

Evaluation of oxidative stress, the activities of paraoxonase and arylesterase in patients with subclinic hypothyroidism

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Abstract. *Introduction:* In subclinical hypothyroidism (SH), serum lipid and lipoprotein concentrations are frequently changed. Compared to the normal population, the levels of oxidized low-density lipoprotein (LDL) cholesterol are higher and the levels of high density lipoprotein (HDL) cholesterol are lower. In SH patients, the mechanism of atherosclerosis may be attributed to the lipid abnormalities. There is evidence showing that, oxidation plays an important role during the process of atherosclerosis, preventing the lipid peroxidation of paraoxonase 1 and thereby, acting against the atherosclerosis. In this study, we evaluated the activity of paraoxonase and arylesterase in subclinical hypothyroidism and investigated its relation with oxidative stress. *Method:* The study enrolled 25 cases with SH and 20 healthy controls. The patient group and the control group were compared in terms of the activity of paraoxonase and arylesterase and the oxidative stress index. *Results:* Between two groups, no significant difference was found in terms of age, gender, total cholesterol, low-molecular weighted lipoprotein, high-molecular weighted lipoprotein. In SH group, the activity of paraoxonase was significantly lower than that observed in the control group ($p=0.01$). Also, the activity of arylesterase was significantly lower in the group with subclinical hypothyroidism ($p=0.03$). Oxidative stress index was found to be significantly higher in the group with subclinical hypothyroidism compared to the healthy controls ($p<0.01$). Oxidative stress index showed a strong positive correlation with the levels of TSH in all cases ($r=0.60$, $p<0.01$). *Conclusion:* Consequently, in SH, the activity of paraoxonase and arylesterase were significantly low and oxidative stress was significantly high. Lower activities of paraoxonase and arylesterase indicated increased oxidative damage in SH. This may be useful to elucidate the mechanism of atherosclerosis in SH. In addition, these findings suggested that the activities of paraoxonase and arylesterase may be used for the determination of therapeutical response and during the follow-up. (www.actabiomedica.it)

Key words: subclinical hypothyroidism, paraoxonase, arylesterase, atherosclerosis, oxidative stress

Introduction

Subclinical hypothyroidism (SH) is the most commonly seen thyroid dysfunction and is defined as the presence of high levels of thyroid stimulating hormone (TSH) when the values of free triiodothyronin (fT3) and free thyroxin (fT4) are within normal range. 30% of the patients which are asymptomatic may show symptoms suggesting the thyroid hormone defi-

ciency. The most marked complaints of the patients are dry skin, weakening of memory and thinking, weakness of muscles, fatigue, muscular cramps, swollen eyes, cold intolerance, constipation and deep voice (1, 2). Definitive insights about the therapeutic modalities are still lacking.

SH is accompanied by left and right ventricular systolic and diastolic dysfunction, increased atherosclerosis and myocardial infarction risk. In the patients

with SH, mechanism of atherosclerosis may be attributed to the lipid abnormalities (1, 3-5).

In thyroid disorders, serum lipid and lipoprotein concentrations are commonly changed. Compared to the normal population, the levels of oxidized low density lipoprotein cholesterol (LDL) are higher and the levels of high density lipoprotein cholesterol (HDL) are lower (1, 3-5). HDL prevents the oxidative changes in LDL. It is reported that, paraoxonase 1 (PON 1), which is an antioxidant enzyme present in the structure of HDL, contributes to the protective effect of HDL, by preventing the LDL oxidation (6, 7). PON 1 is associated with the apolipoprotein A-1 (apoA-1) and apolipoprotein J (apoJ) proteins of HDL (8). ApoA-1 is the structural protein of HDL and stabilizes the linking of PON 1 to the HDL phosphoriles by N-terminal ending. It was suggested that, lower PON1 serum activity despite the normal levels of HDL would lead to a decreased protective effect of HDL against the oxidation of LDL and thereby, to an increased incidence of atherosclerosis. PON 1 is an ester hydrolase that has both arylesterase (ARE) and PON activities (9). As PON, for whom the physiological substrates have not been defined yet, catalyses the hydrolysis of the organophosphate compounds frequently used for the production of insecticide and sarin, it is especially important for the toxicologic studies and for the in vivo xenobiotic metabolism studies. On the other hand, following the determination of the presence of PON in the structure of HDL in the plasma, the studies investigating the physiological functions of PON are gradually increasing. The importance of PON for the cardiovascular physiology, the relation of PON with lipid and lipoprotein metabolism, its potential anti-atherogenic effect and the antioxidant characteristics against peroxidative damage are extensively investigated.

Our aim is to evaluate the activities of PON and ARE in patients with SH and to investigate the status of serum total antioxidants and total oxidative status to see whether there is a relation between PON/ARE activity and oxidative stress. Moreover, we aimed to determine whether PON/ARE activity and total antioxidant status contribute to the development of atherosclerosis in the patients with SH.

Material and method

This study was prospectively designed. The study protocol was approved by the local ethic board of our hospital. Before performing any procedure, all the patients were extensively informed about the study and signed "informed consent form".

From the patients between 18-65 years that admitted to our hospital for various reasons, the patients with SH for whom medical history, physical examination and clinical and laboratory investigations were performed; who were newly diagnosed and who had never received medical therapy until that time were enrolled to the study. Our study group consisted of 25 cases with SH (37±11 years, 72% female) and 20 age and sex-matched healthy subjects (35±9 years, 60% female).

The exclusion criteria were defined as follows: age below 18 or above 65, morbid obesity (body mass index > 35 kg/m²), hyperlipidemia, hypertension, diabetes mellitus, malignancy, hepatic disease, acute or chronic infections, renal failure, autoimmune disease, cardiovascular disease, anemia and smoking.

According to the criteria of the biochemistry laboratory of the hospital, normal serum levels were considered as follows: TSH: 0,27-4,20 mIU/mL, fT3: 1,80-4,60 pg/ml, fT4: 0,93-1,70 ng/dl. Cases with SH were defined as having a TSH level above 4.20 mIU/ml and fT4 level within the normal range. Blood samples were collected from all patients after a 8-12 hours fasting period. For the blood samples for which the serum part would be separated, the tubes with gel and red cap were used. To separate the serum and the plasma, the blood taken into biochemistry tubes were centrifuged at 3000 rpm during 10 minutes. Serum samples were aliquoted and then stored at -80°C.

Systolic and diastolic arterial blood pressures of all the cases were measured using an aired manometer from right brachial artery following a resting period of at least 5 minutes at lying position before the examination. Body weight was measured using a calibrated balance at 0.1 kg sensitivity with light clothes and without shoes. Height measurements were performed in standing position without shoes with a sensitivity of 0.01 m. BMI was calculated by dividing the body weight to the square of the height in meter (kg/m²).

HOMA-IR (homeostasis model assessment of insulin resistance), was calculated using a formula defined by Matthews.

$$\text{HOMA-IR} = \frac{\text{fasting insulin level (microU/ml)} \times \text{Fasting glucose level (mmol/dl)}}{22,5}$$

Measurement of total oxidant level (Total Oxidant Status – TOS)

In the serum samples, TOS levels were investigated using a full-automatized method developed by Erel(10). According to this method, serum oxidants oxidize the complex of ferrous ion-o-dianisidine to ferric ion. Again, the existing molecules of glycerol accelerate the oxidation reaction. Ferric ions form a colored complex with xylenol orange in an acidic environment. The intensity of the color, which is related to the amount of the oxidants present in the serum sample, is measured at 37°C and at a wave length of 530 nm with spectrophotometry. As the calibration of the measurement is done using hydrogen peroxide, the results of the measurement are expressed as micromolar hydrogen peroxide equivalent in one liter ($\mu\text{mol H}_2\text{O}_2$ Equiv./L).

Measurement of Total Antioxidant Capacity (Total Antioxidant Capacity – TAC)

In the serum samples, TAC levels were investigated using a full-automatized method developed by Erel (11). According to this method, as a strong biological radical, ABTS^+ cation radicals are formed. ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] enters to a reaction with peroxidase and H_2O_2 and is oxidized to blue-green ABTS^+ cation radicals. Antioxidants of the serum sample convert the blue-green ABTS^+ cation radicals to the uncolored ABTS form and thereby, decrease the amount of environmental ABTS^+ cation radicals. The change of absorbance at 37°C and 660 nm will be associated with the total antioxidant capacity of the serum sample. As the calibration of the measurement was performed using stable antioxidant standard solution called Trolox Equivalent (an analogue of Vitamine E), the results of the measurement are expressed as $\mu\text{mol Trolox Equiv./L}$.

Evaluation of Oxidative Stress Index (Oxidative Stress Index – OSI)

OSI was used as an indicator of the oxidative stress grade in the patient and control groups. Calculation of OSI using the kits for TAC and TOS was performed according to the formula cited below:

$$\text{OSI (Arbitrary Unit)} = \left[\frac{\text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ equivalent/L})}{\text{TAC } (\mu\text{mol Trolox equivalent/L})} \right] \times 100.$$

Measurement of PON1 Activity

PON1 activity of the serum samples was examined using full-automatized method developed by Rel Assay Diagnostics® (Mega Tıp, Gaziantep, Turkey). According to this method, Paraoxonase activity is measured in medium without NaCl (basal paraoxonase activity) and with NaCl (salt-stimulated paraoxonase activity). Hydrolysis of the paraoxone (diethyl-p-nitrophenylphosphate) is monitored with the follow-up of the increase of absorbance at 37C and 412 nm. The amount of p-nitrophenol resulting from the hydrolysis is calculated using the molar absorption coefficient $17,000 \text{ M}^{-1}\text{cm}^{-1}$ (at pH 8). Net value with enzymatic activity is calculated by subtracting the basal activity value from salt-stimulated activity value. The results are expressed as Unit/Liter which is equal to the hydrolysis of 1 micromol substrate in one minute and one liter.

Measurement of PON 1 Arylesterase Activity

PON 1 arylesterase activity of the serum samples was measured using full-automatized method developed by Rel Assay Diagnostics® (Mega Tıp, Gaziantep, Turkey). According to this method, phenylacetate is used as a substrate for the measurement of arylesterase activity and, with the hydrolysis of phenylacetate, phenol and acetic acid are formed. The resulting phenol joins to 4-aminoantipyrine and potassium ferricyanide and is measured with colorimetric method. ARE enzyme activity is calculated from $4000 \text{ M}^{-1}\text{cm}^{-1}$ which is the molar absorption coefficient of the resulting colored complex. The results are expressed as Unit/Liter which is equal to the hydrolysis of 1 micromol phenylacetate in one minute and in one liter.

Statistical analysis

When evaluating the results obtained from the study, statistical analysis was performed using SPSS (Statistical Package for Social Sciences) for Windows 11.5 software. The variables were expressed as standard deviation and mean or median. The means were compared using Student T Test and median values were compared using Mann Whitney U Test. Data were compared with each other using Spearman correlation test. The incidences were compared using Chi-square and Fisher's Exact tests. In this analysis, $p < 0.05$ was considered as significant.

Results

The study group included 25 cases diagnosed with subclinical hypothyroidism (SCH) and 20 cases with normal test results of thyroid function. The groups formed had similar distribution in terms of age and gender. Of 25 cases of subclinical hypothyroidism, 18 were women and 7 were men. Of 20 healthy cases, 12 were women and 8 were men. The statistical difference between two groups was not significant ($p = 0,52$). Mean age was 37 ± 11 (range, 18 and

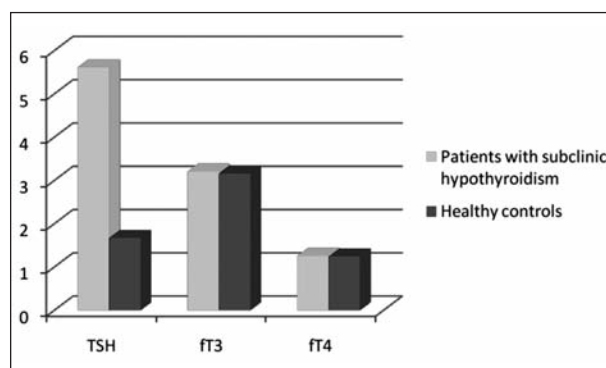


Figure 1. The thyroid function tests of patients and controls

54) in the cases of subclinical hypothyroidism and 35 ± 9 (range, 21 and 51) in the control group. The statistical difference between two groups was not significant ($p = 0,56$). Figure 1 shows the comparison of the thyroid function tests of the cases with subclinical hypothyroidism and healthy cases in our study.

The two groups did not show any significant difference in terms of age, gender, total cholesterol, LDL, HDL, urea, creatinine, HOMA-R, diastolic and systolic blood pressure and heart peak beat.

Paraoxonase activity was 135(82-195) in the group with subclinical hypothyroidism and 190 (124-

Table 1. Clinical-demographic characteristics of the patients and controls

	Subclinical hypothyroidism (n=25)	Healthy control (n=20)	P value
Age (years)	37 ± 11	35 ± 9	0,56 (not significant)
Total cholesterol (mg/dl)	184 ± 35	172 ± 27	0,47(not significant)
LDL (mg/dl)	112 ± 29	102 ± 22	0,32(not significant)
HDL (mg/dl)	50 ± 11	51 ± 10	0,78(not significant)
Triglyceride (mg/dl)	107 ± 37	85 ± 40	0,02(significant)
Paraoxonase (U/L)	135 (82-195)*	190 (124-355)*	0,01(significant)
Arylesterase (kU/L)	232 ± 33	255 ± 40	0,03(significant)
TAC ($\mu\text{mol Trolox equivalent/L}$)	$2,5 \pm 0,2$	$2,3 \pm 0,2$	$< 0,01$ (significant)
TOS ($\mu\text{mol H}_2\text{O}_2$ equivalent/L)	$5,9 (5,4-7,4)^*$	$3,9 \pm 0,8$	$< 0,01$ (significant)
OSI	$239 (194-303)^*$	174 ± 41	$< 0,01$ (significant)
Glucose (mg/dl)	85 ± 7	93 ± 4	0,01 (significant)
Urea (mg/dl)	23 ± 4	25 ± 5	0,15(not significant)
Creatinine (mg/dl)	$0,7 \pm 0,1$	$0,8 \pm 0,07$	0,67(not significant)
BMI (kg/m^2)	26 ± 2	23 ± 3	$< 0,01$ (significant)
HOMA-R	$1,8 \pm 0,8$	$1,5 \pm 0,5$	0,22(not significant)
Diastolic blood pressure (mmHg)	71 ± 8	71 ± 6	0,98(not significant)
Systolic blood pressure (mmHg)	111 ± 12	116 ± 9	0,13(not significant)
Heart's peak beat (beats/min)	76 ± 8	77 ± 7	0,73(not significant)

* Median values between 25-75% confidence interval

355) in the healthy control group. The difference of paraoxonase levels observed in two groups was statistically significant ($p = 0,01$). In the group with subclinical hypothyroidism, paraoxonase showed a slight correlation with total oxidant level ($r = 0.40, p=0.04$). In the group with subclinical hypothyroidism, paraoxonase did not show a correlation with HDL ($r = 0.18, p = 0.37$).

Arylesterase activity was 232 ± 33 in the group with subclinical hypothyroidism and 255 ± 40 in the healthy control group. The difference of ARE activities observed in two groups was statistically significant ($p = 0,03$). Arylesterase showed a moderate correlation with HDL levels in the healthy control group ($r = 0.49, p = 0.02$). ARE showed a slight correlation with triglyceride level in the patients with subclinical hypothyroidism ($r = 0.41, p = 0.03$). ARE showed a moderate correlation with BMI in the patients with subclinical hypothyroidism ($r = 0.58, p < 0.01$). ARE showed a slight correlation with glucose levels in all cases ($r = 0.35, p = 0.01$).

Oxidative stress index was 239 (194-303) in the patients with subclinical hypothyroidism and 174 ± 41 in the healthy controls. A statistically significant difference of OSI levels was observed between two groups ($p = <0,01$). OSI showed a strong correlation with TSH levels in all cases ($r = 0.60, p < 0,01$). OSI showed a strong correlation with TOS levels in all cases ($r = 0.94, p < 0,01$). OSI showed a moderate negative correlation with glucose levels in all cases ($r = -0.52, p < 0,01$).

Total oxidant level was 5.9 (5.4-7.4) in the patients with subclinical hypothyroidism and 3.9 ± 0.8 in the healthy controls. A statistically significant difference of TOS levels was observed between two groups ($p < 0,01$). TOS showed a strong correlation with TSH level in all cases ($r = 0.69, p < 0.01$). TOS levels showed a moderate correlation with total antioxidant capacity in all cases ($r = 0.46, p < 0.01$). TOS showed a moderate negative correlation with glucose levels in all cases ($r = -0.56, p < 0.01$).

Total antioxidant capacity was 2.5 ± 0.2 in the patients with subclinical hypothyroidism and 2.3 ± 0.2 in the healthy controls. A statistically significant difference of TAC levels was observed between two groups ($p < 0,01$). TAC showed a moderate correlation with urea levels in the healthy control group ($r = 0.54, p =$

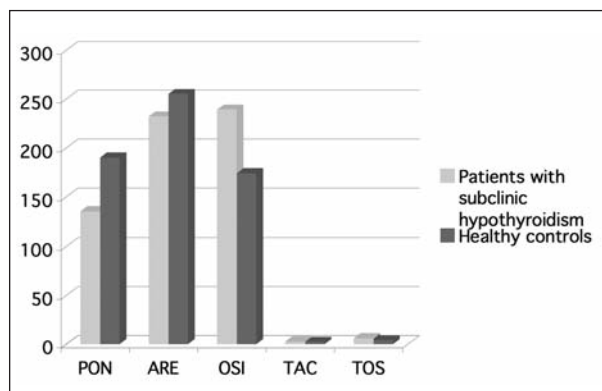


Figure 2. Comparison of paraoxonase activity, arylesterase activities, TAC, TOS, OSI values between the patients with subclinical hypothyroidism and the healthy cases

0.01). TAC showed a slight correlation with heart's peak beat in the group with subclinical hypothyroidism ($r = 0.41, p = 0.04$). TAC showed a slight correlation with TSH levels in all cases ($r = 0.43, p < 0.01$). TAC showed a slight correlation with triglyceride levels in all cases ($r = 0.44, p < 0.01$). TAC showed a moderate correlation with BMI in all cases ($r = 0.46, p < 0.01$).

Figure 2 shows the comparison of paraoxonase activity, arylesterase activity, TAC, TOS, and OSI values of the patients with subclinical hypothyroidism and the healthy cases.

Discussion

SH is the most commonly encountered thyroid dysfunction (12). In patients with SH, the mechanism of atherosclerosis may be attributed to lipid abnormalities (1, 3-5). While some studies revealed higher levels of serum cholesterol in the cases with SH, others showed no differences between SH patients and healthy controls (13-15). Subclinical hypothyroidism is a risk factor for atherosclerosis due to the increased levels of LDL cholesterol and to decreased levels of HDL cholesterol (1, 3-5). In some studies, it was shown that normal serum level of TSH has an inverse effect on the levels of serum lipoproteins (16-18). An increase of 1 mIU/ml in the level of serum TSH increases serum total cholesterol concen-

tration by 0.09 mmol/L (3.5 mg/dl) in women and by 0.16 mmol/L (6.2 mg/dl) in men (16). Due to these lipid abnormalities, subclinical hypothyroidism may be considered as a risk factor for atherosclerosis (19, 20). In the Rotterdam study (61), in the postmenopausal women, a strong correlation was found between subclinical hypothyroidism and atherosclerotic cardiovascular diseases, independent from classical risk factors as smoking, hypertension, hypercholesterolemia and diabetes mellitus. In the study performed by Başkol et al. (22), it was found that lipid peroxidation plays an important role in the pathogenesis of atherosclerosis developed during the hypothyroidism. Many investigators reported the protective effect of HDL against the oxidation of LDL in several studies. PON 1, an antioxidant enzyme found in the structure of HDL, contributes to the protective effect of HDL by preventing the oxidation of LDL (6,7). Durrington et al. (23), in the study in which they aimed to realize the hypothesis that states that PON 1 is responsible to destruct the lipid peroxides before the accumulation on LDL, observed that purified PON 1 is effective in the prevention of the lipid peroxidation of LDL. In the study performed by Aviram et al. (24), they observed that although PON 1 alone has a greater effect for the prevention of LDL oxidation compared to Lecithin Cholesterol acyl transferase (LCAT) and apo A1, the total effect of the combination of PON 1, LCAT and apo A1 was stronger than that of PON 1 alone. Platelet activator factor acetyltransferase (PAFAH) activity observed on LDL prevents coronary artery disease independently from the genetic effect. By contrast, PAFAH activity observed on HDL is not related to the dissociation of the enzymes like in LDL, but to PON 1 (25). It was reported that, if HDL has no PON 1 activity, PAFAH can't protect LDL against lipid peroxidation (9). In many studies, it was shown that HDL-dependent PON prevents not only LDL oxidation but also HDL oxidation (26). This effect is related to the ability of PON to hydrolyze the lipoprotein-mediated peroxides. It was suggested that a low PON1 serum activity despite the normal HDL levels would lead to a decrease in the protective effect of HDL against LDL oxidation and thereby, to an increase in the incidence of atherosclerosis.

During the acute phase reaction, a significant decrease was observed in the PON1 activity (27). Mackness et al. reported that serum PON1 activity and concentration was decreased within 2 hours following the onset of the symptoms of myocardial infarction, that the level of PON1 remained unchanged during 42 days following the myocardial infarction although the acute phase reaction was over, and that this decrease of PON1 activity might be pre-existing (28). These data support the idea that PON 1 may be playing a role in the atherosclerotic process.

In our study, we found that serum paraoxonase activity was significantly lower in the patients with subclinical hypothyroidism compared to control group ($p=0.01$). In the study performed by Başkol et al. (22), PON 1 activity was found to be lower in the pre-treatment patients compared to control group and post-treatment cases of hypothyroidism. Post-treatment mean PON 1 activity for hypothyroidism showed a significant increase but still remained significantly lower compared to control group. In the study conducted by Millionist et al. (29), PON 1 activity was found to be similar among the patients with subclinical hypothyroidism and control group. In another study performed by Azizi et al. (30), both the patients with hyperthyroidism and hypothyroidism showed a lower PON 1 activity compared to control group.

In our study, in addition, paraoxonase did not show any correlation with HDL in the group with subclinical hypothyroidism ($r=0.18$, $p=0.37$). In the study performed by Jayakumari et al. (31), in the healthy controls, while PON1 activity was positively correlated with HDL cholesterol, in the presence of coronary artery disease, they showed a negative correlation. However, in the study performed by enturk et al. (32), no correlation was found between PON 1 activity and HDL levels in the patients with acute coronary syndrome.

PON 1 is an ester hydrolase with both arylesterase and paraoxonase activities. Polymorphism has an effect on its ability to prevent the LDL oxidation. Polymorphism does not show an effect on the arylesterase activity, which is considered as the main indicator of protein concentration independently of the changes of PON 1 activity. In a study performed, it was shown that arylesterase activity of PON 1 was

decreased by approximately 50% during LDL oxidation. In our study, serum arylesterase activity was found to be significantly lower in the patients with subclinical hypothyroidism compared to control group ($p=0.03$). In the study conducted by Coria et al. (33), arylesterase activity did not show a difference among women with obvious hypothyroidism, subclinical hypothyroidism and euthyroidism. In the study performed by Şenturk et al. (32), serum arylesterase activity was lower in the patients with acute coronary syndrome. In the study performed by Gamboa et al. (34), in the Mexican patients with chronic heart disease, both paraoxonase and arylesterase activity were found to be significantly lower compared to control group.

As mentioned before, since PON1 enzyme prevents the oxidation of lipid peroxides considered as a major factor especially in atherosclerosis, it is a part of antioxidant defense system. PON hydrolyzes not only the lipoprotein-derived peroxides but also the hydrogen peroxide. Hydrogen peroxide is the main reactive oxygen species produced by arterial wall cells during the atherogenesis and, under oxidative stress, it is converted to reactive oxygen particle, which causes to LDL oxidation and passes to subendothelium easily. Hydrolyzation of hydrogen peroxide by PON is important in the elimination of potent oxidants that play a role in the atherosclerosis.

The knowledge about the mechanism by which hypothyroidism influence the oxidative stress is limited and conflicting and very few is known about oxidative stress observed in the subclinical hypothyroidism. In the study performed by Resch et al. (35), it was shown that both hyperthyroidism and hypothyroidism were associated with increased oxidative stress involving enzymatic and non-enzymatic antioxidants. In the study performed by Sarandöl et al. (36), increased oxidative stress was observed in subclinical hypothyroidism. In the study performed by Erdamar et al (37), an increase of the development of reactive oxygen species and of impaired antioxidant system in both the patients with hyperthyroidism and hypothyroidism. In the animal study performed by Venditti et al. (38), the rats with hypothyroidism showed an increased predisposition for oxidative stress in the heart and its muscles. In our study, we found significantly increased

oxidative stress compared to control group ($p<0.01$). In addition, oxidative stress index was significantly higher in the group with subclinical hypothyroidism compared to healthy controls ($p<0.01$).

While total antioxidant capacity provides information about all antioxidants existing in the organism, malonylaldehyde (MDA) is a lipid peroxidation marker used in the increased oxidative stress-related lipid peroxidation. In our study, we found significantly increased total antioxidant capacity in the group with subclinical hypothyroidism compared to control group ($p<0.01$). In the study performed by Torun et al. (39), it was investigated how hypothyroidism and subclinical hypothyroidism influence serum MDA and TAC and, as a result, MDA was found to be higher in the patients with hypothyroidism and subclinical hypothyroidism compared to control group but, TAC levels did not show a significant difference between the groups.

The genetic structure of PON 1 varies across the individuals, populations and environmental conditions. PON 1 is influenced by nutritional style. In many studies, the relation between the intake of antioxidant and atherogenic conditions was investigated (40, 41). In the previous studies, it was shown that serum PON activity was lower in obese patients. In the study performed by Ferretti et al. (42), it was shown that increased oxidative stress observed in the obese women might be associated with the decrease of PON1 activity. In another study performed by Tabur et al. (43), no significant difference was found between obese group and healthy control group in terms of PON/ARE activity. In our study, a significant difference was observed between the patients with subclinical hypothyroidism and the healthy cases in terms of BMI (26 ± 2 vs. 23 ± 3) ($p<0.01$). Therefore, although it is thought that obesity contributes to this result of decreased PON/ARE activity, the relation between obesity and PON/ARE is controversial.

Consequently, in the subclinical hypothyroidism, paraoxonase and arylesterase activities were significantly lower and oxidative stress was significantly higher. Again, in subclinical hypothyroidism, lower paraoxonase and arylesterase activities indicated increased oxidative damage. This may be useful to elucidate the formation mechanism of atherosclerosis in

subclinical hypothyroidism. Moreover, these results suggested that paraoxonase and arylesterase activities may be also used for the determination of therapeutic response and during the follow-up. Large, randomized, controlled studies with greater patient sample are warranted.

Limiting points of our study may be listed as follows: small number of patients; significantly higher BMI in the patient group; exclusion of coronary artery disease in our groups in terms of symptoms, anamnesis and electrocardiogram.

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