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OXIDANT/ANTIOXIDANT STATUS IN CASES OF SNAKE BITE

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☐ **Abstract—Background: Snake bites are an important cause of mortality and morbidity worldwide, especially in rural areas. Objective: The aim of this study was to investigate serum paraoxonase (PON), arylesterase (ARLY), ceruloplasmin (Cp), and myeloperoxidase (MPO) activity and lipid hydroperoxide (LOOH) and total sulfhydryl group (-SH) levels in patients with snake venom poisoning. Methods: The study included 49 patients with snake bite envenomation (Group 1) and 39 healthy volunteers as the control group (Group 2). Plasma PON, ARLY, Cp, and MPO activity and LOOH and -SH levels were measured. Laboratory measurements of 20 patients with snake bite envenomation (Group 3) were performed again after treatment. Results: PON and ARLY activity and -SH levels were significantly decreased in Group 1 compared with those in Group 2. Cp and MPO activity and LOOH levels were significantly elevated in Group 1 compared with those in Group 2. PON and ARLY activity were significantly elevated in Group 3 compared with those in Group 1. Cp and MPO activity and LOOH levels were significantly decreased in Group 3 compared with those in Group 1. Conclusions: Patients with snake bite envenomation had increased oxidants (MPO and LOOH) and decreased antioxidants (PON, ARLY, and -SH). Results obtained in this study demonstrate that snake bites are associated with a shift to oxidative status. Therapy with antioxidants can lead to an increase in the antioxidant defense system, and thus improvements in clinical symptoms.** © 2013 Elsevier Inc.

☐ **Keywords—snake bite envenomation; paraoxonase; arylesterase; ceruloplasmin; total sulfhydryl groups; myeloperoxidase; lipid hydroperoxide; oxidant; antioxidant**

INTRODUCTION

Snake bites are common in many regions of the world, particularly in rural areas, and injuries occur most often during the summer months. More than 2.5 million people are bitten by snakes each year (1). The venom of many snake species consists of carbohydrates, lipids, amines, enzymes, and both nontoxic and toxic proteins that have hematotoxic and neurotoxic properties (2). These substances decrease the coagulability of blood; induce bleeding; and can have secondary effects, such as hypovolemic shock and organ damage, or induce thrombosis (1–3). Organisms have antioxidative mechanisms to overwhelm oxidants, however, if there are too many oxidants or too few antioxidants, oxidative stress occurs and can cause chronic and permanent damage (4). As in many other diseases, oxidative stress can play a role in the pathophysiology of snake bite envenomation (5).

Paraoxonase-1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme with three activities: paraoxonase (PON), arylesterase (ARLY), and dyazoxonase (6–8). PON protects low-density lipoprotein (LDL) and HDL from oxidation (9–11). Human serum PON and ARLY are enzymes of the esterase group encoded by the same gene and have similar active centers (12). ARLY is recognized as an antioxidant enzyme because it hydrolyzes lipid peroxides and oxidizes lipoproteins as paraoxonase (7,13). PON and ARLY activity have

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been reported to decrease in many patients diagnosed with hypercholesterolemia, diabetes mellitus, cardiovascular disease, liver and kidney diseases, and iron-deficiency anemia (7,11,14).

Ceruloplasmin (Cp) contains copper atoms and is a plasma protein (15). Cp permits the incorporation of iron into transferrin without the formation of toxic iron products. Under physiologic conditions, Cp is also important in the control of membrane lipid oxidation, probably by direct oxidation of cations, thus preventing their catalysis of lipid peroxidation (16).

Another antioxidant group is sulfhydryl (-SH), which plays a crucial role in protecting cells from oxidative damage by interacting with the electrophilic groups of reactive oxygen species as the first and major member of the physiological antioxidant defense system (17). Decreased levels of -SH in humans have been shown to cause various disorders, such as liver failure, coronary artery disease, stroke, and other neurological disorders (17,18).

Myeloperoxidase (MPO) is a member of the haem peroxidase-cyclooxygenase superfamily released from intracellular granules by activated neutrophils, monocytes, and some macrophages (19,20). MPO plays an important role in neutrophil microbicidal action through catalyzing chloride ion oxidation to hypochlorous acid (19). MPO reacts with a potent pro-oxidant to form water-oxygen and hypochlorous acid-hydroxide radical, respectively, under conditions of elevated oxidative stress (19,21). Elevated systemic levels of MPO have been associated with unfavorable clinical outcomes, such as coronary artery disease, Alzheimer disease, lung cancer, and multiple sclerosis (19).

Lipid hydroperoxides (LOOHs) are highly toxic to biological systems that are important products of enzymatic processes and auto-oxidation products of polyunsaturated fatty acids. LOOHs are the initial products formed when lipids are damaged by oxidants, such as the tyrosyl radical and the nitrogen dioxide radical (22). Circulating levels of LOOH have been shown to be significantly elevated in patients with coronary artery disease and peripheral vascular disease (23–25).

The purpose of this study was to evaluate serum PON, ARLY, and Cp activity and -SH levels as antioxidants, and MPO activity and LOOH levels as an oxidative stress indicator in patients with snake venom poisoning.

METHODS

Study Population and Protocol

The study was conducted at the Departments of Emergency Medicine and Clinical Biochemistry, Gaziantep University, Gaziantep, Turkey from June 2009 to October 2010. The study protocol conforms to the principles of the Helsinki Declaration, and was approved by the Medical Ethics Committee of Gaziantep University.

The study population included 88 individuals (a study group and a control group). The study group (Group 1) consisted of 49 consecutive patients (mean age 39.7 ± 16.1 years; 27 men and 22 women) who were hospitalized for treatment of treat snake bites. The control group (Group 2) consisted of 39 healthy individuals (mean age 40.8 ± 16.2 years; 21 men and 18 women) who were administrative staff at our hospital. Laboratory measurements of 20 patients (Group 3) in the study group were performed again on the third day of treatment.

All enrolled subjects were free of acute or chronic medical disorders and were of normal body habitus. All subjects underwent a detailed medical history and physical examination by the study physicians. Subjects with the possibility of coronary artery disease, hypercholesterolemia, hypertension, neurological disorders, diabetes mellitus, liver and kidney diseases, peripheral vascular disease, Alzheimer disease, lung disease, multiple sclerosis, iron deviancy anemia, Wilson disease, and obesity after medical histories were taken and physical and laboratory examinations were performed were excluded from the study.

Body Mass Index and Blood Sample Collection

Body mass index was calculated by dividing a subject's weight in kilograms by height in meters squared (kg/m^2). Blood samples were withdrawn from a cubital vein into blood tubes and immediately stored on ice at 4°C. The serum was then separated from the cells by centrifugation at 3,000 rpm for 10 min. Serum samples were stored at −80°C until analysis.

Measurement of PON and ARLY Activity

PON activity was measured in the basal activity. The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase in absorbance at 412 nm at 37°C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 17,000 M^{-1} cm^{-1}. Serum PON activity was expressed as U/L (12). Phenylacetate was used as a substrate to measure ARLY activity by monitoring the increase in absorbance at 270 nm at 37°C. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol, 1,310 M^{-1} cm^{-1}. One unit of ARLY activity was defined as 1 μmol phenol generated per minute under the conditions mentioned and expressed as U/L (26).