■Original Article

Paraoxonase 1 activity as a new biochemical marker in the diagnosis of peripheral arterial disease

Periferik arter hastalığı tanısında yeni bir biyokimyasal gösterge olarak paraoksonaz 1 aktivitesi

Hasan Basri SAVAS*[1] ⓘD , Mustafa ETLI[2] ⓘD

[1]Alanya Alaaddin Keykubat University, Faculty of Medicine, Department of Medical Biochemistry, Alanya, Antalya, Turkey
[2]Alanya Keykubat University, Faculty of Medicine, Department of Cardiovascular Surgery, Alanya, Antalya, Turkey

Abstract

Aim: Peripheral artery disease (PAD) is an atherosclerotic disease. It is seen in older ages. It causes cardiovascular morbidity and mortality. PAD may progress without any symptoms. Despite its high frequency, there is no laboratory parameter that directly indicates peripheral arterial disease in routine biochemical tests. The relationship between oxidative stress increase and PAD is known. In this study, it is aimed to show the possible usage of the activities of the antioxidant enzymes paraoxonase 1 and arylesterase as a new marker in the diagnosis of PAD.

Material and Methods: A total of 70 individuals, including 35 in the control group and 35 peripheral artery patients, were included in this study. The collected blood serums were separated and stored at -80 °C. Paraoxonase 1 and arylesterase activities were measured using the spectrophotometric method in the serum which was dissolved at room temperature. The results were subjected to statistical analysis. P <0.05 was accepted as the level of significance.

Results: In the peripheral arterial disease group, the paraoxonase 1 and arylesterase activities were found to be significantly lower than those in the control group (p <0.05). In peripheral arterial disease, paraoxonase 1 and arylesterase activities were shown to decrease.

Conclusion: In peripheral arterial disease, paraoxonase 1 and arylesterase activities were found to decrease significantly. The results of similar studies related to atherosclerosis in the literature were in line with our findings. It would be beneficial to support the results of this study with new studies and evaluate paraoxonase 1 and arylesterase activities in routine biochemistry laboratories for the diagnosis and follow-up of peripheral arterial disease.

Key words: Peripheral arterial disease; biochemical marker; paraoxonase 1.

Öz

Amaç: Periferik arter hastalığı (PAH) aterosklerotik bir hastalıktır. İleri yaşta görülür. Kardiyovasküler morbidite ve mortaliteye sebep olmaktadır. PAH, hiçbir belirti vermeden de seyredebilir. Sıklığı yüksek olmasına rağmen rutin biyokimyasal tetkiklerde direkt olarak periferik arter hastalığını gösteren bir laboratuvar parametresi bulunmamaktadır. Oksidatif stres artışı ile PAH arasındaki ilişki bilinmektedir. Bu çalışmada antioksidan enzimler olan paraoksonaz 1 ve arilesteraz aktivitelerinin PAH tanısında yeni bir marker olarak muhtemel kullanımının gösterilmesi amaçlanmıştır.

Gereç ve Yöntemler: 35 kontrol grubu ve 35 Periferik arter hastası olmak üzere toplam 70 bireyin olurları alınarak çalışmaya dâhil edilmiştir. Toplanan kanlar serumları ayrılarak -80 Co'de saklanmıştır. Oda ısısında çözünen serumlarda ticari kit kullanılarak, spektrofotometrik yöntem ile paraoksonaz 1 ve arilesteraz aktiviteleri ölçülmüştür. Sonuçlar istatistiksel analize tabi tutulmuştur. Anlamlılık düzeyi olarak p<0,05 kabul edilmiştir.

Bulgular: Periferik arter hastalığı grubunda, paraoksonaz 1 ve arilesteraz aktiviteleri, kontrol grubuna göre istatistiksel olarak anlamlı düzeyde düşük bulunmuştur (p<0,05). Periferik arter hastalığında paraoksonaz 1 ve arilesteraz aktivitelerinin azaldığı görülmüştür.

Sonuç: Periferik arter hastalığında paraoksonaz 1 ve arilesteraz aktivitelerinin istatistiksel olarak anlamlı düzeyde azaldığı görülmüştür. Literatürde ateroskleroz ile ilgili benzer çalışmaların sonuçları bulgularımız ile uyumludur. Yeni çalışmalarla desteklenmesi faydalı olmakla birlikte paraoksonaz 1 ve arilesteraz aktivitelerinin rutin biyokimya laboratuvarlarında bakılmasının periferik arter hastalığı tanısında ve takibinde değeri olabilecektir.

Anahtar kelimeler: Periferik arter hastalığı, biyokimyasal belirteç; paraoksonaz 1.

Introduction

Peripheral arterial disease (PAD) is an atherosclerotic disease. It is seen in older ages. It causes cardiovascular morbidity and mortality. PAD may progress without any symptoms [1]. Although its frequency is high, there is no laboratory parameter that directly indicates peripheral arterial disease in routine biochemical tests. The paraoxonase (PON1) enzyme is an esterase enzyme whose antioxidant aspect is evident. It is firmly attached to HDL. Thanks to the PON1 enzyme, it has been demonstrated in studies that HDL reduces lipid peroxides by various enzymatic mechanisms after accumulation of lipid peroxides forming as a result of oxidation of LDL [1]. Paraoxonase 1 activity is variable and affected by environmental factors. It differs based on communities. Paraoxonase 1 and Arylesterase (ARY) are enzymes in the esterase group that are encoded by the same gene whose active centers are similar. The primary importance of ARY is that it is an indicator of the actual protein that is not affected by changes in PON1. Disruption of the balance between oxidative stress and antioxidant capacity and formation of oxidative damage cause many serious diseases [2-8]. The relationship between oxidative stress increase and PAD is known [9]. In this study, it is aimed to show the possible usage of the activities of the antioxidant enzymes paraoxonase 1 and arylesterase as a new marker in the diagnosis of PAD.

Material and Methods

Ethical Considerations

The research was conducted with the approval of Alanya Alaaddin Keykubat University Clinical Research Ethics Committee (ALKÜ-KAEK) dated 26.09.2019 and numbered 10/14. The study was carried out in accordance with the ethical principles in the Declaration of Helsinki, which was adopted by the World Medical Association in 1964 and then updated continuously. All people included in the study signed an informed consent form.

Design

70 individuals, male and female, who visited the Alanya Research and Training Hospital of Alanya Alaaddin Keykubat University, including 35 healthy controls (10 women, 25 men) and 35 peripheral arterial disease patients (10 women, 25 men), were included in the study. The blood samples left over from routine examinations were collected. The collected blood was centrifuged in refrigeration, and its serum was separated and portioned in Eppendorf tubes and stored at -80 °C.

Measurement of Paraoxonase 1 (PON1) and Arylesterase (ARY) Activities

Paraoxonase 1 and arylesterase activities were measured by the spectrophotometric method in the serum using a commercial kit.

Paraoxonase 1 (PON1)

A fully automated and colorimetric commercial kit was used to measure paraoxonase 1 activity (Rel Assay Diagnostics, Gaziantep, Turkey). The basic principle of the measurement method of the kit is based on the measurement of basal paraoxonase 1 activity in the environment without NaCl and stimulated paraoxonase 1 activity in the environment containing NaCl. The absorbance change resulting from the hydrolysis of paraoxone was measured spectrophotometrically at 37 °C and 412 nm. The activity calculation was made by subtracting the basal PON1 activity from the stimulated PON1 activity. The results are given in U / l [10].

Arylesterase (ARY)

A fully automated and colorimetric commercial kit was used to measure arylesterase (ARY) activity (Rel Assay Diagnostics, Gaziantep, Turkey). The basic principle of the measurement method of the kit is the use of phenyl acetate as a substrate for the measurement of ARY activity. Phenol and acetic acid are formed as a result of the hydrolysis of phenyl acetate. The absorbance change resulting from the hydrolysis of phenyl acetate was measured spectrophotometrically. The results are given in U / l [11].

Statistical Analysis

The results were subjected to statistical analysis. ANOVA was applied with the SPSS package program. There were two groups in total, and for this reason, post-hoc tests were not needed. In the statistical analysis, $p<0.05$ was accepted as the level of significance in the comparison between the groups.

Results

The mean age of the peripheral arterial disease group was 67.80 ± 8.70, whereas the mean age of the control group was 68.23 ± 7.80. In the peripheral arterial disease group, the paraoxonase 1 and arylesterase activities were significantly lower than those in the control group ($p<0.05$). The PON1 activity for the control (n = 35) and peripheral artery disease (n = 35) groups was found respectively as 509.54 ± 33.68 and 486.83 ± 30.93 (p = 0.004), while the ARY activity was respectively 612.17 ± 46.26 and 520.71 ± 52.63 ($p<0.001$). The results are shown in Table 1 and Graphic 1 and 2 below.

Table 1. Change in Paraoxonase 1 and Arylesterase Activity in Peripheral Arterial Disease

Parameter (Unit)	Control Group (n=35) (mean±SD)	Peripheral Arterial Disease Group (n=35) (mean±SD)	p
Paraoxonase 1 (PON1) (U/l)	509.54 ± 33.68	486.83 ± 30.93 *	0.004
Arylesterase (ARE) (U/l)	612.17 ± 46.26	520.71 ± 52.63 *	<0.001
Explanation: *: For paraoxonase 1 and arylesterase activity, there was a statistically significant decrease in the peripheral artery disease group in comparison to the control group (p<0.05).			

Graphic 1. Change in Paraoxonase 1 Activity in Peripheral Arterial Disease
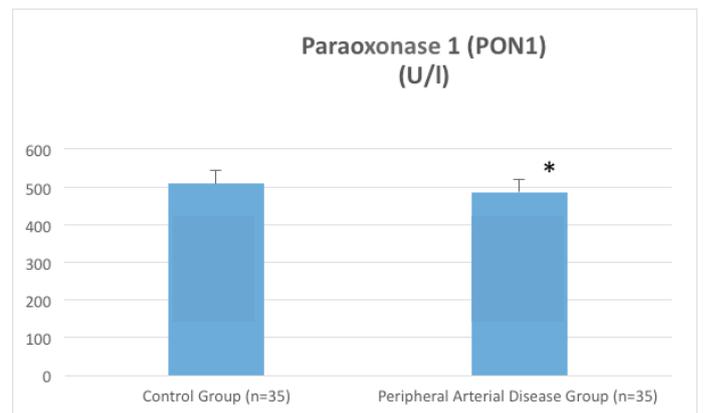
Explanation: *: For paraoxonase 1 activity, there was a statistically significant decrease in the peripheral artery disease group in comparison to the control group (p = 0.004).

Graphic 2. Change in Arylesterase Activity in Peripheral Arterial Disease
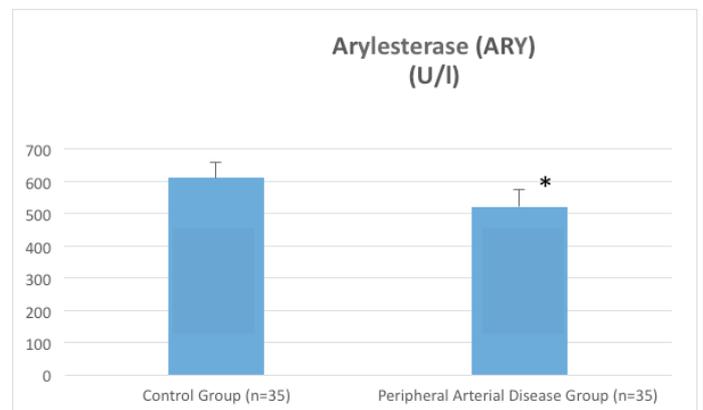
Explanation: *: For arylesterase activity, there was a statistically significant decrease in the peripheral artery disease group in comparison to the control group (p<0.001).

Discussion

Peripheral arterial disease is a pathology that develops in the background of atherosclerosis. Atherosclerosis is an inflammatory disease caused by the combination of oxidized lipid accumulation in the intima layers of the vessels and immune cells. The most important step that initiates atherosclerosis is the migration of LDL cholesterol to the intima layer after its endothelial injury, oxidation and subsequent migration of monocytes and the formation of foam cells. The foam cells then become fat lines in the endothelial layer, and then, form atheromatous plaques [10-15]. An increase in oxidative stress increases foam cell formation and atherosclerosis. There is no parameter that is used in clinical biochemistry practice in the direct diagnosis of peripheral arterial disease. The activity of the paraoxonase 1 enzyme, which is an antioxidant enzyme and not only prevents LDL oxidation but also increases the activity of HDL, may be a biochemical marker in the diagnosis of peripheral arterial disease. According to the results of our study, paraoxonase 1 and arylesterase activities were decreased significantly in peripheral arterial disease ($p<0.05$). In a clinical study, it was previously shown that a decrease in PON1 activity increases carotid atherosclerosis and LDL oxidation [13]. In a study on experimental animal models, it was shown that increased PON1 activity decreased atherosclerosis [14]. The results of these studies on atherosclerosis in the literature were in line with our findings. While it would be beneficial to support this study with new studies, evaluation of the activities of the paraoxonase 1 and arylesterase enzymes in routine biochemistry laboratories may provide evidence for the diagnosis and follow-up of peripheral artery disease.

Conclusion

While deciding about the diagnosis and treatment of a patient, meticulous, clear, and accurate use of the information proven by scientific data is expressed as evidence-based medicine [15]. Therefore, a decrease in the activity of the paraoxonase 1 enzyme in peripheral arterial disease may be an important clinical biochemistry indicator in terms of evidence-based medicine.

Declaration of conflict of interest

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

References

1. Savas HB. Klinik Biyokimyada kanıta dayalı yaklaşım. 1st ed. Ankara: Akademisyen Publishing; 2019.

2. Savran M, Aslankoc R, Ozmen O et al. Agomelatine could prevent brain and cerebellum injury against LPS-induced neuroinflammation in rats. Cytokine 2020; 127: 154957.

3. Savran M, Ozmen O, Erzurumlu Y, Savas HB, Asci S, Kaynak M. The Impact of Prophylactic Lacosamide on LPS-Induced Neuroinflammation in Aged Rats. Inflammation 2019; 42: 1913-24.

4. Seflek HN, Kalkan S, Cuce G, Kılınc I, Sozen ME. Effects of Nigella sativa oil on ovarian volume, oxidant systems, XIAP and NF-kB expression in an experimental model of diabetes. Biotech Histochem 2019; 94: 325-33.

5. Savas HB, Gultekin F, Ciris İM. Positive effects of meal frequency and calorie restriction on antioxidant systems in rats. North Clin Istanbul. 2017; 4(2): 109–116. doi: 10.14744/nci.2017.21548.

6. Gültekin F, Nazıroğlu M, Savaş HB, Çiğ B. Calorie restriction protects against apoptosis, mitochondrial oxidative stress and increased calcium signaling through inhibition of TRPV1 channel in the hippocampus and dorsal root ganglion of rats. Metab Brain Dis. 2018; 33(5): 1761-1774. doi: 10.1007/s11011-018-0289-0.

7. Savaş HB, Yüksel Ö, Şanlidere Aloğlu H, Öner Z, Demir Özer E, Gultekin F. Effects of food based yeast supplementation on oxidative stress in rats fed by high cholesterol diet. Cell Membranes and Free Radical Research. 2013; 5:3: 252-255.

8. Cankara FN, Özmen Ö, Savaş HB, Şahin Y, Günaydın C. Gastroprotective effect of tarantula cubensis extract in the indomethacin-induced peptic ulcer model in rats. Acta Med. Alanya 2020; 4(3): 278-284. doi:10.30565/medalanya.797406.

9. Koutakis P, Ismaeel A, Farmer P et al. Oxidative stress and antioxidant treatment in patients with peripheral arter disease. Physiol Rep 2018; 6: 13650.

10. Bednarz-Misa I, Berdowska I, Zboch M et al. Paraoxonase 1 decline and lipid peroxidation rise reflect a degree of brain atrophy and vascular impairment in dementia. Adv Clin Exp Med 2020; 29: 71-8.

11. Ayan D, Şeneş M, Çaycı AB et al. Evaluation of Paraoxonase, Arylesterase, and Homocysteine Thiolactonase Activities in Patients with Diabetes and Incipient Diabetes Nephropathy. J Med Biochem 2019; 38: 481-88.

12. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. Nature Reviews Immunology 2013; 13: 709.

13. Tavori H, Aviram M, Khatib S et al. Human carotid atherosclerotic plaque increases oxidative state of macrophages and low-density lipoproteins, whereas paraoxonase 1 (PON1) decreases such atherogenic effects. Free Radical Biology and Medicine 2009; 46: 607-15.

14. Tward A, Xia Y-R, Wang X-P et al. Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. Circulation 2002; 106: 484-90.

15. Djulbegovic B, Guyatt GH. Progress in evidence-based medicine: a quarter century on. Lancet 2017: 390: 415-23.