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Oxidative stress biomarkers and cardiac troponin I in Arabian horses with strangles

Wael M. El-Deeb

Ahmed M. Elmoslemany

Short Communication

Oxidative stress biomarkers and cardiac troponin I in Arabian horses with strangles

Wael M. El-Deeb[1,2]* Ahmed M. Elmoslemany[1]

Abstract

In order to investigate the levels of oxidative stress (OS) biomarkers and cardiac troponin I (cTnI) in horses with strangles, fifty horses with strangles and twenty healthy controls were included in this study. Blood and serum samples were tested for levels of cardiac troponin I (cTnI), malondialdehyde (MDA), super oxide dismutase (SOD), glutathione (GSH), nitric oxide (NO) and total antioxidant capacity (TAC). The levels of cTnI were increased in ten diseased cases while normal cTnI levels were found in the others (n=40). The levels of MDA were increased in the horses with strangles when compared with the healthy ones. The levels of SOD, GSH, NO and TAC were lower in the horses with strangles when compared with the controls. Successfully treated cases had lower levels of cTnI and MDA compared to non-responsive ones. Only the SOD level was higher in the successfully treated cases compared to the non-responsive ones. The cases had a moderate and positive correlation with the cTnI and MDA levels. Conversely, the levels of SOD, GSH, NO, and TAC negatively correlated with being a case. Additionally, the treatment success negatively correlated with the cTnI and MDA levels, whereas it positively correlated with the SOD level. In conclusion, OS was detected in horses with strangles. These biomarkers could be used as diagnostic and prognostic tools.

Keywords: cardiac troponin I, malondialdehyde, antioxidant, stress

[1]*Department of Clinical Studies, College of Veterinary Medicine and Animal Resources, King Faisal University, P.O. Box 1757, Al-Ahsa 31982, Saudi Arabia*

[2]*Department of Veterinary Medicine, Infectious Diseases and Fish Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt*

[*]***Correspondence:*** *weldeeb@kfu.edu.sa, drwaeleldeeb@yahoo.com*

Introduction

Strangles is considered as one of the vital challenges facing equine specialists (Timoney et al., 1999). It has many possibly incurable complications and the capability of persistent infection in populations of asymptomatic carrier horses (Sweeney et al., 2005). Cardiac troponin I (cTnI) is a sensitive heart marker for the diagnosis of myocardial damage (Fonfara et al., 2010; Reagan et al., 2013; El-Deeb and Elmoslemany, 2015). Assessment of oxidant & antioxidant levels is essential in horses with strangles. The blood and tissue levels of malondialdehyde (MDA) are used as a precise marker of lipid peroxidation (El-Deeb et al., 2014; El-Deeb and Tharwat, 2015, El-Deeb and Buczinski, 2015). The target of this study was to assess the levels of OS biomarkers and cTnI in cases of strangles in horses, as well as their diagnostic and prognostic importance.

Materials and Methods

Animals: Seventy Arabian male horses (10-18 months old) were selected for this study. They were divided into two groups. Horses of the first group (n=20) were apparently healthy, free of any previous history of strangles and categorized as a control group. The cases (n=50) consisted of horses with clinical signs of strangles. The classification of both groups was based on clinical and laboratory findings.

Sampling protocol

Nasopharyngeal swabs: Nasopharyngeal swabs supplied by Equi-Vets, Denmark were used for collecting samples from all horses. The samples were used for microbiological examination.

Blood samples and sampling protocol: Whole blood and serum samples were harvested from both groups in fresh heparinized and plain vials. Cardiac troponin I was analyzed in the blood samples using a point-of-care analyzer (VetScan i-STAT® 1, Abaxis, CA, USA). The sera were used for estimation of the activities of creatinine kinase (CK), aspartate aminotransferase (AST) and alanine aminotransferase (ALP). The analysis was carried out using a chemistry analyzer (VetScan HM5, Abaxis, CA, USA). In addition, levels of MDA, SOD activity, GSH activity, NO and TAC were estimated using commercially available ELISA kits (Cayman, USA).

Statistical analysis: Descriptive statistics were calculated for each biomarker by three different outcomes (case control status, treatment success or failure, and complication or no complication). Most data did not follow normal distribution and nonparametric test (Wilcoxon Mann-Whitney) was employed at $P < 0.05$ to compare the levels of biomarkers for each outcome of interest. Correlation between different parameters and each outcome was assessed using Spearman's correlation coefficient. Association between each outcome and animal age and sex was evaluated using logistic regression analyses. All analyses were conducted using Stata version 14 (Stata Corp, College Station TX, USA).

Results and Discussion

In this study, the strangles-diseased horses were characterized clinically by fever and serous nasal discharge that transformed to purulent nasal discharge later on. Abscessation of the submandibular lymph nodes was recorded few days after in a number of horses. This clinical picture is in agreement with those formerly reported by Sweeney et al. (2005).

In this study, the recorded complications of strangles in the diseased horses under investigation included pneumonia (n=5), guttural pouch empyema (n=2), colic (n=1), myopathy (n=1) and possible cardiac injury (n=10). Many authors (Sweeney et al., 1987; Sweeney et al., 2005) recorded similar complications previously. The overall complication rate was 26%, which is partially comparable to those previously reported by Ford and Lokai (1980) and Sweeney et al. (1987).

In this study, higher values of cTnI in the diseased horses (n=10) could indicate a possible myocardial injury. The level of increment in serum cTnI has appeared to be associated with the degree of myocardial harm and survival in people and in different animal species (Fonfara et al., 2010, El-Deeb and Elmoslemany, 2015). The levels of cTnI in circulating blood have appeared to correspond well with pathological changes in the heart muscles, cardiovascular pathophysiology, level of cardiac harm, clinical picture and disease outcome (Wells and Sleeper, 2008). It was reported formerly (Ford and Lokai, 1980) that four *Streptococcus equi*-infected horses suffered from cardiovascular complications.

In this investigation, OS was obvious as specified by the more elevated levels of MDA and incitement of cell reinforcement protein frameworks to kill the dynamic free radicals in the strangles-affected horses. Past studies showed larger amount of MDA in horses with *Streptococcus equi ssp equi* infection (Hassanpour, 2013).

This investigation detected low levels of SOD in the horses with strangles, in contrast to the healthy group. The lower serum levels of SOD could be identified with its exhaustion as free radical scavengers amid the oxidative procedure in horses with strangles.

The successfully treated cases had lower levels of cTnI, CK, MDA, ALP and AST compared to the non-responsive ones. Only the SOD level was higher in the successfully treated cases compared to the non-responsive ones. The cases had a moderate and positive correlation with the cTnI, CK, MDA, ALP, AST levels. Conversely, the levels of SOD, GSH, NO, and TAC negatively correlated with being a case. Additionally, the treatment success negatively correlated with the cTnI, CK, MDA, ALP, and AST levels, whereas it positively correlated with the SOD level.

In conclusion, OS biomarkers play a role in the pathogenesis and complications of strangles in horses and their levels could be used as diagnostic and prognostic biomarkers.

Table 1 Statistics of blood biomarker levels in control horses and those with strangles (Cases)

Variable	Control (n=20)				Cases (n=50)				*P-Value
	Mean	Median	Min	Max	Mean	Median	Min	Max	
cTnI (mg/dl)	0.02	0.02	0.02	0.04	0.36	0.03	0.02	2.45	0.0004
CK (IU L^{-1})	195.79	196.36	187.36	204.36	220.33	200.44	188.98	435.34	0.0041
MDA (µmol/L)	1.02	1.03	0.87	1.2	9.67	9.33	7.29	12.33	0.0001
SOD (U/ml)	112.37	112.36	103.25	116.45	71.89	68.59	62.11	141.26	0.0001
GSH (mg/dL)	2.95	2.82	2.53	3.81	1.88	1.45	1.23	6.21	0.0001
NO (µmol/L)	4.03	3.96	3.47	4.63	3.07	2.99	2.82	4.52	0.0001
TAC (µmol/L^{1})	0.69	0.65	0.52	0.85	0.30	0.23	0.21	1.32	0.0001
ALP (IU L^{-1})	412.85	415.23	390.15	429.32	465.33	433.41	399.23	666.23	0.0001
AST (IU L^{-1})	273.72	274.26	260.34	288.54	285.74	278.36	268.35	385.21	0.0046

cTnI=cardiac troponin I; CK=creatinine kinase; MDA=malondialdehyde; SOD=super oxide dismutase; GSH=glutathione; NO=nitric oxide; TAC=total antioxidant capacity; Min=Minimum; Max=Maximum
**P*-value resulting from non-parametric Wilcoxon Mann-Whitney test

Table 2 Statistics of blood biomarker levels by treatment response of horses with strangles

Variable	Success (n=45)				Failure (n=5)				*P-Value
	Mean	Median	Min	Max	Mean	Median	Min	Max	
cTnI (mg/dl)	0.19	0.03	0.02	1.63	2.33	2.37	2.12	2.45	0.0007
CK (IU L^{-1})	204.82	200.23	188.98	435.34	394.75	397.785	350.21	433.21	0.0017
MDA (µmol/L)	9.51	9.32	7.29	12.23	11.48	11.24	11.12	12.33	0.0044
SOD (U/ml)	72.70	68.59	62.35	141.26	62.80	62.98	62.11	63.11	0.0017
GSH (mg/dL)	1.87	1.45	1.23	6.21	2.04	1.32	1.3	4.23	0.3225
NO (µmol/L)	3.07	2.99	2.82	4.52	3.04	3.045	2.97	3.11	0.7132
TAC (µmol/L^{1})	0.30	0.23	0.21	1.32	0.22	0.215	0.21	0.22	0.0624
ALP (IU L^{-1})	454.08	431.25	399.23	650.23	591.92	626.62	448.21	666.23	0.0077
AST (IU L^{-1})	281.14	277.46	268.35	385.21	337.48	329.72	320.125	370.34	0.0023

cTnI=cardiac troponin I; CK=creatinine kinase; MDA=malondialdehyde; SOD=super oxide dismutase; GSH=glutathione; NO=nitric oxide; TAC=total antioxidant capacity; Min=Minimum; Max=Maximum **P*-value resulting from non-parametric Wilcoxon Mann-Whitney test

Table 3 Statistics of blood biomarker levels by case complication (Strangles complications)

Variable	No Complication (n=37)				Complication (n=13)				*P-value
	Mean	Median	Min	Max	Mean	Median	Min	Max	
cTnI (mg/dl)	0.03	0.03	0.02	0.06	1.28	1.34	0.02	2.45	0.0001
CK (IU L^{-1})	199.45	200.23	189.21	204.12	278.14	204.92	188.98	435.34	0.0088
MDA (µmol/L)	9.21	9.28	7.29	11.22	10.95	11.12	8.24	12.33	0.0001
SOD (U/ml)	73.17	69.135	63.11	141.26	68.36	63.12	62.11	123.56	0.0001
GSH (mg/dL)	1.87	1.55	1.23	6.21	1.92	1.33	1.3	5.63	0.0822
NO (µmol/L)	3.03	2.99	2.89	3.62	3.16	3.11	2.82	4.52	0.5233
TAC (µmol/L^{1})	0.32	0.23	0.21	1.32	0.23	0.22	0.21	0.32	0.1423
ALP (IU L^{-1})	437.46	430.21	399.23	552.23	542.51	533.21	429.66	666.23	0.0001
AST (IU L^{-1})	277.85	277.91	268.35	288.54	307.57	281.36	270.34	385.21	0.085

cTnI=cardiac troponin I; CK=creatinine kinase; MDA=malondialdehyde; SOD=super oxide dismutase; GSH=glutathione; NO=nitric oxide; TAC=total antioxidant capacity; Min=Minimum; Max=Maximum
**P*-value resulting from non-parametric Wilcoxon Mann-Whitney test

Table 4 Correlation of blood biomarker levels with case control status, treatment response, and case complication

Variable	Case control	Treatment response
cTnI (mg/dl)	0.43*	-0.49*
CK (IU L^{-1})	0.35*	-0.45*
MDA (µmol/L)	0.79*	-0.41*
SOD (U/ml)	-0.66*	0.45*
GSH (mg/dL)	-0.63*	0.14
NO (µmol/L)	-0.76*	-0.05
TAC (µmol/L^{1})	-0.72*	0.27
ALP (IU L^{-1})	0.54*	-0.38*
AST (IU L^{-1})	0.34*	-0.44*

cTnI=cardiac troponin I; CK=creatinine kinase; MDA=malondialdehyde; SOD=super oxide dismutase; GSH=glutathione; NO=nitric oxide; TAC=total antioxidant capacity; Min=Minimum; Max=Maximum
**P*-value resulting from non-parametric Wilcoxon Mann-Whitney test ($P < 0.05$)

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บทคัดย่อ

ระดับของ Oxidative stress biomarkers และ cardiac troponin I ในม้าพันธุ์อาระเบียที่ป่วยเป็น strangles

วาเอล เอ็ม เอลดีบา[1,2*] อาเหม็ด เอ็ม เอลมอสเลมันยา[1]

การศึกษาครั้งนี้ได้ตรวจหาระดับความเข้มข้นของ oxidative stress (OS) และ cardiac troponin I (cTnI) ในม้าป่วยเป็น strangles โดยทดสอบในตัวอย่างเลือดม้าป่วยเป็น strangles จำนวน 50 ตัว และม้าปกติจำนวน 20 ตัว เพื่อตรวจหาความเข้มข้นของ cardiac troponin I (cTnI), malondialdehyde (MDA), super oxide dismutase (SOD), glutathione (GSH), nitric oxide (NO) และ total antioxidant capacity (TAC) ผลการทดสอบพบค่า cTnI สูงขึ้นในม้าป่วย 10 ตัว และปกติในม้าปกติ (n = 40) และค่า MDA สูงขึ้นในม้าป่วยเมื่อเทียบกับม้าปกติ ในขณะที่พบค่า SOD, GSH, NO และ TAC ต่ำลงในม้าป่วยเป็น strangles เมื่อเทียบกับม้าปกติ นอกจากนี้ในสัตว์ที่ตอบสนองต่อการรักษาจนหายปกติ จะมีค่า cTnI และ MDA ต่ำลงเมื่อเทียบกับสัตว์ที่ไม่ตอบสนองต่อการรักษา และมีค่า SOD สูงขึ้นในสัตว์ที่ได้รับการรักษาเมื่อเทียบกับสัตว์ที่ไม่ตอบสนองต่อการรักษา และพบความสัมพันธ์ทางบวก ระหว่างม้าป่วยกับค่า cTnI และ MDA และในทางตรงกันข้ามพบความสัมพันธ์เชิงลบ ระหว่างม้าป่วยกับค่า SOD, GSH, NO และ TAC นอกจากนี้การตอบสนองต่อการรักษายังมีความสัมพันธ์กับระดับ cTnI MDA และ SOD สรุปได้ว่า OS สามารถนำมาใช้เป็น biomarkers ในม้าป่วยเป็น strangles เพื่อประกอบการวินิจฉัยและการพยากรณ์โรค

คำสำคัญ: cardiac troponin I malondialdehyde สารต้านอนุมูลอิสระ ความเครียด

[1]ภาควิชาการศึกษาทางคลินิก วิทยาลัยสัตวแพทยศาสตร์และทรัพยากรสัตว์, มหาวิทยาลัยคิงไฟซาล, อัล อัลสา 31982, ประเทศซาอุดีอาระเบีย

[2]ภาควิชาอายุรศาสตร์, โรคติดเชื้อและโรคปลา, คณะสัตวแพทยศาสตร์ มหาวิทยาลัยแมนซูรา, แมนซูรา, สาธารณรัฐอาหรับอียิปต์

****ผู้รับผิดชอบบทความ E-mail:** weldeeb@kfu.edu.sa, drwaeleldeeb@yahoo.com*