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Role of the Serum Muscular Enzymes in the Diagnosis of Post-Exertional Rhabdomyolysis in Draft Horses Used in Transplant Rice Seedlings

Yasmin H. Bayoumi*
Animal Medicine Department (Internal Medicine), Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

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Abstract

The present study was done to evaluate the role of serum muscular enzymes in the determination of the workload in healthy horses, and diagnosis of rhabdomyolysis (ER) cases. Twenty-one draft horses were assessed under the field conditions during rice seedling seasons. Animals were under complete history, physical examination and laboratory evaluation of serum muscular enzymes. Horses were categorized into 2 main groups; the first group, healthy horses under rice seedling work (n=10). They were examined and sampled 4times/day work. The second group includes horses with exertional rhabdomyolysis under rice seedlings workload (n=11) and they were examined and sampled on admission. Blood samples were collected and as a minimum, serum creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) were evaluated. The obtained results clarified significant alterations in the vital signs in the exertional rhabdomyolysis group in comparison to healthy horses under the same workload in the four examination periods. Additionally, highly significant increase ($P \leq 0.001$) in serum CK, LDH and AST levels in exertional rhabdomyolysis cases in comparison to the healthy horses and to the normal reference limits. While two or more folds increase in the serum muscular enzymes were recorded in healthy horses during workload and 4 h post-work when compared to the pre-work measurements. This, in turn, reflects the great load in healthy horses during rice seedling seasons.

Keywords: Draft Horses, Rhabdomyolysis, Rice Seedlings, CK, LDH, AST.

Introduction

Among equine diseases, rhabdomyolysis is considered one of the most serious and potentially life-threatening conditions. Previously, equine rhabdomyolysis was considered a single disease described as azoturia or tying-up, it now comprises several different myopathies, which, despite their similarities clinically, they differ significantly in their etiopathology [1]. Recent studies classified rhabdomyolysis in horse into exertional and non-exertional ones. Non-exertional myopathies could be attributed to a number of etiologies that cause significant muscle necrosis, including various infectious agents; viral infections (equine influenza), bacterial infections (*Streptococcus equi* subsp. *equi*), nutritional deficiencies (Vitamin E and Selenium deficiency), toxicities (ivermectin) and inherited (polysaccharide storage myopathy) [1-4]. Exertional rhabdomyolysis "exercise-induced rhabdomyolysis" has been of a rising concern [1,4]. Exertional rhabdomyolysis (ER) occurs in horses performing exercises beyond their conditioning status and in those performing strenuous exercises after a period of rest on full ration [5,6].

In Egypt, draft horses are commonly used to provide draft power for preparation and leveling of the flood, wet-bed rice nurseries and transporting rice seedling in rice cultivation season. Exertional Rhabdomyolysis (ER) is mostly associated with the initiation of this hard work [6]. Recent studies have suggested that many contributing factors to sporadic ER as over-exertion (increase in work intensity without foundation), heat exhaustion (exercising in hot and humid weather), and dietary imbalances (high nonstructural carbohydrates in the diet, inadequate selenium and vitamin E and electrolyte imbalances) [7].

Diagnosis of ER was based on history and clinical signs, as horses typically exhibited stiff gait, excessive sweating, muscle cramp, increased respiratory and heart rates during or after exercise. Biochemically, myoglobinuria

***Corresponding author e-mail: (Yasmin.dream@yahoo.com), Animal Medicine Department (Internal Medicine), Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt.**

and moderate to marked elevations in levels of serum creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) were characteristic, and muscle biopsy with histological lesions was diagnostic [1,8,9].

Creatine kinase (CK) in muscle is important for making Adenosine triphosphate (ATP) available for muscle contraction by the phosphorylation of Adenosine diphosphate (ADP) from creatine phosphate [10]. CK is liberated within few hours of muscle damage or increase the permeability of cell membrane into the extracellular fluid, and it reaches the peak within 4–6 h after the muscle injury [11].

Limited elevations in CK (1000 U/L) may accompany the training or the transport. Extremely exhausted exercise like endurance rides or the cross the country phase may result in serum CK activities being increased to 1000 U/L, but less than 5000 U/L. Under these circumstances, serum CK activities rapidly return to baseline (less than 350 U/L within 24–48 h). While the substantial elevations in the activity of this enzyme from several thousand to hundreds of thousands IU/L may occur with rhabdomyolysis [11].

Elevations in LDH usually occur in horses with rhabdomyolysis, myocardial necrosis, and/or hepatic necrosis. Therefore, concurrent measurement of serum CK is necessary to the rhabdomyolysis cases [11, 12].

Aspartate aminotransferase (AST), is a larger molecular weight protein that has the activity in the skeletal and cardiac muscle as well as the liver, red blood cells, and other tissues. Elevations in AST are not specific for myonecrosis, it could be elevated as a result of hemolysis or muscle, liver, or other organ damage. AST activity rises more slowly in response to myonecrosis than does CK and peaking between 12 and 24 h after the insult. In addition, AST is cleared slowly and may persist for 2–3 weeks after rhabdomyolysis [11, 12]. Combined elevations in CK and AST indicate relatively recent or active muscle necrosis, while the persistent elevation in serum CK indicates that myonecrosis is likely to be continuing. Elevated serum AST level accompanied by decreasing or normal CK level indicates that myonecrosis has ceased [11].

In Egypt, in spite of the considerable range of techniques such as many transplant rice seedling machines may be used. Farmers transplant rice seedlings in fields by the hand with the help of their draft horses, as horses carry seedlings and walk in the flooded field. Walking in the flooded field is so difficult and the work may last for one to two days/field.

Since rhabdomyolysis is being a repeated problem during rice seedling seasons every year, the present study aimed to evaluate the role of serum muscular enzymes in the determination of workloads in healthy horses and those with exertional rhabdomyolysis in rice seedlings seasons.

Materials and Methods

Ethical approve

All procedures used in the present study were approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Animals grouping and study description

Twenty-one horses (10 females and 11 males from 2-6 years old) were included in the present study, of which 10 clinically healthy horses (5 females and 5 males) were assessed under field conditions during rice seedling season. Horses were examined and sampled four times/ day work. Eleven horses (6 females and 5 males) with exertional rhabdomyolysis (during 4 successive rice seedling seasons, 2014 to 2017) under rice seedling workload were examined and sampled on admission (2-16 h of the onset of the clinical signs). The rhabdomyolysis cases were admitted to Veterinary Medical Teaching Hospital, Faculty of Veterinary Medicine, Zagazig University, Egypt or to some private clinics. Horses were belonged to villages in Dakhalia and Sharkia Governorates.

Study conditions

The season of rice nurseries usually starts at the end of May until the end of June every year. Commencement of the work in rice seedling transplant is usually in the early morning, between 5-6 am and ends in the evening. Horsees walk in the flooded field, in hot and humid weather, to transplant rice seedlings. Unexperienced owners usually keep

their horses on carbohydrate-rich diet with or without a period of rest before the work.

Clinical examination

Complete history, thorough physical and clinical examinations were done according to Kelly [13].

Sampling

Blood samples were collected 4 times/day work from the clinically healthy horses; pre-work, during the work, 4 h post work and 12 h post, while animals with exertional rhabdomyolysis were sampled once on admission (2-16 h from the beginning of the symptoms). Blood samples were collected via jugular vein puncture into tubes without anticoagulant. The tubes were centrifuged at 3,000 rpm for 15 min for serum separation, frozen at -20°C till analysis.

Measurement of serum muscular enzymes

Concentrations of serum creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) were measured by a spectrophotometer using commercially available kits (model Slim SEAC, Florence, Italy) according to manufacturers' instructions.

Statistical analysis

Data were statistically analyzed by a software program (SPSS, version 16) using One- way ANOVA to compare between the healthy horses in 4 periods and exertional rhabdomyolysis cases. Mean values ± SE for each parameter were estimated. Results were considered statistically significant at $P < 0.05$ [14].

Results

The recorded temperature, pulse and respiration rates in healthy horses and those with rhabdomyolysis are summarized in Table 1. No abnormalities in healthy horses were detected during different times of clinical examination. All horses have commenced the work with good performance. During the work, there were no apparent problems and they successfully completed the work. Horses with exertional rhabdomyolysis admitted with the history of either being of poor performance, exhausted and did not complete the work without other overt signs. Other horses commenced the work normally for few hours after that stiffness and reluctance to move were observed.

In the healthy horses, pulse rate, respiration rate and rectal temperature significantly increased ($P \leq 0.01$ or 0.05) during the workload (38.20 ± 2.37 beat/min, 17.00 ± 0.45 breath/min and 37.90 ± 0.07°C, respectively) in comparison to the pre-workload values (30.80 ± 0.86 beat/min, 11.20 ± 0.58 breath/min and 37.50 ± 0.04°C respectively). These vital signs were on their way to the normal values at 4 and 12 h post-work. Horses with the exertional rhabdomyolysis showed significant increase ($P \leq 0.01$ or 0.05), comparable to healthy horses at different times, in heart rate (53.20 ± 2.92 beat/min), rectal temperature (38.46 ± 0.26°C) and respiratory rate (13.80 ± 0.37 breath/min) on admission.

Table 1: Alterations in the vital signs in the healthy horses and exertional rhabdomyolysis cases

	Pulse rate (beat/min)			Respiration rate (breath/min)			Rectal temperature (°C)		
	Mean ± SE	Range		Mean ± SE	Range		Mean ± SE	Range	
		Min.	Max.		Min.	Max.		Min.	Max.
Healthy horses									
Pre-workload	30.80 ± 0.86^{c}	28.00	33.00	11.20 ± 0.58^{c}	10.00	13.00	37.50 ± 0.04^{c}	37.40	37.60
During workload	38.20 ± 2.37^{b}	32.00	44.00	17.00 ± 0.45^{a}	16.00	18.00	37.90 ± 0.07^{b}	37.70	38.10
4 h post-workload	33.40 ± 0.87^{bc}	32.00	38.00	12.60 ± 1.29^{bc}	10.00	17.00	37.62 ± 0.06^{bc}	37.50	37.80
12 h post workload	31.80 ± 0.80^{c}	30.00	34.00	11.60 ± 0.51^{bc}	10.00	13.00	37.48 ± 0.04^{c}	37.40	37.60
Rhabdomyolysis horses	53.20 ± 2.92^{a}	47.00	63.00	13.80 ± 0.37^{b}	13.00	15.00	38.46 ± 0.26^{a}	37.80	39.20
Sig.	**			*			**		

SE: Standard Error, Min.: Minimum, Max.: Maximum, Sig.: Significance
All data having different letters are differ significantly at $p < 0.05$

Table 2: Serum muscular enzymes activities in healthy horses and exertional rhabdomyolysis cases

	Serum creatine kinase (CK)			Serum lactate dehydrogenase (LDH)			aspartate aminotransferase (AST)		
	Mean ± SE	Range		Mean ± SE	Range		Mean ± SE	Range	
		Min.	Max.		Min.	Max.		Min.	Max.
Healthy horses									
Pre-workload	192.80 ± 22.86[d]	108.00	240.00	258.60 ± 25.68[d]	194.00	331.00	218.60 ± 8.52[d]	190.00	234.00
During workload	686.08 ± 26.17[b]	608.00	752.00	434.92 ± 23.21[b]	359.00	489.60	490.82 ± 13.68[b]	446.60	517.50
4 h post-workload	525.40 ± 53.70[c]	360.00	665.00	348.80 ± 30.44[c]	240.00	408.00	395.60 ± 23.27[c]	350.00	480.00
12 h post workload	192.80 ± 5.58[d]	192.00	225.00	262.00 ± 5.79[d]	246.00	280.00	222.00 ± 6.12[d]	204.00	245.00
Rhabdomyolysis horses	41416.60 ± 7513.74[a]	28000	70133	16341.80 ± 871.53[a]	13600	18807	3382.40 ± 652.66[a]	1550	5100
Sig.	**			**			**		

SE: Standard Error, Min.: Minimum, Max.: Maximum, Sig.: Significance
All data having different letters are differ significantly at $p < 0.05$

The clinical alteration observed in rhabdomyolysis horses varied in the severity between individuals: poor performance without overt signs (2 of 11), stiffness, short strides, eventually inability to move forward when forced to walk and firm, painful palpation of the gluteal muscle (9 of 11), sweating (2 of 11) and dark brown urine (5 of 11) as shown in Figure 1.

It is worthy to mention that the risk factors for the occurrence of rhabdomyolysis in this study were nervous temperament horses, younger ages (2-4 years old), being females, hoof affections and with full carbohydrate ration during rest period (Figure 2).

The results of serum muscular enzymes are summarized in Table 2. Serum CK, LDH and AST activities in healthy horses were significantly higher ($P \leq 0.001$) during the workload (686.08 ± 26.17 U/L, 434.92 ± 23.21 U/L and 490.82 ± 13.68 U/L, respectively) and 4 h post-workload (525.40 ± 53.70 U/L, 348.80 ± 30.44 U/L and 395.60 ± 23.27 U/L, respectively). Anywhere, the workload multiplied the activity of these enzymes (CK, LDH and AST), when measured during workload and 4 h post-workload, as compared with the pre-workload (192.80 ± 22.86 U/L, 258.60 ± 25.68 U/L and 218.60 ± 8.52 U/L, respectively) and 12 h post-workload (192.80 ± 5.58 U/L, 262.00 ± 5.79 U/L and 222.00 ± 6.12 U/L, respectively). Serum muscular enzymes activities in rhabdomyolysis cases revealed great abnormalities. Serum CK, LDH and AST levels were 41416.60 ± 7513.74 U/L, 16341.80 ± 871.53 U/L and 3382.40 ± 652.66 U/L, respectively. While the reference ranges were 100 to 350 U/L for CK, 150 to 450 U/L for LDH and serum 100 to 600 U/L for AST.

Discussion

Horses have the greatest capacity for physical work but physical exercise usually produces physiological responses and metabolic adaptations [15]**.** In the present study, the healthy horses (under work load of rice seedlings transplant) had higher pulse rate, respiration rate, and rectal temperature during the workload in comparison to the pre-workload state, and their rates were on their way back to normal in 4 h post-work. Unlike the racing horses that under routine training, physical exercise had a lower effect on the vital signs, i.e. lower heart rate was used as a determinant of horse fitness in response to the strenuous effort [16,17]. Therefore, when we lose the basics of the horses training and work (adaptation for the work and nutrition) rhabdomyolysis could be expected.

The development of ER was a common and catastrophic occurrence during the 19th century and early part of the 20th century before cars replaced draft and carriage horses as a means of transportation. Mortality rates as high as 50% were reported in some clinics [18,19]**.**

Figure 1: Dark brown urine in exertional rhabdomyolysis cases.

In the present study, poor performance recorded in two rhabdomyolysis cases may be tentatively attributed to inadequate training for this work, but the elevated muscular enzymes are determinates for rhabdomyolysis, such cases may be named as sub-clinical rhabdomyolysis. Consequently, diagnosis of ER depending only on the clinical signs may be challenging, because clinical signs vary in the severity from mild stiffness during or after work with poor performance to a marked reluctance to move, myoglobinuria, hard, swollen and painful gluteal muscles, beside the elevated pulse, respiratory rates and rectal temperature. Similar clinical findings were previously reported [1,6,9,20]. Regarding to the risk factors, the obtained results were comparable to those previously obtained by other authors [21,22]**.**

Severe exercise can result in serious health problems. The release of serum muscular enzymes, including creatine kinase (CK), aspartate transaminase (AST), and lactate dehydrogenase (LDH), is considered to be a suitable indicator for the muscle injury during exercise [23]**.** Studies have demonstrated the extent of hematobiochemical alteration in horses depends on several factors: type of exercise, the intensity of work (strength, duration, and frequency) and individual variation [24]. On harmony with this statement, the highly significant increase in the activities of CK, LDH, and AST observed in healthy horses during workload and 4 h post-work indicates the intensity of the work and workload can easily double the activity of the enzymes compared to the activity measured at rest [25]. Such increase in the serum muscular enzymes could be related to the increased skeletal muscle membrane leakage following strenuous exercise [26-29]. Meanwhile, the serum muscular activities return to normal (pre-workload) at 12 h post-workload. This suggested that the sampling at least 12-24 h after exercise can be helpful in the diagnosis of muscle damage. This could be helpful to distinguish horses showing a normal physiological response to exercise and those with abnormal or pathologic response to the workload [27]. Serum CK peaks within 4 to 6 h after intense exercise and its half-life is 90-120 min, that makes CK a reliable indicator for detection and monitoring of muscle damage in horses [29-31]. This is confirmed by field observations, that following a short-term effort; the increase in CK is smaller than horses competing in endurance [32-34]**.**

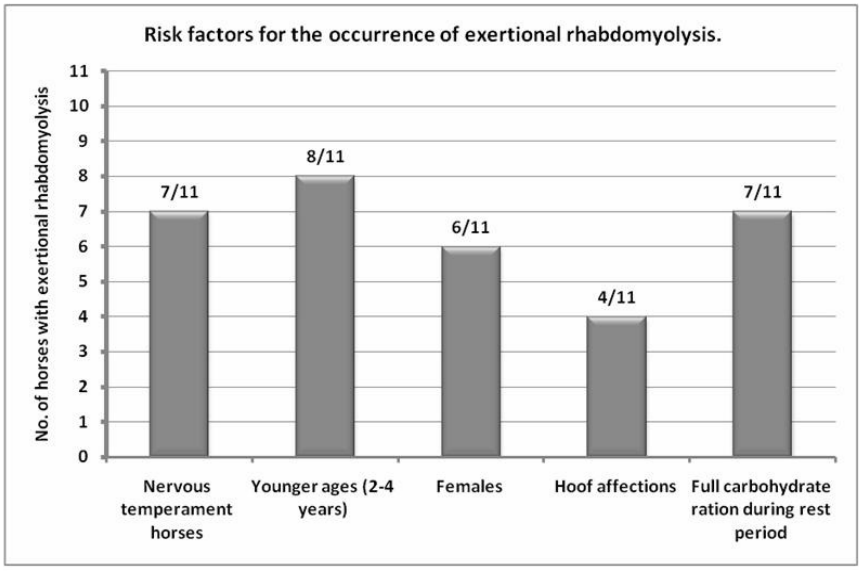

Figure 2: Risk factors for the occurrence of exertional rhabdomyolysis.

Our results regarding the serum muscular enzymes in the rhabdomyolysis cases were similar to those reported by El-Ashker [6], in draft horses in rice seedlings season. The author recorded significant increase in serum CK, LDH and AST in mild (56.103±160, 14.741± 1679 and 2819.4±604 IU/L, respectively) and severe cases of rhabdomyolysis (267.906±11737, 23.373±1440 and 6432.2±823 IU/L, respectively).

In these instances, the over-exertion caused by increased work intensity without a foundation for such type of work is the base for the occurrence of the disease. Overexertion is a well-described cause of ER in polo horses, 81% of cases with ER was attributed to overexertion and 30% occurring after a day of rest [35].

There are large varieties of causes and types for rhabdomyolysis, all leading to muscle breakdown but they seem to lead to a final common feature, which is the breakdown of muscle tissue, destruction of the myocyte and distribution of its components into the circulation. Subsequently, myoglobin, CK, LDH and AST release into the circulation. Besides the old theories in rhabdomyolysis, recent studies indicated that oxidative stress during exercise could be contributed to the formation of oxidative fiber myolysis [36-39]. Free radicals and other reactive oxygen species (ROS) were expected in the pathogenesis of exertional rhabdomyolysis in draft horses used in rice seedlings season [9], the author recorded higher values of lipid peroxides, malondialdehyde, nitric oxide and lower values of antioxidant enzymes in fatal cases of rhabdomyolysis in comparison with both control horses and survivor cases.

Conclusion

Exertional rhabdomyolysis is common at the beginning of rice seedling season in draft horses. In order to obtain better fitness and performance during the season, it is necessary to increase the workload before the season. Feed should be adjusted according to the work done, not according to the work will be done with a period of adaptation for such work and before starting harder work, high fiber and low starch diet is effective to minimize the occurrence of this condition.

Conflict of interest

The author declares no conflict of interest.

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الملخص العربي

دور الإنزيمات العضلية في تشخيص انحلال الربيدات ما بعد الإجهاد في خيول الجر المستخدمة في نقل شتلات الأرز

ياسمين حسن أحمد بيومي

قسم طب الحيوان (مادة الأمراض الباطنه)– كلية الطب البيطري– جامعة الزقازيق

استهدف هذا العمل دراسة دور الإنزيمات العضلية في تقييم عبء العمل في الحيوانات السليمة وكذلك تشخيص الحالات التي تعاني من انحلال الربيدات. ولهذا الغرض تم تقييم ٢١ من خيول الجر تحت الظروف الحقلية أثناء مواسم شتل الأرز. حيث تم أخذ تاريخ الحالات المستخدمة في الدراسة. وتم إجراء الفحص الإكلينيكي والتحليل المعملي لإنزيمات العضلات، وقد قسمت الحيوانات إلي مجموعتين، المجموعة الأولي تضم خيول سليمة مستخدمه في نقل شتلات الأرز (عدد ١٠) حيث تم فحصها وأخذ العينات منها ٤ مرات خلال يوم العمل.بينما اشتملت المجموعة الثانية علي خيول تعاني من انحلال الربيدات نتيجة لاستخدامهم في نقل شتلات الارز (عدد ١١) حيث تم فحصهم وأخذ العينات عند وصولهم. وتم تجميع عينات الدم وقياس مستوي إنزيم الكرياتينين كيناز والإنزيم النازع لهيدروجين اللاكتات وإنزيم ألانين أمينوترانسفيريز في المصل. وقد أوضحت النتائج وجود تغيرات معنوية في المؤشرات الحيوية في الخيول التي تعاني من انحلال الربيدات عند مقارنتها بالخيول السليمة تحت نفس عبء العمل في أربع مرات الفحص. وبالإضافة إلى ذلك فقد تم تسجيل زيادة معنوية جدا في إنزيمات العضلات السابقة في المجموعة التي تعاني من انحلال العضلات عند مقارنتها بالمجموعة السليمة، بينما تضاعف مستوي الإنزيمات العضلية في الحيوانات السليمة أثناء عبء العمل وخلال ٤ ساعات بعد العمل عند مقارنتها بقياسات ما قبل العمل. وهذا بدوره يعكس العبء الكبير علي الحيوانات السليمة أثناء شتل الأرز.