Turkish Journal of Medical Sciences

Volume 42
Number 7 *Sup. 1*

Article 3

1-1-2012

Investigation of serum paraoxonase-1 activity and lipid levels in patients with hyperthyroidism

GÜLDEN BAŞKOL

KIYMET DOLBUN SEÇKİN

FAHRİ BAYRAM

FATİH TANRIVERDİ

TÜBİTAK

Original Article

Turk J Med Sci
2012; 42 (Sup.1): 1166-1171

E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1202-87

Investigation of serum paraoxonase-1 activity and lipid levels in patients with hyperthyroidism

Gülden BAŞKOL[1], Kıymet DOLBUN SEÇKİN[1], Fahri BAYRAM[2], Fatih TANRIVERDİ[2]

Aim: The present study was designed to investigate the relationship among the serum paraoxonase-1 (PON-1) activity, lipid levels, and thyroid hormone status in patients with hyperthyroidism.

Materials and methods: The study group comprised 30 patients with primary hyperthyroidism and 18 normal healthy controls who were matched for age and sex. Serum PON-1 activity was determined by spectrophotometric methods.

Results: Serum PON-1 activity was found to be lower in patients compared to controls ($P < 0.05$). Free triiodothyronine (T_3) and free thyroxine (T_4) levels were significantly higher in the patient group compared to the control group ($P < 0.001$ and $P < 0.001$ respectively). Thyroid-stimulating hormone (TSH) and total cholesterol values were found to be lower in patients than in the controls ($P < 0.001$ and $P < 0.001$ respectively). Low-density lipoprotein cholesterol levels were found to be decreased in patients compared to controls ($P < 0.005$). Triglyceride and high-density lipoprotein cholesterol levels were not significantly different between the 2 groups ($P > 0.05$). Serum PON-1 activity was found to be negatively correlated with free T_4 levels ($r = -0.324$, $P < 0.05$) and positively correlated with total cholesterol ($r = 0.330$, $P < 0.05$) and TSH levels ($r = 0.577$, $P < 0.001$).

Conclusion: Our PON-1 results show that there is decreased antioxidant defense in patients with hyperthyroidism. Therefore, we think that effective antioxidant therapy to increase PON-1 activity may be a therapeutic option in patients with hyperthyroidism.

Key words: Paraoxonase-1 activity, oxidative stress, hyperthyroidism

Introduction

Thyroid hormones play a crucial role in biology, acting on gene transcription and the synthesis and degradation of proteins, regulating the basal metabolic rate and mitochondrial oxidative metabolism, and inducing changes in the antioxidant defense system (1). In excessive production of thyroid hormones, the basal metabolic rate can be increased by 100% (2). Hyperthyroidism induces a hyperdynamic cardiovascular state that is associated with tachycardia, systolic hypertension, atrial fibrillation, and increased cardiovascular mortality (3). Thyroid hormones influence lipid metabolism, including synthesis, mobilization, and degradation (4). Hyperthyroidism triggers lipolysis in subcutaneous tissue, increasing interstitial glycerol levels, lipid oxidation, and circulating fatty acid concentrations (5).

Reactive oxygen species (ROS) normally occur at relatively low levels in all cells and tissues. ROS are highly reactive molecules that, when present in excess, overwhelm the protective systems and result in cell damage, severe metabolic dysfunctions, and damage to biological macromolecules (6). A variety of enzymatic and nonenzymatic mechanisms have evolved to protect cells against ROS. Lipid

Received: 24.02.2012 – Accepted: 30.05.2012
[1] Department of Biochemistry and Clinical Biochemistry, Faculty of Medicine, Erciyes University, Kayseri – TURKEY
[2] Department of Endocrinology and Metabolism, Faculty of Medicine, Erciyes University, Kayseri – TURKEY
Correspondence: *Gülden BAŞKOL, Department of Biochemistry and Clinical Biochemistry, Faculty of Medicine, Erciyes University, Kayseri – TURKEY*
E-mail: gbaskol@erciyes.edu.tr

peroxidation is a well-established mechanism of cellular injury in humans, and it is used as an indicator of oxidative stress in cells and tissues (7).

Paraoxonase-1 (PON-1) is an antioxidant enzyme on high-density lipoproteins (HDLs) that hydrolyzes lipid peroxides in oxidized lipoproteins. PON-1 activity has been suggested to be inversely associated with oxidative stress in serum and macrophages (8). PON-1 has cardioprotective effects that protect low-density lipoprotein (LDL) and HDL from oxidation, induced by either copper ion or free radical generators (9). In addition, it has been reported that PON-1 activity is decreased in some diseases due to ROS pathogenesis under oxidative stress, such as asthma in children or iron deficiency anemia during pregnancy (10,11). Recently there have been increasing experimental and clinical studies that show the role of free radicals in the etiology of many diseases, and there are increasing data supporting the theory that thyroid disease is associated with increased cardiovascular risk. There have been limited studies about PON-1 levels in hyperthyroidism. The present study was designed to investigate the relationship among the serum PON-1 activity, lipid levels, and thyroid hormone status in patients with hyperthyroidism.

Materials and methods

Patients

The study group comprised 30 patients (9 men, 21 women) with primary hyperthyroidism, with a mean age of 42.7 ± 14.3 years. The main causes of hyperthyroidism were Graves disease (22 patients), toxic nodular goiter (7 patients), and toxic adenoma (1 patient). The patients were compared with 18 normal healthy controls (4 men, 14 women), who were matched for age (mean age: 36.5 ± 14.1) and sex.

All laboratory measurements were performed at the research laboratory of the Department of Nuclear Medicine, Faculty of Medicine. Thyroid hormone levels, except thyroid-stimulating hormone (TSH), were measured using radioimmunoassay with the following commercial kits: free triiodothyronine (T_3) (ZenTech, Angleur, Belgium), free thyroxine (T_4) (ZenTech), and immunoradiometric TSH assay (TSH-IRMA; BioSource, Nivelles, Belgium). Total cholesterol (total-C), triglycerides (TG), and glucose were measured with a commercially available enzymatic reagent adapted to an autoanalyzer (Konelab, Vantaa, Finland). HDL-cholesterol (HDL-C) was measured by the cholesterol oxidase–phenol aminophenazone enzymatic method. LDL-cholesterol (LDL-C) was calculated using the Friedewald equation (12).

Samples

Informed consent was obtained from patients prior to the study. The study protocol and the procedures were approved by the Erciyes University ethical committee and were in accordance with the Helsinki Declaration of 1975.

All blood samples were collected in the morning after an overnight fast, and serum samples were stored at –20 °C until assays for analysis.

Chemicals

All chemicals used in this study were from Sigma Chemical Co. (St. Louis, MO, USA) and were of analytical grade or the highest grade available.

Measurement

Assay of paraoxonase activity

Serum PON-1 activity was measured according to a method described elsewhere (13). We measured the rate of hydrolysis of paraoxon by monitoring the increase of absorbance at 405 nm and at 25 °C. The basal assay mixture included 1.0 mM paraoxon and 1.0 mM $CaCl_2$ in 0.05 M glycine buffer, pH 10.5. The definition of 1 unit of paraoxonase activity was taken as 1 millimole of p-nitrophenol formed per minute, and activity was expressed as U/mL of serum.

Statistical analysis

SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. $P \leq 0.05$ was statistically significant. All data were subjected to the Kolmogorov–Smirnov test for normality and are presented as mean ± standard deviation. Differences among the groups were assessed with Student's t-test. Pearson's correlation analysis was used to determine whether significant correlations existed between chosen variables.

Results

There was no statistically significant difference of age distribution between the control and patient group ($P > 0.05$). Free T_3 and free T_4 levels were significantly higher in the patient group compared to the control group ($P < 0.001$ and $P < 0.001$, respectively). TSH and total-C values were found to be lower in the patients than the controls ($P < 0.001$ and $P < 0.001$, respectively). LDL-C levels were found to be decreased in patients compared to controls ($P < 0.005$).

TG and HDL-C levels were not significantly different between the 2 groups ($P > 0.05$). Glucose levels were found to be significantly higher in the patient group ($P < 0.001$). Serum PON-1 activity was found to be lower in patients compared to controls ($P < 0.05$) (Table 1).

Correlations

HDL-C levels were found to be negatively correlated with TG levels ($r = -0.307$, $P < 0.05$). TG levels were found to be positively correlated with total-C levels ($r = 0.559$, $P < 0.001$). Free T_3 and free T_4 levels were found to be negatively correlated with total-C levels ($r = -0.479$, $P < 0.001$; $r = -0.449$, $P < 0.005$). TSH levels were found to be positively correlated with total-C levels ($r = 0.503$, $P < 0.001$). LDL-C levels were found to be positively correlated with total-C levels ($r = 0.825$, $P < 0.001$), TG levels ($r = 0.422$, $P < 0.005$), and TSH levels ($r = 0.505$, $P < 0.001$). LDL-C levels were found to be negatively correlated with free T_3 levels ($r = -0.568$, $P < 0.001$) and free T_4 levels ($r = -0.470$, $P < 0.001$).

Serum PON-1 activity was found to be negatively correlated with free T_4 levels ($r = -0.324$, $P < 0.05$) and positively correlated with total-C levels ($r = 0.330$, $P < 0.05$) and TSH levels ($r = 0.577$, $P < 0.001$) (Table 2).

Discussion

Hyperthyroidism is a relatively common disease and, according to the 6-year National Health and Nutrition Examination Survey III Study, the prevalence of

Table 1. Biochemical features and serum PON-1 activities in patients with hyperthyroidism and healthy controls. Serum PON-1 activity was lower in the patient group than in the controls ($P < 0.05$).

Parameters	Patients with hyperthyroidism	Healthy controls	P
N	30 (9 men, 21 women)	18 (4 men, 14 women)	
Age (years)	42.7 ± 14.3	36.5 ± 14.1	>0.05
Free T_3 (pmol/L) (Normal range: 2.20-4.7)	13.6 ± 8.1	3.1 ± 0.3	<0.001
Free T_4 (pmol/L) (Normal range: 8.0–20.0)	36.3 ± 17.4	13.4 ± 1.7	<0.001
TSH (μU/mL) (Normal range: 0.20–3.20)	0.05 ± 0.06	1.2 ± 0.8	<0.001
TG (mg/dL) (Normal range: 35–160)	123.0 ± 40.0	127.8 ± 90.3	>0.05
Total-C (mg/dL) (Normal range: 120–200)	154.7 ± 29.1	185.8 ± 33.6	<0.001
HDL-C (mg/dL) (Normal range: 35–85)	51.2 ± 17.5	55.5 ± 13.0	>0.05
LDL-C (mg/dL) (Normal range: 60–170)	77.3 ± 27.0	103.6 ± 22.7	<0.005
Glucose (mg/dL) (Normal range: 70–110)	104.8 ± 21.0	82.5 ± 13.4	<0.001
PON-1 (U/mL)	216.7 ± 121.9	316.1 ± 147.9	<0.05

Table 2. Correlation among parameters in hyperthyroidism. Serum PON-1 activity was found to be negatively correlated with free T_4 levels ($r = -0.324$, $P < 0.05$) and positively correlated with total-C levels ($r = 0.330$, $P < 0.05$) and TSH levels ($r = 0.577$, $P < 0.001$).

Parameters	r	P
TG – HDL-C	−0.307	<0.05
TG – total-C	0.559	<0.001
Total-C – free T_3	−0.479	<0.001
Total-C – free T_4	−0.449	<0.005
Total-C – TSH	0.503	<0.001
LDL-C – total-C	0.825	<0.001
LDL-C – TG	0.422	<0.005
LDL-C – TSH	0.505	<0.001
LDL-C – free T_3	−0.568	<0.001
LDL-C – free T_4	−0.470	<0.001
PON-1 – free T_4	−0.324	<0.05
PON-1 – total-C	0.330	<0.05
PON-1 – TSH	0.577	<0.001

hyperthyroidism was 1.3% in a population of 12 years of age or older (14). Thyroid hormones regulate the basal energy expenditure and a hyperthyroid state causes metabolic abnormalities such as insulin resistance, dyslipidemia, and cardiovascular diseases (CVDs) (1). Thyroid hormones play a crucial role in the regulation of antioxidants and accelerate free radical production in the mitochondria (3). Studies have also shown that thyroid hormones appear to exert a prooxidant activity in target cells (15). It has been reported that hyperthyroidism is associated with increased oxidative stress and oxidative damage to lipids and genomic DNA in the aortic wall (16).

Thyroid hormones regulate the expression of enzymes in the lipid metabolism (17). In the literature it has been shown that TG was decreased in hyperthyroid patients (1), but in our study we did not find a significant difference between the 2 groups. We found decreased total-C levels in the patient group compared to the healthy controls. In accordance with our results, decreased total-C levels in patients with hyperthyroidism have been reported in several studies (18). Increased LDL-C levels are associated with high CVD risk. We found decreased levels of LDL-C, which were similar to the findings of other studies (4,19,20). Interestingly in hyperthyroidism, LDL-C is reduced, but the risks of several cardiovascular conditions, such as atrial fibrillation, angina pectoris, tachycardia, systolic hypertension, and palpitations, are increased (21). A hypermetabolic state in hyperthyroidism may be responsible for these conditions.

Thyroid hormones can increase mitochondrial biogenesis, fatty acid oxidation, and tricarboxylic acid cycle activity (22). Thyroid hormones also have a large impact on glucose metabolism, and novel findings support that hyperthyroidism is associated with insulin resistance (23). We found significantly higher glucose levels in the patient group compared to the controls, which may be explained by increased endogenous glucose production through more rapid glycogenolysis and gluconeogenesis, consistent with the literature.

Serum PON-1 is thought to protect lipoproteins against oxidative modification and is accepted as a preatherosclerotic marker (10). It has crucial roles in protecting LDL against oxidation and detoxification of highly toxic substances (24). We found decreased serum PON-1 activities in patients with hyperthyroidism compared to healthy controls. Aviram et al. (25) found that PON-1

reduced oxidized lipids in atherosclerotic lesions that were sampled from a coronary artery or carotid. It has been reported that hyperthyroidism is associated with a higher risk for ischemic stroke among young adults (26). Azizi et al. (27) showed that a significant reduction in PON-1 activity was observed in both hyper- and hypothyroid patients. Raiszadeh et al. (28) reported that reduced PON-1 activity in patients with hyperthyroidism reverts to normal after euthyroidism. Our study results are in accordance with previous studies showing that PON-1 activity is decreased in hyperthyroidism (28,29). Increased production of free oxygen radicals in hyperthyroidism may be responsible for that decrease, or the decreased PON-1 activity may occur as part of an inflammatory response. Serum PON-1 activity was found to be negatively correlated with free T_4 levels as shown in relevant previous studies. Yavuz et al. (30) reported that PON-1 activity was negatively correlated with serum TT_4 and TT_3 levels and positively correlated with insulin sensitivity. Positive correlations between total-C and TSH levels and PON-1 activities were found in the present study. We thought that the increase of free T_4 levels in hyperthyroidism may cause a decrease in PON-1 activity, which is known as an important antioxidant enzyme with cardioprotective effects.

Although hyperthyroidism is usually associated with low total and LDL cholesterol levels, the cardiac abnormalities in this condition are thought to be secondary to the hypermetabolic state. It has been reported in several studies that lipid oxidation and inflammation play a central role in the development of atherosclerosis. We thought that the hypermetabolic state in thyrotoxicosis leads to a decreased antioxidant defense in patients with hyperthyroidism. Further studies are needed to understand the role of PON-1 in this disease pathogenesis. Therefore, we think that effective antioxidant therapy to increase PON-1 activity may be a therapeutic option in patients with hyperthyroidism.

Acknowledgment

The Erciyes University Research Foundation supported this research with contract grant number TA-03-32.

References

1. Asayama K, Dobashi K, Hayashibe H, Megata Y, Kato K. Lipid peroxidation and free radical scavengers in thyroid dysfunction in the rat: a possible mechanism of injury to heart and skeletal muscle in hyperthyroidism. Endocrinology 1987; 121: 2112–8.
2. Gago-Dominguez M, Castelao JE. Role of lipid peroxidation and oxidative stress in the association between thyroid diseases and breast cancer. Crit Rev Oncol Hematol 2008; 68: 107–14.
3. Fazio S, Palmieri EA, Lombardi G, Biondi B. Effects of thyroid hormone on the cardiovascular system. Recent Prog Horm Res 2004; 59: 31–50.
4. Zhu X, Cheng SY. New insights into regulation of lipid metabolism by thyroid hormone. Curr Opin Endocrinol Diabetes Obes 2010; 17: 408–13.
5. Møller N, Nielsen S, Nyholm B, Pørksen N, Alberti KG, Weeke J. Glucose turnover, fuel oxidation and forearm substrate exchange in patients with thyrotoxicosis before and after medical treatment. Clin Endocrinol (Oxf) 1996; 44: 453–9.
6. Halliwell B. Biochemistry of oxidative stress. Biochem Soc Trans 2007; 35: 1147–50.
7. Halliwell B. Lipid peroxidation, antioxidants and cardiovascular disease: how should we move forward? Cardiovasc Res 2000; 47: 410–8.
8. Rajkovic MG, Rumora L, Barisic K. The paraoxonase 1, 2 and 3 in humans. Biochem Med 2011; 21: 122–30.
9. Aviram M, Rosenblat M, Billecke S, Erogul J, Sorenson R, Bisgaier CL et al. Human serum paraoxonase (PON1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. Free Radic Biol Med 1999; 26: 892–904.
10. Can M, Mungan AG., Açıkgöz Ş, Yüksel B, Demirtaş S, Tomaç N. Effect of montelukast treatment on serum paraoxonase activity in asthmatic children. Turk J Med Sci 2007; 37: 373–6.
11. Camuzcuoğlu H, Toy H, Vural M, Camuzcuoğlu A, Taşkın A, Çelik H. Serum paraoxonase and arylesterase activities in iron deficiency anemia during pregnancy. Turk J Med Sci 2011; 41: 185–91.
12. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499–502.
13. Eckerson EW, Romson J, Wyte C, La Du BN. The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. Am J Hum Genet 1983; 35: 214–27.

14. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 2002; 87: 489–99.

15. Videla LA, Fernández V. Thyroid calorigenesis and oxidative stress: modification of the respiratory burst activity in polymorphonuclear leukocytes. Braz J Med Biol Res 1994; 27: 2331–42.

16. Moulakakis KG, Poulakou MV, Paraskevas KI, Dontas I, Vlachos IS, Sokolis DP et al. Hyperthyroidism is associated with increased aortic oxidative DNA damage in a rat model. In Vivo 2007; 21: 1021–6.

17. Rizos CV, Elisaf MS, Liberopoulos EN. Effects of thyroid dysfunction on lipid profile. Open Cardiovasc Med J 2011; 5: 76–84.

18. Abrams JJ, Grundy SM, Ginsberg H. Metabolism of plasma triglycerides in hypothyroidism and hyperthyroidism in man. J Lipid Res 1981; 22: 307–22.

19. Raziel A, Rosenzweig B, Botvinic V, Beigel I, Landau B, Blum I. The influence of thyroid function on serum lipid profile. Atherosclerosis 1982; 41: 321–6.

20. Nishitani H, Okamura K, Noguchi S, Inoue K, Morotomi Y, Fujishima M. Serum lipid levels in thyroid dysfunction with special reference to transient elevation during treatment in hyperthyroid Graves' disease. Horm Metab Res 1990; 22: 490–3.

21. Fadel BM, Ellahham S, Ringel MD, Lindsay J Jr, Wartofsky L, Burman KD. Hyperthyroid heart disease. Clin Cardiol 2000; 23: 402–8.

22. Goglia F, Moreno M, Lanni A. Action of thyroid hormones at the cellular level: the mitochondrial target. FEBS Lett 1999; 452: 115–20.

23. Brenta G. Why can insulin resistance be a natural consequence of thyroid dysfunction? J Thyroid Res 2011; 2011: DOI 10.4061/2011/152850.

24. Costa LG, Cole TB, Furlong CE. Paraoxonase (PON1): from toxicology to cardiovascular medicine. Acta Biomed 2005; 76: 50–7.

25. Aviram M, Rosenblat M, Billecke S, Erogul J, Sorenson R, Bisgaier CL et al. Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. Free Radic Biol Med 1999; 26: 892–904.

26. Sheu JJ, Kang JH, Lin HC, Lin HC. Hyperthyroidism and risk of ischemic stroke in young adults: a 5-year follow-up study. Stroke 2010; 41: 961–6.

27. Azizi F, Raiszadeh F, Solati M, Etemadi A, Rahmani M, Arabi M. Serum paraoxonase 1 activity is decreased in thyroid dysfunction. J Endocrinol Invest 2003; 26: 703–9.

28. Raiszadeh F, Solati M, Etemadi A, Azizi F. Serum paraoxonase activity before and after treatment of thyrotoxicosis. Clin Endocrinol (Oxf) 2004; 60: 75–80.

29. Kinlaw WB. Thyroid disorders and cholesterol: identifying the realm of clinical relevance. Endocrinologist 1995; 5: 148–55.

30. Yavuz DG, Yüksel M, Deyneli O, Ozen Y, Aydin H, Akalin S. Association of serum paraoxonase activity with insulin sensitivity and oxidative stress in hyperthyroid and TSH-suppressed nodular goitre patients. Clin Endocrinol (Oxf) 2004; 61: 515–21.