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Original Research

Oxidative Stress Associated With Spasmodic, Flatulent, and Impaction Colic in Draft Horses

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ABSTRACT

The aim of the present study was to evaluate the oxidative stress level and antioxidant trace elements status associated with spasmodic, flatulent, and impaction colic in draft horses. For this purpose, venous blood samples were obtained from 20 randomly selected clinically healthy draft horses (control group) and 60 draft horses with different types of colic (spasmodic colic, n = 20; flatulent colic, n = 20; and impaction colic, n = 20). In horses with flatulent and impaction colic, there was a significant ($P < .05$) decrease in the total antioxidant capacity and activity of reduced glutathione (GSH) and catalase (CAT) as well as level of selenium, copper, zinc, and iron. However, there was a significant ($P < .05$) increase in the activity of superoxide dismutase (SOD) and in the level of malondialdehyde (MDA), interleukin-6 (IL-6), and manganese. Meanwhile, glutathione reductase (GR) was significantly ($P < .05$) increased in flatulent colic and significantly ($P < .05$) decreased in impaction colic. The oxidative stress index (OSI) was significantly ($P < .05$) increased in horses with flatulent and impaction colic. In horses with flatulent colic, there was a negative correlation between CAT and SOD ($r = -0.446$), MDA and zinc ($r = -0.450$), and IL-6 and zinc ($r = -0.470$). However, those with impaction colic, a negative correlation was recorded between CAT and MDA ($r = -0.602$), copper and GSH ($r = -0.474$), iron and GR ($r = -0.511$), and OSI and GR ($r = -0.662$). The results of the present study indicate that oxidative stress, with alteration of antioxidant trace element levels, is a feature of flatulent and impaction colic in draft horses.

1. Introduction

Colic refers to any cause of abdominal pain and is the leading cause of death in horses [1]. Much of the mortality is associated with ischemic-injured intestine because of the rapid deterioration of the intestinal barrier, absorption of bacterial lipopolysaccharide, and subsequent circulatory collapse [2–4]. Equine colic could be escorted by changes in the morphology and physiology of organs and tissues, including the intestine which could be attributed, at least in part, to the accumulation of oxidative damage products induced by reactive oxygen species (ROS) and reactive nitrogen species, secondary to intestinal ischemia [5].

Oxidants are essentially generated by metabolic enzymes, inflammatory cells, and mitochondrial electron leakage, which have a proinflammatory stimulatory role under certain conditions [6] and may play a role in the tissue damage associated with intestinal ischemia [7].

As a result of ischemia–reperfusion injury and endotoxemia occurring during horses' colic, an increased ROS release could be predicted with an increased burden of oxidative stress in the intestine [8–10]. Consequently, Kooreman et al [8] detected significant oxidant and/or antioxidant disorders in the jejunum but not in the colon in experimentally induced ischemia–reperfusion in the jejunum and large colon of horses. In addition, Grulke et al [6] stated that the plasma myeloperoxidase levels measured in horses

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suffering large intestinal obstruction were increased as a function of strangulation severity, indicating that leukocyte activation and ROS release occurred during colonic ischemia.

Distention of the small intestine proximal to a lesion or distention of the cecum because of dysfunction also causes ischemia. As the intraluminal pressure increases, the capillaries and venules are compressed decreasing the vascular capacitance. The subsequent decrease in perfusion and increase in interstitial fluid cause further vascular compromise [11]. Different cells in the intestine have different susceptibility to ischemia. The mucosal epithelial cells of the intestine are highly energy dependent, and thus, a reduced blood supply and decreased oxygenation of tissues result in rapid injury and death of these cells [12].

Many studies have been shown that reduced glutathione (GSH), a well-known antioxidant [13], protects cells and organs from various forms of injury induced by hypoxia and ischemia [14]. This has been used as evidence for a role of ROS in such injury as GSH metabolism suppresses the cytotoxic effects of these reactive radicals [15]. Nevertheless, GSH is also essential in preserving the ability of the cell to generate adenosine triphosphate and to maintain membrane integrity [16]. So, it has been suggested that GSH may protect cells by mechanisms independent of its antioxidant properties.

In horses, interleukin-6 (IL-6) plays a major role in regulating the inflammatory process induced during oxidative stress associated with exercise as a part of an integrated metabolic regulatory network [17]. When stimulated by oxygen radicals or platelets, endothelial cells generate cytokines, which attract and activate neutrophils during reperfusion injury. The aim of the present study was to evaluate the oxidative stress level and alterations in the antioxidant trace elements status associated with medical colic in draft horses.

2. Materials and Methods

2.1. Animals

A total of 80 draft horses of both sexes at 3–10 years of age were studied. Of all, 60 were exhibiting the signs of colic. In addition, 20 apparently healthy draft horses within the same age were randomly selected as a control group. The present study was carried out between 2012 and 2013 at Dakahlia Governorate, Egypt. This study was approved by the Animal Welfare and Ethics Committee, Mansoura University, on October, 2012.

2.2. Clinical Examination

Data concerned with the case history, clinical findings, and medical record for each horse were recorded. A detailed physical examination of the animals was carried out, and the clinical findings were recorded [18]. The horses were classified to have spasmodic, impaction, and flatulent colic based on the following criteria. First, spasmodic colic had brief attacks of intermittent abdominal pain, the horse rolling, pawing, and kicking for a few minutes, and then shaking itself and standing normally for a few minutes until the next bout of pain occurs. Intestinal sounds are often audible some distance from the horse, and loud rumbling borborygmi are heard on auscultation. The pulse is elevated moderately to about 60 beats/min, and there may be some patchy sweating, but rectal findings are negative, and there is no diarrhea. Second, impaction colic had moderate abdominal pain and relatively normal pulse rate and respiration. The principal manifestation of pain being stretching out and lying down and the bouts of pain are of moderate severity occurring at intervals of up to half an hour. There is anorexia, and the feces are hard covered with thick sticky mucus, passed in small amounts and are absent in some cases. Intestinal sounds are absent or much decreased in intensity in some cases. Rectal findings are impaction of the pelvic flexure of the large colon, and the distended loop of the intestine extends to the pelvic brim and lying on the floor of the abdomen. Third, flatulent colic had evident abdominal distension with acute and severe abdominal pain. Peristaltic sounds are reduced in early stages but become absent in the later stages. On rectal examination, gas-filled loops of large intestine fill the abdominal cavity and make proper examination of its contents impossible. Clinical signs are scored and sum of scores was calculated for each case (Table 1).

2.3. Blood Samples Collection

Two venous blood samples (10 mL each) were collected from each horse via jugular vein puncture. The first blood sample was collected into a clean centrifuge glass tube (Digisystem Laboratory Instrument, His Chih city, Taiwan) containing 5 mg of sodium ethylenediaminetetraacetic acid (EMD Chemicals Inc) as anticoagulant for evaluation of total antioxidant capacity (TAC); activity of GSH, glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD); and level of malondialdehyde (MDA) and IL-6. Meanwhile, the second blood sample was collected into a clean centrifuge glass tube without anticoagulant to separate serum for evaluation of selenium, copper, zinc, manganese, and iron. The separated serum and plasma were kept at −80°C for further biochemical analysis.

2.4. Biochemical Analysis

Total antioxidant capacity; activity of GSH, GR, CAT, SOD; and level of MDA, iron, and zinc were measured spectrophotometrically (Photometer 5010; Germany) following standard methods using commercially available test kits (Biodiagnostics, Cairo, Egypt). The ratio of the total peroxide levels to the TAC gave the oxidative stress index (OSI), an indicator of the degree of oxidative stress [19]. The OSI value was calculated as follows: OSI = [(total peroxide mmol/L)/(TAC mmol/L) × 100]. However, plasma IL-6 level was assayed following standard method using commercially available human IL-6 ELISA test kits (Boster Biological Technology, Pleasanton, Alameda County, CA). Meanwhile, the serum selenium, copper, and manganese levels were determined by established [20] procedures of atomic absorption spectrometry, using a Perkin-Elmer 2380 atomic absorption spectrophotometer instrument (Norwalk, CT). Briefly, after preparation of their stock standard solutions of nitric acid, assaying of selenium, copper, and