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The Role of Heavy Metal Salts in Pathological Biomineralization of Breast Cancer Tissue

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. The process of pathological biomineralization plays an important part in the morphogenesis of tumors. The role of heavy metal salts in the pathological mineralization of breast cancer tissue should not be ruled out, considering their ability to enter into covalent bonds with calcium salt molecules.
Objectives. The aim of the study was to investigate the microelement composition of breast cancer calcifications and the participation of heavy metals in their formation process.
Material and Methods. The material for the study consisted of 20 specimens of breast cancer tissue in which calcifications had been found in histological tests (hematoxylin-eozin and alizarin red S staining). The chemical composition of the calcifications was studied using a scanning electron microscope with an energy-dispersive spectrometer.
Results. Alizarin red S staining detected the presence of concrements in tumor tissue and rings of calcification around these deposits. Examining the biomineralization with energy dispersive spectrometry showed that along with calcium and phosphorus, it contained microelements such as iron, zinc, copper, chromium and nickel, which can replace calcium ions in the exterior part of hydroxyapatite molecules. This causes the hydroxyapatite molecule's molar mass to increase and its solubility to decrease; its chances of being deposited in tumor tissue also increase. This implies that an increased intake of heavy metal salts in organisms can lead to pathological mineralization of breast cancer tissue.
Conclusions. Excessive intake of heavy metal salts into the body leads to their involvement in the pathological mineralization of breast cancer tissue. This happens due to these salts bonding to hydroxyapatite molecules, direct sedimentation of proteins and increasing degenerative-necrotic changes in breast cancer tissue as the mineralization process progresses (**Adv Clin Exp Med 2016, 25, 5, 907–910**).

Key words: breast cancer, heavy metal, mineralization.

The process of pathological biomineralization plays an important role in tumor morphogenesis [1]. It has been established that any calcifications are the result of the opposing action of mineralization activators and inhibitors [2]. The action of klotho protein, matrix gamma-carboxyglutamic acid protein, fibroblast growth factors, pyrophosphates, etc., are described as inhibiting action [3]. Breast cancer tissue biopsies show that bone matrix proteins are excessively expressed under pathologic mineralization, including bone sialoprotein, osteopontin and osteonectin [4].

Inorganic elements are involved in all vital actions of the macro organism: the physiological electrolytic equilibrium and acid-alkali balance control, electron transport, active compound transport, oxidation-reduction reactions and tissue biomineralization [5]. The role of heavy metal salts (elements with an atomic weight exceeding 50) in the pathological mineralization of breast cancer tissue should not be ruled out, considering their ability to enter in covalent bonds with calcium salt molecules. Along with the possibility of their participation in the mineralization progress,

the possibility that they may initiate this process is also under consideration. This matter is most acute in those regions where there is a tendency toward increased pollution of the environment with heavy metal salts.

On the molecular level, microcalcifications in mammary gland tissue are classified in two types that differ in their physical and chemical properties. The first type of calcification contains calcium oxalate and is connected with benign breast tumors. The second type contains calcium phosphate, mainly hydroxyapatite, and most often correlate with invasive forms of breast cancer [6].

Hydroxyapatite – $Ca_{10}(PO_4)_6(OH)_2$ – is a mineral of apatite group, the hydroxy analog of fluorapatite and chlorapatite. It is the core component of the bones and teeth. The calcium to phosphorus ratio in hydroxyapatite is 1.67 (in certain cases it ranges from 1.33 to 2.0). Calcium ions can be substituted with chemical elements with similar properties (isomorphous replacement), including bivalent ions of heavy metals, resulting in calcium to phosphorus ratio changes [7]. That particular property of hydroxyapatite is used to clean water of exogenous contamination with heavy metal salts [8]. Hydroxyapatite's affinity to elements decreases in the following order: Lead → zinc → iron → copper → nickel → manganese → chromium [9].

The main specific feature of biominerals is that they are aggregates in which the mineral and organic components are clearly and inextricably related. They interact according to certain laws, and their ratio is constantly changing with the progression of tumor [1].

The aim of this study was to investigate the microelement composition of breast cancer biominerals and the participation of heavy metals in their formation process. This information could be particularly significant in treating women living in regions with high levels of heavy metals in the environment.

Material and Methods

The material for the research consisted of 20 specimen of breast cancer tissue in which microcalcifications had been found by histological testing (hematoxylin-eozin and alizarin red S staining). Images of the specimens were taken and stored using the SCAN IEX285AK-F IEE-1394 digital image output system (Sumy Electron Optics, Sumy, Ukraine).

Paraffin sections (5 μm thick) were subjected to dewaxing and applied to spectral pure graphite rods. The chemical composition of the microcalcifications was studied by scanning electron microscope with an energy-dispersive spectrometer. Digital images of the specimen and indices of the microelement content in the biomineralization structure were identified with Magellanes software (Sumy, Ukraine) and VCU software (Virginia Commonwealth University, Richmond, USA).

The mathematical and statistical calculations were done using Excel 2010 (Microsoft, Redmond, USA) with AtteStat 12.0.5 software (Moscow, Russia).

Results

The study found biomineralization in the stroma, parenchyma and intraductal tissue. The deposits were of various shapes and sizes (Fig. 1.1).

Alizarin red S staining detected the presence of concrements in the tumor tissue and rings of calcification around these deposits (Fig. 1.2).

Scanning electron microscopy of the tumor tissue with calcifications detected isolated and grouped calcium salt deposits. Studying the microelement composition of the calcifications with energy-dispersive spectrometry showed that along

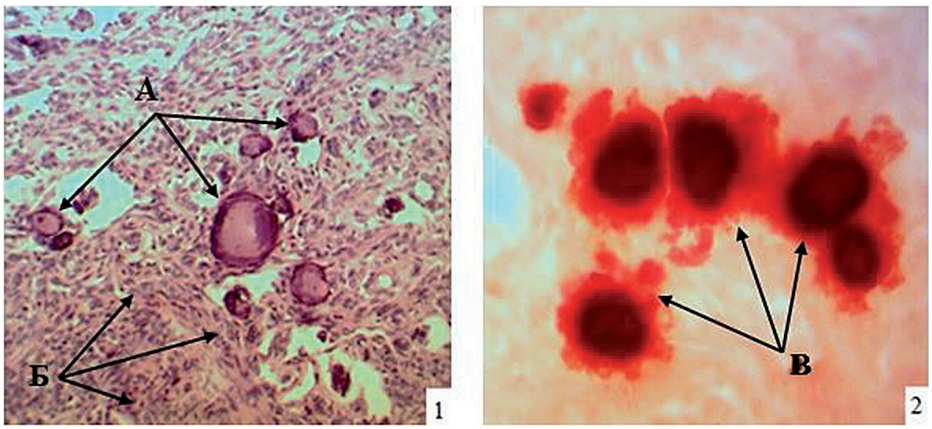
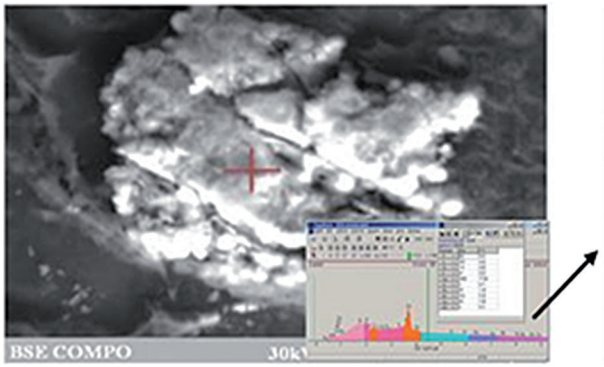


Fig. 1. Calcification in breast cancer tissue; 1. Hematoxylin-eosin staining, ×40 magnification; А – mineralization; Б – tumor tissue; 2. Alizarin red S staining, ×100 magnification; В – ring of calcification around mineralization.



Calcium	57.4 ± 8.34
Phosphorus	34.1 ± 6.47
Iron	2.0 ± 0.26
Copper	1.7 ± 0.12
Zinc	2.7 ± 0.3
Nickel	1.3 ± 0.16
Chromium	0.8 ± 0.9

Fig. 2. Scanning image and chemical composition of biomineralization; ×1360 magnification

with calcium and phosphorus, they contained such microelements as iron, zinc, copper, chromium and nickel (Fig. 2).

Discussion

Histological examinations show that tumor tissue is subject to various morphological changes, including pathological mineralization. This process has a progressive course, as evidenced by the presence of rings of mineralization around calcium salts deposits.

There is no doubt that the mineralization of breast cancer tissue is a polyetiologic process. But the role of excessive intake of heavy metals into the body in the genesis of calcification cannot be excluded. It may be implemented in several ways:

Upon entering tumor tissue, heavy metal ions form a covalent bond with calcium salts. Considering the calcium-phosphoric ratio, which (according to energy-dispersive spectrometry data) equals 1.56–1.73, it is fair to say that tumor tissue mineralizations are hydroxyapatite deposits where calcium ions have been substituted by heavier cations. Such elements as zinc, copper, iron, nickel and chromium can replace calcium ions in the exterior parts of hydroxyapatite molecules. It might happen that calcium atoms in the central nuclei of the mineralization are replaced [10]. This causes the hydroxyapatite molecule's molar mass to increase (zinc, iron, nickel and copper atoms have higher molar masses than calcium, which is 40.078 g/mol) and its solubility decrease; its chances of being deposited in tumor tissue increase as well (Fig. 3).

When the aforementioned elements accumulate excessively in mammary gland tissue, they cause lipid peroxidation and the formation of hydrogen peroxide and superoxide anions. This facilitates the development of inflammation, programmed cell death and necrosis in the tissue, which are prerequisites of pathologic biomineralization (or dystrophic calcification). If the pathology continues, surrounding tissues become involved in the process, which is proved by the ring of mineralization (Fig. 1.2) surrounding most structured deposits of hydroxyapatite.

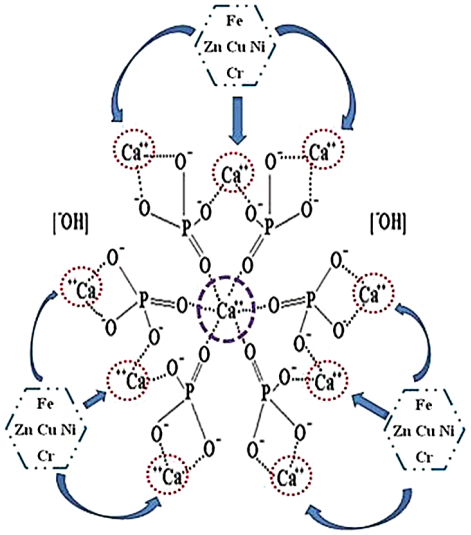


Fig. 3. Diagram of calcium substitution for other elements

It has also been suggested that the influence of heavy metal salts on the biomineralization process could be considered direct deposition connected with the organic matrix (proteins).

It can therefore be said that the presence of heavy metal salts in breast cancer tissue facilitates the process of pathologic mineralization. This in turn contributes to the progression of the neoplastic process, causing a long-lasting focus of inflammation surrounding the "foreign body" [11]. The increasing the ability of tumor cells to bind with hydroxyapatite (due to the synthesis of interleukin 8) leads to stimulation of the emergence of metastases in bone [12]. Cell division and synthesis of matrix metalloproteinase are intensified in the presence of hydroxyapatite in tumor tissue.

The intake of excessive amounts of heavy metal salts into the body causes their involvement in

the progression of pathological mineralization in breast cancer tissue. This happens due to the bonding of heavy metal salts to hydroxyapatite molecules, direct sedimentation of proteins and increasing degenerative-necrotic changes in breast cancer tissue, with their further calcification. All this subsequently leads to tumor progression and a worsening prognosis in breast cancer.

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