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Araştırma Makalesi ***Research Article***

Synovial Fluid Antioxidant Vitamins and Trace Elements in Clinically Healthy and Arthritic Joints of Dromedary Camels

Aliasghar CHALMEH, Khalil BADIEI, Mehrdad POURJAFAR, Saeed NAZIFI*

Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Sorumlu Yazar / Corresponding Author:
Aliasghar CHALMEH
e-mail: achalmeh81@gmail.com

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Abstract

Forty six male dromedary camels (*Camelus dromedarius*), 5 to 10 years of age were entered in this study. Before slaughtering, the animals were visually examined for abnormalities in musculoskeletal system. 33 out of 46 camels did not have any clinical articular abnormalities, whereas 13 ones had gross problems such as lameness and swollen tarsal joints. Based on clinical signs and disease history, these animals were suspected to arthritis. After slaughtering, synovial fluid specimens were taken from tarsal joints of all animals, aseptically and concentrations of zinc, copper, selenium, iron and vitamin A, E and C were assayed. Concentrations of selenium and vitamin C in arthritic joints were significantly lower than clinically healthy camels ($P<0.05$). Zinc concentration of arthritic synovial fluid was significantly higher than normal joints. These data showed that the arthritis could change the synovial fluid vitamins and trace elements in dromedary camels. In conclusion, the results of the current research showed that arthritic joints are in an oxidative stress situation and information regarding the changing patterns of vitamins and trace elements in synovial fluids can be considered as prognostic and diagnostic criteria for articular inflammatory processes.

Introduction

Oxidative stress is a term that is used to describe situations of antioxidant level that produced by the organism. It exceeds the capacity of the organism itself to neutralize them. The result can be damage to cell membranes, lipids, nucleic acids, proteins, and constituents of the extracellular matrix such as proteoglycans and collagens. Oxidative stress by-products such as oxygen free radicals are closely related to a variety of pathological conditions including atherosclerosis, cancer, liver diseases and arthritis. However, the protective mechanisms which inhibit these harmful byproducts are present in animal biological systems. These protective mechanisms include the combined activities of various antioxidants vitamins and trace elements (Hitchon and El-Gabalawy, 2004).

It has been suggested that the prooxidant/ antioxidant imbalance in arthritis may be either due to acceleration of some cellular reactions or insufficiency of the antioxidant defense system (Ozturk et al., 1999). Arthritis, the joint inflammation, refers to a group of diseases that cause pain, swelling, stiffness and loss of motion in the joints (Surapneni and Gopan, 2008). The joints of camels, as in other animals, are susceptible to a variety of infectious and non-infectious disorders that may affect their racing and growing performances. Early diagnosis of articular problems is a principal part of treatment and oxidative stress due to damage the joints by free radicals can influence the response to therapy (Najizadeh et al., 2014). Synovial fluid analysis remains one of the most important diagnostic tools in abnormalities that affect the joint space. 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 fluids can aid in the diagnosis of various joint diseases (Madison et al., 1991). The normal values for synovial fluid analysis in the adult dromedarian camel (Najizadeh et al., 2014; Nazifi et al., 1998), llama and alpaca (Waguespack et al., 2002) have been described. Nonetheless, to the best of the authors' knowledge, there are no reports in the literature regarding concentration of antioxidant vitamins and trace elements in camelids synovial fluid. The purpose of this study was to evaluate the status of synovial fluid antioxidant vitamins (A, C and E) and trace elements

(selenium, copper, zinc and iron) in both clinically healthy and arthritic joints of dromedary camels. Furthermore, the data reported here could be used as reference values for assessing articular abnormalities in this species.

Materials and Methods

The study was carried out in November 2010 on 46 male Dromedary camels (*Camelus dromedarius*), 5 to 10 years of age. The camels were presented for slaughter to the Meibod abattoir, Yazd province, Iran. The slaughterhouse authorities gave permission to use the animals in this study. Before slaughtering, the animals were visually examined for abnormalities in musculoskeletal system. 33 out of 46 camels did not have any clinical articular abnormalities, whereas 13 ones had gross problems such as lameness and swollen tarsal joints. Based on clinical signs and disease history, these animals were suspected to arthritis. An 18 gauge, 1.5 inch needle attached to a 5 milliliters syringe, was used to collect synovial fluid from the healthy and arthritic tarsal joints immediately after the camels were slaughtered. To collect the sample 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. Only blood-free samples were included in the analysis. In cases that blood contamination was suspected based on visual examination, the sample was discarded and a second sampling was attempted at a remote site in the joint. Five milliliters of synovial fluid were collected from each joint and placed in the plain and anticoagulant-coated tubes. Samples of synovial fluids were stored at -20°C until assay. All the samples were digested and analyzed for zinc (Zn), copper (Cu), selenium (Se) and iron (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 Vit A, E and C. The samples were passed through a column filled with a solid adsorbent material. Each component in the sample was 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). Two independent samples t-test was used to compare the synovial fluid parameters between clinically healthy and arthritic tarsal joints. Statistical analyses were performed by SPSS software (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). The level of significance was set at $P<0.05$.

Results

Concentrations (Mean±SEM) of antioxidant vitamins and trace elements in clinically healthy and arthritic joints of Dromedary camels are presented in Table 1. The results of two independent samples t-test showed that concentrations of Se and Vitamin C in arthritic joints were significantly lower than clinically healthy ones ($P<0.05$; Table 1). Zn concentration of arthritic synovial fluid was significantly higher than normal joints.

Discussion

There are several researches on physical, biochemical and cytological properties on normal and arthritic joints of camels (Al-Rukibat et al., 2006; Bani Ismail and Al-Rukibat, 2006; Ellah et al., 2012; Najizadeh et al., 2014; Nazifi et al., 1998), but based on the author's knowledge, there is a lack of data on synovial fluid antioxidant vitamins and trace elements in clinically healthy and arthritic joints of Dromedary camels. Several different pathways can lead to increased formation of reactive oxygen species in inflamed joints (Halliwell et al., 1988). This enhanced oxidation plays a significant role in the tissue damage and inflammation perpetuating process in arthritic joints. Oxygen free radicals lead to lipid peroxidation, which is defined as the oxidative deterioration of unsaturated fatty acids (Tappel, 1973). During health, when reactive oxygen species production is low, lipid peroxidation is inhibited by the combined activities of various antioxidants. The failure of antioxidant defense mechanism to keep pace with oxidant generation may either be due to decrease in antioxidant defense or increased generation of oxidants as is the case in rheumatoid arthritis (Jaswal et al., 2003).

To the best of our knowledge, there is no available document on camelid synovial fluid Se concentration in clinically healthy and arthritic joints. Se concentrations were rarely measured in other body compartments, although such studies would give additional information on the status and distribution of this element. In the study of Yazar et al. (2005) synovial fluid Se concentrations in the rheumatoid arthritis group were significantly lower than those in the healthy subjects ($P<0.05$).

Table 1. Concentrations (Mean±SEM) of antioxidant vitamins and trace elements in clinically healthy (n=33) and arthritic (n=13) joints of dromedary camels.

	Copper (µmol/L)	Zinc (µmol/L)	Iron (µmol/L)	Selenium (ng/mL)	Vitamin A (µg/dL)	Vitamin C (µg/mL)	Vitamin E (µg/mL)
Clinically Healthy Joints	3.24±0.75	9.38±1.52	36.22±3.23	12.32±0.26	6.55±0.50	9.66±0.49	ND
Arthritic Joints	3.91±0.04	13.41±0.20	40.48±0.80	9.51±0.14	6.52±0.08	6.10±0.24	ND
P Value	0.579	0.011*	0.066	0.001*	0.974	0.012*	---

*Stars indicate significant differences between the values of detected parameters in clinically healthy and arthritic joints (P<0.05).
ND: Not detected

In the present study, synovial fluid concentration of Se was significantly lower than normal joints. Yazar et al. (2005) showed that there was highly significant rise in synovial Se during antirheumatic treatment to the level of the control subjects. The fact that low Se status may be a factor in the etiology of arthritis is a plausible hypothesis if one assumes that arthritis is caused by overproduction of peroxides (Kose et al., 1996). The enhancement of adjuvant arthritis observed in rats fed with a Se-deficient diet supported a role for this essential element in rheumatoid arthritis (Parnharm et al., 1983). Therefore, the antiproliferative, anti-inflammatory, and immune modulating effects of Se are of interest (O'dell et al., 1991). Furthermore, several investigators have found depressed plasma or serum Se values in patients with arthritis (Honkanen, 1991; Honkanen et al., 1991; O'dell et al., 1991). Thus, Se deficiency might enhance the development or progression of this problem (O'dell et al., 1991).

The firmly established metabolic role of Se in humans is an essential constituent of the enzyme glutathione peroxidase (Lockitch, 1989), which protects cells from oxidative damage by destroying peroxides (Maddipati and Marnet, 1987). At the active center of this selenoenzyme, Se serves to catalyze the reduction of hydroperoxides produced from oxidized species such as superoxide and lipoperoxides. Therefore, a defective regulation of this Se-containing glutathione peroxidase together with Se could account for some pathological features of the arthritis (Kose et al., 1996).

In the present research, we also detected synovial fluid vitamin C as an antioxidant parameter. The concentration of this vitamin in arthritic joints was significantly lower than clinically normal joints. Jaswal et al. (2003) showed that vitamin C concentrations in patients of rheumatoid arthritis are significantly lower than normal controls. Vitamin C plays a pivotal role in protecting lipids from peroxidative damage initiated either by acquousperoxyl radicals or by activated polymorphonuclear cells (Feri et al., 1988). It has been reported that ascorbate is the first antioxidant to become oxidized immediately upon leukocyte stimulation (Feri et al., 1988). This explains the low concentrations of vitamin C in arthritic synovial fluids. The estimation of vitamin C may also be of diagnostic use as an early indicator of oxidative stress. Blake et al. (1981) estimated total ascorbate concentration in plasma and synovial fluid of arthritis affected patients and reported that they were at the lower end of the normal range. In separate studies conducted by Feri et al. (1988) and Merry et al. (1989), concentrations of vitamin C in patients of rheumatoid arthritis were found to be significantly lower than the controls.

Zn as a trace element is in the structure of superoxide dismutase which scavenges the free radicals and superoxide anions, thus acting as an antioxidant enzyme (Michiels et al., 1994). Based on our findings, Zn in arthiric joints was significantly higher than normal ones. The increased levels of Zn in patients affected with arthritis support the hypothesis of radical-mediated injury in this inflammatory disease. This rise could result from an elevated superoxide dismutase concentration in arthritic synovial fluid, as found by Igari et al. (1982). The first antioxidant response is the enhanced release of superoxide dismutase mainly due to the Zn form of the enzyme. The increased activity of Zn could protect against the exaggerated release of superoxide only if supported by overexpression of other radical scavenging systems (Mazzetti et al., 1996).

These data showed that the arthritis could change the synovial fluid vitamins and trace elements in Dromedary camels. In conclusion, the results of the current research showed that arthritic joints are in an oxidative stress situation and information regarding the changing patterns of vitamins and trace elements in synovial fluids can be considered as prognostic and diagnostic criteria for articular inflammatory processes.

REFERENCES

Al-Rukibat, R.K., Bani Ismail, Z.A., Al-Zghoul, M.B., 2006. Cytologic analysis of synovial fluid in clinically normal tarsal joints of young camels (*Camelus dromedarius*). Veterinary Clinical Pathology 35 (3), 326-328.

Bani Ismail, Z., Al-Rukibat, R., 2006. Synovial fluid cell counts and total protein concentration in clinically normal fetlock joints of young dromedarian camels. Journal of Veterinary Medicine A. Physiology, Pathology, Clinical Medicine 53, 263-265.

Blake, D.R., Hall, N.D., Treby, D.A., Halliwell, B., Gutteridge, J.M., 1981. Protection against superoxide and hydrogen peroxide in synovial fluid from rheumatoid patients. Clinical Science 61 (4), 483-486.

Ellah, M.R.A., Ali, M.M., Semieka, M.A., 2012. Comparative studies on biochemical and cytological constituents of synovial fluids in some farm animals. Comparative Clinical Pathology 21 (5), 901-904.

Feri, B., Stocker, R., Ames, B.N., 1988. Antioxidant defenses and lipid peroxidation in human blood plasma. Proceedings of the National Academy of Sciences USA 85 (24), 9748-9752.

Halliwell, B., Hoult, J.R.S., Blake, D.R., 1988. Oxidants, inflammation and anti-inflammatory drugs. The Journal of the Federation of American Societies for Experimental Biology 2 (13), 2867-2873.

Hitchon, C.A., El-Gabalawy, H.S., 2004. Oxidation in rheumatoid arthritis. Arthritis Research and Therapy 6 (6), 265-278.

Honkanen, V., Konttiren, Y.T., Sorsa, T., Hukkanen, M., Kemppinen, P., Santavirta, S., Saari, H., Westermarok, T., 1991. Serum zinc, copper and selenium in rheumatoid arthritis. Journal of Trace Elements and Electrolytes in Health and Disease 5 (4), 261-263.

Honkanen, V.E., 1991. The factors effecting plasma glutathione peroxidase and selenium in rheumatoid arthritis: a multiple linear regression analyses. Scandinavian Journal of Rheumatology 20 (6), 385-391.

Igari, T., Kaneda, H., Houriuchi, S., Ono, S., 1982. A remarkable increase of superoxide dismutase activity in synovial fluid of patient with rheumatoid arthritis. Clinical Orthopaedics and Related Research 162, 282-287.

Jaswal, S., Mehta, H.C., Sood, A.K., Kaur, J., 2003. Antioxidant status in rheumatoid arthritis and role of antioxidant therapy. Clinica Chimica Acta 338 (1-2), 123-129.

Kose, K., Dogan, P., Kardas, Y., Saraymen, P., 1996. Plasma selenium levels in rheumatoid arthritis. Biological Trace Element Research 53 (1-3), 51-56.

Lockitch, G., 1989. Selenium: clinical significance and analytical concepts. Critical Reviews in Clinical Laboratory Sciences 27 (6), 483-541.

Maddipati, K.R., Marnet, L.J., 1987. Characterization of the major hydroperoxide reducing activity of human plasma. Journal of Biological Chemistry 262 (36), 17398-17403.

Madison, J.R., Sommer, M., Spencer, P.A., 1991. Relations among synovial membrane histopathologic findings, synovial fluid cytologic findings, and bacterial culture results in horses with suspected infectious arthritis: 64 cases (1979-1987). Journal of the American Veterinary Medical Association 198 (9), 1655-1661.

Mazzetti, I., Grigolo, B., Borzì, R.M., Meliconi, R., Facchini, A., 1996. Serum copper/zinc superoxide dismutase levels in patients with rheumatoid arthritis. International Journal of Clinical and Laboratory Research 26 (4), 245-249.

Merry, P., Winyard, P.G., Morris, C.J., Grootveld, M., Blake, D.R., 1989. Oxygen free radicals, inflammation and synovitis: the current status. Annals of the Rheumatic Diseases 48 (10), 864-870.

Michiels, C., Raes, M., Toussaint, O., Remacle, J., 1994. Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free Radical Biology and Medicine 17 (3), 235-248.

Najizadeh, M.H., Pourjafar, M., Chalmeh, A., Badiei, K., Nazifi, S., Naghib, M., 2014. Synovial fluid inflammatory cytokines and proteins in clinically healthy and arthritic joint of dromedary camels. Iranian Journal of Veterinary Medicine 8 (1), 51-55.

Nazifi, S., Rezakhani, A., Gheisari, H.R., 1998. Physical, biochemical and cytologic properties of blood and synovial fluid in clinically normal adult camel (*Camelus dromedarius*). Journal of Veterinary Medicine Series A 45 (3), 155-160.

O'dell, J.R., Lemley-Gillespie, S., Palmer, W.R., Weaver, A.L., Moore, G.F., Klassen, L.W., 1991. Serum selenium concentrations in rheumatoid arthritis. Annals of the Rheumatic Diseases 50 (6), 376-378.

Ozturk, H.S., Cimen, M.Y.B., Cimen, O.B., Kacmaz, M., Drek, J., 1999. Oxidant/antioxidant status of plasma samples from patients with rheumatoid arthritis. Rheumatology International 19 (1-2), 35-37.

Parnharm, M.J., Winkelman, J., Leyck, S., 1983. Macrophage, lymphocyte and chronic inflammation responses in selenium deficient rodents. International Journal of Immunopharmacology 5 (5), 455-461.

Snyder, L.R., Kirkland, J.J., Glajch, J.L., 1997. Practical HPLC 1Method Development, Second Edition. Wiley-Interscience Company, pp. 237-350.

Surapneni, K.M., Gopan,V.Sc., 2008. Lipid peroxidation and antioxidant status in patients with rheumatoid arthritis. Indian Journal of Clinical Biochemistry 23 (1), 41-44.

Tappel, A.L., 1973. Lipid peroxidation damage to cell components. Federation Proceedings 32 (8), 1870-1874.

Waguespack, R.W., Belknap, E.B., Spano, J.S., Wenzel, J.G., Pugh, D., 2002. Analysis of synovial fluid from clinically normal alpaca and llamas. American Journal of Veterinary Research 63 (4), 576-578.

Yazar, M., Sarban, S., Kocyigit, A., Isikan, U.E., 2005. Synovial fluid and plasma selenium, copper, zinc, and iron concentrations in patients with rheumatoid arthritis and osteoarthritis. Biological Trace Element Research 106 (2), 123-132.