INTRODUCTION

Articular cartilage is a thin, smooth, low friction gliding surface with remarkable resiliency to compressive forces. It is only a few millimeters thick yet with excellent wear characteristics. Its mechanical and structural capacity is dependent on the integrity of its extracellular matrix. Effects of immobilization on articular cartilage has been studied in the past. This study was designed to observe inflammatory changes on immobilization and their reversibility on remobilization.

Material & Methods: Ninety male rats of same age were divided into groups and immobilized, remobilized and sacrificed at different periods. The knee joint was cut in sagittal plane and kept in 10% formalin for 48 hours. After processing 10µm sections were cut and stained with Alcian blue to demonstrate proteoglycan content.

Results: Extensive necrotic changes were observed on 4 weeks immobilization. In four of the immobilized animals the entire articular cartilage was eroded. On 4 weeks immobilization staining character in superficial zone demonstrated mild staining in 76% while 24% specimens had lost staining with Alcian blue stain as compared to control group in which 100% specimens were mildly stained. In the group remobilized for 4 weeks, there were focal necrotic areas and splitting of cells from superficial zone.

Conclusion: Immobilization leads to the development of necrosis, clefts and fissures. Remobilization for four weeks does not reverse the changes significantly.

KEY WORDS: Patellar cartilage, Immobilization, Remobilization.

ORIGINAL ARTICLE

IMMOBILIZATION INDUCED EFFECTS IN ARTICULAR CARTILAGE AND THEIR REVERSAL ON REMOBILIZATION IN RATS

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ABSTRACT

Background: Articular cartilage is a thin, smooth, low friction gliding surface with remarkable resiliency to compressive forces. It is only a few millimeters thick yet with excellent wear characteristics. Its mechanical and structural capacity is dependent on the integrity of its extracellular matrix. Effects of immobilization on articular cartilage has been studied in the past. This study was designed to observe inflammatory changes on immobilization and their reversibility on remobilization.

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Conclusion: Immobilization leads to the development of necrosis, clefts and fissures. Remobilization for four weeks does not reverse the changes significantly.

KEY WORDS: Patellar cartilage, Immobilization, Remobilization.
MATERIAL AND METHODS

Ninety male rats belonging to Sprague Dawley strain were procured from National Institute of Health, Islamabad and the study was carried out at the animal house College of Physicians and Surgeons Islamabad.

These animals were divided into three groups. The right hind limbs of rats were immobilized with plaster of Paris cast. Care was taken to cover the knee joint completely. Animals in these groups were immobilized, remobilized and sacrificed at different periods as given below.

Group 1: Control group of 30 animals not immobilized.
Group 2: Experimental group of 30 animals immobilized for four weeks.
Group 3: Experimental group of 30 animals immobilized for four weeks and then remobilized for four weeks.

At the end of experimental period the rats were anaesthetized with chloroform. The skin over knee joint was dissected and the joint along with patella was exposed. The knee joint was cut in sagittal plane and kept in 10% formalin for 48 hours. Specimen was decalcified using ethylene diamine tetra acetic acid (EDTA).

After processing for making paraffin blocks 10µm sections were cut and stained with Alcian blue to demonstrate proteoglycan content.

The necrotic area was measured with the help of computer software image J. This is the software introduced by the National Institute Of Health USA for calculating area and distance of user-defined selections. H&E stained slides were observed under the microscope at 40x magnification. The zone of cartilage was focused and necrosed area identified. Olympus digital camera E115 was used and three photographs were taken (from the eyepiece with the reticule fitted in it). These photographs were then transferred to computer software. The calculated area of one division of ocular micrometer at 40x magnification was fed into computer software "Image J" for reference. Then the irregular necrosed area was outlined by freehand tool. The presence or absence of inflammatory cells (lymphocytes, neutrophils and macrophages) was recorded in the patella-femoral joint space under oil immersion lens in H&E stained sections.

RESULTS

There was reduction of patello-femoral joint space in 10 of the animals in immobilized group as compared to control group. In a few animals hyperextension occurred. Extensive necrotic changes were observed on four weeks immobilization. Erosion began from superficial zone and spread towards the transitional and radial zones. In 4 of the immobilized animals the entire articular cartilage was eroded. (Fig. 1)

On four weeks immobilization staining character in superficial zone demonstrated mild staining in 76% of specimens while 24% specimens had lost staining with Alcian blue stain as compared to control group in which 100% specimens were mildly stained. In three animals fissures and splits were seen. Large population of inflammatory cells

![Patellar cartilage and joint space](image-url)

Figure 1: Photomicrograph showing patellar cartilage and joint space. Arrow showing disruption along the entire thickness of cartilage. Alcian blue stain.
invaded the patella-femoral joint space in immobilized animals. In this group, mostly neutrophils were present within the joint space and in necrotic area. (Table 1)

Some of the cells also invaded the necrotic area. The necrotic area was flooded with inflammatory cells. The necrotic area was 4288.7±27.59 µm².

In 10 animals whole cartilage area was degenerated and loss of staining in surrounding area was noted. Significant number of inflammatory cells mostly neutrophils were observed within the patella-femoral joint space. (Table 2)

In the Group 3 there was reduction of patella-femoral joint space in only 3 animals but segments of split superficial zone were lodging in the space. There were focal necrotic areas filled with debris of fibrous tissue. There was splitting of cells from superficial zone. One or two neutrophils were observed within the patella-femoral joint space. There were focal necrotic areas with average size 2905.5±52.04 µm². (Table 2)

DISCUSSION

The prominent features of necrosis as observed in this study were lesions seen in sections that appeared as clefts and decreased staining of matrix in that area was seen. In some studies it was noted on immobilization, there was a noticeable decrease in the met achrornatic staining, indicative of proteoglycan loss. The first and the most significant sign of immobilization degeneration is a loss of staining leading to softening of the cartilage and it has been correlated with the loss of proteoglycans from the matrix. Results of some other studies showed that casting of the knee joints of beagle dogs for eleven weeks, caused up to 20-48% reduction in proteoglycans concentration of articular cartilage. The proteoglycans were depleted mainly in the superficial zone of articular cartilage. Clefts and fissures were large enough and also cystic areas were observed. In some of the other studies diffuse necrosis of chondrocytes, with pit formation on the surface and disruption and disintegration of the collagen fibers, was seen to some extent in all the animals.

In this study, in some areas, complete loss of tissue was seen. There was penetration of bone. Penetration of bone because of necrosis has been reported in past. Cartilage is a focal point of attack by cellular and molecular elements of the inflammatory response which occurs in arthritic diseases. Neutrophils damage articular cartilage by degrading matrix components and inhibiting their synthesis. In this study, immobilization clearly induced fissuring of the cartilage surface. In some of the studies the fissures, have proven to benefit the attachment of polymorphonuclear cells by creating a larger contact area thus giving better hold for the PMNs. On remobilization there was no regeneration. Instead there was splitting of cells from superficial zone. Necrotic areas were still observed in the cartilage. Penetration of the subchondral bone to expose the blood vessels and generate a blood clot within the defect is very important regarding repair of cartilage. In this experiment formation of clefts as a result of immobilization exposed the bone marrow and hence deficiency of chondroprogenitor cells was fulfilled. Significant population of cells on both immobilization and remobilization consisted of neutrophils. The inflammatory cells eliminate the damaged tissue fragments by phagocytosis, and fibroblasts extensively and markedly elevate production of collagen and other extra cellular matrix components. It is clearly seen that remobilization alone cannot revert the inflammatory damage done on immobilization. Perhaps some better techniques can help in regeneration of cartilage. Currently used clinical methods of stimulating cartilage repair and remodeling include alteration of the loading on degenerated joints (primarily by using osteotomies), introduction of new cartilage-forming cells by perforation of subchondral bone, and soft-tissue arthroplasty. Nevertheless, some research results are sufficiently encouraging to suggest that repair of the degenerating articular cartilage may be possible in the future. The treatment options include conversion of chondral lesions to osteochondral lesions, which facilitates migration of cells from the marrow space to affect repair. In recent years, a greater emphasis has been placed on tissue engineering strategies and thus several new treatment options have been introduced, including the use of cell transplantation. Several tissue sources and cell types can potentially be used for this type of therapy.

**Table 1: Staining grades in various groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>mild</th>
<th>Moderate</th>
<th>Intense</th>
<th>faint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>76%</td>
<td>24%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>80%</td>
<td>20%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Necrotic changes in various groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Necrotic area</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>4288.7±27.59</td>
<td>Neutrophils</td>
</tr>
<tr>
<td>Group 3</td>
<td>2905.5±52.04</td>
<td>Neutrophils</td>
</tr>
</tbody>
</table>
CONCLUSION

Immobilization leads to the development of necrosis, clefts and fissures. Remobilization for four weeks does not reverse the changes significantly. A longer duration of remobilization may be required for reversibility.

REFERENCES


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