

Effects of immobilization on thickness of superficial zone of articular cartilage of patella in rats

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ABSTRACT

Background: Articular cartilage normally functions as a load-bearing resistant material in joints. Patella is composed of hyaline cartilage and spongy bone. Chondrocytes form only 1–5% volume of the articular cartilage. They receive their nutrition by diffusion through the matrix. The alteration in articular cartilage become apparent following immobilization, from 4 to 6 weeks. Until now, focus of research has been the whole cartilage. Zonal changes have not been studied in detail. Since superficial zone bears maximum load and is the first zone to come in contact, the present study was designed to determine changes in thickness on immobilization and remobilization in superficial zone after dividing it into proximal, central, and distal segments.

Materials and Methods: Forty male rats belonging to Sprague Dawley strain were divided into two groups. Group 1 (n=20) subdivided into an experimental subgroup of 10 rats that were immobilized in plaster of Paris (POP) for 4 weeks and a control subgroup of 10 that were not immobilized. Group 2 (n=20) subdivided into an experimental subgroup of 10 rats that were immobilized for 4 weeks and remobilized for next 4 weeks and a control subgroup of 10 animals that were not immobilized. At the end of the experimental period, the knee joint was dissected and was cut in sagittal plane. The section was fixed in 10% formalin for 48 hours. Specimen was decalcified using ethylenediaminetetraacetic acid (EDTA). The paraffin blocks of 7 µm sections were cut and stained by H and E stain for routine histology and Alcian blue stain and Mallory Trichrome for fine structural microscopy. The zones were named as superficial transitional, radial, and hypertrophic according to the shape of cells present in each zone. The superficial zone was divided into superior part, central, and inferior parts. These parts were labeled as central, proximal, and distal segments. The calibrated stage micrometer was used to calibrate the ocular micrometer under objectives of different power. The ocular micrometer was placed inside the ocular lens. It was calibrated with the stage micrometer under objective lenses of different power. The number of divisions of ocular covering each zone was calculated. These divisions were converted into micrometer and the actual thickness was calculated.

Results: The significant decrease in thickness of superficial zone in proximal, central and distal segment was observed in experimental group in comparison to control group. When the experimental subgroup of group 2 was compared with experimental subgroup of group 1 (group immobilized for 4 weeks), no significant reversal was seen in superficial zone and instead significant decrease was observed in distal segment. Fibrous connective tissue was increased adjacent to superficial zone.

Conclusion: Each segment of superficial zone behaves differentially on immobilization and remobilization. Perhaps a much longer duration of remobilization is required to reverse changes of immobilization in articular cartilage and plays a significant role in knee joint movements.

Key words: Articular cartilage thickness, immobilisation, rat

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INTRODUCTION

Immobilization of normal joints for varying periods generally causes the degenerative changes in the articular cartilage as seen in terms of morphology, biochemical composition, and mechanical properties.¹ The patella is composed of spongy bone¹ and hyaline cartilage.² In addition to chondrocytes, the hyaline cartilage contains two major elements: a framework formed by collagen II rich fibrils and a hydrated substance with a high content of the cartilage specific proteoglycan aggrecan.³

Morphologically, there are four named zones starting from

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the zone adjacent to the patellofemoral space are: Superficial zone, Transitional zone, Radial zone and Hypertrophic zone.⁴

The popular concept is that loading and unloading plays a role in nutrition and immobilization causes degenerative changes. These degenerative changes have been studied by researchers over the years.⁵ The changes in articular cartilage become evident on the immobilization for 4 to 6 weeks. The superficial zone of the articular cartilage is affected and, when compression was maintained for longer periods, the cells of the deeper part of the cartilage were also affected, eventually involving the whole thickness, layer after layer, if immobilized for 2 weeks.⁶ The thickness of the condylar cartilage gradually diminishes toward the less weight-bearing, peripheral portions of a joint surface.⁷ The relationship that has been found between the thickness of articular cartilage and the weight is consistent with the hypothesis that this thickness varies with the load applied across the joint.^{4,6} Until now, focus of research has been the cartilage without specifying the zone affected.^{8,9} Zonal changes have not been studied in detail. Since superficial zone is the zone which bears maximum load and is the first zone to come in contact,¹⁰ the present study was designed to determine changes in thickness on immobilization and remobilization in superficial zone after dividing it into proximal, central, and distal segments. The objective of the study was to determine changes in thickness of superficial zone after dividing it into segments so as to determine which of these segments is most vulnerable to injury on immobilization and remobilization.

MATERIALS AND METHODS

Forty male rats of Sprague Dawley strain of 12 weeks of age with mature cartilage (as in 12 weeks of age in the preliminary project, it was seen that three zones were present and weight was adjusted to 2200–2500 g) were procured. The animals were fed on diet prepared at National Institute of Health, Islamabad [Appendix 1]. The feed was in the form of pellets. The rats were fed ad libitum. The daily food consumption was approximately 10 g/day. The animals were weighed before and after the experimental period. These animals were divided into two groups comprising control and experimental 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: (n=20 rats) subdivided into an experimental subgroup of 10 rats that were immobilized by POP Cast for 4 weeks and a control subgroup of 10 animals that were not immobilized.

Group 2: (n=20 rats) subdivided into an experimental

subgroup of 10 rats that were immobilized for 4 weeks and remobilized for 4 weeks and a control subgroup of 10 animals that were not immobilized.

At the end of the experimental period (total duration of immobilization and remobilization was completed starting from day 0), the rats were anesthetized with chloroform. The skin over knee joint was dissected and the knee joint along with patella was exposed. The dissection was done with the scalpel and dissecting knife. The knee joint was cut in sagittal plane. The section included the patella, tibia, and femur as well. It was fixed in 10% formalin for 48 hours. Specimen was decalcified using ethylenediaminetetraacetic acid (EDTA). After processing for making paraffin blocks, 7 μm sections were cut and stained. H and E stain was used for 7 μm thick sections to study routine histology of patellar articular cartilage. Alcian blue stain and Mallory Trichrome was used for fine structural microscopy. H and E stained slides at 40 \times magnification were used to record observations regarding thickness of superficial zone of articular cartilage. The zones were named as superficial, transitional, radial, and hypertrophic according to the shape of cells present in each zone. The superficial zone was recognized as zone with elliptical cells [Figure 1]. The radial zone was one with round to oval, large cells and hypertrophic with oval cells.

Measurement of thickness of different zones of articular cartilage

H and E stained slides at 40 \times magnification were used to record the observations. The zones were divided into superior part, central, and inferior parts (superficial zone in sagittal section). These parts were labeled as central, proximal, and distal segments [Figure 2]. Before carrying out histological examination of the stained slides, the calibrated stage micrometer was used to calibrate the ocular micrometer under objectives of

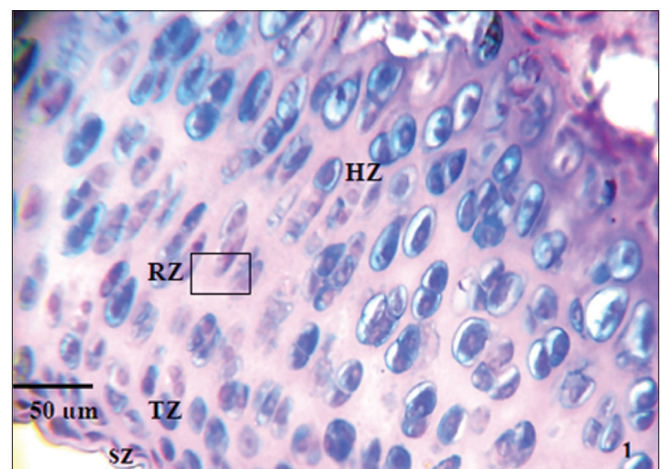


Figure 1: Photomicrograph showing section of articular cartilage of patella in control rat. It shows zones of articular cartilage: superficial (SZ), radial zone (RZ), and hypertrophic zone (HZ). Alcian blue stain. Bar 50 μm

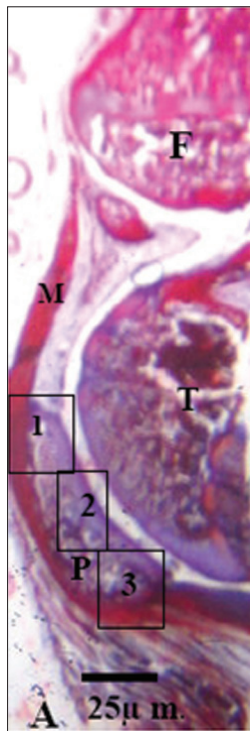


Figure 2: Photomicrograph showing section of patellofemoral joint in control rat, specimen number 12, subgroup 1A. (P) Patella, (F) femur, and (T) tibia can be seen. Other side of patella is covered by skeletal muscle (M). Mallory Trichrome stain. Bar 25 μ m. Areas in boxes 1, 2, and 3 show divisions of cartilaginous part of patella into area 1 - proximal segment, area 2 - central segment and area 3 - distal segment

different power. The ocular micrometer was placed inside the ocular lens. It was calibrated with the stage micrometer under objective lenses of different power. The number of divisions of ocular covering each zone was calculated. These divisions were converted into micrometer and the actual thickness was calculated. Observations were recorded for immobilized as well as remobilized groups and their controls.

The data were analyzed using SPSS version 10. The quantitative data, i.e. thickness, were interpreted with the help of Student's t-test. A P value of ≤ 0.05 was taken as significant and a P value of ≤ 0.001 was taken as highly significant. A P value of > 0.05 was taken as insignificant.

RESULTS

In group 2 control subgroup, the mean thickness of proximal, central, and distal segments in the superficial zone was 30.87 ± 0.41 , 29.23 ± 0.91 and 29.00 ± 0.46 , respectively [Table 1]. In the experimental subgroup, the mean thickness of superficial zone in proximal, central, and distal segments was 20.00 ± 1.24 , 29.23 ± 0.91 , and 16.00 ± 1.58 , respectively. In this zone, in all segments, statistically highly significant decrease was observed ($P < 0.001$) when compared with control subgroup [Table 1].

Table 1: Mean thickness (μ m) of superficial zone of articular cartilage

Group	Subgroup	Proximal segment	Central segment	Distal segment
Group 1	Control	27.27 ± 1.79	26.05 ± 1.44	27.00 ± 1.45
	Experimental	19.67 ± 1.49	24.50 ± 2.21	24.50 ± 2.39
Group 2	Control	30.87 ± 0.41	29.23 ± 0.91	29.00 ± 0.46
	Experimental	20.00 ± 1.24	24.67 ± 1.57	16.00 ± 1.58

In group 1 experimental subgroup, in the superficial zone, the mean thickness in proximal, central, and distal segments was 19.67 ± 1.49 , 24.50 ± 2.21 , and 24.50 ± 2.39 , respectively [Table 1]. In the proximal segment, there was significant decrease ($P < 0.05$) and insignificant decrease ($P > 0.05$) in central and distal segments [Table 1] as compared to the control group. When the experimental subgroup of group 2 was compared with experimental subgroup of group 1 (group immobilized for 4 weeks), no significant reversal was seen in superficial zone and instead significant decrease was observed in distal segment. Fibrous connective tissue was increased adjacent to superficial zone.

DISCUSSION

The pressure necrosis and marked decrease in thickness of superficial zone in cartilage was reported in rabbit after 5 weeks of immobilisation.¹¹ Extreme thinning of superficial zone of cartilage was found with immobilisation of joints for 15 weeks.^{11,12} The erosion of the cartilage, may occur without complete immobilization, i.e. in semiflexion.¹³ In the present study, no significant reversal was seen in superficial zone and instead significant decrease was observed in distal segment. Increased fibrous tissue adjacent to superficial zone was observed in many sections. This has been proved in the past. Cartilage ulceration and subchondral cyst formation have also been demonstrated in some studies even in incomplete immobilization.¹⁴ Again, it appeared to be the friction and pressure superimposed on restricted joint motion and not the degree of limitation of motion that accounted for the cartilage lesion.¹⁵ As regards the patellofemoral joint, increase or decrease in contact area on femur and tibia with patella affects the joint kinematics.¹⁶⁻¹⁸

Division of articulating area into segments has not been studied previously. In the past, in only one study, the articular cartilage from the medial midcondylar region of the knee was obtained, divided into three areas (non-contact area, transitional area, and contact area), and in each area, a degree of degeneration was evaluated by gross observation, histomorphometric grading, and measurements of thickness and number of chondrocytes.¹⁶ This is consistent with the findings of researchers in the past who have shown that different areas of joint respond in different ways to

immobilization.¹⁷ While some researchers believe that the difference in thickness observed on immobilization is related to weight bearing, since opposite changes are seen in the same cartilage in samples taken from weight-bearing versus non weight bearing areas,¹⁸ some others have reported zonal decrease to be a compartment specific response.¹⁹ In one of the studies, it was found that on immobilization of knee joint damage was more in central part as compared to peripheral parts.²⁰ In this experiment on remobilization, when the experimental subgroup of group 2 was compared with experimental subgroup of group 1 (group immobilized for 4 weeks), no significant reversal was seen in superficial zone and instead significant decrease was observed in distal segment. On remobilization, again the response of cartilage in different segments was different. A greater period of remobilization is required for reversal as has been proved in the past.^{9,21} Some researchers claim that immobilization causes long lasting changes in cartilage and no reversal is seen.¹⁵ Early use of injured musculoskeletal tissues increases inflammation and disrupts repair tissue weeks following injury.^{22,23}

CONCLUSION

The superficial cartilage of patella shows significant reduction in immobilization. Each segment of superficial zone behaves differentially on immobilization and remobilization. Perhaps, a much longer duration of remobilization is required to reverse changes of immobilization in articular cartilage.

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