SHIRAZ UNIVERSITY

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EVALUATION OF THE RELATIONSHIP BETWEEN RADIOGRAPHIC, MRI, AND HISTOPATHOLOGIC FINDINGS IN RABBITS WITH EXPERIMENTALLY RUPTURED CRANIAL CRUCIATE LIGAMENT

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September 2009

IN THE NAME OF GOD

Declaration Form

I, Behrooz Nikahval, a veterinary surgery student majored in veterinary surgery from the Faculty of Veterinary Medicine of Shiraz University, declare that this thesis is the result of my research and I had written the exact references and full indication wherever I used others' sources. I also declare that the research and the topic of my thesis are not reduplicative and guarantee that I will not disseminate its accomplishments and not make them accessible to others without the permission of the University. According to the regulations of the mental and spiritual ownership, all rights of this belong to Shiraz University.

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BEHROOZ NIKAHVAL

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Abstract

EVALUATION OF THE RELATIONSHIP BETWEEN RADIOGRAPHIC, MRI, AND HISTOPATHOLOGIC FINDINGS IN RABBITS WITH EXPERIMENTALLY RUPTURED CRANIAL CRUCIATE LIGAMENT

By

Behrooz Nikahval

Osteoarthritis (OA) is a condition that represents a degenerative pathological imbalance involving the whole joint and its component parts. Diagnosis of OA has been a topic of concern for decades, because of the silent nature of disease progression over time. The present study was conducted to induce experimental OA in rabbit knee joint, follow the trend of changes with radiography, MRI and histopathology in the same situations and to evaluate each diagnostic technique in relation to the other and to histopathology as the gold standard method of diagnosis. Twenty adult, Dutch, male rabbits, were randomly divided into two equal groups (groups for short-term and long-term studies, in each group n=10). In half of the animals in each group (n=5) OA was induced by cranial cruciate ligament sectioning (group T: transection) and in the other half only arthrotomy was performed as Sham operation (group A: arthrotomy). Radiography and MRI was performed on days 0, 30 (short-term evaluation group), and on days 0, 90 and 180 on the other group. Gross and histopathological examinations were performed on day 30 on short-term group after the animals were sacrificed and on the other group on day 180. Statistical analysis of the results revealed that the highest slope of changes in different methods was belong to histopathology as well as the highest grade in each studied time, and in the second order was MRI and radiology was in the third place. The slope of changes was 0.01 for histopathology, 0.009 for MRI and was 0.004 for radiology. The ratios of slopes of each technique to others were as follows: His./MRI=1.1, His./Rad.=2.5, MRI/Rad.=2.2. Comparing MRI with radiology revealed that radiology would not show signs of OA when MRI grade is below 0.27 of grade. Comparing both non-invasive techniques with histopathology shows that when histopathological grade for overall OA is below 0.22 of grade, radiology will not show signs of OA involvement while MRI is capable of showing signs of OA involvement when it is more than 0.018 of histopathological grade. We concluded that histopathology is the most sensitive method in detecting OA. MRI is much more sensitive in detecting the early changes in osteoarthritic joints than radiography and show OA progression with higher slope of changes over time and is very close to what happen in joint tissues (histopathology).

TABLE OF CONTENTS

Title **Page**

Title **Page**

LIST OF TABLES

Title **Page**

Title **Page**

Title **Page**

LIST OF FIGURES

Title **Page**

Title **Page**

Title **Page**

1 Introduction

Osteoarthritis (OA) is a condition that represents a pathological imbalance of degradative and reparative processes involving the whole joint and its component parts, with secondary inflammatory changes. OA is characterized by progressive deterioration and localized erosion of articular cartilage, accompanied by remodeling of bone at joint margins. Diagnosis of OA has been a topic of concern for decades, because of the silent nature of its progression over time. There are only signs of involvement in the advanced stages of progression, in which curative procedures usually do not ends in good results but in early stages – if the condition is diagnosed– the results of treatment would be better. The knee joint, is one of the most common affected joints in both human and veterinary medicine. Conventional radiography is the method most frequently used for monitoring the progression of osteoarthritis, however, it may not show osteoarthritic changes of the knee until late in the disease, and mostly may show involvement of only one or two compartments. The two views should be obtained in orthogonal planes to one another (i.e., anteroposterior [AP] and lateral). The radiographic hallmarks of primary osteoarthritis include nonuniform joint space loss, osteophyte formation, cyst formation and subchondral sclerosis. The initial radiographs may not show all of the findings. At first, only minimal, nonuniform joint space narrowing may be present. The involved joint spaces have an asymmetric distribution. As the disease progresses, subluxations may occur and osteophytes may form. Subchondral cystic changes can occur. These cysts may or may not communicate with the joint space, can occur before cartilage loss and have a sclerotic border. Subchondral sclerosis or subchondral bone formation occurs as cartilage loss increases and appears as an area of increased density on the radiograph. In the advanced stage of the disease, a collapse of the joint may occur; however, ankylosis does not usually occur (Gupta et al. 2004).

Magnetic Resonance imaging (MRI) that has recently been introduced into medicine, has direct multiplanar imaging capability and provides a higher soft-tissue contrast than radiography. The pursuit of an accurate non-invasive diagnostic test for internal derangement of the knee has been aided by recent advances in MRI. However, although the advantages of MRI are appealing, its objective accuracy for this purpose is unknown. The results of preliminary investigations have suggested that the structural anatomy of the knee can be delineated by MRI (Soudry et al. 1986). In a retrospective study in which the interpretation of magnetic resonance images was influenced by the clinical history and arthrographic results, Reicher et al. (1986) reported an accuracy of 80 percent in identifying meniscal tears (Reicher et al. 1986). However, they did not offer a precise analysis of injuries of the cruciate ligaments or other intra-articular lesions. Savory et al. (1987) reported that magnetic resonance imaging had a high sensitivity but a poor specificity for identifying meniscal tears, but they did not report the accuracy of the test (Savory et al. 1987). Also, Savory et al. were unable

to identify injuries to the cruciate ligaments or other intra-articular lesions using magnetic resonance imaging.

The most accurate way of diagnosis of OA is histopathological evaluation. This way of diagnosis could not be achieved unless by invasive sampling of the suspected joints that itself may induce more joints tissues damages, depending on the operator expertise. Therefore, it is not applicable unless in special cases that seems necessary or after necropsy.

As yet, ex vivo models cannot simulate the structural changes which occur in joint tissues in animals over months to years. Hence, animal models for osteoarthritis are required to study how the complex structural changes in tissues evolve over time, spontaneously or following experimental injury, and to determine how constitutive, environmental or biomechanical risk factors may initiate, promote, or otherwise regulate these changes (Pritzker 1994). The relatively low incidence and he slow and variable onset of spontaneous OA in mice and dogs lessens the attractiveness of such animals as models for evaluation of OA assessment, but rabbits remains a good choice for this purpose.

Therefore, the present study was conducted in order to induce experimental OA in rabbit's knee joint (stifle), as an animal model, and follow the term of changes in radiography, MRI and histopathology in short- and long term studies. The comparison of early results of the different diagnostic techniques would help comprehensive assessment of the beginning of the observable joint tissues changes and the capability of each technique to diagnose respective changes in either of the techniques. On the other hand, the comparison of the late results (chronic signs) of different techniques would reveal correlation of each technique with histopathological results as a gold standard means of diagnosis. Evaluation of each one of non invasive techniques in the middle of progressive lesions would lead to understand the process of changes for the purpose of diagnostic criteria in each evaluative technique.

2 Literature Review

The first major function of a synovial joint is to facilitate predictable, energy-efficient, and pain-free movement (Todhunter 1996). Translational movement in normal joints is minimal. Such motion is restricted by the joint capsule, ligaments, osteocartilaginous contour, and periarticular tendons and muscles. In addition to flexion-extension and abduction-adduction, muscle-tendon units close to joints control rotational motion. These actions are counter-balanced by opposing tendons and ligaments and the contour of the articular surface (Girgis et al. 1975; Simon and Radin 1997). Cruciate ligaments resist motion in the plane in which the ligaments lie, not across or perpendicular to their major axes. Ligaments augment the stabilizing effect of the bony contour by restricting the degree of rotation (Simon and Radin 1997). Muscle forces acting across the joint also contribute to mechanical stability.

The second major function of joints is to support the musculoskeletal system and transmit load. When weight-bearing movement occurs, joints carry relatively high loads. The synovial joints must distribute and transfer these loads while maintaining the contact stresses across the joint surfaces at acceptably low levels and over a wide range of loads and oscillating speeds. In the human hip or knee, greater than 1- to 3-fold body weight (1 body weight is equivalent to 1 MPa [megapascal]) during walking, and 5-to 10-fold body weight during running, must be transmitted through the body to the grounds (Simon and Radin 1997). Some of this energy associated with movement is dissipated in the bones and muscles; the remainder must be transmitted across the joint surfaces.

2.1 Definition of osteoarthritis

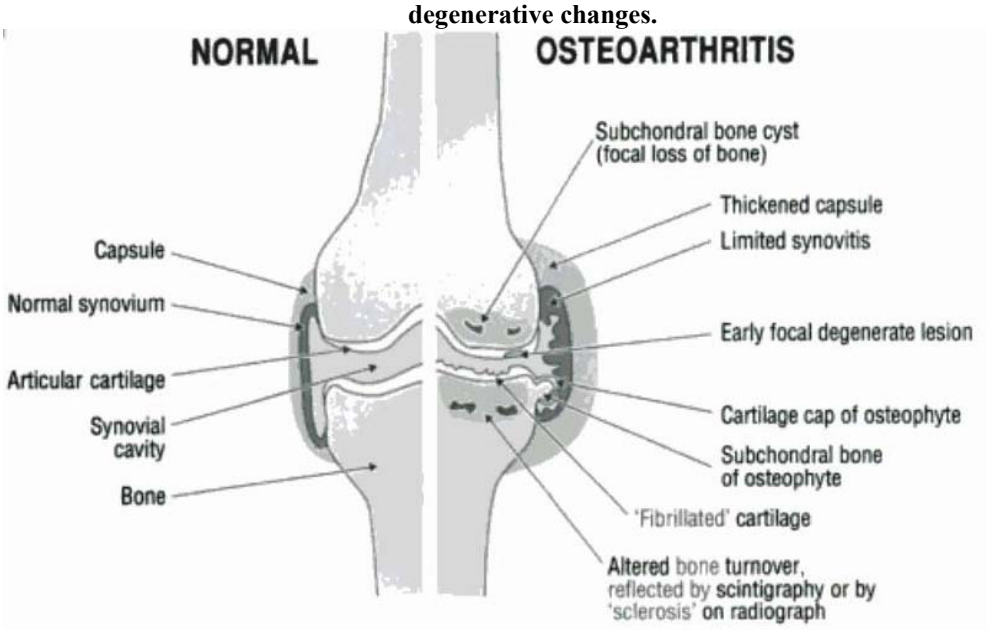
Osteoarthritis (OA) is a condition that represents a pathological imbalance of degradative and reparative processes involving the whole joint and its component parts, with secondary inflammatory changes, particularly in the synovium, but also in the articular cartilage itself (Figure 2-1). Idiopathic primary OA may involve one particular joint, or it may be generalized or involve multiple joints in erosive inflammatory forms (Table 2-1). The presentation of this pathological condition in joints may be a consequence of the biomechanics within the joint which reveal other wise masked systemic genetically determined changes. The mechanical pressures within the joint may therefore reveal weaknesses in tissue maintenance that are more wide-spread than previously considered (Moskowitz et al. 2006).

In 1994 at a workshop entitled 'New Horizons in Osteoarthritis', sponsored by the American Academy of Orthopaedic Surgeons; the National Institute of Arthritis, Musculoskeletal and Skin Diseases; the National Institute on Aging; the

Arthritis Foundation and the Orthopaedic Research and Education Foundation, OA was defined as follows:

Osteoarthritis is a group of overlapping distinct diseases, which may have different etiologies but with similar biologic, morphologic and clinical outcomes. The disease processes not only affect the articular cartilage, but involve the entire joint, including the subchondral bone, ligaments, capsule, synovial membrane and periarticular muscles. Ultimately, the articular cartilage degenerates with fibrillation, fissures, ulceration and full thickness of the joint surface (Moskowitz et al. 2006).

Figure 2-1. Gross pathologic changes observed in OA joints during many years of degenerative changes.



Moskowitz, R., R. Altman, et al. (2006). Osteoarthritis: diagnosis and medical/surgical management, Lippincott Williams & Wilkins.

The above definition emphasizes the concept that OA is not a single disease entity. Depending on the absence or presence of an identifiable local or systemic etiologic factor, OA has been classified into idiopathic (or primary) and secondary forms. Table 2-1 depicts the classification scheme developed in a 1986 international conference on OA (Brandt et al. 1986).

Table 2-1. Classification of osteoarthritis.

Table I Classification of osteoarthritis

Idiopathic

Localized

Hands: Heberden's and Bouchard's nodes (nodal), erosive interphalangeal arthritis (non-nodal), carpal-1st metacarpal joint
Feet: Hallux valgus, hallux rigidus, contracted toes (hammer/cock-up toes), talonavicular joint
Knee: (a) Medial compartment
(b) Lateral compartment
(c) Patellofemoral compartment
Hip: (a) Eccentric (superior)
(b) Concentric (axial, medial)
(c) Diffuse (coxae senilis)
Spine: (a) Apophyseal joints
(b) Intervertebral joints (disk)
(c) Spondylosis (osteophytes)
(d) Ligamentous (hyperostosis, Forestier's disease, diffuse idiopathic skeletal hyperostosis)
Other single sites, e.g. glenohumeral, acromioclavicular, tibiotalar, sacroiliac, temporomandibular joint

Generalized OA includes three or more areas listed above

Secondary

Trauma
Acute
Chronic (occupational, sports)

Congenital or developmental
Localized diseases: Legg–Calvé–Perthes syndrome, congenital hip dislocation, slipped femoral capital epiphysis
Mechanical factors: unequal lower extremity length, valgus/varus deformity, hypermobility syndromes
Bone dysplasias: epiphyseal dysplasia, spondyloepiphyseal dysplasia, osteo-onychochondrodystrophy

Metabolic
Ochronosis (alkaptonuria)
Hemochromatosis
Wilson's disease
Gaucher's disease

Endocrine
Acromegaly
Hyperparathyroidism
Diabetes mellitus
Obesity
Hypothyroidism

Calcium deposition diseases
Calcium pyrophosphate dihydrate deposition
Apatite arthropathy

Other bone and joint diseases
Localized: fracture, avascular necrosis, hyperostosis, infection, gout
Diffuse: rheumatoid (inflammatory) arthritis, Paget's disease, osteopetrosis, osteochondritis

Neuropathic (Charcot joint)

Endemic
Kashin-Beck
Mseleni

Miscellaneous
Frostbite
Caisson disease
Hemoglobinopathies

Reproduced with permission from Brandt KD, Mankin HJ, Shulman LE. Workshop on etiopathogenesis of osteoarthritis. *J Rheumatol* 1986;13:1126–60

Brandt, K., H. Mankin, et al. (1986). "Workshop on etiopathogenesis of osteoarthritis." Journal of Rheumatology 13: 1126-1160

Idiopathic OA is divided into localized and generalized forms. In the latter OA involves three or more joint groups. For example, a patient with OA localized to the hands but involving, one or more distal interphalangeal joints, one or more proximal interphalangeal joints and the thumb base would be classified as having idiopathic generalized OA. As long as it conforms to the above definition, generalized OA may occur with or without hand involvement. It is difficult to apply definitions such as those cited above to the diagnosis of OA in an individual subject in the community or a patient in a clinic setting. Criteria for case definition in community populations have traditionally relied on the presence of radiographic features of OA. However, the use of radiographic criteria alone to define cases for clinical studies of OA has limitations: although a statistically significant association exists between X-ray changes of OA and reported pain in both the hip and knee, in the individual patient the correlation between the severity of X-ray changes and the severity of symptoms is often poor (Brandt et al. 1986).

Over the past decade the Subcommittee on OA of the American College of Rheumatology's Diagnostic and Therapeutic Criteria Committee has published classification criteria for OA of the knee, hand and hip. In each case the classification schemes are based on combinations of symptoms, physical findings and laboratory and radiographic features. The sensitivity, specificity and accuracy of the classification criteria of OA of the knee, hand and hip approaches or exceeds 90%. Because the major inclusion parameter in each case is 'joint pain for

most days of the prior month', the American College of Rheumatology criteria identify patients with clinical OA. This contrasts with the identification of OA based on X-ray features alone. Because most subjects with radiographic evidence of OA do not have joint pain, estimates of the prevalence of OA will be lower when based on the Colleg's criteria than when based on traditional radiographic criteria (Altman et al. 1986; Brandt et al. 1986; Altman et al. 1991; Brandt 2005).

2.2 Osteoarthritis in Veterinary Medicine

Approximately 20% of the 44 million adult dogs in the United States have osteoarthritis (Johnston 1997). Osteoarthritis is a slowly progressive disease; the initiating events in its pathogenesis are obscure (Johnston 1997). Osteoarthritis is characterized clinically by joint pain, limitation of movement, effusion, and variable degrees of local inflammation. It is characterized biochemically by a reduction of proteoglycan concentration in cartilage, alterations in the size and aggregation of proteoglycan increased water content, collagen fibril disruption, and imbalance in the synthesis and degradation of matrix macromolecules. On pathological examination the disease is characterized by irregularly distributed loss of articular cartilage (more frequent in areas of increased load), sclerosis of subchondral bone osteophytes and enthesiophytes, and variable synovial inflammation. On histological evaluation, it is characterized early by decreased uptake of metachromatic stains by the articular cartilage and fragmentation of the cartilage surface (fibrillation); later by vertical clefts in the cartilage clonning of chondrocytes, osteophyte formation indicative of remodeling and repair, and regeneration of the tidemark; and finally by total loss of cartilage, sclerosis, and focal osteonecrosis. Osteoarthritis is biomechanically characterized by alteration of the tensile, compressive, and shear properties and hydraulic permeability of the cartilage. These cartilage changes are accompanied by increased stiffness of the entire subchondral bone. There is no specific therapeutic agent that can restore the articular cartilage to a normal state (Mankin et al. 1986).

2.3 Pathogenesis of osteoarthritis

As previously described, most forms of OA fall into two categories, depending on the predominant background those that are primary, and often idiopathic, with abnormalities of joint biomaterial and biomechanically faulty joint structure that may result from a recognizable mutation, and those that are secondary and result from superimposed risk factors affecting distribution and severity of loading forces acting on specific joints, such as joint injury (Slatter 2003).
Although the most obvious changes in the osteoarthritic joint reside in the cartilage, OA should not be viewed simply as a disease of cartilage. It does not represent the failure of a single tissue, but of an organ, the diarthrodial joint. Just as congestive heart failure may be due to normal, primary disease of the myocardium, pericardium or endocardium, the primary abnormality in OA may reside in the articular cartilage, synovium, subchondral bone, ligaments or neuromuscular apparatus. Nonetheless, given the marked changes that occur in the