Original Article The Eurasian Journal of Medicine EAJM 2012; 44: 153-6

Relationship of Serum Paraoxonase Enzyme Activity and Thermal Burn Injury

Termal Yanık ile Serum Paraoksonaz Enzim Aktiviteleri Arasındaki İlişki

Serap Yildirim[1], Songul Doganay[1], Abdulkadir Yildirim[2], Osman Enver Aydin[3], Akar Karakoc[2], Esra Laloglu[2]

[1]Department of Physiology, Faculty of Medicine, Ataturk University, Erzurum, Turkey

[2]Department of Biochemistry, Faculty of Medicine, Ataturk University, Erzurum, Turkey

[3]Department of Plastic Reconstructive and Aesthetic Surgery, Faculty of Medicine, Ataturk University, Erzurum, Turkey

Abstract

Objective: This study investigated changes in serum oxidative stress parameters in burn cases compared to healthy controls.

Materials and Methods: This study was performed in 41 burn patients with mild to severe thermal burn injuries and 38 healthy volunteers. The burn cases were selected from patients who were hospitalized in the burn unit for the treatment of second- and third-degree burns. Malondialdehyde (MDA) levels and PON-1 paraoxonase and arylesterase activities were measured in patient serum samples.

Results: PON-1 paraoxonase activity and MDA levels in patients with major thermal burn injury were significantly higher than healthy controls, but PON-1 arylesterase activities were lower. A significant negative correlation was observed between the burn percentage of the total body surface area and the PON-1 arylesterase activities in patients.

Conclusion: Human thermal burn injury was associated with an increase in MDA production and a decrease in PON-1 arylesterase activity, which was proportional to the percentage of total burned surface area.

Key Words: Arylesterase, Malondialdehyde, Oxidative stress, Paraoxonase, Thermal burn

Özet

Amaç: Bu çalışmada yanık vakalarında serum oksidatif stres parametrelerinde meydana gelen muhtemel değişikliklerin araştırılması ve sağlıklı kişilerle karşılaştırılması amaçlandı.

Gereç ve Yöntem: Çalışma hafiften şiddetliye 41 termal yanıklı hasta ve 38 sağlıklı gönüllü üzerinde yürütüldü. Vakalar tedavi amaçlı yanık ünitesine yatırılan 2. ve 3. derece yanığı olan hastalar arasından seçildi. Malondialdehit (MDA) düzeyleri ve PON-1 paraoksonaz ve arilesteraz aktiviteleri deneklerden alınan kan örneklerinde ölçüldü.

Bulgular: Sağlıklı kontrollerle karşılaştırıldığında PON-1 paraoksonaz aktivitesi ve MDA seviyesi majör termal yanıklı hastalarda anlamlı düzeyde daha yüksek, PON-1 arilesteraz aktivitesi ise daha düşüktü. Ayrıca hastalarda PON-1 arilesteraz aktivitesi ile vücut yanık yüzey alanı yüzdesi arasında negatif bir korelasyon vardı.

Sonuç: İnsanlarda termal yanık, artmış MDA oluşumu ve azalmış PON-1 arilesteraz aktivitesi ile ilişkilidir. Aynı zamanda azalmış PON-1 arilesteraz aktivitesi yanık yüzey alanının büyüklüğü ile orantılı olarak değişmektedir.

Anahtar Kelimeler: Arilesteraz, Malondialdehit, Oksidatif stress, Paraoksonaz, Termal yanık

Introduction

Burns are a common form of trauma that produce significant patient morbidity and mortality. A severe burn is associated with the release of inflammatory mediators, including reactive oxygen species (ROS) and reactive nitrogen species. Oxidative damage contributes to the local and distant pathophysiological events that occur after burn injuries [1, 2].

The concentration of free radicals and reactive non-radical species are determined by the balance between the rate of production and clearance by various antioxidant compounds and enzymes under normal physiological conditions in the extracellular environment and cells [1]. Oxidative stress refers to an imbalance in the production of ROS, including free radicals, such as superoxide, and non-radicals, such as hydrogen peroxide, and endogenous enzymatic and non-enzymatic antioxidant defenses [3]. However, the generation of free radicals in excess or a defective cellular antioxidant defense system may stimulate chain reactions via interactions with proteins, lipids and nucleic acids, which causes cellular dysfunction and death [4].

Increased concentrations of lipid peroxidation products, such as malondialdehyde (MDA), and decreased activities of antioxidant enzymes, such as catalase, have been identi-

Received: January 19, 2011 / **Accepted:** March 21, 2012

Correspondence to: Serap Yildirim, Department of Physiology, Faculty of Medicine, Ataturk University, 25240 Erzurum, Turkey
Phone: +90 442 231 66 17 Fax: +90 442 236 10 54 e-mail: serapyildirim10@hotmail.com

doi:10.5152/eajm.2012.36

fied in the systemic circulation in experimental and clinical burn patients [5, 6]. Serum paraoxonase-1 (PON-1) is an antioxidant enzyme that has not been evaluated in patients with thermal burns. Human serum PON-1 is an esterase that exhibits paraoxonase and arylesterase activities [3]. We and other investigators have demonstrated that serum PON-1 paraoxonase/arylesterase activities are lower in some diseases that are associated with oxidative stress, such as atherosclerotic heart disease, diabetes mellitus, ischemic stroke, preeclampsia and trauma [7-11]. Previous studies have investigated the relationship between PON-1 activity and oxidative stress in other traumatic diseases, but no data on serum PON-1 activity and MDA levels in burn patients are available. Therefore, we performed a case–control association study in patients with thermal burn and healthy controls and measured serum PON-1 paraoxonase and arylesterase activities and MDA levels.

Patients and Methods

This study was performed in 41 burn patients (24 males and 17 females) with mild to severe thermal burn injury (second- and third-degree burns that covered 5 to 45% of their total body surface area). Burn cases were selected from hospitalized patients in the burn unit of the Department of Plastic Surgery at Ataturk University. Thirty-eight healthy volunteers were matched for age and gender and enrolled in the control group (18 male and 20 female).

Low-Risk Patients, (over 10 years of age or under 50 years) according to the guidelines of the American Burn Association, were included in the study to standardize the burn patient group. Patients with severe illness that may alter serum levels of oxidative stress parameters, such as heart disease, lung disease, renal failure, diabetes mellitus, malignancy and hypertension, were excluded. Wallace's rule of nines was used to assess the burn surface area. Three groups were formed based on the burn percentage of the total body surface area: *Minor burn*, 14 patients with less than 15% burn; *Moderate burn*, 16 patients with 15-25% burn; *Major burn,* 11 patients with more than 25% burn.

Fasting blood samples were obtained from patients with thermal burn injury 24 h after admission. The blood samples were collected in commercially available serum test tubes. The tubes were centrifuged at 3500 rpm for 5 min, and the aliquots of serum were immediately stored at -80°C until analyzed. Serum MDA levels and PON-1 paraoxonase/arylesterase activities were measured spectrophotometrically.

Measurement of PON-1 paraoxonase and arylesterase activities

Serum PON-1 paraoxonase/arylesterase activities were measured using previously described methods [3]. Paraoxon (diethyl-p-nitrophenylphosphate, Sigma Co., UK) was used as the substrate for the determination of paraoxonase activity, and the 4-nitrophenol that was formed from the enzymatic hydrolysis of paraoxon was measured at 405 nm at 25°C using a spectrophotometer. Phenylacetate (Sigma Co., UK) was used as the substrate for the arylesterase activity measurements. Arylesterase activity was measured by the production of phenol using a spectrophotometer. Enzymatic activities for paraoxonase and arylesterase were calculated from the molar absorptivity coefficients of 171000 M^{-1} cm^{-1} and 1310 M^{-1} cm^{-1}, respectively.

Measurement of malondialdehyde (MDA) levels

Measurement of malondialdehyde is a product of lipid peroxidation. Therefore, MDA levels are used to determine oxidative stress in cells and tissues. MDA levels are determined as the spectrophotometric measurement of the pink-colored compound that is formed by the reaction of MDA and thiobarbituric acid (TBA) at 532 nm. Total TBA reactive substances are expressed as MDA levels. 1, 1, 3, 3-Tetraethoxypropane (Aldrich T9889) was used as the standard, and MDA levels are expressed in mmol/liter (μM) [12].

Statistical analysis

Statistical analyses were performed using the SPSS 18.0 statistical package (SPSS, Inc., Chicago, IL, USA). Histogram plots and the Skewness test determined the normal distribution of numerical data. Logarithmic transformation was applied to non-normally distributed variables. The statistical importance of the differences between groups was evaluated using one-way ANOVA and post hoc Tukey's test. Pearson's correlation coefficients compared continuous variables. The data are expressed as the means±standard deviation. A p value less than 0.05 was considered statistically significant.

Results

The PON-1 paraoxonase and arylesterase activities and MDA levels of the patients and healthy controls are presented in Figures 1, 2 and 3. These biochemical parameters were similar in patients with minor burns and healthy controls. However, an increasing trend in the mean values of PON-1 paraoxonase and MDA was observed as the percentage of burn injury of the total body surface area increased. These increases in PON-1 paraoxonase and MDA values were statistically significant for patients with major burns compared to healthy controls (Figures 2 and 3). However, PON-1 arylesterase activities exhibited a decreasing trend based on the burn percentage in patients (Figure 1), which was unlike the other study parameters. A significant negative correlation was observed between serum PON-1 arylesterase activity and

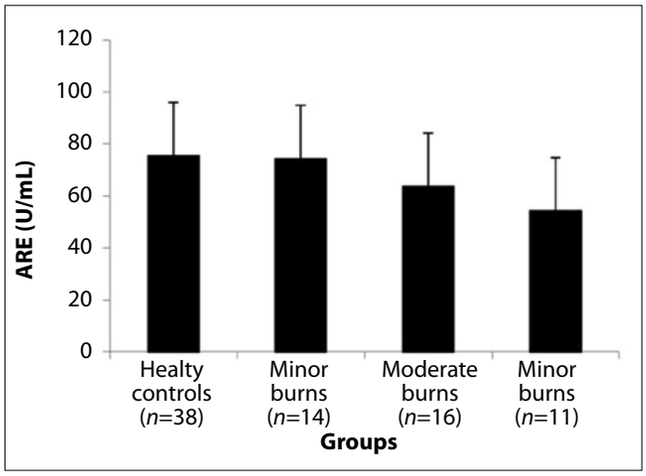

Figure 1. *Serum activities of PON-1 arylesterase (ARE) in patients with thermal burn injury, and healthy controls. Minor burns vs. major burns, p=0.046; major burns vs. healthy controls, p=0.008. (Post hoc Tukey test results).*

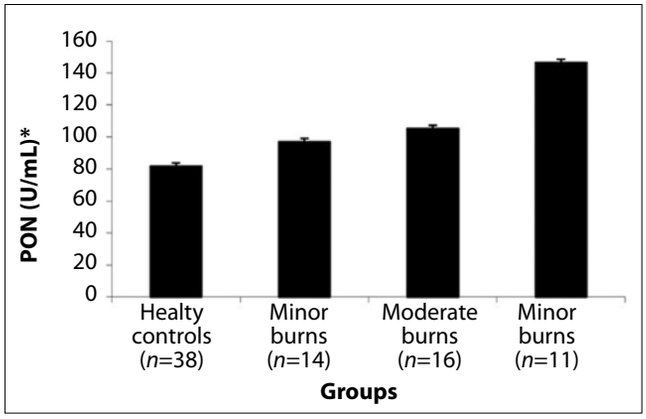

Figure 2. *Serum activities of PON-1 paraoxonase (PON) in patients with thermal burn injury, and healthy controls. Major burns vs. healthy controls, p=0.036 (Post hoc Tukey test results).*
**Log-transformed parameters were used*

the burn percentage of the total body surface area (p<0.001, r=-0.608) (Figure 4).

Discussion

The present study demonstrated that PON-1 paraoxonase activity and MDA levels in patients with major thermal burn injuries were significantly higher than healthy controls, but PON-1 arylesterase activities were lower in burn patients than controls. A significant negative correlation was observed between the burn percentage of the total body surface area and PON-1 arylesterase activity in patients.

Burn injury is accompanied by complex pathophysiological alterations that exert deleterious effects on various organ

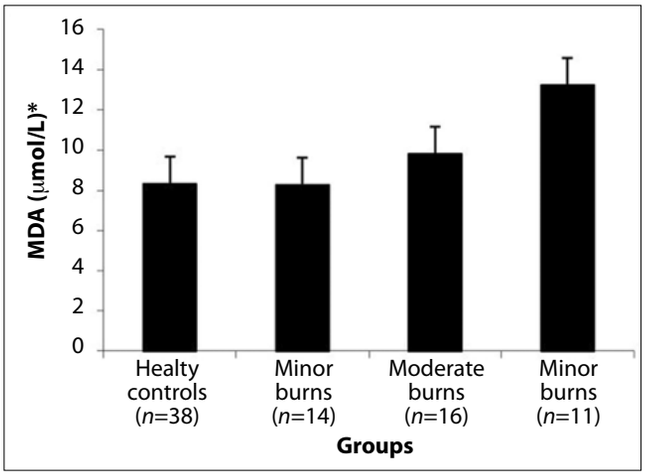

Figure 3. *Serum levels of malondialdehyde (MDA) in patients with thermal burn injury, and healthy controls. Minor burns vs. major burns, p=0.001; moderate burns vs. major burns, p=0.043; major burns vs. healthy controls, p=0.0001 (Post hoc Tukey test results).*
**Log-transformed parameters were used*

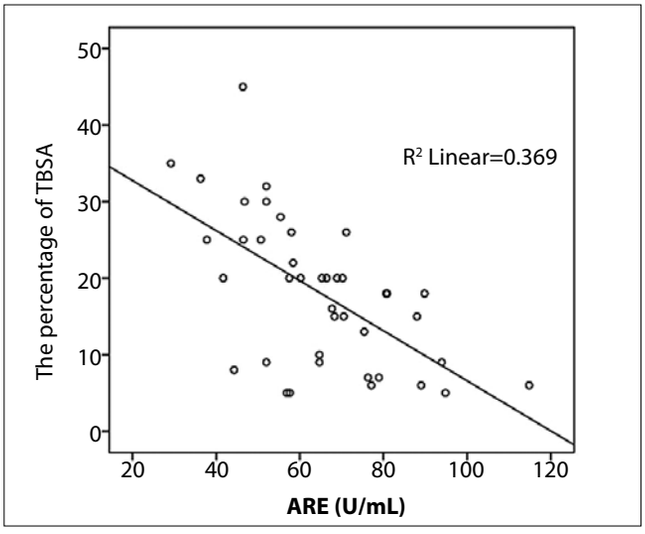

Figure 4. *The correlation between serum PON-1 arylesterase (ARE) activity and the percentage of total body surface area (TBSA) in patients with thermal burn injury (p<0.001, r=-0.608). Values are the Pearson's correlation coefficients.*

systems. Inflammatory shock mediators, such as histamine, serotonin, prostaglandins, thromboxane, interleukins, and oxygen free radicals, are implicated in the pathophysiological processes of burn [13]. Tissue adenosine triphosphate (ATP) levels gradually fall after burn trauma, and the increased adenosine monophosphate (AMP) is converted to hypoxanthine. Hypoxanthine is a substrate for xanthine oxidase, which produces free radicals via a series of reactions that lead to the formation of lipid oxidation products [2, 14]. MDA is one of

these lipid oxidation products, and it is used as an indicator of oxidative stress in tissues and cells [15]. Our current results and previous studies have demonstrated that oxidant stress is inevitable in burn injury. Edema is evident especially in cases where more than 25% of body surface area has been burned, which indicates systemic inflammation. The severity of the injury correlates with the systemic inflammatory response. The oxidant status of the patient determines the immune response to the injury. A greater oxidant stress produces systemic inflammatory response syndrome (SIRS). Capillary leak leads to vascular collapse and death. Clinical management of the patient includes the replacement of this lost volume. Increased MDA and decreased PON-1 arylesterase activities suggest a high oxidant status in burn patients, which is consistent with the clinical situation. Tanaka et al. [16] have demonstrated that antioxidant treatment significantly decreases the need for fluid resuscitation and respiratory support. Khan et al. [17] have also demonstrated that vitamin C treatment decreases the amount of fluid that is required for sufficient resuscitation. This decrease may be related to the decrease in vascular permeability.

The relationship between burn injury and serum MDA levels is well known because previous studies have demonstrated increased MDA levels in burn patients [13, 18]. The results of our study confirm this relationship. An increase in serum MDA levels also paralleled the increase in the burn percentage of the total body surface area.

Oxidative stress is characterized by an imbalance between free radical production, which is principally derived from oxygen, and antioxidant defenses, such as antioxidant enzymes and small molecular weight antioxidants [19]. Therefore, enhanced free radical production is paralleled by impaired antioxidant mechanisms. PON-1 is an antioxidant enzyme that protects lipids against oxidation. A decrease in PON-1 arylesterase activity was observed in burn patients in this study. However, MDA levels in patients with thermal burn injury increased, which suggests that the decrease in PON-1 arylesterase activity compensated for the oxidant stress as a result of its antioxidant nature.

In conclusion, human thermal burn injury was associated with an increase in MDA production and a decrease in PON-1 arylesterase activity, and these changes were proportional to the percentage of total burned body surface area. Therefore, our results aid in the elucidation of the pathogenesis of burn injury.

Conflict of interest statement: The authors declare that they have no conflict of interest to the publication of this article.

References

1. Parihar A, Parihar MS, Milner S, Bhat S. Oxidative stress and anti-oxidative mobilization in burn injury. Burns 2008; 34: 6-17. [CrossRef]
2. Horton JW. Free radicals and lipid peroxidation mediated injury in burn trauma: The role of antioxidant therapy. Toxicology 2003; 189: 75-88. [CrossRef]
3. Yildirim S, Akar S, Kuyucu M, Yildirim A, Dane S, Aygul R. Paraoxonase 1 gene polymorphisms, paraoxonase/arylesterase activities and oxidized low-density lipoprotein levels in patients with migraine. Cell Biochem Funct 2011; 29: 549-54. [CrossRef]
4. Aksoy Y, Yapanoglu T, Aksou H, Yildirim AK. The effect of dehydroepiandrosterone on renal ischemia-reperfusion-induced oxidative stress in rabbits. Urol Res 2004; 32: 93-6. [CrossRef]
5. Demling RH, Lalonde C. Systemic lipid peroxidation and inflammation induced by thermal injury persists into the post-resuscitation period. J Trauma 1990; 30: 69-74. [CrossRef]
6. Demling R, Ikegami K, Lalonde C. Increased lipid peroxidation and decreased antioxidant activity correspond with death after smoke exposure in the rat. J Burn Care Rehabil 1995; 16: 104-10. [CrossRef]
7. Camuzcuoglu H, Toy H, Cakir H, Celik H, Erel O. Decreased paraoxonase and arylesterase activities in the pathogenesis of future atherosclerotic heart disease in women with gestational diabetes mellitus. J Womens Health (Larchmt) 2009;18:1435-9. [CrossRef]
8. Yıldırım A, Aslan Ş, Ocak T, Yıldırım S, Kara F, Şahin YN. Serum paraoxonase, arylesterase activities and malondialdehyde levels in trauma patients. EAJM 2007; 39: 85-8.
9. Sonoki K, Iwase M, Sasaki N, et al. Relations of lysophosphatidylcholine in low-density lipoprotein with serum lipoprotein-associated phospholipase a2, paraoxonase and homocysteine thiolactonase activities in patients with type 2 diabetes mellitus. Diabetes Res Clin Pract 2009; 86: 117-23. [CrossRef]
10. Kim NS, Kang BK, Cha MH, Oh SM, Ko MM, Bang OS. Association between pon1 5′-regulatory region polymorphisms, pon1 activity and ischemic stroke. Clin Biochem 2009; 42: 857-63. [CrossRef]
11. Aksoy AN, Ozturk N, Aksoy H, Akcay F. Paraoxonase and arylesterase activities in patients with preeclampsia. EAJM 2008; 40: 10-3.
12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-8. [CrossRef]
13. Atik B, Tan O, Dülger H, Köseoğlu B, Bekerecioglu M. The time course of serum malondialdehyde levels in burned humans. Eur J Gen Med 2004; 1: 26-7.
14. Acikgoz Z, Bayraktar H, Altan O, Akhisaroglu ST, Kirkpinar F, Altun Z. The effects of moderately oxidised dietary oil with or without vitamin E supplementation on performance, nutrient digestibility, some blood traits, lipid peroxidation and antioxidant defence of male broilers. J Sci Food Agric 2011; 91: 1277-82. [CrossRef]
15. Koruk S. Mizrak A, Kaya R, et al. The effects of dexmedetomidine on ischemia reperfusion injury in patients undergoing arthroscopy under spinal anesthesia. EAJM 2010; 42: 137-41.
16. Tanaka H, Matsuda T, Miyagantani Y, Yukioka T, Matsuda H, Shimazaki S. Reduction of resuscitation fluid volumes in severely burned patients using ascorbic acid administration: A randomized, prospective study. Arch Surg 2000; 135: 326-31. [CrossRef]
17. Kahn SA, Beers RJ, Lentz CW. Resuscitation after severe burn injury using high-dose ascorbic acid: A retrospective review. J Burn Care Res 2011; 32: 110-7. [CrossRef]
18. Zang Q, Maass DL, White J, Horton JW. Cardiac mitochondrial damage and loss of ros defense after burn injury: The beneficial effects of antioxidant therapy. J Appl Physiol 2007; 102: 103-12. [CrossRef]
19. Milatovic D, Aschner M. Measurement of isoprostanes as markers of oxidative stress in neuronal tissue. Curr Protoc Toxicol 2009; Chapter 12: Unit12.14.