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OXIDATIVE STRESS IN PREGNANT AND LACTATING CAMELS

Sajjan Singh, R.K. Dedar[1] and N.V. Patil
National Research Centre on Camels, Jorbeer, Bikaner
[1]E.P.C., National Research Centre on Equines, Bikaner, India

ABSTRACT

Biomarkers of oxidative stress malondialdehyde, catalase, reduced glutathione and plasma vitamin E were evaluated in pregnant, lactating and non pregnant non lactating Indian camels. Whole blood levels of malondialdehyde (lipid peroxidation product) were significantly higher in pregnant camels (28.11±0.44 nanomol/ml) than the non pregnant non lactating (25.86 ±0.91 nanomol/ml). Levels of catalase (antioxidant enzyme) and reduced glutathione (antioxidant protein) were also lower in pregnant and lactating camels than the non pregnant non lactating camels. Study showed that levels of oxidative stress biomarkers are higher in pregnant and lactating camels than the control non lactating non pregnant camels.

Key words: Camels, catalase, lactation, malondialdehyde, oxidative stress, pregnancy, reduced glutathione

Oxidative stress is a disturbance of equilibrium between antioxidants and oxidants in favour of oxidants (Sies, 1991). Electrons leaking from the electron transport chain (ETC) produce reactive oxygen species (ROS) and these molecules then damage ETC components and mitochondrial DNA, leading to further increase in intracellular ROS levels and a decline in mitochondrial function (Wallace, 2005). Redox-sensitive transcriptional factors by oxidative stress causes the up regulation of pro inflammatory gene expression, as a result various pro inflammatory molecules are generated, leading to inflammation processes in various tissues and organs. This inflammatory cascade has been linked with many diseases such as cancer, various cardiovascular diseases, arthritis, and several neuro-degenerative diseases (Chung *et al,* 2006). Oxidative stress has been studied widely in farm animal (pigs, cattle and horses) diseases but there is scarcity of data regarding oxidative stress and their biomarkers in camels.

Both lactation and advanced pregnancy are associated with increased energy requirement and increased chances of reactive oxygen species production. Present study was undertaken to assess the biomarkers of oxidative stress in lactation and pregnancy in camels.

Materials and Methods

Animals and sampling

Study was carried out at National Research Centre on Camels Bikaner. Blood samples in heparinised vacutainers were collected from six pregnant, five lactating and ten non-pregnant non-lactating camels from the animal farm of NRCC Bikaner. Non-pregnant non-lactating camels were used as control animals in the study.

Assay of malondialdehyde

Estimation of whole blood malondialdehyde was carried out by the spectrophotometric method of Okhawa *et al* (1979), Malondialdehyde (MDA) is a secondary product of lipid peroxidation, the reaction of lipid peroxides with thiobarbituric acid (TBA) yields red pigment which can be measured on spectrophotometer at 532 nm. Freshly collected whole blood 0.2 ml was taken and 1.8 ml of 1.15% potassium chloride was added to it, from this 0.2ml was transferred to other test tube. After that 0.2 ml Sodium dodecyl sulphate solution (8.1%), 1.5 ml -20% Acetic Acid (pH adjusted 3.5 by NaOH), 1.5 ml -2 thiobarbituric acid (0.8% aqueous solution) were added to the test tube. The contents were thoroughly mixed and volume was made 4.0 ml with deionised distilled water, test tubes were capped with aluminum foil and kept in water bath for 60 minutes. Test tube were then cooled under tape water immediately and one ml of distilled water was added to each test tube. Each tube was then added 5 ml of n-butanol plus pyridine (15:1 v/v) solution and shaken vigorously and centrifuged at 4000 rpm for 10 minutes. Upper organic pink colour layer

SEND REPRINT REQUEST TO R.K. DEDAR email: rameshdedar@gmail.com

was collected from each test tube and absorbance was read at 532 nm against reagent blank. Solutions of 5,10,15,20,25,30 nnamoles/ml of tetramethoxy propane (TMP) were prepared by diluting it to 1.15% of potassium chloride solution for standard curve.

Assay of catalase

Measurement of catalase was carried out by the spectrophotometric method of Goldblith and Proctor (1950) with slight modifications.

Assay of reduced glutathione

Estimation of reduced glutathione was carried out the by spectrophotometric method of Beutler (1971).

Assay of vitamin E

Estimation of plasma vitamin E was carried out by the method described by Kayden *et al* (1973).

Results

Results are shown in the table, blood malondialdehyde levels were significantly higher in pregnant camels (28.11±0.44 nanomol/ml) than the non pregnant non lactating camels (25.86±0.91 nanomol/ml). Reduced glutathione in pregnant (9.66 ±1.13 mg/dl) and lactating camels (10.44 ±1.58 mg/dl) were lower than the non pregnant non lactating camels (16.06±0.82mg/dl). Blood catalase levels were also lower in pregnant (3569±322 IU/ml) and lactating camels (3251±569 IU/ml) than the non pregnant non lactating camels (5759±174 IU/dl). Plasma vitamin E levels were found lower in pregnant (2.31 ±0.42 mg/l) and lactating (2.75±0.29 mg/l) camels than the control non pregnant non lactating camels, however not statistically significant.

Discussion

Malondialdehyde (MDA) is widely preferred for detection of free oxygen radicals in various pathological conditions (Lazzarino *et al,* 1994), it is a stable byproduct of cell membrane's lipid peroxidation and is an indicator of cell membrane damage (Otamiri, 1988) caused by oxidative stress. Blood levels of malondialdehyde (MDA) were found significantly higher in pregnant camels than the control camels, it shows higher levels of oxidative stress in pregnant camels. Similarly Patil *et al* (2006) found increase in MDA's level with gestational age in women. Blood levels of reduced glutathione and catalase were also found significantly lower in pregnant and lactating camels than the control camels. Reduced glutathione (GSH) plays key role in scavenging t-butyl hydroperoxide, an agent which induces lipid peroxidation (Trotta *et al,* 1982) and catalase is the most important enzymes of antioxidant defence mechanism (Fridovich, 1995). The catalytic activity of catalase allows the transformation of superoxide anion into hydrogen peroxide (H_2O_2) and water and inactivates large amounts of oxidants (Mates, 2000). Results of the present study have shown that pregnancy and lactation both are associated with generation of free radicals and resultant oxidative stress in camels. Pregnancy, mostly because of the mitochondria-rich placenta, is a condition that favors oxidative stress. Transitional metals, especially iron, which is particularly abundant in the placenta, are important in the production of free radicals (Casanueva and Viteri, 2003). Lactating animals undergo substantial metabolic and physiological adaptations during the transition from pregnancy to lactation that contribute to dysfunctional host inflammatory responses (Sordillo, 2005). Physiological stresses associated with rapid differentiation of secretary parenchyma, intense mammary gland growth, and the onset of copious milk synthesis and secretion are accompanied by a high energy demand and an increased oxygen requirement. This increased oxygen demand augments the production of oxygen-derived reactants, collectively termed reactive oxygen species (ROS). Accumulation of ROS can cause cell and tissue injury and can lead to a condition of oxidant stress (Sordillo and Aitken, 2009). Among the biomarkers Malondialdehyde levels in control and lactating animals were equal but catalase and reduced glutathione were significantly low in lactating camels,

Table 1. Biomarkers of oxidative stress in whole blood and plasma vitamin E levels.

	Pregnant (N6)	Lactating(N5)	Non pregnant-non lactating(N10)
Malondialdehyde (nanomol/ml)	28.11±0.44*	26.08±1.40	25.86 ±0.91
Catalase (IU/ml)	3569 ±322*	3251 ±569*	5759±174
Reduced glutathione (mg/dl)	9.66 ±1.13*	10.44 ±1.58*	16.06±0.82
Plasma Vitamin E (mg/l)	2.31 ±0.42	2.75±0.29	3.00±0.13

*significant (<.05) difference than the non pregnant non lactating camels.

it shows that antioxidant defences (Catalase & Reduced glutathione) exhausts earlier than the rise in levels of lipid peroxidation (Malondialdehyde). Plasma vitamin E levels of in Indian camels were similar to the Iranian dromedary camel (2.25 mg/l) as described by Nazifi *et al* (2009). Plasma vitamin E levels were lower in pregnant and lactating camels than the non pregnant non lactating camels, however the difference was not statistically significant. It might be due wide range of vitamin E level in pregnant camels (0.87 to 3.19 mg/l) and lactating (1.5 to 3.78 mg/l). Results of the study showed that pregnant and lactating camel may be fed antioxidant supplementation and further research may be carried out to study the preventive effect of antioxidants on oxidative stress.

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