ORIGINAL ARTICLE

Comparison of the content of some chemical elements in cancerous and intact breast tissue adjacent to the tumor determined by the ICP-AES method: Original data and a mini-review

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ABSTRACT

Objective: In most countries of the world, breast cancer ranks first in the structure of oncological morbidity in women. The etiology of this disease remains largely unclear, although it is known that disturbances in the elemental homeostasis of somatic cells play a certain role in oncogenesis. The purpose of this study was to identify changes in the content of chemical elements during malignant transformation of breast tissue.
Methods: For this purpose, we used the previously developed method of sample preparation, which allows determining the content of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn in micro samples of breast tissue by using atomic emission spectrometry with inductively coupled plasma. Samples of cancerous and visually unchanged breast tissue adjacent to the tumor were examined using the developed technique.
Results: A significantly higher content of all the chemical elements studied, except for Si, was found in cancerous tissue compared to their content in intact tissue.
Conclusions: The detected multiple increase in the content of many minor and trace elements in cancer tissue compared to adjacent intact breast tissue can be used to develop new methods for in vitro and in vivo cancer diagnostics, in which the ratios of chemical elements levels in these tissues will act as tumor markers. Further, more in-depth study and understanding of the discovered phenomenon will allow the development of new methods for the prevention and treatment of breast cancer.

Key Words: Breast cancer, Chemical elements, Inductively coupled plasma atomic emission spectrometry, Cancerous tissue, Intact breast tissue adjacent to tumor

1. INTRODUCTION

The widespread use of modern methods of early diagnostics of breast cancer (BCa) and progressive methods of treatment significantly reduces mortality from this disease.[1] However, despite the progress achieved, BCa remains a global health problem as it continues to occupy a leading position in cancer mortality worldwide.[2] BCa is not only a medical but also a social problem, since the incidence of this disease is steadily

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increasing, and especially rapidly among young women.[1,3] BCa has a complex and still insufficiently studied etiology. It is obvious that the disclosure of the multifaceted nature of breast cancer, a deep understanding of its molecular basis and risk factors will contribute to the development of new highly effective methods of prevention, diagnosis, and treatment of this disease.[4] Numerous epidemiological studies consider many factors that can contribute to the occurrence of breast cancer. These factors can be divided into four groups: 1) genetic predisposition, 2) hormonal status (long-term exposure to estrogens and reproductive history), 3) lifestyle (diet, obesity, physical activity, type of work, bad habits, etc.), and 4) environmental conditions (radiation and chemical exposure). The influence of some of these factors is confirmed by all studies, but many other factors are still controversial, and no consensus has been reached.[4]

In previous studies, we focused on the role of chemical elements (ChEs) of mammary gland tissue in the normal physiology of this organ.[5] In these studies, we proceeded from the idea that a violation of elemental somatic homeostasis (deficiency or excess) can lead to the malignant transformation of mammary gland tissue.[6,7] To continue studying not only normal but also pathologically altered breast tissue, we have developed a sample preparation method that allows us to determine the content of 15 ChEs in small tissue samples using inductively coupled plasma atomic emission spectrometry (ICP-AES). This method made it possible to use puncture biopsy material obtained in the clinic for research.[8]

Earlier, in our studies of malignant tumors of the bones, thyroid and prostate glands, significant differences were found in the content of many ChEs, compared with the levels of these ChEs characteristic of normal bone, thyroid, and prostate tissue, respectively.[9–35] By analogy with the results of these studies, it should be expected that malignant transformation of breast tissue may also be associated with significant changes in the content of some ChEs. Of particular interest in this case was the composition of the intact breast tissue adjacent to the malignant tumor, since it could reflect the initial conditions from which the malignancy of glandular cells began.

To date, there are several publications in which, using various analytical methods, the content of ChEs was studied both in tissue samples of malignant tumors and in samples of visually unchanged breast tissue adjacent to the tumor.[36–58] However, due to the large scatter of published quantitative data, and sometimes their inconsistency, it was not possible to draw unambiguous conclusions about the changes in the content of ChEs in malignant tumors and adjacent visually intact breast tissue. Also, no analytical reviews in the literature on this issue were found that could resolve the existing contradictions and draw adequate conclusions.

The aim of this study was to compare the content of ChEs in malignant breast tumors with the content of the same ChEs in adjacent visually intact breast tissue. To achieve this goal, we used a previously developed technique.[8] To assess the reliability of our results, a systematic analysis of the published data on the content of ChE in malignant tumors and adjacent visually intact breast tissue was carried out. The analysis performed allowed us to determine the median values of the data available in the literature. Determining the medians of the literature data on the mean values of the content of ChE gave us the opportunity to compare them with our results.

2. METHODS

2.1 Tissue samples

Tissue samples obtained from 43 women with breast cancer were examined (age 35 to 77 years, Caucasian race, Caucasian lifestyle) undergoing treatment in the thoracic department of the Medical Radiological Research Center. All patients were diagnosed with breast cancer for the first time and had not yet received any treatment. Pregnant patients, patients with previous surgeries, renal impairment, anemia, diabetes mellitus and other chronic diseases, and those taking mineral micronutrient supplements were excluded from the study. Informed consent was obtained from all patients before collecting breast tissue samples. Under ultrasound control, each patient underwent a core needle puncture biopsy of the breast tumor and adjacent intact tissue for morphological examination and assessment of the ChEs content. In patients operated on for breast cancer, samples of resected material (tumor and adjacent visually healthy tissue) were also used for morphological examination and elemental analysis. In this case, samples of adjacent visually healthy tissue were taken approximately 0.5 cm from the tumor border. In all cases, the diagnosis was confirmed by clinical and morphological results obtained during the examination of biopsies and resected material. Tissue samples intended for elemental analysis were weighed, lyophilized and homogenized.[59]

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk. All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments, or with comparable ethical standards.

2.2 Sample preparation and ICP-AES measurements

To implement this study, we used our own developed method of sample preparation, which allows us to determine the content of several ChEs in small tissue samples by using

inductively coupled plasma atomic emission spectrometry (ICP-AES).[8] This micro method allows us to determine the content of the following elements: aluminum (Al), barium (Ba), calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulfur (S), silicon (Si), strontium (Sr), titanium (Ti), and zinc (Zn) in milligram quantities of the sample under study. The ICP-AES method was chosen due to its high sensitivity, which made it possible to simultaneously determine the content of 15 ChE in a small tissue sample, which is usually obtained during a puncture biopsy.[8] For sample preparation and elemental analysis, deionized water distilled without boiling in a PTFE Subboiler ECO IR Maassen "Water and acid cleaning system" (Germany) and nitric acid for analysis (65%, max. 0.005 ppm Hg) from Merck (Germany) were used. A 2% nitric acid solution was prepared by diluting the initial solution with deionized water and then used to prepare the analyzed solutions.

A single puncture biopsy usually collects material weighing about 10-20 mg. Therefore, we initially developed a method for microwave autoclave acid digestion of small breast tissue samples weighing from 10 mg, which was used in the current study.[60]

Elemental analysis of the samples by inductively coupled plasma atomic emission spectrometry (ICP-AES) was performed using an ICAP-6500 Duo plasma spectrometer (Thermo Scientific). The spectrometer was calibrated using Merck standard multi-element reference solutions (Merck, KGaA, Darmstadt, Germany) and high-purity standards (North Charleston, South Carolina, USA).

The spectral range (166–847 nm) is recorded by a highly sensitive CID semiconductor detector. The optical block of the device is thermally stabilized and purged with argon. High-purity argon (99.993%) is used as a plasma-forming gas. The plasma power is 1,150 W. The rates of the plasma-forming argon flow, transport flow, and cooling flow are 0.5 L/min, 0.55 L/min, and 12 L/min, respectively. The element content in the analyzed solutions was measured using iTEVA analytical software and MS Excel. To verify the accuracy of the obtained results, Polish certified reference materials MODAS-5 (Cod tissue) and MODAS-3 (Herring tissue) and the CRM prepared by the International Atomic Energy Agency IAEA-153 (Milk powder) were used. A more detailed description of the methodology developed and used by us was published earlier.[8,60]

2.3 A systematic mini-review

A systematic search was performed using PubMed, Web of Science, Scopus, and Google Scholar to identify literature published up to February 2025 on the considered elements (Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn) in cancerous and adjacent visually intact breast tissue. The key terms used in the search strategy included "chemical elements" or "trace elements" in combination with "breast cancer", "breast tumor", "breast carcinoma", or "adjacent visually intact breast tissue". In addition, we searched for all results reported in previous reviews and relevant meta-analyses on the topic of interest.

The identified studies were included only if they met the following standards: (1) only studies involving human participants were included; (2) quantitative data on the ChE of interest were presented; (3) in patients with breast cancer, the diagnosis was confirmed morphologically. In some cases, review articles were included in our study if they were relevant to the topic and met the above requirements, but the focus was on original works. There were no restrictions on the language of published papers.

Subsequently, the literature data were collected and classified for each ChE depending on the breast tissue (tumor or adjacent intact tissue). From the published data, the median of the mean values for tumor tissue and adjacent intact breast tissue was found for each specific ChE.

2.4 Statistics

The main statistical parameters such as arithmetic mean, standard deviation, standard error of the mean, minimum and maximum values, median, percentiles with levels of 0.025 and 0.975 for mass fractions of ChE (mg/kg dry weight) were calculated using MS Excel. The significance of differences in the results between the two groups (cancer and adjacent breast tissue) was assessed using the parametric Student's t-test and the nonparametric Wilcoxon-Mann-Whitney U-test. MS Excel was also used to determine the median values of the mean contents of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn in tumor and adjacent breast tissue found in the published articles.

3. RESULTS

3.1 Capabilities and accuracy of the ICP-AES technique used

In our study, we used the ICP-AES method. It was shown that when using breast tissue samples in normal and pathological conditions of small mass (about 10 mg), only 15 ChEs are available for quantitative determination using this method: Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn Since our task included studying changes in all chemical elements available for determination, the content of all the above-mentioned ChEs was determined in samples of malignant tumor and adjacent visually intact tissue of the

mammary gland.

Acceptable agreement of the values of the content of ChEs in the international certified reference materials MODAS-5 (Cod tissue), MODAS-3 (Herring tissue), IAEA-153 (Milk powder) obtained in this study with the data of the corresponding certificates indicated sufficient accuracy of the developed ICP-AES micro method and reliability of the mass fractions of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn in samples of malignant tumor and intact breast tissue adjacent to the lesion,

3.2 Mean mass fraction of the 15 studied ChEs in cancerous and intact breast tissue adjacent to tumor

Figure 1 demonstrates the mean mass fraction and the range of the standard error of the mean (M ± SEM) for each of the 15 studied ChEs in the compared pairs - malignant tumor and adjacent visually intact tissue of the mammary gland.

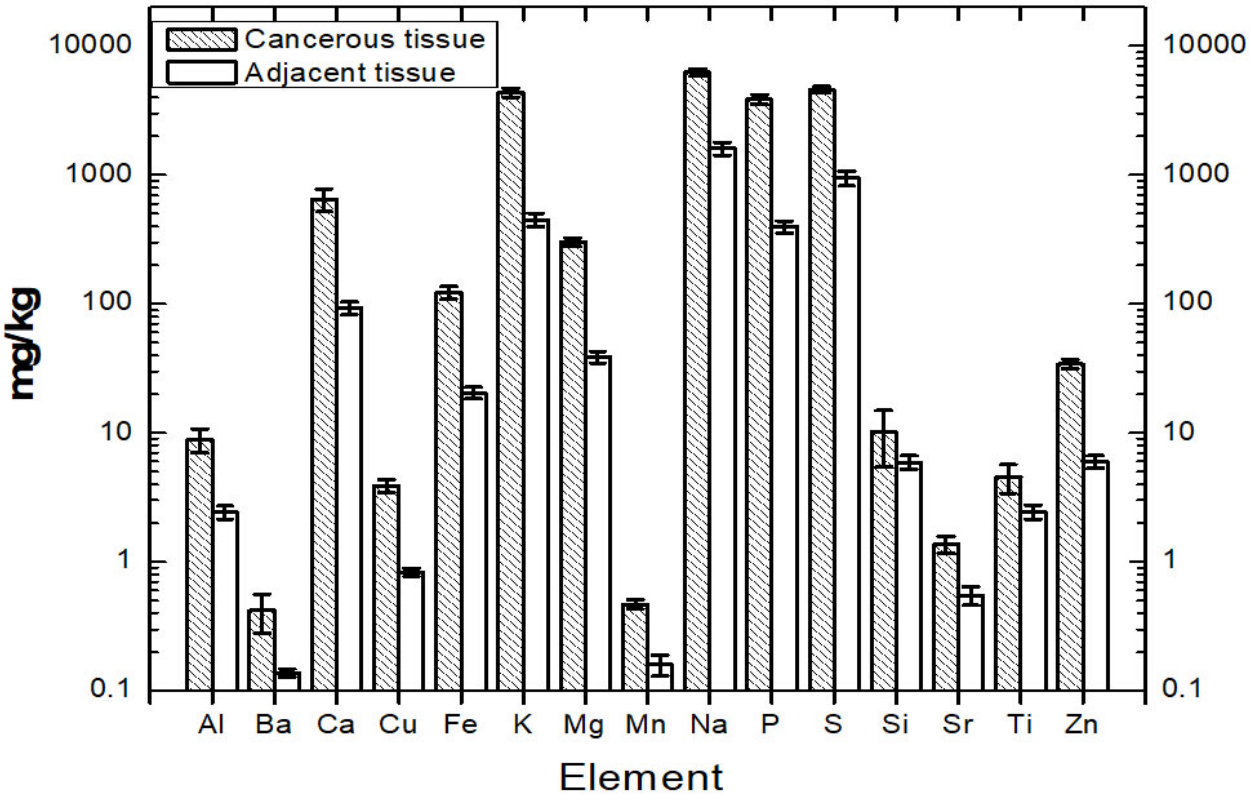

Figure 1. The mean mass fraction (M) and the range of the standard error of the mean (± SEM) for each of the 15 studied ChEs in cancerous and intact breast tissue adjacent to tumor (mg/kg dry tissue)

The mean values of the mass fraction and standard deviation (M± SD) of the ChEs in malignant tumors (MT), obtained using the ICP-AES micro method developed by us, were (mg/kg dry tissue): Al 8.88 ± 12.0; Ba 0.42 ± 0.94; Ca 650 ± 856; Cu 3.90 ± 2.88; Fe 123± 93; K 4,347 ± 2,284; Mg 302 ± 148; Mn 0.47 ± 0.27; Na 6,262 ± 2,771; P 3,867 ± 2,230; S 4,599 ± 1,797; Si 10.2 ± 31.1: Sr 1.37 ± 1.31; Ti 4.55 ± 7.18 and Zn 34.2 ± 18.3, and in the visually intact breast tissue adjacent to the tumor (MA): Al 2.43 ± 1.81; Ba 0.14 ± 0.05; Ca 93.5 ± 71.6; Cu 0.83 ± 0.40; Fe 20.5 ± 14.6; K 449 ± 367; Mg 38.9 ± 27.3: Mn 0.16 ± 0.19; Na 1,607 ± 1,240; P 398 ± 295; S 948 ± 876; Si 5.93 ± 4.84; Sr 0.55 ± 0.65; Ti 2.44 ± 2.07, and Zn 6.00 ± 4.67.

3.3 Comparison of our results on the content of ChEs in cancerous and intact breast tissue adjacent to the tumor

Table 1 depicts the differences between the mean values of the mass fractions of the elements studied in the malignant tumor and the intact breast tissue adjacent to the tumor, assessed using the parametric Student *t*-test and the nonparametric Wilcoxon-Mann-Whitney *U*-test.

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Table 1. Comparison of mean values (M ± SEM) of mass fraction of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn (mg/kg dry tissue) in cancerous breast tissue (M_C) and intact breast tissue adjacent to the tumor (M_A)

Element	Female breast tissue				Ratio
	Cancerous tissue, M_C	Adjacent tissue, M_A	Student's *t*-test *p*	*U*-test *p*	M_C/M_A
Al	8.88 ± 1.84	2.43 ± 0.28	.001*	≤ .01*	3.65
Ba	0.42 ± 0.14	0.14 ± 0.01	.054	≤ .05*	3.00
Ca	650 ± 131	93.5 ± 10.6	.0001*	≤ .01*	6.95
Cu	3.90 ± 0.44	0.83 ± 0.06	< .000001*	≤ .01*	4.70
Fe	123 ± 14	20.5 ± 2.2	< .000001*	≤ .01*	6.00
K	4,347 ± 348	449 ± 54	< .000001*	≤ .01*	9.68
Mg	302 ± 23	38.9 ± 4.0	< .000001*	≤ .01*	7,76
Mn	0.47 ± 0.04	0.16 ± 0.03	< .000001*	≤ .01*	2.94
Na	6,262 ± 423	1607 ± 183	< .000001*	≤ .01*	3.90
P	3,867 ± 340	398 ± 43	< .000001*	≤ .01*	9.72
S	4,599 ± 274	948 ± 126	< .000001*	≤ .01*	4.85
Si	10.2 ± 4.7	5.93 ± 0.70	.30	> .05	1.72
Sr	1.37 ± 0.20	0.55 ± 0.09	.00003*	≤ .01*	2.49
Ti	4.55 ± 1.14	2.44 ± 0.30	.055*	≤ .05*	1.86
Zn	34.2 ± 2.8	6.00 ± 0.67	< .000001*	≤ .01*	5.70

Note. M: Arithmetic mean; SEM: Standard error of mean; *t*-test: Student's *t*-test; *U*-test: Wilcoxon-Mann-Whitney *U*-test; * Significant value $p \leq .05$

Table 2. Median, minimum and maximum value of means of chemical element mass fractions (mg/kg dry tissue) in cancerous breast tissue according to literature data in comparison with this work results

Element	Our results	Published data [Reference]		
	M ± SD *n* = 43	Median of means (*n*)*	Minimum of means M or M ± SD, (*n*)**	Maximum of means M or M ± SD, (*n*)**
Al	8.88 ± 12.0	14.4 (21)	0.157 (100) [36]	564 ± 41 (10) [37]
Ba	0.42 ± 0.94	10.2 (2)	0.13 (17) [38]	20.2 ± 4.4 (-) [39]
Ca	650 ± 856	872 (28)	5 (6) [40]	9,231 (26) [41]
Cu	3.90 ± 2.88	6.12 (43)	0.00062 ± 0.00006 (-) [42]	1,231 ± 154 (-) [43]
Fe	123 ± 93	98 (48)	0.11 (63) [44]	631 ± 200 (-) [39]
K	4347 ± 2284	3,141 (22)	20.8 ± 10.8 (16) [45]	14,400 (15) [46]
Mg	302 ± 148	305 (17)	3 (6) [40]	1,400 ± 400 (6) [47]
Mn	0.47 ± 0.27	1.42 (20)	0.026 (17) [38]	17.3 ± 4.9 (18) [48]
Na	6,262 ± 2,771	5288 (13)	2,258 ± 1,019 (9) [49]	13,230 (15) [46]
P	3,867 ± 2,230	2,223 (13)	270 ± 52 (16) [45]	230,769 ± 23,538 (-) [43]
S	4,599 ± 1,797	3,242 (8)	2,312 ± 21 (10) [37]	11,658 (19) [50]
Si	10.2 ± 31.1	97 (3)	0.0067 ± 0.0008 (-) [42]	1,623 ± 1,223 (7) [51]
Sr	1.37 ± 1.31	2.24 (12)	0.07 (17) [38]	34.6 ± 11.9d (16) [45]
Ti	4.55 ± 7.18	13 (4)	1.0 (17) [38]	20.4 ± 13.1 (16) [45]
Zn	34.2 ± 18.3	49.6 (53)	0.25 (63) [44]	1158 ± 15 (-) [52]

Note. M: Arithmetic mean; SD: Standard deviation; (*n*)*: Number of all found papers for each chemical element; (*n*)**: number of samples

Table 3. Median, minimum and maximum value of means of chemical element mass fractions (mg/kg dry tissue) in intact breast tissue adjacent to malignant breast tumor according to literature data in comparison with this work results

Element	Our results	Published data [Reference]		
	M ± SD *n* = 43	Median of mean (n)*	Minimum of means M or M ± SD, (*n*)**	Maximum of means M or M ± SD, (*n*)**
Al	2.43 ± 1.81	3.5 (16)	0.51 (17) [38]	442 ± 130 (10) [37]
Ba	0.138 ± 0.049	0.93 (2)	0.06 (17) [38]	19.8 ± 19.3 (9) [49]
Ca	93.5 ± 71.6	321 (21)	10.0 ± 3.2 (16) [45]	1,452 (26) [41]
Cu	0.83 ± 0.40	2.0 (30)	0.54 (37) [53]	42.0 ± 6.9 (18) [48]
Fe	20.5 ± 14.6	54.1 (32)	5 ± 2 (1) [54]	300 ± 21 (18) [48]
K	449 ± 367	980 (19)	5.6 ± 3.4 (16) [45]	6,500 ± 3,000 (2) [55]
Mg	38.9 ± 27.3	79.6 (15)	11.9 (80) [56]	1,100 ± 700 (6) [47]
Mn	0.16 ± 0.19	0.88 (14)	0.06 (17) [38]	18.1 ± 2.2 (18) [48]
Na	1,607 ± 1,240	3,475 (11)	722 ± 566 (9) [49]	9,200 (15) [46]
P	398 ± 295	749 (9)	62.6 ± 32.8 (16) [45]	2,860 ± 1,600 (30) [57]
S	948 ± 876	804 (5)	295 ± 120 (1) [54]	3,966 (8) [50]
Si	5.93 ± 4.84	55 (2)	0.00157 ± 0.00050 (-) [42]	111 ± 19 (10) [37]
Sr	0.55 ± 0.65	0.95 (10)	0.03 (17) [38]	8.6 ± 3.0 (16) [45]
Ti	2.44 ± 2.07	10 (3)	0.37 (17) [38]	14.4 ± 2.8 (18) [48]
Zn	6.00 ± 4.67	16.1 (35)	3.38 (37) [53]	99 ± 32 (25) [58]

Note. M: Arithmetic mean; SD: Standard deviation; (*n*)*: Number of all found papers for each chemical element; (*n*)**: number of samples

Table 4. Ratio of median of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn mean mass fractions (mg/kg dry tissue) in cancerous breast tissue (Med_C) and intact breast tissue adjacent to tumor (Med_A) according to literature data in comparison with this work results

Element	Medians of the reported means (*n*)*			This work result
	Cancerous tissue Med_C(*n*)	Adjacent tissue Med_A(*n*)	Ratio Med_C/Med_A	Ratio M_C/M_A
Al	14.4 (21)	3.5 (16)	4.11	3.65
Ba	10.2 (2)	0.93 (2)	11.0	3.00
Ca	872 (28)	321 (21)	2.72	6.95
Cu	6.12 (43)	2.0 (30)	3.06	4.70
Fe	98 (48)	54.1 (32)	1.81	6.00
K	3141 (22)	980 (19)	3.21	9.68
Mg	305 (17)	79.6 (15)	3.83	7,76
Mn	1.42 (20)	0.88 (14)	1.61	2.94
Na	5,288 (13)	3,475 (11)	1.52	3.90
P	2,223 (13)	749 (9)	2.97	9.72
S	3,242 (8)	804 (5)	4.03	4.85
Si	97 (3)	55 (2)	1.76	1.72
Sr	2.24 (12)	0.95 (10)	2.36	2.49
Ti	13 (4)	10 (3)	1.30	1.86
Zn	49.6 (53)	16.1 (35)	3.08	5.70

Note. (*n*)*: Number of all found papers for each chemical element; M_C means mass fraction ChE in cancerous breast tissue; M_A means mass fraction ChE in intact breast tissue adjacent to the tumor

3.4 Comparison of our results with literature data

Comparison of our results with literature data for the mass fractions of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn in cancerous and visually intact breast tissue adjacent to the tumor is shown in Table 2 and 3, respectively. Column 3 of these tables presents the median of the published mean values for each ChE, and in parentheses the number of studies that contained quantitative data on the content of this ChE in cancerous or adjacent to tumor intact breast tissue is indicated. Columns 4 and 5 indicate, respectively, the minimum and maximum values (arithmetic mean $\pm$ standard deviation or median) of the mass fraction of each ChE found from the reported data; the number of samples studied is indicated in parentheses and the corresponding link is given in square brackets.

A comparison of the ratio of the mean mass fraction of ChE in cancerous and intact breast tissue adjacent to tumor obtained in this work with the corresponding ratios calculated from published results are presented in Table 4. To obtain the corresponding ratios according to the literature, we used the median values of the mass fractions of ChE in cancerous and intact breast tissue.

3.5 Individual ratios of ChE content in cancerous and intact breast tissue adjacent to the tumor

Good agreement between the multiple excess of many ChEs in malignant tissue compared to the intact tissue adjacent to the tumor, found in the present study, and the data we obtained from the analysis of published data indicated not only the reliability of the identified phenomenon, but also the prospects for its use for diagnostic purposes. Obviously, in terms of developing new diagnostic methods, the individual ratio of ChE content in cancerous (QC) and intact breast tissue adjacent to the tumor (QA) is of particular importance. Such paired QC/QA ratios were calculated for each patient, and the main statistical characteristics of these ratios, such as the arithmetic mean, standard deviation, standard error of the mean, minimum and maximum values, median, percentiles with levels of 0.025 and 0.975 obtained for the entire group subjects examined are presented in Table 5.

Table 5. Main statistical parameters of individual ratios of the mass fraction of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn (Q_C/Q_A) in cancerous breast tissue (Q_C) to those in intact breast tissue adjacent to the tumor (Q_A)

Element	Mean	SD	SEM	Min	Max	Med.	P0.025	P0.975
Al	4.21	6.14	1.28	0.40	25.0	2.00	0.45	19.5
Ba	2.55	3.68	0.68	0.50	21.0	2.00	0.85	9.8
Ca	12.3	22.7	4.3	0.95	108	4.17	1.32	80.3
Cu	5.44	5.81	1.10	1.31	32.5	3.85	1.34	17.3
Fe	11.1	14.2	2.6	0.90	67.5	5.45	1.11	48.1
K	13.9	14.7	2.8	3.67	78.7	10.0	3.84	47.9
Mg	10.1	9.5	1.8	2.69	42.9	7.18	3.00	37.1
Mn	4.02	3.02	0.56	1.00	16.0	3.50	1.00	10.4
Na	7.51	12.9	2.44	1.10	58.5	3.21	1.25	48.5
P	13.2	11.5	2.2	3.41	48.4	9.48	3.74	45.6
S	9.02	11.5	2.2	2.12	49.7	5.00	2.13	44.8
Si	2.55	4.79	0.91	0.08	20.0	0.94	0.12	17.3
Sr	4.51	5.29	1.00	0.36	25.0	2.47	0.43	18.3
Ti	2.40	3.57	0.70	0.20	18.0	1.25	0.21	11.2
Zn	7.71	5.64	1.07	1.62	24.7	6.10	2.21	23.5

Note. M: arithmetic mean; SD: Standard deviation; SEM: Standard error of mean; Min: Minimum value; Max: Maximum value; Med.: Median; P0.025: Percentile with 0.025 level; P0.975: Percentile with 0.975 level

4. DISCUSSION

Acceptable agreement of the values of the content of ChEs in the international certified reference materials MODAS-5 (Cod tissue), MODAS-3 (Herring tissue), IAEA-153 (Milk powder) obtained in this study with the data of the corresponding certificates indicates sufficient accuracy of the developed ICP-AES micro method[5,8,60] and reliability of the mass fractions of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn in samples of malignant and intact breast tissue, presented in Tables 1-5.

Mass fractions of the ChEs presented in this study (Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti ,and Zn) were

determined in all or most of the samples of both malignant tissue and intact adjacent breast tissue. It allowed us to calculate the main statistical characteristics for the mass fractions of these elements, such as the arithmetic mean (M), standard deviation of the mean (SD), and standard error of the mean (SEM) (see Tables 1-4).

The M, SD, and SEM values are valid only if the results of determination of the content of ChEs in the studied samples are distributed normally. Only after making sure that the distribution of the results within each of the two studied groups (malignant tumor and adjacent intact breast tissue) is normal, is it possible to use M, SD, and SEM for comparison using parametric criteria, for example, Student's *t*-test. However, reliable detection of normal distribution of results with a relatively small number of samples in the presented study ($n = 43$) is impossible, since the existing criteria for detection of the type of distribution of results require a large sample size, usually several hundred samples. Since in our study it was not possible to prove or disprove the "normality" of the distribution of the obtained results due to the small sample size, in addition to the parametric Student's *t*-test, the nonparametric Wilcoxon-Mann-Whitney *U*-test was also used, which applies to any type of distribution of the results of the content of ChE in breast tissue.

To assess the effect of malignant transformation of breast tissue on the content of ChEs in it, a comparison of the elemental composition of cancerous and intact breast tissue adjacent to the tumor was performed (see Table 1). In the malignant tumor, the mass fractions of all the elements studied were higher than the levels characteristic of the adjacent to the tumor unaffected breast tissue. To compare the two groups of samples (malignant tumor and adjacent to the tumor intact breast tissue), both the parametric Student's *t*-test and the nonparametric Wilcoxon-Mann-Whitney *U*-test were used, and both criteria confirmed the reliability of the difference in the mass fractions of all elements except Si.

As a rule, when studying ChE in the mammary gland in normal and pathological conditions, both tissue samples obtained from healthy women and samples of visually unchanged tissue adjacent to the tumor are used as the "norm." However, mixing these two groups of samples is incorrect. For example, we have previously shown that in terms of the ChEs content, intact tissue adjacent to thyroid tumors is not identical to normal thyroid tissue in practically healthy individuals.[61,62] Therefore, in our review of the literature data (see Table 3), only the results obtained in the study of samples of visually intact breast tissue adjacent to a malignant breast tumor were used. Some values of the mass fractions of ChEs were not expressed by the authors of the cited works in terms of dry tissue. However, we calculated these values using literature data on the water content of 50%[63] and ash content of 1% (in dry tissue) [38] in the mammary gland of adult women (see Tables 2 and 3).

When reviewing the literature data, a huge scatter of published data on the content of ChEs in both malignant and non-malignant breast tissue was revealed (see Tables 2 and 3). This scatter is especially clearly manifested when comparing the minimum (column 4) and maximum (column 5) mean value among the detected data. From the data presented in Tables 2 and 3, it is evident that for almost all ChEs, the difference between the extreme values is two, three, or more mathematical orders. In our opinion, such significant discrepancies are primarily due to the lack of regulated sampling technology, insufficient control over losses or the entry of ChEs into the tissue sample being studied at all stages of the technological chain of the analytical process, as well as ignoring the assessment of the quality of the results obtained during the parallel analysis of international certified reference materials. As a result, errors are made at all stages, both in the direction of underestimation and in the direction of overestimation of the analysis result. These errors are random. Therefore, as the number of observations increases, the median of the accumulated data on the content of a particular ChE in both the affected and intact breast tissue should approach the actual value. Such an interpretation of the existing scatter of data accumulated in the literature makes it possible to compare the mean values of the content of each ChE obtained by us (Tables 2 and 3, column 2) with the medians of the published means of mass fractions (see Tables 2 and 3, column 3) for the same ChE.

The values of the mean mass fractions of ChEs in malignant breast tumors that we obtained were in good agreement with the median values of published data (see Table 2). The only exceptions were Ba and Si, the content of which in breast cancer tissue was published in only two and three articles, respectively. As follows from the data in Table 3, the results obtained for most of the studied ChEs were in reasonably good agreement with the medians of previously published mean values of ChEs content in intact breast tissue adjacent to malignant breast tumor and fit within their range (min-max). The only exceptions were Ba, Mn, Si, and Ti. The discrepancy between the results obtained for Ba, Si, and Ti and the medians of published mean values can be explained by the small number of studies (2-3 articles).

If to use the ratios of median values of mean mass fractions of ChE in cancerous tissue and intact breast tissue adjacent to the tumor, found from the analysis of literature data (see Tables 2 and 3, respectively), they can be compared with the

corresponding ratios of mean values of ChEs content in these tissues obtained in the present study and presented in Table 2. Calculation of these ratios showed that the increase in the content of all ChEs studied during malignant transformation of breast tissue discovered in the present study agreed with the results obtained by us from the analysis of published data (see Table 4). Thus, both from the data obtained in the present study and from our calculations made based on literature data, it followed that the content of such elements as Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn in malignant tissue is higher than in intact breast tissue adjacent to the tumor (see Table 4).

For a few ChE (Al, Ba, Ca, Cu, Fe, K, Mg, Na, P, S, and Zn) this increase was multiple (3 or more times). It indicated the potential of using the ratio of ChE content in malignantly transformed tissue (QC) and intact tissue adjacent to the tumor (QA) - QC/QA for diagnostic purposes. To assess the diagnostic significance of the QC/QA ratio for each examined patient, individual QC/QA values were calculated, and the main statistical characteristics obtained for the entire group of patients are presented in Table 5. The "min" values in this table (column 5) show that in all patients (n=43) the QC/QA ratio for Cu, K, Mg, Na, P, S, Zn exceeds 1.0. It follows that if we take QC/QA > 1.0 as the threshold value, then determining the content of any of these ChE in the tumor and adjacent tissue allows us to detect breast cancer with an accuracy of 100%-2%.[64] Considering the P0.025 values (Table 6, column 8), the levels of Ca and Fe can be added to the above-mentioned ChE potentially significant for BCa diagnosis. For these elements, only one patient had a QC/QA ratio of less than 1.0 (0.95 for Ca and 0.90 for Fe). Based on statistical tables,[64] the accuracy of BCa detection based on the level of these ChE in the lesion is $97\% \pm 3\%$. The use of ChEs levels in transformed breast tissue as tumor markers seems to be very promising, since the capabilities of modern analytical chemistry are rapidly increasing. For example, the distribution of such diagnostically promising ChEs as Fe and Zn in the mammary gland can be determined non-invasively using neutron stimulated emission computed tomography (NSECT).[65]

It is known that healthy breast tissue consists of a glandular component and stroma (adipose tissue and ligaments surrounding ducts and lobules, blood and lymphatic vessels).[66] In the mammary gland, the masses of the glandular component and adipose tissue together with the stroma are, on average, related approximately as 1:1.[67] It is also known that the content of many ChEs in adipose tissue is significantly lower than in glandular tissue.[5] Although tumor tissue consists mainly of malignantly transformed glandular cells, even the complete absence of adipose tissue in the tumor cannot increase the mass fractions of ChEs by more than two times. Thus, this factor cannot explain the more than fivefold increase in cancer tissue of such elements as Ca, Cu, Fe, K, Mg, Na, P, S, and Zn (see Table 6). It is known that Ca, K, Mg, and Na ions are the main electrolytes of the body. Na ions are concentrated mainly in the extracellular space, and Ca, K, and Mg ions are concentrated inside the cells. The ions of all these metals regulate intracellular metabolism. Thus, the obtained results indicate colossal changes in intracellular metabolism during the malignant transformation of mammary gland cells. The metals Cu, Fe, and Zn are part of many biologically active substances and are important microelements for the vital functions of the body. Their content inside the cells is under strict homeostatic control, since they form the epigenetic intracellular environment. A multiple increase in the intracellular concentration of these metals can lead to the malignancy of cells.

The observed phenomenon of multiple increase in the content of ChE in tissue of malignant tumor may be caused by different reasons. One of the possible explanations may be related to the fact that during malignancy, there is a violation of the permeability of cell membranes, metabolism of ChEs, and mechanisms of their intracellular transport. These violations cause excessive accumulation of ChEs in cells, and consequently, in the malignant tissues of the mammary gland.

Another possible explanation for the phenomenon discovered may be related to uncontrolled anthropogenic pollution of the environment with ChEs in quantities unusual for the biosphere. Excessive intake of ChEs into the body with food, water and air, which the evolutionary developed homeostasis mechanisms cannot cope with properly, may also cause an increase in the concentration of these elements in cells. It is well known that intracellular concentrations of such metals as Cu, Fe, and Zn are under the control of strict homeostasis. Although there is still no clear idea of how oncogenic transformation occurs, there is already enough data indicating that, for example, disruption of the metabolism of essential electrolytes, as well as the altered homeostasis of exclusively beneficial microelements such as iron, copper, and zinc, can lead to the development of tumors.[68–77]

If the malignant transformation of breast tissue that has occurred is associated with this cause, then an increased ChEs content should be detected not only in cancerous tissue, but also in visually intact tissue adjacent to the tumor. Comparison of ChEs content in samples of visually intact tissue adjacent to the malignant tumor with ChEs content levels characteristic of breast tissue in healthy women may confirm or refute this variant of oncogenesis. We plan to conduct such a comparison in the future.

As for the limitations of the study, first, it should be noted that the relatively small sample size of the studied samples of malignant (n = 43) and adjacent intact breast tissue (n = 43) should be noted. This did not allow us to determine the content of ChE considering the stage of the disease, the histological structure of the malignant tumor and molecular taxonomy, which is of particular interest for diagnostics, prognosis and choice of treatment tactics. Therefore, it is planned to continue collecting samples of malignant and adjacent intact breast tissue and analyzing the obtained material.

The detected multiple increase in the content of ChEs in malignant tumors of the mammary gland opens great prospects for the development of new in vitro and in vivo methods for differential diagnostics of breast tumors, in which the levels of ChE will act as tumor markers. For this purpose, it is necessary to further study the content of ChEs in the tissue of the lesion in benign diseases of the mammary gland and compare the obtained results with the data of this work. We plan to conduct such a study in the future.

5. Conclusions

The developed method of sample preparation allows us to obtain reliable data of the content of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, Zn in samples of cancerous and adjacent intact breast tissue with the help of ICP-AES. An essential feature of the developed method is the ability to determine the content of ChEs in samples weighing only a few milligrams, which allows it to be used for analyzing puncture biopsy materials. In the present study, a significant increase in the content of all the studied minor and trace elements in the breast tissue during its malignant transformation, except for Si, was revealed. All the differences revealed were statistically significant and generally agreed with the results of our analytical review of the literature. The results obtained in this work provide a solid basis for the development of new methods for diagnosing BCa based on the use of the ChEs ratios in the tissue of the lesion and adjacent intact tissue of the mammary gland as a tumor marker.

The study has significant limitations, as it is only the beginning of a larger study of the role of ChEs in the etiology, diagnosis, prevention, and treatment of breast pathologies. The most important of these limitations are the relatively small number of tissue samples examined and the limited list of ChEs determined. Further detailed studies of the content of many ChEs in breast tissues in health and disease are needed to clarify the role of impaired somatic homeostatic ChEs in the etiology and diagnosis of BCa. Our plans include continuing research with an increased sample of malignant breast tumors and the use of a range of different analytical methods, as well as determining the content of ChEs in breast tissues in benign breast lesions.

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Authors contributions

Conception and design of the study, statistical analysis, data analysis and interpretation, manuscript preparation and review: Zaichick V. Experimental studies, data acquisition, diagram design: Dogadkin D. Experimental studies, data acquisition: Gromyak I. Experimental studies, data acquisition: Shirokova V. Manuscript editing, data interpretation, administrative support: Kolotov V.

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Not applicable.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Data sharing statement

The data can be requested from the corresponding author.

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