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Age dependence of chemical element contents in normal human breast investigated using inductively coupled plasma atomic emission spectrometry

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ABSTRACT

Introduction: Breast cancer in women is an actual global medical and social problem. The etiology of this disease remains largely unclear. However, it is well known that the incidence of breast cancer increases with age. In the presented work, for the first time, the age dependence of Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr, and Zn content in the mammary gland of women aged 16-60 years was investigated.
Material and methods: For this purpose, a method of inductively coupled plasma atomic emission spectrometry (ICP-AES) was developed, which makes it possible to determine the content of these elements in microsamples (mass from 10 mg) of breast tissue. With the help of the developed technique, the material obtained during the autopsy of 38 practically healthy women aged 16-60 years who died suddenly was studied.
Results: Using the parametric Student's t-test and the non-parametric Wilcoxon-Mann-Whitney U-test to compare two age groups (16-40 years and 41-60 years), as well as Pearson's correlation coefficients between age and chemical element content, it was found that the level of K, Mg, Na and S in normal breast tissue decrease with age.
Conclusions: The phenomenon of the age-related decrease in the chemical element contents in the normal mammary gland, discovered for the first time, requires further detailed study.

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Estudio mediante espectrometría de emisión atómica de plasma acoplado inductivamente de la dependencia de la edad en el contenido de elementos químicos en las mamas humanas normales

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RESUMEN

Introducción: El cáncer de mama en la mujer es un problema médico y social actual a nivel mundial. La etiología de esta enfermedad sigue sin estar clara. Sin embargo, es bien sabido que la incidencia del cáncer de mama aumenta con la edad. En el trabajo presentado se analiza por primera vez la dependencia de la edad del contenido de Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr y Zn en la glándula mamaria de mujeres de 16 a 60 años. fue investigado.
Material y métodos: Para ello se desarrolló un método de espectrometría de emisión atómica con plasma acoplado inductivamente (ICP-AES), que permite determinar el contenido de estos elementos en micromuestras (masa a partir de 10 mg) de tejido mamario. Con la ayuda de la técnica desarrollada se estudió el material obtenido durante la autopsia de 38 mujeres prácticamente sanas de entre 16 y 60 años que murieron repentinamente.
Resultados: Utilizando la prueba t de Student paramétrica y la prueba U no paramétrica de Wilcoxon-Mann-Whitney para comparar dos grupos de edad (16-40 años y 41-60 años), así como los coeficientes de correlación de Pearson entre edad y elemento químico. contenido, se encontró que el nivel de K, Mg, Na y S en el tejido mamario normal disminuye con la edad.
Conclusiones: El fenómeno de la disminución del contenido de elementos químicos en la glándula mamaria normal relacionado con la edad, descubierto por primera vez, requiere un estudio más detallado.

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1. INTRODUCTION

Cancer is a disease that continues to increasingly aggressively persecute all socioeconomic strata of our modern society [1]. Among all types of cancer, breast cancer (BC) is the most common global malignant neoplasm in women and the leading cause of death among women aged 35–54 [2]. The high morbidity and mortality from BC among able-bodied women makes the problem of diagnosing and treating this disease not only an urgent medical, but also a social task [3]. Despite numerous studies, the etiology of BC remains largely unclear, although many candidates have been found that increase the risk of this disease and, first of all, such as individual genetic characteristics, age, and adverse environmental factors [4]. Since the change in the human gene pool is rather slow, it can be assumed that the alarmingly rapid increase in the incidence of breast cancer is associated primarily with the rate of age-related changes in the body and the transformations taking place in the environment. The age-related incidence of breast cancer in women in Europe and North America shows a continuous growth with a maximum at the age of 40-60 years [5].
The steady development of industry, industrial chemistry and technology in agriculture, food production, pharmaceuticals, medicine, cosmetics, especially over the past 100 years, has led to global changes in the quality of the human environment [6]. These changes also concern the amount of chemical elements (ChE) entering the human body. The ability of the mammary gland during lactation to accumulate significant amounts of ChE for milk production [7] indicates a special elemental composition of this tissue during the dormant period of the mammary gland as well.
Our previous studies have shown that ChE homeostasis plays an important role in the normal and pathophysiology of human bones, thyroid and prostate glands. Moreover, it was found that content of many TE of bones, thyroid and prostate glands depend on age [6, 8-19]. From this can be assumed that the specific physiological factors of the human mammary gland probably play 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. Despite the understanding of the important role of ChE, surprisingly little is known about the involvement of ChE in the normal and pathological physiology of the

human breast.
There are few studies of ChE content in the mammary gland of women by chemical and instrumental methods [20-32]. However, the published data completely lacks information on age-related changes in ChE content in breast tissue.
The main objective of this study was to determine the reliable values of the content of aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulfur (S), silicon (Si), strontium (Sr), and zinc (Zn) in the mammary gland in two age groups (16-40 years and 41-60 years) of healthy women using atomic emission spectrometry with inductively coupled plasma (ICP-AES). The second goal was to evaluate the quality of the results obtained. The third task was to compare the mean mass fractions of Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr and Zn in normal breast tissue obtained during the study with reported data. The final aim was to find differences between mean ChE values obtained for normal breast tissue in two age groups and to evaluate correlations between ChE levels and age.

2. MATERIAL AND METHODS

2.1. SAMPLES

A randomized sample of normal breast tissue was obtained from autopsies of 38 women (age 16 to 60 years, Caucasian race, Caucasian lifestyle) who died suddenly. Autopsies were carried out in the forensic medical examination department of the Obninsk city hospital during the first day after sudden death. Typical causes of death for most women were car accidents and injuries. All of the dead were residents of Obninsk, a small town (about 120,000 inhabitants) in a non-industrial area 105 kilometers southwest of Moscow.
The Ethical Committees of the Medical Radiological Research Centre, Obninsk, approved all studies. 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.
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. Available clinical data were reviewed for each subject. None of the subjects had a history of an intersex condition, endocrine disorder, neoplasm, or other chronic disease that would interfere with normal breast development. None of the subjects received drugs that affect the morphology of the mammary gland and the content of ChE 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 [33]. To reduce the amount of acid used for the sample decomposition an enclose consisting of three mini vessel 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 the vessels to 30^0C the contents of the mini-vessels were quantitatively transferred into 10 ml test tubes and the solutions were adjusted to 10 ml with 2% HNO_3 solution. For measurements, the resulting solutions were additionally diluted in two times with a 2% nitric acid solution.

2.3. 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.

Table 1: ICP-AES data (Mean±SD) of Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr, 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)

Element	MODAS-5		MODAS-3		IAEA-153	
	Certificate	Our result	Certificate	Our result	Certificate	Our result
Al	-	6±1	-	14±1	-	-
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
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
Zn	20.1±1.1	22±2	111±6	111±3	39.6±1.8	38±2

ICP-AES: Inductively coupled plasma atomic emission spectrometry; SD: standard deviation; Al: aluminium; Ca: calcium; Cu: copper; Fe: iron; K: potassium; Mg: magnesium; Mn: manganese; Na: sodium; P: phosphorus; S: sulphur; Si: silicon; Sr: strontium; Zn: zinc.

2.4. STANDARD SOLUTIONS AND INTERNATIONAL REFERENCE MATERIALS

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.
To check the reliability of the results obtained, 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.

2.5. STATISTICAL ANALYSIS

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. 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 construction of "age – ChE mass fraction" diagrams and the estimation of the Pearson correlation coefficient between age and ChE mass fraction the Microsoft Office Excel programs were also used.

3. RESULTS

Table 1 depicts the mass fractions of Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr 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 2 presents the main statistical parameters (arithmetic mean, standard deviation, standard error of the mean, minimum and maximum values, median, percentiles with levels of 0.025 and 0.975) of mass fractions of Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr and Zn in normal breast tissue of healthy women aged 16-40 years and 41-60 years.
Comparison of our results with literature data for the mass fractions of Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr, and Zn in normal breast tissue of adult women is shown in Table 3.

Differences between the mean values of mass fractions of Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr 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 4.

Table 5 shows values of the Pearson correlation coefficient between age and ChE mass fraction

Figure 1 shows individual data sets for the K, Mg, Na, and S mass fraction values in normal breast tissue of healthy females aged from 16 to 60 years.

Table 2: Basic statistical parameters of determined elements mass fraction in the normal breast tissue of two age groups of females aged 16–60 years (mg kg-1, dry tissue)

Group 1 (16-40 years) (n=22)							
Element	**Mean±SD**	**SEM**	**Min**	**Max**	**Med.**	**P0.025**	**P0.975**
Al	4.13±2.88	0.66	1.26	11.1	2.80	1.26	10.3
Ca	85.2±58.6	13.1	15.0	214	76.8	18.6	206
Cu	1.05±0.60	0.14	0.25	2.60	0.87	0.255	2.29
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
Mg	22.1±9.7	2.3	8.10	48.0	19.6	8.91	41.6
Na	882±549	129	156	1827	749	164	1782
P	219±77	18	120	371	204	123	361
S	475±247	58	148	940	491	151	919
Si	9.36±7.47	1.71	2.00	32.1	6.20	2.54	26.3
Sr	0.51±0.27	0.07	0.20	1.06	0,46	0.21	1.02
Zn	3.65±1.99	0.48	1.30	9.60	3.30	1.42	8.32
Group 2 (41-60 years) (n=16)							
Element	**Mean±SD**	**SEM**	**Min**	**Max**	**Med.**	**P0.025**	**P0.975**
Al	2.94±1.49	0.40	1.21	5.56	2.55	1.31	5.55
Ca	67.0±66.7	17.8	11.7	265	49.9	12.9	224
Cu	0.99±1.43	0.36	0.24	5.90	0.66	0.293	4.34
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
Mg	13.9±5.3	1.4	7.66	25.4	12.0	8.42	24.8
Na	434±346	92	140	1325	349	142	1186
P	178±65	17	102	331	166	108	306
S	270±123	33	145	517	225	154	515
Si	7.91±4.08	1.09	2.50	17.1	7.56	2.77	15.2
Sr	0.48±0.19	0.05	0.19	0.75	0.49	0.197	0.738
Zn	2.82±0.91	0.25	1.80	4.30	2.50	1.83	4.24

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; Al: aluminium; Ca: calcium; Cu: copper; Fe: iron; K: potassium; Mg: magnesium; Mn: manganese; Na: sodium; P: phosphorus; S: sulphur; Si: silicon; Sr: strontium; Zn: zinc.

4. DISCUSSION

The developed ICP-AES method makes it possible to determine the content in breast tissues of the following elements: Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr, Zn. Acceptable agreement between the values of the found content of these ChE in the international certified reference materials MODAS-5 (Cod Tissue), MODAS-3 (Herring Tissue) and IAEA-153 (Powdered milk) with the data of the corresponding certificates (Table 1) proves the sufficient accuracy of analysis results accumulated in Tables 2-5.

The content of ChE mentioned above was determined in all or in most of the samples. The mean value of the mass fraction (M), standard deviation (SD), standard error of the mean (SEM), minimum (Min), maximum (Max), median (Med), and percentiles with levels of 0.025 (P 0.025) and 0.975 (P 0.975) was calculated for two age groups (Table 2). 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 the results of determining the content of ChE 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 content in breast tissue.

Table 3: Median, minimum and maximum value of means of chemical element mass fractions (mg kg-1, dry tissue) in normal breast tissue of healthy females according to data from the literature in comparison with this work results

Element	This work results	Published data [reference]		
	Mean±SD n=38	Median of means (*n*)*	Minimum of means M or M±SD, (*n*)**	Maximum of means M or M±SD, (*n*)**
Al	3.62±2.44	6.7 (4)	0.103 (52) [20]	38.4 (20) [21]
Ca	77.7±61.8	262 (7)	52,6±10,6 (-) [22]	680 (2) [23]
Cu	1.03±1.01	2.56 (18)	0.48±0.30 (20) [24]	2280±140 (-) [25]
Fe	13.8±12.3	21.8 (14)	5.1 (46) [26]	75.6 (20) [27]
K	194±114	676 (7)	272 (20) [21]	4600 (-) [28]
Mg	18.5±9.0	85.5 (4)	9.9±1,8 (-) [22]	680 (4) [23]
Na	686±516	2000 (7)	392±56 (-) [22]	5380 (3) [23]
P	201±74	2000 (8)	280 (-) [29]	56000±5460 (-) [25]
S	385±224	4000 (6)	2000 (-) [26]	7600 (-) [28]
Si	8.75±6.22	0.235 (5)	0.00024±0,00003 (-) [30]	0.24±0,39 (16) [31]
Sr	0.50±0.24	0.45 (3)	0.12 (-) [29]	1.4±0.4 (-) [22]
Zn	3.29±1.65	8.3 (17)	2.88 (46) [26]	27.8±5.0 (20) [32]

(n): number of all references; (n)**: number of samples; SD: standard deviation; SEM: standard error of mean; Min: minimum value; Al: aluminium; Ca: calcium; Cu: copper; Fe: iron; K: potassium; Mg: magnesium; Mn: manganese; Na: sodium; P: phosphorus; S: sulphur; Si: silicon; Sr: strontium; Zn: zinc.*

Most often, in studies of 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 [34, 35]. 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 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 (Table 3). The only exceptions are P and S, the content of which is approximately one mathematical order lower than the median of the published data, as well as Si, the average content of which is more than 36 times higher than the median of the previous reports. At the same time, our mean values of the content of P, S, and Si do not even fit into the range of data available in the literature. 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.

The obtained data of the studied ChE content in healthy breast tissue were compared with the reference values of the content of these ChE in blood serum [36, 37], adipose tissue [2, 38], prostate [39] and thyroid [40, 41] human gland. The comparison showed that in terms of the content of Ca, Cu, K, Mg, P, Si and Zn, breast tissue almost does not differ from blood serum, while the content of Al. Fe, and Sr are significantly higher, and, vice versa, the contents of Na and S are noticeably lower. Thus, the ability of breast tissue to absorb trace elements such as Al, Fe, and Sr from the interstitial fluid seems to be quite real. Almost a mathematical order of magnitude lower level of Na compared to serum is due to the fact, that this electrolyte is mainly contained in the extracellular space.

Breast tissue consists of a glandular component and stroma (adipose tissue and ligaments, surrounding ducts and lobules, blood and lymph vessels [2]. On average the ratio by mass of the glandular component and adipose tissue together with the stroma is approximately 1:1 [42]. From a comparison of the data obtained for the mammary gland with adipose tissue, it follows that ChE such as Al, Cu and Sr accumulate mainly in the glandular tissue of the mammary.

Table 4: Differences between mean values (M±SEM) of determined elements mass fraction (mg kg-1, dry tissue) in normal female breast tissue of two age groups (AG)

Element	Female breast tissue				Ratio
	AG1 (16-40 years) n=22	AG2 (41-60 years) n=16	t-test *p*<	U-test *p*	AG2 to AG1
Al	4.13±0.66	2.94±0.40	0.133	>0.05	0.71
Ca	85.2±13.1	67.0±17.8	0.418	>0.05	0.79
Cu	1.05±0.14	0.99±0.36	0.888	>0.05	0.94
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
Mg	22.1±2.3	13.9±1.4	0.005*	□0.01*	0.63
Na	882±129	434±92	0.009*	□0.01*	0.49
P	219±18	178±17	0.114	>0.05	0.81
S	475±58	270±33	0.005*	□0.01*	0.57
Si	9.36±1.71	7.91±1.09	0.481	>0.05	0.85
Sr	0.51±0.07	0.48±0.05	0.745	>0.05	0.94
Zn	3.65±0.48	2.82±0.25	0.140	>0.05	0.77

*SEM: standard error of mean; Min: minimum value; AG1: age groups 1; AG2: age groups 2; Al: aluminium; Ca: calcium; Cu: copper; Fe: iron; K: potassium; Mg: magnesium; Mn: manganese; Na: sodium; P: phosphorus; S: sulphur; Si: silicon; Sr: strontium; Zn: zinc; *: significant values.*

Comparison of the content of Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr and Zn in the mammary gland with the content of these ChE in the prostate and thyroid gland showed that the mass fractions of all studied ChE in the mammary gland are significantly lower than in other glands. This is because approximately half of the breast tissue consists of adipose tissue, in which the ChE content is significantly lower than in glandular tissues.

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 4). In normal mammary glands, we observed a decrease with age in the mass fractions of almost all studied ChE with the exception of Cu and Sr. However, a statistically significant decrease was found only for four elements K, Mg, Na, and S. Thus, in the age group of 41-60 years, the mean value of the contents of these elements were 32%, 37%, 51%, and 43%, respectively, lower than in the age group 16-40 years (Table 4). The distribution of individual data sets for the K, Mg, Na и S mass fractions (Figure 1) and the Pearson correlation coefficients between age and ChE mass fractions (Table 5) confirmed these findings.

Table 5: Correlations between age and determined elements mass fractions in the in normal female breast tissue (r – coefficient of correlation)

Ratio	Element											
	Al	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Na**	**P**	**S**	**Si**	**Sr**	**Zn**
r	-0.21	0.01	-0.04	-0.05	-0.36[a]	-0.46[b]	-0.45[b]	-0.2	-0.42[a]	-0.1	0.1	-0.2

*Al: aluminium; Ca: calcium; Cu: copper; Fe: iron; K: potassium; Mg: magnesium; Mn: manganese; Na: sodium; P: phosphorus; S: sulphur; Si: silicon; Sr: strontium; Zn: zinc; *: significant values.*
[a] $p<0.05$; [b] $p<0.01$.

As mentioned above, the female breast is made up of glandular tissue as well as stroma [2]. Previously, it was shown that the content of many ChE in adipose tissue is significantly lower than in epithelial tissue [43]. 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 [44, 45]. 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 with age of some ChE in the mammary gland.

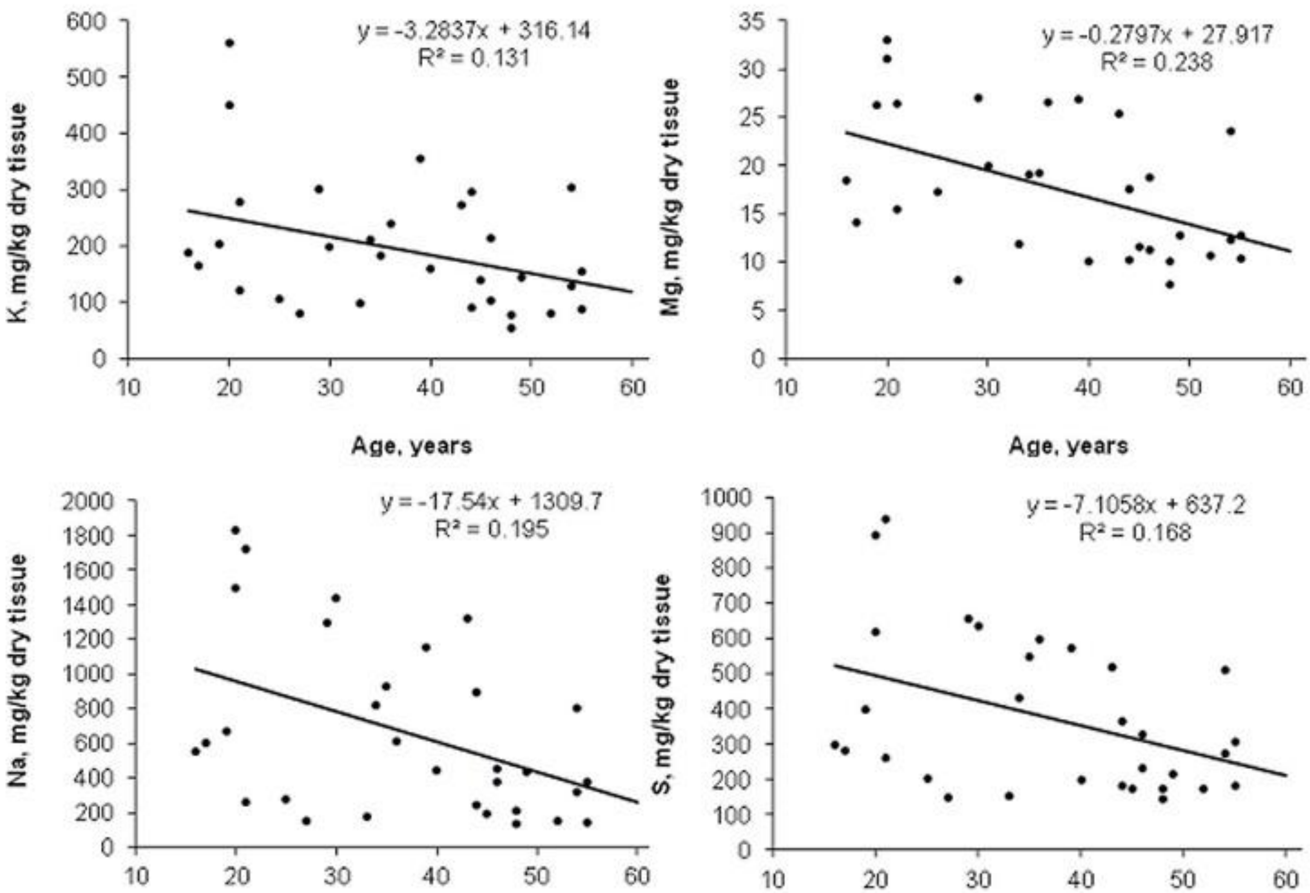

Figure 1: *Data sets of individual potassium (K), magnesium (Mg), sodium (Na), and sulfur (S) mass fraction values and their trend lines in normal breast tissue of healthy females aged from 16 to 60 years.*

This study has several limitations. Firstly, analytical techniques employed in this study measure only twelve ChE (Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr and Zn) mass fractions. Future studies should be directed toward using other analytical methods, which will extend the list of ChE investigated in normal female breast. Moreover, the determination of some elements by different methods with good convergence of the results will strengthen the confidence in the correctness of the results obtained. Secondly, the sample size (n=38) was relatively small and age range (16-60 years) did not include older women. Thirdly, the developed analysis technique was aimed at studying small mass samples that can be obtained by low-traumatic puncture biopsy. This was done with the expectation that the content of some ChE could be a useful biomarker for assessing the pathological conditions of the mammary gland. If these are found, the question arises as to the representativeness of the individual result obtained from the study of such a small tissue sample. Finally, the generalization of our results may be limited to the population of Russia. Despite these limitations, this study demonstrates age-related changes in ChE content in normal breast tissue and indicates the need for further detailed studies of ChE of the mammary gland.

5. CONCLUSIONS

The developed ICP-AES method allows obtaining reliable data on the content of Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr, and Zn in breast tissue samples. An important advantage of the developed technique is the possibility of determining the content of ChE 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 content, it was found that the content of K, Mg, Na, and S in normal breast tissue decrease with age. The results obtained may be useful for a more complete understanding of age-related changes in the physiology and biochemistry of the mammary gland, and probably also for the diagnosis of pathological conditions of this organ.

The phenomenon of the age-related decrease of ChE contents in the normal mammary gland, discovered for the first time, requires further detailed study.

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7. CONFLICT OF INTERESTS

The authors have no conflict of interest to declare. The authors declared that this study has received no financial support.

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