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Morphological Characteristics of the Cartilaginous Tissue of Human Auricle in Different Age Periods

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A complex morphological study of the auricle to determine the human age was performed by evaluating the metric sizes between fixed points in each auricle with axial guidelines. The auricular elastic cartilage in different age periods was characterized by thickening of the cartilaginous plate, different mature and immature cartilage zone ratio, variations in the volume density of the intercellular substance and elastic fibers, and change in the numerical density of individual chondrocytes and isogroups. Aggrecan content in the cartilage was shown to increase in different age periods. Age-related structural changes in the auricular cartilage expand the possibilities of forensic medical examination and hold much promise for the identification of personality.

Key Words: *morphology; auricle; age determination*

The dependence of morphological characteristics of the auricular cartilage on human age is poorly studied [10-12,15]. The data on morphohistochemical features of the human auricular cartilage in different age periods (evaluated by immunohistochemical methods) will expand the knowledge of the age-related morphology and hold much promise for person identification in forensic medicine [1,6,14].

Person identification is one of the major problems in forensic medicine [2,8]. This process plays a key role in the crime solution [2-4,7]. It is not always possible to perform a forensic craniofacial examination and forensic portrait identification of personality in the expert practice (particularly, with the body fragment as an object) [7-9].

The auricle mainly consists of cartilaginous tissue. The structure of the auricle provides its preservation over a long postmortal period, which can be used for age determination in the identification of personality [13]. Hence, the evaluation of morphological characteristics of the auricle for human age determination is an urgent problem of forensic medicine.

This work was designed to study histological features and structure of the auricular cartilaginous tissue in different periods of human life.

MATERIALS AND METHODS

We used the data obtained in studying the morphohistochemical changes in cartilaginous tissue of human auricle. The auricles and cartilage fragments were isolated during autopsy of persons dead from various causes. We examined 580 auricles from men and women of different age groups. The samples were obtained from cadavers in the Departments of Thanatology of the Krasnoyarsk Territorial Bureau of Forensic Medical Examination and Novosibirsk Oblast Clinical Bureau of Forensic Medical Examination (2010-2012).

The samples were classified by age groups in accordance with the scheme of postnatal ontogeny. This procedure was performed in accordance with the classification of the All-Soviet Union Conference on

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Problems of Age Morphology, Physiology, and Biochemistry (Academy of Pedagogical Sciences of the USSR, 1965). A study was performed with the following groups: group 1 (pre-adult age), 17-20 years; group 2 (mature age), 21-35 years; group 3 (1st period of mature age), 36-50 years; group 4 (2nd period of mature age), 51-60 years; and group 5 (elderly age), 61-75 years. All studies were conducted with the written authorizations voluntarily signed by intimates and relatives of dead persons.

Cartilage fragments of the right and left auricles were subjected to a microscopic examination. Cartilage fragments were isolated from the auricles. The auricles were dissected in the site of projection of the triangular fossa, superior and inferior crura of the antihelix, and intertragic notch (8×7 mm, overall width of the cartilage). For a light microscopy, cartilage samples were fixed in 10% aqueous solution of neutral formalin, dehydrated in alcohols of increasing concentrations, and embedded in paraffin. The sections (5-6 μ) were prepared on a sliding microtome and stained with hematoxylin and eosin. These sections were stained with orsein (to identify and study cartilage elastic fibers) or by van Gieson's method (for connective tissue). Each sample was used to prepare 3-4 histological sections.

Morphometric study was performed in accordance with the recommendations [1]. Morphological characteristics of the elastic cartilage were evaluated with a closed test system (100 points, 1.16×10^5 μ^2).

We evaluated the numerical density of chondrocytes (Nai) in the auricular elastic cartilage (per unit test area), diameter of chondrocytes and isogenic groups, volume density (Vv) of chondrocyte zones, and numerical (Nai) and volume density (Vv) of elastic fibers.

Histological sections were examined under an AxioScope A1 light microscope (Carl Zeiss). The test parameters were calculated after a morphometric study.

Numerical density of structures in test area was calculated as follows:

$$Nai=Nti/At,$$

where Nai is the numerical density of structures; and Nti is the number of profiles of study structures in test area (At).

Volume density of structures was calculated as follows:

$$Vvi=Pi/P_t,$$

where Pi is the number of test system points in the profile of study structure; and P_t is the total number of test system points in the profile of the section from study object.

The results were analyzed by methods of variation statistics [5]. The significance of differences between the means was estimated by Student's *t* test. Differences between the means were significant at $p<0.05$. Study signs were characterized by a near-normal distribution.

Immunohistochemical study was performed with a Microm HM 550 device (Carl Zeiss), PBS (washing phosphate-buffered saline), Texas Red antibodies (rabbit anti-mouse), 10% glycerol, DAPI fluorescence staining agent, Biosan thermo-shaker, Axioskop FL40 microscope (Carl Zeiss), and filter set 00 (569/613) for immunofluorescence recording.

Aggrecan content in the cartilage was measured by an indirect immunofluorescence technique. Cryostat sections (10 μ) were prepared from each cartilage fragment of the auricle on a Microm HM 550 device. The sections were placed on poly-L-lysine-coated slides (one plane), incubated with primary anti-aggrecan antibodies (R&D, mouse anti-human) for 60 min, washed 3 times with PBS, and stained with rabbit anti-mouse secondary antibodies (Texas Red). The substrate was maintained in 10% glycerol to provide a close adherence of sections. Moreover, we used the procedure of additional staining with DAPI. Incubation with primary and secondary antibodies was performed on a Biosan thermo-shaker at 24°C. Fluorometry was performed under an Axioskop FL40 microscope, filter set 00 (569/613) under standard conditions at 24°C.

The results of a light microscopic study, histochemical analysis, and morphometric examination were processed with Statistica 5.0 software.

RESULTS

The thickness of the auricular cartilaginous plate, state of the perichondrium, and thickness of immature cartilage (adjacent to mature cartilage) were evaluated during microscopy of sections from the auricular elastic cartilage stained with hematoxylin and eosin.

Cells of the cartilaginous tissue (chondrocytes) were studied during examination of mature cartilage. The numerical density of individual cells and isogroups and count of chondrocytes in isogroups were estimated in various fields of view for each section. The structure of individual chondrocytes and isogroups consisting of different number of cells was determined.

Volume density of the intercellular substance in different age periods was evaluated in sections after staining with hematoxylin and eosin. Our previous studies revealed an age-related increase in the width of the cartilaginous plate in the auricular elastic cartilage [11].

A microscopic study of sections from the elastic cartilage stained with hematoxylin and eosin showed that the zone of immature cartilage in the elderly age (61-75 years) is lower than in the pre-adult age (17-20 years). These differences are probably associated

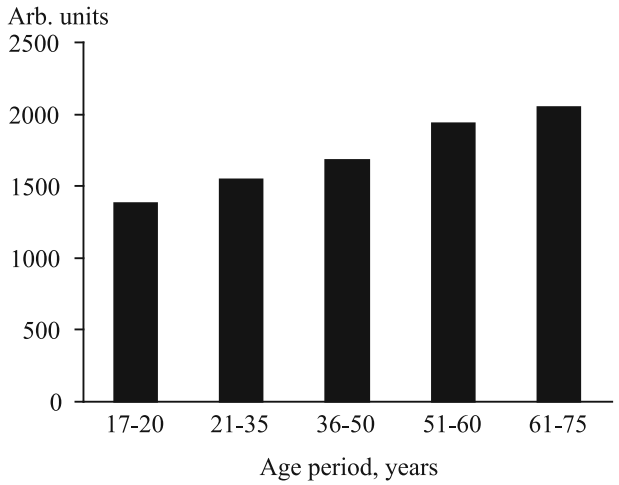

Fig. 1. Histofluorescence of aggrecan in the auricular cartilage in different age groups.

with age-related metabolic changes in the cartilaginous tissue.

Studying the immature cartilage in young subjects revealed a considerable volume density of the intercellular substance and individual chondrocytes. Mitoses were found. Only single isogroups of 4-5 chondrocytes were observed under these conditions (Table 1).

Microscopy showed that structural characteristics of the cartilage in the 1st period of mature age are similar to those in pre-adults. However, we revealed an increase in the count of isogroups with 2-3 chondrocytes and numerical density of isogroups with 4-5 cells. The ratio of mitosis was shown to decrease in this group.

The thickness of the cartilaginous plate in the age group of 36-50 years was greater than in two previous groups. These differences were mainly associated with mature cartilage. The thickness of immature cartilage was reduced in this group. Microscopy revealed a significant increase in the numerical density of isogroups in mature cartilage. The count of individual chondrocytes was also elevated.

The decrease in the zone of immature cartilage was more pronounced at the age of 51-60 years. This age group was characterized by an increase in the thickness of the cartilaginous plate and thickness of mature cartilage. Numerical density of isogroups with 2-3 chondrocytes was slightly reduced in mature cartilage. By contrast, we revealed an increase in the numerical density of isogroups with 4-5 chondrocytes. The count of individual chondrocytes in this age group was lower than in the previous group.

Numerical density of individual chondrocytes was reduced in the elderly age (similarly to the previous period). Numerical density of isogroups with 2-3 cells did not differ from that in the age period of 51-60 years. Moreover, we observed an increase in the count of isogroups with 4-5 cells.

Structural study of the auricular elastic cartilage revealed the presence of well-defined and quite large chondrocytes. Individual cells were shown to prevail. The intercellular substance dominated in the pre-adult age, progressively decreased in the adult period, and was reduced in elderly persons. Aging was accompanied by degenerative changes in the elastic cartilage (focal zones of resorption) and accumulation of collagen fibers.

Morphological analysis based on evaluation of structural characteristics of the auricular elastic cartilage in humans and morphometric examination for numerical density of chondrocytes and volume density of intercellular substance in the cartilaginous plate revealed some features of different age periods. Age-related structural changes in the auricular cartilage were identified in a microscopic study, which hold much promise for the identification of personality.

TABLE 1. Results of a Comparative Morphometric Study for the Numerical Density (Nai) of Chondrocytes in the Auricular Cartilaginous Plate in Humans of Different Age Periods ($M \pm m$)

Nai of chondrocytes, 3.64×10^5 μ²	Age period				
	pre-adult, 17-20 years	1st period of mature age, 21-35 years	2nd period of mature age		Elderly, 61-75 years
			36-50 years	51-60 years	
Solitary	199.06±6.56	201.36±7.13	244.74±8.30*	230.44±9.04	209.76±7.67
Isogroups from 2-3 chondrocytes	17.66±0.52	20.62±0.54*	25.74±0.82*	22.40±0.88*	22.42±0.74
Isogroups from 4-5 chondrocytes	2.54±0.07	5.40±0.17*	7.02±0.24*	7.58±0.25	8.12±0.25

Note. *$p<0.05$ in comparison with the previous age period.

Morphohistochemical study of the cartilaginous tissue revealed morphological changes and parameters of fluorescence reflecting the state and distribution of aggrecan in the auricular cartilaginous tissue (Fig. 1). We observed an age-related increase in aggrecan content in the cartilaginous tissues (from 1385 arb. units at the age of 17-20 years to 2046.3 arb. units at the age of 61-75 years).

We conclude that the auricular elastic cartilage in different age periods is characterized by thickening of the cartilaginous plate, change in the zone ratio of mature and immature cartilages, variations in the volume density of the intercellular substance and elastic fibers, and change in the numerical density of individual chondrocytes and isogroups. Aggrecan content in the cartilage increases in different age periods.

Morphological data on structural characteristics of the auricular cartilage can be used to determine the human age, which expand the possibilities of forensic medical examination in the identification of personality (*e.g.*, from the remains of unknown subjects).

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