Original Article

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Antioxidant Status and Serum Prolidase Activity in Women with Subclinical Hypothroidism

Subklinik Hipotiroidisi Olan Kadınlarda Antioksidan Durum ve Serum Prolidaz Aktivitesi

Mehmet Ali Eren, Suzan Tabur, Ayşe Nur Torun, Turgay Ulaş*, Ömer Faruk Dağ*, Nurten Aksoy**, Tevfik Sabuncu

Harran University Faculty of Medicine, Department of Endocrinology and Metabolism, Şanlıurfa, Turkey
*Harran University Faculty of Medicine, Department of Internal Medicine, Şanlıurfa, Turkey
**Harran University Faculty of Medicine, Department of Clinical Biochemistry, Şanlıurfa, Turkey

Abstract

Purpose: Thyroid hormones are associated with the oxidant-antioxidant balance in the organism. Hypothyroidism is associated with impaired collagen turnover. We aimed to evaluate paraoxonase (PON) and arylesterase (ARE) activities, which prevent oxidative damage and serum prolidase activity (SPA) which is an important marker of collagen turnover in premenopausal women with subclinical hypothyroidism (SH).

Material and Method: Eighteen women with SH (study group) and 18 age-sex-and body mass index-matched controls (control group) were enrolled. PON, ARE and SPA were determined.

Results: PON and ARE activities in the study group were significantly lower than in the control group (149.5 (56.2) U/L, 210.5 (161.2) U/L for PON, respectively; p=0.034 and 198.9 (28.6) U/L, 227.0 (101.3) U/L for ARE, respectively; p=0.044). The study group had significantly higher SPA than the control group (670.0 (9.7) U/L, 664.6 (8.2) U/L, respectively; p=0.027). TSH levels positively correlated with SPA (r=0.404, p=0.015) and PON and negatively with ARE (r=-0.348, p=0.037; r=-0.329, p=0.050, respectively).

Discussion: Women with SH seem to have higher SPA and lower antioxidant enzyme activities when compared with healthy women which may cause an oxidant status in the organism. The underlying mechanisms and the significance of SPA in development of SH need to be further evaluated. *Turk Jem 2015; 19: 38-41*

Key words: Arylesterase, paraoxonase, serum prolidase activity, subclinical hypothyroidism

Conflicts of Interest: The authors reported no conflict of interest related to this article.

Özet

Amaç: Tiroid hormonları vücuttaki oksidan-antioksidan dengesi ile ilişkilidir. Hipotiroidizm bozulmuş kollajen döngüsü ile ilişkilidir. Bizde oksidatif hasardan koruyucu belirteçler olan paraoksonaz (PON) ve arilesteraz (ARE) düzeylerini ve kollajen döngüsünün önemli bir belirteci olan serum prolidaz aktivitesini (SPA) premenapozal subklinik hipotiroidili kadınlarda incelemeyi amaçladık.

Gereç ve Yöntem: Çalışmaya 18 subklinik hipotiroidili (SH) kadın (çalışma grubu) ve 18 yaş, cins ve vücut kitle indeksi uygun (kontrol grup) kadın alındı. PON, ARE ve SPA düzeyleri saptandı.

Bulgular: Çalışma grubunun PON ve ARE aktiviteleri kontrol grubuna göre anlamlı düşüktü (PON için sırasıyla 149,5 (56,2) U/L, 210,5 (161,2) U/L; p=0,034 ve ARE için sırasıyla 198,9 (28,6) U/L, 227,0 (101,3) U/L; p=0,044). Çalışma grubunda SPA kontrol grubuna göre anlamlı yüksekti (sırasıyla 670,0 (9,7) U/L, 664,6 (8,2) U/L; p=0,027). TSH seviyeleri SPA ile pozitif (r=0,404, p=0,015) ve PON ve ARE ile negatif (sırasıyla r=-0,348, p=0,037; r=-0,329, p=0,050) koreleydi.

Tartışma: SH'li kadınlar sağlıklı kadınlara göre organizmadaki oksidan durumdan kaynaklı olabileceği düşünülebilen daha yüksek SPA ve daha düşük antioksidan enzim aktivitelerine sahipti. Altta yatan mekanizmaların ve SH'nin gelişiminde SPA'nın önemini anlamak için daha kapsamlı çalışmalara ihtiyaç vardır. *Turk Jem 2015; 19: 38-41*

Anahtar kelimeler: Arilesteraz, paraoksonaz, serum prolidaz aktivitesi, subklinik hipotiroidizm

Çıkar Çatışması: Yazarlar bu makale ile ilgili olarak herhangi bir çıkar çatışması bildirmemiştir.

Address for Correspondence: Mehmet Ali Eren MD, Harran University Faculty of Medicine, Department of Endocrinology and Metabolism, Şanlıurfa, Turkey
Phone: +90 414 314 29 94 E-mail:drmalieren@hotmail.com **Received:** 07/07/2014 **Accepted:** 26/12/2014

Introduction

Thyroid hormones are associated with the oxidative and antioxidative status of the organism. Thyroid hormones play an important role in free radical production and regulate protein, vitamin and antioxidant enzyme synthesis and degradation (1). There are contradictory reports on the existence of oxidative stress in hypothyroidism; some studies have reported that hypothyroidism was expected to slow the free-radical generation due to lower metabolic rate (1,2), however, the others have shown an increased oxidative stress in overt and subclinical hypothyroidism (SH) (3,4,5,6).

Human serum paraoxonase (PON)-1 is a high-density lipoprotein (HDL)-bound enzyme that has both arylesterase (ARE) and PON activities and protects low-density lipoprotein cholesterol (LDL-C) and HDL cholesterol (HDL-C) against oxidative damage (7,8). It has been showed that the activity of PON and ARE were significantly decreased in patients with SH (9).

Hypothyroidism is associated with decreased rates of collagen catabolism (10). At the same time, the degree of oxidative stress is directly related to the inhibition of collagen production and the relationship between SH and increased oxidative stress is known (11). In the literature, there are limited data on the relationship between SH and the deterioration of the collagen structure (12). Prolidase plays an important role in the recycling of proline for collagen synthesis and is considered as a target enzyme of the collagen production (13). However, there has been no study on the serum prolidase activity (SPA) in patients with SH.

Therefore, this study was mainly planned to evaluate the association between SPA and serum levels of oxidative stress markers, such as PON and ARE. To our knowledge, this is the first study to assess the association between oxidative stress and SPA in SH.

Materials and Methods

Study Population

Eighteen consecutive premenopausal women with newly diagnosed SH and 18 control women with normal thyroid function test satisfying the inclusion criteria were included in the study after giving informed consent for participation. The study protocol conforms to the principles of the Declaration of Helsinki and was approved by the local Medical Ethics Committee.

Subjects

Patients with an active infection, smoking, diabetes, malignancy, pituitary and rheumatologic disease, history of thyroid surgery and usage of drugs that affect the oxidant state or lipid parameters were excluded. The diagnosis of SH was based on the finding of high TSH levels associated with normal free T4 (fT4) levels. The control group consisted of healthy patients without any history of thyroid disease.

Laboratory Measurements

Fasting plasma glucose (FPG), total cholesterol (TC), LDL-C, HDL-C, triglycerides (TG), urea, and creatinine levels were measured in both groups. Sensitive TSH and fT4, free T3 (fT3) were measured with Immulite 2000 by the immunoassay method.

Venous blood samples were collected from all subjects after an overnight fasting to measure PON, ARE and SPA. These were then centrifuged and stored at -80 °C until the day of analysis. Before being analyzed, serum samples were transferred to -20 °C and were dissolved at room temperature.

Measurements of PON and ARE Activities

PON and ARE activities were measured with commercially available kits (Relassay, Gaziantep, Turkey). PON measurement was performed either in the presence (salt-stimulated) or in the absence of NaCl. Paraoxon hydrolysis rate (diethyl-p-nitrophenyl phosphate) was measured by monitoring increased absorption at 412 nm at 37 °C. The amount of generated p-nitrophenol was calculated from the molar absorption coefficient at pH 8.5, which was 18.290/M per cm (14). PON activity was expressed as U/L serum. The coefficient of variation (CV) for individual samples was 1.8%. ARE activity was measured using phenyl acetate as substrate. Enzymatic activity was calculated from the molar absorption coefficient of the produced phenol, 1310/M per cm. One unit of ARE activity was defined as 1 mmol phenol generated per minute under the above conditions and expressed as U/L (15). The CV for individual serum samples was 4.1%. The sensitivities of both tests were over 98%.

Measurements of SPA

SPA was measured after the dilution of serum 40-fold with 2.5 mmol/L Mn^{2+} and 40 mmol/L trizma HCl buffer (pH: 8.0) and pre-incubation at 37 °C for 2 hr. Then, the reaction complex containing 30 mmol/L gly-pro, 40 mmol/L trizma HCl buffer (pH: 8.0) and 100 µl of pre-incubation serum in 1ml was incubated at 37 °C for 30 min. The supernatant was used for the measurement of proline, according to the method of Myara et al., which is a modification of Chinard's method (13,16). The intra-assay CV was 3.8%.

Statistical Analysis

Analyses were conducted using SPSS 11.5 (SPSS Inc., Chicago, Illinois, USA). All variables were expressed as mean ± SD and were compared between the groups using the Mann-Whitney U test. Correlations between PON, ARE and SPA, and other parameters were assessed by the Pearson's correlation coefficient.

Results

As expected, the value of TSH in SH group was significantly higher than in the control group (5.3 (1.5) µIU/mL, 1.9 (1.5) µIU/mL, respectively; $p<0.001$). PON and ARE activities were significantly decreased in patients with SH, compared with the control group (149.5 (56.2) U/L, 210.5 (161.2) for PON, respectively; $p=0.034$ and 198.9 (28.6) U/L, 227.0 (101.3) U/L for ARE, respectively; $p=0.044$). Additionally, SPA was significantly higher in women with SH than in healthy women (670.0 (9.7) U/L, 664.6 (8.2) U/L, respectively; $p=0.027$). Other demographic and clinical characteristics of the groups were similar (Table 1).

In correlation analysis, SPA showed a positive correlation ($r=0.404$, $p=0.015$) and both PON and ARE showed negative correlations with TSH level ($r=-0.348$, $p=0.037$; $r=-0.329$, $p=0.050$, respectively) (Figure 1 a,b,c). Furthermore, SPA showed negative correlations with fT4 and urea ($r=-0.371$, $p=0.026$; $r=-0.347$, $p=0.038$, respectively).

Table 1. Clinical and biochemical characteristics of study population

Parameter	SH group (n=18)	Control group (n=18)	p value
Age (years)	36.9±9.3	36.2±8.2	0.815
BMI (kg/m^2)	26.8±4.5	24.8±2.8	0.252
TSH (μU/mL)	5.3 (1.5)	1.9 (1.5)	<0.001
FT4 (pmol/L)	14.2 (3.9)	14.2 (2.6)	0.226
LDL-C (mmol/L)	3.1 (1.3)	2.7 (1.1)	0.389
HDL-C (mmol/L)	1.3 (0.4)	1.3 (0.4)	0.839
TG (mmol/L)	1.3 (0.8)	1.5 (0.6)	0.481
TC (mmol/L)	4.8 (1.3)	4.6 (1.1)	0.462
FPG (mmol/L)	5.4 (0.6)	5.1 (0.8)	0.126
Urea (mmol/L)	7.3 (1.6)	7.9 (2.3)	0.152
Creatinine (mmol/L)	0.06 (0.01)	0.05 (0.01)	0.085
SPA (U/L)	670.0 (9.7)	664.6 (8.2)	0.027
PON (U/L)	149.5 (56.2)	210.5 (161.2)	0.034
ARE (U/L)	198.9 (28.6)	227.0 (101.3)	0.044

Values are median (interquartile range).
ARE: Arylesterase, BMI: Body mass index, DBP: Diastolic blood pressure, FPG: Fasting plasma glucose, FT4: Free thyroxine, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, PON: Paraoxonase, SBP: Systolic blood pressure, SPA: Serum prolidase activity, TG: Triglyceride, TC: Total cholesterol, TSH: Serum thyrotrophin

Discussion

In the present study, we demonstrated that the two anti-oxidant enzymes, PON and ARE, were significantly decreased, while SPA, a collagen turnover marker, was significantly elevated in women with SH relative to healthy euthyroid women. To the best of our knowledge, this is the first study which evaluated SPA in patients with SH. On the other hand, TSH was significantly correlated with decreased PON and ARE activities and increased SPA.

The most common type of thyroid dysfunction is SH (17). There are limited data on oxidative state in patients with SH. It has been shown that oxidative stress is increased in SH and, more recently, PON and ARE activities have been shown to decrease in SH (6,9). SH may cause lipid abnormalities such as increased TC and LDL-C (18,19), which can explain the mechanism of oxidative stress related with SH. However, this observation was not verified in other studies on SH (9). For example, the Rotterdam study demonstrated a strong correlation between SH and atherosclerosis independent from classical risk factors including hypercholesterolemia (20). We found decreased PON and ARE activities in premenopausal women with SH, while lipid parameters were not comparable, suggesting the latter findings. The altered functions of these antioxidant enzymes may be a causal factor for oxidative stress-related changes in SH causing an increased lipid peroxidation, independent from unaltered lipid composition. The lack of a lipid peroxidation measurement in the present study limits us to get an exact conclusion about this proposed mechanism. Lipid peroxidation may play an important role in the pathogenesis of hypothyroidism-related oxidative stress (21).

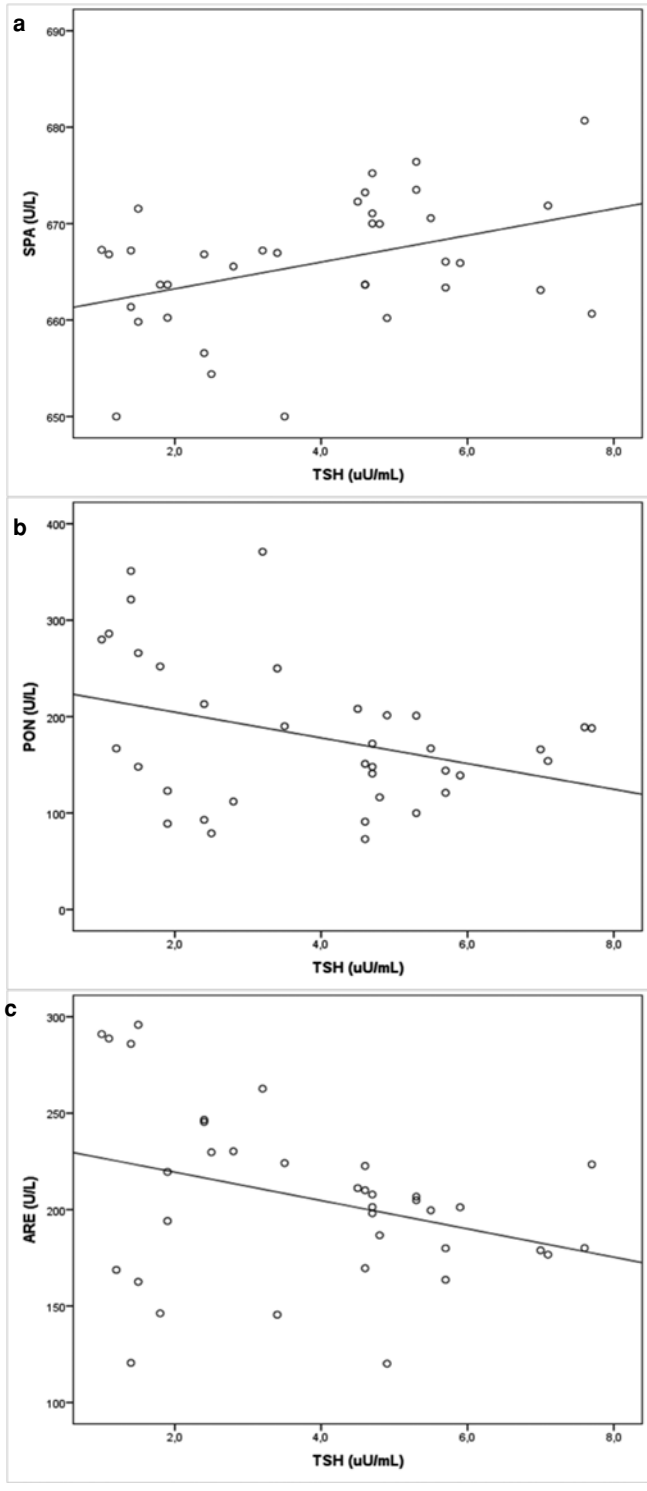

Figure 1a,b,c. The correlations of SPA (1a), PON (1b) and ARE (1c) with TSH

It has been demonstrated that both decreased PON and ARE activities are associated with the presence of coronary artery disease (CAD) (22). The association between elevated SPA and the presence and severity of CAD has been shown previously (23). The alterations of SPA, PON and ARE in our study may have impact on increased CAD risk in SH. The relationship between SH and increased risk of CAD is not fully understood. Collagen turnover

may be key point in the relationship of SH with atherosclerosis and in the exact mechanism of impaired PON1 system in CAD.

The correlation of oxidative stress index with TSH has been demonstrated by Cebeci et al. (9), however, the relationship of SPA with TSH was not known before. In their study, Drobnik et al. have showed that collagen levels were increased in the heart of rats with hypothyroidism (24). The correlation between SPA-as a marker of collagen turnover-and TSH must be evaluated in euthyroid status and in other altered thyroid function.

In our preliminary study, we found increased SPA in premenopausal women with SH. Elevation of SPA may depend on the effects of thyroid hormone on collagen turnover. However, the clinical impact of elevated SPA and the influence of L-thyroxin in SH need to be further evaluated. The cross-sectional design and limited number of subjects are the most important limitations of our study to clarify these points. Thyroid hormones have regulatory effects on connective tissue metabolism (22). Connective tissue and extracellular matrix protein metabolisms have been shown to be altered in hypothyroidism (24,25). Prolidase plays an important role in the proline recycling in collagen synthesis and it seems that prolidase activity may be a regulator in collagen biosynthesis (26). Prolidase activity is essential for collagen breakdown and collagen synthesis has been shown to be decreased in hypothyroidism, but SPA has never been investigated in hypothyroidism or in SH (27,10). Alteration of SPA in several pathological conditions which are limited to an organ system, such as CAD, non-alcoholic steatohepatitis, chronic liver disease, osteoarthritis, Helicobacter pylori infection, wound healing, breast cancer and osteoporosis have been shown previously (23). However, how SPA is affected in different hypothyroid states which are conditions associated with alteration of all body system functions is lacking. This is the first demonstration of SPA, a key enzyme of collagen turnover, is altered in SH which might play a role in possible mechanism of SH-related pathological conditions. Further studies are needed to demonstrate this theoretical relationship.

In conclusion, SPA is increased and PON and ARE activities are decreased in patients with SH which suggest that SH leads to oxidative stress and has an influence on collagen turnover. However, the underlying mechanism of elevated SPA in SH is unclear.

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