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Measurement of serum amyloid A and C-reactive protein in serum and synovial fluids of dogs, following experimental osteoarthritis

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Abstract

Osteoarthritis is a progressive and relatively irreversible condition of articular cartilage which affects the whole joint region at the end. At the beginning, when the injury is limited to the cartilage, it has no or very minimal signs. Once detected, considerable parts of the cartilage have been lost which will not be substituted. Early diagnosis of any cartilage damage is a topic of great interest. Acute phase proteins (APPs) concentrations increase at the beginning of any inflammatory condition or tissue injuries. The present study was conducted to measure the effects of experimental osteoarthritis on Serum Amyloid A (SAA) and C-Reactive Protein (CRP) as two of the most important APPs. For this study four mature, mixed breed dogs weighing about 18 kilograms were selected and their left knees' cranial cruciate ligaments were transected through a 4 mm parapatellar incision. Radiography and measurement of SAA and CRP in both synovial fluid and serum samples were performed before surgery and on days 14, 28, 90 and 180. Statistical analysis of the results showed that there was significant increase in both SAA and CRP after surgery. The data from both synovial and serum samples were more significant when compared with radiography over time, suggesting that measurement of these two parameters in either synovial fluid or serum is diagnostic and better in early detection of OA.

Keywords: Osteoarthritis; knee; dog; amyloid; C-reactive protein

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Introduction

Osteoarthritis (OA), also known as degenerative joint disease (DJD), is defined as the progressive and permanent long-term deterioration of the cartilage surrounding the joints (Martinez, 1997). Arthritis is the medical term for the inflammation of the joints, while osteoarthritis is a term referring to a form of chronic joint inflammation caused by deterioration of joint cartilage (Zachary and McGavin, 2013). There is some controversy as to whether this condition is a true inflammation or not. Synovitis is usually mild and caused by the release of inflammatory mediators by injured chondrocytes (Zachary and McGavin, 2013). Cranial Cruciate Ligament (CrCL) injuries are a major cause of osteoarthritis in dogs (Cook, 2010). Dogs with partial cruciate ligament injuries are challenging to detect in early stages of the injury. In the beginning, they have a mild weight-bearing lameness associated with exercise, which will usually be resolved when the

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dogs have rested. When the degenerative changes get worse and the CrCL continues to tear, the lameness is more evident and the stifle joint becomes more unstable, resting the dog does not resolve the lameness (Tobias and Johnston, 2013).

The acute phase response (APR) is a significant, non-specific, systemic reaction to immunological stress of the body (Ceron et al., 2005; Cray et al., 2009). Pro-inflammatory signals include cytokines IL-1, IL-6 and TNF-α, which will activate the vascular system together with inflammatory cells. Subsequently more cytokines and other inflammatory mediators are produced, which will diffuse to the extra-cellular fluid and to the blood circulation (Cray et al., 2009). Effects of OA on some of these cytokines which have been investigated in previous studies, have resulted in contradictory findings (Kammermann et al., 1996; Stannus et al., 2010; Nikahval et al., 2013)

C- reactive protein (CRP) and serum amyloid A component (SAA) are two main acute phase proteins (APPs) in dogs (Kostro et al., 2002). Indeed, the determination of APP concentrations in humans and animals has proved extremely useful in postoperative monitoring (Conner et al., 1988; Yamamoto et al., 1993; Murata et al., 2004). To the best of our knowledge, there is no reliable source of information in early prediction and diagnosis of osteoarthritis by measurement of APPs. Therefore, the present study was performed to further elucidate the effects of OA on SAA and CRP in both serum and synovial fluid and whether these two markers can be used in early detection of OA.

Materials and Methods

Animals and study design

The present study was carried out using four mature (20±3 months), weighing 18±2 kg, mixed breed female dogs. Thorough clinical, paraclinical and radiographical examinations were performed to exclude any preexisting systemic or musculoskeletal abnormalities. Surgeries and sample collections were carried out under aseptic conditions. The protocol of anesthesia, surgical procedures, postoperative care and sacrifice were identical for all animals. During the experiments, the animals were housed one per cage and were allowed to move freely 2 h a day in a wide fenced area but were not forced to exercise. Animal selection, all experiments, subsequent care, and the sacrificial procedures all adhered to the same guidelines under supervision of Research Council of Shiraz School of Veterinary Medicine.

Animal ethics

This experiment was accomplished under the approval of the State Committee on Animal Ethics, Shiraz University, Shiraz, Iran. The recommendations of European Council Directive (86/609/EC) of November 24, 1986 regarding the standards in the protection of animals used for experimental purposes were also followed.

Surgical procedure

The animals were sedated with Acepromazine (0.1 mg/kg IM) and the whole stifle region was aseptically prepared for surgery. Anesthesia was induced by combination of Ketamine (5mg/kg IV) and Diazepam (0.2 mg/kg IV). The animals were placed on dorsal recumbency, and the Cranial Cruciate Ligament (CrCL) of the left knees was transected through a 4 mm parapatellar stab incision as was previously described for dogs (Pond and Nuki, 1973). Then the incision was sutured in layers. The animals were given appropriate analgesics for three post-operative days.

Samplings

Synovial fluid was aspirated from the stifle joints once before surgery—as baseline—and on days 14, 28, 90, and 180. Medial parapatellar approach was used for synovial fluid aspiration. All synovial samples were centrifuged at 4°C, 4,000g, for 10 min to separate cells and debris. Supernatants were stored at −70°C until assay based on previously described protocols (Fujita et al., 2006). Blood sampling was also done once before surgery and on days 14, 28, 90, and 180 postoperatively from cephalic vein. Radiographic evaluations were also performed on the same days in two standard views for stifle joints. Radiographs were graded based on previously described methods (Kellgren and Lawrence, 1957).

Acute phase proteins (CRP and SAA) determination

C-reactive protein (CRP) was measured using a commercial kit based on enzyme linked immunoassay (ELISA). The analytical sensitivity of this test in serum has been determined as 0.078 µg/ml for CRP by the manufacturer (Cusabio Biotech Company, Wuhan, China). Serum amyloid A (SAA) was measured by a solid phase sandwich-ELISA method with a sensitivity of 0.156 pg/ml (Dog SAA Elisa Kit, Cusabio Biotech Company, Wuhan, China).

Statistical analysis

Significant differences for each measured parameter between different times were evaluated using Repeated Measures Analysis of Variance (ANOVA) and Tukey multiple comparisons as post-hoc test. All values were expressed as mean and standard error (SE), and $P<0.05$ was considered as statistically significant. All data were analyzed using computer software GraphPad Prism for windows version 5.01, 2007 (GraphPad Software, Inc.).

Results

The results of SAA and CRP are depicted in Figures 1-4. Both CRP and SAA showed significant difference between base line (before surgery) and all post operative time points in both synovial fluid and serum samples ($P<0.05$). Fig. 1 shows that SAA measured in serum increased from two weeks and remained high until the end of study. The same pattern was observed for other parameters.

Discussion

For the last three decades, many studies have been conducted in order to discover the ways of early diagnosis of OA and monitoring the progress of the disease. The detection of cartilage defects in the early stages of OA is aimed for diagnostic, prognostic and therapeutic perspectives. Currently, advanced diagnostic methods, such as arthroscopy and magnetic resonance imaging are costly and can only be applied to animals in general anesthetic state and display the results only when a large area of the articular cartilage is injured or the cartilage fibrillation occurred (Tobias and Johnston, 2013).

Acute phase proteins are circulating proteins in blood, which have a significant part in acute phase response. Serum amyloid A is one of the major APPs in dogs and it is a valuable diagnostic marker for systemic inflammation (Ceron et al., 2005; Christensen et al., 2012). Although the major source for SAA production is hepatocytes, it has been shown that it is produced in other organs such as joints (Jacobsen et al., 2005). In veterinary medicine, SAA has been noted as a valuable biomarker, especially for prognostic applications, but more scientific research was proposed to be still required to specify the practical usability of SAA and other APPs (Ceron et al., 2005). It has been indicated that SAA from serum or synovial fluid was not detected from healthy dogs, but it was detected from the serum of dogs with inflammatory disease and from synovial fluid from dogs with inflamed joints (Kjelgaard-Hansen et al., 2007). In the present study, SAA in both serum and synovial fluid followed the same increasing pattern over the course of study, indicating that it could be used as a diagnostic value, which reflects the inflammation within the joints. CRP showed the same pattern as SAA in both synovial fluid and serum. CRP is a protein produced by the liver. Its concentration is increased in the case of inflammation or the healthy tissues injury. The main benefit obtained from the measurement of CRP concentration is to assess the presence of inflammatory activity. Also, it is useful to verify the effectiveness of the treatment or experimental procedures-by CRP concentration measurement before treatment, during it and afterward (Noreikaite-Bulotiene and Bizokas, 2014).

The results of the present study show that SAA and CRP in both synovial fluid and serums can reflect OA in joints. The values were even higher in serum than synovial fluid. Surprisingly, baseline levels were higher in serum than in SF for both CRP and SAA, indicating that non joint sources are added to blood stream which is not present in SF. However, its less invasiveness and being easy to obtain make it more practical.

Comparing radiographic signs with other measured criteria shows that prediction of the presence of OA based on the detection of APPs is not only possible but it is even more reliable and diagnostic. Early diagnosis is the most important feature of biochemical detection of OA. Based on the data seen in radiography, there were no observable signs until about one month post-surgical mild changes in just one case on day 28, which can easily be neglected particularly by inexperienced practitioners. The most definitive and diagnostic features were observed from the third month onwards which is relatively late to initiate treatment (Nikahval et al., 2012).

In detection of OA in femoropatellar joint which is a compartment of knee joint OA in human, skyline and lateromedial radiographs are often essential. However, there are some controversies in this respect (Chaisson et al., 2000). The optimal detection of abnormalities could be achieved by obtaining three (or even more) images of each knee, but this is not always practicable. The study conducted by Chaisson et al. (2000), stated the difficulty to obtain all required radiographs with high precision. The conditions in animals are even worse. For example, weight bearing radiographs which are essential to diagnose some features of this disorder like joint space narrowing is neither possible in most of the patients nor can it be done in anesthetized dogs. Based on these two markers increase seen in this research, it can be proposed that measurement of these two markers can be diagnostic in early detection of OA. However, the present study's shortcomings should not be neglected. It is recommended that similar study be performed on more patients and with naturally occurring OA or natural CrCL rupture or on only arthrotomized patients. Also, other forms of OA and other joints involvement can be assessed.

In conclusion, it is recommended that in future CRP and SAA in either synovial fluid or serum be used as diagnostic biomarkers in osteoarthritis. These markers are able to reflect the OA in the stifle joints more quickly than traditional radiographic examination of the joint. Radiographic signs of OA are merely detectable when there are observable bony changes to joints which are relatively late in the disease process. However, any pre- or co-existing inflammatory condition should be ruled out first.

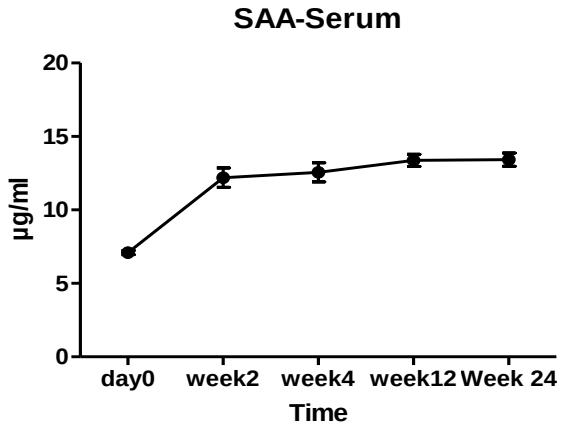

Fig. 1: Serum Amyloid A measured in days after surgery showed significant increase as compared with that of before surgery as a baseline.

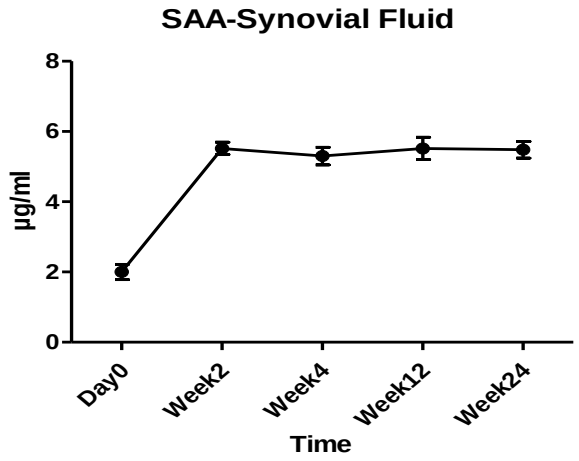

Fig. 2: Serum Amyloid A showed a significant increase after cranial cruciate ligament rupture and continued to increase over the course of study when compared with before surgery.

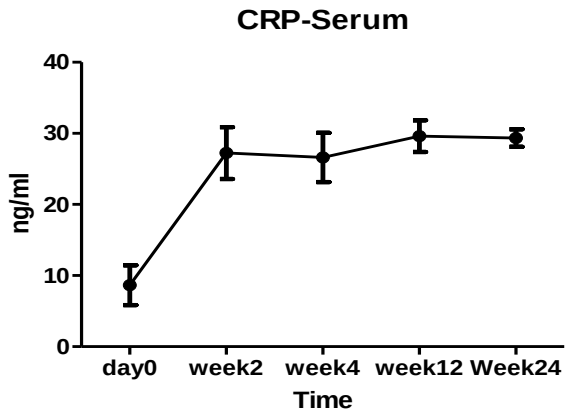

Fig. 3: C-Reactive Protein measured in serum showed significant increase after surgery as compared to before it and remained high throughout the study.

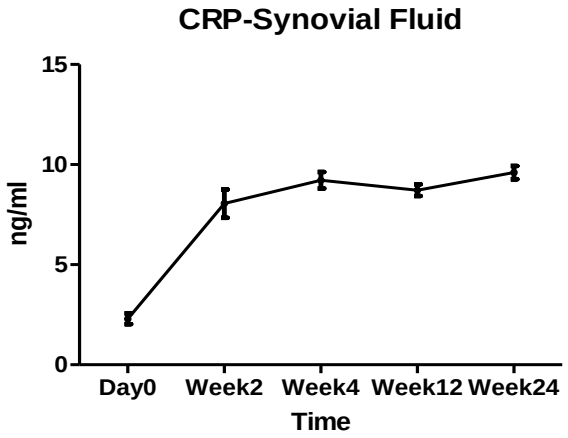

Fig. 4: CRP measured in synovial fluid showed a significant increase over the course of study as compared with before surgery.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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