Age-related changes in the structure of myocardial collagen network of auricle of the right atrium in healthy persons and ischemic heart disease patients

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Key words: heart, myocardial collagen network, ischemic heart disease, age, video morphometric analysis.

Summary. The objective of the study was to examine and evaluate morphometrically age-related changes in the structure of myocardium collagen network of auricle of the right atrium in control group persons, who were not diagnosed with cardiac pathology leading to heart lesion or overload, and in ischemic heart disease patients.

Material of 56 persons of both genders aged 20–94 years was used for study purposes. Biopsy material of 17 healthy persons (control group, average age 60.53±9.89 years) and autopsy material of 39 ischemic heart disease patients (average age 63.83±15.67 years) taken from the basis of auricle of the right atrium (specimen size – 2 mm × 2 mm) were examined. Morphometric analysis of collagen network was performed using histologic and video morphometric methods. After this investigation we evaluated quantitative parameters of the bundles of collagen net – namely area, number, perimeter. The percentile occupied area of bundles in control group was 17.6±2.5%; ischemic heart disease patients group – 26.8±2.9%, number of bundles was 4179±1073 and 2523±867; perimeter – 24163±3308 μm and 23426±409 μm, respectively.

After investigation of age-related changes of collagen network in control group and ischemic heart disease patients’ group, which did not statistically significantly differed by age, we determined that collagen network area in auricle of the right atrium increased with age in both groups, however, spatial distribution of collagen network was different. Collagen network area enlarged with lengthening of its fibers along cardiomyocytes in control group. In ischemic heart disease group, it enlarged both in parallel to cardiomyocytes and by separate collagen fibers merging into bigger bundles. Fibrillar collagen network area and its total perimeter of healthy persons increased with age, and number of fibers did not change. Consequently, collagen fiber area of one location increased with age: its shape, judging by in parallel increasing total perimeter, became branchier, i.e. proliferated in endomysium in parallel with cardiomyocytes. In ischemic heart disease group fibrillar collagen network percentage area increased with age, however, total perimeter and number of separate fibers in visual field decreased. Consequently, in ischemic heart disease group separate collagen fibers merged, their locations enlarged, taking an integral structure, which allowed assuming development of interstitial fibrosis.

Introduction
Recently more attention is being paid to prevention and treatment of cardiovascular diseases; scientists work intensively, performing research in remodeling of cardiomyocytes and collagen. Collagen is one of the most important structures of the connective tissue. It is the main structural protein in the interstitium. Currently functional significance of myocardial collagen is emphasized due to its importance for contraction and nutrition of cardiomyocytes (1–4). Five molecular isoforms of collagen are found in the heart: type I, III, IV, V and VI collagens. Fibrillar types of I and III collagens are the most abundant and constitute 90% of total amount in the myocardium. Type I collagen consists of thick and tensile fibers. Type III collagen forms thin reticular, more elastic network than type I collagen, and usually is found together with type I collagen, forming collagen fibers in myocardium between myocytes and muscle fibers (5). Many publications could be found on remodeling

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mechanisms, changes in collagen network in different cases of heart pathology, and on experimental research using different preparations, which influence remodeling of collagen network (1, 6–11).

Currently research studies of changes occurring during the remodeling process of cardiomyocytes and collagen in cases of arterial hypertension, myocarditis and dilated cardiomyopathy predominate (3, 5, 8, 12, 13). In more detail remodeling processes are examined in aspect of changes in cardiomyocytes and contractile collagen network in patients suffering from ischemic heart disease and after myocardial infarction (14–16). However, very few clinical trials of this type are dedicated for analysis of age-related remodeling processes. There are few extensive morphometric studies, which evaluate fibrillar collagen network of human atrial myocardium. Even though many studies were performed investigating structural, functional and biochemical changes of collagen, the main attention was paid to changes of collagen in ventricles but not in atria. No studies were performed with the aim to evaluate age-related changes of contractile collagen network of human atrial myocardium (17).

The objective of the study was to examine and evaluate morphometrically age-related changes in the structure of myocardium collagen network of auricle of the right atrium in control group persons, who were not diagnosed cardiac pathology leading to heart lesion or overload, and in ischemic heart disease patients.

Material and methods

Study material consisted of 56 persons (control group) and ischemic heart disease (IHD) patients of both genders aged 20–94 years. 17 healthy people aged 20–94 years (average age 60.53±9.89 years) and 39 IHD patients, aged 40–80 years (average age 63.83±15.67 years) were investigated.

Material for study was taken from the basis of auricle of the right atrium. It was always taken from the same location. Either autopsy or biopsy material was examined. For control group, autopsy material was taken no later than 3–4 hours after death at the Clinic of Pathologic Anatomy of Kaunas University of Medicine. For IHD group biopsy material was taken during coronary artery bypass grafting operation at the Clinic of Cardiac Surgery of Kaunas University of Medicine.

Morphometric analysis of collagen network was performed using histologic and video morphometric methods (18). Study material (specimen size – 2 mm × 2 mm) was fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 1–2 days, embedded in paraffin and then sectioned into 6 μm slices using microtome. Collagen was stained with Picrosirius red. Structure of collagen fibers was enhanced using polarizing light microscopy (Fig. 1).

Preparations were analyzed using optic microscope “Micros” (Austria), magnifying 20x. Ten images of each person were registered for examination of myocardium collagen network. Images were selected visually based on optimal brightness of examined structures. Area of one visual field was 0.25 mm². Totally 560 atrial myocardium fields were examined using semi-automated image analysis system. These images were recorded onto personal computer using color digital video camera “Pixera” and analyzed using special image processing program “Image-Pro Plus v. 4”.

Using this program color image is transformed to binary image, i. e. black and white image. For enhancement of structures of collagen network we used 255 halftone image levels (255=white, 0=black). Detailed quantitative analysis of myocardium collagen network was performed: bundle area, their perimeter and number were calculated. Area was expressed in percent as comparative value between examined structures and the whole visual field. Data was processed and statistically analyzed using “Microsoft Excel” program.

Distribution of morphologic examination data of collagen network in both groups was analyzed using Kolmogorov-Smirnov criterion. It was determined that null hypothesis on normal distribution of analyzed data should not be rejected, therefore sample average approximation method and dispersion analysis were used. Sample average and dispersion confidence intervals of morphometric parameters were evaluated. Established short interval length as well as small ratio between standard deviation and parameter average (for all parameters less than 5%) show sufficient sample size and that it well reflects general population.

For comparison of averages of control group and IHD group, two-factor variance analysis (ANOVA) was performed, evaluating parameter dispersions of group and case (nested design). Correlation between age of study participants and percentage area, number and perimeter of collagen structures was determined using regressive analysis.

Results

Video morphometric analysis revealed that area of collagen bundles in control group was 17.6±2.5%
and in IHD group – 26.8±2.9%; number of bundles was 4179±1073 and 2523±867, respectively; perimeter was 24163±3308 μm and 23426±409 μm, respectively. Area of collagen bundles was 51.7% bigger in IHD group than in control one (p<0.001); number of bundles was 39.6% smaller (p<0.001), and perimeter of collagen network was only 3.1% smaller in IHD group than in control group (p<0.05) (Fig. 2).

**Fig. 1.** Evaluation of quantitative changes of myocardium collagen network preparations stained with Picrosirius red using standard light microscopy (left) and polarized light microscopy (right)

**Fig. 2.** Comparison of collagen network area, number of bundles and perimeter in control group and IHD group (average and standard deviation, *p<0.001, **p<0.05)
Linear correlation between fibrillar collagen network area of myocardium of auricle of the right atrium and number of its separate bundles was weak both in control and in IHD group (Fig. 3, 4). Correlation between collagen network area and perimeter was close to the second-degree polynomial, however, in control group with an increase of area, perimeter also increased ($r=0.74$, $p<0.001$). In IHD group it was opposite: with an increase of area, perimeter decreased, however, this correlation was weak ($r=0.23$, $p<0.001$).

Correlation between collagen parameters and age in control group is presented in Figure 5. Even though collagen bundle area, expressed in percent, increased linearly with age ($r=0.95$, $p<0.001$), change in number of bundles was not observed ($p>0.05$). Collagen network perimeter increased linearly with age ($r=0.73$, $p<0.001$).

In IHD group (40–80 years) collagen bundle area increased linearly with age ($r=0.85$, $p<0.001$), number of bundles ($r=0.53$, $p<0.001$) and perimeter decreased.

**Fig. 3. Correlation between auricle of the right atrium myocardium collagen network area, number of bundles and perimeter in control group**

**Fig. 4. Correlation between auricle of the right atrium myocardium collagen network area, number of bundles and perimeter in IHD group**

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Fig. 5. Relationship of collagen network area, number of bundles and perimeter with age in control group

$y = 0.16x + 7.58$; 
r = 0.95;  
p = 0.00001.

$y = 2.69x + 3948.99$;  
p = 0.8

$y = 130.2x + 15710.8$; 
r = 0.73;  
p = 0.00079.
After investigation of age-related changes in collagen network in control and IHD groups, which did not statistically significantly differed by age, we determined that in both groups fibrillar collagen network of auricle of the right atrium increased with age (Fig. 7, 8), however spatial distribution of collagen network differed. In control group collagen area increased via lengthening of its fibers along cardiomyocytes; in IHD group it increased both in parallel to cardiomyocytes, and by merging of col-

\[
y = 0.25x + 11.66; \\
r = 0.85; \\
p = 0.0001. 
\]

\[
y = -46.47x + 53336.65; \\
r = 0.53; \\
p = 0.00052. 
\]

\[
y = -152.7x + 32669; \\
r = 0.44; \\
p = 0.0047. 
\]

**Fig. 6.** Relationship of collagen network area, number of bundles and perimeter with age in IHD group
Fig. 7. Age-related quantitative changes in myocardium collagen network of auricle of the right atrium in control group patients
A – normal (40 year old person), B – slightly increased (65 year old person), C – moderately increased (81 year old person).

Fig. 8. Age-related quantitative changes in myocardium collagen network of auricle of the right atrium in IHD patients
A – slightly increased (40 year old person), B – moderately increased (65 year old person), C – markedly increased (81 year old person).

Age-related changes in the structure of myocardial collagen network in health and ischemic heart disease

Age-related quantitative changes in myocardium collagen network of auricle of the right atrium in IHD patients
A – slightly increased (40 year old person), B – moderately increased (65 year old person), C – markedly increased (81 year old person).

In control group fibrillar collagen network percentage area increased with age, however total perimeter and number of fibers in the visual field decreased. Consequently in IHD group separate collagen fibers merged, their location areas increased taking integral structure, which allowed assuming development of interstitial fibrosis. This confirms that fibrillar collagen network of myocardium of auricle of the right atrium changes with age. Both in control and IHD group fi-
brillar collagen network percentage area equally increases starting with the fifth decade of life.

**Discussion**

Collagen is the only human protein, physico-chemical characteristics of which change with age, its amount in myocardium decreases, even though its area increases. Similar age-related development of fibrillar collagen network is determined in the studies performed by other authors (10). Literature data show that decrease of average optical density with age is related to increased amount of binding tissue (proteoglycans) among collagen fibrils (main structural unit, which forms collagen fiber), even though diameter of fibers also increases in advanced age (17).

With an increase of amount of proteoglycans, amount of collagen and elasticity of myocardium decrease. Increased total fibrillar collagen network percentage capacity and bigger separate location areas increase resilience of myocardium, change direction of force vectors and disturb its contractile function (7, 10, 18, 19).

Even though for IHD patients hemodynamic overload falls on the right atrium in the last place of all heart parts, we determined that its myocardium collagen network percentage area was bigger, perimeter of collagen fibers did not differ, and perimeter of separate bundles was smaller. Consequently, in IHD group amount of collagen in auricle of the right atrium myocardium not only increased, but also its fibers merged into bigger bundles. This reactive interstitial fibrosis develops when synthesis of type I and III collagens increases, and when their degradation is unchanged or decreased. Together increased collagen concentration is defined as fibrosis, which can start both in case when cardiomyocyte hypertrophy is present or not. Collagen accumulation is determined by volume and pressure overload (12, 20, 21).

There is very little data on remodeling of atria and age-related changes of collagen network. We could not find similar studies, which would analyze age-related changes of collagen network of the atrium. There are more studies analyzing structural and functional changes in case of heart diseases: hypertension, valve pathology and ischemic heart disease (3, 9, 12, 16, 20, 22).

It is determined that origin of atrial fibrosis is influenced by extracellular signal-regulated protein kinase and angiotensin receptors when atrial fibrillation is present. Renin-angiotensin-aldosterone activation determines cell growth, fibroblast proliferation and atrial fibrosis (8, 11, 14, 23). Due to increased atrial strain, natriuretic peptides are excreted. In experiments with rats, analyzing model of heart fibrosis, it was determined that when type B natriuretic peptide was not excreted, ventricular fibrosis developed (24–26). In IHD patients, we did not observe atrial fibrillation, however area of collagen network was 50% bigger compared with healthy patients; i.e. myocardial ischemia is related not only to changes in collagen network of ventricles but also of atria.

Supposedly, in cases of short-term ischemia episodes, reserve, flow and contractile function changes of compensative character occur in atrium. Increased flow function at first compensates disturbances of atrial systole; later changes in these functions become more severe and insufficiency develops (21).

In case of ischemia reverse correlation exists between activity of ventricle and atrium; when ventricular stroke volume index decreases, atrial stroke volume index increases. With decrease in ventricular ejection fraction, atrial pump or contractile function increases (3, 21, 26, 27). Strengthening of contractile function might be a response to an increased preload and post load due to dilated ventricular tightness.

In addition that collagen network area was determined, perimeter of its fibers and number of bundles were also determined, which not only widens possibilities for quantitative assessment, but also provides objective information on qualitative characteristics of collagen network. Similar collagen network perimeter of IHD patients and healthy persons and in case of IHD smaller number of collagen bundles indicates the presence of bigger and more rounded collagen fiber bundles. D. Pangonyté et al performed analogous measurements investigating changes in myocardial collagen network of the left ventricle (14, 15). However, in myocardium of left ventricle in IHD patients they found bigger perimeter of collagen network as well as number of bundles, i.e. forming of collagen fibers in parallel to cardiomyocytes.

**Conclusions**

Quantitative parameters of collagen network bundles: area, number and perimeter, evaluated using video microscopic system, provide additional information on structural changes occurring in myocardium of auricle of right atrium and their correlation with ischemic heart disease. Remodeling of collagen network of auricle of right atrium occurs both due to age and myocardial ischemia. In ischemic heart disease group percentage area (51.7%) of collagen network bundles was bigger; number (39.6%) and perimeter (3.1%) were smaller compared with data of control group. Collagen network bundle percentage area increases

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Sveikų žmonių ir sergančiųjų širdies liga dešiniojo priešūrdžio ausytės miokardo kolageno tinklo sandaros pokyčiai, susiję su amžiumi

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Age-related changes both in healthy and ischemic heart disease patients, starting with the fifth decade of life. In control group number of collagen bundles does not change with age, however, their perimeter increases; in ischemic heart disease patients both number of collagen bundles and perimeter decrease.

Sveikų žmonių ir sergančiųjų širdies liga dešiniojo priešūrdžio ausytės miokardo kolageno tinklo sandaros pokyčiai, susiję su amžiumi

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Raktažodžiai: širdis, miokardo kolageno tinklas, išeminė širdies liga, amžius, vaizdo morfometrinis tyrimas.

Santrauka. Darbo tikslas. Įvertinti ir morfometriškai įvertinti dešiniojo priešūrdžio ausytės miokardo kolageno tinklo sandaros pokyčius, susijusius su amžiumi, bei sergančių širdies liga amžių.

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