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APPLICATION OF X-RAY FLUORESCENCE ANALYSIS TO DETERMINE THE ELEMENTAL COMPOSITION OF TISSUES FROM DIFFERENT OVARIAN NEOPLASMS

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We present the results of x-ray fluorescence analysis of tissues from healthy ovaries and from ovaries with different pathologies: benign and borderline tumors, mucinous and endometrioid cancers, serous carcinomas. We determine the average copper, zinc, calcium, selenium, cadmium, lead, and mercury levels. We observed that in the benign ovarian tumors, we see a significant decrease in the cadmium, mercury, and lead levels compared with healthy tissues. In the borderline neoplasms, the copper level is reduced relative to zinc (Cu/Zn), cadmium, mercury, and lead, and also the zinc concentration is increased. In the ovarian carcinomas, we observed changes in the ratio of the chemical elements in the tumor tissues, depending on the histologic type. The results obtained can be used for differentiation, diagnosis, and adjustment of treatment for different ovarian neoplasms.

Keywords: *x-ray fluorescence analysis, endometrioid cancer, mucinous cancer, serous carcinoma, ovarian tissues, microelements.*

Introduction. Stability of chemical composition is one of the most important and necessary conditions for normal functioning of the body. Deviation of the level of different microelements from normal values results in either the appearance of disease or risk of disease. Furthermore, a change in the balance of microelements in the body can be due to a specific disease or disturbance of the digestive process. Therefore a promising direction in modern medicine is identification and assessment of changes in the levels of microelements and macroelements, followed by correction of these levels.

Ovarian cancer is a pressing problem in gynecologic oncology, since in 70%–80% of the cases, the tumor is diagnosed in clinical stage III or IV of the disease [1]. The leading reasons for the low survival rate of patients with malignant ovarian tumors include the asymptomatic course of the disease in the early stages and the lack of adequate diagnostic methods. Despite some successes in diagnosis of ovarian carcinoma, most cases are recognized in the later stages of the disease, when long-term treatment results are not very reassuring and the five-year survival rate for such patients is <40%, even when sophisticated medical care is available. Expansion of studies in the area of the biology of tumor growth makes it possible to identify factors of practical value for understanding the mechanisms not only of development but also metastasis and recurrence of ovarian carcinoma, and also provides a theoretical foundation for introducing new approaches to diagnosis and treatment of this disease.

In some papers, development of ovarian neoplasms is associated with hormonal and genetic factors, diet, age, number of children, anthropometric characteristics (height, excess body weight), lifestyle. Environmental factors may play a not unimportant role in the appearance of ovarian tumors. Epidemiological studies indicate that genetic hereditary history is one of the important risk factors for development of ovarian cancer. Hereditary ovarian cancer, for which mutations of established genes play a major role in the etiology, accounts for up to 10–15% of the total incidence rate of the disease. In the overwhelming majority of cases of sporadic cancer, it is a complicated problem to determine the causal role of individual genetic defects. In such cases, over the course of a lifetime various mutations accumulate, located in oncogenes or suppressor genes, which leads to progressive transformation of benign or borderline tumors to malignant tumors

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characterized by an unfavorable clinical prognosis. A definite role in malignant transformation of cells is played by gene polymorphism, determining the particular details of metabolism of steroid hormones and environmental carcinogens and thereby predisposing to exposure to endogenous and exogenous risk factors. The role of the microelemental composition of cells is also rather large. The diverse impact of microelements on the body is determined by the fact that they enter into the composition or participate in synthesis of a vast number of substances, including hormones, vitamins, proteins, enzymes, etc. Researchers believe that hormonal imbalance is often affected by nothing else but insufficient levels of microelements needed for synthesis of hormones in a woman's body and as we know, the ovary is the major hormonal organ in the female reproductive system.

In order to estimate the microelement levels in the human body, different biological substrates such as blood, urine, skin appendages (nails, hair) are used. Currently hair is often used for diagnosis, since it provides considerable information. The microelemental composition of blood reacts first to an increase in the heavy metal level, but may not reflect their true level in the whole body or its individual organs. Therefore it is important to study such biological substrates as biological tissues, which most fully reflect the elemental status in the entire body or in an individual organ.

The use of x-ray fluorescence for medical analysis is promising due to its availability and speed, accuracy, and high reproducibility in estimating the content of the analyte elements to 10^{-2}%. The method is divided into two major approaches: qualitative and quantitative analysis of solid and liquid test samples [2]. Modern equipment for x-ray fluorescence analysis (XFA) and software for the method allow us to rapidly identify the object of investigation while simultaneously recording the fluorescence spectrum of the elements, an objective characteristic of the sample.

The x-ray fluorescence method is based on analysis of the characteristic spectrum of secondary fluorescent emission by the test sample, which arises on exposure to harder x-rays. The spectral composition of the secondary emission adequately reflects the elemental composition of the analyte sample, since the atoms of the chemical elements have their own characteristic lines that are individual for the given element. The presence of characteristic lines in the spectrum indicates the qualitative composition of the test sample, while measurement of the intensity of these lines allows us to quantitatively evaluate the concentration of the substance. However, no reliable database exists today for the microelemental content in development of specific diseases, the comparative microelement distribution pattern in the human body under normal and pathological conditions.

The aim of this work was to use XFA methods for differentiation of ovarian cells undergoing pathological changes.

The Experiment. The studies were conducted in 2013–2014 on material from patients who were operated on for various ovarian neoplasms. As a control, we used autopsy material (ovary) taken from women ($n = 5$) who died from accidental causes. Clinical data on the patients were obtained from medical documentation (history of the illness, clinical records) and the cancer registry. In the morphology study, we identified 17 cases of malignant carcinomas of different types (serous, endometrioid, and mucinous cancer [3]) and nine benign tumors (cystadenomas), and also several cases of borderline tumors. The women were 40–65 years old. All tissues were subjected to standard treatment and analysis used in pathomorphology, and also a procedure for preparing samples for XFA.

In accordance with an adapted procedure for determining the weight fraction of chemical elements in samples of plant and animal origin by the XFA method on an SER-01 ElvaX (registered as MVI.MN 3272-2009), we prepared the samples (by wet ashing, GOST 29629-94) and made measurements on samples from histological tissue sections. The object of investigation was ground down with a scalpel (by the scraping method) on a glass plate and thoroughly mixed. The weighed material was dried at 105°C for 24 h until constant weight was achieved, and was ground in a porcelain mortar. To the weighed sample obtained after drying, we added (based on calculations) 1 mL distilled water to moisten, 3 mL of 60% nitric acid (GOST 12.1.005-88), and 1 mL of 30% hydrogen peroxide (GOST 177-88). Complete drying was carried out at a temperature of 170–190°C for 60 minutes. The ash obtained was glued together with a special glue based on polychlorinated resin and acetone (solvent), dried in a laboratory muffle furnace (for removal of excess solvent), and pressed into pellets for XFA. Then measurements were made on the samples from histological tissue sections. Using the software, we obtained and analyzed the x-ray fluorescence spectra of the microelements.

Results and Discussion. Copper is a very important microelement which not only regulates physiological functions and has pronounced anti-inflammatory properties but also has a negative effect on DNA, leading to mutations which subsequently may cause formation of some types of cancer. Along with iron, copper participates in all oxidation–reduction processes. Copper is incorporated into blood plasma as protein complexes. Copper deficiency disrupts iron uptake. Recent studies [4] have shown that a large percentage of melanomas and some other types of tumors, including leukemia and thyroid cancer, are stimulated by mutations in the *BRAF* gene. The probability of this mutation depends on the amount of copper in the blood.

An increase in the number of copper atoms in the blood forces cancer cells to actively "breathe", as a result of which their growth rate increases. It has been observed [5] that blocking the copper supply to cancer cells decreases tumor growth in mouse and human cells.

In the studied samples, the copper content is found in the range 18.41–21.32 μg/g in healthy tissues, 15.72–20.21 for benign tumors, 19.22–22.54 for mucinous cancer, 34.35–36.28 for serous carcinoma, 20.05–22.87 for endometrioid cancer, and 15.44–17.32 μg/g in borderline tumor tissues. Figure 1a shows the average copper content in healthy, benign, and cancerous tissues. We see that compared with healthy tissues, in the cells of benign and borderline neoplasms we observe a slight decrease in the copper level. At the same time, the copper concentration in the cancerous tissues increases (mucinous cancer is an exception), and the copper content increases in serous cancer cells by a factor of 1.7. Such differences in copper content are probably due to the particulars of the cell damage, depending on the type of cancer. The data obtained on the change in copper content correlate well with studies by other authors who monitored the content of this element in the body. For example, an increase in the blood copper level is observed in colon cancer, breast cancer, prostate cancer, lung cancer, liver cancer, and brain cancer [6].

Zinc is a cofactor for the structure and function of a broad range of cell proteins, including enzymes, transcription factors, and structural proteins [7, 8]. Recent studies show [9] that zinc plays an important role in development of different cancers. It causes apoptosis in many types of mammalian cells, such as epithelial cells of the prostate, neurons and glial cells, ovarian surface epithelium, epithelial cells of the esophagus, etc. In contrast, in other cells (such as in the chest, epithelial cells of the lungs, kidney epithelium, pancreas) zinc has anti-apoptotic effects. How and why these opposing tendencies appear, depending on the particular features of the tumor, still remains an important unanswered question.

The zinc content also changes in different types of cancer. For example, in the prostate, normal epithelial cells accumulate zinc and contain high levels of cellular zinc. In contrast, malignant cells lose the ability to accumulate zinc, and the content of this element in malignant cells is low. At the same time, it has been established that in breast cancer, the zinc level markedly increases in cancer tissue cells compared with healthy tissue cells [10].

Based on XFA, the zinc concentration in cellular dry matter is found in the range 107.34–122.38 μg/g in healthy tissues, 108.85–116.71 for benign tumors, 127.12–132.24 for mucinous cancer, 198.34–204.06 for serous carcinoma, 120.33–125.24 for endometrioid cancer, and 150.43–155.31 μg/g in borderline tissues. Figure 1b shows the average Zn concentrations in the tissues.

The zinc concentration increases in ovarian carcinomas of different histologic structures, and as for the copper content, the zinc content significantly increases for serous neoplasms. The Zn content in borderline tumor cells is intermediate between that in healthy tissues and in serous cancer (Fig. 1b). We must note that in cells of benign neoplasms, the zinc content decreases only slightly compared with healthy cells. However, in analysis of blood from patients with ovarian cancer, we observe a decrease in the zinc concentration [11]. Thus in ovarian cancer, a redistribution of the zinc occurs in the body: its concentration increases in the most damaged organ and decreases in the patient's blood. We also note an enzyme in the human body which prevents oxidation-reduction reactions of metals and therefore prevents DNA damage: Cu/Zn superoxide dismutase [23]. The Cu/Zn ratio decreases in cells of benign neoplasms (0.158) and mucinous cancer cells (0.159) compared with healthy tissues (0.179). The copper content remains practically unchanged in cells of healthy tissues (average value, 20.6 μg/g) and mucinous cancer cells (average value, 20.7 μg/g), while the zinc content in mucinous cancer cells increases by 11% compared with healthy tissues. In cases of benign neoplasms, the copper and zinc content decreases compared with healthy tissues, but the copper content decreases more substantially (by 17%).

In the case of borderline tumor cells, the ratio Cu/Zn significantly decreases (down to 0.123): the copper content drops on the average by 13%, while zinc increases by 30% compared with healthy cells. We should note that the indicated tendency in the change in copper content relative to zinc content is apparent not only as a result of statistical treatment, but also for all the samples we studied, taken individually. The identified change in copper and zinc content and in the Cu/Zn ratio may be taken as one of the most sensitive parameters in monitoring the ecological pattern of the body and will help in developing new methods for diagnosis of ovarian cancer. In blood serum of patients with ovarian cancer, a significant increase in Cu/Zn has also been noted compared with the serum of patients with benign formations [13].

Calcium is a microelement playing an important regulatory and structural role for the human body, and also participating in key physiological and biochemical processes in cells. Calcium ions participate in blood clotting processes, act as a universal secondary messenger within cells, and regulate different intracellular processes: muscle contraction, exocytosis, including secretion of hormones and neuromediators.

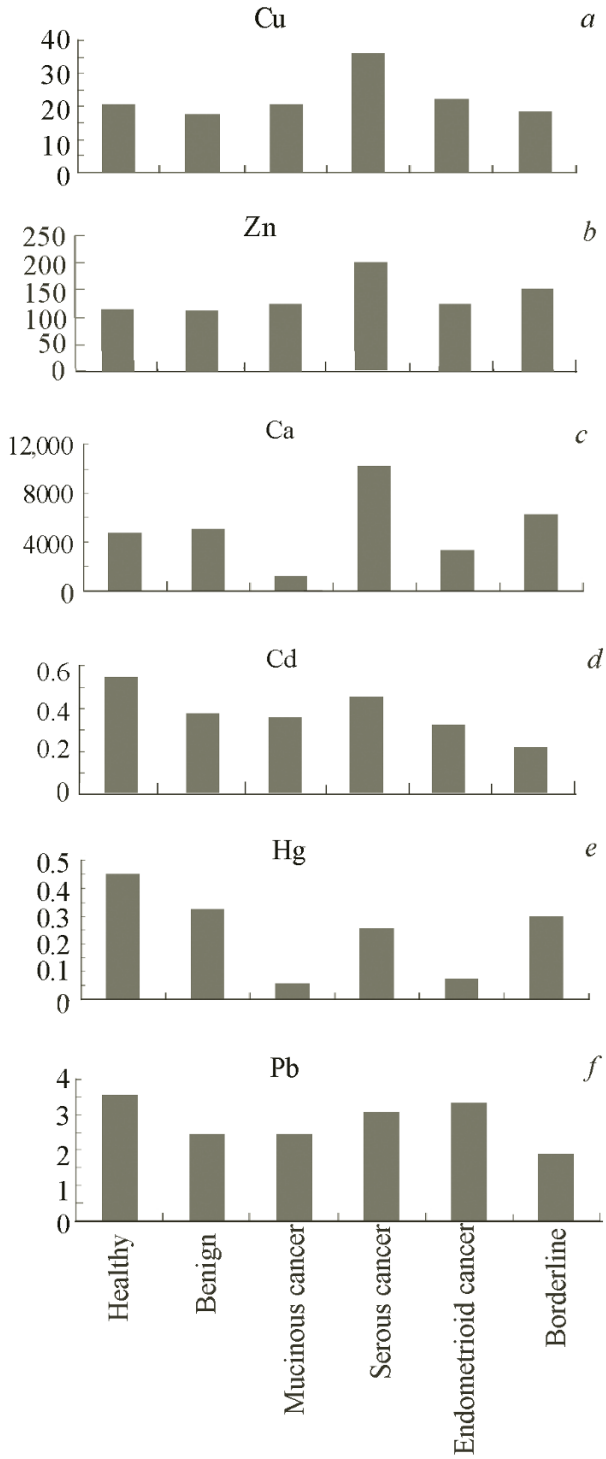

Fig. 1. Average concentrations of Cu (a), Zn (b), Ca (c), Cd (d), Hg (e), Pb (f) in tissues with different levels of ovarian pathology (in μg/g).

Figure 1c shows the histograms for the average calcium concentrations in ovarian tissues, obtained based on XFA. The spread in the relative values is <10%. The calcium level remains practically unchanged in benign neoplasms compared with healthy tissues, while in different forms of cancerous tumors we observe different tendencies: the calcium content increases by a factor of two for serous carcinoma, decreases by a factor of 3 and 1.5 for mucinous and endometrioid cancer.

We especially note the microelement selenium (Se). Modern studies show [14] that Se is promising as a drug for many types of cancer. Using XFA on the tissues, we determined the selenium concentration on a dry basis: 0.34–0.36 μg/g in healthy tissues, 0.28–0.32 for benign tumors, 0.21–0.24 for mucinous cancer, 0.25–0.26 for serous carcinoma, 0.20–0.21 for endometrioid cancer, and 0.19–0.21 μg/g in borderline tissues. The average selenium content in ovarian tissues for cancer patients is decreased compared with healthy patients. This suggests that either the risk of carcinoma formation increases when there is a selenium deficiency in the human body, or that cancer cells are depleted in selenium as a result of some mechanisms. Comprehensive monitoring of selenium content in blood, hair, and ovarian tissues is necessary to make a definite choice between the first or second hypothesis.

Cadmium, mercury, and lead are well-known carcinogens. Figure 1d–f shows the average levels of these elements in ovarian tissues for different levels of pathology. The cadmium level decreases as we go from healthy tissues to cancerous tissues (Fig. 1d). The XFA data for ovarian tissues match studies in [15] of the concentration of these microelements in the blood of patients with ovarian cancer. The concentrations of cadmium on a dry basis are found in the range 0.50–0.60 μg/g in healthy tissues, 0.35–0.41 μg/g in benign tumors, 0.34–0.40 μg/g in mucinous cancer, 0.41–0.47 μg/g in serous carcinoma, 0.31–0.35 μg/g in endometrioid cancer, and 0.2–0.24 μg/g in borderline tissues. In all the tumor tissues, the mercury level decreases compared with healthy tissues (Fig. 1e), and the greatest decrease is observed in the case of mucinous cancer (by a factor of 9) and endometrioid cancer (by a factor of 8). In analysis of the lead level in the samples, we observed a decrease in lead concentration in all the tissues compared with the healthy tissues (Fig. 1f). In healthy tissues, the lead level is found in the range 3.3–3.7 μg/g; in benign tissues and for mucinous cancer, the range is 2.4–2.6 μg/g; for serous cancer, 2.9–3.2 μg/g; for endometrioid cancer, 3.2–3.5 μg/g; and in borderline tissues, 1.8–2.1 μg/g.

The significant decrease in cadmium, mercury, and lead levels in pathological cells compared with cells from healthy patients is probably due to the characteristic features of the genesis of these cells. This is possibly connected with the fact that cancer cells divide rapidly and cannot accumulate toxic elements, although the content of these elements increases in the blood serum of cancer patients [16].

Conclusions. X-ray fluorescence analysis is a method that is sensitive to the smallest changes in the ecology of biological tissues in development of pathology. It can be recommended as the most inexpensive and sensitive invasive diagnostic method, compared with histology methods and as a supplement to these methods. A number of identified characteristics in the relative content of microelements such as copper, zinc, selenium, cadmium, mercury, cadmium, and lead provide a basis for more intensive study of the mechanisms for differentiation of tissues and development of pathology at the molecular and cellular level.

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