Evaluation of Total Oxidant / Antioxidant Capacity and Ceruloplasmin Levels in Horses with Inflammatory Airway Disease

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ABSTRACT

Background: Respiratory disease negatively affects the physiological performance of racehorses. Inflammatory Airway Disease (IAD) is a typical respiratory disease that affects young horses. The pathogenesis of IAD remains incompletely defined but it is thought to be associated with the inhalation of irritants such as barn dust and air pollution. Although non-infectious agents are likely to be central to the development of IAD, infectious agents can be associated with the disease. The main objective of the present study was to determine the relationships between IAD and antioxidant capacity, ceruloplasmin levels and bacterial and fungal infection status, in racehorses.

Materials, Methods & Results: In this study, 25 thoroughbred race horses with exercise intolerance and respiratory system symptoms were evaluated as the study group and 10 healthy horses were included in the control group. Clinical examinations, endoscopic evaluation and tracheal lavage were administered to all horses. Mucus accumulation in the trachea was scored and the horses having a mucus score of ≥3 were included the study group. Tracheal lavage fluids were investigated to determine the microbiological status of all animals. In addition, blood samples were collected and total oxidant capacity (TOC), total antioxidant capacity (TAC) and ceruloplasmin (Cp) levels were determined with a spectroscopic method. Exercise intolerance and various respiratory system symptoms such as coughing, nasal discharge and noisy breathing, were the common findings from horses in the study group. Fungal culture was negative and only Streptococcus equi subsp. zooepidemicus strain was isolated from 6 samples. No statistical difference was determined between the TOC, TAC and Cp levels of the groups (P > .05).

Discussion: Racehorses with IAD typically exhibit poor performance, chronic coughing and/or nasal discharge. This study showed little association between coughing and the isolation of bacterial species from tracheal aspirates, with only S. zooepidemicus strains isolated from 6 samples. Endoscopic examination of the airways after exercise and detection of excess mucus accumulation is an important diagnostic tool. In the present study, all animals of the study group had a mucus score of ≥3 (=excessive) mucus accumulation in the trachea. Excessive oxidant generation or antioxidant insufficiency can lead to oxidative stress (OS). A regular training regime helps horses to adapt to the higher levels of oxidative substances during exercise. Similarly, although there were IAD symptoms in the horses in the present study, no differences were detected in TAC and TOC levels between the study and control groups. Serum Cp is defined as an acute-phase protein (APP) and antioxidant. The APP concentration was not detectably different between exercise intolerant racehorses, with and without IAD in an earlier study of racehorses. Similarly, in our study, no differences were detected for Cp levels between the control and study group. These results may be pointing to non-systemic infection in the horses with respiratory symptoms. In conclusion, based on the clinical, endoscopic and microbiological findings in the present study, IAD was diagnosed in 25 racehorses. Probably, due to high adaptation ability of racehorses a systemic response was not observed in IAD which causes only mild to moderate pulmonary disorders. However, further studies involving a microscopic evaluation of tracheal aspirates for inflammatory cells should be performed for a detailed evaluation of acute phase proteins and oxidative stress parameters in racehorses with respiratory diseases and poor performance.

Keywords: Inflammatory Airway Disease, ceruloplasmin, total antioxidant capacity, total oxidant capacity, horse.
INTRODUCTION

Inflammatory Airway Disease (IAD) is a typical respiratory disease which affects young horses. Poor performance, coughing, excess mucus accumulation observed within the airways during endoscopic examination are characteristic symptoms of IAD. This disease differs from other respiratory conditions in horses like heaves because horses affected by IAD typically do not show increased respiratory effort at rest [9].

Although non-infectious agents are likely to be central to the development of IAD, the disease can be related to tracheal microorganisms such as Streptococcus pneumoniae, S. equi subsp. zooepidemicus, Actinobacillus spp., Mycoplasma equirhinis and Equine Herpesviruses 1 and 4 [20,30,32,34]. S. equi subsp. zooepidemicus is a multi-host pathogen that has been associated with infections in sheep, dogs, cats, poultry, rabbits, swine, and has zoonotic potential [1,3,11,19,22,25,26,33].

Oxidative stress (OS) is thought to be associated with some important infectious diseases in farm animals, such as pneumonia and enteritis. The oxidant/antioxidant equilibrium in racehorses can also become unbalanced in stressful physiological situations such as reproduction and exercise [5,10,18,23]. In addition, the level of acute phase proteins (APPs) can increase in the blood with non-infectious and infectious respiratory conditions [16,21].

In this study, the possible infectious agents, total oxidant/antioxidant capacity and Cp levels in horses with exercise intolerance and IAD symptoms were investigated. Specifically, the aims were to evaluate the relationships between IAD, and antioxidant capacity, Cp levels and bacterial and fungal infection status, in racehorses.

MATERIALS AND METHODS

Animals

A total of 35 thorough bred racehorses which were stabled together were used in the study. Twenty five of the horses with respiratory system symptoms (mean age 2.60 ± 0.65), such as coughing, nasal discharge, noisy breathing, and exercise intolerance, were used as the study group. The remaining ten horses, which were healthy, were included in the control group.

Clinical examination

All horses were physiologically examined and clinical symptoms were recorded. In addition, blood samples were collected from the jugular vein of all horses into sterile, empty tubes for the analysis of serum Cp, TAC and TOC levels.

Endoscopic Evaluation

Mucus accumulation in the trachea was scored as 0: none (clean singular), 1: little (multiple small blobs), 2: moderate (larger blobs), 3: marked (confluent, stream-forming), 4 large: (pool forming) and 5 extreme: (profuse amounts). The horses with a mucus score of ≥3 mucus were included the study [15].

Transendoscopic tracheal aspiration

Tracheal lavage was applied to all horses to obtain an aseptically-collected, diagnostic specimen from the lower trachea after training. Horses were restrained in stocks and a twitch was applied to the upper lip. A video endoscope (Olympus CF 401) was passed via one nostril to the pharynx and through the rimaglottidis into the trachea. The endoscope was advanced through the straight, mid-cervical trachea until the curvature of the distal trachea was observed. A single lumen endoscope flushing catheter (135mmx160 mm) was then advanced through the endoscope biopsy channel and passed beyond the end of the endoscope until the catheter tip was positioned just proximal to the tracheal bifurcation. Ten mL of sterile 0.9% saline solution was injected into the tracheal lumen and as much fluid as possible was aspirated under endoscopic visualisation [13].

Microbiological examination

A total of 25 tracheal aspirate fluid samples were obtained from clinically suspect horses and 10 samples from healthy horses. In order to detect bacterial contamination, samples were cultured at 37°C in aerobic, microaerobic and anaerobic conditions to determine the presence of any aerobic and facultative anaerobic bacteria, including Acheloplasma and Mycoplasma. Samples were cultured in sheep blood agar and PPLO agar supplemented with and without 20% (v/v) horse serum and incubated at 22°C and 37°C for Acheloplasma, Mycoplasma and other possible bacteria [4]. Fungal culture was also performed on Saboraud dextrose agar at 22°C in humidified atmosphere for 7 days. Identification of organisms was based on standard procedures including colony characteristics, reaction to Gram-staining and biochemical tests [8]. The PPLO agars were examined for colony formation with the aid of a stereo microscope during 10 days of incubation.
Biochemical Analysis

Serum Cp levels

Serum Cp was estimated with a colorimetric enzymatic assay based on oxidation of the dye, paraphenylenediamine (Genesys™ 10S UV-Vis Spectrophotometer)\(^2\). The obtained optical densities were calculated in mg % [28].

Total Oxidant Capacity (TOC)

A commercial kit was used for the determination of TOC (Rel Assay Diagnostics®)\(^3\). The initial solution containing reagent 1 (assay buffer) and the sample or standard was read at 530 nm for the first absorbance value. After that, prochromogen solution was added and incubated for 10 min at room temperature or 5 min at 37°C to produce colour complexing between the ferric ions and chromogen which can be measured spectrophotometrically and related to the total oxidant concentration. After incubation, the solution was read again at 530 nm (DAS Plate Reader)\(^4\).

Total Antioxidant Capacity (TAC)

For the measurement of TAC (Rel Assay Diagnostics®)\(^3\), the initial solution contained reagent 1 (assay buffer) and the sample or standard absorbance was read at 660 nm for the first value. After that, ABTS radical solution was added and the mixture was incubated for 10 min at room temperature or 5 min at 37°C to allow the antioxidants to reduce the dark blue-green ABTS to form colourless, reduced ABTS before the absorbance was read again at 660 nm (DAS Plate Reader)\(^4\).

Statistical Analysis

Normality was evaluated with the Shapiro-Wilk test and all data showed normal distribution. The Independent Samples T test was used for comparison of the control and study groups. All the data were expressed as mean ± standard deviation (SD). Differences were considered significant when \(P\) values were less than .05.

RESULTS

Clinical findings

In the study group, exercise intolerance was the common finding reported by trainers such as lower speeds of exercise and delayed recovery of respiratory rate after exercise. In addition, coughing and nasal discharge were detected in 15 horses, only coughing was observed in 4 horses, only exercise intolerance were detected in 3 horses and noisy breathing determined by auscultation was observed in 3 horses. Body temperature was normal in all horses and other clinical signs were not detected. No clinical signs were observed in the control animals.

Microbiological findings

Fungal culture was negative except for a few contaminants. In addition, Mycoplasma and Acheloplasm colonies were not detected, with only Streptococcus strains isolated from 6 samples only. The Streptococcus strain was identified by its biochemical characteristics including heamolysis and fermentation of lactose, trehalose and sorbitolas S. equi subsp. zooepidemicus. No bacteria or fungi were cultured from the control horses.

In the light of the clinical, microbiological and biochemical findings, IAD was diagnosed in 25 horses of the study group.

Biochemical analysis

The results of biochemical analysis are shown in Table 1. No statistical difference was determined between TOC, TAC and Cp levels of the symptomatic/study and control groups (\(P > .05\)).

<table>
<thead>
<tr>
<th>Group</th>
<th>TOC</th>
<th>TAC</th>
<th>Cp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>22.86 ± 6.38</td>
<td>1.17 ± 0.82</td>
<td>10.35 ± 3.05</td>
</tr>
<tr>
<td>Control</td>
<td>24.25 ± 12.51</td>
<td>1.03 ± 0.51</td>
<td>9.15 ± 4.04</td>
</tr>
</tbody>
</table>

There is no statistical difference between groups (\(P > .05\)).
DISCUSSION

Poor athletic performance of racehorses is a major problem in the racing industry. Locomotory and respiratory conditions have been identified as the two main causes of training disruption and interruption of racing in thoroughbred horses. Determination of the definitive reason for poor performance is however a real diagnostic challenge since many of the causative conditions are multifactorial and may only be manifested during exercise. In general, poor performance in horses is attributed to viral infections; environment, especially stabling environment; and *Mycoplasma* and other bacterial infections in equine respiratory disease [7,29,34].

Horses with IAD typically exhibit poor performance, chronic coughing and/or nasal discharge [9]. Diagnostic tests are required to differentiate IAD from other diseases such as heaves, bacterial infections, viruses and sometimes parasitic infections that can cause similar symptoms. One common test is an endoscopic examination of airways after exercise to see whether excess mucus has accumulated [9,34]. In the present study, mucus accumulation was evaluated with a scoring system from 0 to 5. Endoscopic examination showed excessive mucus accumulation (3 - 5 score) in the trachea of all animals of the study group. All horses had received vaccination against viral diseases (Equine herpes viruses 1 and 4 and Equine influenza virus), and antiparasite treatment had been administered periodically.

The present study showed little association between coughing and the isolation of bacterial species from tracheal washes. Only *Streptococcus equi* subsp. *zooepidemicus* strains were isolated from 6 samples. Moreover, there was no association with *Mycoplasma*, *Acheloplasma* and fungi. Isolation of low numbers of bacteria may represent a transient population that is cleared rapidly from the trachea without being associated with disease or contamination of the sample. Alternatively, it is possible that low numbers of bacteria are associated with the disease, but the count was insufficient to detect their effect; certain strains of *S. zooepidemicus* may be more adapted than others to infect the upper airways of horses and cause outbreaks of upper respiratory disease [17]. Equilibrium between the pro-oxidative burden and the antioxidant defence system seems to favour health, well-being and, potentially, performance [14]. Niedzwiedz and Jaworski [23] evaluated the oxidant-antioxidant status in horses with symptomatic recurrent airway obstruction (RAO) and detected a significant increase in the activities of GPx and SOD in RAO-affected horses compared with the control animals. Furthermore, there is clear evidence that OS is involved in the pathophysiology of airway inflammation. Endogenous and exogenous antioxidants counterbalance the oxidative processes and so maintain the oxidant/antioxidant equilibrium. Excessive oxidant generation or antioxidant insufficiency can lead to OS [14].

However, Fazio et al. [10] indicated that a regular training regime helps horses to adapt to the higher levels of oxidative substances generated during exercise. This regime makes horses more resistant to OS and the training also has an influence on the 24 h cycle in the physiological processes of oxidant and antioxidant parameters [10]. Similarly, although there were IAD symptoms in the horses in the present study, no differences were detected in TAC and TOC levels between the study and control groups.

The plasma protein, Cp, has been implicated as an anti-inflammatory agent [6] and serum Cp was defined as an acute-phase, reactive protein that is detected at higher levels in the intermediate or later phases of acute inflammation in horses [24]. Cp serves as a scavenger of toxic, free oxygen radicals produced during inflammation, minimizing damage to the host’s tissues [31]. Therefore it has an antioxidant, protective function [12]. Also, serum Cp levels increase in the acute phase of strangles [27].

In contrast, Leclere et al. [16] reported that APP (serum amyloid A (SAA), CRP, and haptoglobin) concentrations were not detectably different between exercise intolerant horses, with and without IAD, in a racehorse population. Similarly, in our study, Cp was evaluated in horses, together with TOC and TAC, and no differences were detected for all parameters between the control and study group. These results may be pointing to non-systemic infection in the horses with respiratory symptoms. The horses investigated in the study had poor performance, according to the trainers’ reports delayed recovery of respiratory rate after exercise and lower speeds of exercise, coughing and nasal discharge signs, which are indicative of IAD. In addition, according to our clinical and microbiologic investigations and the vaccination records, there is no evidence for severe respiratory disease.
Previous and present findings showed that Cp and haptoglobin are unlikely to be useful markers of IAD because horses with IAD have only mild to moderate pulmonary function impairment (i.e., they do not display respiratory difficulty at rest) expressed clinically by poor performance, exercise intolerance, or cough [16].

CONCLUSIONS

In conclusion, based on the clinical, endoscopic and microbiological findings in the present study, IAD was diagnosed in 25 race horses. Probably, due to high adaptation ability of racehorses a systemic response was not observed in IAD which causes only mild to moderate pulmonary disorders. A weakness of the study was that microscopic evaluation could not made from tracheal aspirates due to circumstances beyond the control of the authors. However, all indicators for the horses such as age range environmental conditions, clinical and endoscopic findings confirmed the diagnosis of IAD. Owners should change stabling conditions to provide optimal circumstances for horses such as fresh air circulation; sawdust and other inhalable particles should not be used. The horses should also be protected from air pollutants as well. Further studies should be performed for a more detailed evaluation of APPs and OS parameters in racehorses with poor performance and respiratory diseases

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Ethical approval. All procedures, treatments and animal care were in compliance with the international and Turkish guidelines for the care and use of animals. The study design was approved by The Local Ethical Committee for Animal Experiments of Ondokuz Mayis University (No: 2015-75).

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

REFERENCES


