Studies on oxidant-antioxidant and trace minerals alteration in equine upper respiratory affection

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Abstract

In equine field, respiratory affections are common diagnostic problems, strangles is a bacterial disease causing airway affection in young horses. This study aimed to evaluate oxidant/antioxidant status in strangles affected horses. Fifteen horses (15) were evaluated in this study, the levels of SOD, GPx, Catalase, MDA, hydrogen peroxide and antioxidant trace minerals (Zn and Cu) were estimated. Significant decrease in SOD, GPx, Zn along with significant increase in MDA and hydrogen peroxide levels were recorded. Oxidative process has been associated with strangles infection in horses leading to oxidant-antioxidant imbalance and its effect extends to associate antioxidant trace minerals, thus, a future thinking of adding antioxidant supplementation to regular treatment regimen may be rewarding.

Keywords: horse; strangles; oxidant; antioxidant; trace minerals

Introduction

Respiratory affections are considered a main cause of morbidity and even fatality in foals (Carr, 2007). *Streptcoccus equi* is organism known to cause upper respiratory affections in foals worldwide (Waller and Jolley, 2007). One of the most important respiratory affection of young horses is strangles, a gram positive cocci (Sweeney, 1996). Strangles is also known as equine distemper (Arias, 2013) is characterized of variety of respiratory signs including pyrexia, muco-purulent to purulent nasal discharge and abscessiated lymph nodes (Sweeney et al., 2005). With diagnosis depends basically on detection of micro-organism in pus of affected lymph nodes via bacteriological culture, strangles came as one of the most feasible diagnostic affection in horses (Sweeney et al., 2005; Laus et al., 2007).

The consequence of creating imbalance between reactive oxygen species (ROS) generation and scavenging mechanism is the oxidative stress (Sikka 2001), these modifications apt injuries outcomes (Mandelker and Vojdovich, 2011). More than a few mechanisms are intricate in protection against devastating nature of oxidants, Glutathione peroxidase, Superoxide dismutase (among several) are two of them (Aviram, 2003). The enzymatic antioxidant system actions banks on certain trace minerals (Kleczkowski et al., 2004), Cu and Zn play a major role in body antioxidant mechanism by averting radical-induced injury (Nazifi et al., 2011).

In equine practice, correlation between oxidant/antioxidant balances has been discussed in heaves (Deaton et al., 2005), however, studies regarding them in other topics including strangles are limited (Hassanpour, 2013). The present study was performed on foals with confirmed strangles affection to evaluate oxidant/antioxidant status in the affected patients.

Material and Methods

Fifteen Arabian horses (15) with age range from 4 month to 7 month old were evaluated in this study. The study was performed in Giza government; all the fifteen horses were previously confirmed positive to have strangles on basis of history, clinical and bacteriological means within 2-3 days after appearance of clinical signs.

From each horse a blood sample was taken from jugular vein and divided into EDTA-tube, heparin containing tube and plain tube to collect RBCs lysate, heparinized plasma and serum respectively (Weiss and Wardrop 2010). The blood from EDTA containing tubes was used to prepare erythrocytes lysates for estimation levels of glutathione peroxidase (GPX) and superoxide dismutase (SOD) enzymes using respective test kits (Bio-Diagnostic Company-Egypt) according to manufacture instructions, the resultant erythrocyte lysate was stored in -80ºC till use. Heparinized plasma samples were used for estimation of catalase, Malondialdehyde "MDA" and Hydrogen peroxide, using respective test kits (Bio-Diagnostic Company-Egypt), the parameters were estimated immediately after plasma separation. The collected sera were used to estimate anti-oxidant trace minerals "copper and zinc" levels using respective test kits (Spectrum Diagnostic-Egypt). Cu and Zn were estimated immediately. All parameters were evaluated colorimetrically (APEN , PD-303S, Japan).

Control animal were used to establish control data, criteria for inclusion were: the same age and breed group, apparently healthy animal, fecal samples were examined for exclusion of parasitic infestation and nasal swap were taken to confirm they are strangles-free.

Data of diseased horses were compared with data of control horses using student T-test for Calculation of mean ± SE, $p \leq 0.05$ considered significant.

Results

The alterations in antioxidant agents and antioxidant trace minerals are recorded in table 1 and graph 1. Significant decrease in SOD and GPx were observed in diseased animals compared to control group. Though catalase enzyme showed decrease in activity in diseased group compared to control group, this difference is considered to be not quite statistically significant.

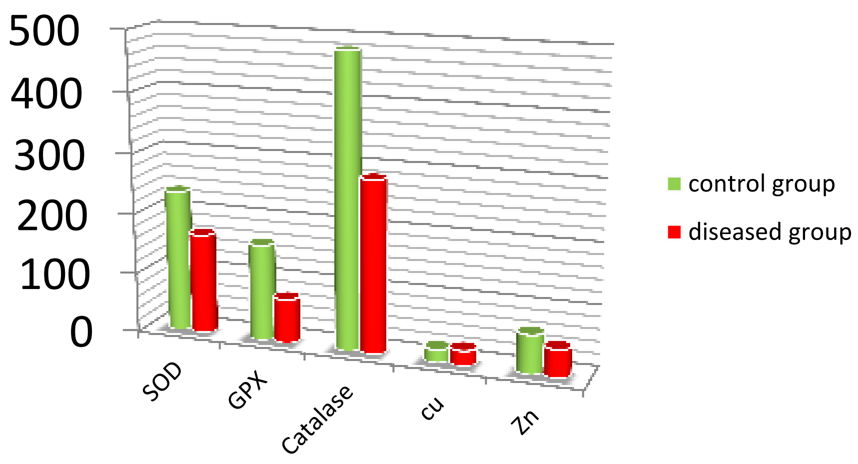
Trace minerals activity, namely Zn and Cu showed significant decrease in zinc activity in diseased group while Cu level showed an increase in activity however this increase is considered not statistically significant.

The variations in non-enzymatic oxidant parameters are shown in Table 1. Both Malondialdehyde "MDA" and Hydrogen peroxide showed significant increase in their activities in diseased animals compared to control group.

Table 1. Oxidative stress biomarkers in diseased and control horses

Parameters		Control horse (A)	Diseases horses (B)	P value
Enzymatic anti-oxidant	SOD (u/ml)	235.190±11.34	166±9.04	0.002
	GPx(mu/ml)	159.75±12.22	73.34±16.20	0.0049
	Catalase(u/l)	482.00±58	284.038± 71	0.0793
Non-Enzymatic	Cu (umol/l)	21.300±1.96	24.76±1.63	0.2
	Zn (ug/dl)	64.084±2.23	45.983±2.23	0.043
Non enzymatic oxidant system	MDA (nmol/ml)	1.426±0.171	3.188±0.58	0.0266
	Hydrogen Peroxide(mM/l)	0.0075±0.00623	0.0180±0.00229	0.0228

Fig. 1 Oxidative stress biomarkers in diseased and control horses



Discussion

In recent years, studying oxidant-antioxidant mechanisms have increased popularity and focus in both human and veterinary medicine (Ayala et al., 2014). In equine practice, oxidative biomarkers gained attention especially in topics dealt with anemia (Deger et al., 2009, Bolfă et al., 2012).

In the current study significant decrease in both SOD and GPx activities with slight decrease in Catalase activity were observed, these results were recorded in other equine diseases (Bolfă et al., 2012). These decreased activities may indicate disruption in oxidant/antioxidant equilibrium, these enzymes poses the ability to transform anions into H_2O_2 and water making them easily removed (Kirschvink and Lekeux 2008). In a study dealt with bacterial infection to reproductive tract in mares, infection appears to have a direct effect on both SOD and MDA in affected mares (Abo-Maaty et al., 2014). Increase lipoperoxide products and decreased enzymatic antioxidant activities were recorded in the blood of race horses following strenuous exercise (Maranon et al., 2008). Loss of enzymatic antioxidant components may weaken the cellular defenses and buildup of ROS is expected (Kolodziejczyk et al., 2005). It seems like first role of GPx is to detoxify the "organic peroxides" (Johnson et al., 2000). Catalase is vital enzyme in antioxidant cycle, its importance come from the role it plays in destruction of H_2O_2 (Kirkman and Gaetani, 2007), aid in protection of somatic cells especially in inflammation sites against damaging effect of H_2O_2 (Agar et al. , 1986).

Cu and Zn play a major role in body antioxidant mechanism by averting radical-induced injury (Nazifi et al., 2011). Zinc plays an important role in formation of SOD and Cu in the structure of ceruloplasmin (Ciftci et al., 2003).Significant decrease in zinc activities accompanied with non-significant increase in Cu levels were observed, similar findings were recorded in strangles affected foals in Iran (Rad et al., 2013), also the same observation were reported in *T.equi* infected horses (Salem and El-sherif, 2015), however in a study dealt with EHV-1, a significant reduction in both Cu and Zn were recorded (Yeter et al., 2007). The decrease in zinc level may affect the immune system (Shankar and Prasad, 1998), because body requires zinc to activate T-lymphocytes (Wintergerst et al., 2007). It has been postulated that in inflammatory process, level of ceruloplasmin "an acute phase protein" increased and consequently, leads to increase Cu levels (Healy and Tipton, 2007).

Lipid peroxidation process produced by free radicals in body, the peroxidation of polyunsaturated fatty acids results in MDA, a widely used marker for oxidative stress (Gawel et al., 2004). Significant increase in MDA level was recorded, estimation levels of MDA permits detection of lipid peroxidation and the indirect determination of free oxygen radicals' levels (Esterbauer, 1996). When the free radical levels exceed the counter antioxidant enzymes, these radicals assault cells and membrane, it has been concluded that measurement of MDA is highly substantial in oxidative stress pathology (Grotto et al., 2009). Significant increase in levels of hydrogen peroxide was observed in the diseased horses, in animals, a level of 50µM or higher considered cytotoxix (Halliwell and Gutteridge, 1999), therefore, enzymes like catalase and GPx are employed for the elimination (Halliwell et al., 2000). In human study, it has been hypothesized that high plasma level H_2O_2 can be detected if condition for removal was prohibited (Varma and Devamanoharan, 1991).

In conclusion, oxidative process has been associated with strangles infection in horses leading to oxidant-antioxidant imbalance and its effect extends to associate antioxidant trace minerals, thus, a future thinking of adding antioxidant supplementation to regular treatment regimen may be rewarding.

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