Synovial fluid inflammatory cytokines and proteins in clinically healthy and arthritic joint of dromedary camels (*Camelus dromedarius*)

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Abstract:

BACKGROUND: Background: Analyzing the synovial fluid is one of the common methods to diagnose the articular problems to detect the stage and express the prognosis. Such gross, cytological, and biochemical analysis of synovial fluids can aid in the diagnosis of various joint diseases. **OBJECTIVES:** Normal values for synovial fluid in the camels have been described previously; however, there are no reports regarding concentration of acute phase proteins and inflammatory cytokines in camelids synovial fluid. Hence, the present study tried to compare the synovial fluid inflammatory cytokines and acute phase proteins in clinically healthy and arthritic tarsal joints of dromedary camels. **METHODS:** 46 male dromedary camels, 5 to 10 years old, were used in this study. 33 camels did not have any clinical articular abnormalities while 13 camels had gross articular problems such as lameness and swollen tarsal joints. Collecting the synovial fluids was extracted from the healthy and arthritic tarsal joints immediately after slaughter. Then, the concentration of haptoglobin, serum amyloid A, tumor necrosis factor-alpha, and interferon-gamma were measured in samples. **RESULTS:** Concentration of all measured parameters in arthritic joints were significantly higher than clinically healthy joints ($p<0.05$). The synovial fluid concentration of serum amyloid A, haptoglobin, tumor necrosis factor-alpha, and interferon-gamma were 5.379, 4.285, 25.503, and 1.904 times higher in arthritic joints than normal joints, respectively. **CONCLUSIONS:** The articular inflammatory processes can increase the synovial fluid concentration of acute phase proteins and inflammatory cytokines. Information about the normal values of these parameters and their changing patterns may help camel rearing systems during arthrititis by assessing the health status of joints in the camels; in addition, the information about normal values can be diagnostically valuable when considering diseased animals.

Introduction

The joints of camels, as in other animals, are susceptible to a variety of infectious and noninfectious disorders that may affect their racing performance. In addition, camels serve as an important food source in many parts of the world. Early diagnosis of articular problems is a principal part of treatment. 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). Synovial fluid is a plasma dialysate modified by constituents secreted by the joint tissues; hence, alterations in synovial fluid are indications of articular problems. Such gross and cytological analysis of synovial fluids 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). The normal values for synovial fluid analysis in the adult dromedarian camel (Nazifi et al., 1998) and 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 acute phase proteins and inflammatory cytokines in camelids synovial fluid.

The purpose of the present study was to determine and compare the concentrations of acute phase proteins (serum amyloid A and haptoglubin) and inflammatory cytokines (tumor necrosis factor-alpha and interferon-gamma) in synovial fluid from the clinically healthy and arthritic tarsal joint of adult male 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. 46 male dromedary camels (Camelus dromedarius), 5 to 10 years of age, were used in this study. 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. From all animals, 33 camels did not have any clinical articular abnormalities whereas 13 camels 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.

Haptoglobin (Hp) was measured according to prevention of peroxidase activity of hemoglobin, which is directly proportional to the amount of Hp (Tridelta Development Plc, Wicklow, Ireland). Serum amyloid A (SAA) was measured by a solid phase sandwich ELISA (Tridelta Development Plc, Wicklow, Ireland). Tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) were measured by a solid phase sandwich ELISA (AbC 606 and AbC 607, respectively; Votre fournisseur AbCys S.A. Paris, France).

Data were expressed as mean ± standard deviation (SD). 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

The values (mean±SD) of synovial fluid concentration of SAA, Hp, TNF-α and IFN-γ in clinically healthy and arthritic tarsal joints of male dromedary camels are presented in Table 1. Concentration of these parameters in arthritic joints was significantly higher than clinically healthy joints ($p<0.05$). Based on our findings, the synovial fluid concentration of SAA, Hp, TNF-α and IFN-γ in arthritic joints were 5.379, 4.285, 25.503, and 1.904 times higher than normal joints, respectively.

Discussion

Currently, reference values for synovial fluid concentration of inflammatory cytokines and acute phase proteins from camels are interpreted based on values from ruminants (Bani Ismail and Al-Rukibat,

Table 1. Synovial fluid concentrations (mean±SD) of acute phase proteins and inflammatory cytokines in clinically healthy and arthritic tarsal joints of male dromedary camels.

	Clinically healthy tarsal joints (n=33)	Arthritic tarsal joints (n=13)
Serum amyloid A (µg/mL)	0.124±0.015	0.667±0.016
Haptoglubin (g/L)	0.007±0.001	0.030±0.008
Tumor necrosis factor-alpha (pg/dL)	7.474±0.737	19.061±4.318
Interferon-gamma (pg/dL)	1.351±0.191	2.573±0.375

2006). There are literatures on physical, biochemical, and cytologic properties of blood and synovial fluid in clinically normal adult camels (Nazifi et al., 1998; Bani Ismail and Al-Rukibat, 2006; Al-Rukibat et al., 2006); however, there are few or no information about synovial fluid inflammatory cytokines, acute phase proteins, and their changes in arthritic joints in dromedary camels. Bani Ismail et al. (2007) studied the synovial fluid analysis and bacterial population in clinically arthritic joints of juvenile male camel calves; however, synovial fluid concentration of inflammatory cytokines and acute phase proteins were not determined in their study. In the present study, we described, for the first time, the reference values of synovial fluid concentration of inflammatory cytokines and acute phase proteins from the tarsal joints of clinically healthy adult male dromedary camels and their comparison with the arthritic joints.

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 hematological 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 as 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 increases over 100-fold 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 and Roberts, 1994). To the best of the authors' knowledge, there are no reports on synovial fluid concentration of SAA and Hp in clinically healthy and arthritic joints of dromedary camels. Based on the findings of the present study, synovial fluid concentration of acute phase proteins in arthritic tarsal joints were significantly ($p<0.05$), and several times, higher than clinically healthy similar joints.

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). In the present study, for the first time, we compared the synovial fluid concentration of TNF-α and IFN-γ in clinically healthy dromedary camels with these cytokines in arthritic synovial fluid. The results of the present study showed that the synovial fluid concentration of inflammatory cytokines in clinically healthy tarsal joints was significantly ($p<0.05$), and several times, lower than similar arthritic joints.

Several studies on measuring acute phase response proteins and cytokines have mentioned that increasing the amount of these factors takes place immediately after commencement of inflammatory processes (Nazifi et al., 2008; Chalmeh et al., 2013a-c). Hence, it could be stated that evaluating acute

phase response biomarkers can be used for early diagnosis of camel inflammatory joint diseases.

In conclusion, the data provided here are the first reference values of synovial fluid concentration of acute phase proteins and inflammatory cytokines in clinically healthy tarsal joints of dromedary camels. These data showed that the articular inflammatory processes could increase the synovial fluid acute phase response biomarkers such as acute phase proteins and inflammatory cytokines. Information about the normal values of these parameters and their changing patterns may help camel rearing systems during arthritis by assessing the health status of joints in the camels; in addition, the information about normal values can be diagnostically valuable when considering diseased animals.

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سیتوکین‌ها و پروتئین‌های التهابی مایع مفصلی سالم و ملتهب شترهای یک‌کوهانه

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چکیده

زمینهٔ مطالعه: ارزیابی مایع مفصلی، یکی از شیوه‌های رایج در تشخیص اختلالات مفصلی در جهت پیش‌آگاهی و تعیین میزان آسیب به مفاصل است. برخی آزمون‌های ظاهری، سلول‌شناسی و بیوشیمیایی مایع مفصلی در تشخیص شماری از بیماریهای مفصلی کمک‌رسان است. **هدف:** برخی مقادیر طبیعی مایع مفصلی شتر قبلاً گزارش شده‌اند؛ اما گزارشاتی مبتنی بر غلظت پروتئین‌های فاز حاد و سیتوکین‌های التهابی در مایع مفصلی شترسانان به چشم نمی‌خورد. از این‌رو، مطالعهٔ حاضر، به مقایسهٔ این فراسنجه‌ها بین مایع مفصلی مفاصل تارس به ظاهر سالم و ملتهب شترهای یک‌کوهانه پرداخته است. **روش کار:** در این مطالعه از ۴۶ نفر شتر یک‌کوهانه در سنین ۵ تا ۱۰ ساله استفاده شد. ۳۳ نفر از این تعداد از دید بالینی هیچ‌گونه اختلال مفصلی نداشتند و ۱۳ شتر مشکلات ظاهری مفصلی نظیر لنگش و تورم مفاصل تارس را بروز می‌دادند. بلافاصله پس از کشتار، مایعات مفصلی از مفاصل تارس سالم و ملتهب اخذ و غلظت هاپتوگلوبین، سرم‌آمیلوئیدآ، فاکتور نکروزکنندهٔ توموری آلفا و اینترفرون گاما در نمونه‌ها اندازه‌گیری شد. **نتایج:** غلظت تمام فراسنجه‌ها در مفاصل ملتهب به طور معنی‌داری بیش از مفاصل به ظاهر سالم بود ($p<0/05$). غلظت سرم‌آمیلوئیدآ، هاپتوگلوبین، فاکتور نکروزکنندهٔ توموری آلفا و اینترفرون گاما در مایع مفصلی مفاصل ملتهب به ترتیب ۵/۳۷۹، ۴/۲۸۵، ۲۵/۵۰۳ و ۱/۹۰۴ برابر بیشتر از مفاصل به ظاهر سالم بود. **نتیجه‌گیری نهایی:** روندهای التهابی مفصلی غلظت‌های پروتئین‌های فاز حاد و سیتوکین‌های التهابی را در مایعات مفصلی افزایش می‌دهد. آگاهی از مقادیر طبیعی این فراسنجه‌ها و الگوی تغییرات آنها در خلال التهاب مفاصل، ممکن است در ارزیابی وضعیت سلامت مفاصل شترها به سیستم‌های پرورش دهندهٔ شتر کمک کند و در مواجهه با حیوان بیمار از ارزش تشخیصی برخوردار باشد.

واژه‌های کلیدی: پروتئین‌های فاز حاد، التهاب مفاصل، شتر یک‌کوهانه، سیتوکین‌های التهابی، مایع مفصلی

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