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Acute Phase Proteins, Trace Elements and Cytokines Expression as A Diagnostic and Prognostic Biomarker in Diseased Camel

Hany Y. Hassan[1*], Shaaban M. Gadallah[2], Ahmed Kamr[1], Ali Abdelazeim[1]

(1) Department of Animal Medicine and Infectious Diseases (Animal Internal Medicine). Faculty of Veterinary Medicine, University of Sadat City, 32897, Egypt.

(2) Department of Surgery, Anesthesiology and Radiology. Faculty of Veterinary Medicine, University of Sadat City, 32897, Egypt.

**corresponding author:* hanyhassan1959@gmail.com

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ABSTRACT

The present study was carried out to evaluate the diagnostic value of inflammatory biomarkers on diseased camels via exploring the possible effects on oxidants and antioxidants status in addition to characterizing the profiles of acute phase proteins and inflammatory cytokines. Forty-eight adult male and female dromedary camels (Camelus dromedaries) were examined, according to general health conditions, clinical and laboratory diagnosis they were split into 3 groups. Group 1: Included 20 apparently healthy camels act as a control group, Group 2: Included 12 camels suffered from trypanosomiasis, Group 3: Included 16 camels suffered from mange. Our results revealed that the mean values of copper and zinc were significantly decreased with statistical elevation of MDA and statistical lowering of catalase and total antioxidant capacity in cases of trypanosomiasis and mange compared to apparently clinically healthy camels (P value <0.05), there is a pearson negative correlation between copper and MDA (r=-0.6; P<0.05) in diseased camels, The mean values of acute phase proteins (haptoglobin, ceruloplasmin and serum amyloid A) and inflammatory cytokines (IL-1, IL-6, IL-10 and TNF-α) were significantly elevated in diseased camels at (Trypanosomiasis and mange) than apparently clinically healthy camels (P value <0.05).

Key words: *Acute phase proteins; Camel; Cytokines; Oxidants and antioxidants*.

INTRODUCTION

Surra disease in camels is transmitted mechanically by flies biting (Gill, 1991), and characterized clinically by pyrexia, loss of appetite, edema in different parts of body and anemia (Parsani *et al.,* 2008), while, camel mange was caused by *Sarcoptes scabiei var cameli* (Al-Rawashdeh *et al.,* 2000), which was spread through direct contact with contaminated or infected animals (Khan *et al.,* 2003), and expressed clinically by irregular areas of hair loss, itching, formation of scales and the skin becomes thick and hard (Saleh *et al.,* 2011).

Free radical is defined as any species with one or more unpaired electrons capable of independent existence, reactive oxygen species (ROS) can attack all major bimolecular classes, ROS has multiple normal physiological functions, but oxidative stress will occur when the excess production of antioxidants cannot be counteracted, potentially leading to pathological changes (Dröge, 2002).

Acute phase proteins, proinflammatory cytokines and oxidative stress parameters could be used as biomarkers of *T. evansi* infection in dromedary camel (EL-Bahr and EL-Deeb, 2016).

Acute phase proteins (APP) are plasma proteins which, in response to infections, inflammation, and internal or external challenge, increase or decrease in concentration. The monitoring of APPs has been shown to provide valuable information for diagnosis and prognosis during infection (Eckersall, 2000).

Inflammatory cytokines are typically those expressed early in disease or injury process in an antigen-independent manner. The bulk of inflammatory cytokine expression is due to macrophages and monocytes cells, the lipopolysaccharide (LPS) in the cell wall of gm (-ve) bacteria is perhaps the most famous and characteristic inductor of inflammatory cytokines. Tumor-necrosis factor (TNF), interleukin IL-1, IL-6, IL-10 are the main inflammatory cytokines. TNF is a cytokine pleiotropic to modify physiological and immunological conditions (Michael *et al.,* 1996). The acute phase response is a complicated mechanism for early defense of trauma, infection, tissue harm, swelling, stress or neoplasm activated responses. The hepatic synthesis of some plasma proteins known collectively as acute phase proteins is one of the most significant components for this reaction. The discovery of these new biomarkers has led to the clinical monitoring and clinical implementation of distinct illnesses, and the diagnosis, assessment, therapy, prognostic and therapy of a number of distinct illnesses (Tothova *et al.,* 2014).

The aims of this study were to evaluate the diagnostic value of inflammatory biomarkers in camels via concerning on the possible effect of different diseased conditions on oxidants and antioxidants status in addition to changes in copper and zinc levels, exploring the relationship between oxidants and antioxidants markers and copper and zinc deficiency in diseased camels and characterizing the profiles of cytokines inflammatory mediators, acute phase proteins to explore their importance as a diagnostic value of diseased camels.

MATERIALS AND METHODS

Animals

Forty-eight adult male and female dromedary camels (*Camelus dromedaries*) were examined in separate farms and locations in Menofia and Behera Governorates, Egypt. The age was varied between 2–8 years old. According to general health conditions, clinical and laboratory examination of the camels, they were classified into 3 groups. Group 1 (Control group): Included twenty apparently clinically healthy camels free from external and internal parasites, act as a control; Group 2 (Camels suffered from trypanosomiasis) Included 12 camels suffered from trypanosomiasis characterized by edema in lower parts of abdomen and legs, swelling of lymph nodes, severe debilitating conditions, presence of petichae on ocular mucus membrane and positive blood film (Fig. 1); Group 3 (Manged camels) Included 16 dromedary camels suffered from irregular large area of alopecia with scales formation with pruritis and confirmed by skin scraping test (Fig. 2).

Blood samples

Ten ml of blood gathered from each animal via a jugular venous puncture was split into two aliquotes. A tube of 5 mg ethylenediaminetraacetic acid (EDTA) was added to the first aliquot for blood film examination. In a dry, clean centrifuge tube the second aliquot was collected and stored in an inclined position for 10 minutes were centrifugated at 3000 rpm to separate only clear non hemolyzed sera stored at -20 (°**C**) until used for biochemical analysis.

Blood film examination, skin scraping examination

Thin blood film examination and skin scrapings examinations were carried out according to method reported by (Hoare, 1972; Bayou, 2005), respectively

Determination of trace elements

Commercial kits (Bio-Diagnostic, Giza, Egypt), were used for spectrophotometric determination of copper and zinc according to methods specified by (Ventura and King, 1951; Hayakawa, 1961), respectively

Oxidant and antioxidant status

Malondialdehyde (MDA) was measured in serum samples as previously described by (Ohkawa *et al.,* 1979) using a kit from Bio Diagnostics company in which MDA reacts directly at optimum pH (3.5) with thiobarbituric acid to produce a spectrophotometrically determined red color. Catalase was measured in serum sample as previously described by (Aebi,

1984) using a kit from Bio Diagnostics company. In the presence of peroxidase, remaining H2O2 reacts with 3,5-Dichloro -2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample. Total antioxidant capacity was determined according to the method described by (Koracevic *et al.,* 2001), using a kit from Bio Diagnostics company in which a Fe-EDTA complex reacts by a fenton-type reaction with hydrogen peroxide, resulting in the formation of hydroxyl radicals that degrade benzoate, followed by the release of reactive substances (thiobarbituric acid).

Acute phase proteins and cytokines

Ceruloplasmin (CP/CER), Serum amyloid A (SAA), Haptoglobin (Hpt/HP), and cytokines Interleukin 1(IL-1), Interleukin 6 (IL-6) and Tumor necrosis factor α (TNF-α) Serum levels were identified by using ELISA kits of Shanghai Coon Koon Biotech., Ltd (China). All procedures were performed as described in the instructions of the manufactures.

Statistical Analysis

Data from healthy and diseased camels were compared by means of one-way ANOVA by using the statistical package for social science (SPSS) for windows (Version 16.0; SPSS INC., Chicago, Ill. Results were expressed as the mean ± standard error (SEM). **IBM SPSS Bootstrapping 24. 2016.**

RESULTS

Trace elements, oxidant and antioxidant status determination

The mean values of serum copper were statically decreased in camels suffered from trypanosomiasis and camels with mange compared to control ones (P value < 0.05), while the mean values of zinc were significantly reduced in camels suffered from mange compared to control and camels suffered from trypanosomiasis. The mean values of malondialdehyde were statically increased , the mean values of catalase and total antioxidant capacity significantly decreased in camels suffered from trypanosomiasis and mange compared to control ones (P value < 0.05), and there is statistical variations for these parameters between camels suffered from trypanosomiasis and camels with mange lesions (P value < 0.05). Table 1

Relationship between oxidants and antioxidants markers and trace elements deficiency (copper and zinc deficiency)

In the present study, there is a pearson negative correlation between copper and MDA (r=-0.6; P<0.05) in diseased camels (Fig 3), copper deficient camels were associated with increased MDA levels. On the other hand, there is no a pearson correlation between zinc and MDA levels (r=0.062; P>0.05) in diseased camels (Fig 4).

Acute phase proteins

The mean values of ceruloplasmin were significantly increased in both diseased group (Trypanosomiasis and mange) compared to control ones (P value< 0.05) with statistical increase in trypanosomiasis than manged camels (P value < 0.05) while the mean values of serum amyloid A and haptoglobin were significantly higher in cases of diseased camels with trypanosomiasis and mange in comparison to control ones (P value < 0.05), without statistical difference for theses parameters between mange and trypanosomiasis (P value > 0.05). Table 2

Cytokines expression

The mean values of interleukin 1, interleukin 6 and Tumor necrosis factor α were statically elevated in camels suffered from trypanosomiasis and camels suffered from mange compared to apparently healthy ones (P value <0.05), while there is no statistical variations for values of IL-1, IL-6 and TNF- α between camels with trypanosomiasis and camels suffered from mange (P value >0.05). Table 3

Table 1: Trace elements, oxidant antioxidant status in apparently clinically healthy and diseased camels.

Variables	Control n=20	Trypanosomiasis n=12	Mange n=16
Copper (µg/dl)	93.7±1.4 [a]	84.1±1.1 [b]	84.19±19 [b]
Zinc (µg/dl)	121.9±1.57[a]	120.8±1.6 [a]	116.4±0.8 [b]
Malondialdehyde (nmol/ml)	2.6±0.09[a]	7.56±0.24[b]	4.6±0.263[c]
Catalase (mM/L)	78.3±0.4[a]	71.19±0. 39[b]	74.45±0.2[c]
TAC (U / L)	0.81±0.02[a]	0.139±0.02[b]	0.44±0.04[c]

Means within the same row having the different superscripts differ significantly different at (P<0.05).

Table 2: Acute phase protein levels in apparently clinically healthy and diseased camels.

Variables	Control n=20	Trypanosomiasis n=12	Mange n=16
Ceruloplasmin (ug/ml)	37.28±1.9 [a]	81.32±3.48 [b]	88.7±1.62[c]
Serum amyloid A (g/ml)	0.51±0.038 [a]	0.91±0.039 [b]	1.28±0.15 [b]
Haptoglobin (ug/ml)	77.38±1.11[a]	109±4.86 [b]	103.3±2.147[b]

Means within the same row having the different superscripts differ significantly different at (P<0.05).

Table 3: Cytokines expression in apparently clinically healthy and diseased camels.

Variables	Control n=20	Trypanosomiasis n=12	Mange n=16
Interleukin 1 (pg/ml)	44.9±1.6 [a]	89.27±3.16 [b]	90.9±4.4 [b]
Interleukin 6 (ng/L)	28.29±1.5 [a]	57.99±4.9 [b]	63.34±4.5 [b]
Tumor necrosis factor α (ng/L)	19.19±1.05 [a]	36.51±1.8 [b]	33.88±4.2 [b]

Means within the same row having the different superscripts differ significantly different at (P<0.05).

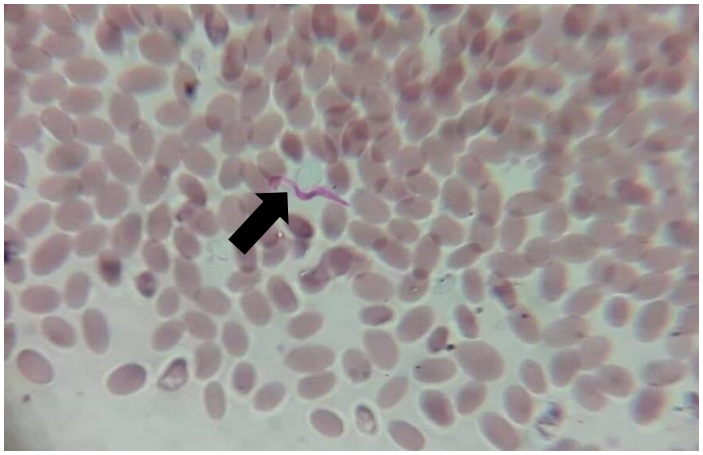

Fig. 1. Presence of trypanosoma evansi between red blood cells of infected camel.

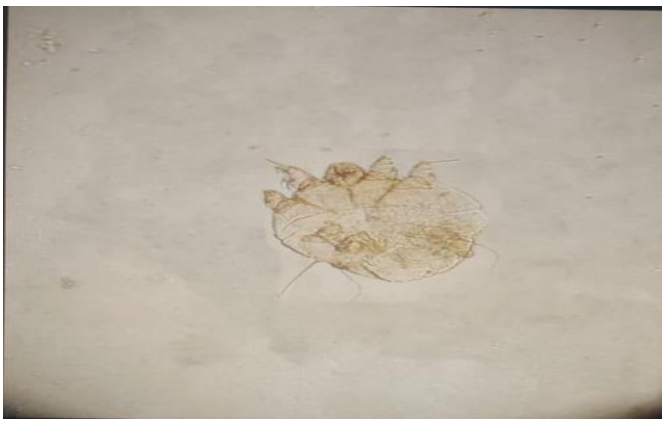

Fig. 2. Sarcoptic scabie var cameli.

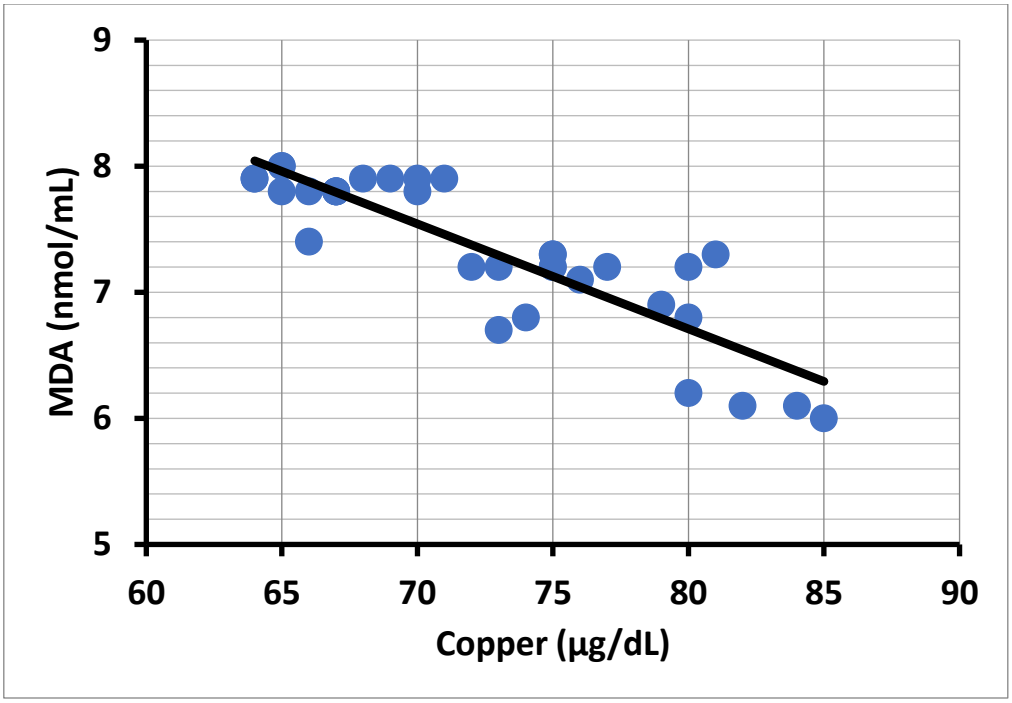

Fig. 3. Pearson negative correlation between copper and MDA (r=-0.6; P<0.05) in diseased camels.

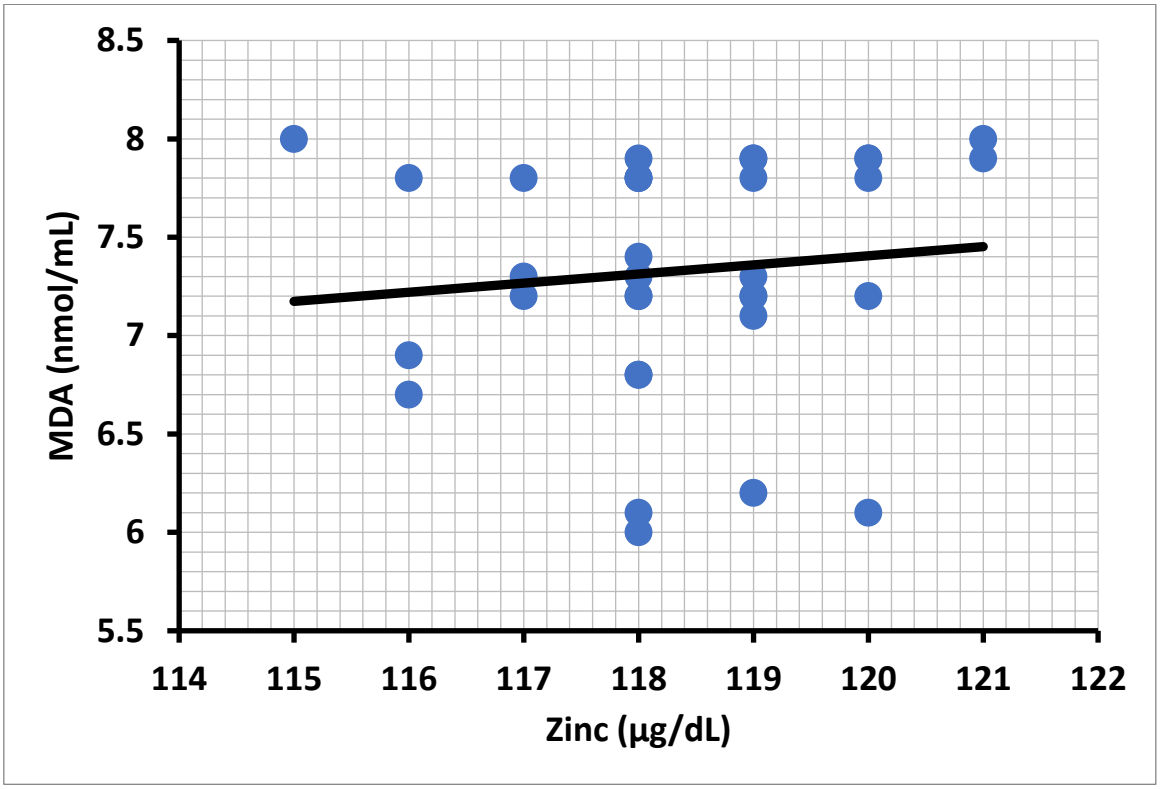

Fig. 4. Pearson correlation showed that no correlation between MDA and zinc (r=0.062; P>0.05) in diseased camels

DISCUSSION

Regarding to trace elements, oxidant and antioxidant estimations, there is significant lowering of levels of copper in trypanosomiasis and mange and significant diminishing of zinc levels in camels with mange, our results were nearly with results obtained by (Hassan *et al.*, 2018), also there is statistical elevation of MDA with statistical decrease of catalase and total antioxidant capacity in diseased camels, Our results were nearly in the line with the results reported by (Dimri *et al.*, 2010; Saleh *et al.*, 2011). Lowered copper status may be due to the fact that copper had anti-inflammatory and anti-oxidant characters, so copper may be consumed

due to its performance in this function. copper is associated with delayed immune response (Puls, 1994). Zinc deficiency resulted in growth failure and feed intake and loss of hair so zinc deficiency facilitated the pathways for different microorganism to penetrate the animal body and infection incidence (Fraker, *et al.,*2003). Oxidative stress occurred when an oxygen molecule splits into single atoms with unpaired electrons which are could free radicals. Free radicals are the natural byproduct of chemical process such as metabolism, free radical are essential to life, the body,s ability to turn air and food into chemical energy depends on chain reaction of free radicals, oxidative stress which broke cell membrane occur when there are too many free radicals and too much cell damage as occur in Trypansoma evansi infection and the lipid peroxidation end product is MDA (Bernabucci *et al.,* 2005; Rezaei and Dalir-Naghadeh, 2006; Chaudhuri *et al.,* 2008). The severity of the blood parasite infection depends on the degree of parasitemia, the direct correlation between the percentage of parasitemia and MDA concentration suggests that the severity of the disease is directly associated with erythrocyte lipid peroxidation (Sevinc *et al.,* 2007). The increase in the generation of MDA reflects increased membrane lipid peroxidation and provides additional evidence of increased free radical generation in erythrocytes of camels infested with sarcoptic scabies (Saleh *et al.,* 2009). In skin disease, significantly higher levels of MDA indicated a decline in antioxidant defense and improved oxidative damage to dermal tissues in animals infested with Psoroptic mange, our results are in agreement with those obtained by (Dimri *et al.,* 2010). Reduction in total antioxidant capacity levels may be attributed to the use of antioxidant enzymes as free radical scavengers in camels during the oxidative process of trypanosomiasis.

Regarding to pearson correlation between MDA and trace elements deficiency, there is a pearson negative correlation between copper and MDA, while there is no a pearson correlation between zinc and MDA levels in diseased camels, our results in agreement with obtained by (Zhang *et al.,* 2012) whom indicated that copper deficient goats were associated with increased MDA levels and increased oxidative damage, while supplemental Cu increased the activities of antioxidant enzymes and decreased the serum MDA content in cashmere goats. In our research study, there was significant decrease in level of copper. Nearly results were obtained by (Saleha *et al.,* 2008), the inhibited antioxidant activity and oxidative damage that occurred in hypocuperosis were manifested as the decreased activities of total antioxidant capacity and catalase and the increased MDA level.

Regarding to acute phase and cytokines expression, the mean values of ceruloplasmin, serum amyloid A, haptoglobin, IL-1, IL-6 and TNF-α were statically higher in cases of trypanosomiasis and mange compared to control camels, our results agreed with results obtained by (Saleh *et al.,* 2011; EL-Bahr and EL-Deeb, 2016). In sarcoptic mange cases, acute phase proteins could be increased increase either by immune response, pro-inflammatory cytokines secretion or systemic inflammation associated with large lesions of skin (Rahman *et al.,* 2010). An increased concentration of serum amyloids A was linked to secondary pathological amyloidosis (Ceciliani *et al.,* 2002), that is a common finding that leads to organ failure in animals seriously affected by mange (Arlian *et al.,* 1990).

Elevated levels of Il-1, TNF-α and interferon gamma in trypanosoma evansi infected rats had been involved in anemia development (Paim *et al.,* 2011). This elevation was accompanied with the immune response regulation against the parasite (Graham *et al.,* 2001), also the high concentrations of proinflammatory cytokines and APPs in parasitemia, could be attributed to hemorrhages, lymphocytic infiltration and necrosis on abomasum, liver, kidney, intestine, lung and bone marrow, indicating the widely spread inflammatory reaction, nearly suggestions were provided by (Nazifi *et al.,* 2010). Increased cytokine levels (IL-6, TNF) in diseased camels compared to control ones may be accredited by Psoroptes mange for a severe allergic inflammation that promotes the release of already secreted and newly synthesized cytokines and contributes to pathology in the parasites (Majewska *et al.,* 2016), scabies extracts can contribute additionally to cytokine pool since they have been displayed to up-regulate the production of interlukin-1 (Arlian *et al.,* 2003).

CONCLUSION

Cytokines inflammatory mediators and acute phase proteins have a diagnostic and prognostic value in camels suffering from trypanosomiasis or mange which are considered the most serious and wide spread diseases among this animals which associated with trace elements deficiency and oxidative stress, so adequate antioxidant and trace elements as supportive therapy should be adequate with access amount to counteract against oxidative stressors which increased in these diseases.

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