

Radiographic Follow up of Early Phases of Osteoarthritis

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Abstract: The purpose of the present study was to induce experimental osteoarthritis (OA) in the rabbit knee joint and to describe the radiographic changes on 30 and 90 days post OA induction. Ten adult, male rabbits were randomly divided into two equal groups. In one group cranial cruciate ligament (CCL) was sectioned (Transected group; TG), in the other group only arthrotomy performed through the same approach, but the CCL was remained intact (Arthrotomy group; AG). Radiography was performed in two standard views on days 0, 30 and 90 after operation. Image analysis consisted of evaluation of four diagnostic parameters including joint space narrowing, subchondral sclerosis, osteophytes and subchondral cysts and finally an overall OA assessment was performed. CCL transection induced OA in rabbit knee. The results showed significant differences in joint space narrowing and overall OA grades among different days. The differences between TG and AG were significant on day 90 post-operation in joint space narrowing and overall OA. The earliest sign of OA progression was joint space narrowing which was observable 30 days post-operation. Subchondral sclerosis and osteophytes formation appeared in radiographs 30 and 90 post-operative days respectively. Subchondral cyst formation was not detectable over a 90 days period of this study.

Key words: Radiography • Experimental • Osteoarthritis • Rabbit

INTRODUCTION

Osteoarthritis (OA) is characterized by progressive deterioration and localized erosion of articular cartilage, accompanied by remodeling of bone at the joint margins [1]. Conventional radiography is the method most frequently used for monitoring progression of osteoarthritis. However, it is usually used in clinical cases in both veterinary and human medicine, in which the disease is well progressed. Although it has been used for many years, but most of its application was limited to natural disease with clinical sign indicating of OA and there is limited information about the radiographic feature of the early stages of this disease. To fully understand the radiographic appearance of OA a controlled study of experimental OA is mandatory. There are many techniques to produce experimental OA, among which is transecting the Cranial (Anterior) Cruciate Ligament (CCL) of the knee joint [2]. Transection of the CCL of the knee produces

laxity of the joint which leads to physiological changes resembling those of natural OA.

To the author's knowledge there are little published information describing chronological radiographic changes in knee joint following OA induction. The objective of the present study was to induce experimental OA in the rabbit knee (stifle) joint by a technique already been described for dogs [2] and to describe the radiographic changes in 30 and 90 days post OA induction.

MATERIALS AND METHODS

Animals, Preparation and Operative Procedures: A total number of 10 adult (13 ± 3 months), Dutch, male rabbits, weighing 2.7 ± 0.4 kg was used in this study and randomly divided into two equal groups. All the rabbits were physically and radiographically assessed for signs of OA at the beginning of the study. CCL of the left knee of five rabbits were sectioned through a 3-4 mm parapatellar stab

incision (Transected group; TG) for dogs [2]. In other five rabbit arthrotomy performed through the same approach but the CCL was remained intact and this group considered as Sham operation (AG group). The whole stifle region was aseptically prepared for surgery. All the rabbits were anesthetized by injection of Xylazine (3 mg kg^{-1} , IM) (Rompun®, Alfasan International B.V. Woerden, Nederland) and Ketamine (50 mg kg^{-1} , IM) (Alfamine®, Alfasan International B.V.; Woerden, Nederland). After anesthesia the animals were placed on dorsal recumbency. Carprofen (Rimadyl®, Pfizer Inc.; Zaventem, Belgium) was injected to all animals (4 mg/kg SC) once just before the end of operation for pre-emptive analgesia and every 24 h for 3 days postoperatively. The rabbits were kept in standard cages with controlled environmental conditions, same as they were before the operation. They were allowed to move freely in groups of five rabbits for 2h a day in a 100 m^2 fenced area throughout the experiment but were not forced to exercise.

This study was approved by the ethical committee of Veterinary School of the Shiraz University and the principles of laboratory animal care (NIH publication NO. 86-23, revised 1985) were followed.

Imaging Techniques and Studied Criteria: On days 0, 30 and 90 post OA induction, radiography was performed under general anesthesia. Radiography on day 0 was performed to rule out preexisting diseases and considered as control. The animals positioned in lateral recumbency and medio-lateral radiographs were taken. Anterior-posterior radiographs were also obtained from all animal in dorsal recumbency. Image analysis consisted of evaluation of four diagnostic parameters including joint space narrowing, subchondral sclerosis, osteophytes and subchondral cysts and overall OA assessment [3, 4].

The extent of the disease was evaluated separately for the medio-lateral and anterior-posterior compartments. The severity of the osteoarthritic changes of the knee was graded from 0= normal; 1= mild OA; 2= moderate OA and 3= severe OA [5]. Subchondral sclerosis was noted as 0 = absent; 1 = mild, or localized eburation; 2 = moderate increase in density; 3 = severe, widespread sclerosis [4]. Joint space narrowing was graded as 0= normal; 1= mild narrowing (less than 30% narrowing compared to normal); 2= moderate narrowing (between 30-60% narrowing compared to normal) and 3= severe narrowing (more than 60% narrowing compared to normal). Osteophytes were graded as 0 = absent; 1 = small beaklike osteophyte; 2 = intermediate-size osteophyte, between those of grades 1 and 3; 3 = proliferative or mushroomlike osteophyte [6]. Subchondral cysts were evaluated as 0 = absent; 1 = one to two small cysts; 2 = single large or multiple small cysts; or 3 = many large cysts [4]. In the event of non-uniform involvement of a single compartment, the most severe degree of change was used for grading.

Statistical Analysis: Non parametric two independent samples tests were used to compare the differences between TG and AG in the same days, using Mann Whitney U test. Two related samples tests were used in order to compare the results of different days of the same group, to see the significant differences over time. Differences were considered statistically significant when $p=0.05$, using computer software SPSS version 11.5 for windows (SPSS Inc., Chicago, IL., USA).

RESULTS

As is shown in table 1, there were significant differences in joint narrowing and overall OA grades but the differences in subchondral sclerosis, osteophytes

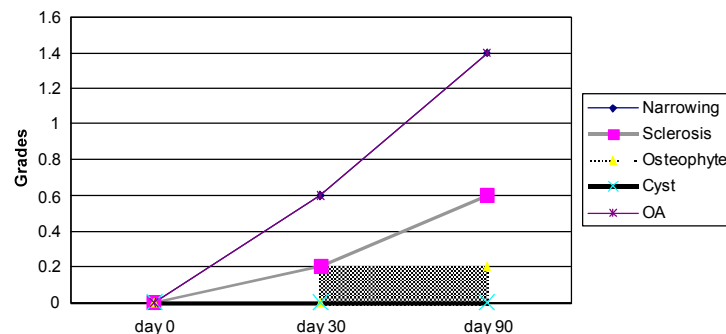


Fig. 1: Changes in joint space narrowing, subchondral sclerosis, subchondral cysts, osteophytes formation and overall osteoarthritis grading of the CCL-transected radiography over time.

The term of changes of joint space narrowing and osteoarthritis (OA) were significant over time ($p<0.05$) (The graphs of OA and narrowing are the same). The changes in subchondral sclerosis and cysts and osteophytes in radiography were not significant ($p>0.05$).

Table 1: The grades of the joint space narrowing, subchondral sclerosis, cysts, osteophytes changes and overall osteoarthritis of the arthrotomized and CCL-transected joints on days 30 and 90.

Rabbits	Joint narrowing ^a		Subchondral sclerosis		Osteophyte Formation		Subchondral Cysts		Overall Osteoarthritis ^b	
	30d	90d	30d	90d	30d	90d	30d	90d	30d	90d
A1	0	0	0	0	0	0	0	0	0	0
A2	0	0	0	0	0	0	0	0	0	0
A3	0	0	0	0	0	0	0	0	0	0
A4	0	1	0	0	0	0	0	0	0	0
A5	1	1	0	1	0	0	0	0	1	1
T1	1	1	0	0	0	0	0	0	1	1
T2	1	2	1	2	0	1	0	0	1	2
T3	1	2	0	1	0	0	0	0	1	2
T4	0	1	0	0	0	0	0	0	0	1
T5	0	1	0	0	0	0	0	0	0	1

A1-5 refer to the arthrotomized joints; T1-5 refer to CCL-transected joints, NL= normal, G= grade of changes. The severity of the osteoarthritic changes (Joint space narrowing, subchondral sclerosis, osteophytes changes, subchondral cyst and overall osteoarthritis) of the knee was graded from 0= normal; 1= mild OA; 2= moderate OA and 3= severe OA. In the event of non-uniform involvement of a single compartment, the most severe degree of change was used for grading. ^a there were significant differences in joint space narrowing of the TG (T1-5) between days 90 with 30 and 0 and there was a significant difference between TG and AG (A1-5) on day 90 post-operation ($p<0.05$). ^b there were significant differences in osteoarthritis of the TG (T1-5) between days 90, 30 and 0 and there was a significant difference between TG and AG (A1-5) on day 90 post-operation ($p<0.05$).



Fig. 2: A) A CCL-transected joints 30 days post-operation. The differences in joint narrowing between operated and control (day 0-not shown) was not significant. B) A CCL-transected joints 90 days post-operation. The arrow refers to subchondral sclerosis. The arrowhead refers to joint space narrowing which can be compared to contra-lateral intact joint and to the operated joint 30 days post injury (part A left knee). R: right, L: left.

formation and subchondral cysts formation were not significant in different days (Figure 1). The differences between TG and AG were significant on 90 days post-operation in joint narrowing and overall OA. None of the normal (intact control) joints showed radiographic changes on days 0, 30 and 90.

DISCUSSION

The degenerative changes which take place in rabbit knee joints after CCL section, resemble those of naturally occurring OA. The changes which occurred in the articular cartilage have been studied in the same experimental model [1]. Very briefly, these changes that were reported may be summarized as an abnormal distribution of chondrocytes, the appearance of tangential and vertical clefts in the cartilage and also loss of metachromasia as shown by special staining techniques. The pathological findings were not parts of the present study, but our radiological findings are in accordance with previous pathological studies. Three criteria (joint space narrowing, subchondral sclerosis and osteophytes) were found out of the four studied radiological criteria indicative of OA progression.

The earliest radiographic sign was joint narrowing which was more evident and seen in more cases than other criteria. Although it was measured qualitatively but it seems to be the first sign indicative of OA. By radiography, the cartilage was assessed indirectly by evaluating the width of the joint space [4].

Cartilage loss appeared frequently as joint space narrowing (Table 1) and as was shown, three of the CCL-transected joints showed grade 1 of joint narrowing 30 days post-operation and all of them showed joint narrowing 90 days after transaction (Figure 2). Although it could be well indicative of OA but weight bearing radiography was not accessible in rabbit, so higher grades of joint space narrowing could be achieved in other animal species and human, where weight-bearing projections are possible where body weight can compress the joint space more. Radiography primarily detects cartilage loss in the medial compartments in both the 30 and 90 days. This may be due to cartilage loss in the medial compartment that forces the lateral joint space to open 1 -2 mm as weight is shifted medially [7]. Considering the significant difference between TG and AG on day 90 in joint space narrowing and OA as well as the significant terms of changes over time in joint narrowing and OA of the TG group reflects the time related nature of OA progression, in which the longer time is passed the more evidence of OA, is seen. This is in accordance to the findings of almost all the previously performed experimental OA studies in different animal species [8, 9], which have shown more pathological or radiological changes over the time.

A case of osteophytes formation was seen 90 days post-CCL transection which implies the time of progression of the detectable changes within the operated joints or its margins to be later than other studied criteria. Presence of osteophytes has been well documented in previous investigations and has been shown markedly variable from earlier to the later stages of the OA development [10, 11]. It has been reported that the onset of osteophytes formation is from 2 to 8 weeks and the new bone is relatively radiolucent with a spongy appearance [11]. In some cases it can be seen to start as a deposition of mineral outside the pre-existing bone cortex. Other investigators have suggested that repeated stretching of the synovial membrane at its insertion may stimulate proliferation of the vascular, undifferentiated tissue at this site and hence lead to osteophytes formation [11]. To prove this would be difficult, but the fact of a mechanical instability resulting, either directly or indirectly, could be the result of such findings [12].

Although it was not statistically significant, but more cases of subchondral sclerosis were seen on day 90 in the TG than day 30 post-operation, which reflects more evidence of OA progression over time. Radiography later than allocated 90 days would prove more significant results. In other works it has been shown that there was a good correlation between joint space narrowing and

subchondral sclerosis due to same weight dependant pathogenesis and reported to be more evident in the joints where more weight is exerted [13]. This is also a sign of progressive and time related nature of OA.

We did not observe any radiological signs of subchondral bone cyst during the 90 days of this study. It was reported that subchondral cysts results from pre-existing regions of subchondral bone marrow edema-like signals detected by magnetic resonance imaging (MRI) [14]. In other investigation cyst like lesions were seen on medial aspect of tibia by MRI, 12 weeks post CCL-transection in 4 of the 6 studied dogs, however, no subchondral cysts were seen in the tibia on radiographs at the same time [15], indicating that MRI is a better way to detect cyst like lesions within subchondral bones in early OA.

In conclusion, the most evident and earliest sign of OA in early radiography would be joint space narrowing which was observable 30 days post-operation. Subchondral sclerosis and osteophytes formation appeared in radiographs of 30 and 90 post-operative days, respectively.

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