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REVIEW ARTICLE

COMPARISON OF THE MASS FRACTION OF 35 TRACE ELEMENTS IN BENIGNLY TRANSFORMED AND INTACT BREAST TISSUE ADJACENT TO A BENIGN LESION

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Abstract

Background: Many women around the world suffer from benign breast diseases. The causes of this disease are not entirely clear, although it is known that disturbances in the homeostasis of chemical elements in the breast tissue play a significant role in the etiology of diseases of this organ. The present study aimed to identify changes in the mass fractions of 35 trace elements in benign transformation of breast tissue compared to the levels of these microelements characteristic of the norm.
Method: We achieved this goal using a previously developed sample preparation technique that allows us to determine the content of 35 microelements in breast tissue samples weighing approximately 10 mg using inductively coupled plasma mass spectrometry. Using the developed technique, samples of benignly transformed and intact breast tissue adjacent to a benign lesion were examined.
Results: It was found that in benignly altered tissue, the content of studied elements, except for Ba, Cr, Nb, and Ti exceeded the levels characteristic of intact breast tissue adjacent to the lesion.
Conclusion: The observed significant increase in the content of many trace elements in benignly altered tissue compared to intact adjacent breast tissue can be used to develop new *in vitro* and *in vivo* diagnostic methods in which trace element levels in the breast lesion will act as markers. The discovered phenomenon, which opens up new possibilities in the prevention, diagnosis, and treatment of benign breast diseases, requires further, more in-depth study.
Keywords: Benign breast diseases, benignly transformed breast tissue, inductively coupled plasma mass spectrometry, trace elements.

INTRODUCTION

Benign breast diseases (BBD) include a wide range of clinical changes in the mammary gland[1,2]. Many women around the world suffer from BBD. For example, conditions such as fibrocystic changes and fibroadenomas occur in 50% and 25% of women aged 30 years and older, respectively, and are the most common benign breast lesions[1]. BBD are often accompanied by malignant transformation of mammary gland tissue. For example, complex cysts carry a risk of malignancy from 23% to 31%, and papillary lesions 16%[2]. Therefore, all types of BBD are generally recognized as a risk factor for breast cancer (BC)[3,4]. Screening tests for early detection of BC typically use full-field digital mammography and ultrasound. In complex cases, if there are doubts, histological confirmation is resorted to using percutaneous core needle biopsy. Since a biopsy is a great psychological trauma for every woman, sometimes more complex examination methods, such as contrast magnetic resonance imaging (MRI), are used before this procedure[5,6]. However, although contrast-enhanced MRI has demonstrated higher sensitivity than mammography and ultrasonography, its specificity is insufficient, leading to the problem of false-positive results - approximately 70–80% of biopsies performed after contrast MRI give a false positive result[5]. Therefore, the search for specific characteristics of benign and malignantly transformed breast tissues that could be used for differential diagnosis of these pathologies continues. Previously, in our studies, it was shown that benign and the malignantly transformed bones[7-14], prostate[15-22], and thyroid[23-33] glands differ significantly in the level of many trace elements (TEs), which made it possible to use these differences for

differential diagnosis of these pathologies. These results, as well as the fact that almost 50% of benign breast disease subtypes are associated with micro-calcifications[34], suggest the presence of specific levels of TEs characteristic of benignly transformed breast tissue.

Information about the TEs composition of benignly altered breast tissue is of interest not only from the point of view of searching for diagnostic indicators. It can also reflect the causes of pathology. It is known that the global spread of BBD is influenced by a complex interaction of genetic, environmental and lifestyle factors. One of the environmental factors is TEs that enter the human body with food, drinking water and air. Earlier, we paid special attention to the role of TEs in the normal physiology of the mammary gland[35,36]. In the present study, we proceeded from the fact that a violation of TEs somatic homeostasis (deficiency or excess) can provoke pathological transformation of mammary gland[37-40]. To enable the study of pathologically altered breast tissue, we have developed a sample preparation method that allows the determination of 35 TEs content in small tissue samples using inductively coupled plasma mass spectrometry (ICP-MS). This method made it possible to use tissue samples obtained using percutaneous core needle biopsy[40]. To date, several papers have been published in which the content of TEs in benignly transformed breast tissue was studied using various analytical methods[41-51]. However, due to the large scatter of published quantitative data, and sometimes their inconsistency, it is not possible to draw unambiguous conclusions about the changes in the TEs composition occurring in BBD. Also, no systematic reviews on this topic were found in the literature that could resolve the existing contradictions and draw adequate conclusions.

The present study was aimed at comparing the content of TEs in benignly transformed breast tissue with the content of the same TEs in the intact breast tissue adjacent to a benign lesion. To determine the content of TEs, we used a previously developed technique[40]. To assess the reliability of our results, a systematic analysis of the published data on the content of TEs in benign transformed and intact breast tissue adjacent to a benign lesion was carried out. The analysis performed allowed us to determine the median values of the data available in the literature and made it possible to compare the identified median values with our results.

MATERIALS AND METHODS

Tissue samples

The study used a collection of benignly transformed and intact breast tissue adjacent to benign lesion samples obtained by percutaneous core needle biopsy. The collection was made by surgeons of the thoracic department of the Medical Radiological Research Center (Obninsk) in the 1990s. After biopsy, the obtained material was weighed, lyophilized[52], and weighed again. Then, each dried tissue sample was sealed in polyethylene film pre-treated with rectified ethyl alcohol, and in sealed form placed in a numbered polyethylene capsule. Samples were stored in a fume hood at room temperature. As our studies have shown, lyophilized tissue samples in this form can be stored for decades without changing the levels of TEs in them[53,54]. Eleven pairs of tissue samples (benign transformed and intact adjacent) were obtained from women with fibrocystic breast disease, and six paired samples represented cases with fibroadenoma. The women's age ranged from 18 to 43 years. All patients were Caucasian.

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-MS measurements

The sample preparation procedure, ICP-MS equipment, software, and chemicals used, measurement modes, calibration and interference elimination methods, and the achieved detection limits (DL) have been presented in details in our previous publications[55,56].

To verify the accuracy of the obtained results, Polish certified reference materials (CRM) such as 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 systematic search was performed using Google Scholar, Web of Science, PubMed, and Scopus to identify articles published up to March 2025 that provided quantitative data on the content of the 35 trace elements of interest in benignly transformed and intact breast tissue adjacent to a benign lesion. The key terms used included “trace elements” or “chemical elements” in combination with “intact breast tissue adjacent to benign lesion”, “intactbreast tissue”, “intactadjacent breast tissue”, or “benign breast disease”,“benign breast tumor”, “breast fibroadenoma”, and “fibrocystic breast disease”. 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 TEs of interest were presented; (3) in patients with benign breast disease, 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 TE depending on the breast tissue (benignly transformed or intact breast tissue adjacent to benign lesion). From the published data, the median of the mean values for benignly transformed breast tissue was found for each specific TE.

Statistical analysis

The basic statistical characteristics such as arithmetic mean (M), standard deviation (SD), and standard error

of the mean (SEM) for mass fraction of TEs (mg/kg dry tissue) were calculated by MS Excel program. The significance of differences in the results between the two groups (benign transformed and intact breast tissue adjacent to benign lesion 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 value of the mean contents for each TE in benignly transformed breast tissue found in the published papers.

RESULTS

The results of determination of mass fraction of 35 TEs in three different CRM MODAS-5, MODAS-3, and IAEA-153 obtained using our developed ICP-AES method are presented in Table 1.

Table 1: ICP-MS data (Mean±SD) of trace elements mass fraction (mg kg^{-1}, dry mass basis) in certified reference material MODAS-5 (Cod Tissue), MODAS-3 (Herring Tissue), and IAEA-153 (Powdered milk) compared to their certifiedvalues.

El	MODAS-5		MODAS-3		IAEA-153	
	Certificate	Obtained result	Certificate	Obtained result	Certificate	Obtained result
Al	-	6±1	-	14±1	-	-
As	1.64±0.27	1.7±0.1	9.24±0.81	8.8±0.4	-	-
B	-	0.34±0.05	-	9.0±0.3	-	2.03±0.07
Ba	0.162±0.028	0.18±0.02	2.71±0.28	2.6±0.1	-	0.67±0.04
Bi	0.007	0.006±0.001	-	-	-	-
Cd	0.005	0.0046±0.0004	0.33±0.03	0.32±0.01	-	-
Ce	-	0.006±0.002	-	0.021±0.008	-	-
Co	0.014	0.012±0.001	0.08±0.01	0.110±0.003	-	0.016±0.001
Cr	0.201	0.3±0.1	0.90±0.11	0.9±0.2	-	-
Cs	0.059±0.005	0.059±0.002	0.085±0.008	0.086±0.005	-	-
Cu	1.38±0.09	1.5±0.1	3.19±0.22	3.2±0.1	0.6±0.2	0.42±0.03
Ga	-	0.012±0.001	-	0.036±0.002	-	-
Ge	-	0.006±0.001	-	0.018±0.002	-	-
La	-	0.007±0.002	-	0.017±0.005	-	-
Li	0.026	0.030±0.002	0.90±0.11	0.76±0.03	-	0.034±0.005
Mg	1200±200	1178±38	3000±200	2739±75	1060±75	1023±19
Mn	0.92±0.08	0.89±0.05	5.78±0.61	5.3±0.1	-	0.22±0.04
Mo	-	-	0.13±0.02	0.14±0.01	0.3± 0.3	0.228±0.004
Nb	-	-	-	0.006±0.002	-	-
Nd	-	-	-	0.006±0.003	-	-
Ni	0.136	0.14±0.02	0.32±0.05	0.5±0.1	-	0.13±0.02
Pb	0.045	0.05±0.01	0.104±0.013	0.13±0.01	-	-
Rb	4.54±0.33	4.5±0.1	2.33±0.20	2.24±0.07	14.0±1.9	14.9±0.4
Sb	-	-	0.016±0.004	0.017±0.002	-	-
Se	1.33±0.1	1.2±0.1	2.63±0.2	2.8±0.1	-	-
Sm	-	-	0.0018	0.0015±0.0003	-	-
Sn	-	0.14±0.01	-	0.23±0.02	-	0.05±0.02
Sr	4.07±0.36	3.5±0.4	192±15	180±6	4.1±0.6	3.76±0.07
Th	-	0.002±0.001		0.003±0.001	-	0.0009±0.0008
Ti	-	<0.9	-	<2.1		<0.2
Tl	-	0.0013±0.0002	-	0.0014±0.0005	-	-
U	-	-	0.075±0.008	0.063±0.002	-	-
V	-	-	0.78±0.11	0.62±0.01	-	-
W	-	0.024±0.008	-	-	-	-
Zn	20.1±1.1	21±1	111±6	114±3	39.5±1.8	33±1
Zr	-	0.10±0.02	-	0.09±0.03	-	0.014±0.008

El – Element, Mean - arithmetical mean, SD - standard deviation

Figure 1 demonstrates the mean mass fraction and the range of the standard error of the mean (M±SEM) for each of the 35 studied TEs in the compared pairs - benignly transformed and intact breast tissue adjacent to a benign lesion. The mean values of the mass fraction and standard deviation (M±SD) of the TEs in benignly transformed beast tissue, obtained using the ICP-MS micro method developed by us, were (mg/kg dry tissue): Al 11.9±7.4, As 0.061±0.027, B 0.73±0.46, Ba 0.17±0.11, Bi 0.0105±0.0079, Cd 0.182±0.198, Ce 0.0092±0.0054, Co 0.0078±0.0043, Cr 0.38±0.20, Cs 0.0119±0.0069, Cu 3.04±1.07, Ga (0.0052), Ge (0.0057), La 0.0079±0.0079, Li 0.017±0.012, Mg 265 ±159, Mn 0.33±0.20, Mo 0.031±0.022, Nb 0.0051± 0.0053, Nd 0.0042±0.0061, Ni 0.136±0.106, Pb 0.165± 0.136, Rb 5.59±3.72, Sb 0.0032± 0.0021, Se 0.43± 0.16, Sn 0.042±0.026, Sr 1.19±0.59, Th (0.0017), Ti 2.45±2.13, Tl 0.0014±0.0008, U 0.0024±0.0034, V 0.188±0.145, W 0.033±0.030, Zn 35.4±18.0, and Zr 0.016±0.012, and in the intact breast tissue adjacent to benign lesion (M_{IA}): Al 6.88±4.63, As 0.044±0.015, B 0.58±0.19, Ba 0.21±0.17, Bi 0.0029±0.0029, Cd 0.054 ±0.037, Ce 0.0061±0.0042, Co 0.0042±0.0023, Cr 0.56 ±0.52, Cs 0.0026±0.0017, Cu 1.09±0.48, Ga <0.0020*, Ge <0.0010*, La 0.0040±0.0043, Li 0.0065 ±0.0050, Mg 63.6±32.1, Mn 0.152±0.061, Mo 0.0117± 0.0058,

Nb 0.33±0.74, Nd 0.0029±0.0042, Ni 0.102±0.086, Pb 0.123±0.115, Rb 1.13±0.65, Sb 0.0032±0.0040, Se 0.183±0.061, Sn 0.033±0.017, Sr 0.53±0.22, Th (0.00086), Ti** 2.44±2.10, Tl (0.00030), U (0.00075), V 0.050±0.039, W 0.015±0.014, Zn 15.7±10.3, Zr 0.011±0.006 (*detection limit, in parentheses - possible upper limit of the mean). The Ga, Ge, and Th mass fractions in the benignly transformed breast tissue and Th, Tl, and U in the intact breast tissue adjacent to benign lesion were determined just in few samples.

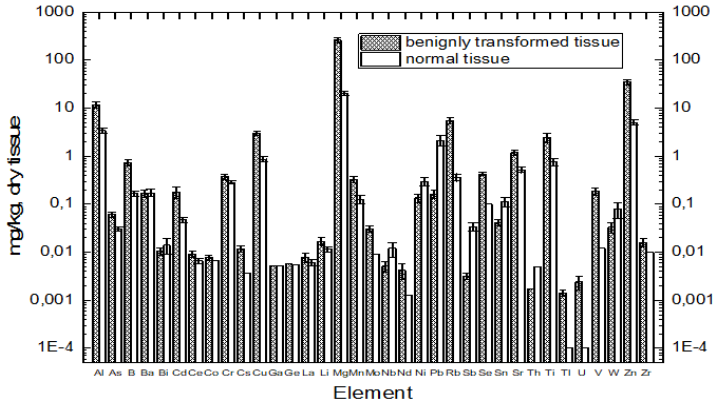


Figure 1: The mean mass fraction (M) and the range of the standard error of the mean (±SEM) for each of the 35 studied TEs in benignly transformed and intact breast tissue adjacent to benign lesion.

Table 2: Comparison of mean values (M±SEM) of trace elements mass fraction (mg/kg dry tissue) in the benignly transformed (BT) and intact adjacent breast tissue (AT) of females.

Element	Female breast tissue				Ratio
	BT n=17	AT n=17	t-test *p*	U-test *p*	BT to AT
Al	11.9±1.8	6.9±1.0	0.020*	<0.01*	1.72
As	0.061±0.007	0.044±0.003	0.030*	<0.01*	1.39
B	0.73±0.11	0.58±0.04	0.196	>0.05	1.26
Ba	0.17±0.03	0.21±0.04	0.457	>0.05	0.81
Bi	0.0105±0.0019	0.0029±0.0006	0.0014*	<0.01*	3.62
Cd	0.182±0.047	0.054±0.008	0.018*	<0.01*	3.37
Ce	0.0092±0.0013	0.0061±0.0009	0.058	<0.05*	1.51
Co	0.0078±0.0010	0.0042±0.0005	0.0040*	<0.01*	1.86
Cr	0.38±0.05	0.56±0.11	0.135	>0.05	0.68
Cs	0.0119±0.0017	0.0026±0.0004	0.00004*	<0.01*	4.58
Cu	3.04±0.26	1.09±0.10	<0.00001*	<0.01*	2.79
Ga	0.0052	<0.0020	-	-	>2.60
Ge	0.0057	<0.0010	-	-	>5.7
La	0.0079±0.0019	0.0040±0.0009	0.078	>0.05	1.98
Li	0.0170±0.0030	0.0065±0.0011	0.0029*	<0.01*	2.62
Mg	265±38	63.6±6.7	0.00008*	<0.01*	4.17
Mn	0.330±0.050	0.152±0.013	0.0021*	<0.01*	2.17
Mo	0.0310±0.0050	0.0117±0.0012	0.0024*	<0.01*	2.65
Nb	0.005±0.001	0.33±0.15	0.046*	<0.01*	0.015
Nd	0.0042±0.0015	0.0029±0.0009	0.461	>0.05	1.45
Ni	0.136±0.026	0.102±0.018	0.294	>0.05	1.33
Pb	0.165±0.035	0.123±0.025	0.340	>0.05	1.34
Rb	5.59±0.90	1.13±0.14	0.00014*	<0.01*	4.95
Sb	0.0032±0.0005	0.0032±0.0008	0.936	>0.05	1.00
Se	0.43±0.04	0.18±0.01	<0.00001*	<0.01*	2.39
Sn	0.042±0.006	0.033±0.004	0.239	>0.05	1.27
Sr	1.19±0.14	0.53±0.05	0.00032*	<0.01*	2.25
Th	0.0017	0.00086	-	-	1.98
Ti**	2.45±0.55	2.44±0.44	0.991	>0.05	1.00
Tl	0.0014±0.0002	0.00030	0.00007*	<0.01*	4.67
U	0.0024±0.0008	0.00075	0.058	<0.05*	3.20
V	0.188±0.036	0.050±0.008	0.0019*	<0.01*	3.76
W	0.033±0.008	0.015±0.003	0.048*	<0.01*	2.20
Zn	35.4±4.4	15.7±2.2	0.00047*	<0.01*	2.25
Zr	0.016±0.003	0.011±0.001	0.124	>0.05	1.45

M – arithmetic mean, SEM – standard error of mean, *t*-test - Student's *t*-test,
U-test - Wilcoxon-Mann-Whitney *U*-test, * Significant values.

Table 3: Median, minimum and maximum value of means of trace element mass fractions (mg kg^{-1} dry tissue) in benignly transformed breast tissue of females according to data from the literature in comparison with this work results.

Element	This work results	Published data [Reference]		
	M±SD n=17	Median of means (*n*)*	Minimum of means M or M±SD, (*n*)**	Maximum of means M or M±SD, (*n*)**
Al	11.9±7.4	50.5 (1)	50.5±23.9 (61) [41]	50.5±23.9 (61) [41]
As	0.061±0.027	-	-	-
B	0.73±0.46	-	-	-
Ba	0.17±0.11	-	-	-
Bi	0.0105±0.0079	-	-	-
Cd	0.182±0.198	0.58 (3)	0.185 (50) [42]	231±456 (14) [43]
Ce	0.0092±0.0054	-	-	-
Co	0.0078±0.0043	0.090 (3)	0,027±0,025 (23) [44]	6,75±9,85 (61) [41]
Cr	0.38±0.20	0.845 (3)	0.80±0.11d (68) [45]	1.85±2.90 (61) [41]
Cs	0.012±0.007	0.010 (1)	0.0088±0.0072 (-) [44]	0.017±0.012 (-) [44]
Cu	3.04±1.07	6.00 (9)	1.80±0.41 (18) [46]	99±114 (14) [43]
Ga	0.0052	-	-	-
Ge	0.0057	0.69 (1)	0.69±0.39 (68) [47]	0.69±0.39 (68) [47]
La	0.0079±0.0079	-	-	-
Li	0.017±0.012	3.4 (1)	3.4±3.2 (61) [41]	3.4±3.2 (61) [41]
Mg	265±159	168 (2)	19.6±5.4 (11) [48]	317±438 (61) [41]
Mn	0.33±0.20	1.07 (3)	0.90±1.05 (61) [41]	1.25±0.50 (50) [49]
Mo	0.031±0.022	7.85 (1)	7.85±7.70 (61) [41]	7.85±7.70 (61) [41]
Nb	0.0051±0.0053	-	-	-
Nd	0.0042±0.0061	-	-	-
Ni	0.136±0.106	0.49 (3)	0.47±0.10 (68) [45]	10.4±15.1 (61) [41]
Pb	0.165±0.136	2.42 (4)	1.13±0.13 (68) [45]	37.1±119.5 (61) [41]
Rb	5.59±3.72	2.2 (3)	0.50±0.09 (68) [45]	8.8±5.8 (-) [44]
Sb	0.0032±0.0021	0.35 (2)	0.064±0.049 (-) [44]	21.3±11.2d (61) [41]
Se	0.43±0.16	0.515 (7)	0.315±0.090 (22) [50]	1.62 (7) [51]
Sn	0.042±0.026	-	-	-
Sr	1.19±0.59	20.6 (1)	20.6±28.2 (61) [41]	20.6±28.2 (61) [41]
Th	0.0017	-	-	-
Ti	2.45±2.13	-	-	-
Tl	0.0014±0.0008	-	-	-
U	0.0024±0.0034	-	-	-
V	0.188±0.145	-	-	-
W	0.033±0.030	-	-	-
Zn	35.4±18.0	35.6 (10)	15±8 [44]	253±179 (14) [43]
Zr	0.016±0.012	-	-	-

M - arithmetic mean, SD – standard deviation,

The possible upper limit of the mean (M_{max}) for these TEs was calculated as the mean mass fraction, using the value of DL instead of the individual value when the latter was found to be below the DL:

$$M_{max} = (\sum_{1}^{n_i} C_i + DL \times n_j)/n \quad \text{.....(1)}$$

Where *Ci* is the TE mass fraction in sample *i*, n_i is the number of samples with TE content above DL, n_j is the number of samples with TE content below DL, and $n = n_i + n_j$ is the number of samples studied.

Table 2 depicts the differences between the mean values of the mass fractions of the studied TEs in the benignly transformed and in the intact breast tissue adjacent to benign lesion. A comparison of the results of the presented work with the data of our literature review on the mass fractions of TEs in the benignly transformed beast tissue is shown in Table 3.

Obviously, in terms of developing new diagnostic methods, the individual ratio of TEs content in benignly transformed (Q_{BT}) and intact breast tissue adjacent to the lesion (Q_{IA}) is of particular importance. Such paired Q_{BT}/Q_{IA} ratios were calculated for each patient, and the main statistical characteristics of these ratios, such as M, SD, SEM, min, max, median, percentiles with levels of 0.025 and 0.975 obtained for the entire group subjects examined are presented in Table 4.

DISCUSSION

Acceptable agreement of the values of the content of TEs in the international CRM MODAS-5, MODAS-3, and IAEA-153 obtained in this study with the data of the corresponding certificates (Table 1) indicates sufficient accuracy of the developed ICP-MS micro method and reliability of the mass fractions of 35 TEs in samples of benignly transformed and intact breast tissue adjacent to the lesion, presented in Figure 1 and Table 2-Table 4.

Mass fractions of the overwhelming majority of TEs were determined in all or most samples of both benign transformed and intact breast tissue adjacent to the lesion. This allowed us to calculate the basic statistical

characteristics for the contents of these TEs, such as M, SD and SEM (Table 2 - Table 4).

In samples of the intact tissue adjacent to the lesion the contents of Ga and Ge were below the DL of our ICP-MS method, while the levels of Th> Tl, and U were detected only in a few samples. In all benignly transformed breast tissue samples, only Ga, Ge, and Th content were determined in several samples. This allowed us to calculate the M_{max} value for these TEs using equation 1. The M, SD and SEM values are valid only if the TE content data in the samples under study are normally distributed. Only after ensuring that the distribution of data in the two groups (benign and normal breast tissue adjacent to the lesion) is normal, can M, SD, and SEM be used for comparison using parametric tests such as Student's t-test. However, reliable detection of normal distribution of results with a relatively small sample size (n=17 in each group) is impossible, since the existing criteria for detection of the type of distribution of results require several hundred samples. Since in our study it was not possible to prove or disprove the "normality" of the distribution of the obtained results, in addition to the Student's t-test, the nonparametric Wilcoxon-Mann-Whitney U-test was also used, which is applicable to any type of distribution of the obtained results.

Table 4: Main statistical parameters of individual ratios of the mass fraction of trace elements in the benignly transformed breast tissue to those in intact breast tissue adjacent to benign lesion.

Element	Mean	SD	SEM	Min	Max	Med.	P0.025	P0.975
Al	1.73	1.34	0.37	0.38	4.50	1.50	0.38	4.68
As	1.46	0.70	0.19	0.60	3.00	1.50	0.61	2.80
B	1.42	0.80	0.22	0.60	3.24	1.09	0.63	3.02
Ba	1.06	0.94	0.27	0,10	3.00	0.73	0.10	2.74
Bi	4.56	4.61	1.28	0.32	13.5	2,67	0.38	13.4
Cd	5.13	7.96	2.21	0.14	23.4	1.50	0.16	22.2
Ce	1.98	1.81	0.50	0.40	6.67	1.25	0.45	5.87
Co	2.12	1.17	0.34	0.71	5.00	2.00	0.74	4.54
Cr	0.89	0.71	0.20	0.14	2.50	0.82	0.17	2.43
Cs	9.6	10.6	2.9	2.00	33.3	6.50	2.15	33.1
Cu	3.29	1.91	0.53	1.59	9.12	2.93	1.70	7.64
Ga	2.22	-	-	0.40	10.0	-	-	-
Ge	2.53	-	-	0.20	10.0	-	-	-
La	2.48	2.90	0.84	0.40	10.0	1.18	0.46	8.76
Li	3.22	2.58	0.71	0.50	10.0	2.50	0.65	8.50
Mg	5.05	3.38	0.94	1.65	15.3	4.07	2.02	12.6
Mn	2.64	1.89	0.52	0.97	7.58	2.08	0.99	6.67
Mo	3.25	2.88	0.80	0.36	11.7	2.67	0.55	9.67
Nb	1.26	1.51	0.44	0.01	4.67	0.83	0.01	4.48
Nd	1.29	1.37	0.39	0.22	5.00	0.88	0.26	4.45
Ni	3.14	5.06	1.40	0.07	16.5	0.93	0.11	15.1
Pb	1.15	0.94	0.27	0.13	3.13	0.78	0.18	2.99
Rb	5.23	2.66	0.77	1.05	11.1	5.54	1.55	9.99
Sb	1.63	1.31	0.36	0.09	5.00	1.20	0.21	4.40
Se	2.67	1.09	0.30	1.00	4.55	2.50	1.10	4.38
Sn	1.71	1.68	0.47	0.37	6.00	1.00	0.46	5.55
Sr	2.93	1.33	0.37	1.50	5.18	2.60	1.52	5.18
Th	3.22	3.77	1.05	0.05	12.5	1.67	0.14	11.3
Ti	1.46	2.14	0.65	0.16	7.71	1.01	0.19	6.27
Tl	6.80	6.48	1.80	0.80	20.0	4.00	0.98	20.0
U	4.02	6.21	1.72	0.13	22.0	2.00	0.24	19.1
V	8.89	9.62	2.78	0.78	29.9	3.94	0.79	27.1
W	4.32	7.99	2.31	0.11	29.0	1.14	0.22	22.4
Zn	3.35	1.86	0.52	1.19	7.43	3.05	1.32	7.11
Zr	2.00	2.45	0.68	0.26	8.00	1.00	0.34	7.60

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.

To assess the effect of benign transformation of breast tissue on the TE contents in it, a comparison of the composition of benign and intact tissue adjacent to the lesion was performed (Table 2). In benignly altered tissue, the contents of all studied TEs, except for Ba, Cr, Nb, and Ti exceeded the levels characteristic of intact tissue adjacent to the lesion. To compare the benignly transformed and intact adjacent breast tissue, both the Student's t-test and the Wilcoxon-Mann-Whitney U-test were used, and both criteria confirmed the reliability of the difference in the mass fractions of such TEs as Al, As, Bi, Cd, Ce, Co, Cs, Cu, Li, Mg, Mn, Mo, Rb, Se, Sr, Tl, U, V, W, and Zn.

The data from our systematic review of publications on the TE contents in benign breast lesions are presented in Table 3. Some published TE values were not expressed on a dry tissue basis. However, we recalculated these values using literature data showing 50% water[57] and 1% ash (on a dry tissue basis)[58] in the adult female mammary gland. When examining the literature data in Table 3, attention is drawn to the huge difference between the min (column 4) and max

(column 5) values, which for almost all TEs is one order of magnitude or more. In our opinion, the wide range of data in the literature is mainly due to insufficient attention to changes in the levels of TE content that occur in the sample during its selection and preparation for analysis, as well as proper quality control of the data obtained. As a result, random errors arise, leading to both underestimation and over-estimation of the TE content in samples. The random nature of the inaccuracies that arise and their opposite direction allows us to approach the true value as the number of observations increases, focusing on the median of the accumulated data on the content of TEs in the breast tissue. This approach to leveling the existing scatter of literature data allows us to compare the results we obtained for each TE (column 2) with the medians of the published mean values (column 3). The values we obtained for the mean values of TE content in benignly transformed breast tissue are in good agreement with the medians of the published data (Table 3).The only exceptions were Co, Ge, Li, Mo, Pb, Sb, and Sr. The results we obtained for Co and Pb were almost 10 times lower, and for Sb almost 100 times lower than the corresponding median values found from published data. For Ge, Li, Mo, Pb, Sb, and Sr content we found only one paper in the literature. In the available literature, no data on the mass fraction of As, B, Ba, Bi, Ce, Ga, La, Nb, Nd, Sn, Th, Ti, Tl, U, V, W, and Zr in benignly transformed breast tissue were found.

Usually, when determining the content of TEs in the mammary gland in norm and pathology, tissue samples taken from healthy women as well as samples of undamaged tissue adjacent to the lesion are used as the "norm". However, mixing these two groups of samples is incorrect. For example, in an earlier study of intact tissue adjacent to thyroid tumors, it was shown that in terms of TE content, it is not identical to the thyroid tissue of healthy women[59,60]. Therefore, in our review of published data, only results related to TE contents in visually intact tissue adjacent to a benign lesion were used. As for the published data on the content of the studied TEs in the visually intact breast tissue adjacent to the benign lesion, in the literature available to us we found only one paper in which the Zn content was determined[61]. In the cited work, only 4 tissue samples were studied, and the published result of 16.1±1.2 mg/kg dry tissue is in good agreement with our data of 12.7±2.0 mg/kg dry tissue.

For some TEs (Bi, Cd, Cs, Cu, Li, Mg, Mn, Mo, Rb, Se, Sr, Tl, U, V, W and Zn), as can be seen from the data in Table 2, the increase in their content in the benign transformed tissue compared to the content of these TEs in the intact tissue adjacent to the lesion was multiple (2 or more times). This indicated the potential of using the ratio of TEs content in benign transformed tissue (Q_{BT}) and in intact breast tissue adjacent to the lesion (Q_{IA}) – Q_{BT}/Q_{IA} for diagnostic purposes. To assess the diagnostic significance of the Q_{BT}/Q_{IA} ratio for each examined patient, individual Q_{BT}/Q_{IA} values were calculated and the main statistical characteristics obtained for the entire group of patients are presented in Table 4. The "min" values in (column 5) showed that in all patients (n=17) the Q_{BT}/Q_{IA} ratio for Cs, Cu, Mg, Rb, Sr, and Zn was higher than 1.0. It followed that if we take Q_{BT}/Q_{IA}> 1.0 as the threshold value, then determining the content of any of these TEs in the lesion and adjacent tissue allows us to detect BBD with an accuracy of 100-6%[62]. The use of TEs levels in transformed breast tissue as biological markers seems to be very promising, since the capabilities of modern nuclear medicine are rapidly increasing. For example, the distribution of such diagnostically promising TE as Zn in the mammary gland can be determined non-invasively using neutron stimulated emission computed tomography[63].

One of the possible explanations for the observed phenomenon of multiple increase in the content of TEs in benignly transformed tissue may be associated with structural changes in the tissue, since the content of TEs in various morphological structures is different, as well as with disturbances in the intracellular metabolism of TEs that occur when the mechanisms regulating the proliferation of cells break down. As a result of such disturbances, a change in the permeability of cell membranes and subsequent excessive accumulation of TEs in cells may occur.

It is also possible that the discovered phenomenon is associated with excessive intake of TEs into the body with food, water and air due to uncontrolled changes in the content of TEs in the environment. Even a slight increase in the intracellular concentration of such metals as Cu and Zn, the levels of which are under conditions of strict homeostasis, can provoke the process of excessive proliferation of cells. A similar effect can also be caused by increased levels of potentially tumorigenic metals, such as Cd. In this case, an increase in the TE contents should be detected not only in transformed tissue, but also in intact tissue adjacent to the lesion of the mammary gland. To confirm or refute this variant of the BBD development, it is necessary to compare the TE contents in intact tissue adjacent to the benign lesion with the TE levels in breast tissue of healthy women.

The limitations of the present study include the small sample size of benign (n=17) and intact breast tissue adjacent to the benign lesion (n=17).This did not allow us to determine the content of TEs considering the histological structure of the transformed tissue, which is of particular interest for diagnostics, prognosis and choice of treatment tactics. Therefore, we plan to continue collecting samples and analyzing the material obtained.

The revealed multiple increase in the content of TEs in benignly transformed breast tissue opens great prospects for the development of new *in vitro* and *in vivo* methods for differential diagnostics of BBD and BC, in which TEs levels will act as tumor markers. For this purpose, further study of the TEs content in the tissue of the lesion in benign and malignant diseases of the mammary gland and comparison of the obtained results are necessary. We plan to conduct such studies in the future.

CONCLUSIONS

The developed sample preparation method allows obtaining reliable information on the content of 35 TE in samples of benignly transformed and intact tissue adjacent to benign lesion with the help of ICP-MS. An important feature of the developed method is the ability to determine the content of TE in samples with a small mass (only a few milligrams), which can be obtained using a puncture biopsy. In the present study, a significant increase in the content of all the studied TEs in the breast tissue during its benign transformation, except for Ba, Cr, Nb, and Ti, was revealed. The results obtained in this work provide a solid basis for new methods for diagnosing breast diseases that will use the level of TEs in the tissue of the lesion. Further studies are needed to obtain additional information on the role of TEs accumulation in benignly transformed tissues in the etiology of BBD. In our planned future studies, we will increase the sample size and determine the TE contents in malignant breast tumors.

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AUTHOR'S CONTRIBUTION

Zichick V: writing original draft. **Dogadkin D:** writing methodology. **Irina G:** formal analysis. **Valentina S:** data curation. **Kolotov V:** conceptualization. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

Data will be made available on request.

CONFLICTS OF INTEREST

No conflict of interest associated with this work.

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