**Hematological, biochemical, and skeletal muscle metabolic responses to exercise related myopathy in endurance horses**

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**Abstract:**

Equine exertional rhabdomyolysis (ER) is a well-recognized disease affecting racehorses therefore this study was performed to evaluate haemato-biochemical alterations in 20 adult Arabian horses after 120 km race with trial of treatment. Horses were classified into 2 main groups: the first group (n:5) included healthy control horses while the second group(n:15) included horses showed signs of distress, moderately depressed, muscle stiffness, shifting hind limb lameness, elevated respiratory rate, sweating, firm painful hindquarter muscles, dark red urine and reluctance to move that lasts for several hours after ending of race. Based on the history and the through clinical examination of diseased horses, they were tentatively diagnosed as suffering from ER syndrome. Whole blood and serum samples were collected from both healthy control horses and diseased ones before and after treatment. Hemogram of examined horses showed only significant increased PCV (%) values of diseased horses with more prominent thrombocytopenia but with treatment their values showed marked improvement, where other haematological parameters showed non-significant changes in both groups either before or after treatment. Serum analysis of ER affected horses showed a higher serum enzymatic activities of creatine phosphokinase (CK) and aspartate aminotransferase (AST) with significantly increased values of total proteins, albumin, globulins, total bilirubin, glucose, creatinine and potassium. But with effective treatment protocol, these higher values began to be improved towards normal values again. While non-significant changes were observed in serum levels of Gamma-glutamyl transpeptidase (GGT), calcium, sodium and blood urea nitrogen (BUN) in ER affected horses. Based on obtained results, ER has negative impact on haemato-biochemical biomarkers with remarkable serum elevation of skeletal muscular specific enzymes, as well as by paralytic myoglobinuria.

**Key words**: Exercise related myopathy,Endurance horses**,** Exertional rhabdomyolysis

1. **Introduction:**

Rhabdomyolysis is defined as the breakdown of muscle fibres that results in the release of muscle proteins and other cellular constituents into the blood circulation. Exercise, particularly when strenuous and unaccustomed, causes damage and subsequent muscle fibre breakdown, known as exertional rhabdomyolysis (**Clarkson and Hubal, 2002**)

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. Recent studies classified rhabdomyolysis in horses into exertional and non-exertional ones. Non exertional myopathies could be attributed to several 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 (**Quist et al., 2011**).

Exertional rhabdomyolysis was known as Monday horse sickness which is considered one of the most serious and potentially life-threatening conditions affecting horses. Exertional rhabdomyolysis (ER) is the general term for muscle breakdown (**Hunt et al.,2008**). ER occurs in horses performing exercises beyond their conditioning status and in those performing strenuous exercises after a period of rest on full ration (Reed et al, 2010).

**Valentine et al. (1999)** 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 non-structural carbohydrates in the diet, inadequate selenium and vitamin E and electrolyte imbalances).

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 (**McGowan, 2002**).

Common clinical signs of ER in horses occur during or shortly after exercise, or occasionally in anticipation of exercise, and include excessive sweating, stiffness, increased heart rate, trembling of the muscles (particularly in the hindquarters), and reluctance to move or to continue exercise. Severely affected horses may become immobile or recumbent, with darkly coloured orange, red, or brown urine as a result of myoglobin released from damaged muscle tissue collecting in the urine **(Reed et al., 2010).**

Increased permeability or destruction of muscle cell membranes in horses with ER also leads to leakage of potassium, phosphorus, and the enzyme creatine kinase into the blood **(Aleman, 2008 and Valberg,2012).**

A number of diagnostic tools are used to identify ER in horses. Most commonly, the occurrence of rhabdomyolysis is supported through measurements of serum muscle enzyme activities including CK and aspartate transaminase (AST). Serum CK is considered to be a muscle specific enzyme and its activity typically peaks within four to six hours after a myocellular insult, returning to normal within 24 to 72 hours, unless ongoing or additional muscle damage occurs (**Hinchcliff, 2008**).

Aspartate transaminase (AST) is also an indicator of rhabdomyolysis; however, it is also released from other damaged tissues, particularly the liver and erythrocytes. The serum half-life of AST is much longer than serum CK, and it typically peaks in activity 24 to 48 hours after muscle injury occurs, remaining elevated for several weeks after severe rhabdomyolysis **(Reed, 2010 and Valberg, 2012)**

The current study was designed to throw some lights on the clinical, haemato-biochemical alterations with skeletal muscle metabolic responses of Arabian racehorses suffered from exertional rhabdomyolysis after 120 km race.

**2- Material and methods:**

**2.1. Animals:**

 During October 2021, this study was performed on twenty adult male Arabian racehorses belonged to a private farm in Alexandria governerate , with age range of 3-6 years and weighted 400-500 kg. Five of them were clinically healthy and enrolled as healthy control group where other 15 horses on admission, were suffering from stiff gait, lameness, sweating, reluctance to move, muscle rigidity, swelling of the gluteal muscle and exhibited dark brown urine after 120km race. The diseased horses had a history of preforming a hard work above the foundation level of training. Exertional rhabdomyolysis was tentatively diagnosed depending on the history, clinical and physical examination of the affected horses and confirmed by laboratory investigations.

**2.2. Clinical examination:**

 Full case history and complete thorough clinical examination of all horses under investigation was performed according to the method described by **(Kelly, 1984).**

**2.3. Sampling and Measurements:**

Blood samples were collected via jugular vein puncture of each horse and divided into two tubes. First, EDTA-containing tube was used for hematologic analysis including: Red blood Cell count (RBCs X106/µl), haemoglobin concentration (Hb g/l), packed cell volume (PCV%), mean corpuscular volume (MCV fl), mean corpuscular haemoglobin (MCH pg), mean corpuscular haemoglobin concentration (MCHC g/dl), WBCs count (X103/µl) and differential leucocytic count all were determined by using of fully automated veterinary haematology analyser, Exigo, Boule medical AB., Sweden in the central laboratory, Faculty of veterinary medicine, Alexandria University.

Second, plain tubes without anticoagulant for serum analysis. Determination of serum concentrations of sodium(Na), potassium(K), calcium(Ca+2) , glucose, AST, GGT, Total bilirubin(TBIL), Total proteins and Albumin all were carried out by using commercial test kits supplied by (Bio-labo, France) while analysis of CK, BUN and creatinine were carried out by using commercial test kits supplied by (Ben-Biochemical Enterprise, Italy) all were measured spectrophotometrically following standard methods mentioned in the leaflet of the manufacturer. Serum globulins was calculated by substraction of the amount of serum albumin from the amount of total serum proteins.

**2.4. Treatment trial:**

Before starting treatment, each animal was kept immobile and it was not allowed to move until it was cooled off, rehydrated, and adequately recovered. While recumbent horses should be deeply bedded and repositioned by rolling every 2-4 h. severely affected horses should not be forced to stand.

 All clinical cases was treated with I.V balanced electrolyte solution (lactated ringer solution®, supplied by El-Nasr pharmaceuticals chemicals co. Abuzaabal, Egypt) given according degree of dehydration, pain relief using a non-steroidal anti-inflammatory agent as flunixin meglumine (Flunixin® supplied by Norbrook co., Northern Ireland) I/V at dose rate of 10 mg kg-1, muscle relaxant as Metocarbamol (metocarbamol® supplied by Vetec S.A. Argentina) 5-25mg/kg slowly I.V every 6 hours as needed .

**2.5. Statistical analysis:**

Data were analysed using the packaged SPSS program for windows version 10.01 **(SPSS, 2000 Inc., Chicago, IL)**. Data were presented as mean ±standard Error (SE). Differences between groups were determined by the one-way analysis of variance (ANOVA). Significance level was set at P≤ 0.05.

**3- Results:**

**3.1. Clinical examination:**

 On admission, diseased horses were presented with lameness, stiffness of gait, short strides, inability to move forward when forced to walk, firm and painful palpation of the gluteal muscle, excess sweating, constipation with red colored urine **(fig.1,2)**. The severely affected horses were being recumbent. Compatible thorough clinical examination reveals significant elevated body temperature, tachycardia, significant tachypnea with congested mucous membrane in ER affected horses compared to healthy control ones **(Table 1).**



Fig. 1,2: A horse suffering from ER showing signs of red urine

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Control | Diseased(pre-treatment) | Post-treatment |
| Pulse (beat/min.) | 30.77±0.81b | 61.37±2..62a | 33.52±1.4b |
| Respiration rate(breath/min) | 11.35±0.43b | 15.69±1.02a | 11.82±0.57 b |
| Rectal temp. (Cº) | 37.83±0.25 b  | 39.05±0.23a | 37.9±0.36 b |

**Table (1):** Mean values (±SE) of some vital signs in healthy control horses and ER affected horses before and after treatment:

 Means bearing different letters within the same row are significant at (P<0.05).

**3.2. Hematological analysis:**

 The obtained results regarding haematological analysis of examined horses were summarized in (Table 2), showed a significant increase in PCV (%) and reduced Platelets count in ER affected horses compared to others. With non-significant changes in the remaining haematological values.

**Table (2):** Mean values (±SE) of some haematological parameters in healthy control horses and ER affected horses before and after treatment:

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Control | Diseased (pre-treatment) | Post- treatment |
| RBCS (X106/µl) | 10.07±0.98a | 9.87±0.34a | 10.02±0.13a |
| Hb (g/dl) | 14.09±0.86a |  13.69±0.52a | 13.86±0.35a |
| PCV (%) | 41.35±1.55c | 52.45±1.12a | 45.83±2.74b |
| MCH (*Pg.*) | 16.93±0.34a | 16.58±0.38a | 16.62±0.28a |
| MCHC (g/dl) | 35.15±0.53a | 34.80±0.32a | 34.93±0.37a |
| MCV (*fl*) | 47.08±1.92a | 46.86±0.98a | 46.95±1.50a |
| WBCS (X103/µl) | 12.16±1.34a | 11.56±0.91a | 11.95±0.87a |
| Lymphocytes (X103/µl) | 4.54±0.87a | 3.86±0.66a | 4.22±0.63a |
| Neutrophils (X103/µl) | 7.42±0.58a | 7.61±0.67a | 7.54±0.54a |
| Platelets (X103/µl) | 150.32±4.14a | 105±3.05c | 140.93±5.17b |

 Means bearing different letters within the same row are significant at (P<0.05).

**3.3. Biochemical analysis:**

Regarding to the serum biochemical analysis of ER affected horses; there was a significant increase of serum enzymatic activities of CK and AST. Moreover, the diseased horses had higher values of total plasma proteins, albumin, globulins, glucose creatinine and total bilirubin. While BUN and GGT was non- significantly changed between different groups of horses.

The serum electrolytes pattern showed a significant increase in serum potassium level with non-significant changes in serum calcium and sodium levels in diseased horses as compared with levels in affected horses after treatment and in healthy control ones. (Table 3).

**Table (3):** Mean values (±SE) of some biochemical parameters in healthy control horses and ER affected horses before and after treatment:

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Control | Diseased(pre-treatment) | Post- treatment |
| CK (u/l) | 290±12.06c | 38221±80.76a | 722.54±34.34b |
| AST (u/l) | 175.37±6.98c | 6313±23.29a | 816.58±11.73b |
| GGT (u/l) | 21.72±0.6a | 19.42±0.43b | 20.37±0.55a |
| Total Proteins (g/dl) | 6.21±1.05b | 7.72±1.2a | 6.57±1.61b |
| Albumin (g/dl) | 3.5±0.59b | 4.22±0.61a | 3.72±0.43b |
| Globulins (g/dl) | 2.71±0.26b | 3.5±0.34a | 3.11±0.25a |
| TBIL (mg/dl) | 1.4±0.12c | 3.8±0.55a | 2.26±0.18b |
| Glucose (mg/dl) | 87.5±1.23b | 105.8±2.34a | 89.11±.1.84b |
| Urea (mg/dl) |  21.39±0.64a | 20.45±0.45a | 21.02±0.21a |
| Creatinine (mg/dl) | 1.36±0.16b | 1.49±0.26a | 1.39±0.11b |
| Na (mmol/l) | 134.99±3.2a | 133.5±2.63a | 134.2±3.51a |
| K (mmol/l) | 4.06±0.62b | 5.43±0.14a | 4.65±0.36b |
| Ca+2 (mg/dl) | 11.04±0.48a | 11.47±0.52a | 11.26±0.23a |

 Means bearing different letters within the same row are significant at (P<0.05).

# **4- Discussion:**

 ER is a common and economically relevant problem of endurance horses **(Hunt et al., 2008)**. It occurs in horses performing exercises beyond their conditioning status and in horses performing strenuous exercises after a period of rest and inactivity while being fed a high-grain diet (**Valberg, 2009).** It also represents a major problem in performance horses by limiting or preventing training and inhibiting peak performance **(Knoepfli, 2002).**

 In this study, ER was tentatively diagnosed depending upon case history of completing a 120 km. race, physical examination findings and confirmed by higher serum enzymatic activities of creatine phosphokinase and aspartate aminotransferase with light to dark red colored urine according to level of released myoglobin in the urine which is similar to those previously described by **(El-Ashker, 2011).**

According to our compatible medical history, the affected horses were suddenly preformed a hard work after a period of rest while being fed a high carbohydrate rich food. Furthermore, the observed clinical signs of affected horses were stiffness of gait, reluctance to move, muscle stiffness and cramping with firm and painful palpation of gluteal muscles and red coloured urine. These results agreed with those reported by **El-Ashker (2012).** Horses suffered from ER showed a significant increase in vital signs (pulse rate, respiration rate and rectal temp.) as compared with control healthy horses which comes in agreement with **(Yasmin, 2018).**

Regarding erythrogram of ER affected horses there was a significant increase of PCV (%) as compared with healthy control ones and its level after treatment. This may be attributed to the dehydration state associated with ER which agreed with those findings of **Muñoz et al. (2010).**

 In accordance with **Criddle (2003)** ER affected horses revealed significant thrombocytopenia in comparison to the mean values of healthy horses which may infrequently attributed to development of disseminated intravascular coagulation (DIC) specially in severe cases of rhabdomyolysis due to the release of thromboplastin and other prothrombotic substances from the damaged muscle leading to increased platelets utilization.

The biochemical analysis of diseased horses revealed a significant increase of plasma CK level in diseased horses as compared with levels post treatment and in healthy horses. CK is liberated within a 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 **(Hunt, 2008).** Our results agreed with **Banasik et al. (2008)** who attributed that increase to destruction of muscle cells in the damaged area which elaborated their enzyme content.

Concerning serum AST activities, our results revealed a significant increase in its level in ER affected horses as compared with its level after treatment and in healthy control horses. Our results agreed with those of **Yasmin (2018)** who observed a high increase in AST levels in ER affected horses. **Valberg et al. (1993)** reported that 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 peaks between 12 and 24 hrs. after the insult. In addition, AST is cleared slowly and may persist for 2–3 weeks after rhabdomyolysis**.** 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. An elevated serum AST level accompanied by decreasing or normal CK level indicates that myonecrosis has ceased **(El-Ashker, 2011).**

Serum levels of creatinine were significantly increased in ER affected horses compared to others which was agreeable with those findings of **El-Ashkar (2011)** who reported a significant a higher serum creatinine levels in horses with sever rhabdomyolysis due to excess muscle damage.

Serum concentrations of total proteins, albumin and globulins were significantly higher in in horses suffering from ER as compared with their levels post-treatment or in healthy control horses, these results agreed with (**Youssef et al., 2018)** who reported that horses with rhabdomyolysis were suffering from hyperproteinemia and hyperalbuminemia. **El-Ashker (2011) and Sharma et al. (1999)** mentioned that dehydration could be the cause of hyperproteinemia and hyperalbuminemia specially in the early stage of the disease, later on when inflammation supervene besides the potential ischemic nephropathy, it could result in protein loss into urine which in turn participate to the state of hypoproteinemia and hypoalbuminemia that is observed in severely affected horses. Furthermore,a highly significant increased total bilirubin values may be attributed to rapid turnover of elevated serum concentration of myoglobin released from damaged muscles into bilirubin **(Khan,2009)**.

Blood glucose level of ER affected horses showed a significant increase than its level in healthy control ones and tends to decrease towards normal values after being treated. These results agreed with those of **Youssef et al. (2018)** who attributed this increment to either stress or to the inflammatory reaction that might induce glycogenolysis and enhance gluconeogenesis.

Regarding serum electrolytes pattern in ER affected horses, our results showed hyperkalemia with non-significant changes in serum sodium and calcium levels. Hyperkalemia may be attributed to the release of intracellular electrolytes contents into plasma particularly K. These results agreed with **Youssef et al. (2018)**.

# **5- Conclusion:**

Endurance and heavy draft horses must be supplied with water, electrolytes and antioxidants to keep normal hydration status and electrolyte balance and to maintain the integrity of the muscle cell. Additionally, correct and rapid treatment must be performed as soon as possible to avoid the common complications of this syndrome.

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