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Morphological and Age-Related Features of the Structure of the Articular Cartilage

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Abstract: Nowadays, age-related changes in the mechanisms of articular cartilage and meniscus formation have been poorly studied. The study respectively included the subjects of the mature in the age period 36-60 years and the elderly aged 61-74 years. The morphological characteristics of the articular cartilage were learned by the methods of morphometry, cyto and karyometry, histology, histochemistry. There is an accumulation of glycosaminoglycans inside the cells and their absence in the main substance also leads to a decrease in the secreted synovial fluid, which serves as a lubricant for the articular surfaces, and increases friction. During the study, age-related changes were found: an increase in the cytoplasmic-nuclear ratio (CNR), cell volume, intra-articular friction, which are a consequence of a deeper process of cellular adaptation, when the latter becomes aimed only at preserving the cells themselves, and not at ensuring the function of articular cartilage. Articular cartilage in old age is characterized by a decrease in the number of cells in the field of view, which occur in an amount from 6 to 1, their swelling, loosening, detachment, rupture of the surface layers of tangential fibers, the presence of focal seals, an increase in intra-articular friction of the articular cartilage. All this leads to deformation of the free surface of the articular cartilage in old age.

Keywords: articular cartilage, age, mature, elderly, morphological characteristics, morphometry, cyto- and karyometry, glycosaminoglycan, cells.

Introduction

Urbanization of life and the growth of hypokinemia in modern conditions lead to an increase in the number of deforming diseases of the joints of the lower extremities of a person

[1,2,3,11,12]. Successful treatment of pathological processes of the knee joint is impossible without knowledge of its normal development [3,4,8,13]. The study of the regularities of the formation of the cartilaginous elements of the articular surfaces of the knee joint in humans in connection with age and intensity of function is also necessary due to the wide spread of pathological processes of a degenerative-dystrophic nature: deforming arthrosis, hypoplasia, dysplasia, dislocation, etc. [4,5,6,7,8,9,10,13].

However, previous studies do not sufficiently reveal the mechanism of age-related rearrangement of cartilaginous tissues, do not allow identifying specific causes of the development of pathological processes. Most of the conducted studies are devoted to certain aspects of the life of cartilage tissue.

Now, the changes in the mechanisms of the formation of articular cartilage and menisci with age have been little studied.

The lack of a sufficient number of complex morphological and functional studies aimed at revealing the qualitative and quantitative patterns of growth and development of the cartilaginous elements of the joint was one of the reasons for this study. The lack of data on the qualitative and quantitative relationship of functional loads with the structural features and chemistry of tissue complicates the disclosure of mechanisms and ways of influencing the morphofunctional properties of cartilage tissue for preventive and therapeutic purposes. All this prompted us to study the patterns of formation of the cartilaginous elements of the knee joint in people of mature and elderly age using modern morphological methods.

Material and methods

In total, the study included the subjects of the mature in the age period 36-60 years and the elderly aged 61-74, respectively.

The object of the study was the articular cartilage of the distal femoral epiphysis. The dynamics of the volume of cells of the articular cartilage was studied in layers (Tables 1-3).

The morphological characteristics of the articular cartilage were studied by the methods of morphometry, cyto- and karyometry, histology, histochemistry.Along with qualitative descriptions, quantitative (organometry, morphometry, cytokaryometry) methods of studying the age-related dynamics of the cartilaginous elements of the knee joint were also used in the work, especially since a layer-by-layer study of articular cartilage in the literature available to us was not found, this allowed age (in mature and old age) to express the dynamics through quantitative morphological patterns. On the other hand, the identification of the quantitative patterns of intercellular interaction allows us to take a fresh look at the process of differentiation, growth and age-related changes in the articular cartilage of the knee joint, to quantitatively assess the degree

of influence of both age on cartilage parameters and the intensity of loads. The cytoplasmic-nuclear relationships of cells were also studied to assess their metabolic activity in people of mature and old age.

At the tissue and cellular level, the material was studied by histological and histochemical methods: staining the sections with hematoxylin and eosin van Gieson, orsein-light green, toluidol blue, Schiff reactionand Hale. We used such quantitative methods as morphometry of tissue elements cyto-and karyometry according to G.G. Avtandilov.

Cyto- and karyometry was performed on sections stained with hematoxylin-eosin, on the basis of which calculated values were determined, such as cytoplasm volume, cell volume, nucleus volume, and cytoplasmic-nuclear ratios.

Fibrous structures were studied on sections stained by the Van Gieson method, picrofusin, light green or orsein light green. To quantitatively determine the dynamics of the formation of the articular cartilage of the knee joint, morphometry of the content of the basic substance, the number of cellular elements, the number of fibers and the cross-sectional area of blood vessels was performed on histological sections. Morphometry was carried out using a standard eyepiece grid on a Biolam R-7 microscope. According to the recommendation of G.G. Avtandilov, counted the nodes of the eyepiece grid, projected onto the above tissue elements. Within the same field of view, the area occupied by the main substance, cells, fibers and vessels was simultaneously calculated: 20 fields were taken on one specimen.

The constituents of the basic substance and cells, neutral and acidic mucopolysaccharides (glycosaminoglycans) were studied by histochemical methods.

Results and discussion

The articular cartilage of the distal femoral epiphysis and the proximal tibial epiphysis at this age have a homogeneous base substance, which is stained with hematoxylin, basophilic with foci of eosinophilia. Attention is drawn to the fact that the number of cellular elements in the articular cartilage in this age period sharply decreases, this is especially noticeable in the 2nd - the surface layer and in the 5th layer - the zone of columns, where there are many empty cells with acellular content or with decaying cellular detritus (Fig.1).

In the 1st layer - in the acellular zone throughout the morphology as in the previous age, i.e. it has the form of an interlayer and has a finely lumpy rough surface. These lumps are lightly colored with eosin; branching, short fibers are visible towards the lumen of the joint space.

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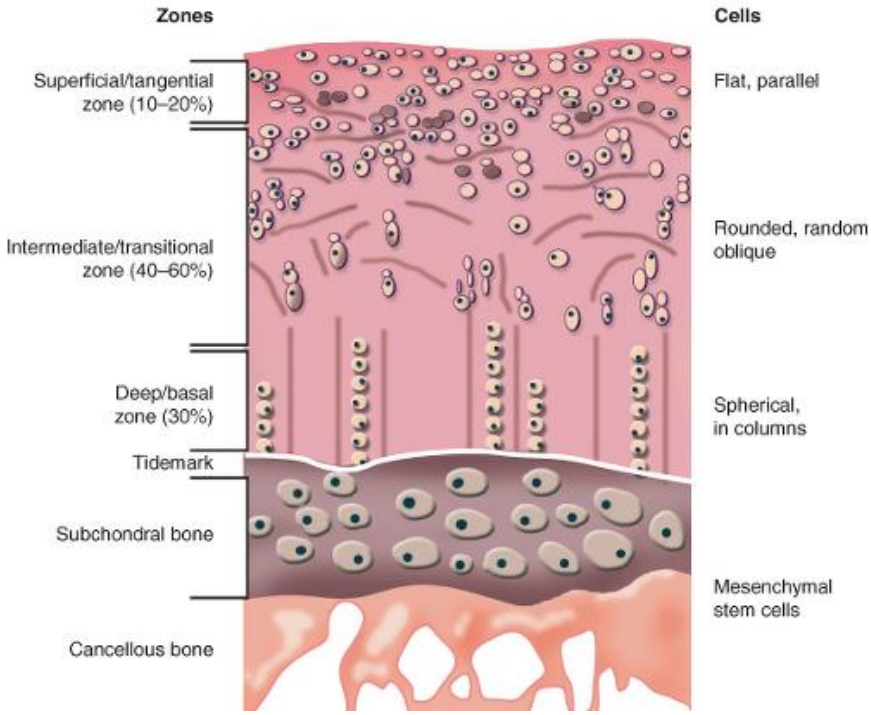

Figure 1. The morphology of articular cartilage [14].

The second surface layer has the structure of a dense fibrous layer, the fibers merge homogeneously, and this layer contains almost no cellular elements. The latter are very rare here. It can be seen that collagen fibers in this layer are also directed along the articular surface, and individual cellular elements lying deep in the old surface layer are located in the form of isogenic pairs. These cells have oval, rounded and polygonal nuclei, densely packed in cells. The cytoplasm is moderate (Fig.1).

Deeper than the surface layer lies the third layer - a transition zone, which also has a sharp decrease in the number of cells in comparison with previous age periods, apparently due to cell death, since empty cells with dying cells are often found. The remaining cells are oriented predominantly in different directions and are generally round, oval in shape. There are fewer isogenic groups here than in the previous age groups. The main substance is dense, eosinophilia predominates.

In the zone of isogenic groups - in the 4th layer, the cells are 2-3 in one cell. Their distinctive feature is that the cytoplasm is more pronounced (Fig.1).

The zone of the columns - the 5th layer at this age has a dense base substance with predominant eosinophilia. The cell chains are located between the columns of the main substance. They differ in that most of them are in the stage of destruction. Compared to the previous age period, the volume of the nuclei seems to be smaller (Table 1), they are denser and chromasia in the nuclei is not visible.

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Table 1.

Dynamics of the volume of cells of the articular cartilage (layer by layer) of the distal epiphysis of the femur (M ± m)

Age	Layers				
	II	III	IV	V	VI
	M±m	M±m	M±m	M±m	M±m
36-60 лет	23,68±0,56	19,68±0,47	23,99±0,53	28,50±0,37	41,04±0,39
61-74 года	24,39±0,61	30,01±0,59	36,75±0,47	50,76±0,40	84,41±0,75

It seems that in this layer of the cell is in unfavorable conditions, because they are all deformed to some extent. It is not possible to find such classical rounded isogenic groups of cells as in previous age periods. Even in the same cell, cells of different sizes. Such a polymorphism of nuclei and cellular elements, an increased number of decaying cells, a decrease in the volume of the nucleus, distinguish this layer at this age.

In the zone of hypertrophied cells - in the 6th layer, the main substance, as before, naturally becomes more eosinophilic, the number of cells is significantly reduced, and among the cells there are many dying and in the process of lysis of nuclei. The surviving cells are 3-5 in one isogenic group. The latter are of different sizes, which is typical for a given age (Table 1). The nuclei of cells in this layer are also reduced: the cells are round, their cytoplasm is increased in volume, the boundaries of the cells are quite clear, the polymorphism of the nuclei is also characteristic of this 6th layer. The cells in one cell are of different sizes, i.e. in this there are some signs of degenerative changes (Fig.1,Tables 2 and 3).

Table 2

Dynamics of the cytoplasmic-nuclear ratio (CNR) of articular cartilage cells (M ± m)

Age	Layers				
	II	III	IV	V	VI
	M±m	M±m	M±m	M±m	M±m
36-60 лет	6,99±0,18	5,54±0,11	7,45±0,09	6,20±0,12	12,63±0,14
61-74 года	6,11±0,13	9,57±0,14	11,29±0,14	13,76±0,14	24,27±0,25

In the calcification zone - in the 7th layer at this age, there is a sharp, intense basophilic staining. In the cartilage, there are round-shaped cells, and cells with small nuclei, with a narrow cytoplasm, lie closer to the bone tissue. This zone has the shape of a curved curve and is usually surrounded by the basophilic material of the cell chain; the main substance between them remains

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eosinophilic.

Table 3

Dynamics of the volume of nuclei of cells of articular cartilage (layer by layer) of the femur (M ± m)

Age	Layers				
	II	III	IV	V	VI
	M±m	M±m	M±m	M±m	M±m
36-60 лет	2,99±0,09	3,01±0,10	2,84±0,09	3,96±0,12	3,01±0,14
61-74 года	3,43±0,10	2,84±0,12	2,99±0,08	3,44±0,13	3,34±0,13

Summarizing the morphological picture of the articular cartilage of the knee joint, it can be noted that in the mature age period there are significant degenerative changes in the cellular material. A decrease in their number occurs mainly in the second and fifth layers.

As the age increases - in the period of 36-60 years, the structure of the articular cartilage becomes less clear, the cellular structure also becomes blurred and signs of a decrease in the synthesis of glycosaminoglycans appear, the number of metachromic material in the cytoplasm of cells decreases.

In the mature age period - 36-60 years, the dynamics of the Hale-positive structures is close to the dynamics of the metachromatic reaction, and here, too, the Hale-positive substance is revealed more intensively in the pressure zone. In the mature age period, the CHIC reaction is characterized by the appearance of poorly colored islets of the basic substance of the articular cartilage, this is especially pronounced in the pericellular zone around the cells, both in the deep and in the surface layers.

Thus, as studies show, the intensity of the PIC reaction in the articular cartilage decreases with age, both in the main substance and in the cytoplasm of cells. Obviously, the decrease in the activity of this reaction reflects the decrease in metabolic processes with age.Age period 61-74 years - old

The articular cartilage of the knee becomes thinner at this age. The study of histological sections shows that in this age period there are quite noticeable morphological differences in cartilage in comparison with the previous age. A distinctive feature is the absence of a cell-free zone on cartilage sections, oval cells with light cytoplasm lie very superficially and above them there is a thin layer of fibrous cartilage with collagen fibers. These light cells come so close to the articular surface that tears form in the areas where these cells are located. In all likelihood, when the articular surface is erased, the cells of the surface layer are exposed. These light cells of the

surface layer lie on collagen fibers; there are "breaks" in the surface area of the articular cartilage. In the surface zone and under it, shadow cells are very often observed.

Collagen fibers of this layer are characterized by a heterogeneous color. Some parts of the zone are more eosinophilic, others acquire a weak basophilia. Uneven coloration of fibers along the surface layer is also a characteristic sign of old age.

Along with the thinning of the cell-free zone and the second surface layer, many empty gaps are observed. The remaining cells of the transition zone have a light cytoplasm: the basophilia disappears. The shape of the cells is predominantly round; the cells are light with a reduced nucleus stained with hematoxylin. The main substance is weakly basophilic, with areas of weak eosinophilia, i.e. this is the heterochromism of the basic substance, which is also a characteristic feature of this age.

In the zone of isogenic groups, cells are also characterized by light cytoplasm. The cell nuclei are much smaller and are intensely basophilic stained (Table 2).

The same pattern is observed in the cellular elements of the column zone. In this zone, there is also the absence or rather weak basophilia of the cytoplasm, the presence of a large number of empty lacunae, which may indicate that there is an increased cell disintegration due to lysis of nuclei. The base substance is unevenly colored.

In addition, the basic substance of the zone of hypertrophied cells corresponding to the 6th layer has a weak electivity. The cells of this layer are larger in size compared to other layers, but around them at the boundaries of these cells there is a sharp eosinophilia. A number of cells contain "decaying" contents - detritus.

The calcification zone is also less basophilic compared to previous ages.

Further, in old age - 61-75 years, the main substance loses gamma metachromasia, becomes orthochromic. In the cellular elements, the number of metachromic granules decreases, only in the deep layers, in particular, at the level of the 5-6th layers, the alpha and beta metachromasia of the main substance is preserved. The surface layer is colored orthochromic, the fibers and the main substance are blue, metachromasia here appears only around the cells of the transition layer. In some areas, the main substance in a given age period has heterochromasia, i.e. there are irregularly colored areas of alpha and beta metachromasia, the boundaries of the cells are blurred, the cellular elements also contain significantly less metachromatic material inside and around the cell. There are cells that are completely free of metachromatic material in and around them. There is an increase in the number of large cells, with homogeneous metachromasia of the cytoplasm; there is no characteristic enhancement of matachromasia around the cells. Thus, by the age of 74, the secretion of metachromatic material drops sharply, as evidenced by their absence in

the main substance. Apparently, this accumulation led to an increase in cell cytoplasm, eccentric expulsion nuclei, and there are a lot of such figures.

The hydrophilicity of glycosaminoglycans leads to the absorption of a significant volume of water, resulting in cell edema. Perhaps this is an age-related adaptive mechanism, i.e. a decrease in the histological properties of the main substance leads to an increase in the compression of cartilaginous elements, cartilage cells, accumulating metachromatic material - glycosaminoglycans inside the cytoplasm and soldering water in them, acquire additional hydrostatic stability, i.e. a decrease in the elasticity of the basic substance leads to a compensatory increase in the rigidity of the cellular elements and their resistance to pressure. In its likelihood, age-related adaptation occurs in the same way - realized due to the predominantly intracellular synthesis of glycosaminoglycans inside the cell, against the background of its absence of extracellular synthesis. This process leads to undesirable phenomena, but, apparently, this adaptation also has its reason in the position of cellular metabolism and the preservation of cellular elements, as it were, to maintain vital activity, and not to maintain the functional properties of the entire articular cartilage. A decrease in the adaptability of all tissues of the articular cartilage to stress at the cellular level is an irrational type of adaptation.

The accumulation of glycosaminoglycans inside cells and their absence in the basic substance also leads to a decrease in the secreted synovial fluid, which serves as a lubricant for the articular surfaces, and increases friction. Consequently, the accumulation of glycosaminoglycans inside the cell is also an adaptation at the cellular level, but does not provide an adaptation effect at the tissue level and at the level of the organ: the knee joint, which leads to the fact that, in general, both cushioning properties decrease with age, and friction in the joint increases. ... Consequently, the age-related changes that we discovered earlier: an increase in the CNR, cell volume, intra-articular friction, cannot be considered one-sided, as soon as negative negative phenomena; they are a consequence of a deeper process of cellular adaptation, when the latter becomes aimed only at preserving the cells themselves, and not at ensuring the function of the articular cartilage as a tissue and the knee joint as an organ as a whole. This is the adaptation of low structures aimed at preserving themselves at the cell level, which leads to undesirable phenomena with age - to an aging decrease in the functional parameters of the knee joint, which then turns out to be on the adaptability of the entire joint and the body as a whole. Because a decrease in the functional activity of the joint leads to a deterioration in motor properties.

The qualities noted above are more pronounced in the articular cartilage of the femur than in the articular cartilage of the tibia involved in the formation of the knee joint.

In old age, not only is there a razvlecheniya articular surface of the cartilage, thinning of

the layers, the appearance of large light areas of the main substance.

After 61 years of age, the intensity of the PIC reaction in the basic substance of the articular cartilage decreases. In the deep layers, the PIC-positive material is retained, albeit in small quantities, while in the surface layers this reaction is reduced to a minimum.

Conclusions

1. In the mature age period there are significant degenerative changes in the cellular material: a decrease in the number of cellular elements is noted, mainly in the second and fifth layers of the articular cartilage.

2. As the age increases in the age range of 36-60 years, the structure of the articular cartilage becomes less distinct, the cellular structure also becomes blurred and signs of a decrease in glycosaminoglycan synthesis appear, and the number of metachromic material in the cytoplasm of cells decreases.

3. In old age, one can observe not only the disfiguring of the articular surface of the cartilage and thinning of the layers, but also the appearance of large light areas of the main substance.

4. Articular cartilage in old age is characterized by a decrease in the number of cells in the field of view, which occur in numbers from 6 to 1, their swelling, loosening, detachment, rupture of the surface layers of tangential fibers, the presence of focal seals, an increase in intra-articular friction of the articular cartilage. All this leads to deformation of the free surface of the articular cartilage in old age.

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