop the reaction. The absorbance of the formazone dye formed due to the reaction was measured at 560 nm. All samples were studied in three replicates, and the results were calculated from the prepared SOD standard chart and expressed as nM/minute/mg tissue.

Measurement of Total Glutathione (GSH) Levels in Ovarian Tissues: Total glutathione level in ovarian tissues was determined using a modified version of the method developed by Sedlak and Lindsay (1968)(31). According to the protocol of this experiment, 50 mg of tissue was homogenized with 20 mM EDTA, 1 mL Tris-HCl (50 mM, pH 7.4), and then centrifuged at 12000xg for 4 min. From the supernatant obtained after centrifugation, 500 µL supernatant, 1500 µL Tris-HCl (200 mM, pH 8.2), 100

µL DTNB, and 7900 µL methanol were pipetted into the capped tubes prepared for each sample. After pipetting, they were incubated at 37 °C for 30 minutes, and then their absorbance was measured at 412 nm. All samples were studied in three replicates, and the results were calculated from the prepared GSH standard graph and expressed as nmol/g tissue.

Measurement of Lipid Peroxidation (LPO) Levels in Ovarian Tissues: Lipid peroxidation level in ovarian tissues was determined by Ohkawa et al(32). According to the protocol of this experiment, 50 mg of tissue will be homogenized with 4.5 mL KCl (10%). After the homogenization process, it was centrifuged at 5000xg for 20 minutes. From the supernatant obtained after centrifugation, 250 µL supernatant, 100 µL sodium lauryl sulfate (8%), 750 µL acetic acid (20%), 750 µL TBA (0.8%), and 150 µL distilled water were added into the capped tubes prepared for each sample. Pipetted. Following the pipetting process, it was mixed with the help of a vortex and incubated in a shaking water bath at 100 °C for 60 minutes. At the end of the incubation period, the sample tubes were cooled to room temperature under tap water. After cooling, 2.5 mL of n-butanol: pyridine (15:1) was pipetted and then centrifuged at 4000xg for 30 min, and then the absorbance was measured at 532 nm. All samples were studied in three replicates, and the results were calculated from the prepared MDA standard chart and expressed as nmol/g tissue.

Western Blot Analysis: The tissue samples were homogenized using a tissue lyser device (Qiagen, USA) at 30 Hz for 30 sec to the extraction of proteins for western blot analysis after being weighted and crushed in nitrogen gas, treated with radioimmunoprecipitation (RIPA buffer, Ecotech Bio, Turkey), supplemented with protease and phosphatase inhibitors, and treated with protease and phosphatase inhibitors. The relative protein expressions of Bcl-2, Caspase-3, P2X7R, mTOR, IL-1β, and TNF-α were then determined by quantifying the proteins using a commercial kit (Pierce BCA, Thermo Sci., USA). The protein loaded membranes were incubated at 4 °C overnight with the appropriate primary antibodies ((IL-1β (sc-52012, Santa Cruz), TNF-α (sc-52746, Santa Cruz), P2X7R antibody (11144-1-AP, Proteintech), Caspase-3 (sc-56053, Santa Cruz), Bcl-2 (sc-7382, Santa Cruz), NfkB-p65 (AF5006, Affinity biotech) and mTOR (AF6308, Affinity Biotech), and Beta-actin (sc-47778, Santa Cruz). secondary antibody. After primary antibody incubation, the PVDF membranes were washed with TBST and then incubated for 90 minutes at room temperature with the second antibody (Santa Cruz, sc-2004/sc-2005) coupled to horseradish peroxidase. Then, the protein bands were captured using the enhanced chemiluminescence reagent Western ECL substrate (Thermo, 3405), visualized, and analyzed by Image Lab™ Software (Bio-Rad, Hercules, CA, USA).

Statistical Analysis: The analyses were performed depending on whether the data were parametric or not. While the Shapiro-Wilk test was used to determine the normality of data distributions, the homogeneity of variance of the groups was determined by the Levene test. One-way analysis of variance was used for data that met parametric assumptions, and Mann Whitney U test was used for multiple comparisons if the Kruskal Wallis test was significant for data that did not meet parametric assumptions. SPSS (IBM SPSS Statistics Desktop 20.0) program was used while performing the tests.

Results

Histopathological Evaluations: In the histopathological examination performed in the control group, follicles and corpus luteum of different sizes and periods were detected in the cortex and were observed to be normal. The germinal epithelium was normal and regular (Figure 1 a). In the hypothyroid group, it was observed that the vessels were severely

dilated, there were many atretic and irregular follicles, and the germinal epithelium was irregular and damaged (Figure 1 b). In the treatment group, it was observed that atretic and irregular follicles decreased, the deterioration in vascular structures was less, and the germinal epithelium was regular (Figure 1c).

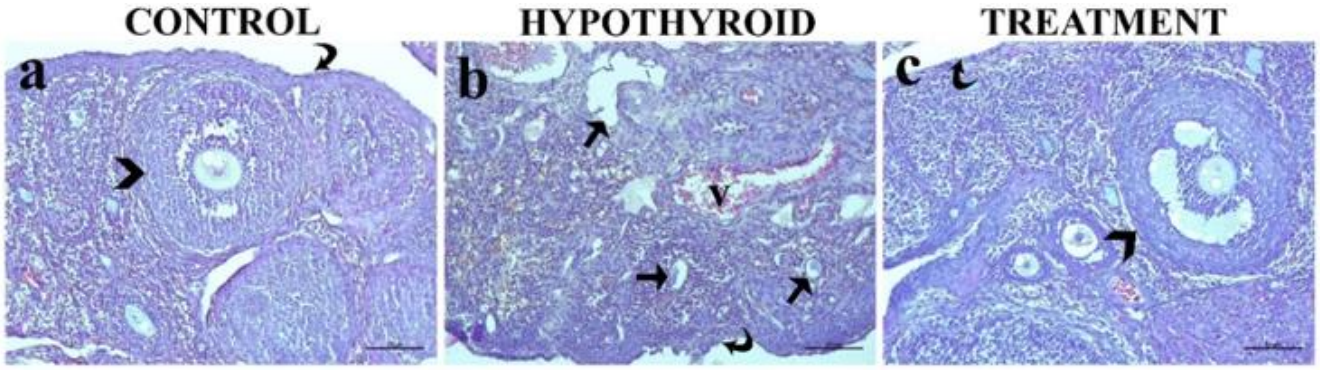

Figure 1. Illustration of histologic ovarian section all groups. Arrow head; secondary follicle, curved arrow; germinal epithelium, Arrow; atretic follicle, v; dilated vein. Crossman Modified Triple Staining, Magnification 200X.

When the numbers of antral follicles were compared among the three groups, a significant difference was observed (Table 1).

Table 1. Antral follicle count by group in sections evaluated after H&E staining.

		Left Ovary	Right Ovary
		Antral follicle	Antral follicle
CONTROL	M ±SD	6,71±1,25	5,14±1,77
LT	M ±SD	3,43±0,98	2,85±1,07
HT	M ±SD	2,14±0,81	1,28±0,95
P-VALUE	M ±SD	0,001	0,001

Mean (M), Standard Deviation (SD)

Biochemical Results: In the ovarian tissue biochemical analysis, the SOD activity was higher in the Control and LT groups but dramatically decreased in the HT group ($p<0.05$). GSH levels were higher in the Control and LT groups but slightly decreased in the HT groups ($p>0.05$). Tissue MDA level was higher in the HT group but significantly reduced in the Control and LT groups ($p<0.05$). The levels and comparisons of tissue biochemical analysis are presented in Table 2.

Table 2. Tissue biochemical analysis results for all groups: Superoxide dismutase (SOD), Glutathione (GSH), and Malondialdehyde (MDA).

	Sod Activity (U/Ml)	Gsh Activity (Mm/ Mg Protein)	Mda Level (µm/Mg Protein)
CONTROL	21.44±4.23^{a}	7,05±2.15^{a}	2,11±0.56^{a}
LT	19,33±3.21^{a}	6,60±2.45^{a}	2,96±0.44^{a}
HT	7,71±1.35^{b}	5,55±1.43^{a}	3,55±0.77^{b}

The letters (a,b) indicates the statistical differences among the groups, $p<0.05$ accepted.

Western Blot Results: In the analysis of relative protein expressions, Caspase-3, mTOR, P2X7R, NfkB, IL-1β, and TNF-α levels lower in the Control and LT groups, but the relative protein expression of Caspase-3, mTOR, P2X7R, NfkB, IL-1β, and TNF-α were found as higher in the HT group. The protein expression of Bcl-2 level was found higher in the Control and LT groups but increased in the HT group. The bands and levels of relative protein expressions for all groups are presented in the Figure 2.

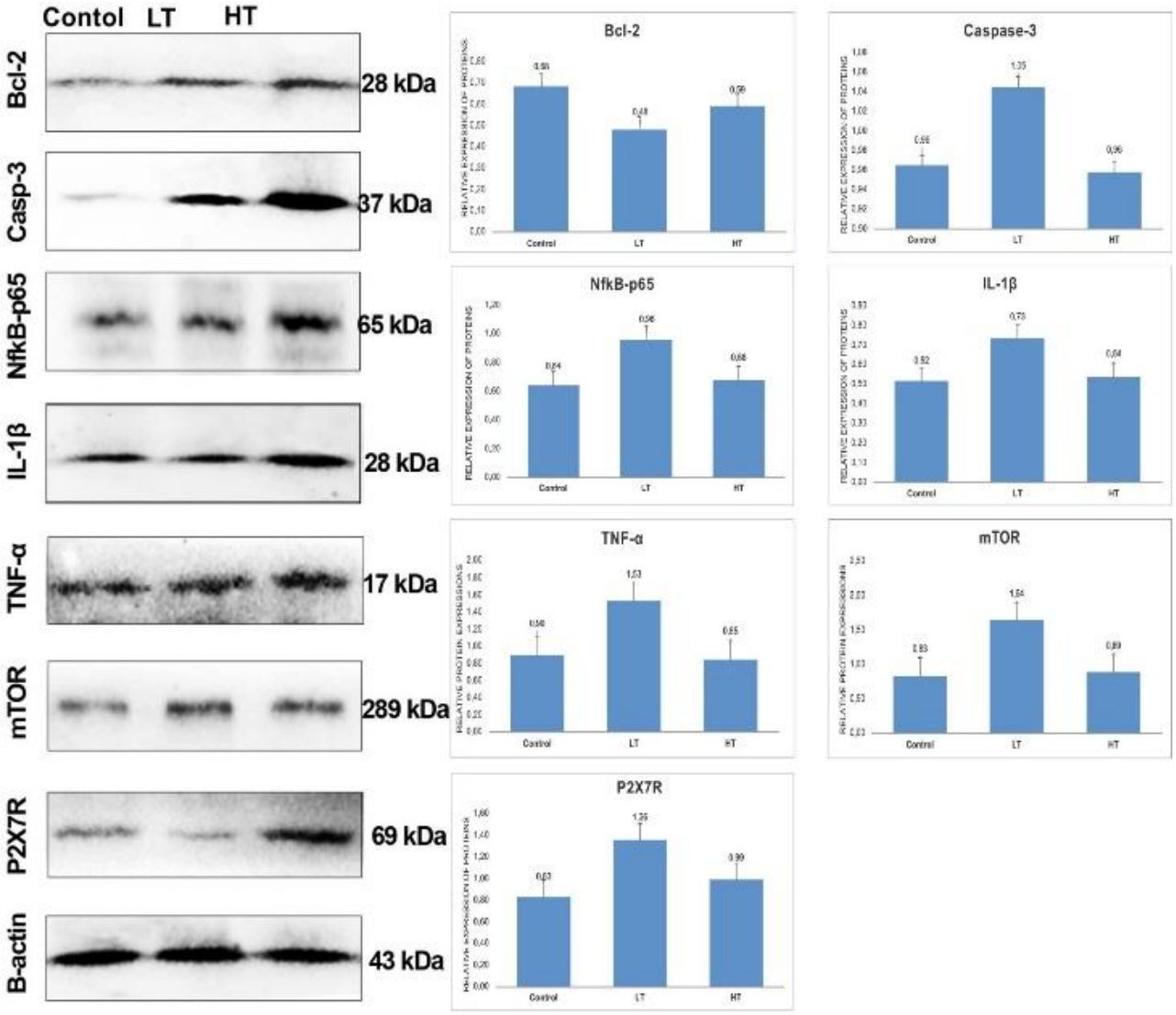

Figure 2. The analysis of relative protein expressions, Caspase-3, mTOR, P2X7R, NfkB, IL-1β, and TNF-α levels.

Discussion

In this study, ovarian tissue damage, oxidative stress level and apoptotic activity were analyzed in detail in adult female rats exposed to chronic hypothyroid conditions for a long time. In hypothyroid ovaries, tissue damage, decreased follicle reserve, and oxidative parameters and apoptotic activity increased significantly compared to controls. Decreased antioxidant levels and increased apoptotic activity in the hypothyroid group probably lead to a decrease in follicle reserve through cellular damage.

When experimental hypothyroidism is induced in female rats, degenerative changes occur in their ovaries, and the estrous cycle is altered (33). In the conducted studies, untreated mature hypothyroid rats were observed to have no signs of estrus and no ovulation. Histological examination showed that most follicles were atretic, and corpus luteum was not observed. Gonadotropin treatment triggered ovulation, but the number of eggs ovulated was lower than that in normal control groups. These findings were consistent with the results of previous studies on hypothyroid rats and chickens, demonstrating that hypothyroidism inhibits follicular development and ovulation (34,35). In the ovaries of animals treated with PTU, compared to rats not treated with PTU, there were more

secondary follicles and fewer antral follicles, while non-atretic antral follicles were smaller, and the number of atretic follicles was higher. However, corpus luteum was not observed. This indicates that folliculogenesis is disrupted, and this disruption is related to the diameters of antral follicles (20). In another study aiming to investigate the effects of long-term hypothyroidism on ovarian follicle reserve in adult rats, the total number of follicles in each ovarian was determined. Hypothyroid females showed an approximate 60% decrease in primordial follicle count compared to age-matched control groups after 16 weeks of hypothyroidism. Additionally, a roughly 40% reduction in primary and preantral follicle counts compared to control groups was observed in hypothyroid rats, and there was a noticeable decreasing trend in antral follicle counts, although this decrease was not statistically significant. Consistent with these observations, there was a decreasing trend in the total number of Corpus Luteum (CL) per ovarian in hypothyroid females, but this decrease was also found not to be statistically significant (36). The findings observed in the hypothyroid group were quite pronounced. In this group, there was a significant dilation of blood vessels and the presence of numerous atretic follicles and irregularly shaped follicles. Additionally, the germinal epithelium was irregular and damaged. These results appear to be in line with other studies in the literature. Previous research has also demonstrated that hypothyroidism leads to similar changes in ovarian tissue and has adverse effects on follicular development. Therefore, the findings of our study contribute to the broader context of evaluating the effects of hypothyroidism on ovarian tissue and demonstrate consistency with previous research.

It is known that antioxidant levels in the serum can decrease in the case of hypothyroidism. This decrease has been observed to weaken the body's protective abilities against reactive oxygen species (ROS) and increase oxidative stress (37). Specifically, hypothyroid individuals have been observed to have low levels of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and thioredoxin reductase (Txnrd1) (38). It is known that excessive TSH directly increases oxidative stress (OS) (39). Other studies have confirmed increased lipid peroxidation in overt hypothyroidism and subclinical hypothyroidism, demonstrated by elevated levels of MDA. Protein oxidation has also been reported, with increased levels of protein carbonyls observed (40). The correlation analysis conducted in this study suggests that both the increase in TSH and the elevation in MDA contribute to protein damage. Finally, various studies have reported increased levels of NO (41,42). In the biochemical analysis of ovarian tissue, SOD activity was higher in the Control and LT groups but dramatically decreased in the HT group. GSH levels were higher in the Control and LT groups but showed a slight decrease in the HT group. Tissue MDA levels were higher in the HT group but significantly decreased in the Control and LT groups. These findings appear to be consistent with similar studies in the literature and support that hypothyroidism leads to increased oxidative stress and reduced antioxidant activity in ovarian tissue.

This study, by showing increased IL-1β levels associated with subclinical hypothyroidism (SH), a subclinical form of hypothyroidism, has obtained results consistent with similar findings in the literature. This suggests that hypothyroidism may increase inflammation markers and emphasizes the need for further research in this area. In conclusion, increased serum IL-1β levels were observed in rats with SH (43). In our study, levels of Il-1β and TNF-α, which are inflammation parameters, were high in the hypothyroid group.

The results of this study conducted on ovarian tissue are consistent with previous research on hypothyroidism and Bcl-2 in the literature. Other studies in the literature have suggested that

hypothyroidism can affect Bcl-2 gene expression and that this effect can regulate cellular apoptosis. Therefore, the findings of this study confirm the impact of hypothyroidism on Bcl-2 in line with these literature data. In the hypothyroidism model created with methimazole, an increased autophagic process has been detected in the cerebellum. Additionally, it has been observed that the levels of p-AKT/AKT, p-mTOR/mTOR, and p-ULK1/ULK1 in the cerebellum have decreased due to hypothyroidism (44). In the same study, it was also observed that hypothyroidism led to an increase in c-Caspase-3 and TUNEL-positive (apoptotic) cell counts in cerebellar neurons (44). Our results show that while Caspase, NFKB, and mTOR protein expressions were increased in the hypothyroid group, the increase in apoptotic activity is compatible with findings previously reported in the literature.

In light of the results, this study shows that long-term mild hypothyroidism causes ovarian damage, oxidative stress, inflammation, and apoptosis, which may negatively affect fertility.

Declaration of Interest: The author declares that there is no conflict of interest regarding the publication of this paper.

Acknowledgements: Self funded.

Author Contribution: SG and NAC contributed to the conception and design of the study. SG organized the database. SNP and EA performed the statistical analysis. NAC wrote the first draft of the manuscript. NAC, SNP, and EA wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

ORCID:

Nevra AYDEMİR CELEP 0000-0003-1608-5881

Semin GEDİKLİ 0000-0001-8238-7226

Seçil Nazife PARLAK 0000-0001-9577-986X

Elvin ALİYEV 0000-0003-3755-5846

References

1. Pearce EN. Thyroid dysfunction in perimenopausal and postmenopausal women. Menopause international 2007;13:8-13.
2. Vanderpas J. Nutritional epidemiology and thyroid hormone metabolism. Annu Rev Nutr 2006;26:293-322.
3. Ozkanlar S, Kara H, Gür C, Gedikli S, Kara A, Ozudogru Z, et al. Effects of photoperiod on thyroid gland development and function in growing chicks: a biochemical and morphometric study. Animal Production Science 2021;61:1652-8.
4. Vissenberg R, Manders V, Mastenbroek S, Fliers E, Afink G, Ris-Stalpers C, et al. Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction. Human reproduction update 2015;21:378-87.
5. Abalovich M, Mitelberg L, Allami C, Gutierrez S, Alcaraz G, Otero P, et al. Subclinical hypothyroidism and thyroid autoimmunity in women with infertility. Gynecological Endocrinology 2007;23:279-83.
6. Joshi J, Bhandarkar S, Chadha M, Balaiah D, Shah R. Menstrual irregularities and lactation failure may precede thyroid dysfunction or goitre. Journal of postgraduate medicine 1993;39:137.
7. Krassas G, Pontikides N, Kaltsas T, Papadopoulou P, Paunkovic J, Paunkovic N, et al. Disturbances of menstruation in hypothyroidism. Clinical endocrinology 1999;50:655-9.
8. DORRINGTON J, CHUMA AV, BENDELL JJ. Transforming growth factor β and follicle-stimulating hormone promote rat granulosa cell proliferation. Endocrinology 1988;123:353-9.
9. Teerds KJ, Dorrington JH. Immunohistochemical localization of transforming growth factor-β1 and-β2 during follicular development in the adult rat ovary. Molecular and cellular endocrinology 1992;84:R7-R13.
10. Danilovich N, Wernsing D, Coschigano K, Kopchick J, Bartke A. Deficits in female reproductive function in GH-R-KO mice; role of IGF-I. Endocrinology 1999;140:2637-40.
11. Almog B, Gold R, Tajima K, Dantes A, Salim K, Rubinstein M, et al. Leptin attenuates follicular apoptosis and accelerates the onset of puberty in immature rats. Molecular and Cellular Endocrinology 2001;183:179-91.
12. Lagaly DV, Aad PY, Grado-Ahuir JA, Hulsey LB, Spicer LJ. Role of adiponectin in regulating ovarian theca and granulosa cell function. Molecular and Cellular Endocrinology 2008;284:38-45.
13. Kobayashi N, Orisaka M, Cao M, Kotsuji F, Leader A, Sakuragi N, et al. Growth differentiation factor-9 mediates follicle-stimulating hormone-thyroid hormone interaction in the regulation of rat preantral follicular development. Endocrinology 2009;150:5566-74.
14. Zhang C, Guo L, Zhu B, Feng Y, Yu S, An N, et al. Effects of 3, 5, 3′-triiodothyronine (t3) and follicle stimulating hormone on apoptosis and proliferation of rat ovarian granulosa cells. Chin J Physiol 2013;56:298-305.
15. Erbaş E, Gedikli S. Investigation of the endometrial receptivity status in experimental hypothyroid-induced female rats. Iranian Journal of Basic Medical Sciences 2022;25.
16. Devdhar M, Ousman Y, Burman K. Hypothyroidism Endocrinol Metab Clin North Am, 36 (2007). View PDF View article View in Scopus:595-615.
17. Krassas G, Poppe K, Glinoer D. Thyroid function and human reproductive health. Endocrine reviews 2010;31:702-55.
18. Soliman FA, Reineke E. Influence of variations in environmental temperature and thyroid status on sexual function in young female mice. American Journal of Physiology-Legacy Content 1952;168:400-5.
19. Rivkees SA, Mattison DR. Propylthiouracil (PTU) hepatoxicity in children and recommendations for discontinuation of use. International journal of pediatric endocrinology 2009;2009:1-8.
20. Dijkstra G, de Rooij DG, de Jong FH, van den Hurk R. Effect of hypothyroidism on ovarian follicular development, granulosa cell proliferation and peripheral hormone levels in the prepubertal rat. European Journal of Endocrinology 1996;134:649-54.

21. Fedail JS, Zheng K, Wei Q, Kong L, Shi F. Roles of thyroid hormones in follicular development in the ovary of neonatal and immature rats. Endocrine 2014;46:594-604.
22. Dobrzyńska MM, Baumgartner A, Anderson D. Antioxidants modulate thyroid hormone-and noradrenaline-induced DNA damage in human sperm. Mutagenesis 2004;19:325-30.
23. Polat S, Topal H, Celep NA, Erbaş E, Adem K. Melatonin and cisplatin synergistically enhance apoptosis via autophagy-dependent alteration of P53 transcription in human colorectal cancer cells. Eurasian Journal of Molecular and Biochemical Sciences 2022;1:11-8.
24. Zuo L, Zhou T, Pannell B, Ziegler A, Best TM. Biological and physiological role of reactive oxygen species–the good, the bad and the ugly. Acta physiologica 2015;214:329-48.
25. Behrman HR, Kodaman PH, Preston SL, Gao S. Oxidative stress and the ovary. Journal of the Society for Gynecologic Investigation 2001;8:S40-S2.
26. Takami M, Preston S, Toyloy V, Behrman HR. Antioxidants reversibly inhibit the spontaneous resumption of meiosis. American Journal of Physiology-Endocrinology and Metabolism 1999;276:E684-E8.
27. Ruder EH, Hartman TJ, Blumberg J, Goldman MB. Oxidative stress and antioxidants: exposure and impact on female fertility. Human reproduction update 2008;14:345-57.
28. Armada-Dias L, Carvalho J, Breitenbach M, Franci CR, Moura EG. Is the infertility in hypothyroidism mainly due to ovarian or pituitary functional changes? Brazilian Journal of Medical and Biological Research 2001;34:1209-15.
29. Moulakakis KG, Poulakou MV, Dosios T, Dontas I, Sokolis DP, Vlachos IS, et al. Hypothyroidism and the aorta. evidence of increased oxidative DNA damage to the aorta of hypothyroid rats. in vivo 2008;22:603-8.
30. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clinical chemistry 1988;34:497-500.
31. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical biochemistry 1968;25:192-205.
32. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry 1979;95:351-8.
33. Dalton AJ, Morris HP, Dubnik CS. Changes in the organs of female C3H mice receiving thiourea. JNCI: Journal of the National Cancer Institute 1945;5:451-4.
34. You HH, Song G. Review of endocrine disruptors on male and female reproductive systems. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 2021;244:109002.
35. Jiang J, Umezu M, Sato E. Improvement of follicular development rather than gonadotrophin secretion by thyroxine treatment in infertile immature hypothyroid rdw rats. Journal of reproduction and fertility 2000;119:193-200.
36. Meng L, Rijntjes E, Swarts HJ, Keijer J, Teerds KJ. Prolonged hypothyroidism severely reduces ovarian follicular reserve in adult rats. Journal of ovarian research 2017;10:1-8.
37. Gedikli S, Gelen V, Sengul E, Ozkanlar S, Gur C, Agırbas O, et al. Therapeutic effects of melatonin on liver and kidney damages in intensive exercise model of rats. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders) 2015;15:308-14.
38. Meng L, Rijntjes E, Swarts H, Bunschoten A, van der Stelt I, Keijer J, et al. Dietary-induced chronic hypothyroidism negatively affects rat follicular development and ovulation rate and is associated with oxidative stress. Biology of reproduction 2016;94:90, 1-11.
39. Dardano A, Ghiadoni L, Plantinga Y, Caraccio N, Bemi A, Duranti E, et al. Recombinant human thyrotropin reduces endothelium-dependent vasodilation in patients monitored for differentiated thyroid carcinoma. The Journal of Clinical Endocrinology & Metabolism 2006;91:4175-8.
40. Haribabu A, Reddy VS, Pallavi C, Bitla AR, Sachan A, Pullaiah P, et al. Evaluation of protein oxidation and its association with lipid peroxidation and thyrotropin levels in overt and subclinical hypothyroidism. Endocrine 2013;44:152-7.
41. Coria MJ, Pastrán AI, Gimenez MS. Serum oxidative stress parameters of women with hypothyroidism. 2009.
42. Erdamar H, Demirci H, Yaman H, Erbil MK, Yakar T, Sancak B, et al. The effect of hypothyroidism, hyperthyroidism, and their treatment on parameters of oxidative stress and antioxidant status. Clinical chemistry and laboratory medicine 2008;46:1004-10.
43. He J, Zhou Y, Zhang T, Zou Y, Huang H. Effect of Bushen Antai recipe on pyroptosis mechanism of subclinical hypothyroidism decidual cells in early pregnancy. Annals of Translational Medicine 2022;10.
44. Vishwakarma J, Gupta K, Mishra J, Garg A, Malik R, Kashyap A, et al. Hypothyroidism induces motor deficit via altered cerebellar HB-EGF/EGFR and autophagy. The Journal of endocrinology 2023;257:e220338.