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A Multiple Increase in the Content of Some Chemical Elements in Breast Tissue After its Malignant Transformation. Original Data and Mini Review

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ABSTRACT

The etiology of breast cancer remains largely unclear, although it is known that disturbances of somatic elemental homeostasis play a certain role in oncogenesis. This study aimed to identify changes in the content of chemical elements during malignant transformation of breast tissue. For this purpose, an effective method of small samples (mass from 10 mg) analysis by means of inductively coupled plasma atomic emission spectrometry was developed. With the help of the developed technique, the samples of cancerous (n=43) and normal (n=38) breast tissues were studied. Differences in the content of chemical elements were analyzed using Student's t-test and Wilcoxon-Mann-Whitney U-test. In patients with breast cancer the mean mass fractions of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn in malignant breast tissue were (mg kg-1 dry tissue) 8.88, 0.42, 650, 3.90, 123, 4347, 302, 0.47, 6262, 3867, 4599, 10.2, 1.37, 4.55, and 34.2, respectively, and were 2.45, >4.2, 8.37, 3.79, 8.91, 22.4, 16.3, >2.35, 9.13, 19.2, 11.9, 1.17, 2.74, >4.55, and 10.4 times higher than in healthy gland tissue. All differences identified were statistically significant except for Si. The phenomenon of the multiple increase in the Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Sr, Ti. and Zn contents in the malignant breast tumors requires further detailed study.

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Introduction

Despite significant advances in early diagnosis and the introduction of various novel therapeutic strategies for female breast cancer (BCa), this disease remains one of the most common cancers worldwide and continues to hold a leading position in mortality globally [1]. The etiology and pathogenesis of this disease remains largely unclear. Numerous epidemiological studies have identified a number of genetic and non-genetic factors that are associated with the risk of BCa [1-4]. These factors can be divided into three groups related to genetics, lifestyle (including behavior, diet, physical activity, type of work, bad habits and others) and environmental conditions. There are still ongoing debates around many of the identified factors, but some of them are confirmed by all studies. Among these indisputable factors, first, we can name gender (in 99% of cases BCa occurs in women) and age, since the risk of BCa increases with a woman's age, although it is noted that BCa is "getting younger" and the proportion of young women suffering from BCa is constantly increasing.

In our previous study, we determined age-related changes in the content of certain chemical elements (ChE) in the mammary gland of healthy women, guided by the idea that disruption of elemental somatic homeostasis (deficiency or excess) can provoke malignant transformation of breast tissue [5-7]. To implement this research, we developed a method of inductively coupled plasma atomic emission spectrometry (ICP-AES), which makes it possible to determine the content of the 15 ChE in the samples of breast tissue of small mass [8].

We have previously shown that malignant tumors of bones, prostate and thyroid glands differ significantly in the content of many ChE from the normal tissues from which tumors originate [9-35]. By analogy, we hypothesized that malignant transformation of breast tissue is also associated with significant changes in the content of certain chemical elements. To date, several studies have been published that examined the ChE content in normal and malignant breast tissues [36-61]. These studies also noted high concentrations of some ChE and, especially metals, in cancerous tissue. Despite the relatively large number of studies of the elemental composition of the mammary gland in normal and cancerous tissue, due to the large scatter of published data, and sometimes their contradictions, reliable information on the content of ChE in mammary gland tissue in the norm and during its malignant transformation is absent. There are still no analytical reviews of the literature on this issue that could resolve the existing contradictions and determine the most realistic results.

The main goal of our study was to compare age-related changes in the ChE content in breast tissue with the changes that occur during its malignancy. To achieve this goal, we conducted our own study of the ChE content in the tissue of malignant breast tumors using a previously developed technique [8]. To verify the reality of the results obtained, we reviewed published data on the ChE content in breast tissue in normal and cancerous conditions, determined the median values of the data available in the literature, and compared these median values with our results. For those elements for which good agreement was obtained with the medians of published data, which could with a high probability be considered as close to the true values, we compared age-related changes in the ChE content in breast tissue with the changes that occur during its malignancy.

Materials and Methods

Tissue samples

All patients suffering from breast cancer (n = 43, age from 35 to 77 years, Caucasian race, Caucasian lifestyle) were hospitalized in the thoracic department of the Medical Radiological Research Center. All of them were diagnosed with breast cancer for the first time and had not yet received any treatment. Pregnant patients, as well as patients with previous surgeries, kidney dysfunction, anemia, diabetes and other chronic diseases, as well as those taking micronutrient supplements, were excluded from the study. Informed consent was obtained from all patients before taking breast tissue samples. Each patient underwent a thick-needle puncture biopsy of affected area of breast for morphological examination and assessment of ChE content. In surgically operated patients with breast cancer, samples of resected material were also used for morphological examination and elemental analysis. In all cases, the diagnosis was confirmed by clinical and morphological results obtained from the study of biopsy and resected material.

Randomized samples of normal breast tissue were obtained from autopsies of 38 women (age 16 to 60 years, Caucasian) who died suddenly. An autopsy was performed in the forensic medical examination department of the Obninsk city hospital on the first day after the sudden death. The typical causes of death for most women were automobile accidents and injuries. Available clinical data were reviewed for each victim. None of them had a history of intersex diseases, endocrine diseases, neoplasms or other chronic diseases that would interfere with normal breast development. None of the subjects received drugs that affected the morphology of the mammary gland and the content of ChE in the gland. Morphologically, each breast tissue sample taken corresponded to the age norm. After the samples intended for elemental analysis were weighed, they were freeze-dried and homogenized [62].

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.

Sample Preparation and ICP-AES Measurements

To implement this study, we used the method of inductively coupled plasma atomic emission spectrometry (ICP-AES), which we developed. This method makes it possible to determine the content of the listed 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 test sample.

The ICP-AES method was chosen due to its high sensitivity, which allowed the simultaneous determination of the content of 15 ChE in small mass sample of tissue which may be obtained by puncture biopsy [8].

Deionized water distilled without boiling in a PTFE Subboiler ECO IR Maassen "Water and acid purification system" (Germany) and nitric acid for analysis (65%, max 0.005 ppm Hg) from Merck (Germany) were used for sample preparation and element analysis. 2% solution of nitric acid was prepared by dilution of initial one with deionized water and used further for preparation of solutions to be analyzed.

When examining a patient with a single puncture biopsy, a material mass about 10-20 mg is usually available. Therefore, we initially developed a technique for microwave autoclave acid digestion of breast tissue samples of small mass from 10 mg which was applied in the current study [63].

Element analysis of the samples by inductively coupled plasma atomic emission spectrometry (ICP-AES) was performed using an ICAP-6500 Duo plasma spectrometer (Thermo Scientific). To calibrate spectrometer, standard multielement reference solutions by Merck (Merck, KGaA, Darmstadt, Germany) and High-Purity standards (High-Purity Standards, North Charleston, SC, USA) were used.

The spectral range (166–847 nm) is recorded by a highly sensitive CID semiconductor detector. The optical unit of the instrument is thermally stabilized and purged with argon. High purity 99.993% argon is used as the plasma gas. The plasma power is 1150 W The rates of the plasma-forming argon flow, transport flow and the cooling flow are 0.5 L/min, 0.55 L/min, and 12 L/min correspondingly. Measurements of the element's content in the analyzed solutions were carried out using the iTEVA analytical software and MS Excel program.

To check the accuracy of the results obtained, the Polish certified reference materials MODAS-5 (Cod Tissue) and MODAS-3 (Herring Tissue), as well as the CRM prepared by the International Atomic Energy Agency IAEA-153 (Powdered milk) were used. A more detailed description of the methodology we developed and used was published by us earlier [8,63].

Systematic Mini Review

A systematic search was conducted using databases such as PubMed, Web of Science, Scopus and Google Scholar to identify literature published up to June 2024 for the elements under consideration (Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn) in normal and malignant breast tissue. Key search terms used in the search strategy included "chemical elements" or "trace elements" in combination with "breast", "breast cancer", "breast tumor", "breast carcinoma", or "cancer of mammary gland". Additionally, we searched all results presented in previous reviews and relevant meta-analyses on our topic of interest.

Identified studies were included only if they met the following standards:

- Only studies involving human participants were included;
- Quantitative data on the ChE of interest were presented;
- Control groups used breast tissue samples from healthy women;
- In patients suffering from 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 work. There were no restrictions on the language of published works.

Subsequently, literature data were collected and classified for each ChE depending on the condition of the breast tissue (normal or cancer). Among the published data on the mean values of a particular ChE, the median of the collected mean values for healthy and tumor breast tissue was found.

Statistic
The main statistical parameters, 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 for mass fractions of ChE (mg kg-1 of dry mass) were calculated using the MS Excel program. The reliability of difference in the results between two groups (normal and cancerous breast tissue) was evaluated by the parametric Student's t-test and nonparametric Wilcoxon-Mann-Whitney U-test. MS Excel was also used to determine the median values of the mean content of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn in normal and malignant breast tissue found in published articles.

Results
Table 1 depicts the mass fractions of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn obtained by us using the developed ICP-AES method in three different international certified reference materials MODAS-5 (Cod Tissue), MODAS-3 (Herring Tissue) and IAEA-153 (Powdered milk).

Table 1: ICP-AES data (Mean±SD) of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn mass fraction in certified reference material MODAS-5 (Cod Tissue), MODAS-3 (Herring Tissue), and IAEA-153 (Powdered milk) compared to their certified values (mg kg-1, dry mass basis)

El	MODAS-5		MODAS-3		IAEA-153	
	Certificate	Our result	Certificate	Our result	Certificate	Our result
Al	-	6±1	-	14±1	-	-
Ba	0.162±0.028	0.18±0.02	2.71±0.28	2.6±0.1	-	0.67±0.04
Ca	1100	1200±100	36900	39800±900	12870±320	12900±600
Cu	1.38±0.09	1.5±0.1	3.19±0.22	3.3±0.1	0.57±0.20	0.48±0.03
Fe	13.2±1.1	14.5±2.3	190±13	210±30	2.53±0.91	3.4±1.8
K	19300±1200	18100±700	11800±1300	10700±500	16480±1140	16400±800
Mg	1200±200	1111±43	3000±200	2522±74	1060±75	948±48
Mn	0.92±0.08	0.89±0.05	5.78±0.61	5.3±0.1	-	0.22±0.04
Na	3400±200	3100±100	19400±1700	16200±700	4180±290	3700±200
P	9600±1200	10000±400	23500±3900	26100±600	10100±1020	9600±500
S	10500±1600	12200±400	9300±1000	10900±400	-	-
Si	-	-	-	-	17±12	-
Sr	4.07±0.36	3.9±0.3	192±15	177±5	4.09±0.62	4.1±0.2
Ti	-	0.9±0.1	-	2.1±0.3	-	0.22±0.02
Zn	20.1±1.1	22±2	111±6	111±3	39.6±1.8	38±2

El – Chemical Element, Mean - Arithmetical Mean, SD - Standard Deviation

The data on the mass fractions of the ChE (Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn) in normal and cancerous breast tissue obtained using the method we developed are presented in Table 2. The table contains a set of data 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.

Table 2: Basic Statistical Parameters of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn mass fraction (mg kg-1 dry tissue) in the normal and cancerous breast tissue of females

Tissue	Element	Mean	SD	SEM	Min	Max	Med.	P0.025	P0.975
Normal	Al	3.62	2.44	0.42	1.21	11.1	2.69	1.25	9.60
	Ba	<0.1*	-	-	-	-	-	-	-
	Ca	77.7	61.8	10.6	11.7	265	60.8	14.4	223
	Cu	1.03	1.01	0.18	0.24	5.90	0.75	0.25	3.26
	Fe	13.8	12.3	2.1	4.51	66.0	10.1	5.01	43.6
	K	194	114	20	53.6	560	173	72.5	475
	Mg	18.5	9.0	1.6	7.66	48.0	17.4	8.00	36.4
	Mn	<0.2*	-	-	-	-	-	-	-
	Na	686	516	91	140	1827	502	145	1745
	P	201	74	13	102	371	193	114	352

	S	385	224	40	145	940	301	147	902
	Si	8.75	6.22	1.08	2.00	32.1	7.33	2.40	21.7
	Sr	0.50	0.24	0.04	0.19	1.06	0.46	0.20	0.98
	Ti	<1.0*	-	-	-	-	-	-	-
	Zn	3.29	1.65	0.30	1.30	9.60	2.95	1.52	7.28
Cancerous	Al	8.88	12.0	1.84	1.0	63	3.0	1.0	32
	Ba	0.42	0.94	0.14	0.10	6.10	0.20	0.10	2.03
	Ca	650	856	131	149	5154	364	151	2357
	Cu	3.90	2.88	0.44	1.50	15.0	3.20	1.61	12.9
	Fe	123	93	14	9.0	416	113	15.8	394
	K	4347	2284	348	1563	11794	3614	1704	8805
	Mg	302	148	23	112	751	244	120	649
	Mn	0.47	0.27	0.04	0.20	1.60	0.40	0.20	0.90
	Na	6262	2771	423	1704	13412	6056	2479	13128
	P	3867	2230	340	1108	11681	3043	1281	9239
	S	4599	1797	274	1901	8225	3999	2469	8105
	Si	10.2	31.1	4.7	1.0	207	4.0	1.0	19.8
	Sr	1.37	1.31	0.20	0.30	7.50	0.90	0.40	4.30
	Ti	4.55	7.18	1.14	1.0	43.0	2.0	1.0	18.6
	Zn	34.2	18.3	2.8	11.0	114	30	12.2	65.7

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. * Detection Limit.

Table 3 depicts differences between the mean values of mass fractions of the studied elements in normal and cancerous breast tissue evaluated by the parametric Student's t-test and nonparametric Wilcoxon-Mann-Whitney U-test.

Element	Female breast tissue				Ratio
	Cancerous tissue	Normal tissue	Student's t-test p	U-test* p	Cancerous to normal tissue
Al	8.88±1.84	3.62±0.42	0.0077*	<0.01*	2.45
Ba	0.42±0.14	<0.1	0.0299*	<0.01*	>4.2
Ca	650±131	77.7±10.6	0.00007*	<0.01*	8.37
Cu	3.90±0.44	1.03±0.18	0.00006*	<0.01*	3.79
Fe	123±14	13.8±2.1	0.000000002*	<0.01*	8.91
K	4347±348	194±20	<0.000000001*	<0.01*	22.4
Mg	302±23	18.5±1.6	<0.000000001*	<0.01*	16.3
Mn	0.47±0.04	<0.2	0.00000007*	<0.01*	>2.35
Na	6262±423	686±91	<0.000000001*	<0.01*	9.13
P	3867±340	201±13	<0.000000001*	<0.01*	19.2
S	4599±274	385±40	<0.000000001*	<0.01*	11.9
Si	10.2±4.7	8.75±1.08	0.76	>0.05	1.17
Sr	1.37±0.20	0.50±0.04	0.0002*	<0.01*	2.74
Ti	4.55±1.14	<1.0	0.003*	<0.01*	>4.55
Zn	34.2±2.8	3.29±0.30	<0.000000001*	<0.01*	10.4

M – Arithmetic Mean, SEM – Standard Error of Mean, t-test - Student's t-test, U-test - Wilcoxon-MANN-Whitney U-test, * Ssignificant Values.

Comparison of the mean values of the content of minor and trace elements in cancerous and normal breast tissue, for which a statistically significant difference was found, are presented in Figure 1 and 2, respectively.

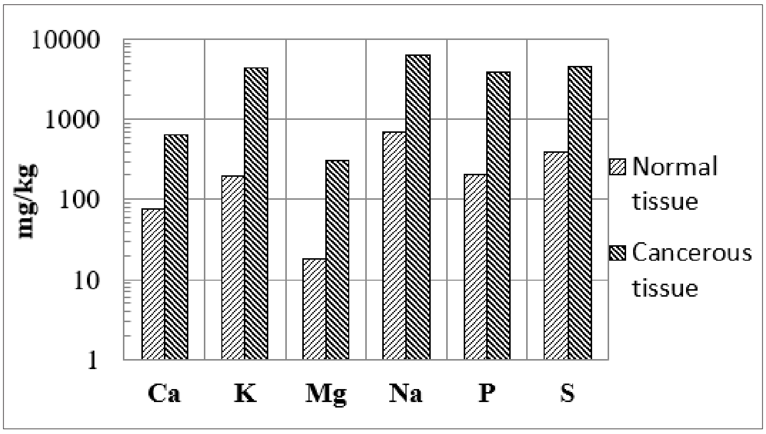

Figure 1: Comparison of Mean Values of the Content of Minor Chemical Elements such as Ca, K, Mg, Na, P, and S in Malignant Breast Tumors and Normal Breast Tissue of Apparently Healthy Females

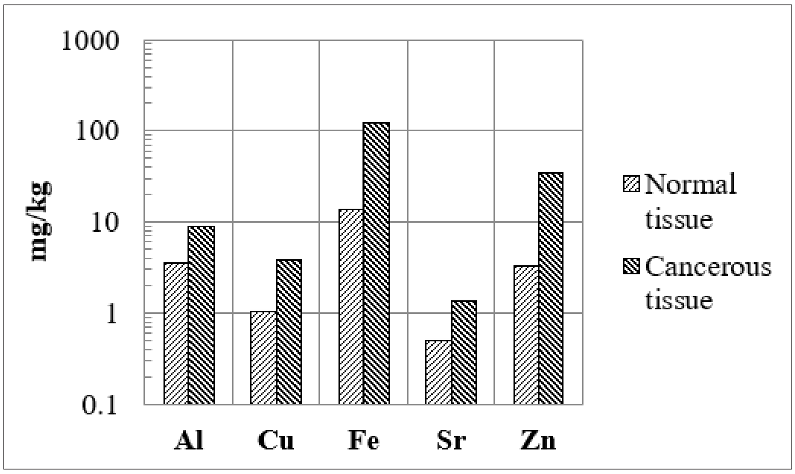

Figure 2: Comparison of Average Values of the Content of Trace Elements such as Al, Cu, Fe, Sr, and Zn in Malignant Breast Tumors and Normal Breast Tissue of Apparently Healthy Females

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 normal and cancerous breast tissue is shown in Table 4. Column 3 of this table 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 normal and/or cancerous breast tissue is indicated. Columns 4 and 5 indicate, respectively, the minimum and maximum values (arithmetic mean ± standard deviation or median) of the mass fraction of each CE found from the reported data; the number of samples studied is indicated in parentheses and the corresponding link is given. in square brackets.

Table 4: Median, Minimum and Maximum Value of Means of Chemical Element Mass Fractions (mg kg-1 dry tissue) in the Normal and Cancerous Breast Tissue According to Literature Data in Comparison with this Work Results

Tissue /Element	Our results	Published data [Reference]		
	M±SD	Median of means (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M or M±SD, (n)**
Normal				
Al	3.62±2.44	0.53 (5)	0.103 (52) [36]	38.4 (20) [37]
Ba	<0.1	0.084 (3)	0.03 (-) [38]	6.24±0.59 (-) [39]
Ca	77.7±61.8	262 (7)	52.6±10.6 (-) [39]	680 (2) [40]
Cu	1.03±1.01	1.3 (21)	0.137 (63) [41]	2280±140 (-) [42]
Fe	13.8±12.3	17.9 (17)	0.056 (63) [41]	75.6 (20) [43]
K	194±114	676 (7)	272 (20) [37]	4600 (-) [44]
Mg	18.5±9.0	85.5 (4)	9.9±1.8 (-) [39]	680 (4) [40]
Mn	<0.2	0.35 (8)	0.06 (-) [38]	3.74 (4) [40]
Na	686±516	2000 (7)	392±56 (-) [39]	5380 (3) [40]

P	201±74	2000 (8)	280 (-) [38]	56000±5460 (-) [42]
S	385±224	4000 (6)	2000 (-) [45]	7600 (-) [44]
Si	8.75±6.22	0.235 (5)	0.00024±0.00003 (-) [46]	0.24±0.39 (16) [47]
Sr	0.50±0.24	0.45 (3)	0.12 (-) [38]	1.4±0.4 (-) [39]
Ti	<1.0	<0.10 (3)	<0.04 (8) [48]	0.16 (1) [49]
Zn	3.29±1.65	7.48 (19)	0.21 (63) [41]	27.8±5.0 (20) [50]
Cancerous				
Al	8.88±12.0	14.4 (21)	0.157 (100) [36]	564±41 (10) [51]
Ba	0.42±0.94	10.2 (2)	0.13 (17) [52]	20.2±4.4 (-) [39]
Ca	650±856	872 (28)	5 (6) [53]	9231 (26) [54]
Cu	3.90±2.88	6.12 (43)	0.00062±0.00006 (-) [46]	1231±154 (-) [41]
Fe	123±93	98 (48)	0.11 (63) [41]	631±200 (-) [39]
K	4347±2284	3141 (22)	20.8±10.8 (16) [55]	14400 (15) [56]
Mg	302±148	305 (17)	3 (6) [53]	1400±400 (6) [57]
Mn	0.47±0.27	1.42 (20)	0.026 (17) [52]	17.3±4.9 (18) [58]
Na	6262±2771	5288 (13)	2258±1019 (9) [59]	13230 (15) [56]
P	3867±2230	2223 (13)	270±52 (16) [55]	230769±23538 (-) [42]
S	4599±1797	3242 (8)	2312±21 (10) [51]	11658 (19) [37]
Si	10.2±31.1	97 (3)	0.0067±0.0008 (-) [46]	1623±1223 (7) [60]
Sr	1.37±1.31	2.24 (12)	0.07 (17) [52]	34.6±11.9d (16) [55]
Ti	4.55±7.18	13 (4)	1.0 (17) [52]	20.4±13.1 (16) [55]
Zn	34.2±18.3	49.6 (53)	0.25 (63) [41]	1158±15 (-) [61]

M - Arithmetic Mean, SD – Standard Deviation, (n)* – the Number of all Found Articles for each Chemical Element, (n)** - Number of Samples.

A comparison of the ratio of the mass fraction of ChE in cancerous and healthy breast tissue obtained in this work with the corresponding ratios calculated from published results are presented in Table 5. To obtain the corresponding ratios according to the literature, we used the median values of the mass fractions of ChE in cancerous and healthy breast tissue.

Table 5: 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-1 dry tissue) in the Normal and Cancerous Breast Tissue According to Literature Data in Comparison with this Work Results

Element	Medians of the reported means (n)*		This work result	
	Cancerous tissue		Ratio cancerous to normal tissue	Ratio cancerous to normal tissue
Al	14.4 (21)	0.53 (5)	27.2	2.45
Ba	10.2 (2)	0.084 (3)	121	>4.2
Ca	872 (28)	262 (7)	3.33	8.37
Cu	6.12 (43)	1.3 (21)	4.71	3.79
Fe	98 (48)	17.9 (17)	5.47	8.91
K	3141 (22)	676 (7)	4.65	22.4
Mg	305 (17)	85.5 (4)	3.57	16.3
Mn	1.42 (20)	0.35 (8)	4.06	>2.35
Na	5288 (13)	2000 (7)	2.64	9.13
P	2223 (13)	2000 (8)	1.11	19.2
S	3242 (8)	4000 (6)	0.81	11.9
Si	97 (3)	0.235 (5)	413	1.17
Sr	2.24 (12)	0.45 (3)	4.98	2.74
Ti	13 (4)	<0.10 (3)	>130	>4.55
Zn	49.6 (53)	7.48 (19)	6.63	10.4

(n)* – the Number of all Found Articles for each Chemical Element.

Discussion

The acceptable agreement between the obtained values of the content of these ChE in the international certified reference materials MODAS-5 (Cod Tissue), MODAS-3 (Herring Tissue), IAEA-153 (Powdered milk) and the data of the corresponding certificates (Table 1) indicated sufficient accuracy of the results of measuring the mass fractions of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti and Zn in breast tissue samples using the developed ICP-AES technique, presented in Tables 2-5 [5,8,63].

In all samples of normal breast tissue, the Ba, Mn, and Ti content was below the detection limit (DL). The content of all other elements (Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr, and Zn) was determined in all or most of the samples, which made it possible to calculate all the main statistical characteristics for the mass fractions of these elements (Table 2). In cancerous breast tissue, the content of all elements, including Ba, Mn, and Ti, was determined in all samples, which made it possible to calculate all the main statistical characteristics for the mass fractions of all studied elements (Table 2).

The values of M, SD and SEM are valid only if the results of determining the ChE content in the studied samples are normally distributed. Only after making sure that the distribution of results within each of the two studied groups of samples (normal and cancer) is normal, can M, SD and SEM be used for comparison, for example using the student's t-test. However, reliable identification of the normal distribution of results with relatively small numbers of samples in the presented study (normal group n=38 and cancer group n=43) is not possible, since existing criteria for identifying the distribution type of results require larger sampling, usually several hundred samples. Since in our study it was not possible to prove or disprove the "normality" of the distribution of the results obtained due to the smaller sampling, in addition to the values of M, SD and SEM, we calculated such statistical characteristics as median, range (minimum-maximum) and percentiles with level 0.025 and 0.0975, and also used nonparametric Wilcoxon-Mann-Whitney U-test, which are valid for any distribution type of the results of the ChE content in breast tissue.

To assess the effect of malignant transformation of breast tissue on the ChE content in it, a comparison of the elemental composition of cancerous and normal breast tissue was carried out (Table 3). In a malignant tumor, the mass fractions of all studied elements, except for Si, were many times higher than the levels characteristic of normal tissue. An indirect assessment of the presence of a normal distribution of the results obtained can be made by the proximity of the Mean and Median values. As can be seen from the data in Table 1 for normal breast tissue, the differences between the Mean and Median mass fractions of all studied elements, as a rule, do not exceed 20-25%. Malignant breast tumor samples clearly show no normal distribution of results for mass fractions of such ChE as Al, Ba, Ca, Si, Sr, and Ti. The clear violation of the normal distribution of results in a group of samples of malignant breast tumors confirms the need for the use of nonparametric tests for these elements. To compare two groups of samples (normal and malignant tumors), we used both parametric Student's t-test and nonparametric Wilcoxon-Mann-Whitney U-test and both criteria confirmed the reliability of the difference in the mass fractions of all elements. except Si.

Usually, when studying ChE in the mammary gland, samples of visually intact tissue adjacent to the tumor are used as the "norm". However, such a replacement is incorrect. For example, we have previously shown that, in terms of the level of ChE content, intact tissue adjacent to thyroid tumors is not identical to normal thyroid tissue of practically healthy individuals [64, 65]. Therefore, in our review of published data (Table 4), only results obtained from the study of normal mammary glands of apparently healthy women were used. Some values of the mass fractions of ChE were not expressed in terms of dry tissue by the authors of the cited references. However, we calculated these values using literature data on the content of water - 50% and ash - 1% (in dry tissue) in breast of adult women [52, 66].

When considering the published data in Table 4, attention is drawn to the huge difference between the minimum (column 4) and maximum (column 5) values, which for almost all ChE amounts to two, three or more orders of magnitude. Such a wide range of published data, in our opinion, is mainly due to the insufficient attention of many authors to proper quality control of their results including sampling. The lack of proper control allows for random errors both in the direction of underestimation and in the direction of overestimation of the analysis result. Since errors are random in nature, as the number of observations increases, the median of accumulated data on the content of one or another ChE in breast tissue should approach the true value. This interpretation of the existing spread of accumulated data allows us to compare our results (column 2) with the medians of published mean mass fractions (column 3) for each ChE. As follows from the data in Table 4 the results obtained for most of the investigated ChE are in good agreement with the medians of the previously published mean values of the content of ChE in healthy breast tissue. The only exceptions are P and S, the content of which is approximately one order of magnitude lower than the median of the published data, as well as Si, the average content of which is about 40 times higher than the median of the previous reports. In addition, our mean values of the content of P, S, and Si in the normal breast tissue do not even fit into the range of data available in the literature. Studies of ChE content in malignant tumors were carried out much more often than in normal breast tissue, so our results are in significantly better agreement with the median values of published data. The only exceptions are Ba and Si, the content of which in cancerous breast tissue was published in only two and three articles, respectively.

Trends in changes in the ChE content in malignant breast tumors compared to the norm can also be traced according to literature data, if we use the ratio of median values of mean mass fractions for cancerous and normal tissue. The calculation of these ratios (Table 5) showed that during malignant transformation of breast tissue there is a multiple increase in the content of all studied elements, except for P and S. Thus, both from the data obtained in our study and from the literature data it follows that the content of such elements as Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Sr, Ti, and Zn in cancerous tissue is many times higher than in normal breast tissue (Tables 3 and 5).

Normal breast tissue consists of a glandular component and stroma (adipose tissue and ligaments, surrounding ducts and lobules, blood and lymph vessels [67]. On average the ratio by mass of the glandular component and adipose tissue together with the stroma is approximately 1:1 [68]. It is known that the content of many ChE in adipose tissue is significantly lower than in the glandular component [5]. Tumor tissue consists mainly of transformed glandular cells. However, even the complete absence of adipose tissue in the tumor cannot increase the mass fractions of ChE by more than twice. Thus, this factor cannot explain the multiple increase of the mentioned above ChE in cancer tissue.

A study of the age-related dynamics of the content of Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr, and Zn in the breast tissue of healthy women revealed a tendency towards a decrease in the mass fractions of all these elements, and for such elements as K, Mg, Na, and S, this decrease was statistically significant and amounted to 30-50% [5]. Thus, the multiple increase in ChE mass fractions found in the presented work in malignantly transformed breast tissue is not a logical consequence of the normal age-related physiology of mammalian gland.

One of the possible explanations for the observed phenomenon of a multiple increase in ChE content in tumor tissue may be associated with disturbances in the mechanisms of intracellular transport and metabolism of ChE that occur during malignancy of glandular cells. Such disturbances can lead to changes in the permeability of cell membranes and excessive accumulation of ChE in cells.

Another possible explanation may be related to the excessive intake of ChE into the body through food, water and air due to uncontrolled changes in the ChE content in the environment. Excessive accumulation of ChE in glandular cells can lead to their malignancy. In this case, an increase in the content of many ChE should be detected not only in cancerous tissue, but also in visually intact tissue adjacent to the tumor. To confirm or refute the possibility of such a variant of breast tumorigenesis, we plan to study the ChE content in samples of visually intact tissue adjacent to the tumor and compare the obtained data with the ChE content levels characteristic of breast tissue of healthy women.

Regarding the limitations of the study, the first thing to note is the relatively small (n=43) sample size of cancer tissue samples examined. 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, the collection of cancerous tissue samples and analysis of the resulting material will continue.

The detected multiple increase in the content of ChE in malignant tumors of the mammary gland opens great prospects for the development of new methods for in vitro and in vivo BCa diagnostics, in which ChE levels will act as biomarkers. To do this, it is necessary to study the content of ChE in the tissue of the lesion in benign breast diseases and compare the obtained results with the data of this study. We plan to conduct such a study. Further, more detailed research into the role of excess concentrations of some of the ChE found in this study will help develop measures to prevent BCa and outline new approaches to treating this disease.

Conclusion

The developed ICP-AES method makes it possible to obtain reliable data on the content of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn in breast tissue samples. An important advantage of the developed method is the ability to determine the ChE content in samples weighing only a few milligrams, which makes it possible to use it for the analysis of puncture tissue biopsy materials. The present study revealed a multiple increase in the content of many minor and trace elements in the breast tissue during its malignant transformation. Thus, according to the data obtained, in patients with BCa, the content of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Sr, Ti, and Zn in samples of malignant breast tissue was 2.45, >4.2, 8.37, 3.79, 8.91, 22.4, 16.3, >2.35, 9.13, 19.2, 11.9, 2.74, >4.55, and 10.4 times, respectively, higher than in healthy gland tissue. All identified differences were statistically significant and generally consistent with the results of our analytical review of the literature. Further detailed studies are needed to investigate the role of the accumulation of many ChEs in malignant tissue in the etiology and pathogenesis of BCa. Our future studies will be aimed at increasing the sample size of malignant breast tumors, as well as at investigating the content of ChE in adjacent intact breast tissue and in benign breast tumors.

The results obtained in this work provide a solid basis for the development of new methods for the prevention, diagnosis and treatment of breast cancer.

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Conflicts of Interest
The authors declare no conflicts of interest.

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