Journal of Equine Veterinary Science 34 (2014) 484–488

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Selected Biochemical Indicators of Equine Rhabdomyolysis in Arabian Horses: Acute Phase Proteins and Trace Elements

Wael M. EL-Deeb BSc, MSc, PhD [a,b], Sabry M. El-Bahr BSc, MSc, DVM, PhD [c,d,*]

[a] Department of Clinical Studies, College of Veterinary Medicine and Animal Resources, King Faisal University, Al-Ahsa, Saudi Arabia
[b] Departments of Veterinary Medicine, Infectious Diseases, and Fish Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt
[c] Department of Physiology, Biochemistry and Pharmacology (Biochemistry), College of Veterinary Medicine and Animal Resources, King Faisal University, Al-Ahsa, Saudi Arabia
[d] Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt

ARTICLE INFO

Article history:
Received 6 March 2013
Received in revised form 15 August 2013
Accepted 20 September 2013
Available online 13 January 2014

Keywords:
Serum amyloid
Haptoglobin
Iron
Ceruloplasmin
Horse
Rhabdomyolysis

ABSTRACT

Although creatine kinase (CK), aspartate transaminase (AST), cytokines, and oxidative stress parameters were shown to be useful biomarkers for diagnosis of equine rhabdomyolysis, additional biomarkers of the disease may be of interest to indicate prognosis of the disease. Therefore, the present study investigated acute phase proteins and trace elements as additional biomarkers of ER. Sixty male horses (4-6 years old) of 2 equal groups were used. Horses of the first group were clinically healthy and served as a control group, whereas horses of the second group were ER-diseased animals. Harvested sera were used for estimation of the activities of CK, AST, lactate dehydrogenase (LDH), serum amyloid A (SAA), haptoglobin (Hp), ceruloplasmin (Cp), copper, iron, and zinc, whereas plasma samples were used for determination of fibrinogen. The present findings revealed a significant ($P \leq .05$) increase in values of CK, AST, and LDH in diseased horses compared to those in control values. In addition, a significant ($P \leq .05$) increase in SAA (162.6 ± 5.32 mg/L), Hp (3.6 ± 0.54 g/L), Cp (39.32 ± 2.31 mg/L), and copper (28.36 ± 1.23 μmol/L) along with a significant ($P \leq .05$) reduction in levels of iron (9.32 ± 0.23 μmol/L) and zinc (8.65 ± 1.02 μmol/L) was recorded in diseased horses compare to that in controls (11.3 ± 2.2 mg/L, 0.8 ± 0.2 g/L, 24.23 ± 1.32 mg/L, 18.41 ± 1.03 μmol/L, and 14.2 ± 0.42 μmol/L, respectively). In conclusion, SAA, Hp, Cp, copper, and zinc were useful prognostic biomarkers for the diagnosis of ER in Arabian horses.

1. Introduction

Exertional rhabdomyolysis was known as Monday morning disease, a disease related to work horses that were given a day of rest after a week of hard work. The affected horses developed stiffness and pain in the hindquarter musculature [1]. The first form of the disease was described in coldblood horses, whereas the second form was described as postexercise in race horses [2]. Later on the disease was known as tying-up [3]. Due to confusion arising from the terminology regarding equine exercise-induced rhabdomyolysis, scientists all over the world accepted the term equine exertional rhabdomyolysis (ER) to describe such problem in horses. This term indicates muscle disorder in the equine resulting from exercise. Therefore, tying-up, azoturia, setfast, Monday morning disease, Kreuzslag, and coupe de sang are considered synonyms for ER. Arabian horses were affected by rhabdomyolysis [4-6], and the cause is thought to involve carbohydrate metabolism [7]. Diagnosis of rhabdomyolysis depends greatly on

* Corresponding author at Dr. Sabry M. El-Bahr, BSc, MSc, DVM, PhD, Department of Physiology, Biochemistryand Pharmacology (Biochemistry), College of Veterinary Medicine and Animal Resources, King Faisal University, PO Box 1757, Al-Ahsa, 31982, Saudi Arabia.
E-mail address: sabryelbahr@hotmail.com (S.M. El-Bahr).

0737-0806/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2013.09.012

history, clinical signs, and biochemical analysis of horse blood. Clinical signs differ in mild cases compared with those in advanced rhabdomyolysis, which are characterized by shortened gait, muscle stiffness, and bad performance in racing horses [8,9]. Differential diagnosis is of great importance to exclude colic, laminitis, and aortoiliac thrombosis. Biochemical evaluation of creatine kinase (CK) and aspartate transaminase (AST) activities is a diagnostic tool for muscle disorder. An elevation of their activities indicates muscle damage and myolysis. Recently, proinflammatory cytokines, tumor necrosis factor-alpha (TNF-α), interlukein-6 (IL-6), and prostaglandin were introduced as effective biomarkers for diagnosis of ER in Arabian horses [6]. Acute phase protein (APP) responses were monitored extensively in animals for clinical purposes in the last 2 decades [10,11]. Therefore, many quantitative APP assays have been established. Acute phase proteins constitute negative (albumin and transferrin) and positive (haptoglobin [Hp], C-reactive proteins, serum amyloid A [SAA], ceruloplasmin [Cp], fibrinogen [Fb], and α-1-acid glycoprotein) proteins whose activity levels decreased or increased, respectively, in response to stimuli [12]. Acute phase proteins are synthesized in liver, and their synthesis is mediated by IL-6, TNF-α, and IL-1, which are released mainly by macrophages. However, extrahepatic synthesis was also reported in mammalian species [13]. Stimuli such as inflammation, infection, or tissue damage trigger cytokine release by defense-oriented cells, thereby inducing APP synthesis. Induction of positive APP in hepatocytes by cytokines [14] was associated mainly with reduction in negative APP biosynthesis [10]. Few publications demonstrated that SAA concentration and other APP were increased in blood of horses suffering from bacterial or viral infection, neoplasm, trauma, and surgery [15]. Earlier research reported that SAA was a major APP in horses, whereas both Hp and Fb act as moderate APPs [16]. Iron is a negative acute phase reactant in horses and other species [17,18]. Abnormally low iron values were associated with different diseases and tissue injury [19]. A defense mechanism of the body against bacterial infection was encountered by decreasing the availability of iron to bacteria via sequestering of iron in mononuclear phagocytes, inducing bacterial growth inhibition [17,18,20]. Ceruloplasmin is one APP that has the ability to scavenge toxins, free oxygen radicals produced during inflammation, and to protect the host against tissue damage [21]. In addition, Cp allows binding between iron and transferrin. Copper is the rate-limiting element in the biosynthesis of Cp. Correlation between copper and Cp was reported in humans and animals [22-24].

The antioxidant effect of zinc is documented [25]. The concentration of zinc is always associated with high cytokine and peroxidation levels [26,27]. It is well known that AST and CK are imperfect indicators whose values are not correlated with the severity of clinical signs, but they are the only criteria available to determine the severity of the disease and to decide when to return the horse to work. Although, cytokines and oxidative stress parameters were shown to be useful biomarkers for diagnosis of ER, additional biomarkers of the disease may be of interest to indicate the severity and prognosis of the disease. Therefore, the present study investigated APP and trace elements as additional biomarkers of ER.

2. Material and Methods

2.1. Animals and Sampling Protocol

A total of 60 male horses (4-6 years old) were used in our previous work [6]. They were divided into 2 equal groups. Horses of the first group (n = 30) were clinically healthy, with no history of ER, and served as the control group. Horses of the second group (n = 30) were ER-diseased horses. They were selected on the basis of clinical examination and history of overexercise after a period of rest and overfeeding of nonstructural carbohydrates (grains). In addition, biochemical analyses of CK, AST, and lactate dehydrogenase LDH indicated a significant increase in the activities of these enzymes in ER-diseased horses. Blood samples were collected from the jugular vein from all horses in both groups (samples from the second group were collected shortly after exercise) in fresh plain and heparinized vials for serum and plasma collection, respectively. Part of the serum samples were used in our previous work for estimation of proinflammatory cytokines and oxidative stress biomarkers. The remaining serum samples and plasma were kept frozen at −20°C until their use in the current experiment for determination of SAA, HP, Cp, iron, copper, and zinc, whereas plasma samples were used for determination of Fb.

2.2. Determination of Enzymes Activities

Enzymatic methods were used for colorimetric determination of serum AST, CK, and LDH (Bio-diagnostic, Cairo, Egypt) according to manufacturer's instructions.

2.3. Determination of APP

Fibrinogen concentration in plasma was determined [28]. Serum Hp was determined using the hemoglobin binding assay described previously [29]. Serum amyloid A was measured with a commercially available enzyme-linked immunosorbent assay kit (Phase SAA kit; Tridelta Ltd, Ireland). Moreover, a commercially available kit (catalog no. 4096-1000; Biovision Inc, CA) was used for determination of serum Cp by *p*-phenylenediamine oxidization [30].

2.4. Determination of Trace Elements

Serum iron concentration was determined by colorimetric spectrophotometry using commercially available kit (catalog no. EZ-0067; Assay Biotechnology Co, CA) [31]. Commercially available kits were used for determination of serum copper (catalog no. DICU-250; BioAssay; Quantichrom, CA) [32] and zinc (catalog no. K387-100; Biovision) [33].