Vertebral End-Plate Changes With Aging of Human Vertebrae

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The present study describes the sequential age changes within the growth and articular layers of the cartilaginous end-plates of vertebrae from humans varying in age from birth to 73 years. There is a gradual reduction in the width of the growth cartilage up to 16–20 years of age. During adulthood and progressing into old age (60–73 years), the end-plates consist of only articular cartilage which undergoes calcification followed by resorption and replacement by bone. Age changes are observed in the arterioles, capillaries, and venules found in the nutrient canals or spaces of the bone adjacent to the cartilage or disc. The calcification of the articular cartilage and vascular changes seen in the older vertebrae would impede the passage of nutrients from the blood to the disc proper. Collagen fibers are observed arising from the older vertebral end-plates to course into the midregion of the disc. [Key words: age changes, end-plates, microvasculature, calcification]

Previous studies confirming the avascularity of the intervertebral disc have indicated that the nutrition of the disc is mainly dependent upon the diffusion of nutrients and other substances through the central part of the cartilaginous end-plates.1,4,5,9 It has also been shown that the permeability of the articular cartilage decreases with age.9,11,12 In spite of these studies, there has been only speculation of the morphologic basis to explain the loss of diffusion through the end-plates. It has been suggested that there may also be an undefined reduction in blood flow to the bone, end-plates, and disc proper.

In a recent study by the authors,3 sequential changes within the layers of the end-plate cartilage of vertebrae were described in marmosets from birth to old age, showing a gradual narrowing with alternate disappearance of the growth cartilage and simultaneous histochemical changes in the articular cartilage, leading to mineralization of this layer. It was assumed that the calcification of the articular cartilage would impede the diffusion of nutrients from the bone microvasculature through the calcified cartilage zone to reach the disc.

The present investigation was undertaken to demonstrate the age changes in human lumbar spine from birth to old age, with special emphasis on the end-plates and the microvascular components in the bone adjacent to the articular cartilage.

MATERIALS AND METHODS

Lumbar vertebrae from humans varying in age from birth to 73 years were studied. Specimens were obtained from both male and female individuals. Two adjacent lumbar vertebrae and the intervertebral disc were removed at the time of autopsy and fixed in an alcohol–formol acetic acid solution. Following decalcification in 10% nitric acid in 10% formalin, the specimens were embedded in nitrocellulose in the routine manner. The embedded blocks were sectioned 14 to 20 μm in thickness, and stained with hematoxylin and trisvin, PAS-hematoxylin, alcian blue (pH 2.5) PAS, and silver nitrate impregnation.

RESULTS

In the following description of the findings, a positive PAS reaction (red in color) indicates the presence of neutral polysaccharides (proteoglycans) and silic
acid in the matrices of cartilage or bone. A pink-staining matrix with the PAS reaction confirmed by a positive alcian blue staining demonstrates the existence of glycosaminoglycans (chondroitin -4 (6)-sulfate). An arylphilic fiber as visualized by silver nitrate impregnation is a collagen fiber.

The end-plates of the lumbar vertebrae from new-borns consisted of an inner growth zone and a articular region bordering on the intervertebral disc. The growth cartilage was characterized by parallel columns of proliferating and hypertrophic cells. A thin, calcified cartilage zone was present adjacent to the diaphyseal trabeculae. The outer articular cartilage consisted of spindle-shaped chondrocytes scattered throughout the matrix. There was a difference in the staining response between the growth and articular cartilages following exposure to the PAS reaction. The growth cartilage appeared pink, whereas the articular zone was red in color, representing the variations in the type of mucopolysaccharide contents of the matrix in each zone (Figure 1a). This variance in staining response was also demonstrated with silver nitrate impregnation, in which the growth cartilage exhibited negative staining in contrast to the grayish-black appearance of the articular cartilage (Figure 1b).

In vertebrae from individuals 10 to 15 years of age, there was a reduction in the width of the growth zone with a decrease in the number of irregularly arranged proliferating cells. The matrix still stained pink in color following exposure to the PAS reaction. The articular cartilage was relatively thicker and stained intensely PAS-positive (Figure 2a). The articular cartilage was arylphilic in nature whereas the growth cartilage still stained negative after silver nitrate impregnation (Figure 2b).

Specimens obtained from individuals 17 to 20 years of age exhibited a sealing-off of the cartilage by bone. The remnants of the growth cartilage stained basophilic with hematoxylin and appeared homogenous in nature. Cracks were found within the calcified mass.
The articular cartilage, on the other hand, stained intensely with the PAS reaction (Figure 3).

The end-plates of vertebrae from adults 20 to 40 years of age consisted only of an articular cartilage layer that stained deep red after the PAS reaction. A pink matrix containing chondrocytes was observed in the region of the disc adjacent to the articular cartilage. Pinkish-staining fibers were arising from the articular cartilage and coursing through the pink matrix of the disc (Figure 4a). The matrix of the articular cartilage, following silver nitrate impregnation, showed fine silver granules deposited throughout intercellular regions. The pinkish-staining fibers seen in Figure 4a were found to be argyrophilic, indicating that they were collagenous in nature (Figure 4b). With higher magnification, fine collagenous fibers were demonstrable in the matrix; other areas showed the intimate relationship between the collagen fibers and silver granules; and in other regions only heavy deposits of silver granules were seen "masking" over the collagenous elements as well as the ground substance (Figure 5).

No staining differences were noted in the articular cartilage in vertebrae from individuals 45 to 73 years of age. In the 45 to 60 year age group, there was resorption of the calcified articular cartilage with replacement by bone (Figure 6). By the age of 65 years, only a narrow strip of articular cartilage separated the disc from the underlying bone (Figure 7a). With silver nitrate impregnation, collagenous fibers were seen arising from both the cartilage and bone passing into the disc in an interweaving pattern (Figure 7b).

Figure 8 shows an end-plate and underlying bone from a 73-year-old female. Only a very thin, incomplete bony plate was observed adjacent to the narrowed calcified articular cartilage. Severe osteoporosis of the body of the vertebra was apparent.

Vascular changes were seen in the bone adjacent to the articular cartilage and disc in individuals over 45 years of age. The nutrient canals and spaces in the bone adjacent to the cartilage were partially or completely occluded with an intensely PAS-positive material (Figure 9). The walls of the arterioles and venules within these spaces were homogenous in nature and deep red in color. In contrast, the canals and spaces in

Fig 4. Lumbar vertebra from a female in her middle 30s. Notice that the bone is directly adjacent to the articular cartilage. The articular cartilage appears deep red after PAS exposure (a). At the cartilage disc border, a pinkish material containing chondrocyte-like cells is present in the nucleus pulposus. In addition, pink colored fibers are radiating into the nucleus. b. An alternate section exposed to silver nitrate impregnation reveals that the articular cartilage is strongly argyrophilic, and the fibers are collagenous in nature. A = articular cartilage, B = bone; C = collagen; N.P. = nucleus pulposus (photographically reproduced at 85% of original magnification ×50).

Fig 5. Higher magnification of the end-plate illustrated in Figure 4b. Notice the presence of (A) fine collagenous fibers, (B) silver granules that are associated with the collagenous fibers as well as being free in the matrix, and (C) the heavy concentration of granules and fibers in the capsular areas (silver nitrate impregnation) (photographically reproduced at 82% of original magnification ×250).

Fig 6. Lumbar vertebra from a 55-year-old male. Note the invasion of bone into the articular cartilage. A = articular cartilage; B = bone (PAS hematoxylin; photographically reproduced at 96.5% of original magnification ×50).
the vertebral endplates in transporting nutrients to the avascular intervertebral discs. It is generally agreed that the discs in adults are nourished by diffusion of substances through the cartilaginous end-plates and that there is a decrease in permeability through the end-plates in vertebrae from aging animals and man.4,9,11,12 Questions prevail about whether the loss of permeability is due to physical and/or chemical change in the articular cartilage or due to changes in the vascular supply of the underlying bone.

Bernick et al3 have recently demonstrated progressive age changes in the marmoset vertebrae from birth to old age. In the old animal, as demonstrated by histochemical staining, there was a gradual decrease in the stainable sulfated glycosaminoglycan, chondroitin sulfate, and keratin sulfate in the articular cartilage. In adult and aged animals, the articular cartilage became intensely PAS-positive, indicating the presence of neutral mucopolysaccharides and sialic acid. The cartilaginous matrix of the end-plate in growing animals contained fine fibrillar collagen elements (type 2) that were masked by the carbohydrate moiety so that the matrix appeared homogenous following silver nitrate impregnation. At the time of vertebral maturity, fine arylphophilic collagen fibers were demonstrable with silver nitrate impregnation. The presence of arylphophilic collagen fibers, neutral mucopolysaccharides, and a depolymerization of GAG provided the environment for mineralization of the matrix. The mineralization of the articular cartilage not only makes it susceptible to osteoclastic activity but would restrict the diffusion of substances.

Similar changes were observed in the present study on human vertebrae. The sequence in the aging of the human lumbar spine from birth to old age is summarized in Figure 11. Beginning in adults (age 20–40 years), there was a gradual mineralization of the articular cartilage. As with the marmoset vertebrae, there was resorption of the calcified cartilage during middle and old age and a replacement with bone so that in the vertebrae from individuals over the age of 60 years only a thin layer of calcified cartilage or bone separated the disc from the body of the vertebrae. The presence of calcified cartilage would therefore interfere with movement of nutrients through the end-plates and substantiates the findings of Marasdas et al,7 Marasuda et al,8 and Brown5 that there is a decrease in diffusion of substances in aging end-plates.

One can subscribe to the thesis that calcified arti-
carilage in aged vertebrae impedes diffusion of substances into the nucleus pulposus. Other factors must be considered. In animals and man, before sexual maturity, blood vessels are in contact with the bone cartilage interface, permitting age of substances through the cartilage. These cartilage buds allow nutrients to pass from the blood to the cartilage and into the nucleus pulposus. In the young vertebrae, the progressive resorption of the fibrous cartilage allows the invasion of bone to reach the border of the disc. The microvasculature within the nutrient spaces or canals would permit materials to pass eventually into the nucleus pulposus. Examination of the boy nutrient spaces or canals adjacent to the disc in the aged vertebrae revealed that the walls of the arterioles, capillaries, and venules within these spaces were thickened and intensely PAS-positive. In addition, the spaces themselves contained varying degrees of PAS-positive material. Many were completely obliterated with the carbohydrate moiety or even calcified. The changes to the microvasculature as well as obliteration of the

Fig 11. Schematic drawing summarizing age changes of the lumbar vertebral end-plates. a. The growth stage consisted of an active growth cartilage zone characterized by regular columns of proliferating and hypertrophic chondrocytes. b. The maturation stage. There is a decrease in the number of proliferating chondrocytes, leading to a narrower, irregularly arranged growth cartilage. Bone is sealing off the cartilage border. The growth cartilage has completely disappeared by stage c, and bone is directly apposed to the articular cartilage. The articular cartilage is undergoing calcification. Collagen fibers arising from the articular cartilage course into the nucleus pulposus. In stages d and e there is a progressive resorption of the articular cartilage with replacement by bone so that during stage e only a very thin layer of cartilage separates the underlying bone from the disc.
nutrient spaces or canals would retard passage of substances from the blood into the bone, cartilage, and disc. These age changes in the vasculature are not restricted to the vessels of bone but are found throughout the soft tissues, as shown by Ballard et al\textsuperscript{2} and deMignard and Bernick.\textsuperscript{6} The findings of the present study contradict the views of Brown et al\textsuperscript{5} and Amato et al,\textsuperscript{1} who, on the basis of their own studies, found no change in the vasculature.

An interesting incidental finding of this study was the demonstration of collagen fibers radiating from the articular cartilage into the nucleus pulposus. The appearance of these collagen fibers were first noted in the vertebrae having reached skeletal maturity. These collagen fibers were independent of the anchoring fibers of the annulus. The attachment of these fibers into the disc may give stability to the disc at times when the articular cartilage is calcified or replaced by bone.

REFERENCES


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