Selenium and iodine are important trace elements in human metabolism and are also essential for thyroid function. Iodine is the main component of thyroid hormones, namely, thyroxine (T4) and triiodothyronine (T3). Selenium is present in the thyroid gland in high concentrations, and this suggests that selenoproteins may contribute significantly to the integrity and functioning of the gland.1–3

Selenium-dependent glutathione reductase and selenoproteins are important for their antioxidant activity, which is vital for the protection of the organism. Selenium affects the metabolic pathways by changing the activity of selenoproteins and plays a role in cellular defense against oxidative stress. Selenium concentration regulates the expression of selenoproteins. Different selenium concentrations may affect immunity and energy metabolism diversely.4

There are different chemical forms of selenium. In particular, sodium selenate (Se⁴⁺) directly activates natural killer cells, and has anti-leukemic effects and anti-cancer properties.5,6 These anti-cancer properties have been proven by clinical and experimental studies.7–9 Selenium deficiency deteriorates the immune system by affecting the thymus, which is responsible for the production of macrophages and lymphocytes. In contrast, excess selenium is also toxic. Hair loss, skin and nail lesions, bone weakening, nausea, and diarrhea have been reported with intake above 400 µg/day.10,11 Selenium may be derived from plant and animal sources. The main sources of selenium in the diet are cereals, meat, fish, shellfish, milk, and nuts.4

Thyroid diseases are manifested when the thyroid gland is unable to provide the body with sufficient hormones. A goiter is an enlargement of the thyroid, and the most common cause of the condition has been accepted as an inadequate intake of iodine in the daily diet. Multinodular goiter disease is a common condition that is characterized by the slow growth of soft nodules in the thyroid.12
Anxiety and depression are psychiatric disorders that are commonly observed in society and have been associated with chronic diseases such as thyroid function disorders. The negative effects of chronic diseases are known to affect mental health. Hypothyroidism and hyperthyroidism are associated with an increased risk of depression. Previous studies have evaluated the relationship of trace element levels with anxiety and depression in nodular goiter. However, the association of trace elements with anxiety and depression, specifically in patients with euthyroid nodular goiter (ENG), has yet to be evaluated.

We aimed to evaluate the relationship between selenium and iodine levels with anxiety and depression in patients with ENG.

METHODS
A total of 102 participants (92 female and 10 male) between the ages of 18 and 80, who applied to the endocrine outpatient clinic between January 2018 and June 2018, were included in this cross-sectional study. Bozok University Clinical Research Ethics Committee approved the study (2017-KAEK-189_2017.12.21_16), and informed consent was obtained from all individual participants included in the study. The study was conducted following the principles of the Declaration of Helsinki (Edinburgh 2000 revision). After a physical examination, height, weight, body mass index (BMI), medications, smoking habits, and family history of thyroid disease and psychiatric disease were noted. Analyses of free T3 (FT3), free T4 (FT4), thyroid-stimulating hormone (TSH), anti-thyroglobulin, and anti-thyroid peroxidase were performed on DxI 800 Access Immunoassay (Beckman Coulter Inc., Brea, CA, USA) using a direct chemiluminescence detection system. Euthyroidism was defined as TSH, FT3, and FT4 levels are in normal range. Urinary iodine concentration (UIC) was measured by Sandell Coldhoff method with spectrophotometry. Thyroid ultrasonography was performed using Logic 700 (GE medical sys, Milwaukee, USA) with a 7 MHz superficial probe.

Venous blood samples for selenium were obtained after 10–12 hours fasting, and samples were centrifuged at room temperature for five minutes at 5000 RPM. The extracted serum was kept in ice bags and stored in deep freezers at -80 °C. Serum (1 mL) samples were taken and placed into Teflon tubes belonging to microwave digestion system (Milestone Start D-Microwave Digestion System). Then, 5 mL suprapur nitric acid (HNO₃, 65%) and 5 mL of ultrapure water were added to the blood samples and digested in the microwave system. The samples were transferred to 50 mL polypolypropylene tubes, and the total volume was completed to 20 mL with ultrapure water. The selenium levels of the digested samples in the microwave system were determined by the Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) system; Thermo Scientific ICAP QC, USA). The operating parameters were set as follows: RF power 1550 W, nebulizer gas 0.90 L/min, plasma gas 0.80 L/min, nebulizer pressure 3.00 bar, dwell time 0.01, and spray chamber temperature 2.7 °C. The sampler probe was washed between injections by rinsing with ultrapure water for 30 seconds, followed by washing with 2% HNO₃ for 45 seconds, then rinsing with ultrapure water for 45 seconds. After the wash steps, the instrument automatically ran the next sample. The r² value of the calibration curve was calculated as 0.9999, and the interval of the calibration was set 0.5–1000 µg/L for selenium. The limit of detection of selenium was determined based on the standard deviation (SD) of the response and the slope of the calibration curves and was 0.204 mg/L. The sample and standard of measurements were repeated three times. Method validations were performed with certified reference material (CRM)-Seronorm™ Trace Elements Whole Blood L-2 (for selenium range 128–193 mg/L). CRM was measured five times on the same day and different days. Moreover, the average of the repeated measurements was used for the validation of the method, whereby the relative SD of the values did not exceed 5%.

After thyroid assessment was finished in the endocrine outpatient clinic, patients were referred to the psychiatry outpatient clinic. The Beck Anxiety Inventory (BAI) and Hamilton Depression Rating Scale (HDRS) were completed by the same psychiatrist. A 21-item BAI was scored from 0 to 3 points, depending on the severity of each symptom. The final score (range: 0–63 points) was calculated by summing the results for all items. A psychiatrist completed the HDRS score by observing the patients and asking the questions on the scale. Then, the points of the items were collected. The highest possible score was 53.
Patients were excluded from the study in cases of thyrotoxicosis, hypothyroid, euthyroid with antithyroid drugs, euthyroid with thyroid replacement therapy, selenium and iodine supplement use, positive thyroid antibody, chronic heart disease, renal failure, pregnancy, inflammatory diseases, malignancy, and diagnosis of psychiatric disease.

The study population was dichotomized according to the median values of selenium and urinary iodine. Statistical analyses were calculated using SPSS Statistics (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). The Kolmogorov-Smirnov test was used for normality. Comparisons among the groups were performed using an independent samples t-test for variables distributed normally and Mann-Whitney U test for non-normally distributed variables. Correlation of the study parameters was assessed by Pearson or Spearman’s correlation analysis, as appropriate. A p-value < 0.050 was considered statistically significant.

RESULTS

The general demographics and clinical characteristics of the participants are summarized in Table 1. Selenium and UIC values were non-normally distributed. The median values of serum selenium and UIC were 63.17 ng/mL (38.36–118.42) and 93.45 mg/day (21.80–600.00), respectively.

The participants were divided into two groups according to their median selenium levels: low (selenium level ≤ 63.17 ng/mL) and high (selenium level > 63.17 ng/mL). The groups were similar in terms of age, weight, height, BMI, FT3, FT4, TSH, UIC, and thyroid volume. BAI and HDRS scores were significantly higher in the low selenium group ($p = 0.032$, $p = 0.042$, respectively) [Table 2].

The participants were divided into two groups according to their median UIC: low (UIC ≤ 93.45 mg/day) and high (UIC > 93.45 mg/day). The groups were similar in terms of age, weight, height, BMI, Us.

| Parameters       | Low serum selenium $n = 51$ | High serum selenium $n = 51$ | p-value |
|------------------|---------------------------|-----------------------------|---------|
| Age, year        | 45.7 ± 12.5               | 43.4 ± 10.6                 | 0.319   |
| Weight, kg       | 73.8 ± 15.4               | 79.6 ± 14.9                 | 0.056   |
| Height, m        | 158.7 ± 7.5               | 159.5 ± 7.1                 | 0.06    |
| BMI, kg/m²       | 29.2 ± 5.8                | 31.3 ± 5.8                  | 0.086   |
| Frec T3, ng/dL   | 2.7 ± 0.3                 | 2.7 ± 0.3                   | 0.921   |
| Frec T4, ng/dL   | 1.0 ± 0.1                 | 1.0 ± 0.1                   | 0.643   |
| TSH, mIU/L       | 1.50 (0.35–4.90)          | 1.22 (0.38–4.60)            | 0.482   |
| Urinary iodine, mg/day | 88.90 (21.8–600)   | 100.20 (26.30–600.00)       | 0.385   |
| Thyroid volume, cm³ | 9.30 (4.87–33.00)   | 9.74 (5.70–32.15)           | 0.385   |
| BAI              | 19.6 ± 8.2                | 15.9 ± 9.2                  | 0.032   |
| HDRS             | 10.00 (2.00–53.00)        | 8.00 (0.00–25.00)           | 0.042   |

BMI: body mass index; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone; BAI: Beck Anxiety Inventory; HDRS: Hamilton Depression Rating Scale.

Values are presented as the mean±standard deviation (SD) and median (minimum–maximum), p-values < 0.050 are statistically significant.
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FT3, FT4, TSH, serum selenium, thyroid volume, and HDRS score. BAI score was significantly higher in the low UIC group than the high UIC group ($p = 0.007$) [Table 3].

Serum selenium levels were negatively correlated with the BAI ($r = -0.262$, $p = 0.008$) and HDRS ($r = -0.149$, $p = 0.010$). The UIC was negatively correlated with the BAI score ($r = -0.248$, $p = 0.013$).

**DISCUSSION**

Anxiety and depression scores were significantly higher in the low selenium group, and anxiety scores were significantly higher in the low iodine group in patients with ENG. Furthermore, serum selenium was negatively correlated with anxiety and depression scores, and iodine level was negatively correlated with the anxiety score.

Selenocysteine, as a form of selenium, is included in the structure of selenoproteins. Glutathione peroxidase, thioredoxin reductase, and selenoprotein P protect tissues from lipoperoxidation and oxidative damage. Increased levels of stress biomarkers have been reported in depression in recent studies, and this suggests that oxidative stress may be an important factor in the pathogenesis of depression. High underground water selenium levels were associated with lesser depression symptoms.

High selenium levels have been associated with low geriatric depression scale scores in another study. Furthermore, selenium supplementation has been reported to prevent postpartum depression, which affects about 6.5–12.9% of women in the first postpartum year.

Selenium is required for the synthesis and metabolism of thyroid hormones since it is included in the structure of iodothyronine deiodinase. Thyroid hormones have long been associated with neuropsychiatric findings such as mood disorders, cognitive function disorders, and other psychiatric symptoms. Patients with ENG were enrolled in this study to avoid the thyroid hormone effect. As a result, thyroid hormone levels were similar in both groups, whereas BAI and HDRS scores were significantly higher in the low selenium group. Our results suggest that, in patients with ENG, selenium may be associated with anxiety and depression independent of thyroid hormone levels. Selenium may have a protective role against anxiety and depression, possibly due to its protective effect on oxidative stress.

A new thyroid follicle cell formation has to be stimulated to develop a goiter. The thyroid gland increases its uptake of iodine in the event of insufficient iodine in the diet. In particular, T4 hormone production decreases in favor of T3, and this change causes increased TSH. Minor increases in TSH can cause thyroid enlarging effects, especially in regions of iodine deficiency. Iodine deficiency is the most frequent cause of hypothyroidism. T4 is converted to T3 by type 2 deiodinase, a selenoprotein produced by the glial cells in the brain.

| Parameters       | Low UIC (n = 50) | High UIC (n = 50) | p-value |
|------------------|------------------|------------------|---------|
| Age, years       | 46.0 ± 10.9      | 44.0 ± 11.7      | 0.382   |
| Weight, kg       | 78.0 ± 15.2      | 75.6 ± 15.8      | 0.450   |
| Height, m        | 158.8 ± 8.1      | 159.1 ± 6.2      | 0.836   |
| BMI, kg/m²       | 30.8 ± 5.5       | 29.9 ± 6.3       | 0.452   |
| Free T3, ng/dL   | 2.6 ± 0.3        | 2.7 ± 0.3        | 0.368   |
| Free T4, ng/dL   | 1.0 ± 0.1        | 1.0 ± 0.1        | 0.256   |
| TSH, mIU/L       | 1.46 (0.44–4.49) | 1.32 (0.35–4.90) | 0.425   |
| Serum selenium, ng/mL | 62.80 (38.36–111.07) | 63.63 (44.27–118.42) | 0.326   |
| Thyroid volume, cm³ | 9.40 (4.80–32.15) | 9.70 (4.90–33.00) | 0.326   |
| BAI              | 20.0 ± 8.1       | 15.2 ± 9.1       | 0.007   |
| HDRS             | 9.00 (0.00–53.00) | 7.50 (0.00–42.00) | 0.425   |

UIC: urinary iodine concentration, low ≤ 93.45 mg/day and high > 93.45 mg/day; BMI: body mass index; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone; BAI: Beck Anxiety Inventory; HDRS: Hamilton Depression Rating Scale. Values are presented as the mean ± standard deviation (SD) and median (minimum–maximum), p-values < 0.050 are statistically significant.
In population-based studies, increased prevalence of depression and anxiety was observed in patients with hypothyroidism and hyperthyroidism. Another study detected no association between thyroid hormones and anxiety and depression in the general population, and in patients with hyperthyroidism secondary to Graves’ disease, respectively. Although the TSH, FT4, and FT3 levels were similar between the groups, anxiety scores were significantly higher in the lower urinary iodine group in our study. While our results indicated an inverse relationship between urinary iodine and anxiety scores, no significant relationship was observed between depression scores and iodine.

Our results suggest that in patients with ENG, selenium may be inversely related to anxiety and depression, and iodine may be inversely related to anxiety regardless of the effect of selenium and iodine on thyroid functions. The inverse relationship of selenium with anxiety and depression can be explained by the protective effect of selenium on oxidative stress. The mechanism of the inverse relationship between iodine and anxiety is not clear. Prospective, large scale, randomized clinical trials are needed to elucidate the relation of these elements with anxiety and depression.

Our study has several limitations. Firstly, it is a cross-sectional study. Therefore, we could make assumptions about only possible etiological relationships. Longitudinal studies may be designed to determine the long-term influence of these parameters. Secondly, the sample size of this study population was relatively small. Thirdly, it reflects a single-center experience.

**CONCLUSION**

Low selenium and iodine levels may contribute to the development of anxiety and depression, independent of thyroid functions, in patients with ENG. In these patients, selenium and iodine replacement may be useful for the prevention of anxiety and depression, especially in deficient regions. Longitudinal studies may be designed to determine the long-term influence of these parameters on anxiety and depression.

**Disclosure**

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**REFERENCES**

1. Aaseth J, Frey H, Glatte E, Norheim G, Ringstad J, Thomassen Y. Selenium concentrations in the human thyroid gland. Biol Trace Elem Res 1990 Feb;24(2):147-152.
2. Schmutzler C, Mentrup B, Schomburg L, Hoang-Vu C, Herzog V, Köhle J. Selenoproteins of the thyroid gland: expression, localization and possible function of glutathione peroxidase 3. Biol Chem 2007 Oct;388(10):1053-1059.
3. Köhle J, Jakob F, Contempre B, Dumont JE. Selenium, the thyroid, and the endocrine system. Endocr Rev 2005 Dec;26(7):944-984.
4. Kieliszek M. Selenium-fascinating microelement, properties and sources in food. Molecules 2019 Apr;24(7):1298.
5. Kieliszek M, Lipinski B. Pathophysiological significance of protein hydrophobic interactions: an emerging hypothesis. Med Hypotheses 2018 Jan;110:15-22.
6. Kieliszek M, Lipinski B, Blażejak S. Application of sodium selenite in the prevention and treatment of cancers. Cells 2017 Oct;6(4):39.
7. Ip C, Hayes C, Budnick RM, Ganther HE. Chemical form of selenium, critical metabolites, and cancer prevention. Cancer Res 1991 Jan;51(2):595-600.
8. Frenkel GD, Falvey D, MacVicar C. Products of the reaction of selenite with intracellular sulphhydryl compounds. Biol Trace Elem Res 1991 Jul;30(1):9-18.
9. Lipinski B, Pretorius E. Iron-induced fibrin in cardiovascular disease. Curr Neurol Neurosci Res 2013 Aug;10(3):269-274.
10. Fordyce F. Selenium geochemistry and health. Ambio 2007 Feb;36(1):94-97.
11. Kieliszek M, Blażejak S. Current knowledge on the importance of selenium in food for living organisms: a review. Molecules 2016 May;21(5):609.
12. Tauro LF, Lobo GJ, Fernandes H, George C, Aithala PS, Shenoy D, et al. A comparative study on fine needle aspiration cytology versus fine needle capillary cytology in thyroid nodules. Oman Med J 2012 Mar;27(2):151-156.
13. Bunevicius R, Prange AJ Jr. Thyroid disease and mental disorders: cause and effect or only comorbidity? Curr Opin Psychiatry 2010 Jul;23(4):363-368.
14. Shoaar S, Naderan M, Aghajani M, Sahimi-Izadian E, Hosseini-Araghi N, Khorgami Z. Prevalence and determinants of depression and anxiety symptoms in surgical patients. Oman Med J 2016 May;31(3):176-181.
15. Thvilum M, Brandt F, Almind D, Christensen K, Brix TH, Hegedüs L. Increased psychiatric morbidity before and after the diagnosis of hypothyroidism: a nationwide register study. Thyroid 2014 May;24(5):802-808.
16. Brandt F, Thvilum M, Almind D, Christensen K, Green A, Hegedüs L, et al. Morbidity before and after the diagnosis of hyperthyroidism: a nationwide register-based study. PLoS One 2013 Jun;8(6):e66711.
17. Aliyev V, Kayaalti Z, Kaplan B, Soylemezoglu T. Effect of GST polymorphisms on As levels of placental and maternal biological samples. Toxicol Lett 2012;211(5):567.
18. Turksyo VA, Tütünum L. Changing levels of selenium and zinc in cadmium-exposed workers: probable association with the intensity of inflammation. Molecular biology reports 2019;46(5):5455-5464.
19. Ulusoy M, Sahin NH, Erken H. The beck anxiety inventory: psychometric properties. J Cogn Psychother 1998;12(2):163-172.
20. Williams JB. A structured interview guide for the Hamilton depression rating scale. Arch Gen Psychiatry 1988 Aug;45(8):742-747.
21. Steinbrenner H, Sies H. Selenium homeostasis and antioxidant selenoproteins in brain: implications for disorders in the central nervous system. Arch Biochem
22. Vargas HO, Nunes SO, de Castro MR, Vargas MM, Barbosa DS, Bortolasci CC, et al. Oxidative stress and inflammatory markers are associated with depression and nicotine dependence. Neurosci Lett 2013 Jun;544:136-140.

23. Lee S-Y, Lee S-J, Han C, Parkar AA, Masand PS, Pae C-U. Oxidative/nitrosative stress and antidepressants: targets for novel antidepressants. Prog Neuropsychopharmacol Biol Psychiatry 2013 Oct;46:224-235.

24. Johnson LA, Phillips JA, Mauer C, Edwards M, Balldin VH, Hall JR, et al. The impact of GPX1 on the association of groundwater selenium and depression: a Project FRONTIER study. BMC Psychiatry 2013 Jan;13(1):7.

25. Gao S, Jin y, Unverzagt FW, Liang C, Hall KS, Cao J, et al. Selenium level and depressive symptoms in a rural elderly Chinese cohort. BMC Psychiatry 2012 Jul;12(1):72.

26. Gavin NI, Gaynes BN, Lohr KN, Meltzer-Brody S, Gartlehner G, Swinson T. Perinatal depression: a systematic review of prevalence and incidence. Obstet Gynecol 2005 Nov;106(5 Pt 1):1071-1083.

27. Bauer M, Goetz T, Glenn T, Whybrow PC. The thyroid-brain interaction in thyroid disorders and mood disorders. J Neuroendocrinol 2008 Oct;20(10):1101-1114.

28. Bahn RS, Castro MR. Approach to the patient with nontoxic multinodular goiter. J Clin Endocrinol Metab 2011 May;96(5):1202-1212.

29. Hegedüs L, Brix TH, Paschke R. Etiology of simple goiter. Mary Ann Liebert, Inc; 2009.

30. Wu E-L, Chien I-C, Lin C-H, Chou Y-J, Chou P. Increased risk of hypothyroidism and hyperthyroidism in patients with major depressive disorder: a population-based study. J Psychiatr Res 2013 Mar;47(3):233-237.

31. Ittermann T, Völzke H, Baumeister SE, Appel K, Grabe HJ. Diagnosed thyroid disorders are associated with depression and anxiety. Soc Psychiatry Psychiatr Epidemiol 2015 Sep;50(9):1417-1425.

32. Trzepacz PT, Klein I, Roberts M, Greenhouse J, Levey GS. Graves’ disease: an analysis of thyroid hormone levels and hyperthyroid signs and symptoms. Am J Med 1989 Nov;87(5):558-561.