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Age related histological changes in the articular cartilage of acetabulum of hip joint in buffalos (*Bubalus bubalis*)

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Abstract

Microscopic features of acetabular articular cartilage showed that it was entirely cartilaginous in prenatal stage of Group-I since articular cartilage was not yet differentiated. Articular surface was smooth with numerous chondrocytes in random arrangement in second zone. Large chondrocytes were in homogenous cartilage matrix in mid zone amongst cartilage canals. Acetabular articular cartilage was appreciable in all post-natal groups since it was clearly differentiated from underlying sub-chondral bone. Ligamentous tissue of acetabular labrum infiltrated into the articular cartilage on one side in specimens of groups II, III and IV. Deeper zone closer to tide mark showed larger chondrocytes in isogenous groups and calcified zone showed atrophied chondrocytes with few areas of calcification. In group III and IV specimens of five and half and eight years old surface of acetabular articular cartilage was uneven in outline. Second zone showed slightly large and oval chondrocytes in lacunae and deeper zone above the calcified part consisted elongated chondrocytes arranged in perpendicular columns. Acetabular labrum blended with articular cartilage of acetabulum at one side invaded by bundles of collagen. Superficial zone was devoid of chondrocytes in articular cartilage of Gr-IV specimens.

Keywords: Acetabulum, articular cartilage, buffalo, hip joint

1. Introduction

The acetabulum is the concave cotyloid cavity with 'C'-shaped articular surface, the lunate surface, which is lined by hyaline cartilage. The rim of the acetabulum is lined with a fibrocartilagenous acetabular labrum, part of which bridges across the acetabular notch as the transverse acetabular ligament. Articular cartilage plays an important role in joint diseases especially in osteoarthritis where there are specific changes in it and also its repair and remodelling (Taylor *et al*., 2011) [8]. A predictable pattern of age-dependent degeneration on both femoral head and acetabulum in human hip joints was observed by Bullough *et al*. (1973) [1]. They noted possible relationship between age-dependent degenerative changes and senile degenerative joint disease in humans. Gupta and Goyal (2016) [3] studied the microstructure of femoral cartilage in humans of different ages and stated that four clear zones were demarcated with advancing age. They inferred that signs of degeneration were seen beyond forty years of age in humans. Information about age related changes in the acetabular articular cartilage was scanty. Preseent study was taken up to provide data about changes in the articular cartilage of acetabulum in buffaloes.

2. Materials and Methods

Intact hip joint specimens of twenty-four (24) apparently healthy buffaloes irrespective of their breed, sex and nutritional status were procured from GHMC Modern Abbattoir, Chengicherla and local slaughter houses in and around Hyderabad. Collected specimens were divided into two primary groups *viz.*, one Prenatal stage - Group I (as per the formula of Soliman 1975) [7] and three Post-natal stagesas per their approximate age based on dentition pattern by FAO (1994) [2] such as Group II (neonatal), Group III (young adult) and Group IV (aged). Samples were collected from both hind limbs by cutting at the level shaft portions of the three bones oscoxae (Ilium, ischium and pubis) to collect intact acetabulum.

Ageing of prenatal specimens was determined by measuring the CVRL (Curved Crown Rump length) of the foetus and its approximate age was estimated by the formula of Soliman (1975) [7] *i.e.*, $Y=28.66 \pm 4.496 X$ (if CVRL is ≤ 20 cm) and $Y= 73.544 \pm 2.256 X$ (if CVRL is ≥ 20 cm).

For post natal specimens the dentition pattern of the animals before slaughter was carefully noted and approximate age assessed as per (FAO, 1994) [2]. Soon after collection they were first cleaned with wet cloth to remove blood stains and pressed firmly to drain excess fluids after which they were packed neatly in polythene bags and kept in ice box for immediate transportation to the laboratory.
For histological studies, tissue pieces of articular cartiage acetabulum of all four groups were collected and fixed in 10% NBF (Singh and Sulochana 1997) [6]. The fixed specimens were processed either un-decalcified or decalcified wherever necessary for routine paraffin processing technique and sections of 6 – 8 µm were cut and subjected to the following staining methods:

1. H & E staining for detailed micro-architecture (Singh and Sulochana, 1997) [6].
2. Van Gieson’s technique for collagen fibers (Luna, 1970)
3. Masson’s Trichrome for metachromasia of cartilage matrix and collagen fiber differentiation (Singh and Sulochana, 1997) [6].
4. Safranin ‘O’ method for cartilage matrix (Singh and Sulochana, 1997) [6]

Stained histological slides of all four groups were examined and read to note any anatomical changes in articuar cartilage of acetabulum with regards to routine micro-architecture and histological changes if any. Results were noted and photographed accordingly.

3. Results and Discussion

In three and four month old fetuses of prenatal Gr- I, acetabular articular cartilage was not yet differentiated. Articular surface was smooth and regular in outline below, which numerous chondrocytes were seen in random arrangement in second zone (Fig. 1). Large chondrocytes were seen in homogenous cartilage matrix in mid zone amongst cartilage canals with different blood vessels (Fig.2). Similar reports regarding prenatal articular cartilage by several authors were made in articular cartilage of animals and humans, which was mainly hyaline cartilage during development. In the literature reviewed so far most studies existed on acetabular labrum rather than articular cartilage of acetabulum.
The acetabular cartilage was appreciable in all post natal groups. It was clearly differentiated from underlying sub-chondral bone. Ligamentous tissue of acetabular labrum infiltrated into the articular cartilage on one side of the articular cartilage in specimens of groups II, III and IV. Superficial zone of articular cartilage was thin and comprised of small elliptical shaped chondrocytes arranged parallel to articular surface in specimens aged approximately 1.5 yrs. The next zone was wider with paired chondrocytes arranged almost in columnar fashion (Fig. 3). Ligamentous tissue of acetabular labrum infiltrated into the AC on one side. Deeper zone closer to tide mark showed larger chondrocytes in isogenous groups and calcified zone showed atrophied chondrocytes with few areas of calcification (Fig. 4). These findings are in agreement with reports of Seldes *et al.* (2001) [5] in adult human hips regarding the fibro-cartilaginous labrum. They stated that it was contiguous with the acetabular articular cartilage through 1.0 to 2.0 mm zone of transition. They also stated that a consistent projection of bone extended from bony acetabulum into the substance of the labrum that is attached via a zone of calcified cartilage and a well-defined tidemark.

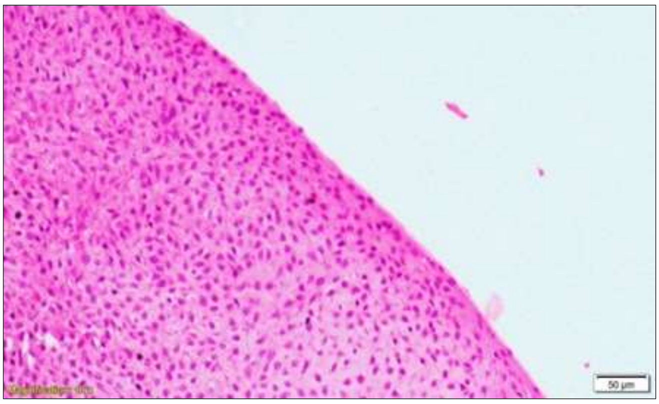

Fig 1: Photomicrograph of surface of acetabular cartilage showing high cellularity of chondrocytes (black arrow) in prenatal groups aged 3 months (Group-I). H&E 10X (Cross section)

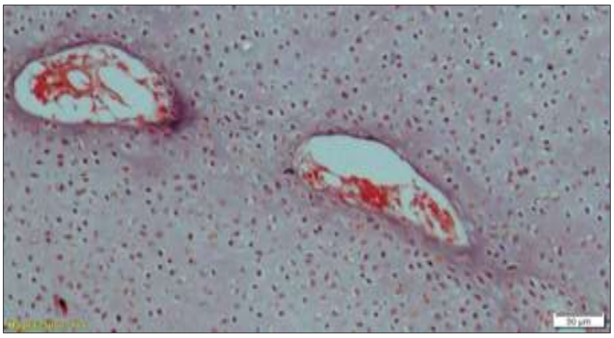

Fig 2: Photomicrograph ofacetabular cartilage showing cartilage canals (CC) in deeper zone in prenatal groups aged 4months (Group-I). Safranin O stain10X (Cross section)

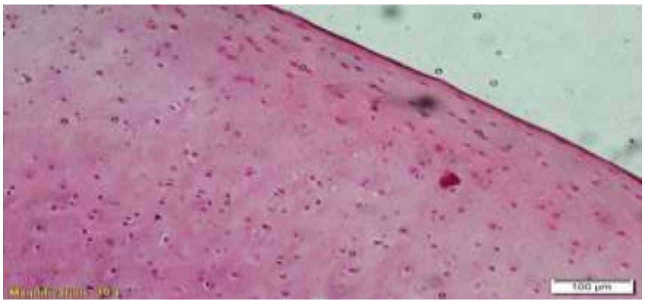

Fig 3: Photomicrographofacetabular cartilage showing superficial zone (zone I) and mid zone (zone II) in 1.5 years old buffalo (Group-II). H&E 10X (Cross section)

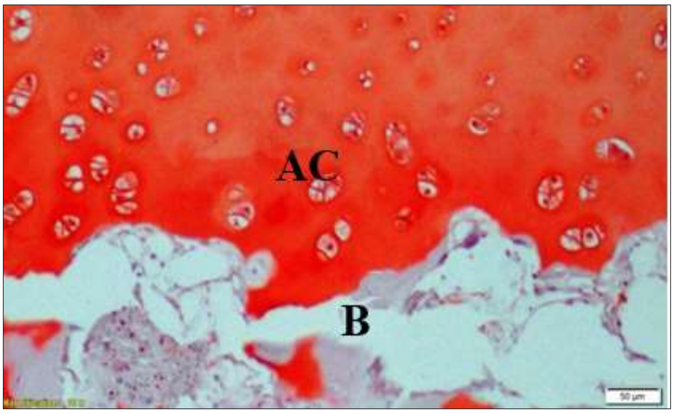

Fig 4: Photomicrograph of Acetabulum showing deep zone (AC) and subchondral bone (B)in 2.5 years old buffalo (Group-II).

Safranin O stain10X (Cross section)

In group III and IV specimens of five and half and eight years old in this study the surface of acetabular articular cartilage was uneven in outline. Second zone showed slightly large and oval chondrocytes in lacunae and deeper zone above the calcified part consisted elongated chondrocytes arranged in columns in perpendicular fashion to the surface of articular cartilage. Calcified zone showed few degenerating cells and was clearly demarcated by a tide mark from underlying sub-chondral bone (Fig. 5). Acetabular labrum blended with the articular cartilage of acetabulum at one side wherein bundles of collagen fibres extended into cartilage matrix. Superficial zone was almost devoid of chondrocytes in articular cartilage of Gr-IV specimens.

In view of the close association of acetabular labrum with articular cartilage of acetabulum, Kim *et al.* (2019) stated that damage to acetabular labrum is associated with cartilage degeneration in porcine hip joint in their studies regarding effects of labrectomy on articular cartilage contact area. Their results showed that changes in articular cartilage followed labrectomy wherein degenerative process of cartilage was initiated.

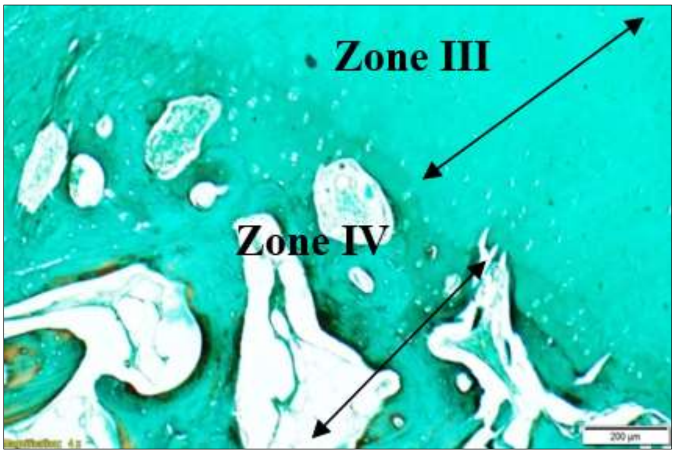

Fig 5: Photomicrograph of acetabular AC showing deep zone (zone III) and calcified zone (zone IV) in 8.0 years old buffalo Group-IV). Masson's trichrome stain 4X (cross section)

4. Conclusion

The results of this investigation infer that acetabular articular surface is well protected by the labrum around its margin. Only the inner portion *i.e.*, closer to the pubic bone, the articular cartilage showed some signs of wear and tear were observed.

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