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MUSCLE SPECIFIC ENZYMES AND α-TOCOPHEROL AS WELL AS ERYTHROCYTIC GLUTATHIONE PEROXIDASE AND SERUM MAGNESIUM LEVELS IN PARALYTIC MYOGLOBINURIA AFFECTED HORSES

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ITRODUCTION

Paralytic myoglobinuria, azoturia, Monday morning disease, Typing-up, myositis and extertional rhabdomyloysis, all are synonyms of disease affecting horses mainly during exercise after a period of inactivity on full working rations (Blood et al., 1983 and Hodgson, 1987). This disease occurs as a result of an umber of predisposing factors that may act individually or incombination. These factors include diet/exercise factors, endocrine factors, genertic factors in addition to number of other unidentified or poorly understood factors (Hodgson, 1987). Actual or induced vitamin E-selenium deficiency are believed to cause a wide range of symptoms in horses and other equines. Myoglobinuria paralytica or azoturia is among conditions that are either known or suspected to be caused or predisposed to by deficiency of vitamin E and selenium (Blaxter, 1975; Dyson et al., 1975 and Gedek, 1975).

On the other hand, while possible role of dietary deficiency of vitamin E in this disease has been suggested, selenium deficiency, althaugh does occur in in horses, there is no evidence that it plays any

part in paralytic myoglobinuria (Blood et al., 1983). For these resons it is of considerable importance not only to throw more light on enzymatic assays specially those of muscle specific types, but also on its possible etiological or predisposing causes as well as results of treatment trials of this disease among draught horses in Egypt.

In this study due to difficulty of selenium estimation and in addition to the high positive correlation between selenium concentration in blood in horses glutathione peroxidase activity (Robinson et al.,1987), it was determined as useful alternative means of defining selenium status of horses. On the other hand, the disease was known to be accompanied with or charactrised by musculer degenration (Blaxter, 1975; Dyson et al., 1975;Gedek, 1975 Blood et al., 1983 and Hodgson, 1987) and muscle specific group of enzymes (SGPT, LDH and CPK) were determined both as diagnostic and also as an indication of degree of muscular degeneration. It is also of great interest in the present study to include the determination of serum vitamin E both as related to selenium and also to clarify its possible etiological or predisposing role in this disease. serum magnesium determination was also included.

MATERIAL AND METHODS

Twenty adult male draught horses were used in the present investigation. They were local breeds aging between 4-7 years and of about 300-450 kg body weight. Ten of them were diseased animals admitted to Vet. Med. Clinic, Vet Med. Dep., Fac. Vet. Med. Cairo University in the period from 1979 to 1982. All animals were admitted with a complaint of locomotor disturbances ranged from profuse sweating, stiffness in gait, and reluctant to move beside voiding dark reddish-brown urine with absence of fever. Some admitted in recumbant position carried on a car. In most cases there was a hisotry of period of complete rest for

about 2 days or more, during which the animal was put on high carbohydrate working rations immediatly precedding the onset of symptoms which developed about 15 minutes to one hour after beginning of work. Most affected horses were those draughting heavy loads. Diagnosis was based on case history and clinical symptoms in addition to clinical examination and was confirmed in the laboratory by blood and urine examination.

Whole blood "Jugular vein puncture" and urine "by catheterizatio" samples were collected from the 10 affected horses before beginning of treatment trials. Whole blood samples were collected on ACD anticoagulant (El-Neweehy, 1982) and without anticoagulant for serum collection. Whole blood samples collected on ACD anticoagulant were used for estimation of glutathione perioxidase activity in erythrocytes, while serum samples were used for estimation of GPT, GOT, LDH, CPK, o-tocopherol and magnesium levels.

Determination of glutathione peroxidase (GSH-PX) in erythrocytes was performed followign the method described by Paglia and Valentine (1967). Serum transaminases (GPT, GOT) were determined using colorimetric method according to Reitman and Frankel (1957). Serum LDH activity was measured using the methos described by Von F Wreblewski and La Duean (1955). Serum CPK activity was determined using colorimetric method according to Forster et al., (1970). Serum α-tocopherol was determined according to the macro-method described by Hashim and Schnuttringer (1966). Serum magnesium level was determined by the titan yellow method according to Neil and Neely (1956).

On the other hand, although specific identification of myoglobin in urine is a complex procedure, urinalysis test strips "Combur-9*" desgined to detect the presence of haemoglobin will also yield a positive

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* Combur-9 Boehringer Corporation (London) Ltd Bell Lane Lewes, East Sussex X BN 71 LG.

reaction to myoglobin, so it was used not only to detect presence of myoglobin pigments but also to detect all the other abnormal constituents that may be present.

Treatment trials began after collection of samples and included 25% Dextrose 500 ml I/V followed by Cal. D. Mag* 500 ml bottle by I/V both twice daily till disapperance of both locomotor disturbance and abnormality in colour of urine. Antihistamincs (6 amp. Avil** S/C) daily for three successive days. Both Vitamin E and B_1 preparations were recommonded by the author in dose rate of 10 amp. Ephynal-ROCHE (1 gm DL-o tocopherol acetate) and Eca-Vit. B_1-ROCHE in dose rate of 10 amp. both given by deep-I/M route and daily for three successive days. Boiled barely water was offered to affected animals continuously.

Another ten adult apparently healthy male draught horses of nearly the same age and body weight were used as control group.

RESULTS AND DISCUSSION

When muscle tissues are damaged, there is a leakage of celluler constituents into the surrounding tissues and subsequently into circulation. By measuring the levels of specific enzymes within the serum, an assessment of the degree of myodegeneration is possible (Hodgson, 1987).

The results obtained are shown in Table (1) which indicated that both SGPT and SGOT were significantly higher ($P < 0.001$) in affected horses when compared with healthy ones. These findings agree with those reported by Lindholm (1974), Lindohlm and Johansson (1977), Lindholm et al., (1974); Blood et al., (1983) and Hodgson (1987), who attributed this elevation to hepatocellular or red blood cell damage in addition to degenration occurred in muscle cells.

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* Cal.D. Mag injectable Solu. Containing 23% calcium glyconat. 2% magnesium chloride, 10% Dexrose, Pfizer-Egypt.

** Avil® Pheniramine hydrogen maleate 1 ampoule (2 ml) 45.5 mg pheniramine hydrogen maleate Hochest.

Table (1): Serum GPT, GOT, LDH, CPK, o -Tocopherol, magnesium and blood glutathone Peroxidase in normal and paralytic myoglobinuria affected horses.

		Number	S-GPT (μ/L)	S-GOT (μ/l)	S-LDH (μ/L)	S-CPK (μ/L)	GSH-PX EU/gmHb	α-Toco-pherol mg/100 ml	Serum Magnesium (mg/100 ml)
Apparently	Range	10	3.4-5.8	98.13-106.07	341.589-70.411	119.99-190.61	8.608-11.570	0.25217-0.47669	2.276-3.383
Healthy	Mean	10	4.6***	102.4***	406.0***	155.30***	10.089	0.36443***	2.830
Horses	S.E.	10	0.379	1.350	20.37	11.167	0.4683	0.0355	0.17508
Paralytic myoglobrinuria	Range	10	181.395-307.396	1769.945-2546.261	1953.645-2831.283	2563.706-3853.354	7.668-11.242	0.0733-0.2221	2.156-3.308
Affected	Mean	10	244.396***	2158.103***	2392.464***	3208.53***	9.455	0.1477***	2.732
Horses	S.E.	10	19.924	122.757	138.778	203.929	0.565	0.02352	0.182

* P<0.05 ** P<0.01 *** P<0.001

Due to difficulty in separation of LDH to its isoenzymes fractions and no need to determine whether cardiac or skeltal muscles are severely affected, the enzyme was determined totally, as indicated in Table (1) which showed that paralytic myoglobinuria was accompanied with great elevation (5.5 fold increase) when compared with its level in normal horses. These findings agree with those reported by Hodgson (1987), who added that LDH tends to peak within 12 hours and remain elevated for up 7-10 days in horses suffering from paralytic myoglobinuria.

Simillarly serum CPK was significantly very high ($P< 0.001$) in affected horses when compared with control ones. These findings clearly agree with those reported by Gerber (1969), Lindholm (1974), Lindholm and Johansson (1974), Lindholm et al (1974), Blood et al., (1983) and Hodgson (1987), who added that CPK is the most sensitive and more specific indicator of muscle pathology in horses. It rose rapidly to peak within six hours and elevated from 1000 to greater than 400.00 i.u./L.

One of interest and unexpected finding in our study is the significant decrease ($P<0.001$) in serum o-tocopherol level observed in paralytic myogrobinuria affected horses (o.1477$\pm$0.0235 mg/100 ml) when compared with normal ones (0.3644 $\pm$0.0355 mg/100 ml). This finding agrees with those reported by Si-Kwang Liu et al., (1983), who found that plasma α-tocopherol concentration in 5 affected horses ranged from <0.03 to 0.08 (Mean, 0.04$\pm$0.01 mg/ml) but reported relatively lower level in normal horses. Adams (1972), reported that the normal plasma o-tocopherol concentration in horses is >0.5 mg/100 ml and >0.3 mg/100 ml is considered deficient. On the other hand, Robinson et al. (1987), mentioned that expected values of Vitamin E in horses are 300-600 µg/100 ml and considered 120 µg/100 ml as indication of deficiency in foals. Furthermore, they attributed protective role of Vitamin E in this disease to prevention of peroxidation of the lipids of cell membrane and

thus preserve the structural integrity of muscle cells. On the other hand blood et al., (1984), suggested possible role of dietary deficiency of vitamin E in paralytic myoglobinuria, while Hodgson (1987), included selenium and/or vitamine deficiency among factors implicated in the etiology of azoturia in equines.

No significant difference was found in the level of erythrocytic seleno-enzyme glutathione peroxidase (GSH-PX) between affected and normal horses, a finding which may indicate that selenium does not play neither etiological nor predisposing role in this disease in Egypt. Our findings agree with those reported by Blood et al., (1983), who mentioned that selenium deficiency does occur in horses but there is no evidence that it plays any part in paralytic myoglobinuria. The adequacy of selenium level in soil in Egypt ($\leq$ 0.5 ppm) (El-Neweehy, 1982), Explains the cause of normality of GSH-PX level in both affected and normal horses. In addition, Robinson et al. (1987), indicated that grains and forages grown in selenium deficient area tends to be low in selenium and selenium deficiency results when animals fed solely on feed grown in selenium deficient area.

Although we can conclude that vitamin E but not selenium appears to be among predisposing or even etiological factors of paralytic myoglobinuria among horses in Egypt, a number of studies show that vitamin E-selenium injections can be considered almost specific for treating or preventing this syndrome. (Cooper, 1966; Buescher, 1972; Lindholm & Johansson, 1974 and Lindholm et al., 1974).

No significant difference was found in seurm magnesium level between paralytic myoblobinuria affected and normal horses; a finding which may indicate that magnesium does not play any role in this diseases.

SUMMARY

The present investigation was carried out to study changes in serum muscle specific enzymes (SGPR, SGOT, LDH and CPK) and selenoenzyme glutathione peroxidase (GSH-PX) as well as serum α-tocopherol and magnesium levels in horses suspected to be suffering from paralytic myoglobinuria. Significant increase occurred in all estimated muscle specific enzymes in affected horses. While serum α-tocopherol level was significantly decreased, no significant changes were found in both erythrocytic glutathione peroxidase activity and serum magnesium level. It could be concluded that vitamin E but not selenium appear to be among predisposing or even etiological factors of paralytic myoglobinuria in draught horses in Egypt.

RFERENCES

1. Adams, C.R. (1972): Vitamin nutrition of the horse. Stud Manger Hanbook 8, 37-41.

2. Blaxter, C.S. (1975): A case of muscular dystrophy. Vet. Rec. 97, 172.

3. Blood, D.C.; Radostitis, O.M. Henderson, J.A.; Arundel, J.H. and Gay, C.C. (1983): Veterinary Medicine: A text book of the disease of cattle, sheep, pigs, goats. (6th Ed), Bailliere Tindall, London.

4. Buescher, D.W. (1972): Das Doping Diss., Hannover.

5. Copper, C.D. (1966): Selenium-tocopherol therapy in horses. Med. Vet. Pract. 47, 79-80.

6. Dyson, D.A.; Gibson, J.C. and Gibson, I.R. (1975): Cardiac Myopathy in a donkey foal. Vet. Rec. 97, 295-296.

7 . El-Neweehy, T.K.A. (1982): Studies on white muscle disease in suckling Egyptian buffalo-calves in A.R.E. Ph.D. Thesis, Vet. Med., Cairo University.

8 . Forester, G. et al. (1970): Creatin-Kinase. Bestimmung mit creatinphosphat als Substrat. Page 755 ff. in H.U. Bergmayer, ed. Methoden der Enzymatischen Analyse, 2nd German ed. Verlag Chemic Weinhein 2 Vols.

9 . Gedek, B. (1975): Leistungsdepressionen und Erkrankungen durch Keimhaltige Furttermittel. Kraftfutter 58, 300, 302, 304.

10. Gerber, H. (1969): Equ. Vet. J., 1, 120. Citgd by Blood et al., 81983).

11. Hashim, S.A. and Schuttringer, G.R. (1966): Rapid determination of tocopherol in macro and microquantities of plasma. Results obtained in Various Nutrition and Metabolic Studies. Am. J. Clin Nutr 19. 137.

12. Hodgson, D.R. (1987): Current therapy In Equine Medicine-2, 1st. Edition, W.B., Saunders Company, Harcourt Brace Jovanovich, Inc. Philadelphia, Pa 19106 P. 487-489.

13. Lindholm, A. (1974): Muscle morphology and metabolism in standardbred horses at rest and during exercise. Stockholm.

14. Lindholm, A. and Johansson, H.E. (1974): "Tying-up" eller "equine rhabdomyolysis". Proc. 12th Nordic Vet. Congr. Reykjavik, pp. 121-124.

15. Lindholm, A.; Johansson, H.E. and Kjaersgaard, P. 1974): Acute rhabdomyloysis (Tying-up) in Standardbred horses. Acta Vet. Scand. 15, 325-339.

16. Niel, D.W. and Neely, R.A.J. (1956): Titen yellow methd for estimation of serum magnesium. J. Clin. Path. 9, 162.

17. Paglia, D.E. and Valentine, W.N. (1967): Studies on the quantitative characterization of erythrocytes glutamthione peroxidase. J. Lab. Clin. Med. 70, 158.

18. Reitman, S. and Frankel, S. (1957): A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Path. 28, 56.

19. Robinson, N.E.; Schryver, H.F. and Hintz, H.F. (1987): Current therapy in Equine Medicine-2 1st Edition, W.B. Saunders Company, Harcourt Brace Jovanovich, Inc. Philadelphia, PA 19106. P. 402-404, 408-409.

20. Si-Kwang Liu, Dolensek, E.P.; Adams, C.R. and Tappe, J.P. (1983): Myelopathy and Vitamin E deficiency in six Mongolian wild horses. J. AVWA, 183, No. 11, 1266-1268.

21. Wreblewski, F. Und L.S. La Duean (1955): Bestimmung der Akitivitat der Lactat-dehydrogenase, Proc. Soc. Exp. Biol. Med. 90, 210.