

Investigation of anulus fibrosus morphology in human fetus intervertebral discs

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ABSTRACT

Objective: To determine whether the thickness of the anulus fibrosus (AF) structure of the intervertebral disc plays a role in disc herniation etiology, an important pathology.

Methods: We performed this study between February 2000 and May 2001 at Karadeniz Technical University Medical Faculty, Turkey. We used 20 human fetus cadavers with intrauterine ages between 22 and 41 weeks, taking into consideration the highest, lowest, and average frequencies, with 3 each of cervical, thoracic, and lumbar parts of the vertebral column, using a total of 9 intervertebral discs. We measured the thickness of the AF layer in each intervertebral disc in anterior, posterior, postero-dexter, and postero-sinister locations. We performed comparisons among the values obtained at the

same level, and of comparative thicknesses at the same location at other levels.

Results: It was determined that there was no difference between the thickness of the AF in the same location at the various levels of intervertebral discs, but that at almost all levels, the thickness in posterior and anterior locations was significantly less compared to that in posterolateral locations.

Conclusion: Anulus fibrosus thickness is not directly related to the incidence of disk hernia at different levels of the vertebral column according to the values obtained from this study.

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Health problems currently represent one of the main reasons for workforce losses. Disc herniation is important as it both causes symptoms and requires prolonged treatment. Intervertebral discs are 23 formations existing between adjacent surfaces of the vertebral bodies from the axis to the sacrum.¹ The area surrounding the nucleus pulposus consists of fibers, which include a number of chondrocytes. Therefore, the tissue is known as "fibrocartilage". Disc herniation is a tearing of the anulus fibrosus (AF) as a result of a trauma, and degenerative changes, and a herniated nucleus often causes neurological symptoms, pressing a nerve root in the intervertebral foramen or vertebral canal.^{2,3} Macroscopic and microscopic structures of

intervertebral discs have been investigated in fetus studies performed to date, but their relations to the formation of disc herniation have not been examined. In addition, postural muscle weakness, and traumatic causes have also been investigated with regards to disk hernia etiology, while the role of AF thickness of the intervertebral disc in disc herniation etiology was ignored. The aim of this study was to determine whether the thickness of the AF structure of the intervertebral disc plays a role in disc herniation etiology, an important pathology.

Methods. This study was performed between February 2000 and May 2001 at Karadeniz

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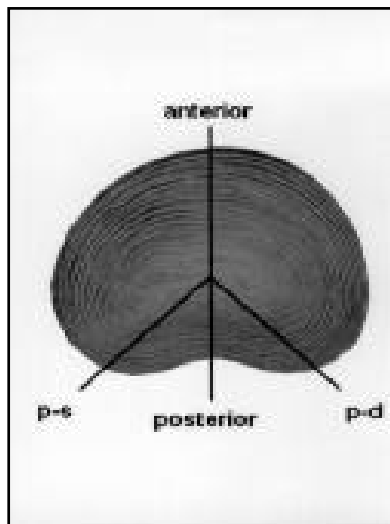


Figure 1 - Localizations of sections. (PD - postero-dexter, PS - postero-sinister).

Technical University Medical Faculty, Turkey, on intervertebral discs obtained from C₂-C₃, C₄-C₅, C₆-C₇, T₁-T₂, T₆-T₇, T₁₁-T₁₂, L₁-L₂, L₃-L₄, and L₄-L₅ intervals from the vertebral columns of 20 human fetus cadavers whose intrauterine ages varied from 22-41 weeks. Levels were determined considering the highest, lowest, and mean observed rates of disc herniation frequency. Intervertebral discs were initially kept in 10% formaldehyde solution for 10 days. Each disc was first cut into 2, from front to back through the midline and then cut towards the sides at an angle of 45° from the midpoint of this line towards the back, and was then divided into 4 sections for investigation; anterior (A), posterior (P), postero-dexter (PD), postero-sinister (PS) (Figure 1). Tissue observations were performed on each part and determined materials were inserted into paraffin blocks. Sections were taken from the prepared blocks using a microtome and placed on slides. (Specimens were prepared for investigation by applying hematoxylin-eosin stain to the sections). The thickness of the AF layer in the specimens (the measurement of the conjugation point of the AF, and nucleus pulposus, and external interval) (Figure 2) was measured using a microscope and 100-section linear scaled micrometer installed in the eyepiece. The A, P, PD, and PS, AF thicknesses measured in intervertebral discs were compared not only with each other in the same level, but also with the measurements from the same parts from different levels. The ANOVA was used in the statistical analysis of the data, and the Tukey test in post-hoc comparisons. Significance level was taken as $p < 0.05$. Data are given as mean \pm standard deviation.^{4,5}

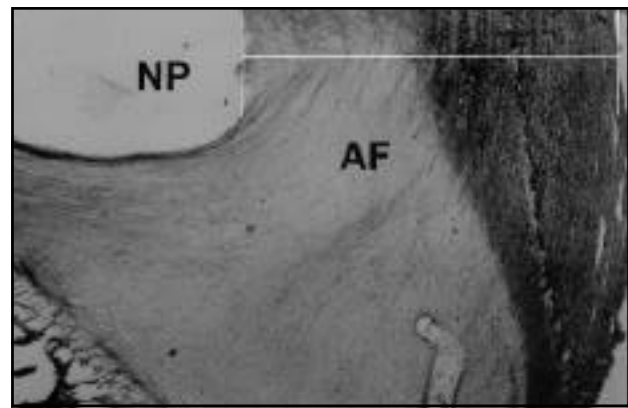


Figure 2 - The thickness of the annulus fibrosus layer (hematoxylin x eosin x 4). (NP - nucleus pulposus, AF - annulus fibrosus).

Results. The values for the cervical are given in (Table 1), thoracic in (Table 2), and lumbar sections in (Table 3). Comparison of the thicknesses of the sections in the discus intervertebralis showed that the thickness in the C₂-C₃ level was statistically, and significantly less than that in C₆-C₇, T₆-T₇, L₁-L₂, and L₃-L₄. However, it was determined that A section thicknesses in other levels were not statistically and statistically different from each other ($p > 0.05$).

In comparison of the P sections in the groups it was determined that the P section thickness in the C₂-C₃ level was significantly lower than the P section thicknesses in T₁₁-T₁₂, L₁-L₂, L₃-L₄, and L₄-L₅. Moreover, P section thickness in the C₄-C₅ level was significantly lower than that in the T₁₁-T₁₂, and L₃-L₄ levels. However, the P section thicknesses in other levels were not statistically and significantly different ($p > 0.05$).

Comparison of the PD section thicknesses of intervertebral discs in all levels showed that the PD section in the C₂-C₃ level was significantly thinner than those in the L₁-L₂, and L₃-L₄ levels, while PD thicknesses in other levels were not statistically and significantly different from each other ($p > 0.05$). When the same comparison was performed with PS section thicknesses, those in the C₂-C₃ level were significantly thinner than those in T₁₁-T₁₂, L₁-L₂, and L₃-L₄. However, PS sections thicknesses in other levels were not significantly different from each other ($p > 0.05$).

Discussion. Disk hernia is the rupturing of the nucleus pulposus, tearing the AF, under the influence of many factors.⁶ As the cause of disc hernia, the role of AF morphology has been taken into consideration for many years, and scientists with different points of view have carried out many investigations into this subject. In this study, the thickness of AF in different levels in the columna

Table 1 - Results of measurement of the cervical part of the vertebral column.

Level	Anterior mean \pm SD	Posterior mean \pm SD	Postero-dexter mean \pm SD	Postero-sinister mean \pm SD	P value
C2 - C3	1.23 \pm 0.35	1.12 \pm 0.40	1.88 \pm 0.54	1.85 \pm 0.58	0.000
C4 - C5	1.52 \pm 0.44	1.20 \pm 0.23	1.99 \pm 0.49	1.98 \pm 0.40	0.000
C6 - C7	1.60 \pm 0.44	1.44 \pm 0.59	2.09 \pm 0.60	2.17 \pm 0.78	0.000
P value	0.018	0.055	0.479	0.259	
SD - standard deviation					

Table 2 - Results of measurement of the thoracal part of the vertebral column.

Level	Anterior mean \pm SD	Posterior mean \pm SD	Postero-dexter mean \pm SD	Postero-sinister mean \pm SD	P value
T1 - T2	1.73 \pm 0.46	1.59 \pm 0.36	2.47 \pm 1.03	2.45 \pm 1.00	0.000
T6 - T7	1.88 \pm 0.68	1.62 \pm 0.44	2.43 \pm 1.01	2.60 \pm 0.92	0.000
T11 - T12	1.74 \pm 0.52	1.78 \pm 0.82	2.77 \pm 1.06	2.90 \pm 1.05	0.000
P value	0.632	0.538	0.543	0.345	
SD - standard deviation					

Table 3 - Results of measurement of the lumbar part of the vertebral column.

Level	Anterior mean \pm SD	Posterior mean \pm SD	Postero-dexter mean \pm SD	Postero-sinister mean \pm SD	P value
L1 - L2	1.84 \pm 0.48	1.76 \pm 0.63	2.92 \pm 1.31	2.83 \pm 1.20	0.000
L3 - L4	2.02 \pm 0.89	1.80 \pm 0.94	2.85 \pm 1.26	2.89 \pm 1.16	0.002
L4 - L5	1.75 \pm 0.59	1.71 \pm 0.56	2.52 \pm 0.81	2.69 \pm 1.12	0.000
P value	0.436	0.918	0.505	0.848	
SD - standard deviation					

vertebralis was correlated with the frequency of disk hernia cases. Hickey and Hukins⁷ maintained that the laminar structure of the AF is the same in both fetuses and adults, and added that collagen fibrils occur approximately in the 10th week of fetal life, whereas Cotten et al⁸ maintained that they occur in the 13th week.

In this study, human fetus cadavers were used, since these were not exposed to external influences and are not structurally different from those of adults. In many studies, it is suggested that disk hernia is seen more frequently in cervical, and lumbar sections than in thoracic sections. We determined that all the measurements in the C₂-C₃ level were significantly smaller than those of lumbar section when the sections were compared with each other in terms of A, P, PD and PS thicknesses investigated in intervertebral disks. However, no information was found in the literature to support this result. This, therefore, indicates that sectional differences in AF thickness are not the most important factor in the more frequent occurrence of disc herniation in cervical and lumbar sections. On the other hand, we may say that the most important factor in disc hernia cases is that disks in different sections are exposed to different pressures. Lumbar section disks are an example of this, as the body exerts greater pressure on them.

The AF in cervical sections does not have the same characteristics as that in lumbar sections according to investigations into disk herniation in the former. It is known that collagen structure is thick in the anterior and thins increasingly towards the sides. It is also weaker in posterolateral parts.⁹ Marchand and Ahmed¹⁰ determined that the laminar structure of the AF is more irregular in posterolateral parts of the lumbar section discus intervertebralis than in other sections, and that laminar thickness varies widely. We were unable to find any statistically significant difference in the comparison of A, P and posterolateral parts in each section. Weakness of the AF and mechanical wear in daily life are risk factors for rupture. Tsuji et al¹¹ determined that the laminar structure of the AF in P and postero-lateral areas is different from that in A areas.

At the end of the study, A and P section thicknesses in all levels in the lumbar section were determined to be statistically less than those in the PD and PS sections. It was concluded that in the lumbar section, AF thickness can be an important factor in determining the localization of disk herniation, as in cervical sections, but that it is not an effective factor in determining its vertebral level. The internal pressure of the nucleus pulposus rises when exposed to greater pressure, and the nucleus pulposus transmits this to each point of the AF equally. On the other hand, there may also be differences in the pressure on the AF due to sudden

changes such as flexion, extension and rotation, which can occur in body posture. In this case, disk hernia will occur, as the weak parts in the anulus cannot withstand that pressure. In addition, with regard to the question of why disk herniation is seen more in P than in A parts, it was considered that this was owing to the wide and different structure of the ligamentum longitudinale anterior, although there was no statistically significant difference between A and P parts.

The importance of the values obtained from the measurements in the occurrence of disk herniation was not indicated primarily. However, it was secondarily considered that AF morphology is important due to the functional structure of the vertebral column and the effects to, which it is exposed. The AF thickness in disk herniation is related to external mechanical effects, trauma, and growing disk degeneration based on age. Lee et al¹² maintained in one of their studies that degenerative disk change is the major cause of in 73% disk hernia of cases. Discus intervertebralis degeneration is closely related to AF morphology. Moore et al⁶ indicated that intervertebral disk degeneration causes disk herniation. Nevertheless, we also pointed out that the reason for the disk herniation seen in low numbers in young people is that the swollen nucleus pulposus leaves the AF due to traumatic tearing. Degeneration can be seen in different locations in the discus intervertebralis. It was determined that 70.3% of AF degenerations were paramedian, 9.45% median, and 20.25% posterolateral in the L₄-L₅ level.¹³

In conclusion, AF thickness is not directly related to the incidence of disk hernia in different levels of the vertebral column according to the values obtained from this study. However, it may be an effective factor in determining disc herniation localization in intervertebral discs.

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