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RESEARCH ARTICLE

SERUM CHEMERIN, VASPIN, OXIDATIVE STRESS AND INFLAMMATION MARKERS IN SUBCLINICAL HYPOTHYROIDISM/ HYPERTHYROIDISM

Sümeyye Tamer[1], Taylan Turan[1], Tuba Taşkan[1], Mehmet Ayhan Karakoç[2], Emre Arslan[2], Aymelek Gönenç[1*]

[1] Gazi University, Faculty of Pharmacy, Department of Biochemistry, Ankara, Turkey
[2] Gazi University, Faculty of Medicine, Department of Endocrinology and Metabolism, Ankara, Turkey

ORCID: S.T. 0000-0003-4956-2015; T.T. 0000-0001-7335-1213; T.T. 0000-0003-1677-5356; M.A.K. 0000-0002-0601-6893; E.A. 0000-0003-4460-8598; A.G. 0000-0001-9661-8291

Corresponding author: Aymelek Gönenç, **E-mail:** aymelek@gazi.edu.tr
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ABSTRACT

Purpose: Subclinical thyroid diseases constitute the first stage of clinical thyroid, so it is important to investigate underlying mechanisms. Clinical studies have revealed changes in some adipokines concerning thyroid disorders. Relationship chemerin and vaspin adipokines with thyroid hormones needs to crarify. So, it was aimed to evaluate chemerin, vaspin, oxidative stress, and inflammation markers in subclinical hypothyroidism/hyperthyroidism.
Material and Methods: The study included 38 subclinical hyperthyroidism, 31 subclinical hypothyroidism, and 44 healthy controls. Serum chemerin, vaspin, interleukin-10, C-reactive protein, and Oxidized LDL were measured with ELISA method, while total antioxidant status, and total oxidant status were spectrophotometric methods.
Results: Serum chemerin levels were higher in the subclinical hypothyroidism group, whereas lower in the subclinical hyperthyroidism compared to the controls. Vaspin levels of subclinical thyroid patients were lower than the controls. Interleukin-10 levels of subclinical hyperthyroidism were lower, conversely C-reactive protein levels were higher in both patient groups than the control group. Total antioxidant status were higher in the subclinical hypothyroidism group; total oxidant status and oxidative stress index were lower in subclinical hyperthyroidism patients.
Conclusion: Increased total antioxidant and C-reactive protein levels in the subclinical hypothyroidism group and decreased total oxidants, interleukin-10, and oxidative stress index in the subclinical hyperthyroidism group indicated that oxidant-antioxidant balance is impaired, suggesting that subclinical thyroid diseases may cause changes in inflammation and defense mechanism. The decreases in chemerin levels in the subclinical hyperthyroidism patients and vaspin levels in the both patient groups show that chemerin and vaspin may be candidates as biomarkers in subclinical thyroid diseases.

Keywords: Chemerin, vaspin, oxidative stress markers, inflammation markers, subclinical hypothyroidism/hyperthyroidism

INTRODUCTION

Thyroid hormones have functions on metabolic rate, heat production, cell differentiation and development, response to other hormones, carbohydrate, protein, and lipid metabolism (1). Thyroid-stimulating hormone (TSH) directly provides the synthesis and secretion of adipokines. The positive relationship between TSH level and adiposity is biologically important. Clinical studies reveal changes in some adipokine levels accompanying thyroid diseases (2,3). The most recently discovered chemerin, vaspin are members of adipokines this group. Chemerin is

encoded by the G protein-coupled receptor 1 (GPR1) gene and, acts as an endogenous ligand for GPR and is known to have pro-inflammatory and insulin resistance inducing properties. It was found that physiological amounts of chemerin are secreted from adipose tissues in early adipocyte differentiation, and the level of secreted chemerin increases with the maturation of cells. Another adipokine synthesized in adipose tissue, vaspin is known for its insülin-sensitizing effects and modulator role on glucose tolerance. There are limited studies in the literature investigating the role of serum levels of chemerin and vaspin in thyroid diseases. Serum chemerin levels were suggested to be significantly higher in patients with hyperthyroidism compared to controls (4). Vaspin mRNA levels were shown as significantly lower in rats with hyperthyroidism while higher in rats with hypothyroidism compared to rats with euthyroidism (5).

Cytokines are pro-inflammatory mediators which play a central role in inflammatory and immune processes. Interleukin-10 (IL-10) is recognized as one of the most important anti-inflammatory immunomodulatory cytokines. It has been reported that IL-10 mRNA expression has an important role in thyroid autoimmune diseases (6). C-reactive protein (CRP) is involved in inflammatory processes in some thyroid disorders. Serum CRP values were shown as high in patients with subclinical hypothyroidism (SubHypo) compared to controls (7). Oxidized LDL (Ox-LDL) has vasoconstrictor, mitogenic, and proinflammatory properties. It has been reported that dysfunction of thyroid hormones may cause an increase in Ox-LDL levels (8). Therefore, it is essential to specify the possible risk factors for increased Ox-LDL. Recent studies have suggested that Ox-LDL may trigger endoplasmic reticulum stress in endothelial cells and macrophages (9), and this may have an effect on the secretion of some adipokines (10). Measurement of the Ankle Brachial Index (ABI) is the most used method in diagnosing peripheral artery disease (PAD). There are few studies in the literature to explain the relationship between thyroid dysfunction and PAD determined by ABI measurement (11).

Metabolic changes accompanying thyroid dysfunction may cause changes in the antioxidant defense of the organism. Cebeci et al. found reduced antioxidant defense in SubHypo patients (12). Supportively, a decrease in serum antioxidant activity was observed due to the decrease in body antioxidant defense system capacity in patients with hyperthyroidism by Marcocci et al. (13). Total antioxidant status (TAS), total oxidant status (TOS) and Oxidative Stress Index (OSI) are among the critical parameters that can be used to assess the redox status. OSI is an indicator of the degree of oxidative damage. Several studies have indicated that OSI is responsible for the formation of endothelial dysfunction (14,15).

In the light of these informations, it is crucial to investigate the roles of some new adipokines, proinflammatory, and oxidant/antioxidant indicators in the progression of subclinical thyroid to clinical thyroid diseases. So, it was aimed to measure chemerin, vaspin, IL-10, CRP, Ox-LDL, ABI, TAS, TOS, and OSI in subclinical hyperthyroidism (SubHyper) and SubHypo.

MATERIAL AND METHODS

Characteristics of Study Participants

The study group consisted of 38 SubHyper, 31 SubHypo patients, and 44 healthy individuals. The patient group consisted of applied to the Thyroid Clinic of Gazi University Medical Faculty Hospital, were over the age of 18, were newly diagnosed, and did not receive treatment. Patients were excluded from the study if they had a history of diabetes, chronic hyperlipidemia, Chronic Artery Disease (CAD), or PAD. The control group composed of healthy volunteers had never transmitted a systemic and/or thyroid disease.

Ethical Consideration

The study was approved by the Ethics Committee of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital with decision number 2017-03/06, and all participants or their relatives gave their informed consent before participating in the study.

Anthropometric Measurements

Body weight was measured with a TANITA weighing device while hungry in the morning and after defecation with less clothed, dry, and bare feet. The height of individuals was measured with a TANITA brand portable wall stadiometer with feet side by side and head on the Frankfort plane, with eye triangle and top of the auricle aligned. Body Mass Index (BMI) was calculated as weight (kg) divided by the square of height (m2). Waist circumference (WC) of individuals; with arms on both sides and feet together, the middle point of the area between the lowest rib and navel

Table 1. Characteristic features and biochemical measurements of the study group

Parameter	SubHyper (N=38)	SubHypo (N=31)	Control (N=44)
Age (X±SD)	46.37±2.28	44.39±2.55	45.41±1.95
Gender (F/M)	31/7	25/6	12/32
BMI (kg/m^2)	26.52±0.78	27.77±0.68	26.14±0.78
WC (cm)	79.94±1.98	92.16±2.49§	86.11±1.93
WC/HC (X±SD)	0.82±0.01	0.88±0.27	0.84±0.02
SBP (mmHg)	112.68±0.56	116.03±4.67*,§	113.77±0.59
DBP (mmHg)	77.24±0.80*	68.55±0.44*,§	72.95±0.89
FBG (mg/dL)	99.78±2.62*	93.47±1.80	91.95±1.98
TC (mg/dL)	146.07±7.29	168.71±7.29	162.77±6.16
HDL-C (mg/dL)	45.62±1.02	47.11±1.64	44.93±1.15
LDL-C (mg/dL)	110.24±4.53	111.73±6.01	104.40±4.64
TG (mg/dL)	148.91±10.04	127.73±8.99	140.12±11.73
fT$_3$ (pg/mL)	3.46±0.07**	3.26±0.05**	2.87±0.06
fT$_4$ (ng/dL)	0.93±0.02**	0.84±0.28**	1.18±0.04
TSH (mIU/mL)	0.20±0.02**	7.68±0.64**,§§	1.93±0.13

*Significant difference from control, p<0.05, **significant difference from control, p<0.01, §Significant difference from SubHyper, p<0.05, §§significant difference from SubHyper, p<0.01. SubHyper (Subclinical Hyperthyroidism), SubHypo (Subclinical Hypothyroidism), BMI (Body Mass Index), WC (Waist circumference), HC (Hip circumference), SBP (Systolic Blood Pressure), DBP (Diastolic Blood Pressure), FBG (Fasting Blood Glucose), TC (Total Cholesterol), HDL-C (HDL cholesterol), LDL-C (LDL cholesterol), TG (Triglycerides), fT$_3$ (Free Triiodothyronine), fT$_4$ (Free Thyroxine) TSH (Thyroid-stimulating hormone)

was determined and measured on a flat surface. Hip circumference (HC) was measured with the arms of the individuals on their sides, their feet side by side and standing upright, individual's gaze was directed towards the front and parallel to the ground by a non-stretch tape from the highest point. Blood pressure was measured two times intermittently with an Omron brand blood pressure device after all individuals had been in rest for at least 20 minutes in a sitting position and, the average of the two measurements was taken to determine the result. Limit values were determined according to NCEP ATP III diagnostic criteria.

Biochemical Measurements

5 ml of peripheral venous blood was taken from patient groups and the healthy controls into red-capped flat serum tubes with the help of professional health personnel. The blood samples were immediately transported to the Gazi University Faculty of Pharmacy Biochemistry Department Laboratory under appropriate storage conditions and centrifuged at +4ºC, 3000 rpm, for 15 minutes.

Serums were kept in deep freeze at -80ºC until to be analyzed. The biochemical parameters of fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), LDL cholesterol (LDL-C), and HDL cholesterol (HDL-C) were analyzed in Gazi Hospital Biochemistry Laboratory from the residue part of blood samples. FBG was measured with the hexokinase method and BeckmanCoulter kit, AU5800 analyzer (Beckman Coulter, CA, USA). TG, TC, LDL-C, and HDL-C analyses were performed by using the spectrophotometric method with the same device. TSH, free triiodothyronine (fT3), and free thyroxine (fT4) were measured by using a Beckman Coulter kit with the CLIA method (Abbott Architecht I200 autoanalyzer, USA).

Serum Chemerin/Vaspin/IL-10/CRP/Ox-LDL Concentrations

Human serum chemerin levels were measured spectrophotometrically using a commercial ELISA kit (Elabscience Biotechnology Co. Ltd, USA, E-EL-H0698) according to the manufacturer's instructions. The sensitivity of the chemerin ELISA assay is 0.10 ng/mL, and the detection range is 0.16-10 ng/mL. The intra-assay CV is <4.8%, and inter-assay precision is <5.3%.

Using a sandwich-ELISA (Elabscience Biotechnology Co. Ltd, USA, E-EL-H1762), vaspin levels in serum were measured. The detection range is 62.50-4000 pg/mL, and the sensitivity of the vaspin sandwich-ELISA assay is 37.50 pg/mL. The intra-assay CV is <5.3%, and inter-assay precision is <4.7%.

Detection of human IL-10 levels in serum was performed with an ELISA kit (Elabscience Biotechnology Co. Ltd, USA, E-EL-H0103). Kit sensitivity for IL-10 is 4.69 pg/mL, and the detection range is between 7.81 and 500 pg/mL. Intra-assay CV is <5.4%, and inter-assay precision is <4.7%.

Serum CRP concentrations were determined with a commercially available ELISA (Elabscience Biotechnology Co. Ltd, USA, E-EL-H0043) according to the manufacturer's instructions. Human CRP ELISA kit has 0.23 ng/mL sensitivity with a detection range of 0.39-25 ng/mL. Intra-assay CV is <3.9%, and inter-assay precision is <6.1%.

Commercial ELISA kits (Elabscience Biotechnology Co. Ltd, USA, E-EL-H0124) were used for the quantification of serum levels of Ox-LDL. All the tests were performed according to the manufacturer's instructions. Intra-assay CV is <5.3%, and inter-assay precision is <5.0%.

Measurements of ABI Value

The ABI value of participants was inflated to 220 mmHg by tying cuff to the left upper arm or 20-30 mmHg above expected Systolic Blood Pressure (SBP). Air of cuff was decreased 10 mmHg in 5 seconds by placing a Doppler device on the brachial artery, and SBP was recorded. Later, it was inflated to 220 mmHg by tying cuff under the left knee cap or 20-30 mmHg above the expected SBP. Doppler device was placed on the dorsalis pedis, and air of cuff was evacuated in 5 seconds with a decrease of 10 mmHg. Pulsed SBP was noted when a pulse was taken on a Doppler device. Two SBPs are divided with pressure in the arm at the top. The figure obtained has been noted as ABI (11). ABI Value=Ankle Systolic $Pressure_{(max)}$/Brachial Systolic $Pressure_{(max)}$.

Serum TAS/TOS/OSI Concentrations

TAS measurement in serum was performed according to the decolorization method of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radical modified by Re et al. (16). In the total antioxidant measurement method, suppression of the generated radical by antioxidants in serum was determined using a spectrophotometer. Absorbance measurements were done at 734 nm in 6th minute. The phosphate buffer solution was used in measurements as a blank solution.

10 µL of serum was added to 1 ml of ABTS radical solution whose absorbance was adjusted to be 0.70(±0.02), and absorbance was read at 6th minute. Based on the results, %Inhibition was calculated by the formula: $\%Inhibition=100–[(Abs_{sample}/Abs_{ABTS})x100]$

TOS measurement in serum was made according to the fully automatic colorimetric method developed by Erel et al. (17). Oxidants present in the sample oxidize ferrous ion-o-dianisidine complex to ferric ion. Glycerol in the environment accelerates this reaction approximately three times. Ferric ions form a colorful complex with xylenol orange in an acidic environment. The intensity of the color associated with the amount of oxidants in the sample is measured spectrophotometrically. By calibrating the measurement with hydrogen peroxide, results are expressed in micromolar hydrogen peroxide equivalent per liter (µmol H2O2 Equivalent/L) (17). OSI value was calculated as the ratio of TOS to TAS level. Specifically, OSI (arbitrary unit)=TOS (µmol H2O2 Eq/L)/TAS (µmol Trolox Eq/L).

Statistical Analysis

Statistical analysis of data was performed using the SPSS statistical package program (version 22 software, SPSS Inc. Chicago, Illinois, USA). The normality of the data was tested by Kolmogorov-Smirnov. Differences among the three groups were evaluated using one-way analysis of variance ANOVA followed by Post Hoc and Tukey test for multiple comparisons between groups. To evaluate the correlation between parametric and nonparametric variables, Pearson and Spearman correlation tests were used, respectively. Results were considered statistically significant at $p<0.05$ at 95% confidence interval.

RESULTS

The clinical characteristics and some biochemical parameters of the study group were given in Table. Statistical differences were found between SubHyper and control groups in Diastolic Blood Pressure (DBP), FBG, fT3, fT4, and TSH levels. DBP, FBG, and fT3 levels were significantly higher ($p<0.05$, $p<0.05$, $p<0.01$, respectively), while fT4, and TSH were lower in SubHyper when compared to control ($p<0.01$, $p<0.01$, respectively). Similarly, significant differences were found between SubHypo and control groups in SBP, DBP, fT3, fT4 and TSH levels. SBP, fT3, and TSH levels were higher ($p<0.05$, $p<0.01$, $p<0.01$, respectively), while DBP and fT4 were lower in SubHypo than control ($p<0.05$, $p<0.01$). SBP, WC,

and TSH levels were higher (p<0.05, p<0.05, p<0.01, respectively), whereas DBP was lower in SubHypo compared to SubHyper (p<0.05). No significant differences were found between groups in age, BMI, WC/HC, TC, HDL-C, LDL-C, and TG levels (p>0.05). Serum chemerin levels of SubHyper, SubHypo, and control groups are given in Figure 1. In the study, serum chemerin levels were found 2.58±0.14 ng/mL in SubHypo, 1.42±0.19 ng/mL in SubHyper, and 2.22±0.16 ng/mL in the control group. Serum chemerin levels were significantly lower in the SubHyper group compared to the control group (p<0.01). Chemerin levels in patients with SubHypo were found to be higher than patients with SubHyper (p<0.01). No significant difference was found between SubHypo, and the healthy control group in terms of serum chemerin levels (p>0.05). Figure 2

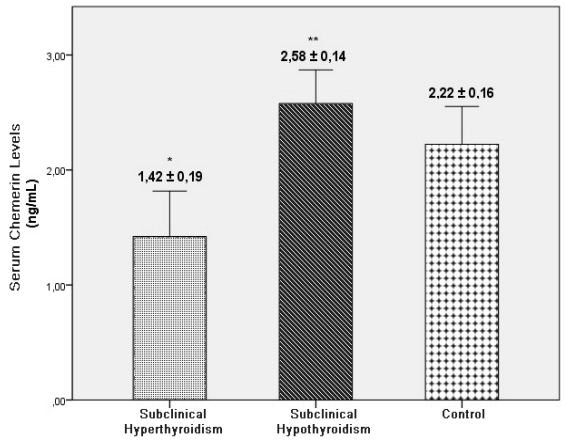

Figure 1. Comparison of serum chemerin levels in study groups. Boxes represent the means of the serum Chemerin levels and error bars indicate±standart deviation. Significant difference from control group and from SubHyper group were shown with an asterisk and double asterisks, respectively. The value of p<0.05 was considered as statistically significant, in all statistical analyses. *Significant difference from control group, p<0.01. **Significant difference from SubHyper, p<0.01.

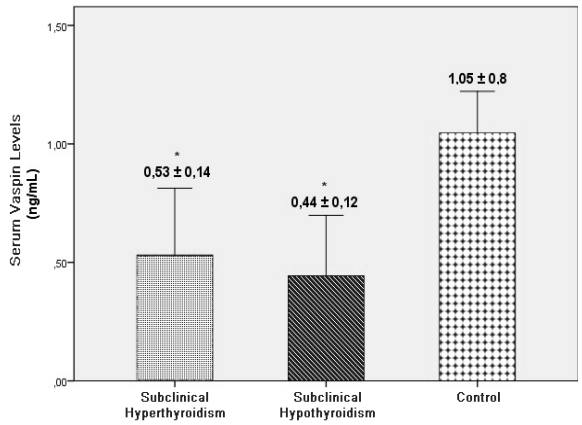

Figure 2. Comparison of serum vaspin levels in study groups. Boxes represent the means of the serum vaspin levels and error bars indicate±standart deviation. Significant difference from control group was shown with an asterisk. The value of p<0.05 was considered as statistically significant, in all statistical analyses. *Significant difference from control group, p<0.01.

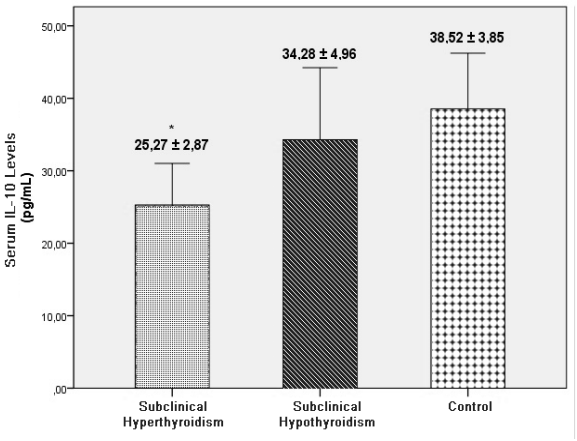

Figure 3. Comparison of serum IL-10 levels in study groups. Boxes represent the means of the serum IL-10 levels and error bars indicate±standart deviation. Significant difference from control group was shown with an asterisk. The value of p<0.05 was considered as statistically significant, in all statistical analyses. *Significant difference from control group, p<0.01, IL-10 (Interleukin-10).

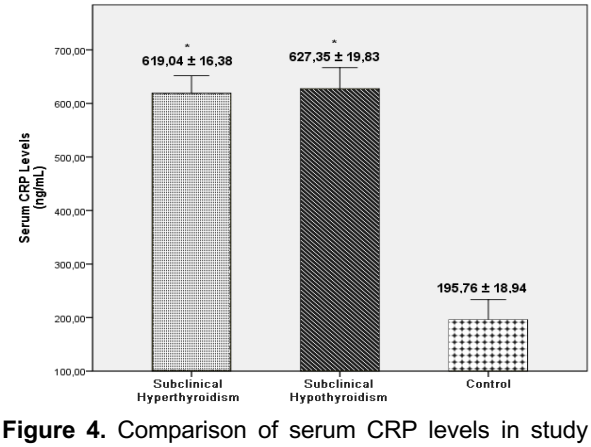

Figure 4. Comparison of serum CRP levels in study groups. Boxes represent the means of the serum CRP levels and error bars indicate±standart deviation. Significant difference from control group was shown with an asterisk. The value of p<0.05 was considered as statistically significant, in all statistical analyses. *Significant difference from control group, p<0.01, CRP (C-reactive protein).

shows serum vaspin levels in SubHyper, SubHypo and healthy control groups. Serum vaspin levels were found as 0.44±0.12 ng/mL in patients with SubHypo, 0.53±0.14 ng/mL in patients with SubHyper and, 1.05±0.8 ng/mL in the control group. Serum vaspin levels were found to be statistically lower in both patients with SubHypo and SubHyper as compared to control ($p<0.01$). There was no significant difference in serum vaspin levels between SubHyper and SubHypo ($p>0.05$).

In Figure 3, serum IL-10 levels of SubHyper, SubHypo, and control groups are given. Serum IL-10 levels were found to be 34.28±4.96 pg/mL in SubHypo patients, 25.27±2.87 pg/mL in SubHyper patients, and 38.52±3.85 pg/mL in controls, respectively. Serum IL-10 levels were found lower in SubHyper patients compared to the control group. There was no significant difference in serum IL-10 levels between SubHypo and healthy control ($p>0.05$), SubHyper and SubHypo ($p>0.05$). Serum CRP levels in SubHyper, SubHypo, and control groups are given in Figure 4. In our study, serum CRP levels were found as 627.35±19.83 ng/mL in SubHypo, 619.04±16.38 ng/mL in SubHyper, and 195.76±18.94 ng/mL in the control group. Serum CRP levels were found to be importantly higher in patients with SubHypo and SubHyper compared to controls ($p<0.01$).

In Figure 5, serum Ox-LDL levels are given in study groups. Serum Ox-LDL levels were found to be 8.33±0.11 ng/mL in patients with SubHypo, 8.27±0.66 ng/mL in patients with SubHyper, and 8.34±0.13 ng/mL in the control group, respectively. There was no difference between all groups in serum Ox-LDL levels ($p>0.05$).

Figure 6 shows the ABI values in SubHyper, SubHypo, and healthy control groups. ABI values were found 1.055±0.02 in SubHypo, 1.020±0.02 in SubHyper, and 1.040±0.02 in the control group, in our study. No statistically significant difference was observed between the groups in terms of ABI values ($p>0.05$).

Figure 7 demonstrates serum TAS levels in study groups. Serum TAS levels were 2.27±0.26 mmol/L in SubHypo, 2.20±0.58 mmol/L in SubHyper, and 2.07±0.57 mmol/L in the control group, respectively. Serum TAS levels were significantly higher in patients with SubHypo compared to control ($p<0.01$). Although TAS levels of patients with SubHyper were higher than the control group, were not significant ($p>0.05$). The comparison of TOS levels in SubHyper, SubHypo, and healthy controls were given in Figure 8. TOS levels were found 10.43±0.63 µmol/L in patients with SubHypo, 4.44±0.31 µmol/L in patients with SubHyper, and 9.90±0.52 µmol/L in the control group, respectively. Serum TOS levels were found to be significantly lower in patients with SubHyper compared to the control group ($p<0.01$). There was no statistically significant difference between SubHypo and healthy control groups in terms of serum TOS levels ($p>0.05$). Figure 9 shows the serum OSI values in SubHyper, SubHypo, and healthy control groups. Serum OSI values were calculated as 4.58±0.26 in patients with SubHypo, 2.00±0.15 in patients with SubHyper, and 5.06±0.36 in the control group, respectively. Serum OSI values were significantly lower in patients with SubHyper compared to controls ($p<0.01$). No statistically significant difference was obtained between SubHypo and healthy control groups in terms of serum OSI values ($p>0.05$).

Using bivariate correlation analysis among measured parameters in patients with SubHyper, a positive correlation was found between TOS and OSI ($r=0.887$, $p<0.01$), and a negative correlation was found between IL-10 and TOS ($r=-0.318$, $p<0.05$). Positive correlation was found between TOS and OSI ($r=0.978$, $p<0.01$), and negative correlations were found between IL-10 and Ox-LDL ($r=-0.470$, $p<0.01$) in patients with SubHypo. In healthy controls, positive correlations were found between vaspin and TOS ($r=0.303$, $p<0.05$), Ox-LDL and TOS ($r=0.344$, $p<0.05$), TOS and OSI ($r=0.479$, $p<0.01$), and negative correlations were found between IL-10 and Ox-LDL ($r=-0.439$, $p<0.01$), IL-10 and TOS ($r=-0.395$, $p<0.01$), TAS and OSI ($r=-0.685$, $p<0.01$).

DISCUSSION

There is a tight interaction between thyroid hormones and adipokines (3). Clinical studies have revealed changes in apelin, adiponectin, and leptin adipokines accompanying thyroid disorders (2,3,18). The pathophysiological role of thyroid hormones regulating chemerin and vaspin in subclinical thyroid diseases has not been clarified yet. Thus, we mainly aimed to demonstrate the clinical significance of chemerin and vaspin adipokines in SubHyper and SubHypo. Additionally, serum levels of IL-10, CRP, Ox-LDL, TAS, and TOS were measured to show the changes in inflammation and oxidative stress in subclinic thyroid disorders.

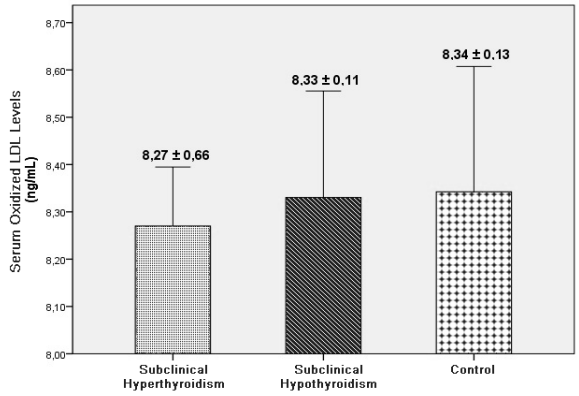

Figure 5. Comparison of serum Ox-LDL levels in study groups, Ox-LDL (Oxidized LDL). Boxes represent the means of the serum Ox-LDL levels and error bars indicate±standart deviation. The value of p<0.05 was considered as statistically significant, in all statistical analyses.

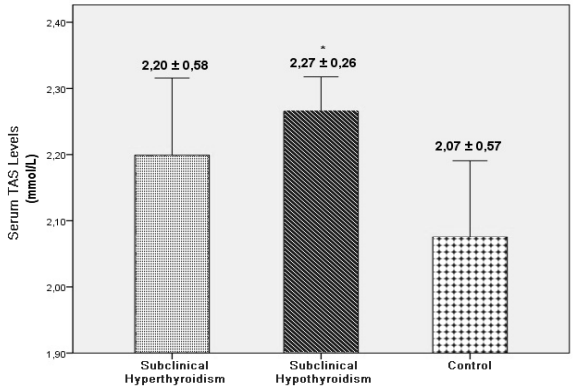

Figure 7. Comparison of serum TAS levels in study groups. Boxes represent the means of the serum TAS levels and error bars indicate±standart deviation. Significant difference from control group was shown with an asterisk. The value of p<0.05 was considered as statistically significant, in all statistical analyses. *Significant difference from control group, p<0.01, TAS (Total Antioxidant Status)

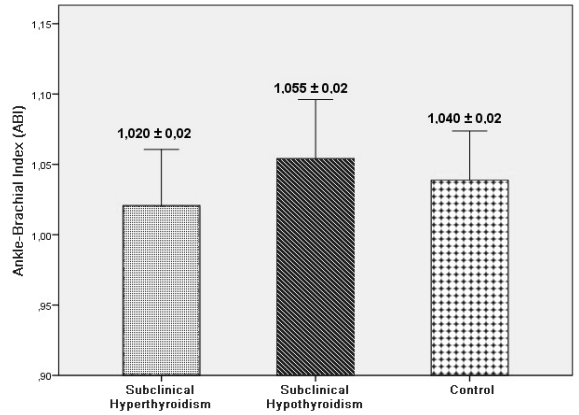

Figure 6. Comparison of serum ABI levels in study groups, ABI (Ankle Brachial Index). Boxes represent the means of the serum ABI levels and error bars indicate±standart deviation. The value of p<0.05 was considered as statistically significant, in all statistical analyses.

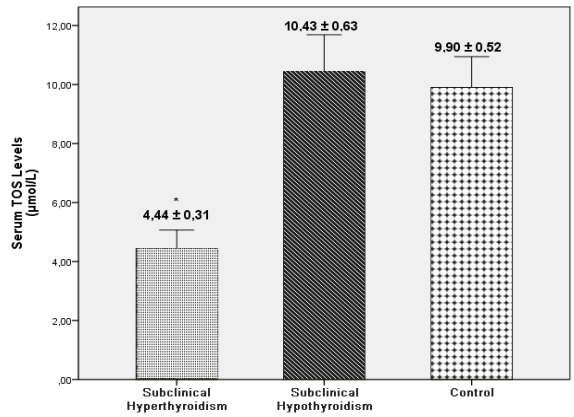

Figure 8. Comparison of serum TOS levels in study groups. Boxes represent the means of the serum TOS levels and error bars indicate±standart deviation. Significant difference from control group was shown with an asterisk. The value of p<0.05 was considered as statistically significant, in all statistical analyses. *Significant difference from control group, p<0.01, TOS (Total Oxidant Status).

Many inflammatory molecules circulating in the blood are might be a potential markers. In many studies conducted in recent years, it has been shown that human plasma chemerin levels have a significant relationship with BMI, inflammation, and metabolic syndrome (19,20). Nevertheless, there are limited studies in the literature investigating the role of serum levels of chemerin and vaspin in thyroid diseases. Berta et al. evaluated serum chemerin levels in patients with Hashimoto's Thyroiditis (HT) and found no differences between patients and controls. It was also reported by the authors that no correlation was observed between chemerin and TSH, fT_3, and fT_4 levels (21). On the other hand, Alshaikh et al. stated that serum chemerin levels were higher in patients with hyperthyroidism compared to controls. Moreover, serum chemerin concentrations were positively correlated with total triiodothyronine (TT_3), total thyroxine (TT_4), and fT_3 and negatively correlated with TSH and fT_4 (4). In a study conducted on rats with experimentally induced thyroid dysfunction, a significant increase in chemerin was

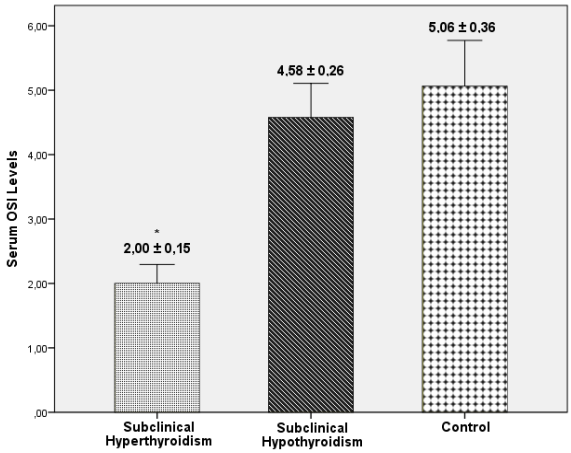

Figure 9. Comparison of serum OSI values in study groups. Boxes represent the means of the serum OSI levels and error bars indicate±standart deviation. Significant difference from control group was shown with an asterisk. The value of $p<0.05$ was considered as statistically significant, in all statistical analyses. *****Significant difference from control group, $p<0.01$, OSI (Oxidative Stress Index).

observed in clinical hypothyroidism, while a decrease in hyperthyroidism. Additionally, a positive correlation was found between chemerin and TSH in hyperthyroidism, hypothyroidism, and healthy control groups. They indicated that chemerin may be a marker in thyroid hormone disorders since experimentally induced thyroid dysfunction affects chemerin (22). Similarly, higher serum chemerin levels were found in three groups of SubHypo rats administered increasing doses of methimazole compared to euthyroid rats. Additionally, it has been reported that there is a positive relationship between chemerin levels and TSH in study groups (23). In another study with different doses of methimazole, gene expression of chemerin and serum levels were measured. Compatible results with the previous studies in terms of serum chemerin levels and expressions were obtained (24). Considering the literature, there are very few clinical studies examining the relationship between chemerin and thyroid diseases. Our study was the first to investigate chemerin levels in SubHyper and SubHypo cases. In the study, serum chemerin levels were significantly higher in patients with SubHypo compared to patients with SubHyper. Serum chemerin was significantly lower in patients with SubHyper compared to healthy controls. Although there were higher chemerin levels in SubHypo patients compared to healthy controls, no significant difference was found.

Higher/lower serum vaspin levels were obtained in hypothyroidism/hyperthyroidism compared to the control group in the study of Jowari et al.. They thought that these changes in vaspin levels may be due to the effect of thyroid hormone disorder (25). Çınar et al. analyzed serum vaspin in clinical hypothyroidism, SubHypo patients, and euthyroid healthy controls. They found higher serum vaspin levels in SubHypo and clinical hypothyroidism compared to controls, but there were no significant differences between groups. Also, there was no correlation between vaspin levels and TSH (26). Salam et al. examined the effects of experimentally induced hyperthyroidism and hypothyroidism on vaspin, adiponectin, and visfatin levels in rats. They reported increased/decreased vaspin levels in hypothyroidism/hyperthyroidism compared to controls. In addition, serum vaspin levels were found negatively correlated with T_3 and T_4 levels and positively correlated with TSH levels in all study groups. They concluded that the decrease in vaspin production in hyperthyroid rats might be due to the dominant effect of T_3 on adipocytes (27). Likewise, Gonzalez et al. stated that vaspin mRNA levels were significantly decreased in rats with hyperthyroidism compared to rats with euthyroidism and increased significantly in rats with hypothyroidism although there was no change in glucose and insulin levels, and this was explained as thyroid dysfunction may affect vaspin expression (5). In our study, serum vaspin levels were found to be significantly lower in patients with SubHypo and SubHyper compared to the control group. While the data obtained from our study are consistent with the results obtained by Salam et al., Jowari et al., and Gonzalez et al. in terms of decreasing vaspin levels in the hyperthyroid model, decreasing serum vaspin levels in patients with SubHypo were first shown.

IL-10 is a mediator that regulates the systemic inflammatory response. It has an important role in autoimmune thyroid diseases and regulates the growth and development of both normal and neoplastic thyroid cells (28). CRP, which is a nonspecific marker of inflammation, is synthesized by the liver. The changes in serum CRP levels have not been routinely used to monitor thyroid disease, whereas inflammation occurs in many thyroid diseases. In a study conducted by Marchiori et al., serum CRP and IL-10 levels were measured at the end of 6 and 12 months to examine the changes in some inflammatory markers in hypothyroid patients

receiving levothyroxine treatment. Researchers found an increase in serum IL-10 levels in measurements after 6 and 12 months of levothyroxine treatment, but no significant difference was observed in CRP over time. They postulated that the changes in cytokine levels may be due to thyroid hormones (29). The serum concentration of IL-10 levels was measured in patients with extreme obesity in a study performed by Gómez-Zamudio et al. (30). For analyzing the effect of the presence of hypothyroidism on IL-10, patients with hyperthyroidism were excluded from the study, and euthyroid and hypothyroidism patients were compared. They reported that serum IL-10 levels did not differ in patients with hypothyroidism compared to euthyroid individuals (30). Tuzcu et al. aimed to determine CRP concentrations in SubHypo. It was shown that serum CRP values were higher in patients with SubHypo compared to the control group (7). According to the study of Kvetny et al. SubHypo was associated with higher concentrations of CRP (31). In our study, significantly decreased serum IL-10 levels were obtained in patients with SubHyper compared to the control group. This decline in IL-10 levels may be related to the increased antioxidant defense mechanism in patients with SubHypo. Serum CRP levels were found as statistically high in patients with both SubHypo and SubHyper compared to controls. Serum CRP results observed in SubHypo from our study are consistent with studies of Tuzcu et al. and Kvetny et al. We think that the increased serum CRP levels in the study are due to the increased inflammation in both subclinical groups.

Many studies to date have demonstrated that high oxidative stress supports oxidative modification of LDL levels. There are studies showing that excessive secretion of thyroid hormones may cause an increase in Ox-LDL levels (8,32). Itterman et al. measured the Ox-LDL levels of participants to determine the relationship between serum TSH and Ox-LDL levels and noted a positive relationship between them. They also suggested that Ox-LDL levels elevated with the increase in serum TSH levels, especially in the range of subclinical thyroid disease (33). Uçan et al. measured serum Ox-LDL before and after thyroid hormone therapy in HT patients and controls. They found no difference in serum Ox-LDL levels between patient and control groups (34). In another study, plasma Ox-LDL levels were evaluated in SubHypo, clinical hypothyroidism, and healthy individuals. It was reported that plasma Ox-LDL levels were significantly higher in clinical hypothyroid patients compared to the control group. They also found that plasma Ox-LDL was higher in SubHypo patients compared to controls but not significant. It was suggested that thyroid hormone deficiency affects Ox-LDL levels (35). In our study, there was no significant difference in serum Ox-LDL levels among individuals with SubHyper, SubHypo, and control. Our results are consistent with data obtained from the study of Uçan et al. and Duntas et al.

There are few studies in the literature investigating the relationship among thyroid dysfunction, ABI measurement, and PAD. In a study by Ittermannet al., ABI values were measured, and no significant relationship was found between TSH concentrations and ABI values (11). Compatible with this study, no significant difference was found between the groups in terms of ABI values in our study.

In thyroid diseases, TAS plays an essential role in protecting the organism from damage caused by oxidative stress and is an important parameter that can be used to assess redox status with TOS and OSI. In a study involving SubHypo and healthy individuals, TAS, TOS, and OSI were determined. TAS and OSI were found higher in SubHypo patients compared to controls. They reported that OSI showed a strong positive correlation with TSH levels in both groups (12). In another study, Cheserek et al. examined oxidative stress status in SubHypo and euthyroid individuals. Although studies detected higher serum levels of TAS in SubHypo patients compared to the control group, they did not find a significant difference between the groups (36). Ateş et al. studied the association between HT and oxidative stress parameters in euthyroid, subclinical, and overt hypothyroid stages. It was noted that TOS and OSI levels were higher, while TAS levels were lower in the overt hypothyroid group, whereas it was similar in control, euthyroid, and SubHypo groups. Also, it was shown that TOS and OSI were higher in SubHypo compared to controls, while TAS levels were similar in these groups (37). Aslan et al. evaluated the oxidative status of patients with hyperthyroidism and healthy controls. Serum TAS levels were found significantly lower in the hyperthyroidism group than controls, while serum TOS and OSI levels were significantly higher (38). In our study, serum TAS levels were found significantly higher in the SubHypo group compared to controls. While our results are compatible with the results of Cebeci et al. (12), there is a study with opposing-view

by reporting that there is no significant difference between groups (36). Changes in serum TAS levels in SubHypo may have occurred due to increased oxidative stress in SubHypo. We found that TOS and OSI were found to be significantly lower in SubHyper patients as compared to healthy controls. These controversial results in the study obtained by Aslan et al. may be due to increased oxidative stress.

Limitations

There is no doubt that the limitations of the original research were insufficient sample size, so these findings should be confirmed with further studies, especially with a larger population.

CONCLUSION

In conclusion, our study is the first in the literature to investigate the levels of serum chemerin and vaspin adipokines in subclinical thyroid diseases in humans. Although the conditions that affect the parameters evaluated in the study were excluded in the selection of the patient group, decreases were observed in Subhyper in serum chemerin levels and in both Subhyper and Subhypo in vaspin levels. CRP levels increased in both groups in subclinical thyroid patients as expected due to inflammation, while IL-10 levels decreased only in the Subhyper group. High TAS levels in the subhypo group and low TOS and OSI levels in the subhyper group indicate that the oxidative balance is impaired in subclinical thyroid diseases.

It is thought that new studies to be conducted with more patients in which serum chemerin and vaspin levels are investigated will contribute to evaluating the diagnosis and prognosis of thyroid diseases in which many factors participate in the etiology.

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