ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at: www.ijmacr.com

Volume – 4, Issue – 5, September – October - 2021, Page No. : 81 – 86

Embryogenesis of human lumbar intervertebral disc

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How to citation this article: Dr Shahnaz Choudhary, Dr Sheetal Chandel, Dr Sonia Jandial, Dr Misbah, Dr Parveen Akhter, "Embryogenesis of human lumbar intervertebral disc", IJMACR- September – October - 2021, Vol – 4, Issue - 5, P. No. 81 – 86.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

The present study is designed to gain insight into the pattern of sequential development of various components of the intervertebral disc, like the nucleus pulposus, annulus fibrosis and their vascularisation. Study of the intervertebral disc of lumbar region was carried out on 35 foetuses ranging from 53mm crown rump length to 300mm crown rump length. An examination of the literature showed that not many studies have been conducted on the development of intervertebral disc in humans. The scanty literature and the difference in opinion regarding the development of intervertebral disc was a prompting factor to undertake this study.

Key words: Spinal cord, intervertebral disc, CRL (crown rump length).

Introduction: Human vertebral column consists of 33 bony blocks known as vertebrae, which sit one on the top of another and there are flexible cushions between the bodies of the vertebrae. These cushions are called the intervertebral discs. Intervertebral disc forms approximately 25% of the length of the vertebral column above sacrum and 33% of length of the lumbar column. Disc composition changes during development, growth, aging and degeneration and this in turn alters how disc responds to mechanical stress (Christopher et al. 2003).

There are 23 intervertebral discs in the normal vertebral column. They are thinnest in the thoracic region and thickest in the lumbar region. The structure of all the intervertebral discs is relatively the same (Coventry et al. 1945). Intervertebral disc grossly consists of two parts-an outer fibrocartilage nous ring known as annulus fibrosus and a central soft mucoid portion, the nucleus pulposus. The annulus fibrosis consists of concentric layers of collagen fibers running obliquely from one vertebral body to another. The angle of the collagen bundles alternates between successive lamellae thus forming a cross-woven

and reinforced structure (Hukins 1984). The nucleus of the disc act as shock absorber absorbing the impact of the body activities and keeping the two vertebrae separated (Mccann MR etal.2012).

The lumbar vertebral region bears most of the strain of the spinal cord and is therefore the region of interest for the present study.

Materials and methods:

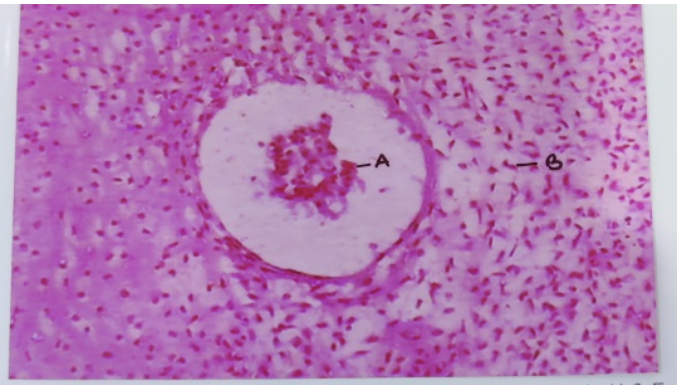
The present study was done on 35 foetuses of varying gestational age ranging from 53mm to 300mm crown rump length. Foetuses with any type of congenital malformation were not included in the study. Gestational age was determined on crown rump length of the foetus and according to the rule as described by Hamilton, Boyd and Mossman (1976) which states that at 32 days of intrauterine life, embryo is 5mm in crown rump length. For each additional day upto 55th day, the embryo grows at the rate of 1mm per day and after 55 days, embryonic growth is 1.5mm per day with respect to crown rump length.

The lumbar region of the vertebral column was dissected out. Collected specimen was kept in jars containing 10% formalin for large specimen and Bouin's solution for small specimen. After histological processing of the tissue, serial sections of 7micrometre were cut in transverse and longitudinal manner and stained with Haematoxylin and Eosin and Masson's trichrome stains and then seen under microscope and photographs taken.

Result:

An effort has been made to present the results of the prenatal developing intervertebral disc in an organised way in this report. Developmental changes at different ages and various components of intervertebral disc have been classified into following categories: annulus fibrosus, nucleus pulposus, time of appearance of vascularisation and appearance of changes in vertebral bodies.

53 mm CR Length to 58 mm CR Length (72 days to 75 days) foetuses: The intervertebral disc appears almost biconcave and consists of a central portion and a peripheral part. The central part, which is smaller in diameter, is composed of notochordal cells (fig 1). Spaces are seen between these cells which gives a network-like appearance to this tissue which is called 'chorda-reticulum. This region represents the nucleus pulposus of the intervertebral disc. The peripheral part of the intervertebral disc is made of mesodermal tissue and represents the annulus fibrosus. The circumferential part of the disc shows well defined fibres with spindle shaped cells along long axis of fibres. No signs of vascularity observed at this stage. The vertebral bodies are made of cartilage cells in lacunae at this stage.



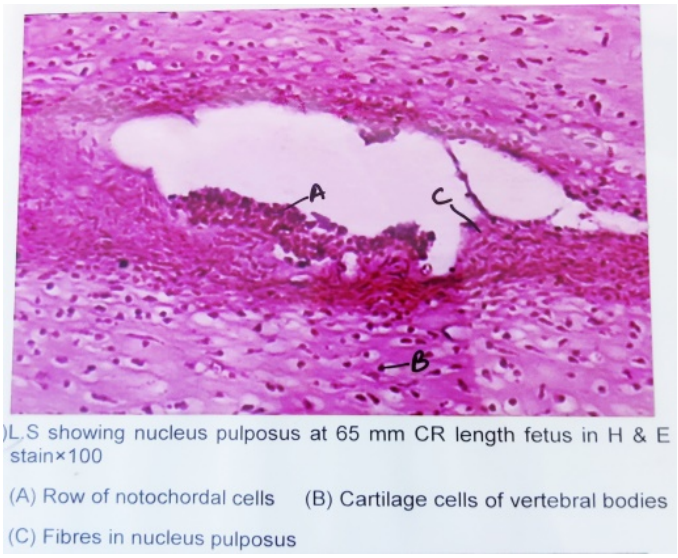
T.S showing intervertebral disc at 53 mm CR length fetus in H & E stain×100

(A) Notochordal cells in nucleus pulposus
(B) Spindle shaped cells

Fig 1

65 mm CR length to 76 mm CR Length (80 days to 88 days) foetuses: At this stage the intervertebral disc is seen bulging out beyond vertebral bodies. At the circumference i.e in the region of annulus fibrosus, well defined fibres are present which are running in parallel rows and their terminations are lost in the adjacent vertebrae. The

innermost fibres are halfway as long as the outermost fibres. Small spindle shaped cells with condensed nuclei appear to lie along the long axis of fibres. The cartilage canals are seen in the peripheral region of the vertebral bodies, but no vascularisation of intervertebral disc is seen at this stage of development (fig 2).



)L.S showing nucleus pulposus at 65 mm CR length fetus in H & E stain×100

(A) Row of notochordal cells (B) Cartilage cells of vertebral bodies

(C) Fibres in nucleus pulposus

Fig. 2

95 mm CR Length-110 mm CR Length (100 days to 110 days) foetuses: There is increase in the thickness of intervertebral disc. The fibres in annulus fibrosis are arranged in form of bundles and they form the lamellae. The lamellae towards the periphery are wider and more discrete and those towards the centre are less prominent and more concentrically arranged. Vertebral bodies show well defined centres of ossification. Along the peripheral fibres of the annulus fibrosis, a few small blood vessels are present in the dorsolateral areas of the disc but no vascularisation of the inner part is seen.

In 115mm CR Length- 125mm CR Length (113 days to 120 days) foetuses: The intervertebral discs as well as the vertebral bodies are growing with cranio-caudal convexity. The notochordal area is seen expanded with chorda-reticulum and intercellular spaces containing a homogenous substance and the notochordal cells being scattered in a uniform manner (fig 3). Some blood vessels are present in the dorsolateral area of the disc, but there is no vascularisation of the inner area. The cartilage canals of vertebral bodies have increased in number and size.

In 130mm CR Length – 145mm CR Length (123 days to 133 days) foetuses: The region of annulus fibrosus show splitting and joining of the fibres of different lamellae and an interlocking of various lamellae. Ventrally there are fibres of the anterior longitudinal ligament. However on the whole, the fibres of the annulus fibrosus remain more condensed at the periphery of the disc and concentrically arranged in the centre. The vertebral bodies show well defined centres of ossification and large number of cartilage canals. vascularization is seen along the dorsolateral areas of the disc but not of the inner part.

In 155mm CR Length- 172mm CR Length (142 days to 151 days) foetuses: The fibrous component of individual disc has increased in volume and the disc is thicker at its circumference than at its centre. As we go towards the centre, close to nucleus pulposus, the characterstic feature observed is that the cells in the annulus fibrosus seem to occupy lacunae parallel to fibre bundles. The lacunae are small, each containing single cell. The majority of cells being spindle shaped resembling cartilage and referred as specialized embryonic cartilage. The peripheral fibres of the annulus fibrosus show blood vessels where as central portion is still avascular.

In 180mm CR Length- 200mm CR Length (158days to 170 days) foetuses: The intervertebral disc is well demarcated now due to increase in its thickness. The vertebral bodies show well formed spongy bone with trabeculae and spaces containing the bone marrow. Vascularisation is evident only in the outer part of annulus fibrosis.

In 220mm CR Length- 240mm CR Length (183 days to 196 days): At this stage principal change is in the nucleus pulposus. This has enlarged and in a medial saggital section is elongated to occupy half of the length of intervertebral region. The remains of chorda-reticulum are present. The cartilage plates are well developed showing features of hyaline cartilage. The blood vessels are seen only in the peripheral portion of annulus upto fibrocartilagenous tissue. The vertebral bodies are showing the centres of ossification and trabeculae with marrow spaces.

In 280mm CR Length - 300mm CR Length (223 days to 236 days) foetuses: (fig 3) The intervertebral disc is seen bulging beyond the vertebral bodies. As seen ventrodorsally there are fibres of the anterior longitudinal ligament, deep to which are several very dense lamellae. Towards the nucleus pulposus, the fibres are arranged in a homogenous matrix. The nucleus pulposus is extensive and occupied by fibrous elements which invade from adjoining annulus fibrosus. The centres of ossification are present in the vertebral bodies. Vascular canals are present, as in the previous specimen, but no vessels are seen to enter the notochordal area.

L.S showing fibres of annulus fibrosus ending in cartilage plates at 300 mm CR length fetus in H & E stain×100

(A) Lamellae (B) Cartilage plates

(C) Vertebral body

Fig. 3

Discussion

The study was aimed to get information regarding the histological structure of intervertebral disc at different ages of foetuses. The histological sequence of intervertebral disc of foetuses under consideration shows progressive differentiation of the primitive embryonic mesenchyme in the region of developing lumbar intervertebral disc into two distinct types of tissues, the peripheral annulus fibrosus with well-formed collagenous tissue and the central nucleus pulposus of a homogenous matrix containing a few notochordal cells which gradually decreases with advancing age of the foetus.

In the present study, in the region of annulus fibrosis, there is gradual increase in thickness due to increase in collagen bundles at the periphery of annulus fibrosus. Bundles are arranged in lamellaes and fibres lying parallel to one another in each lamella. These findings are in accordance with Peacock (1951) and Hukins (1984) who also observed the lamellar pattern.

Observations in relatively older foetuses of 155mm CR Length onwards, reveal that individual cells close to nucleus pulposus occupy lacunae and is presumabily the first step in the formation of future fibrocartilage. One can conclude from these features that it is the middle zone of intervertebral disc where earliest attempt at differentiation of the mesenchymal tissue into primitive fibrocartilagenous tissue is taking place. Simultaneously other cells closer to nucleus pulposus are forming lacunae around them, in an attempt to form the future cartilaginous cells. Keys et al (1932) observed the appearance of fibrocartilage in the inner zone of annulus fibrosus at 130mm CRL stage where as Peacock (1951) reported the appearance of cells in lacunae at much earlier stage at 60mm CRL.Present study is approximately in conformity with the findings of Keys et al (1932).

As far as the mode of attachment of the intervertebral disc with the vertebral bodies is concerned, the present study reveals that the fibres of the annulus fibrosus are ending in adjoining parts of the vertebral bodies which lie along the boundaries of intervertebral disc. These findings are in accordance with Beadle (1931) and Peacock (1951) who had observed similar mode of termination of fibres.

The notochordal area in the present study reveals that the notochordal cells show a gradual process of degeneration starting from 155mm CR Length and continues upto 300mm CR Length. By 300mm CR Length, most of these cells show degenerative changes. These findings differ from those of Keys et al (1932) who noticed that the notochordal cells actually do multiply and take part in formation of nucleus pulposus in growing embryo. Prader (1947) recorded mitosis in the cells of notochord, but at very early stage foetus.

The vascularisation of the intervertebral disc is observed in the present series in 115mm CR Length foetuses and then onwards it is progressive. But the vascularisation in present only upto the outer part of annulus fibrosis and confined to the dorsal region mainly. Ubermuth (1929) described peripheral vessels in the cartilage plates only, but he failed to find any bloodvessels in the annulus fibrosis or nucleus pulposus. Bohmig (1930) stated that bloodvessels supply cartilage plates, annulus fibrosus and nucleus pulposus. Peacock (1951) noticed vascularisation at 93mm CR Length stage onwards in the outer part of annulus fibrosus, which is in concordance to the present study. The vascularisation of nucleus pulposus and inner part of annulus fibrosis was neither observed by Peacock (1951) nor in the present study.

Conclusion

Following conclusions are drawn from the present study. The annulus fibrosus at its periphery shows parallel bundles of collagen fibres with cells arranged along their long axis with progressive collagenisation as the age of the foetus advances. The inner zone of annulus fibrosus changes from a primitive mesenchymal tissue at 53mm CR Length stage and is thereafter progressive. The nucleus fibrosus shows structureless mucoid matrix, with the notochordal cells embedded in it. These cells are undergoing gradual degenerative changes during intrauterine life. In later life the fibrous elements invade it from the junctional region between nucleus pulposus and annulus fibrosus.

The nucleus pulposus is avascular during foetal life, while annulus fibrosus is vascularised only in its outer lamellae especially dorsally. The vertebrae are freely vascularised during foetal life and some blood vessels enter the cartilage plates only.

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