Relationship of Serum Paraoxonase Enzyme Activity and Thermal Burn Injury

Termal Yanık ile Serum Paraoksonaz Enzim Aktiviteleri Arasındaki İlişki

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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

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-
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 activity 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 mean±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
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 arylerase 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 arylerase activity in patients.

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

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