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RELATIONSHIP BETWEEN ERYTHROCYTIC OXIDATIVE INDICES AND HYPERTRIGLYCERIDEMIA IN CHRONICALLY SICK MULES

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Abstract: In India, mules were mostly used as draught animals and their health management is highly depends on the owner's socio-economic status. The aim of the study was to find out the correlation/relationship between hyperlipedimia and erythocytic oxidative stress in clinically ill mules for various diseases. Heparinized blood samples were collected from 21 clinically ill mules and biochemical analysis were carried out by UV spectrophotometer. From our study, hypertriglyceridemia was common in mules like other equids in diseased condition. Chronically sick mules had anemia. There was a significant increase in lipid peroxide and glutathione values in hypertriglyceridemic mules. The relationship/correlation between the plasma triglyceride and erythrocytic lipid peroxidation (r = +0.594) and erythrocytic super-oxide dismutase (r = +0.607) was positive.

Keywords: Mules; Hyperlipedimia; Hypertriglyceridemia; Oxidative indices

Introduction

Mules are sterile animals, widely used for carting and transportation purposes in hilly terrains where motorized vehicles cannot reach (Yash Pal and Legha, 2008). Mule population in India is 137 thousands with the annual growth rate of 6.05% (17th livestock census 2007). Most of the mule producers are landless and illiterate peoples (only 6% of them were literate). Majority of the equine owners (80%) were ignorant of equine diseases and their prophylactic control. The sick equines were being treated by equine owners themselves through previous prescriptions of same or other equine and with used needles and syringes. Chances of morbidity were higher when the equines were left uncared for grazing (Yash Pal and Legha, 2008).

Hyperlipidemias are common metabolic disorders of miniature horses, ponies and donkeys. It is defined as elevated lipid concentrations in blood, periods of negative energy balance and physiologic stress (McKenzie, 2011). Hyperlipidemia is an elevation of serum triglycerides concentration upto 500 mg/ml, without lactescent plasma or fatty infiltration of the liver

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(Seifi et al 2002). When the triglyceride concentrations exceed the normal range (100 mg/dl) without the evidence of clinical disease is called as hypertriglyceridmia (Naylor, 1982). Severe hypertriglyceridmia occurs more commonly in clinically ill horses without evidence of serum opacity (Dunkel and McKenzie, 2003). In ponies and donkeys, hyperlipaemia is a primary disease process (Hughes *et al.,* 2004). A positive correlation was observed between the plasma insulin and serum triglyceride concentration in various clinical conditions of hyperlipaemia/hyperlipidaemia cases of donkeys (Forhead *et al.,* 1994). Systemic inflammation is commonly associated with insulin resistance in clinically ill horses (McKenzie, 2011).

In farm animals, oxidative stress has been reported in many diseases on recent years. During infection, immune reactions may contribute for the generation of oxidants. Since micro-organisms were able to generate oxidants directly, some of them induce secondary oxidant formation and release via immune system (Lykkesfeldt and Svendsen, 2007). In horses, lot of research has been carried out in pathological conditions such as lower airway diseases, exercise-induced pulmonary haemorrhage, laminitis, arthritis, neurological disorders, muscle disorders and perfusion-related disorders (Kirschvink *et al*., 2008).

As it is the common phenomenon that clinically ill donkeys and ponies were hyperlipidemic and have oxidative stress, the relationship or correlation between them was unknown. The aim of the study was to find out the correlation/relationship between hyperlipedimia and oxidative stress in clinically ill mules for various diseases.

Materials and Methods

Location of study and sampling

The study was conducted at Division of Medicine, IVRI, Izatnagar (UP). Blood samples were collected from 21 clinically ill mules at Army Cantonment and villages near by the Institute, Izatnagar. Animals chronically (more than a month) suffered with various diseases were chosen randomly and blood samples collected from jugular vein and shifted to heparinized glass stoppered tubes for biochemical analysis. Ten healthy mules maintained in Army Cantonment Bareilly were acted as healthy control. The health status was analysed through their records and clinical examination.

Biochemical assays

Lipid profile assays

Plasma samples were analysed for triglyceride concentration by glycerol phosphate oxidase p-amino-phenazone method and total cholesterol (TC) concentration by the cholesterol

oxidase p-amino phenazone method using a UV/visible spectrophotometer. All the kits were purchased from Span Diagnostics, Surat, India. The erythrocytic count was carried out in automatic cell counter (Vetscan HM-5-Abaxis- CA) within 4 h of collection.

Oxidative parameters

Erythrocytic Lipid peroxidation (LPO) was estimated as per the method of Placer *et al.* (1966). The concentration of malonaldehyde (MDA) in nanomoles per millilitre of erythrocytic hemolysate was derived using 1.56 x 10^5 Lmol/cm as extinction coefficient (Utley *et al.,* 1967). The haemoglobin in the hemolysate was estimated by the cyanmethemoglobin method and the LPO concentration in the erythrocytes was expressed in nmol MDA/mg Hb/ml. The glutathione (GSH) level in erythrocytic hemolysate was determined according to the method of Prins and Loos (1969). The superoxide dismutase (SOD) activity was determined by the method of Menami and Yoshikawa (1979) which is a modification of the method given by Marklund and Marklund (1974). Each unit of SOD activity is defined as the quantity of enzyme that inhibited autooxidation of pyrogallol by 50 % under suitable experimental conditions. Activity was expressed as units/mg Hb/mL. The activity of catalase (CAT) was determined from RBC hemolysate as per the method described by Cohen *et al.* (1970). Decomposition of H_2O_2 was followed directly by the decrease in absorbance per min/ mg Hb and it was taken as a measure of the CAT activity.

Statistical analysis

Statistical analysis was done by using SPSS software, version 15 (SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412). The means were compared by independent students T-test and the relationship was compared by Pearson's correlation.

Results

In spite of various diseases, none of the animals were gross lipaemic. The mean ± SE values of serum biochemical and erythrocytic oxidative stress have been presented in Table 1. There was a significant ($P \leq 0.05$) reduction in total erythrocytic count in diseased mules when compared to healthy control. Serum triglyceride level was significantly ($P \leq 0.001$) increased in diseased mules than that of healthy animals. Similarly The increase in lipid peroxide and glutathione levels were significant ($P \leq 0.05$) when compare to healthy control. There was a significant positive correlation (r=+0.594) between lipid peroxidation and triglyceride levels. The correlation between superoxide dismutase and triglyceride was also significantly positive (r=+0.607).

Discussion

In equines, hyperlipidemia is a form of dyslipidemia, which is a disorder of lipid metabolism with abnormal amounts of circulating lipids (McKenzie, 2011). Severe hypertriglyceridaemia is more common in inappetant and clinically ill horses (Dunkel and McKenzie, 2003). Our study proves that, mules were also hyperlipidemic in diseased condition like, ponies, horses and donkeys. Existence of oxidative stress was proved in many diseases on humans (Durackova, 2010) and in horses (reviewed in Kirschvink *et al.,* 2008). One fraction of oxidant increase during the inflammation is due to respiratory burst of inflammatory cells (Moslen 1994). Oxidants released by neutrophils and macrophages were act as a main stem of the non-specific immune response against invading micro-orgnisms. They kill the micro-organism through various ways (Kowaltowski and Vercesi, 1999; Kobayashi *et al.,* 2001). In acute phase of the disease, the hypertriglyceridemia was from increased very low density lipoproteins (Oikawa *et al.,* 2005). In response to the endotoxin liver increases it's secretion and synthesis of TGC laden VLDL, by altering the lipid metabolism. High blood endotoxin concentrations may also inhibit clearance of TGC by LPL in peripheral tissues (Feingold *et al.,* 1992). Further the increased levels of circulating cytokines, tumor necrosis factor (TNF) and interleukin (IL-1, IL-6) associated with a systemic inflammatory response may contribute to the development of severe hypertriglyceridaemia (Moore *et al.,* 1994). When the histone deacylase (HDAC) and whole transcription were inhibited due to oxidative stress, acylation of histones and transcription of pro-inflammatory molecules begins and resulting in progression of inflammation, even in the presence of corticosteroids. Arachidonic acid is a suitable substrate for oxidation (Durackova, 2010) and its metabolites are playing an important role in inflammation (Adams, 2001). In response to lippopolysaccharides, TNF seems to increase serum triglyceride and IL-1 and IL-6 are suspected to influence lipid metabolism (Fingold and Grunfeld, 1992; Feingold *et al.,* 1992). The correlation between lipidperoxidation and triglyceride might be due to the increased levels of pro-inflammatory cytokines due to oxidants and its subsequent change in lipid metabolism. The increased level of super-oxide production might be a reason for the increased level of Super-oxide dismutase. The significant increase in level of glutathione may also indicate the higher level of oxidants in diseased mules. Insulin antagonism can be induced by the actions of catabolic hormones released in stressful situations (Lager, 1991). Positive correlation has been proved to be existing between plasma triglyceride and insulin concentrations. Williams (2008) suggested that more research is needed to evaluate systemic

inflammation and oxidative stress of horses with laminitis. It indicates that the relationship between the oxidative stress and systemic inflammation was not fully evolved and requires more research.

In conclusion from our study, hypertriglyceridemia was common in mules like other equids in diseased animals. Chronically sick mules had anemia. There was a significant increase in lipid peroxide values and glutathione values in hypertriglyceridemic mules. The relationship/correlation between the plasma triglyceride and erythrocytic lipid peroxidation and erythrocytic super-oxide dismutase was positive.

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Table 1. Mean ± SE values of Hematology & serum biochemical changes

S.No.	Parametes	Healthy control (n=10)	Chronically sick animals (n=21)
1.	TEC (10^6/cumm)	6.73±0.24	5.20±0.40*
2.	HCT %	30.30±1.48	26.91±1.99
3.	LPO (nmol/mgHb/ml)	2.66±0.16	4.49±0.65*
4.	GSH (mM/ml)	0.36±0.02	1.12±0.14*
5.	SOD (Units/mgHb)	0.91±0.21	1.56±0.24
6.	Catalase (Units/mgHb)	0.40±0.10	0.61±0.08
7.	Triglycerides (mg/dl)	41.69±1.49	105.11±10.15**
8.	Total cholesterol (mg/dl)	118.64±5.28	80.03±12.95

*Significant (P<0.05), ** Highly significant (P<0.001)