

Age-related characteristic of human intervertebral discs from normal anatomy's point of view

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Abstract

Aim. To compare morphological characteristics between anterior and posterior parts of human intervertebral discs, taking into account age.

Methods. Fragments of 36 intervertebral discs C₅C₆, D₅D₆, L₅S₁ anterior parts taken from deceased persons aged 34 to 94 years, median age 61.0 (50.5; 71.8) years were examined. The comparison group consists of histopathological material obtained from 12 patients with radicular syndrome during planned L₅S₁ microdiscectomies aged 35–77 years, median age 48.5 (43.0; 58.8) years. There were no statistically significant differences in age between the studied groups ($p=0.126023$). All materials were divided into subgroups depending on the age of the deceased/operated: 34–52 and 60–94 years for material obtained from the deceased; 35–51 and 58–77 years — for material obtained after surgery. The differences between the three groups were examined by the Kruskal–Wallis test, and quantitative indicators in the two groups were compared by Mann–Whitney U-test.

Results. In the anterior part of the intervertebral discs, signs of degenerative-dystrophic changes were noted in all studied samples. All samples of nucleus pulposus and annulus fibrosus were fibrocartilage with no inflammation. Statistically significant differences ($p=0.0283$) were obtained in the number of isogenous groups of chondrocytes in intervertebral discs C₅C₆ anterior part compared with D₅D₆, L₅S₁ in individuals aged 34–52. Age subgroups (34–52 and 60–94 years old) differed significantly ($p=0.0219$) in the number of single chondrocytes according to results of morphometry of anterior part of intervertebral discs L₅S₁. Anterior and posterior parts of intervertebral discs L₅S₁ differed statistically significant in the number of isogenous groups of chondrocytes when comparing the subgroup of operated patients aged 35–51 years ($p=0.008475$) with the subgroup of deceased persons aged 34–52 years and the subgroup of operated patients aged 58–77 years ($p=0.033753$) with the subgroup of deceased aged 60–94 years.

Conclusion. Anterior and posterior part of intervertebral discs L₅S₁ had similar qualitative histological characteristics; however, the number of isogenous groups of chondrocytes in the posterior part of intervertebral discs L₅S₁ samples indicated a greater effect of compression loading compared to anterior part of the same spinal motion segment.

Keywords: intervertebral disc, fibrous cartilage, isogenic group of chondrocytes, chondrocyte.

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Background. Despite the variety of contemporary methods available for the conservative and surgical treatment of vertebral–neurological diseases, the prevalence of these diseases and the economic burden they impart to public healthcare systems and employers continues to increase annually [1, 2]. To date, no consensus on the pathogenesis of degenerative–dystrophic diseases of the intervertebral discs has yet been formed. The wide spectrum

of clinical manifestations of vertebral–neurological diseases is the result of simultaneous action of two or more factors. Because the intervertebral discs are constantly subjected to static and dynamic compression loads during the day, assessing changes in the structural characteristics of these discs in the postnatal period of life is advisable to clarify the effect of age on the intensity of degenerative–dystrophic changes in the spine.

In the normal anatomy, an intervertebral disc represents a continuous connection of bones through cartilage tissue, that is, synchondrosis. The disc includes three components, namely, the cartilaginous endplate, the nucleus pulposus, and the annulus fibrosus. The cartilaginous endplate is composed of hyaline cartilage, and the nucleus pulposus and annulus fibrosus are composed of fibrous cartilage [3]. Several structural changes occur in the nucleus pulposus during the postnatal period. For example, the nucleus pulposus is liquid in newborns but gel-like in childhood and early adolescence. From a histological point of view, the nucleus pulposus can be attributed to hyaline cartilage. By the age of 15 years, the nucleus pulposus becomes fibrous in nature, and its histological pattern is similar to that of the annulus fibrosus. Further increases in age enhance the fibrous nature of the nucleus pulposus [4].

Biomechanically, the vertical straightening of the body, which is associated with the transition to an upright posture, is the result of not only the backward tilting of the pelvis but also the extension of the spinal column. During this type of movement, the spinal curvature adjusts to balance the load distribution on each point of support in different planes of motion and increase the resistance to axial compression [5]. Each vertebra represents a lever, and the facet joints act as rotation points [6,7]. This functional system enables the absorption of passive axial compression at the level of the intervertebral disc. In this system, the intervertebral disc functions as a shock-absorbing layer, and its gel-like nucleus pulposus is similar to a hinge [8].

Given the complex spatial biomechanics of spinal movements, the posterior and anterior parts of the intervertebral disc simultaneously undergo reversible structural changes under physiological stress. The resulting vector of a compression force displaces the nucleus pulposus in the opposite direction, thereby increasing the tension of the fibers of the annulus fibrosus in this area. Displacement of the nucleus pulposus promotes the rupture of the annulus fibrosus and prolapse of the nucleus pulposus in unfavorable environmental conditions, which, in turn, lead to the development of hernias and protrusions [9,10]. However, the clinical manifestations of vertebral–neurological diseases described in the vast majority of the available literature on this topic are associated with the posterior part of the intervertebral disc [9,11]. In these works, the anterior part of the intervertebral disc is hardly ever mentioned.

A number of questions naturally arise. What morphological changes occur in the anterior part of the intervertebral discs during the postnatal

period? How do the biomechanics of the intervertebral disc affect the structural characteristics of the anterior and posterior parts?

The present study aimed to compare the morphological characteristics of the anterior and posterior parts of the intervertebral discs as a function of age.

Materials and methods of research. This research represents a cross-sectional, descriptive, and analytical study, with a one-stage design. The study protocol was approved by the Ethics Committee of the South Ural State Medical University of the Ministry of Health of Russia (Protocol No. 10 dated November 15, 2019).

Fragments of the anterior part of 36 intervertebral discs C_5C_6 , D_5D_6 , and L_5S_1 , including the annulus fibrosus and nucleus pulposus, were obtained over the course of 12 autopsies conducted at the Chelyabinsk Regional Office of the Chief Medical Examiner in 2019. The criteria for exclusion from the study were trauma and infectious inflammatory diseases of the spine. The age of the deceased ranged from 34 years to 94 years, and the median age was 61.0 (50.5; 71.8) years.

Fragments of the posterior part of these discs are represented by histopathological materials obtained from 12 patients with radicular syndrome during scheduled microdiscectomies of L_5S_1 in the neurosurgical department of Regional Clinical Hospital No. 3 of Chelyabinsk in 2019. The patients were aged 35–77 years, and the median age was 48.5 (43.0; 58.8) years.

No statistically significant difference in age was noted among the study groups ($p = 0.126023$).

The materials were fixed in 10% neutral buffered formalin solution (ErgoProduction, Russia) for 24 hours. All histological samples analyzed in this study were prepared at the Chelyabinsk Regional Pathoanatomical Bureau. Embedding into paraffin wax (GISTOMIKS, ErgoProduction, Russia; melting point, 52°C–54°C) was performed according to the standard procedure on a Microm EC-350 embedding station (Microm, Germany). All paraffin sections were obtained on an HM 200 Ergostar microtome equipped with an STS section transfer system (Microm, Germany).

Histological preparations were stained with hematoxylin and eosin (Mayer's hematoxylin, BioVitrum, Russia; eosin, 1% alcohol solution, BioVitrum) and van Gieson's stain (BioVitrum) according to the standard technique on an automatic histological multistainer (Tissue-Tek DRS 2000; Sakura Seiki Co. Ltd., Japan). Silver impregnation staining was performed according to the standard technique using a ready-made reagent kit (BioVitrum) on a Leica Autostainer XL ST5010 staining

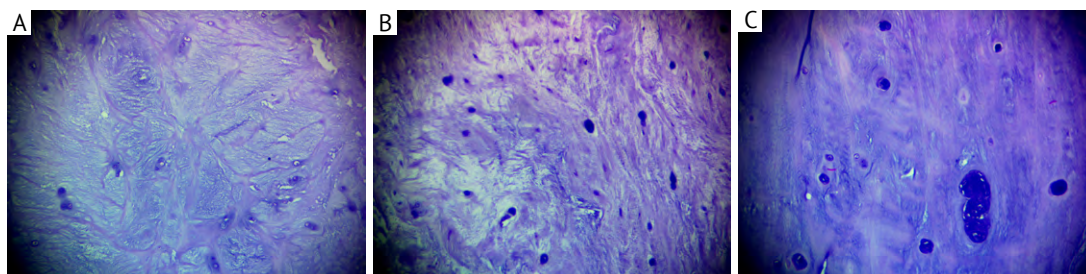


Fig. 1. Anterior parts of intervertebral discs and fibrous cartilages showing single chondrocytes and their isogenic groups. (A) C5C6 of a deceased person aged 43 years old. (B) D5D6 of a deceased person aged 52 years. (C) L5S1 of a deceased person aged 68 years. Hematoxylin–eosin staining; lens magnification, $\times 10$.

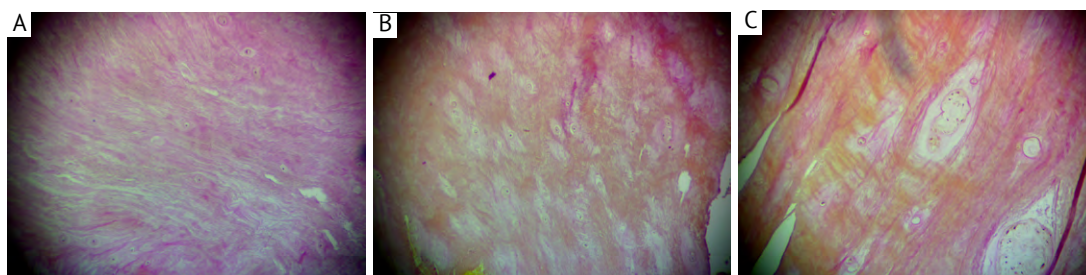


Fig. 2. Anterior parts of intervertebral discs and fibrous cartilages showing single chondrocytes, their isogenic groups, and collagen fibers. (A) C5C6 of a deceased person aged 34 years. (B) D5D6 of a deceased person aged 52 years. (C) L5S1 of a deceased person aged 68 years old. Van Gieson staining; lens magnification, $\times 10$.

machine (Leica Biosystems Nussloch GmbH, Germany).

The microslides were analyzed under a Levenhuk 870T microscope (Levenhuk Inc., USA) using objectives with magnifications of $\times 4$, $\times 10$, and $\times 40$. Microphotography was performed with a Toupcam UCMOS08000KPB-Special USB2.0 digital camera (ToupTek, China) using the ToupLite software package (ToupTek) included in the kit. Visual and quantitative assessment of the photomicrographs was performed using ImageJ 2.0.0-rc-43/1.50e software with a calibration slide (X/Y OPLENIC, China; scale graduation, 0.01 mm) [12].

The results were statistically processed using Statistica 10.0 software, and the data were presented as medians and interquartile ranges (25th and 75th percentiles). Comparisons among the three groups were performed using the Kruskal–Wallis test, and comparisons between two groups were conducted using the Mann–Whitney test. Differences were considered statistically significant at $p \leq 0.05$.

Results. The group of deceased persons was divided into two subgroups to compare the age-related morphological characteristics of the intervertebral discs. Subgroup 1 included young and middle-aged persons (34–52 years), while subgroup 2 included elderly and senile persons (60–94 years). The group of operated patients was also divided into two subgroups whose age ranges were close to those of the deceased persons (subgroup 1, 35–51 years; subgroup 2, 58–77 years).

Previous studies revealed that the border between the annulus fibrosus and nucleus pulposus is distinguishable in children. By contrast, in adults, the annulus fibrosus and nucleus pulposus cannot be differed macro- and microscopically [3]. In the present study, all of the histological specimens obtained during microscopy were morphologically homogeneous, consistent with the findings of other authors.

Histological sections of the anterior part of C₅C₆ stained with hematoxylin and eosin revealed various shades of purple. These sections showed convoluted fibers arranged in a disordered manner (Fig. 1).

Specimens stained using the van Gieson method were colored pink or yellowish-pink (Fig. 2). Van Gieson staining allowed the classification of the fibers noted in the sections as type I collagen. The presence of a yellow tint may be attributed to the rearrangement of fibrous cartilage components in response to a compression load.

Reparative regeneration involves the formation of argyrophilic reticular fibers. Impregnation of all specimens with silver produced colors ranging from golden yellow to brown or gray–brown (Fig. 3).

The results thus far confirm that the fibers noted in the sections are composed of type I collagen. Reticular fibers containing type III collagen and exhibiting the characteristics of granulation tissue were not found in C₅C₆ samples.

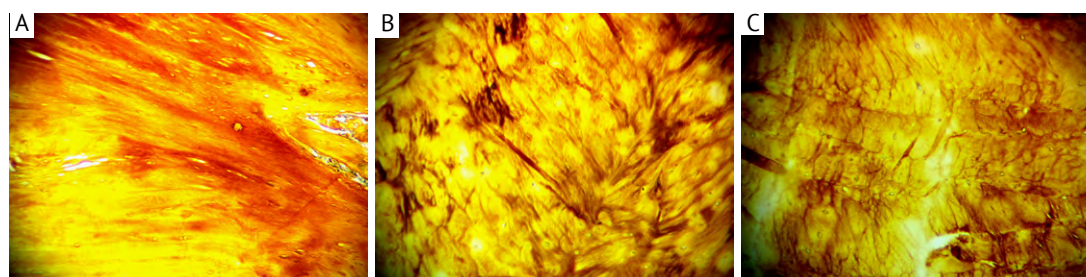


Fig. 3. Anterior parts of intervertebral discs and fibrous cartilages showing single chondrocytes, their isogenic groups, and collagen fibers. (A) C5C6 of a deceased person aged 68 years. (B) D5D6 of a deceased person aged 46 years. (C) L5S1 of a deceased person aged 91 years old. Silver impregnation staining; lens magnification, $\times 10$.

The data obtained thus far may be explained by the effects of a compression load on the intervertebral discs associated with an upright posture. Because the intervertebral disc samples belonged to persons over 30 years of age, the histological patterns obtained reflect the outcome of the structural reorganization of fibrous cartilage that was clearly extended over time; such patterns are usually indicative of degenerative and dystrophic changes.

Besides collagen fibers, the sections revealed single chondrocytes and their isogenic groups. Empty lacunes were also noted. The nuclei of the cells, as well as the contours of isogenic groups of chondrocytes, were strongly stained with hematoxylin and eosin. The absence of fibroblasts and fibrocytes can be explained as follows. As mentioned earlier, the annulus fibrosus and nucleus pulposus of adults do not differ morphologically and are composed of fibrous cartilage. Because the fibrous cartilage of the intervertebral disc is of mesenchymal origin, cells giving rise to fibroblasts and chondrocytes can coexist in this cartilage for a long time. However, the presence of fibroblasts and fibrocytes in either the annulus fibrosus or nucleus pulposus is not mandatory [3].

Infiltration of intercellular substances, which typically indicates an inflammatory reaction, was not observed in the sections under study. In 4 out of 12 cases (the ages of the deceased are 34, 52, 60, and 61 years), slit spaces filled with erythrocytes and leukocyte cells were noted. Pericytes and endothelial cells were not found in the peripheral portions of the slit spaces.

The histological patterns of the anterior parts of D₅D₆ and L₅S₁ were identical to those noted in C₅C₆ (Fig. 1–3). However, sections at the D₅D₆ level revealed slit spaces filled with erythrocytes in 8 out of 12 cases (the ages of the deceased are 34, 46, 52, 60, 61 [three cases], and 94 years); sections at the L₅S₁ level revealed these features in 5 out of 12 cases (the ages of the deceased persons are 46, 60, and 61 [three cases] years).

A notable difference between the anterior part of L₅S₁ and those at higher locations is the presence of single fibroblasts in the discs of deceased persons aged 34–52 years. The median values of the quantitative indicators of C₅C₆, D₅D₆, and L₅S₁ of the different subgroups under study are presented in Tables 1–3. Analysis of the data obtained (Tables 1,2) indicated significant differences only in terms of the number of isogenic groups of chondrocytes per 1 mm² among the different spine regions of persons who died at the age of 34–52 years. In the older age subgroup (60–94 years), the number of isogenic groups of chondrocytes per 1 mm² did not differ among the different spine regions studied.

No differences in the number of single chondrocytes in the anterior parts of C₅C₆ and D₅D₆ were observed among the different age subgroups. By contrast, the number of single chondrocytes per 1 mm² in the anterior part of L₅S₁ differed among the age subgroups. Specifically, the number of single chondrocytes per 1 mm² in the younger age subgroup was twice that in the older age subgroup (Table 3).

The fragments of the anterior part of the intervertebral discs examined appeared to be composed of fibrous cartilage. The histological patterns obtained reflect degenerative–dystrophic changes without the signs of inflammation.

The posterior part of L₅S₁ generally showed no difference compared with the anterior part (Fig.4), despite the fact that the samples of the posterior part of L₅S₁ are histopathological materials obtained during an exacerbation of vertebral–neurological diseases accompanied by radiculopathy and severe pain syndrome. However, degenerative and dystrophic changes in the form of a disordered arrangement of collagen fibers were more pronounced in the posterior part than in the anterior part. In 3 out of 12 cases (the ages of the operated patients are 46, 51, and 70 years), fibroblast aggregates typically indicating scar connective tissue were found.

Table 1. Morphometric indicators of the anterior parts of the intervertebral discs of the age subgroup 34–52 years (per 1 mm²).

Indicator	C ₅ C ₆ anterior part	D ₅ D ₆ anterior part	L ₅ S ₁ anterior part	p
Number of isogenic groups	4.0 (3.5; 4.6)	1.1 (0.3; 2.0)	0.5 (0.4; 0.6)	0.0283*
Maximum number of cells in an isogenic group	2.5 (2.0; 4.0)	3.0 (2.0; 4.3)	2.0 (1.5; 2.3)	0.3948
Number of single chondrocytes	16.7 (15.1; 18.7)	16.6 (11.8; 21.4)	20.3 (18.6; 22.4)	0.6252

Note: *differences are statistically significant.

Table 2. Morphometric parameters of the anterior parts of the intervertebral discs of the age subgroup 60–94 years (per 1 mm²).

Indicator	C ₅ C ₆ anterior part	D ₅ D ₆ anterior part	L ₅ S ₁ anterior part	p
Number of isogenic groups	2.7 (0.6; 4.3)	1.0 (0.0; 2.0)	2.0 (0.7; 3.2)	0.4215
Maximum number of cells in an isogenic group	4.0 (2.8; 6.5)	3.0 (0.0; 4.5)	6.5 (4.3; 8.8)	0.2183
Number of single chondrocytes	21.9 (7.3; 52.3)	12.6 (5.7; 24.4)	11.1 (8.6; 14.8)	0.614

Table 3. Differences in the morphometric parameters of the anterior parts of L₅S₁ of deceased persons aged 34–52 and 60–94 years (per 1 mm²).

Indicator	34–52 years, n = 4	60–94 years, n = 8	p
Number of isogenic groups	0.5 (0.4; 0.6)	2.0 (0.7; 3.2)	0.1488
Maximum number of cells in an isogenic group	2.0 (1.5; 2.3)	6.5 (4.3; 8.8)	0.0894
Number of single chondrocytes	20.3 (18.6; 22.4)	11.1 (8.6; 14.8)	0.0219*

Note: *differences are statistically significant.

Differences in the numbers of single chondrocytes and their isogenic groups were also noted among the different subgroups (Tables 4–6).

Another notable finding in the posterior part of L₅S₁ among the different age subgroups is the absence of statistically significant differences in the number of single chondrocytes, isogenic groups of chondrocytes, and the maximum number of cells in these isogenic group (Table 4).

The number of isogenic groups of chondrocytes per 1 mm² (Tables 5, 6) in the posterior part of L₅S₁ of patients operated on at the age of 35–51 years was 12 times greater than that in persons who died at the age of 34–52 years. Similarly, the number of isogenic groups of chondrocytes per 1 mm² in the posterior part of L₅S₁ of patients operated on at the age of 58–77 years was 4.4 times greater than that in persons who died at the age of 60–94 years. The maximum number of chondrocytes in the isogenic groups of the posterior part of L₅S₁ of patients operated on at the age of 35–51 years was 4.5 times greater than that of persons who died at the age of 34–52 years. The numbers of single chondrocytes per 1 mm² in the anterior and posterior parts of L₅S₁ did not differ among different age subgroups studied (Tables 5, 6).

Discussion. The response of cartilage tissue to any static and dynamic load, regardless of its magnitude and point of application, is the activation of proliferative processes in the form of an increase

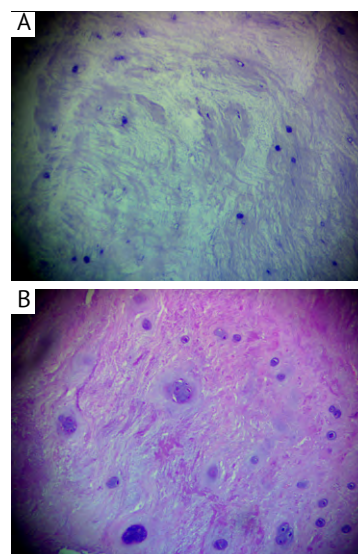


Fig. 4. Comparison of the histological patterns of L5S1 showing isogenic groups of chondrocytes and a disordered arrangement of collagen fibers. (A) Anterior part of L5S1 of a deceased person aged 34 years. (B) Posterior part of L5S1 of an operated patient aged 35 years. Hematoxylin–eosin staining; lens magnification, $\times 10$.

not only in the number of isogenic groups of chondrocytes but also in the number of daughter cells in these isogenic groups [3]. According to the concept of the three-point stability of the spinal column, the maximum load falls on the middle and posterior supporting structures regardless of physiological range and plane of application. Consequently,

Table 4. Differences in the morphometric parameters of the posterior part of L₅S₁ of persons aged 35–51 and 58–77 years (per 1 mm²).

Indicator	35–51 years, <i>n</i> = 8	58–77 years, <i>n</i> = 4	<i>p</i>
Number of isogenic groups	6.2 (4.0; 9.7)	8.8 (5.7; 12.0)	0.444697
Maximum number of cells in an isogenic group	9.0 (5.8; 11.3)	9.0 (7.5; 13.0)	0.734095
Number of single chondrocytes	13.6 (2.0; 16.8)	8.2 (6.0; 13.8)	0.865135

Table 5. Morphometric parameters of L₅S₁ of the age subgroups 34–52 years (anterior part of the disc) and 35–51 years (posterior part of the disc) (per 1 mm²).

Indicator	Anterior part, <i>n</i> = 4	Posterior part, <i>n</i> = 8	<i>p</i>
Number of isogenic groups	0.5 (0.4; 0.6)	6.2 (4.0; 9.7)	0.008475*
Maximum number of cells in an isogenic group	2.0 (1.5; 2.3)	9.0 (5.8; 11.3)	0.021857*
Number of single chondrocytes	20.3 (18.6; 22.4)	13.6 (2.0; 16.8)	0.148838

Note: * differences are statistically significant.

Table 6. Morphometric parameters of L₅S₁ of the age subgroups 60–94 years (anterior part of the disc) and 58–77 years (posterior part of the disc) (per 1 mm²).

Indicator	Anterior part, <i>n</i> = 8	Posterior part, <i>n</i> = 4	<i>p</i>
Number of isogenic groups	2.0 (0.7; 3.2)	8.8 (5.7; 12.0)	0.033753*
Maximum number of cells in an isogenic group	6.5 (4.3; 8.8)	9.0 (7.5; 13.0)	0.234483
Number of single chondrocytes	11.1 (8.6; 14.8)	8.2 (6.0; 13.8)	0.350238

Note: * differences are statistically significant.

regardless of age, the greatest compression load is experienced by the posterior part of the intervertebral discs because these structures provide serves as a part of the middle supporting structure [13]. Considering that the force of a compression load in the spinal column increases from top to bottom, the posterior part of the lumbar intervertebral discs is exposed to the greatest compression load, regardless of the physiological range and point of application [5]. These previous findings were confirmed by the results obtained in L₅S₁ of the younger age subgroups.

The absence of granulation tissue in all of the studied samples of the anterior and posterior parts of the intervertebral discs may indicate that the acting static and dynamic compression loads do not reach critical values leading to the destruction of fibrous cartilage regardless of the point of application. Inflammatory infiltration did not develop, and no reticular fibers were observed in the samples analyzed in this work. In this case, reparative processes must have occurred at the expense of the cartilaginous tissue itself [2,3]. This supposition is supported by the presence of isogenic groups of chondrocytes containing a large number of cells.

Despite the fact that the axis of distribution of the vertical compression load in the thoracic spine should fall on the anterior part of the intervertebral discs due to kyphosis [8,14], the numbers of single chondrocytes and their isogenic groups were nearly

equal in the anterior part of the intervertebral discs of the thoracic spine of the age subgroups of 34–52 and 60–94 years. Earlier studies by A.I. Kapanji and N.Ozkaya et al. revealed that thoracic spine experiences the least stress among the different regions of the spine because it is fixed by the ribs and less involved in movements; moreover, part of the load is distributed to the sternum and ribs [5,8]. These facts could explain why the compression load on the thoracic spine is fairly stable and does not have a remarkable effect on the number of chondrocytes and their isogenic groups in this region.

Compared with the samples of the anterior part of D₅D₆ and L₅S₁, the samples of the anterior part of C₅C₆ of persons who died at the age range of 34–52 years showed a greater number of isogenic groups of chondrocytes. This observation may be due to the greater mobility of the cervical spine.

Conclusion. Signs of degenerative–dystrophic changes were noted in the anterior parts of the intervertebral discs. At the age of 34–52 years, the anterior part of C₅C₆ was more functionally involved than the anterior part of D₅D₆ and L₅S₁; this finding was confirmed by the finding of statistically significant differences in the number of isogenic chondrocyte groups among specimens. The anterior and posterior parts of L₅S₁ of the two age ranges studied (i.e., 34–94 and 35–77 years) revealed similar qualitative histological characteristics; however, the number of isogenic groups of chon-

drocytes in samples of the posterior part of L₅S₁ indicated a greater effect of compression loads compared with the anterior part of the same spinal motion segment (values in the age subgroup 35–51 years were 12 times greater than those in the age subgroup 34–52 years; values in the age subgroup 58–77 years were 4.4 times greater than those in the age subgroup 60–94 years). These findings are preliminary and require further study.

Author contributions. V.V.M. created the study concept and design, processed the materials, analyzed the data obtained, wrote the text, designed the illustrations, and revised the manuscript. M.V.V. prepared the histological materials.

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Conflict of interest. The authors declare no conflict of interest.

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