

## OSTEOCYTE POPULATION SHIFT IN RESPONSE TO INJURY IN THEIR IMMEDIATE VICINITY: A QUANTITATIVE STUDY

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### ABSTRACT

**Background and Objectives:** Osteocytes are the main cells of the mature bone. They undergo apoptosis for many reasons. Literature is found deficient on the effects of injury after injury in the area surrounding the bone. This study was aimed to see the effect of injury on the osteocyte number when there is healing process going on in the immediate vicinity of injury. Intervertebral disc area was selected for this purpose as it is enclosed by the vertebral bone.

**Method:** Rabbits were used as experimental animals and their intervertebral disc were injured by open surgery. Animals were divided into two subgroups with 22 animals in each ( $N = 44$ ). Day 5 and 7 were selected for harvesting the experimental tissue, as cellularity of injured area increases gradually after injury due to recruitment of inflammatory cells and beginning of granulation. Vertebral bones with intervertebral discs were obtained after sacrificing the animals, decalcified and prepared for paraffin embedding. Slides were stained with hematoxylin & eosin and alcian blue stain. Osteocytes were counted in a defined unit area under high power in the two groups and the average of the two groups was compared.

**Results:** Osteocyte number decreased significantly ( $p < 0.001$ ) on the day 7 as compared to the cell count on day 5. It was observed that osteocyte population followed a reverse population pattern as compared to cells of inflammation.

**Conclusion:** It is gained from the study that chemical mediators released by inflammation may be causing the regression of osteocytes by apoptosis, which may be one of the forms for the bone remodeling after injury in the area near the healthy bone.

**Key words:** Cancellous bone, injury, intervertebral disc, inflammation, osteocytes, vertebra.

### INTRODUCTION

Mature bone is mainly formed by the osteocytes. Bone remodeling and mineral homeostasis is initiated by these cells. Osteocytes have a specific cell shape and their molecular structure is also unique. They are stellate in shape and possess many long, thin dendritic processes. Body of these cells is found in the bone lacuna and the slender dendritic processes protrude into the canaliculi to neighboring osteocytes<sup>1</sup> (Figure 1). The bony lacunae are tinyholes within the bone matrix. Their special arrangement can alter the mechanical properties of the this tissue.<sup>2</sup>

After injury to any tissue starts the process of healing with inflammation that is a chemically mediated process. The process of healing starts at the same time as the injury. It is a complex chemically mediated growth process beginning with hemostasis and involving accumulation of inflammatory cells, laying down of granulation tissue and neovascularization.<sup>3</sup> In the previous studies by the author, it had been found that the injury affects the surrounding tissue in a variety of

ways. Literature was found deficient in observing the effects of injury on the bone cells.

It is presumed that these mediators may also affect the population curve of osteocytes near the site of injury. The present study was designed and conducted to see the response of osteocyte population to the injury in the surrounding region. Rabbits were selected as animal models since their intervertebral disc resembles human discs.<sup>4</sup>

### ANIMALS AND METHODS

The research work was done at Department of Anatomy, College of Physicians and Surgeons of Pakistan, Islamabad, using its ancillary facilities for research.

Healthy young rabbits were chosen as experimental animals for the study. All the animals were housed and treated ethically as per the guidelines of National institute of health sciences animal house Islamabad for the experimental animals.

Animals ( $N = 44$ ) were divided into two groups A1 and A2 with 22 animals in each group.

Young male healthy 4 – 6 month old (0.9 – 1.5 kg) rabbits of “Domestic rabbit (*Oryctolagus cuniculus*)” were used in the study. Animals that did not fulfill the following criteria were excluded from the study;

- i. Diseased rabbits/rabbits with any visible skeletal deformity.
- ii. Rabbits developing wound infection during the course of study period, i.e. from the time of operation till the day of sacrifice.
- iii. Rabbits showing any signs of spinal cord injury after the operation.
- iv. Species other than the domestic rabbit.

To begin with the surgery, animals were anaesthetized by a combination of ketamine (KETLAR 500) and xylazine (XYLAZ).

A midline cut was given on the shaved ventral abdomen of the rabbit. Abdominal was explored for spine and vertebral column located. White Intervertebral disc was stab-wounded with a sharp blade-up to a depth of 4mm. Abdomen was then stitched together in layers. Wound was kept dry and clean and observed for any infection.

Animals in group A1 were sacrificed after 5 days and ones in A2 were sacrificed after 7 days of surgery.

Adjacent injured vertebrae with intervening intervertebral disc were obtained and fixed in 4% buffered formalin. The tissues were then decalcified and prepared for embedding into paraffin blocks. 10µm thick coronal sections of the specimen were made.

Tissue was stained with hematoxylin and eosin and Alcian blue stain for observing the cell numbers.

Quantitative data was noted for number of osteocytes in a defined per unit area of the slide under 100X. Osteocytes were counted in the area that was nearer to the area of injury i.e, towards the intervertebral disc side (Figure 2).

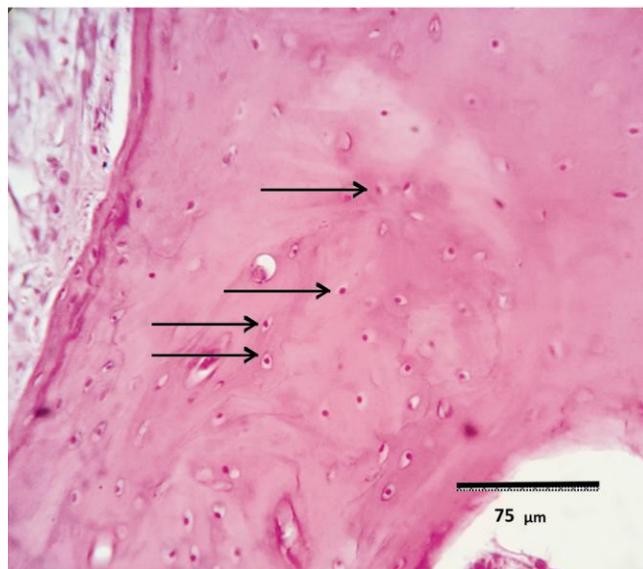
Data thus obtained was analyzed using the SPSS version 16. T-test was applied to compare the means of osteocytes of two groups. A p-value of equal to or less than 0.05 was taken as statistically significant level of difference between the means of the cell population.

## RESULTS

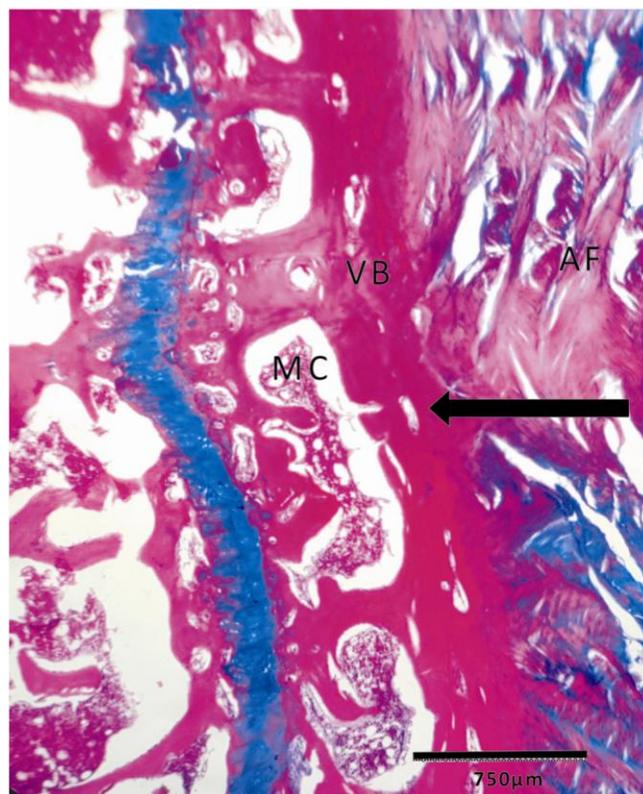
The total population of osteocytes in vertebral bone was found to be reduced significantly ( $P < 0.001$ ) in animals that were sacrificed on the 7th day of experiment as compared to the ones on the 5th day of experiment (Table 1). Alcian blue stain improved the contrast by staining the ground substance.

## DISCUSSION

Since long the bone-bound cells, osteocytes, had been considered a comparatively quiet cell with a yet to be explored part in bone. Recently it has been reported that they secrete a number of chemicals that control the native mineralization and differentiation of bone cells (Figure 3).



**Fig. 1:** Osteocytes (arrows) population in A2 group as seen by H&E stain under 40X. Cells can be seen in the lacunae.



**Fig. 2:** A low powered photomicrograph showing the area of bone where the osteocytes were counted (broad arrow) at 4X. Annulus fibrosus (AF), Cellulous bone (VB) and bone marrow cavity (MC) is also shown. Blue stained areas is full of collagen stained by Alcian blue. H&E and Alcian blue stain.

**Table 1:** Statistical data of the osteocytes and their calculated p-value.

GROUP STATISTICS					
Sub-Group	N	Mean	± S.D	± S.E.M	p-value
A1	22	17.3168	3.04608	0.64943	< 0.001
A2	22	9.9682	3.40998	0.72701	

However, the explanation for the progress in the biological significance of osteocyte preprogrammed death, is yet to be completely understood.<sup>5</sup>The finding that in case of rapid multiplication of these bone cells, as occurs in growth or remodeling during healing of injury to bones, renewed interest in osteocytes.<sup>6</sup>

In the previous studies by the author,<sup>7,8,9</sup> it has been found that injury and inflammation has profound effects in the vertebrae and its adjacent areas, from bone marrow, cartilaginous growth plates etc., to the intervertebral discs itself.

The current study is amongst the series designed to understand the spread of inflammatory process and its effects on cell population in the surrounding areas. In the process of inflammation it is known that the cells of inflammation increase steadily from the day of injury, gradually reaching a maximum and then there is a gradual decline in their population. However, in this study we found that, in case of osteocytes, the curve was opposite as compared to inflammatory cells.

This finding may be due to a negative feedback of the inflammatory mediators on the osteocyte growth and multiplication. Also, the chemical mediators of inflammation may be sending signals for apoptosis to the osteocytes. This may be one of the pathways of remodeling of the bone in case of injury to tissue surrounding the bone.

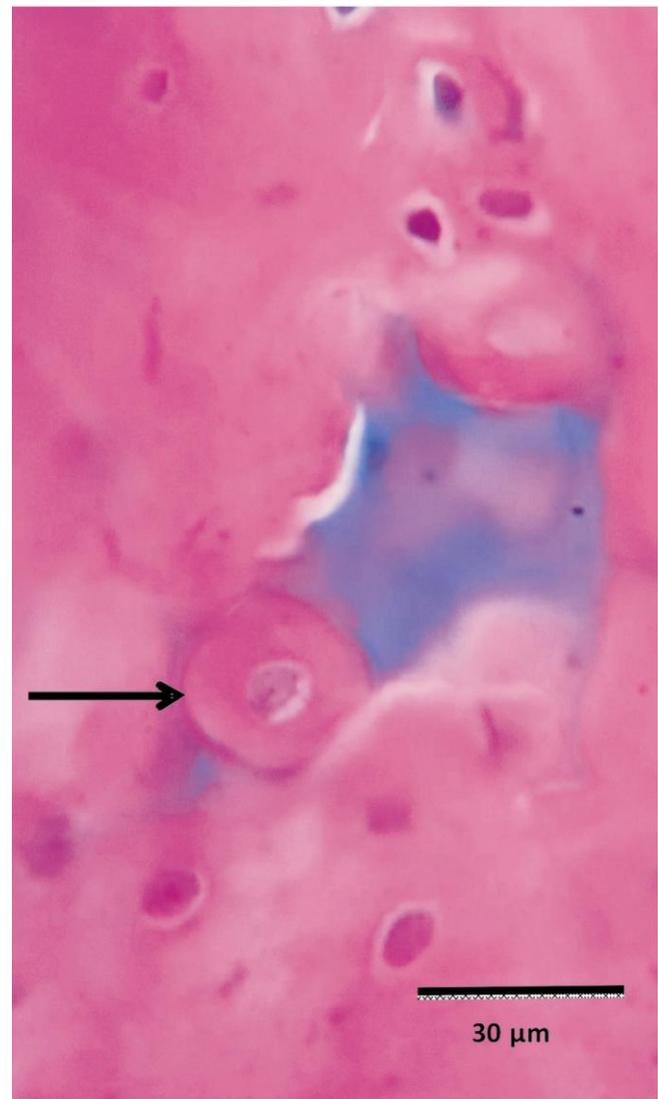
The study **concluded** that the injury in the region surrounding the bones, and not directly to the bone, itself can alter the population of the osteocytes. As this was a basic study, its findings may be elaborated in a histochemical study to outline the mediators or markers for the process. The effects of osteocyte demographic shift need to be elucidated in further studies.

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#### Authors' Contribution

WAC:



**Fig. 3:** An osteocyte with a freshly laid down osteoid (arrow) around it in group A2. Blue colored staining of matrix with alcian blue can also be seen.

#### Conflict of Interest

Nil.

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