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Abstract

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PATHOLOGICAL BIOMINERALIZATION IN HEART VALVES AFFECTED BY ATHEROSCLEROSIS

Due to the aging of Ukrainian population the prevalence of growing of atherosclerotic affection of heart valve structures is observed, it affects significantly the life quality and prognosis for patients. Pathological biomineralization of heart valves has an adverse prognostic value for the course of coronary heart disease and it is accompanied by increase of frequency of sudden coronary death.

The aim of the work is the morphological study of mineralized tissue of aortic and mitral heart valves, which were affected by atherosclerosis.

During the study we used macroscopic description, histology and histochemistry, scanning electron microscopy with X-ray difraction. Material of 49 section cases was investigated, in which 30 patients had the combination of aortic valve (AV) and mitral valve (MV) affection, 16 and 3 had isolated affection of only MV and AV, respectively.

The average age of death people with atherosclerotic affection of MV was 69.09 ± 1.34 years, with AV affection – 68.84 ± 1.54 years. MV affection was equal due to the gender (50 % men and 50 % women), in the case of AV affection women (54.6 %) dominated insignificantly.

During macroscopic study of heart valves it was found that biomineral deposits were located in the cusps or in the fibrous ring in the case of atherosclerotic valve affection. Histological examination shows that thickening of the fibrous layer and elastic fibers, focal deposition of lipids, myxomatous changes, edema were found in the affected valve components.

The presence of calcium compounds in the identified biominerals was confirmed by histochemical staining with alizarine red and due to the method of von Kossa.

Study of the specific mineral component in the tissue of various heart valves (HV) showed the following results. MV contained in average 17.35 ± 2.08 % of calcified substance, 18.84 ± 2.23 % of biominerals was found in AV ($p > 0.46$).

During SEM with X-ray microanalysis mineralized HV elements were detected as bright objects with white and gray color, in the form of blocks, lumps, small powdered particles, it didn't depend on the localization – MV or AV. Biomineral part of leaves and fibrous ring was inlaid into the histological structure of valves and it is associated closely with connective tissue component of the organ. In some places delamination of connective tissue and elastic fibers was observed, in other locations biomineral tissue passed fluently into the surrounding stroma. X-ray diffraction of mineralized components of all HV locations shows a similar chemical composition, which is close to the ratio of calcium and phosphorus, most of which corresponded to hydroxyapatite.

Keywords: heart valves, hydroxypatite, morphological changes, atherosclerosis, biomineralization.

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Резюме

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ПАТОЛОГІЧНА БІОМІНЕРАЛІЗАЦІЯ В СЕРЦЕВИХ КЛАПАНАХ, УРАЖЕНИХ АТЕРОСКЛЕРОЗОМ

У зв’язку зі старінням популяції України відбувається ріст поширеності атеросклеротичного ураження клапанних структур серця, що суттєво погіршує якість життя та прогноз для пацієнтів. Патологічна біомінералізація клапанного апарату серця має несприятливе прогностичне значення для перебігу ішемічної хвороби серця та супроводжується збільшенням частоти раптової коронарної смерті.

Метою роботи є морфологічне дослідження мінералізованих тканини серцевих клапанів, уражених атеросклерозом.

Під час дослідження використали гістологічні, гістохімічні методи та скануючу електронну мікроскопію з рентгенівською дифракцією. Всього було досліджено 49 пацієнтів, з них у 30 – поєднання ураження аортальних клапанів (АК) та мітральних клапанів (МК), у 16 та 3 – відповідно ізольоване ушкодження лише МК та АК.

Середній вік померлих з атеросклеротичним ураженням МК становив 69,09 ± 1,34 року, з ураженням АК – 68,84 ± 1,54 років. За гендерною ознакою спостерігалася рівність при ураженні МК (50 % чоловіки і 50 % жінки), при ураженні АК незначно переважали жінки (54,6 %).

При макроскопічному дослідженні серцевих клапанів виявлено, що при атеросклеротичному ураженні біомінеральні депозити локалізувалися в стулках або в фіброзному кільці. Гістологічне дослідження показує, що в уражених компонентах клапанного апарату виявляється потовщення фіброзного шару та еластичних волокон, вогнищеве відкладення ліпідів, міксоматозні зміни, явища набряку. Присутність сполук кальцію у виявлених біомінералах підтверджувалася за допомогою гістохімічних забарвлень алізаріновим червоним та методом фон Косса.

Дослідження питомої частки мінерального компоненту в тканині різних СК показало наступні результати. МК у середньому містили 17,35 ± 2,08 % кальцифікованої речовини, в АК виявлено 18,84 ± 2,23 % біомінералів.

При СЕМ із рентгенівським мікроаналізом мінералізовані елементи СК, незалежно від локалізації – МК, АК виявлялися як яскраві об’єкти біло-сірого кольору у вигляді брил, грудок, дрібних порошкоподібних частинок. Біомінеральна частина листків та фіброзного кільця була інкрустована в гістологічну структуру клапанів і тісно пов’язана зі сполучнотканинним компонентом органу. В окремих місцях спостерігалось розшаровування еластичних і сполучнотканинних волокон, в інших локаціях біомінеральна тканина плавно переходила в навколишню строму. Рентгенівські дифрактограми мінералізованих компонентів усіх локалізацій СК показували подібний хімічний склад, близький за співвідношенням кальцію та фосфору, переважна більшість яких відповідала гідроксиапатитам.

Ключові слова: серцеві клапани, гідроксиапатит, морфологічні зміни, атеросклероз, біомінералізація.

Резюме

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ПАТОЛОГИЧЕСКАЯ БИОМИНЕРАЛИЗАЦИЯ СЕРДЕЧНЫХ КЛАПАНОВ, ПОРАЖЕННЫХ АТЕРОСКЛЕРОЗОМ

В связи со старением популяции Украины происходит рост распространенности атеросклеротического поражения клапанных структур сердца, что существенно ухудшает качество жизни и прогноз для пациентов. Патологическая биоминерализация клапанного аппарата сердца имеет неблагоприятное прогностическое значение для течения ишемической болезни сердца и сопровождается увеличением частоты внезапной коронарной смерти.

Целью работы является морфологическое исследование минерализованных ткани сердечных клапанов, пораженных атеросклерозом.

Во время исследования использовали гистологические, гистохимические методы и сканирующую электронную микроскопию с рентгеновской дифракцией. Всего было исследовано 49 пациентов, из них у 30 – сочетание поражения аортальных клапанов (АК) и митральных клапанов (МК), в 16 и 3 – соответственно изолированное повреждение только МК и АК.

Средний возраст умерших с атеросклеротическим поражением МК составил 69,09 ± 1,34 года, с поражением АК – 68,84 ± 1,54 лет. По гендерному признаку наблюдалась равенство при поражении МК (50 % мужчины и 50 % женщины), при поражении АК незначительно преобладали женщины (54,6 %).

При макроскопическом исследовании сердечных клапанов выявлено, что при атеросклеротическом поражении биоминеральные депозиты локализовались в створках или в фиброзном кольце. Гистологическое исследование показывает, что в пораженных компонентах клапанного аппарата оказывается утолщение фиброзного слоя и эластических волокон, очаговое отложение липидов, миксоматозной изменения, явления отека. Присутствие соединений кальция в выявленных биоминералы подтверждалась с помощью гистохимических окрасок ализариновим красным и методом фон Косса.

Исследование удельной доли минерального компонента в ткани различных сердечных клапанов (СК) показало следующие результаты. МК в среднем содержали 17,35 ± 2,08 % кальцифицированными вещества, в АК выявлено 18,84 ± 2,23 % биоминералов.

При сканирующей электронной микроскопии (СЭМ) с рентгеновским микроанализом минерализованные элементы СК, независимо от локализации – МК, АК оказывались как яркие объекты бело-серого цвета в виде глыбок, комков, мелких порошкообразных частиц. Биоминеральная часть лепестков и фиброзного кольца была инкрустирована в гистологическую структуру клапанов и тесно связана с соединительнотканным компонентом органа. В отдельных местах наблюдалось расслаивание эластичных и соединительных волокон, в других локациях биоминерального ткань плавно переходила в окружающую строму. Рентгеновские дифрактограммы минерализованных компонентов всех локализаций СК показывали сходный химический состав по соотношению кальция и фосфора. Подавляющее большинство биоматериалов по фазовому составу отвечала гидроксиапатитам.

Ключевые слова: сердечные клапаны, гидроксиапатит, морфологические изменения, атеросклероз, биоминерализация.

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Introduction

Structural changes of the demographic situation in Europe countries and Ukraine have the tendency to the growth of population in older age groups, it leads to an increase in degenerative and metabolic diseases [1]. That's why there is a prevalence of growing of atherosclerotic affection of heart valve structures, which worsens significantly the life quality and prognosis for patients [2]. The prevalence of heart valves affection with different origin is 20–30 % of patients aged over 65 years and reaches 48–57 % in patients in the age of 80 years [3].

An aortic valve (AV) damage with valvular calcification is the most common disease of heart valves in Europe and in the USA, especially in the older group of patients [4]. Thus, in developed countries near 50 000 replacing heart valves operations are carried out because of this disease each year [1].

The aim of the work is the morphological study of mineralized tissue of aortic and mitral heart valves, which were affected by atherosclerosis.

Materials and methods

Protocol of ethics committee. Conducting of the study was approved by the ethics committee of Medical Institute of Sumy State University (Protocol №1, 14.01.14).

Study was conducted on the section material, which were obtained during autopsies in Sumy Regional Postmortem Office. Heart valves with signs of atherosclerotic affection and biomineralization were selected for the study. 46 mitral (MV) and 33 aortic (AV) heart valves were studied, they were obtained from the dead people with different somatic pathology and manifestations of atherosclerotic affection of cardiovascular system. Tissue of heart valves was investigated by the methods of macroscopic description, histology and histochemistry, scanning electron microscopy with microanalysis to detect pathological biomineralization.

Histological and histochemical methods.

For histological study heart valves material was fixed in 10 % solution of neutral formalin for 24 hours. Paraffin blocks making was performed with the usage of the conventional method. Paraffin serial sections were produced on rotary microtome Shandon Finnesse 325 (Thermo Scientific) with the thickness of 4–5 microns, they were stained with hematoxylin-eosin, alizarine red and due to the method of von Kossa.

Scanning electron microscopy.

Sample preparation. Histological sections from paraffin waxes preparations with the thickness of 6–7 microns were placed on graphite object tables. Preparations were kept for 30 minutes in an incubator at 60 ºC. Then paraffin sections were covered with xylene for 5 minutes three times, then they were covered with 96 % ethanol for 5 min three times, they were rinsed with distilled water. Made preparations were investigated on the scanning microscope REMMA 100U with energy dispersible X-ray spectrometer (Selmi, Ukraine).

Mineral component determination

To establish the amount of mineral content in the samples of heart valves tissue were processed with the usage of thermal treatment in a muffle furnace at 200 °C for 1 hour. It gave a possibility to remove free water and the organic part of organic-mineral aggregates, and to keep the unchanged mineral structure. The ashes of organic tissues are separated easily mechanically from the thick tissue of biominerals during such a low temperature of thermal treatment.

Statistical analysis

Data processing was carried out by applied statistical methods, using the Microsoft Excel 2010 with add-in program AtteStat 12.0.5. Results of the research have been checked for normal distribution (ND) using Shapiro–Wilk test. Most measurement results had incorrect distribution that's why non-parametric Wilcoxon signed-rank test was used to evaluate statistical significance. In the cases of correct normal distribution of sample data, their comparing was performed by using parametric Student's t-test, in the condition of the confirmation of chance differences ($F_{crit} > F_{exp}$) according to Fisher's exact test. If $t_{exp} \geq t_{krit}$, the difference was considered to be reliable.

Research results

Material of 49 section cases was investigated, in which 30 patients had the combination of aortic valve (AV) and mitral valve (MV) affection, 16 and 3 had isolated affection of only MV and AV, respectively.

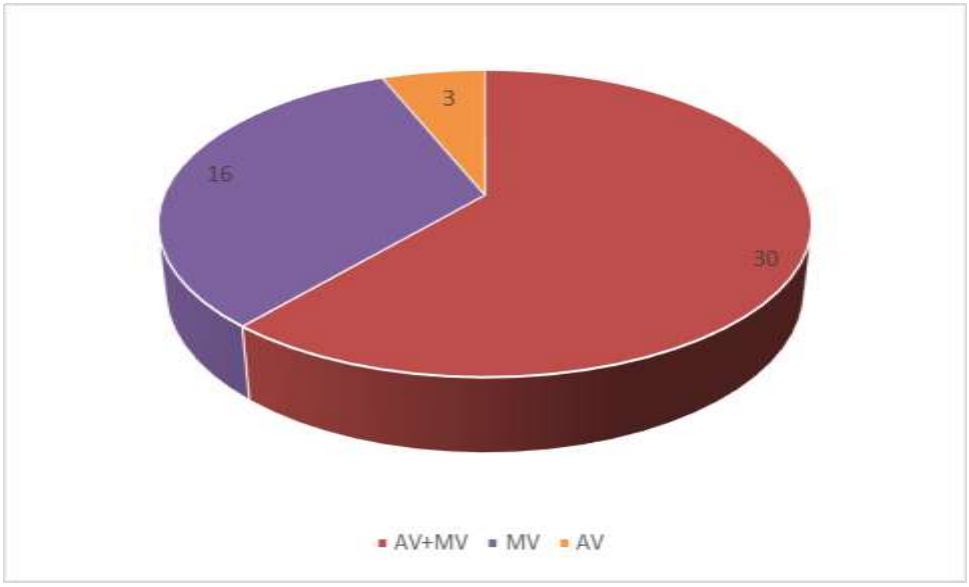

Figure1 – Localization of heart valves affection. AV – aortic valve, MV – mitral valve, AV + MV – involvement of both valves into the pathological process

The average age of death people with atherosclerotic affection of MV was 69.09 ± 1.34 years, with AV affection – 68.84 ± 1.54 years. MV affection was equal due to the gender (50 % men and 50 % women), in the case of AV affection women (54.6 %) dominated insignificantly.

Signs of atherosclerotic affection and different mineralized fragments of the pathologically altered tissue are detected easily during the macroscopic study of heart valves. Visually, the affected valve cusps were thickened, opaque or dusky, they had whitish or grayish color with yellow shades. Atherosclerotic plaques manifested in size from 0.2 to 1.2 cm on the leaflets of the investigated valves. Some valve cusps were deformated, sometimes ulcerated. Left atrioventricular aperture, aortic bulb had a tendency to the narrowing, fibrous valve ring was tight, it was cut difficult and with typical crunch. Often biomineral deposits were located in the cusps or in the fibrous ring in the case of atherosclerotic valve affection.

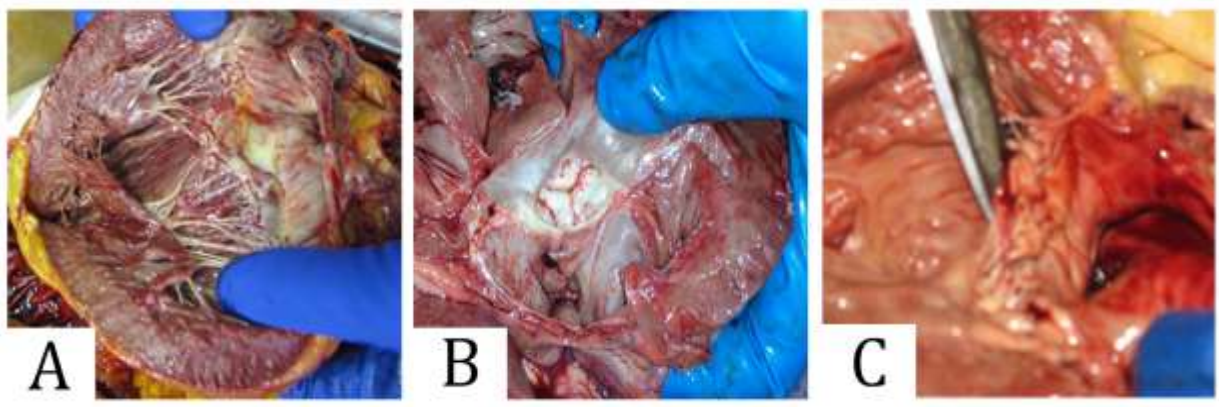

Figure 2 – Macropreparations of heart valves with atherosclerotic affection. A. Mitral valve, leaflets thickening. B. Aortic valve. C. Mitral valve, biomineralization of valve fibrous ring (under the knife).

Histological study shows the presence of atherosclerotic plaques with detritus formation, biominerals, connective tissue and inflammatory infiltration in heart valves tissue (Fig. 3 A–B). The level of atherosclerotic process evidence in the HV varies, but the thickening of the fibrous layer and elastic fibers (Fig. 3 C–D), focal lipids deposition, sometimes myxomatous changes, edema are revealed in the affected components of valve. Pathological biomineralization was revealed well in the HV micropreparations, which were stained with hematoxylin-eosin (as purple or dark blue deposits).

The presence of calcium compounds in the identified biominerals was confirmed by special histochemical staining with alizarine red and due to the method of von Kossa (Fig. 3 E–F).

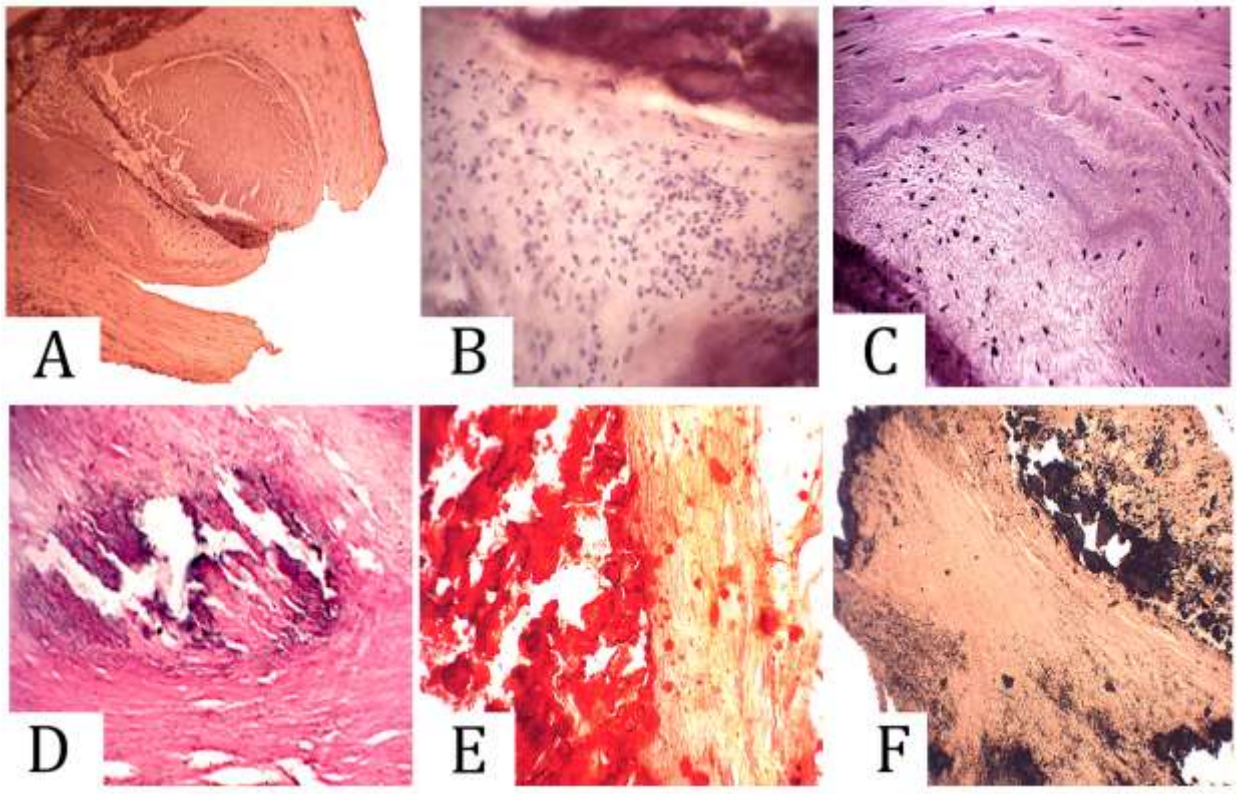

Figure 3 – Histological changes in heart valves affected by atherosclerosis. A. Aortic valve with the formation of atherosclerotic plaque. Magnification x40. B. Mitral valve with inflammatory infiltration in the tissue around calcifications. Magnification x400. C. Thickening of elastic fibers in the mitral valve cusp. Magnification x400. A–C – hematoxylin-eosin staining. D. Fibrous tissue around the calcified plaque in heart valve. Magnification x100. The staining due to the Malori method. E. Mineralized tissue of the mitral valve. Magnification x100. The staining with alizarine red. F. Mineralized tissue of aortic valve base. Magnification x100. The staining due to the method of von Kossa.

Parts of deposits of calcium compounds in the cusps and in the fibrous rings of valves had mainly irregular polygonal shape, indeterminate outlines. Often biomineral deposits were separated from surrounding tissues by clear "fibrous" border and, in many cases, by the inflammatory infiltrate (Fig. 3 B, 3 D). It is noticeable in micropreparations that biominerals are located as amorphous masses, lumps, spherolits, plates, solid masses (Fig. 3 E–F). It should be noted that biomineralized tissue crumbles, is was destroyed during the samples preparation on microtome that's why there are artifacts and defects in the micropreparations .

During SEM with X-ray microanalysis mineralized HV elements were detected as bright objects with white and gray color, in the form of blocks, lumps, small powdered particles, it didn't depend on the localization – MV or AV (Fig. 4). Big biomineral formations have the signs of destruction and fragmentation, which originate from a damage of samples during microtome section making. Biomineral part of leaves and fibrous ring was inlaid into the histological structure of valves and it is associated closely with connective tissue component of the organ. In some places delamination of connective tissue and elastic fibers was observed, in other locations biomineral tissue passed fluently into the surrounding stroma. Small mineral objects were detected in the valve tissue as a "decoration", entwining organically into a connective tissue skeleton.

X-ray diffraction of mineralized components of all HV locations shows a similar chemical composition, which is close to the ratio of calcium and phosphorus.

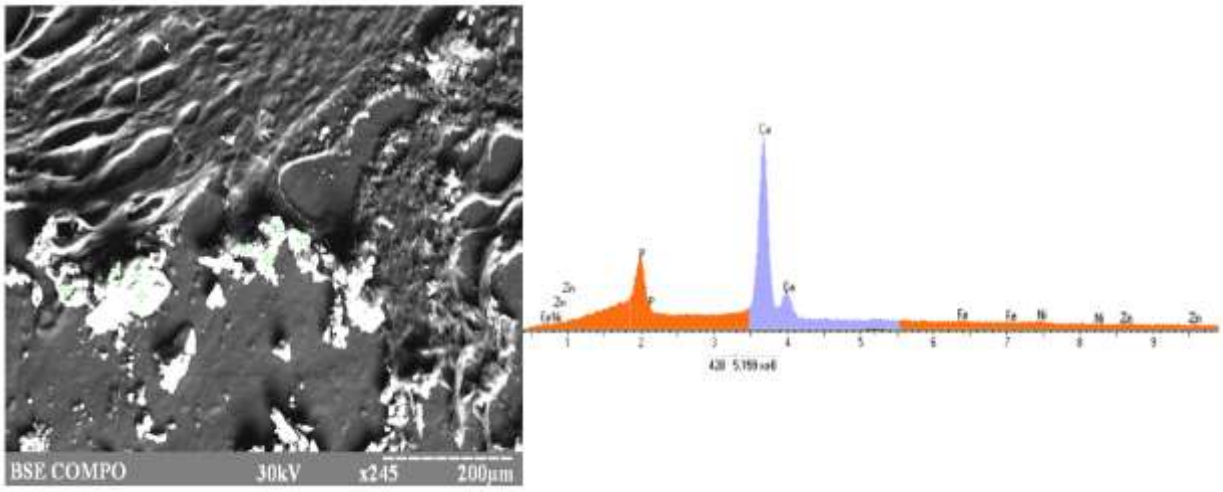

Figure 4 – Scanning electron microscopy with microanalysis. A. Aortic HV with biomineral deposits. Places of microanalysis are indicated by green crosses. B. X-ray diffraction of the same mineral component. Magnification and marker are shown in the right bottom corner of the micrographs.

Study of the specific mineral component of various HV showed the following results: MV tissue contained in average 17.35 ± 2.08 % of calcified substance, 18.84 ± 2.23 % of biomineralized substance was found in AV ($p > 0.46$).

Discussion

The origin and development of pathological biomineralization in heart valves, which are affected by atherosclerotic process, take time from the moment of the beginning of a primary disease. In the classical pathological anatomy atherosclerotic affection in the cardiovascular system with the presence of calcification is considered to be a manifestation of complicated atherosclerosis, but microcalcifications begin to form during the lipid stage [5]. In our study this is confirmed by the average age of the patients, which corresponds to 68.91 ± 1.49 (AV) and 69.09 ± 1.37 (MV) ($p > 0.05$) years.

Macroscopic study of both types of heart valves showed that biomineral deposits were located in the cusps or in the fibrous ring in the case of the atherosclerotic affection. Histological examination has found typical pathological changes in the heart valves tissues, such as thickening of the fibrous layer and elastic fibers, focal deposition of lipids, myxomatous changes, edema, calcium deposition of various size and morphology, chronic inflammatory infiltration. Similar histopathological signs accompany the atherosclerotic process, secondary inflammation joins them often, as a response to the atheromatous detritus and pathological biomineralization. In the last case a typical «circulus vitious» forms: calcium formations deposit in the form of grains, plungers or granules under the influence of pro-inflammatory stimuli, they irritate the surrounding tissues, causing inflammation [6; 7].

Pathological biomineralization begins often in aortic valve, and then the process extends to the MV, to the left ventricle and interventricular septum. Biomineralization changes drastically the elasticity and extensibility of valve tissue that contributes to the development of its failure, left ventricular hypertrophy and reduce of contractility of the heart muscle [8].

It is known that cardiovascular calcification is an unfavorable sign of the atherosclerosis development and it is a predictor of cardiovascular pathology and patients mortality [8]. Especially dangerous phenomenon can be the destruction of atherosclerotic plaque, located on the valve leaflets or in the area of connection with the muscles [9]. There is an interesting idea that microcalcifications have a higher damage potential of the tissue rupture [8]. Especially dangerous situation arises in the case of close-localization of two deposits-microcalcificates [10]. Conversely, the formation of leaf-like calcifications ("macrocalcification") stabilizes an atherosclerotic plaque and can be a barrier against inflammation [8]. The molecular mechanism, which regulates the development of a certain type of pathological biomineralization, is exactly unknown.

SEM with X-ray microanalysis confirmed that biomineral part of AV and MV consists mainly of hydroxyapatite. Material science research of HV biominerals that was conducted earlier, had clarified their structure. It was found that the majority of

AVs consist of β- three-calcium-magnesium phosphate (β-TCMP) (approximately 80 %), while this mineral is found in the MV only in near 20 % of cases [11]. An interesting fact is also a close correlation between valve mineral and organic parts with the structure formation in the type of biocomposit material.

Conclusions

Morphological study of heart valves shows a typical pathological changes in the tissues of the heart valves, such as thickening of the fibrous layer and elastic fibers, focal deposition of lipids, myxomatous changes, edema, calcium deposition of various size and morphology, chronic inflammatory infiltration.

During the comparing of the obtained results for AV and MV, we did not reveal a significant differences between patients age, their gender, morphology and localization of calcifications, chemical composition.

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