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Research Article

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Study of the Age Dependence of 30 Chemical Element Contents in the Female Mammary Gland Using Inductively Coupled Plasma Atomic Emission Spectrometry and Mass Spectrometry

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Abstract

Background

The etiology of breast cancer remains largely unclear. However, it is well known that the incidence of this disease increases with age. In the presented work, for the first time, the age-related changes of 30 chemical elements: Al, As, B, Ba, Bi, Ca, Cd, Ce, Cr, Cu, Fe, K, La, Li, Mg, Mn, Na, Nb, Ni, P, Pb, Rb, S, Sb, Si, Sn, Sr, Ti, W, and Zn content in the mammary gland of women aged 16-60 years was investigated.

Methods

A method based on inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry was employed, which makes it possible to determine the mass fractions of these chemical elements in microsamples (mass from 10 mg) of breast tissue. With the help of this method, the material obtained during the autopsy of 38 practically healthy women aged 16-60 years who died suddenly was studied. Chemical element mass fractions were determined in two age groups of women: 16-40 and 41-60 years old.

Results

Using the parametric Student's t-test and the non-parametric Wilcoxon-Mann-Whitney U-test to compare two age groups, as well as Pearson's correlation coefficients between age and chemical elements mass fractions, it was found that the K, Mg, Na, Rb, and S mass fractions in normal breast tissue decrease with age, while the As mass fraction increase.

Conclusion

The first discovered phenomenon of age-related changes in the mass fractions of chemical elements in the normal mammary gland is of great interest for a more complete understanding of the age-related physiology and biochemistry of breast tissue and requires further detailed study.

Keywords: Woman Mammary Gland, Age-Related Changes, Chemical Elements, Inductively Coupled Plasma Atomic Emission Spectrometry, Inductively Coupled Plasma Mass Spectrometry

1. Introduction

In many countries, breast cancer (BC) in young women aged 35-54 years in terms of prevalence and mortality occupies a leading position among malignant neoplasms of other localizations [1,2]. Since BC affects women of working age, solving the problem of diagnosing and treating this disease is not only an urgent medical, but also an important social task [3]. Since there have been no breakthrough changes in the methods of diagnosing BC over the past decade, the constant improvement of early diagnostic methods is not at least the main factor influencing the steady increase in the incidence of this pathology. Despite the discovery of many factors that increase the risk of BC, the etiology of this disease remains largely unclear. Among the considered risk factors for BC, genetic characteristics, unfavorable environmental factors, and age are of particular importance based on the results of numerous studies [4]. The rapid increase in the incidence of BC cannot be explained by a genetic factor since the human genetic apparatus changes very slowly. Most likely, the alarmingly rapid increase in the BC incidence may be associated with changes in breast tissue that occur by 40 -60 years of age, since this is the age when BC incidence peaks in women in Europe and North America [5].

The rapid development of industry, chemistry, agriculture, food production, pharmaceuticals, medicine, cosmetics, which has occurred over the past 100 years and has steadily continued, has led to global changes in the quality of the human environment [6]. These changes cause a violation of the evolutionarily established quantities of chemical elements (ChE) and especially trace elements (TE), entering the human body from the environment mainly with food, water, and air. The functional feature of the breast, associated with the production of milk during lactation, determines the ability to accumulate significant amounts of ChE and the specific content of ChE in the gland tissue [7]. Therefore, changes in the environment can cause disturbances in the elemental homeostasis of breast tissue, initiating tumor growth.

Previously, we proved the existence of somatic homeostasis of ChE and demonstrated that disruption of this homeostasis plays an important role in the pathophysiology of bone tissue, prostate, and thyroid glands. In addition, age-related dynamism of elemental homeostasis was revealed, meaning that at different age periods the normal levels of ChE content in the tissue may be different [6,8-39].

By analogy, it should be assumed that the specific elemental composition of the breast, determined by its function, plays a key role not only in the normal physiology of the mammary gland, but also in the etiology of various diseases of this organ, including BC. However, the number of studies on the content of ChE in breast tissue is extremely small [40-54], and there are no data on age-related changes in the content of ChE in this gland.

The main goal of this study was to determine the ChE content in the breast tissue of healthy women of two age groups (16-40 years and 41-60 years), using the technique we developed, which is based on the combined use of inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS). The study also included an assessment of the reliability of the data obtained and their comparison with published results. The final goal was to identify differences between the mean mass fractions of ChE in the two age groups, as well as correlations between age and mass fractions of ChE obtained for normal breast tissue.

2. Material and Methods

2.1 Samples

Frozen breast tissue samples supplied by the Forensic Medical Examination Department of the City Hospital, Obninsk, Russia were investigated. A randomized sample of normal breast tissue was obtained from autopsies of 38 women (age 16 to 60 years, Caucasian race, Caucasian lifestyle) during the first day after sudden death. Typical causes of death for most women were car accidents and injuries. All the dead women were residents of Obninsk, a small town (about 130,000 inhabitants) in a non-industrial area 105 kilometers southwest of Moscow. Written informed consent was obtained from relatives of the victims during sampling. The study was performed according to the standards of the Institutional Ethics Committee and the Helsinki declaration of 1975, as revised in 1983, and was approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

Tissue samples from all victims weighing about 10 g were taken in the right mammary gland in its lower inner quadrant. A scalpel made of high-purity titanium was used for sampling [55]. Available clinical data were reviewed for each subject. None of the individuals had a history of an intersex condition, endocrine disorder, neoplasm, or other chronic disease that would interfere with normal breast development. None of the individuals received drugs that affect the morphology of the mammary gland and ChE mass fraction in the gland. The collection of samples was divided into two age groups of females: 16-40 and 41-60 years old.

2.2 Sample Preparation

One of the goals of our studies of the content of ChE in the mammary gland in normal and pathological conditions is the search for markers of BC and the development of new diagnostic methods by determining the content of ChE in puncture biopsies of the lesion. When examining a patient with a single puncture biopsy, a material weighing about 10-20 mg can be obtained. Therefore, we initially developed a technique for microwave (MW) acid digestion of breast tissue samples of small mass from 10 mg for subsequent determination of the ChE content in them using ICP-AES and ICP-MS analytical methods [56]. To reduce the amount of acid used for the sample decomposition an enclose consisting of three mini vessels has been developed. The enclosure is intended for the standard EasyPrep (100 cm^3) autoclave of the MARS-5 MW oven. Analyzed sample of 10 mg and more mass were placed in mini vessels. In each mini-vessel 1.4 ml of high-purity nitric acid was added. The mini vessels were closed with a stopper, the stopper was fixed with a lid, and a Teflon condenser tube was inserted into the common hole. Three assemblies of these mini-vessels were enclosed in autoclave. The nitric acid (12.5 ml) of pure for analysis grade was added to the autoclave to provide a vapor pressure equal to the pressure of acids in mini-vessels. The autoclaves with mini-vessels were then placed on the microwave system rotor. One of the autoclaves contained temperature and pressure sensors, as well as a hollow fluoroplast cylinder, the volume of which corresponded to that of the enclosure. The samples were heated to 150°C for 15 min and hold for 20 min at this temperature. The radiation power in MW was 800 watts at a frequency of 2450 Hz. After cooling to 30^0C the contents of the mini-vessels were quantitatively transferred into 10 ml tubes and the solutions were adjusted to 10 ml with 2% (v/v) HNO_3 solution. For ICP-AES measurements, the resulting solutions were additionally diluted two times with a 2% (v/v) HNO_3 solution. For ICP-MS measurements of Zn, As and Se, the sample solutions obtained were diluted five times with distilled water. For measurements of the rest of elements, the solutions were diluted twice with distilled water.

2.3 Reagents, Calibration, and Certified Reference Materials

ICP-AES: To plot calibration dependences, standard reference solutions by Merck (Merck, KGaA, Darmstadt, Germany) and High-Purity standards (High-Purity Standards, North Charleston, SC, USA) of elements were used. Merck solutions contain the following set of elements Al, Ba, Ca, Cd Co, Cr, Cu, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Zn (solution IV), Mo, V, Ti (solution XVI), and Zr (solution XVII). For P and S calibration dependences we used single-element reference solutions by High-Purity standards. Working calibration solutions in interval 0.1-10 mg/l were prepared by serial dilutions of initial ones.

ICP-MS: Deionized water distilled without boiling in a PTFE Subboiler ECO IR Maassen (from Germany) and HNO_3 (65% m/m) from Merck (Germany) were used to prepare solutions and samples. This acid was purified by sub boiling distillation using the PTFE Subboiler ECO IR Maassen system. The calibration solutions prepared by serial dilution of reference solutions supplied by High-Purity Standards (High-Purity Standards, North Charleston, SC, USA), Element Standards ICP-MS-68A (Solution A and Solution B, 68 elements) and single-element solutions of B, Mg, Al, Mn, Ni, Cu, Zn, Se, Rb, Sr, Cs, Ba.

Solutions containing 10, 25 and 50 μg/L of specific elements were prepared from solution A (10 mg/L), solutions containing 5 and 10 μg/L were from solution B. Single-element solutions (10 mg/L) were diluted to 500 μg/L for B, Mg, Al, Mn, Ni, Cu, Zn, Sr, Ba and to 20, 75 and 100 μg/L for Se, Rb, Cs. In all cases, 2%(v/v) HNO3 was used as a diluent. The solutions were diluted before measurement in disposable polypropylene test tubes of the volume 10 or 50 mL (Litaplast-Med, Belarus). The gravimetric method was used to determine the degree of dilution. To control the signal drift during the measurements using ICP-MS indium (In) was used as an internal standard, which was added to the calibration and sample solutions, at a concentration of 10 μg/L. Trace Elements in Drinking Water Standard CRM-TMDW (26 elements) were analyzed to check the ICP-MS performance. The CRM-TMDW was used as received.

To check the reliability of the results obtained, three certified reference materials (CRMs) were analyzed; the Polish certified reference materials MODAS-5 (Cod Tissue) and MODAS-3 (Herring Tissue), as well as the reference material prepared by the International Atomic Energy Agency IAEA-153 (Powdered milk) were used.

2.4 ICP-AES Measurements

Determination of the content of ChE in the studied samples by inductively coupled plasma atomic emission spectrometry (ICP-AES) was carried out using an ICAP-6500 Duo plasma spectrometer (Thermo Scientific). 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 was used as the plasma gas. The plasma power was 1150 W, the rate of the plasma-forming argon flow was 0.5 L/min, the transport flow was 0.55 L/min, and the cooling flow was 12 L/min. Measurements of ChE in the analyzed solutions were carried out using the iTEVA analytical software.

2.5 ICP-MS Measurements

A X Series II inductively coupled plasma quadrupole mass spectrometer (Thermo Scientific, Germany) equipped with a concentric atomizer nebulizer and a quartz cyclonic spray chamber cooled by a Peltier element (2°C) was employed. The parameters were as follows. Plasma power: 1400 W, plasma-forminggas (argon) flow rate: 13 L/min, auxiliary gas (argon) flow rate: 1.25 L/min, nebulizer gas (argon) flow rate: 0.88 L/min, plasma sampling depth: 105 rel. units and sample flow rate: 1 mL/min. Mass spectra were acquired using two scanning modes: (1) panoramic (Survey Scan) with 5 passes from 5 to 244 m/z and (2) at points (Peak Jumping) with 1 channel per weight, the integration time of 20 ms, and with 25 passes. All measurements were performed using PlasmaScreen software. Subject to all the device settings, the level of oxide ions CeO+/Ce+ is no more than 2%, and the level of doubly charged ions (Ba2+/Ba+) is no more than 3%.

The ICP–MS data were processed using the iPlasmaProQuad software developed in the laboratory of Vernadsky Institute [57]. This program was designed to process information output from the X Series II quadrupole mass spectrometer. The program involves data from calibration, element concentrations calculation, and corrections (the mass spectrometer is used only as an isotope mass detector). This approach gives complete control over the processing and estimation of the uncertainty of the measurement results, which is important both for the subsequent use of the results of analysis in other fields and for monitoring the analysis performance.

2.6 Statistics

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 Microsoft Office Excel program. Since the content of five CEs, such as Al, Cu, Mg, Sr and Zn, was measured by two methods of ICP-AES and ICP-MS, the average result obtained by both methods for each sample was used in the final calculation. The reliability of difference in the results between two age groups was evaluated by the parametric Student's t-test and nonparametric Wilcoxon-Mann-Whitney U-test. For the estimation of the Pearson correlation coefficient between age and ChE mass fraction the Microsoft Office Excel program was also used.

3. Results

Table 1 depicts the mass fractions of ChE determined in the CRM MODAS-5 (Cod Tissue), MODAS-3 (Herring Tissue) and IAEA-153 (Powdered milk).

El	MODAS-5		MODAS-3		IAEA-153	
	Certified	Found	Certified	Found	Certified	Found
Ag	-	-	0.04±0.01	0.039±0.003	-	-
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	-	-	-	-
Ca	1100	1200±100	36900	39800±900	12870±320	12900±600
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
Fe	13.2±1.1	14.5±2.3	190±13	210±30	2.53±0.91	3.4±1.8
Ga	-	0.012±0.001	-	0.036±0.002	-	-
Ge	-	0.006±0.001	-	0.018±0.002	-	-
K	19300±1200	18100±700	11800±1300	10700±500	16480±1140	16400±800
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
Na	3400±200	3100±100	19400±1700	16200±700	4180±290	3700±200
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
P	9600±1200	10000±400	23500±3900	26100±600	10100±1020	9600±500
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
S	10500±1600	12200±400	9300±1000	10900±400	-	-
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	-	-
Si	-	-	-	-	17±12	-
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
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	-	-	-	-
Y	-	-	0.0096	0.009±0.003	-	-
Zn	20.1±1.1	21±1	111±6	114±3	39.5±1.8	33±1

Table 1: This work ICP-AES and ICP-MS data (Mean±SD) for chemical 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 certified values El – Element, Mean - arithmetical mean, SD - standard deviation.

The contents of five ChE, such as Al, Cu, Mg, Sr and Zn, were measured by two methods ICP-AES and ICP-MS. A comparison of the mean values of the mass fractions of these ChE in the normal female mammary gland is presented in Table 2

Element	ICP-AES M_1	ICP-MS M_2	Δ, %
Al	3.63±2.40	3.60±2.43	0.8
Cu	1.29±1.72	1.39±1.38	-7.2
Mg	18.5±9.0	20.8±13.0	-11.0
Sr	0.55±0.24	0.52±0.24	5.8
Zn	3.29±1.65	3.39±1.54	-2.9

Table 2: Comparison of mean (M±SD) mass fractions of Al, Cu, Mg, Sr and Zn (mg kg^{-1}, dry tissue) in normal breast tissue of women aged 16–60 years determined using both ICP-AES. and ICP-MS methods M – arithmetic mean, SD – standard deviation, $\Delta = [(M_1\text{-}M_2)/M2]100\%$

Arithmetic mean of Al, As, B, Ba, Bi, Ca, Cd, Ce, Cr, Cu, Fe, K, La, Li, Mg, Mn, Na, Nb, Ni, P, Pb, Rb, S, Sb, Si, Sn, Sr, Ti, W and Zn. mass fractions in normal breast tissue of healthy women aged 16-40 years and 41-60 years are presented in tables 3 and 4, respectively. In addition to the arithmetic mean, some other statistical indicators were included in these tables, such as standard deviation, standard error of the mean, minimum and maximum values, median, percentiles with levels of 0.025 and 0.975.

Element	Mean	SD	SEM	Min	Max	Med.	P0.025	P0.975
Al	4.29	2.74	0.61	1.27	11.0	3.91	1.37	10.2
As	0.018	0.014	0.004	0.0010	0.0430	0.018	0.0010	0.0423
B	0.186	0.100	0.033	0.068	0.380	0.164	0.0730	0.364
Ba	0.202	0.171	0.046	0.0270	0.601	0.160	0.0296	0.580
Bi	0.015	0.021	0.007	0.0010	0.0620	0.0072	0.00115	0.0562
Ca	85.2	58.6	13.1	15.0	214	76.8	18.6	206
Cd	0.043	0.033	0.008	0.0102	0.126	0.0340	0.0114	0.123
Ce	0.0074	0.0045	0.0012	0.0018	0.0160	0.0075	0.00193	0.0154
Cr	0.294	0.173	0.039	0.0697	0.661	0.291	0.0836	0.643
Cu	1.14	0.57	0.13	0.285	2.63	1.10	0.287	2.33
Fe	16.2	15.4	3.44	5.12	66.0	10.1	5.21	53.1
K	226	125	29	80.0	560	194	87.2	513
La	0.0070	0.0058	0.0016	0.00180	0.0240	0.0044	0.00210	0.0199
Li	0.0132	0.0043	0.0019	0.00690	0.0190	0.0133	0.00744	0.0185
Mg	22.8	9.6	2.3	9.1	48.0	20.1	9.91	42.1
Mn	0.151	0.171	0.039	0.0460	0.774	0.095	0.0460	0.611
Na	882	549	129	156	1827	749	164	1782
Nb	0.0085	0.0031	0.0014	0.0050	0.0122	0.0099	0.00505	0.0120

Ni	0.138	0.076	0.017	0.0330	0.303	0.116	0.0340	0.288
P	219	77	18	120	371	204	123	361
Pb	1.74	1.65	0.38	0.200	5.74	1.19	0.227	5.43
Rb	0.502	0.421	0.097	0.111	1.71	0.359	0.126	1.53
S	475	247	58	148	940	491	151	919
Sb	0.0265	0.0247	0.0064	0.0100	0.101	0.0170	0.0100	0.0846
Si	9.36	7.47	1.71	2.00	32.1	6.20	2.54	26.3
Sn	0.106	0.071	0.019	0.0360	0.251	0.0775	0.0386	0.243
Sr	0.514	0.273	0.064	0.060	1.07	0.480	0.113	1.01
Ti	1.01	0.62	0.23	0.190	1.93	0.830	0.261	1.90
W	0.100	0.132	0.054	0.00200	0.335	0.031	0.00438	0.316
Zn	3.65	1.89	0.44	1.45	9.45	3.43	1.51	8.07

Table 3: Basic statistical parameters of 30 chemical elements mass fraction (mg kg^{-1}, dry tissue) in the normal breast tissue of females 16–40 years old 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.

Element	Mean	SD	SEM	Min	Max	Med.	P0.025	P0.975
Al	2.96	1.54	0.41	1.10	5.56	2.55	1.23	5.55
As	0.036	0.017	0.005	0.0100	0.0640	0.038	0.0116	0.0615
B	0.149	0.057	0.021	0.069	0.212	0.169	0.0737	0.211
Ba	0.129	0.096	0.032	0.0370	0.356	0.100	0.0398	0.321
Bi	0.013	0.015	0.006	0.0020	0.0429	0.0081	0.00218	0.0393
Ca	67.0	66.7	17.8	11.7	265	49.9	12.9	224
Cd	0.054	0.033	0.010	0.0140	0.114	0.049	0.0157	0.112
Ce	0.0055	0.0023	0.0007	0.0029	0.0090	0.0054	0.00293	0.00885
Cr	0.278	0.138	0.037	0.0420	0.496	0.256	0.0644	0.478
Cu	1.66	1.96	0.52	0.265	6.1	0.815	0.341	6.1
Fe	10.3	4.3	1.2	4.51	18.7	9.84	4.97	18.1
K	153	85	23	53.6	304	134	61.5	302
La	0.0052	0.0027	0.0008	0.0016	0.0094	0.0051	0.00168	0.00931
Li	0.0103	0.0050	0.0022	0.0057	0.0186	0.0088	0.00587	0.0178
Mg	14.5	5.3	1.4	8.80	25.7	12.3	9.35	25.2
Na	434	346	92	140	1325	349	142	1186
Mn	0.091	0.042	0.012	0.042	0.200	0.086	0.0423	0.178
Nb	0.013	0.017	0.006	0.0033	0.0551	0.0069	0.00332	0.0485
Ni	0.154	0.105	0.029	0.027	0.319	0.109	0.0308	0.316
P	178	65	17	102	331	166	108	306
Pb	2.89	3.94	1.19	0.443	13.0	0.94	0.474	11.7
Rb	0.187	0.105	0.028	0.0920	0.467	0.163	0.0936	0.424
S	270	123	33	145	517	225	154	515
Sb	0.044	0.045	0.013	0.0090	0.145	0.021	0.00983	0.139
Si	7.91	4.08	1.09	2.50	17.1	7.56	2.77	15.2
Sn	0.122	0.160	0.051	0.035	0.564	0.061	0.0355	0.474
Sr	0.553	0.218	0.058	0.160	1.07	0.580	0.220	0.958

Ti	0.64	0.53	0.20	0.240	1.77	0.45	0.257	1.63
W	0.060	0.073	0.030	0.0087	0.201	0.036	0.00911	0.184
Zn	2.91	0.88	0.24	1.95	4.35	2.65	2.00	4.29

Table 4: Basic statistical parameters of 30 chemical elements mass fraction (mg kg^{-1}, dry tissue) in the normal breast tissue of females 41–60 years old 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.

Comparison of the results of the present work with data reported for the mass fractions of Al, As, B, Ba, Bi, Ca, Cd, Ce, Cr, Cu, Fe, K, La, Li, Mg, Mn, Na, Nb, Ni, P, Pb, Rb, S, Sb, Si, Sn, Sr, Ti, W and Zn. in normal breast tissue of adult women is shown in Table 5.

Element	Published data [Reference]			This work
	Median of means (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M or M±SD, (n)**	M±SD n=38
Al	6.7 (4)	0.103 (52) [40]	38.4 (20) [41]	3.6±2.4
As	0.48 (3)	0.095 (3) [42]	<5 (-) [43]	0.030±0.015
B	<0.16 (1)	<0.16 (-) [43]	<0.16 (-) [43]	0.17±0.08
Ba	3.1 (2)	0.030(-) [43]	6.24±0.59 (-) [44]	0.17±0.15
Bi	<0.06 (1)	<0.06 (-) [43]	<0.06 (-) [43]	0.014±0.018
Ca	262 (7)	52.6±10.6 (-) [44]	680 (2) [45]	77.7±61.8
Cd	0.034 (5)	0.0310 (8) [46]	<0.4 (-) [43]	0.047±0.033
Ce	0.0012 (1)	0.0012 (1) [47]	0.0012 (1) [47]	0.0066±0.0038
Cr	0.088 (7)	0.0012(1) [47]	2.44±0.23 (-) [44]	0.29±0.16
Cu	2.56 (19)	0.4(1) [47]	2280±140 (-) [48]	1.36±1.35
Fe	21.8 (14)	5.1 (46) [49]	75.6 (20) [46]	13.8±12.3
K	676 (7)	272 (20) [41]	4600 (-) [50]	194±114
La	<0.6 (1)	<0.6 (-) [43]	<0.6 (-) [43]	0.0062±0.0047
Li	-	-	-	0.012±0.005
Mg	85.5 (4)	4.5±0.9 (-) [44]	680 (4) [49]	19.2±8.9
Mn	0.5 (7)	0.06 (-) [43]	3.74 (4) [45]	0.13±0.14
Na	2000 (7)	392±56 (-) [44]	5380 (3) [45]	686±516
Nb	<0.3 (2)	0.0004(1) [47]	<0.6 (-) [43]	0.012±0.014
Ni	0.16 (7)	0.01(1) [47]	1.14 (20) [41]	0.144±0.087
P	2000 (8)	280 (-) [50]	56000±5460 (-) [48]	201±74
Pb	0.128 (6)	0.0081(1) [47]	3.21±2.15 (16) [44]	2.2±2.7
Rb	626 (2)	0.2(1) [47]	2504 (4) [45]	0.37±0.36
S	4000 (6)	2000 (-) [51]	7600 (-) [50]	385±224
Sb	0.044 (2)	0.030-0.044 (2) [42]	5.0 (-) [43]	0.034±0.036
Si	0.235 (5)	0.00024±0.00003 (-) [52]	0.24±0.39 (16) [53]	8.75±6.22
Sn	0.52 (1)	0.52 (-) [43]	0.52 (-) [43]	0.11±0.11
Sr	0.2 (4)	0.12 (-) [43]	0.70±0.22 (16) [44]	0.53±0.25
Ti	0.13 (2)	<0.1 (-) [43]	0.16(1) [47]	0.83±0.59
W	-	-	-	0.080±0.104
Zn	8.3 (17)	2.88 (46) [49]	27.8±5.0 (20) [54]	3.34±1.56

Table 5: Median, minimum and maximum value of means of chemical element mass fractions (mg kg^{-1}, dry tissue) in normal

breast tissue of adult females according to data from the literature in comparison with this work results M - arithmetic mean, SD – standard deviation, (n)* – number of all references; (n) - number of samples.**

Differences between the mean values of mass fractions of Al, As, B, Ba, Bi, Ca, Cd, Ce, Cr, Cu, Fe, K, La, Li, Mg, Mn, Na, Nb, Ni, P, Pb, Rb, S, Sb, Si, Sn, Sr, Ti, W and Zn.in normal breast tissue of healthy women aged 16-40 and 41-60 years evaluated by the parametric Student's t-test and nonparametric Wilcoxon-Mann-Whitney U-test are presented in Table 6.

Element	Female "normal" breast tissue				Ratio
	AG1 16-40 years n=22	AG2 41-60 years n=16	t *p£*-test	U-test p	AG2 to AG1
Al	4.29±0.61	2.96±0.41	0.095	>0.05	0.69
As	0.018±0.004	0.036±0.005	0.013*	<0.01*	2.00
B	0.186±0.033	0.149±0.021	0.356	>0.05	0.80
Ba	0.202±0.046	0.129±0.032	0.205	>0.05	0.64
Bi	0.015±0.007	0.013±0.006	0.846	>0.05	0.87
Ca	85.2±13.1	67.0±17.8	0.418	>0.05	0.79
Cd	0.043±0.008	0.054±0.010	0.394	>0.05	1.26
Ce	0.0074±0.0012	0.0055±0.0007	0.206	>0.05	0.74
Cr	0.294±0.039	0.278±0.037	0.756	>0.05	0.95
Cu	1.14±0.13	1.66±0.52	0.343	>0.05	1.46
Fe	16.2±3.4	10.3±1.2	0.117	>0.05	0.64
K	226±29	153±23	0.059	□0.05*	0.68
La	0.0070±0.0016	0.0052±0.0008	0.355	>0.05	0.74
Li	0.0132±0.0019	0.0103±0.0022	0.361	>0.05	0.78
Mg	22.8±2.3	14.5±1.4	0.0045*	<0.01*	0.64
Mn	0.151±0.039	0.091±0.012	0.155	>0.05	0.60
Na	882±129	434±92	0.009*	□0.01*	0.49
Nb	0.0085±0.0014	0.013±0.006	0.421	>0.05	1.53
Ni	0.138±0.017	0.154±0.029	0.629	>0.05	1.12
P	219±18	178±17	0.114	>0.05	0.81
Pb	1.74±0.38	2.89±1.19	0.373	>0.05	1.66
Rb	0.502±0.097	0.187±0.028	0.005*	<0.01*	0.37
S	475±58	270±33	0.005*	□0.01*	0.57
Sb	0.0265±0.0064	0.0440±0.0130	0.240	>0.05	1.66
Si	9.36±1.71	7.91±1.09	0.481	>0.05	0.85
Sn	0.106±0.019	0.122±0.051	0.778	>0.05	1.15
Sr	0.514±0.064	0.553±0.058	0.659	>0.05	1.08
Ti	1.01±0.023	0.640±0.200	0.255	>0.05	0.63
W	0.100±0.054	0.060±0.030	0.535	>0.05	0.60
Zn	3.65±0.44	2.91±0.24	0.161	>0.05	0.80

Table 6: Comparison of mean values (M±SEM) of Al, As, B, Ba, Bi, Ca, Cd, Ce, Cr, Cu, Fe, K, La, Li, Mg, Mn, Na, Nb, Ni, P, Pb, Rb, S, Sb, Si, Sn, Sr, Ti, W, and Zn mass fraction (mg kg^{-1}, dry tissue) in normal female breast tissue of two age groups (AG) M – arithmetic mean, SEM – standard error of mean, AG1 - age groups 1, AG2 - age groups 2, t-test - Student's t-test, U-test - Wilcoxon-Mann-Whitney U-test, * Significant values.

Table 7 shows values of the Pearson correlation coefficient between age and ChE mass fraction.

Element	**Al**	**As**	**B**	**Ba**	**Bi**	**Ca**	**Cd**	**Ce**	**Cr**	**Cu**
r	-0.22	0.38[a]	0.15	-0.17	0.17	0.01	0.25	-0.29	-0.08	0.19
Element	Fe	K	La	Li	Mg	Mn	Na	Nb	Ni	P
r	-0.05	-0.36[a]	-0.01	0.11	-0.46[b]	-0.11	-0.45[b]	0.36	0.20	-0.20
Element	Pb	Rb	S	Sb	Si	Sn	Sr	Ti	W	Zn
r	0.11	-0.28[a]	-0.42[a]	0.27	-0.10	0.05	0.01	-0.39	-0.21	-0.15

Table 7: Correlations between age and chemical element mass fractions in the in normal female breast tissue (r – coefficient of correlation) Significant values: [a]$p<0.05$. [b]$p<0.01$.

4. Discussion

The mass fractions of Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr and Zn were obtained by us using the developed ICP-AES method, while the mass fractions of Al, As, B, Ba, Bi, Cd, Ce, Cr, Cu, La, Li, Mg, Mn, Nb, Ni, Pb, Rb, Sb, Sn, Sr, Ti, W, and Zn - using the developed ICP-MS method. Acceptable agreement between the certified mass fractionation values of Al, As, B, Ba, Bi, Ca, Cd, Ce, Cr, Cu, Fe, K, La, Li, Mg, Mn, Na, Nb, Ni, P, Pb, Rb, S, Sb, Si, Sn, Sr, Ti, W and Zn. and those found in the present work can be seen in Table 1. The absence of significant differences in the mean values of the Al, Cu, Mg, Sr and Zn mass fractions obtained by both IKPAES and ICPMS methods is shown in Table. 2. These findings testified to the sufficient accuracy of the methods used.

The Al, As, B, Ba, Bi, Ca, Cd, Ce, Cr, Cu, Fe, K, La, Li, Mg, Mn, Na, Nb, Ni, P, Pb, Rb, S, Sb, Si, Sn, Sr, Ti, W and Zn mass fractions were determined in all or in most of the samples, therefore, for these ChE, the mean value of the mass fraction (M), standard deviation (SD), standard error of the mean (SEM), minimum, maximum, median, and percentiles with levels of 0.025 and 0.0975 was calculated for two age groups (Tables 3 and 4). The values of M, SD, and SEM can be used to compare data for different groups of samples only under the condition of a normal distribution of determining the ChE mass fractions in the samples under study. Statistically reliable identification of the law of distribution of results requires large sample sizes, usually several hundred samples, and therefore is rarely used in biomedical research. In the conducted study, we could not prove or disprove the "normality" of the distribution of the results obtained due to the insufficient number of samples studied. Therefore, in addition to the M, SD, and SEM values, such statistical characteristics as the median, range (minimum-maximum) and percentiles with the level of 0.025 and 0.0975 were calculated, which are valid for any law of distribution of the results of ChE in breast tissue.

Most often, in studies about ChE in the mammary gland, samples of visually intact tissue adjacent to the tumor are used. However, we have previously shown that the intact tissue adjacent to the thyroid tumors in terms of the level of ChE content is not identical to the normal thyroid gland tissue of apparently healthy individuals [58,59]. Therefore, in our review of reported data, only results obtained from the study of normal mammary glands of apparently healthy women were used. The results obtained for Al, B, Bi, Ca, Cd, Ce, Fe, K, La, Na, Ni, Sb, Sn, Sr, and Zn were in good agreement with the medians of previously published means of ChE mass fractions (Table 5), whereas Ba, Cr, Cu, Mg, Mn, Nb, Pb and Rb mass fractions were within the reported ranges of means. At the same time, the As mean was below the reported mean mass fractions, while the Ti mean was higher. The content of P and S was approximately one mathematical order lower than the median of the published data, as well as Si, the mean content of which was more than 36 times higher than the median of the previous reports. Moreover, our mean values of the content of P, S, and Si do not even fit into the range of data available in the literature. Reported data on Li and W were not found (Table 5). However, it should be noted that the variations of published mean values for some of the studied ChE are very large and amounts to several mathematical orders (Table 5).

To assess the effect of age on the mass fractions of ChE in the normal mammary gland of healthy women, two age groups described above were studied (Table 6). In normal mammary glands the mass fractions of almost all studied ChE decreases with the age, with the exception of As, Cd, Cu, Nb, Ni, Pb, Sb, and Sn. However, the increase was statistically significant only for As, while the decrease was only for K, Mg, Na, Rb, and S. Thus, for the age group of 41-60 years, the mean K, Mg, Na, Rb, and S. mass fractions were 32%, 36%, 51%, 63%, and 43%, respectively, lower than for the age group 16-40 years (Table 6). In contrast to this, the As mass fraction for the age group of 41-60 years was two times higher ($p<0.01$) than for the age group of 16-40 years. The Pearson correlation coefficients between age and ChE mass fractions confirmed these findings (Table 7).

The female breast is made up of mammary glands (glandular tissue) as well as stroma (adipose tissue and ligaments, surrounding ducts and lobules, blood and lymph vessels) [2]. Previously it was shown that the concentration of many ChE in adipose tissue is significantly lower than in epithelial tissue [61,62]. It is known that morphological changes, occur in the mammary gland with age, expressed in the loss of both epithelial and adipose tissue, but the relative rates of mass loss of these components have not been measured. If the rate of loss of the relative mass of epithelial tissue is higher than that of adipose tissue, this may be one of the reasons for the decline of some ChE in the mammary gland with age increase. Of particular interest is age-related increase of As mass fraction found in the breast tissue in the present study. This phenomenon can also be associated with age-related changes in the ratio of glandular and adipose tissue, if As predominantly accumulates in adipose tissue. However, all these issues require furthermore detailed study.

5. Conclusion

The combination of ICP-AES and ICP-MS methods allowed obtaining reliable quantitative data on the Al, As, B, Ba, Bi, Ca, Cd, Ce, Cr, Cu, Fe, K, La, Li, Mg, Mn, Na, Nb, Ni, P, Pb, Rb, S, Sb, Si, Sn, Sr, Ti, W, and Zn mass fractions in breast tissue samples. An important advantage of the method employed is the possibility of determining the ChE mass fractions in samples weighing only a few milligrams, which makes it possible to use materials from puncture tissue biopsies for analysis.

Using the parametric Student's t-test and the non-parametric Wilcoxon-Mann-Whitney U-test to compare two age groups, as well as Pearson's correlation coefficients between age and ChE mass fractions, it was found that the K, Mg, Na, Rb, and S mass fractions in normal breast tissue decrease with age, while the As mass fraction increases. The phenomenon of the age-related changes of these ChE mass fractions in the normal mammary gland, discovered for the first time, requires further detailed study.

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