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*Original article*

# CORRELATIONS AMONG SYNOVIAL FLUID BIOMARKERS IN CLINICALLY HEALTHY DROMEDARY CAMELS

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**Summary**

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Synovial fluids were taken from 33 adult male dromedary camels (*Camelus dromedarius*), after slaughtering. In these samples, the concentrations of haptoglobin, serum amyloid A, tumor necrosis factor-alpha, interferon-gamma, zinc, copper, selenium, iron and vitamin A, E and C were assayed. Iron had positive and significant correlations with vitamin A, haptoglobin and tumor necrosis factor-alpha ($P<0.05$). The correlation between iron and vitamin C was negative. Zinc was correlated significantly with haptoglobin, serum amyloid A, tumor necrosis factor-alpha and interferon-gamma, ($P<0.05$). The relationships between copper and haptoglobin and serum amyloid A were negatively and positively significant, respectively. Selenium was closely correlated to haptoglobin, tumor necrosis factor-alpha and interferon-gamma ($P<0.05$). The relationship between vitamin C and haptoglobin was negatively significant in studied animals. There are relationships among oxidative stress biomarkers in synovial fluid of dromedary camels. In synovial fluid, each detected biomarker can affect others and the presence and activity of each parameter it can be estimated by measuring another one.

**Key words**: correlation, dromedary camels, oxidative stress biomarkers, synovial fluid

## INTRODUCTION

Oxidative stress plays a key role in the pathogenesis of several infectious and non-infectious stress conditions in domestic animals (Lykkesfeldt & Svendsen, 2007). Oxidative stress commonly does not exhibit clinical signs and it can be extremely dangerous; hence, early diagnosis of this condition may be used as an effective method to detect, control and prevent of subsequent disorders such as degenerative damage of cellular structures (Matsuo & Kaneko, 2000; McCord, 2000). Measuring oxidative stress allows estimation of the real status of physiological defense and prevention of the appearance of correlated pathologies (Piccione *et al.,* 2007).

Oxidative stress can interfere with the healthiness of joints of camels similar to other animals. Several infectious and non-infectious stress factors as oxidative stressors can alter the racing and growing performances of camels by affecting their joints. A synovial fluid analysis is one of

the most important diagnostic tools to early diagnosis of oxidative stress in articular tissues. It also provides valuable information about the stage and prognosis of the articular abnormalities (Al-Rukibat *et al.,* 2006). Such gross and cytological analysis of synovial fluid can aid in the diagnosis of various joint diseases, including ligament damage, trauma, neoplasia, infectious and non-infectious synovitis and arthritis, osteoarthritis, and immune-mediated polyarthritis (Madison *et al.,* 1991).

Trace elements such as zinc (Zn), copper (Cu), selenium (Se) and iron (Fe) are the main part in the structure of anti-oxidant enzymes, so their evaluation can reflect the activity of those enzymes. These elements play a key role in oxidative stress processes. Determination of serum concentrations of anti-oxidant and free radical scavenger vitamins such as vitamin A, E and C has a key role in evaluating oxidative stress status in animals (Liu, 2003). Oxidative stress is a part of acute phase response and this response can be evaluated via assessing the acute phase proteins and inflammatory cytokines to reflect the oxidative stress conditions (Chalmeh *et al.,* 2013a, b, c).

Some researchers indicated that oxidative stress plays an important role in inducing arthritis and that molecular targeting therapy, such as vitamin E and glutathione, can be used in the role of anti-oxidants to treat arthritis (Machtey & Ouaknine 1978; Blankenhorn, 1986; Jordan *et al.,* 2004). However, some reports mentioned that these therapies are controversial (Brand *et al.,* 2001; Wluka *et al.,* 2002). There is little information about synovial fluid biomarkers in dromedary camels. Knowledge of the normal synovial levels of these parameters and their relationships together may help the arthritis diagnosis and response to treatment in the future.

Except for oxidative stress biomarkers, the normal values for synovial fluid analysis in the adult dromedary camel (Nazifi *et al.,* 1998) and llama and alpaca (Waguespack *et al.,* 2002) have been described. Therefore, the data reported here could be used as reference values for assessing articular abnormalities in this species. Furthermore, the relationships among these parameters together can represent the effect of each parameter on the other ones. Information regarding their relationships can assist veterinarians to estimate the changes of each parameter based on evaluating another.

## MATERIALS AND METHODS

Thirty tree adult male dromedary camels (*Camelus dromedarius*) were used in this study after slaughter in the Meibod abattoir, Yazd province, Iran, in November 2010. The clinical healthiness of the animals was proved before slaughtering and all animals did not have any clinical and gross articular abnormalities. Synovial fluids were collected by 18 gauge, 1.5 inch needle attached to a 5 milliliters syringe, from the healthy tarsal joints immediately after the camels were slaughtered. To collect the samples aseptically, the skin covering each joint was clipped and scrubbed using povidone-iodine solution. The needle was inserted into the medial pouch of the tarsal joint. Five milliliters of synovial fluid were collected from each joint and placed in plain and anticoagulant-coated tubes. Samples of synovial fluids were stored at –22°C until assay.

Haptoglobin (Hp) and serum amyloid A (SAA) were measured by using commercial kits (Tridelta Development Plc, Wicklow, Ireland). Tumor necrosis factor-

alpha (TNF-α) and interferon-gamma (IFN-γ) were measured based on factory instructions (AbC606 and AbC 607, respectively; Votre fournisseurAbCys S.A. Paris, France). All the samples were digested and analyzed for Zn, Cu, Se and Fe using atomic absorption spectrophotometry (Shimadzu-AA-670, Kyoto, Japan). In order to analyze the specimens, the samples were atomized. The atoms then were irradiated by optical radiation. The radiations then were passed through a monochromator in order to separate the element-specific radiation from any other radiation emitted by the radiation source, which was finally measured by a detector. High-performance liquid chromatography (HPLC) was used to determine the serum values of vitamins A, E and C. The samples were passed through a column filled with a solid adsorbent. Each component in the sample interacted slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column (Snyder *et al.,* 1997).

Data were expressed as mean ± standard error of mean (SEM). Correlations among all studied parameters were evaluated by Pearson's correlation test. In the present study, the correlation coefficient greater than 0.8 was considered strong, whereas a correlation less than 0.5 described as weak. Statistical analyses were performed by SPSS software (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois) and the level of significance was set at $P<0.05$.

## RESULTS

Synovial fluid concentrations (mean ± SEM) of oxidative stress biomarkers in clinically healthy adult male dromedary camels are presented in Tables 1 and 2.

**Table 1.** Synovial fluid concentrations (mean± SEM) of trace elements and vitamins in clinically healthy male dromedary camels (n=33)

| Biomarker | Synovial fluid concentration |
|---|---|
| Iron (μmol/L) | 36.22±3.23 |
| Zinc (μmol/L) | 9.38±1.52 |
| Copper (μmol/L) | 3.24±0.75 |
| Selenium (μmol/L) | 12.32±0.26 |
| Vitamin A (μmol/L) | 6.55±0.50 |
| Vitamin C (μmol/L) | 9.66±0.49 |
| Vitamin E (μmol/L) | not detected |

**Table 2.** Synovial fluid concentrations (mean± SEM) of acute phase proteins and inflammatory cytokines in clinically healthy male dromedary camels (n=33)

| Biomarker | Synovial fluid concentration |
|---|---|
| Haptoglobin (g/L) | 0.124±0.002 |
| Serum amyloid A (μg/mL) | 0.007±0.001 |
| Interferon-γ (pg/dL) | 1.351±0.033 |
| Tumor necrosis factor-α (pg/dL) | 7.474±0.129 |

The correlations among synovial fluid oxidative stress biomarkers in studied animals are shown in Table 3. Fe had positive and significant correlations with vitamin A, Hp and TNF-α ($P<0.05$). The correlation between Fe and vitamin C was negative. Zn was significantly correlated with acute phase proteins (Hp and SAA) and inflammatory cytokines (TNF-α and IFN-γ) ($P<0.05$). The relationships between Cu and Hp and SAA were negatively and positively significant, respectively. Se was significantly correlated to Hp, TNF-α and IFN-γ ($P<0.05$).

**Table 3.** Correlations among synovial fluid oxidative stress biomarkers in clinically healthy male dromedary camels (n=33)

| | Fe | Zn | Cu | Se | Vit. A | Vit. C | Hp | SAA | TNF-α |
|---|---|---|---|---|---|---|---|---|---|
| Zn | 0.160 | | | | | | | | |
| Cu | 0.282 | 0.032 | | | | | | | |
| Se | 0.200 | –0.193 | 0.320 | | | | | | |
| Vit. A | 0.426* | 0.194 | –0.043 | –0.017 | | | | | |
| Vit. C | –0.486* | –0.174 | –0.341 | –0.197 | –0.272 | | | | |
| Hp | 0.544* | 0.887* | –0.543* | 0.799* | 0.038 | –0.538* | | | |
| SAA | 0.032 | 0.960* | 0.800* | –0.184 | –0.121 | –0.239 | 0.016 | | |
| TNF-α | 0.623* | 0.533* | 0.177 | 0.571* | 0.003 | –0.012 | –0.201 | 0.072 | |
| IFN-γ | 0.138 | 0.691* | –0.190 | 0.686* | 0.120 | –0.036 | 0.113 | –0.150 | 0.090 |

* statistical significance at $P<0.05$; Hp=haptoglobin; SAA=serum amyloid A; TNF- α=tumour necrosis factor α.

The relationship between vitamin C and Hp was negatively significant in studied animals.

## DISCUSSION

Evaluating oxidative stress biomarkers in synovial fluid can be used as a diagnostic method to detect the inflammatory processes in affected joints. Acute phase response is a term defining the response to inflammatory agents and the concentrations of acute phase proteins, cytokines and enzymes increase in this process (Chalmeh *et al.,* 2013b). Assessing the acute phase proteins as a part of acute phase response can be used to detect oxidative stress processes. Both Hp and SAA are two main acute phase proteins in ruminants. Acute phase proteins and their changes have been intensively studied in response to various inflammatory and non-inflammatory conditions in many animal species (Eckersall, 2000; Petersen *et al.,* 2004; Murata, 2007). Acute phase proteins assessment is more sensitive than haematological and clinical tests for diagnosis of diseases. Furthermore, acute phase proteins increase during the progressive stage of disease and decrease in the recovery stage; therefore, it helps to diagnose the disease in the early stages (Nazifi *et al.,* 2008). SAA and Hp as well as other acute phase proteins have been proposed as stress markers in animals (Pineiro *et al.,* 2007). SAA is an apolipoprotein of high-density lipoprotein and is considered one of the major acute phase proteins in vertebrates. Determination and evaluation of SAA showed that this protein could be a valuable factor in the diagnosis of infection (Gruys *et al.,* 1994). Hp is an alpha2-globulin synthesized in the liver and is used as another major acute phase protein in numerous species of productive and companion animals. In ruminants, the level of circulating Hp is negligible in normal animals but it increases over 100 times with immune stimulation (Feldman *et al.,* 2000). Furthermore, Hp is a clinically useful parameter for the evaluation of the occurrence and severity of inflammatory diseases in large animals (Skinner & Roberts, 1994).

The results of the present study showed that Hp was significantly correlated with Fe, Zn, Cu, Se and vitamin C (Table 3; $P<0.05$). Fagoonee *et al.* (2005) revealed that plasma protein Hp modulates renal Fe loading. They suggested that Hp metabolism is well related to Fe concentrations. Liu *et al.* (2014) showed that Zn can regulate the acute phase response through gene signalling. The high concentration of Zn is a controller factor in induction of acute phase response and production of acute phase proteins. Other researchers have been suggested that the high concentrations of Cu can decrease the production of Hp in postpartum dairy cows (Anton *et al.,* 2013). Our findings also revealed the significant and negative relationship between Cu and Hp in synovial fluid of dromedary camels. Mahn *et al.* (2009) showed that the high concentrations of Se in the diet of rats increased their plasma Hp levels. Their findings can explain the positive and significant correlation between Se and Hp in our study. The correlation between Vit C and Hp in the results of Langlois *et al.* (1997) was negative which was due to the effects of vitamin C on preventing oxidative stress processes.

Based on our findings, SAA was significantly correlated with Zn and Cu (Table 3; $P<0.05$). The regulatory effects of Zn on acute phase response and acute phase proteins such as SAA have been revealed by Liu *et al.* (2014). They mentioned that

the high concentration of Zn is a controller factor in induction of acute phase response via a gene signalling process. Wang & Colón (2007) suggested that the high concentrations of Zn protect the structure and stability of SAA by balancing the homeostasis.

Evaluating the inflammatory cytokines such as TNF-α and IFN-γ can be used to assess inflammatory and oxidative processes. TNF-α is a cytokine involved in systemic inflammation and a member of a group of cytokines that stimulate the acute phase response. In the liver, TNF-α stimulates the acute phase response, leading to an increase in acute phase proteins. TNF-α, in particular, has been amply implicated in deleterious host responses (Heinzel, 1990). IFN-γ is a dimerized soluble cytokine that is the only member of the type II class of interferons. IFN-γ is a cytokine that is critical for innate and adaptive immunity against viral and intracellular bacterial infections. Endotoxin activates macrophage microbicidal effector functions and production of proinflammatory cytokines such as IFN-γ (Schroder *et al.,* 2004). The ability of IFN-γ has been described to increase macrophage TNF-α production by both transcriptional and translational mechanisms (Burchett *et al.,* 1988).

The results of the present study showed that TNF-α and IFN-γ were significantly correlated with Fe, Zn and Se (Table 3; $P<0.05$). There was no significant correlation between IFN-γ and Fe in studied dromedary camels. Koorts *et al.* (2011) showed that Fe can modulate the inflammatory responses via the stimulation of cytokines production. Liu *et al.* (2014) revealed that Zn regulates the acute phase response via gene signalling to produce acute phase proteins and cytokines. Broome *et al.* (2004) mentioned that Se deficiencies interfere with immune responses. Based on their results, Se is a promoter agent balancing the immune function and production of inflammatory cytokines such as TNF-α and IFN-γ.

As a trace element, Se is an essential factor of cellular protection against oxidative injuries. Se metabolism in camel is not well known and few references are available. Gluthatione peroxidase, one of the primary antioxidant enzymes, is an important component in the protection against free radical damage to cells and, thus, is crucial to cell survival. Usually, gluthatione peroxidase activity was considered as an indicator of Se status in a variety of species (Ganther *et al.,* 1976). As for other species, gluthatione peroxidase is a good indicator of the Se status of camels (Faye & Seboussi, 2009). A linear relationship between erythrocyte glutathione peroxidase and whole blood Se concentration was already described in camels (Abdel Rahim, 2005).

Cu is another trace element that participates in anti-oxidative function of enzymes such as superoxide dismutase (Liu, 2003). It is known that camels graze more forage-trees than grasses (Rutagwenda *et al.,* 1990), and leaves from those trees are generally richer in Cu than pasture plants, which are the main source of vitamin A (Faye & Tisserand, 1989).

One of the major roles of the water-soluble vitamin C is its antioxidant property. This function is accomplished by inactivating harmful free radicals produced through normal cellular activity and mediated through various stressors. Under stress conditions, the status of vitamin C is greatly reduced (Chew, 1995). The favourable effect of vitamin C appears to occur only in the presence of sufficient quantities of the antioxidant, vitamin E (Pruiett *et al.,* 1989). Stress increases the demand

for vitamin C (Newberne & Conner, 1989).

Vitamin E, known for its antioxidant properties, is usually found in feedstuffs and supplements. However, even in cases where vitamin E is abundant (fresh hay for example), its level decreases rapidly after storage, which leads to deficiencies (Seboussi *et al.,* 2010). Seboussi *et al.* (2008) reported a slight negative effect of Se supplementation on vitamin E concentration in plasma, with a negative correlation between gluthatione peroxidase and vitamin E and found no correlation between Se and vitamin E in camels.

Iron compounds are involved in the lipid peroxidation processes; hence Fe is a prooxidant agent (Ahn & Kim, 1998). However, the catalytic effects of free ionic Fe, bound Fe, and heme pigments on lipid oxidation, and the mechanisms by which the lipid peroxidation is catalysed, are still controversial. Kanner *et al.* (1988) reported that free ionic Fe is the major catalyst of lipid oxidation. Johns *et al.* (1989), however, found that all forms of inorganic Fe have little prooxidant activity. The work of Halliwell & Gutteridge (1990) indicates that all simple Fe complexes are capable of decomposing hydrogen peroxide to form hydroxyl radicals.

The results of the present study showed significant and positive correlation between Fe and vitamin A. Stab *et al.* (1984) mentioned that vitamin A had a direct effect on Fe and high dietary concentrations of vitamin A induced high levels of hepatic Fe. Their data confirmed that vitamin A is involved in the regulation of Fe release from the liver. There were negative and significant relationship between Fe and vitamin C. Cook & Reddy (2001) revealed that dietary Fe had no effect on intestinal absorption of vitamin C in different diets.

In conclusion, the results of the present study showed that there are relationships among oxidative stress biomarkers in synovial fluid of dromedary camels. In synovial fluid, each detected biomarker can affect others. The activity of each parameter can be estimated by measuring another one.

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