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Trace Element Levels in Prostate Gland as Carcinoma's Markers

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Abstract

Objectives: The aim of this study was to evaluate the changes in the prostatic levels of trace elements in the malignant human prostate. **Methods**: Contents of 43 trace elements in normal (N, n = 37), benign hypertrophic (BPH, n = 32) and cancerous (PCa, n = 60) prostate were investigated. Measurements were performed using instrumental neutron activation analysis and inductively coupled plasma mass spectrometry. **Results**: The mass fractions of all trace elements with the exception of La, Nb, and Yb show significant variations in cancerous prostate when compared with normal and BPH prostate. The contents of Co, Hg, Rb, Sc, Se, and Zn were significantly lower and those of Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Cs, Dy, Er, Gd, Ho, Li, Mn, Mo, Nd, Ni, Pb, Pr, Sb, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, and Zr were significantly higher in PCa than in BPH tissues. When trace elements of cancerous prostate were compared with those in normal prostatic parenchyma, contents of Cd, Rb, Sc, Se, and Zn were significantly lower and Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Dy, Er, Fe, Gd, Hg, Ho, Li, Mn, Nd, Ni, Pr, Sb, Sm, Sn, Tb, Th, Tl, Y, and Zr were significantly higher. **Conclusion**: The Ag, Al, B, Br, Li, Mn, Ni, and Zn mass fraction in a needle-biopsy core can be used as the informative indicators for distinguishing malignant from benign prostate. Sensitivity, specificity, and accuracy of these tests were in range 72% - 100%, 66% - 100%, and 74% - 98%, respectively.

Keywords

Trace Elements, Prostate, Benign Prostatic Hypertrophy, Prostatic Carcinoma, Neutron Activation Analysis, Inductively Coupled Plasma Mass Spectrometry

1. Introduction

The prostate gland may be a source of many health problems in men past middle age, such as the most common benign prostatic hyperplasia (BPH), and prostatic carcinoma (PCa). BPH is a noncancerous enlargement of the prostate gland leading to obstruction of the urethra and can significantly impair quality of life. The prevalence of histological BPH is found in approximately 50% - 60% of males age 40 - 50, in over 70% at 60 years old and in greater than 90% of men over 70 [1] [2]. In many Western industrialized countries, including North America, PCa is the most frequently diagnosed form of noncutaneous malignancy in males. Except for lung cancer, PCa is the leading cause of death from cancer [3]-[8]. Although the etiology of BPH and PCa is unknown, some trace elements have been highlighted in the literature in relation to the development of these prostate diseases [9]-[29].

Trace elements have essential physiological functions such as maintenance and regulation of cell function and signalling, gene regulation, activation or inhibition of enzymatic reactions, neurotransmission, and regulation of membrane function. Essential or toxic (mutagenic, carcinogenic) properties of trace elements depend on tissue-specific need or tolerance, respectively [30]. Excessive accumulation, deficiency or an imbalance of the trace elements may disturb the cell functions and may result in cellular degeneration, death and malignant transformation [30].

In earlier reported studies [31]-[64] significant changes of some trace element contents in hyperplastic and cancerous prostate in comparison with those in the normal prostatic tissue were observed. Moreover, a significant informative value of Zn content as a tumor marker for PCa diagnostics was shown by us [65] [66]. Hence some other trace elements besides Zn probably can be used as tumor markers for distinguishing between benign and malignant prostate.

Currently a number of methods were applied for the measurement of chemical elements contents in samples of human tissue. Among these methods, the instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) is non-destructive and one of the most sensitive techniques. It allows to measure the trace element contents in few milligrams tissue without any treatment of sample. Analytical studies of the Ag, Co, Cr, Fe, Hg, Sb, Sc, Se, and Zn contents in normal, BPH and PCa tissue were done by us using INAA-LLR [15] [20] [28] [60] [62]. Nondestructive method of analysis avoids the possibility of changing the content of trace elements in the studied samples [67] [68] [69] [70], which allows for the first time to obtain reliable results. In particular, it was shown that the average mass fraction of Co, Cr, Hg, Sb, and Se in BPH was higher than normal levels [66]. In PCa tissues the mean values of Ag, Cr, Fe, Hg, and Sb were higher while those of Co, Rb, Sc, and Zn were lower than in healthy prostatic tissue [60] [66]. Obtained results formed the basis for a new method for differential diagnosis of BPH and PCa, the essence of which was to determine the content of trace elements in the material of needle biopsy of prostate indurated site.

It is obvious that the most effective methods will be non-destructive analytical methods because they involve a minimal treatment of sample since the chances of significant loss or contamination would be decreased. However, the INAA-LLR allow only to determine the mean mass fractions of 9 - 10 trace elements in the samples of normal and cancerous prostate glands [15] [20] [28] [60] [66]. The inductively coupled plasma mass spectrometry (ICP-MS) is a more power analytical tool than INAA-LLR [18] but sample digestion is a critical step in elemental analysis by this method. In the present study both analytical methods were used and the results obtained for some trace elements by ICP-MS were under the control of INAA-LLR data.

The present study had three aims. The main objective was to obtain reliable results about the 43 trace elements: Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr contents in intact prostate of healthy men and in the prostate gland of BPH and PCa patients combining in consecutive order non-destructive INAA-LLR with destructive ICP-MS. The second aim was to compare the levels of trace elements in normal, hyperplastic, and cancerous prostate, and the third aim was to evaluate the trace element contents for diagnosis of prostate cancer.

All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

2. Materials and Methods

2.1. Materials

The patients studied (n = 92) were hospitalized in the Urological Department of the Medical Radiological Research Centre (Obninsk, Russia). All of them were European-Caucasian, citizens of Moscow and Obninsk (a small city in a non-industrial region 105 km south-west of Moscow). Transrectal puncture biopsy of suspicious indurated regions of the prostate was performed for every patient, to permit morphological study of prostatic tissue at these sites and to estimate their chemical element contents. For all patients the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. The age of 32 patients with BPH ranged from 56 to 78 years, the mean being 66 ± 6 (M ± SD) years. The 60 patients aged 40 - 79 suffered from PCa (stage T1 - T4). Their mean age was 65 ± 10 (M ± SD) years. There were no any inclusion and exclusion criteria of patients. Samples of prostate tissue were obtained from all patients with BPH and PCa, who were in the hospital of Medical Radiological Research Center in period of this study.

Intact prostates (N) were removed at necropsy from 37 men aged 41 - 87 who had died suddenly. All deceased were European-Caucasian, citizens of Moscow. Their mean age was 55 ± 11 (M ± SD) years. The majority of deaths were due to trauma. Tissue samples were collected from the peripheral zone of dorsal and lateral lobes of their prostates, within 2 days of death and then the samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis. A histological examination was used to control the age norm conformity, as well as to confirm the absence of microadenomatosis and latent cancer [15] [20] [28].

All tissue samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis. After the samples intended for trace element analysis were weighed, they were freeze-dried and homogenized. The sample weighing about 10 mg (for biopsy materials) and 50 - 100 mg (for resected materials) was used for trace element measurement by INAA-LLR. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with double rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

After NAA-LLR investigation the prostate samples were taken out and used for ICP-MS. The samples were decomposed in autoclaves; 1.5 mL of concentrated HNO_3 (nitric acid at 65%, maximum (max) of 0.0000005% Hg; GR, ISO, Merck) and 0.3 mL of H_2O_2 (pure for analysis) were added to prostate tissue samples, placed in one-chamber autoclaves (Ancon-AT2, Ltd., Russia) and then heated for 3 h at 160˚C - 200˚C. After autoclaving, they were cooled to room temperature and solutions from the decomposed samples were diluted with deionized water (up to 20 mL) and transferred to plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without tissue samples (only HNO_3 + H_2O_2 + deionized water), and the resultant solutions were used as control samples.

2.2. Methods

A vertical channel of a nuclear reactor was applied to determine the trace element mass fractions by NAA-LLR. The quartz ampoule with prostate samples and certified reference materials was soldered, positioned in a transport aluminum container and exposed to a 24-hour neutron irradiation in a vertical channel with a neutron flux of $1.3 \cdot 10^{13}$ $n \cdot cm^{-2} \cdot s^{-1}$. Ten days after irradiation samples were reweighed and repacked. The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 min to 10 hours subject to pulse counting rate. The gamma spectrometer included the 100 cm^3 Ge (Li) detector and on-line computer-based multichannel analyzer. The spectrometer provided a resolution of 1.9 keV on the ^{60}Co 1332 keV line.

An ICP-MS Thermo-Fisher "X-7" Spectrometer (Thermo Electron, USA) was used to determine the content of trace elements by ICP-MS. The element concentrations in aqueous solutions were determined by the quantitative method using multi elemental calibration solutions ICP-MS-68A and ICP-AM-6-A produced by High-Purity Standards (Charleston, SC 29423, USA). Indium was used as an internal standard in all measurements.

Information detailing with the NAA-LLR and ICP-MS methods used and other details of the analysis was presented in our previous publication [15] [20] [28] [60] [66].

For quality control, ten subsamples of the certified reference materials IAEA H-4 Animal muscle from the International Atomic Energy Agency (IAEA), as well as five sub-samples INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2 Mixed Polish Herbs from the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) were analyzed simultaneously with the investigated prostate tissue samples. All samples of CRM were treated in the same way as the prostate samples. Detailed results of this quality assurance program were presented in earlier publications [15] [18] [22].

A dedicated computer program for INAA mode optimization was used [71]. All prostate samples for INAA-LLR were prepared in duplicate and mean values of chemical element contents were used in final calculation. For elements investigated by INAA-LLR and ICP-MS the mean of all results was used. Using the Microsoft Office Excel software arithmetic mean, standard deviation, and standard error of mean was calculated for trace element mass fraction in normal, benign hyperplastic and cancerous prostate tissue. The difference in the results between BPH and Norm, PCa and Norm, as well as PCA and BPH was evaluated by parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test. Values of $p < 0.05$ were considered to be statistically significant. For the construction of "individual data sets for trace element mass fraction in normal, benign hypertrophic and cancerous prostate" diagrams the Microsoft Office Excel software was also used.

3. Results

Table 1 depicts mean values ± standard error of mean (M ± SEM) of the Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr mass fraction in normal, benign hypertrophic and cancerous prostate.

The ratios of means and the difference between mean values of Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr mass fraction in normal, benign hypertrophic and cancerous prostate are presented in **Table 2**.

Individual data sets for Ag, Al, B, Br, Li, Mn, Ni and Zn mass fraction in all investigated samples of normal, benign hypertrophic and cancerous prostate, respectively, are shown in **Figure 1**.

Table 3 contains parameters of the importance (sensitivity, specificity and accuracy) of Ag, Al, B, Br, Li, Mn, Ni and Zn mass fraction for the diagnosis of PCa calculated in this work.

4. Discussion

As was shown by us [15] [18] [22], the use of CRM IAEA H-4, INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs as certified reference materials for the analysis of samples of prostate tissue can be seen as quite acceptable. Good agreement of the Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr contents analyzed by INAA-LLR and ICP-MS with the certified data of reference materials indicates

Table 1. Comparison of mean values (M ± SEM) of the trace element mass fraction (mg/kg, dry mass basis) in normal (N), benign hypertrophic (BPH) and cancerous prostate (PCa).

Element	Symbol	Prostatic tissue		
		Normal 41 - 87 year (n = 37)	BPH 56 - 78 year (n = 32)	PCa 40 - 79 year (n = 60)
Silver	Ag	0.038 ± 0.006	0.0415 ± 0.0090	0.252 ± 0.030
Aluminum	Al	34.2 ± 3.5	24.4 ± 3.2	328 ± 73
Gold	Au	0.0041 ± 0.0008	0.00257 ± 0.00077	0.0297 ± 0.0056
Boron	B	1.04 ± 0.18	1.51 ± 0.26	12.6 ± 3,7
Berillium	Be	0.00094 ± 0.00007	0.000918 ± 0.000042	0.0137 ± 0.0022
Bismuth	Bi	0.029 ± 0.011	0.140 ± 0.042	1.75 ± 0.27
Bromine	Br	27.9 ± 2.9	30.6 ± 3.4	99.9 ± 8.9
Cadmium	Cd	1.12 ± 0.13	1.07 ± 0.43	0.425 ± 0.099
Cerium	Ce	0.0309 ± 0.0050	0.0128 ± 0.0019	0.101 ± 0.013
Cobalt	Co	0.0467 ± 0.0064	0.0617 ± 0.0084	0.0336 ± 0.0040
Cromium	Cr	0.56 ± 0.08	1.00 ± 0.10	2.34 ± 0.32
Cesium	Cs	0.0339 ± 0.0033	0.0235 ± 0.0025	0.0389 ± 0.0039
Dysprosium	Dy	0.00293 ± 0.00049	0.00156 ± 0.00024	0.00771 ± 0.00110
Erbium	Er	0.00148 ± 0.00023	0.00072 ± 0.00013	0.00297 ± 0.00038
Iron	Fe	111 ± 9	133 ± 11	165 ± 15
Gadolinium	Gd	0.00290 ± 0.00041	0.00153 ± 0.00027	0.00945 ± 0.00173
Mercury	Hg	0.052 ± 0.008	0.259 ± 0.029	0.122 ± 0.019
Holmium	Ho	0.00057 ± 0.00008	0.00032 ± 0.00005	0.00178 ± 0.00022
Lanthanum	La	0.080 ± 0.020	0.0385 ± 0.0073	0.969 ± 0.537
Lithium	Li	0.0419 ± 0.0055	0.0385 ± 0.0073	0.251 ± 0.054
Manganese	Mn	1.34 ± 0.08	1.19 ± 0.09	6.99 ± 1.35
Molybdenum	Mo	0.282 ± 0.038	0.167 ± 0.009	0.298 ± 0.035
Niobium	Nb	0.0054 ± 0.0012	0.0102 ± 0.0079	0.0052 ± 0.0002
Neodymium	Nd	0.0137 ± 0.0021	0.0062 ± 0.0009	0.0413 ± 0.0065
Nickel	Ni	3.10 ± 0.51	3.22 ± 1.06	6.96 ± 1.04
Lead	Pb	2.39 ± 0.56	0.69 ± 0.16	1.81 ± 0.35
Praseodymium	Pr	0.00353 ± 0.00053	0.00149 ± 0.00027	0.00973 ± 0.00174
Rubidium	Rb	13.3 ± 0.9	14.3 ± 0.8	8.71 ± 0.66
Antimony	Sb	0.043 ± 0.006	0.163 ± 0.036	0.490 ± 0.059
Scandium	Sc	0.0294 ± 0.0053	0.0257 ± 0.0040	0.0116 ± 0.0015
Selenium	Se	0.75 ± 0.05	1.11 ± 0.07	0.56 ± 0.08
Samarium	Sm	0.0027 ± 0.0004	0.0014 ± 0.0004	0.0095 ± 0.0029
Tin	Sn	0.32 ± 0.06	0.108 ± 0.029	1.28 ± 0.24
Terbium	Tb	0.00039 ± 0.00006	0.00017 ± 0.00003	0.00089 ± 0.00012
Thorium	Th	0.0033 ± 0.0007	0.0018 ± 0.0003	0.0495 ± 0.0123
Titanium*	Ti*	2.82 ± 0.64	1.52 ± 0.20	8.60 ± 2.20
Thallium	Tl	0.0014 ± 0.0001	0.00202 ± 0.00057	0.0219 ± 0.0056
Thulium	Tm	0.00024 ± 0.00003	0.000151 ± 0.000021	0.000535 ± 0.000111
Uranium	U	0.0070 ± 0.0021	0.0021 ± 0.0009	0.0068 ± 0.0013
Yttrium	Y	0.0187 ± 0.0043	0.0071 ± 0.0012	0.0340 ± 0.0038
Ytterbium	Yb	0.00141 ± 0.00025	0.00083 ± 0.00020	0.00174 ± 0.00039
Zinc	Zn	1031 ± 129	1271 ± 102	136 ± 10
Zirconium	Zr	0.036 ± 0.006	0.091 ± 0.036	2.13 ± 0.89

M—arithmetic mean, SEM—standard error of mean, *Titanium tools were used for sampling and sample preparation.

Table 2. Ratio of means and the difference between mean values of trace element mass fractions in normal (N), benign hypertrophic (BPH) and cancerous prostate (PCa).

	BPH and Normal (N)			PCa and Normal (N)			PCa and BPH		
	Ratio BPH/N	$p \le$ t-test	p U-test	Ratio PCa/N	$p \le$ t-test	p U-test	Ratio PCa/BPH	$p \le$ t-test	p U-test
Ag	1.09	0.74	>0.05	6.63	**0.001**	**<0.01**	6.07	**0.001**	**<0.01**
Al	0.71	0.052	>0.05	9.59	**0.003**	**<0.01**	13.4	**0.002**	**<0.01**
Au	0.63	0.17	>0.05	7.24	**0.001**	**<0.01**	11.6	**0.001**	**<0.01**
B	1.45	0.16	>0.05	12.1	**0.012**	**<0.01**	8.34	**0.015**	**<0.01**
Be	0.98	0.78	>0.05	14.6	**0.001**	**<0.01**	14.9	**0.001**	**<0.01**
Bi	4.83	**0.026**	>0.05	60.3	**0.001**	**<0.01**	12.5	**0.001**	**<0.01**
Br	1.10	0.53	>0.05	3.58	**0.001**	**<0.01**	3.26	**0.001**	**<0.01**
Cd	0.96	0.95	>0.05	0.38	**0.001**	**<0.05**	0.40	0.17	>0.05
Ce	0.41	**0.002**	>0.05	3,27	**0.001**	**<0.01**	7.89	**0.001**	**<0.01**
Co	1.32	0.16	>0.05	0.72	0.087	>0.05	0.54	**0.005**	>0.05
Cr	1.79	**0.002**	**<0.01**	4.18	**0.001**	**<0.01**	2.34	**0.001**	**<0.01**
Cs	0.69	**0.017**	>0.05	1.15	0.33	>0.05	1.66	**0.004**	**<0.01**
Dy	0.53	**0.018**	>0.05	2.63	**0.003**	**<0.05**	4.94	**0.001**	**<0.01**
Er	0.49	**0.007**	>0.05	2.01	**0.004**	**<0.05**	4.13	**0.001**	**<0.01**
Fe	1.20	0.13	>0.05	1.49	**0.004**	**<0.05**	1.24	0.10	>0.05
Gd	0.53	**0.008**	>0.05	3.26	**0.004**	**<0.01**	6.18	**0.001**	**<0.01**
Hg	4.98	**0.001**	**<0.01**	2.35	**0.002**	**<0.01**	0.47	**0.004**	**<0.01**
Ho	0.56	**0.012**	>0.05	3.12	**0.001**	**<0.01**	5.56	**0.001**	**<0.01**
La	0.48	**0.005**	>0.05	12.1	0.132	>0.05	25.2	0.11	**<0.01**
Li	0.92	0.71	>0.05	5.99	**0.003**	**<0.01**	6.52	**0.003**	**<0.01**
Mn	0.89	0.25	>0.05	5.22	**0.002**	**<0.01**	5.87	**0.002**	**<0.01**
Mo	0.59	**0.007**	**<0.05**	1.06	0.76	>0.05	1.78	**0.018**	**=0.05**
Nb	1.89	0.56	>0.05	0.96	0.82	>0.05	0.51	0.53	>0.05
Nd	0.45	**0.003**	**<0.01**	3.01	**0.002**	**<0.01**	6.66	**0.001**	**<0.01**
Ni	1.04	0.92	>0.05	2.25	**0.005**	**<0.01**	2.16	**0.020**	**<0.01**
Pb	0.29	**0.007**	>0.05	0.76	0.38	>0.05	2.62	**0.011**	**<0.01**
Pr	0.42	**0.002**	**<0.05**	2.76	**0.005**	**<0.01**	6.53	**0.001**	**<0.01**
Rb	1.08	0.42	>0.05	0.65	**0.001**	**<0.01**	0.61	**0.001**	**<0.01**
Sb	3.79	**0.004**	>0.05	11.4	**0.001**	**<0.01**	3.01	**0.001**	**<0.01**
Sc	0.87	0.58	>0.05	0.39	**0.004**	**<0.01**	0.45	**0.004**	**<0.01**
Se	1.48	**0.001**	**<0.05**	0.75	**0.041**	**<0.05**	0.50	**0.001**	**<0.01**
Sm	0.52	**0.023**	>0.05	3.52	**0.039**	**<0.05**	6.79	**0.019**	**<0.05**
Sn	0.34	**0.005**	**<0.01**	4.00	**0.002**	**<0.01**	11.9	**0.001**	**<0.01**
Tb	0.44	**0.002**	**<0.05**	2.28	**0.008**	**<0.01**	5.24	**0.003**	**<0.01**
Th	0.55	0.066	>0.05	15.0	**0.004**	**<0.01**	27.5	**0.003**	**<0.01**
Ti*	0.53	0.064	>0.05	3.05	0.056	>0.05	5.73	**0.032**	>0.05
Tl	1.44	0.30	>0.05	15.6	**0.004**	**<0.01**	10.8	**0.005**	**<0.01**
Tm	0.63	**0.033**	>0.05	2.23	0.072	**<0.05**	3.54	**0.038**	**<0.01**
U	0.30	**0.041**	>0.05	0.97	0.95	>0.05	3.24	**0.008**	**<0.01**
Y	0.38	**0.014**	>0.05	1.82	**0.012**	**<0.01**	4.79	**0.001**	**<0.01**
Yb	0.59	0.074	>0.05	1.23	0.50	>0.05	2.10	0.080	>0.05
Zn	1.23	0.15	>0.05	0.13	**0.001**	**<0.01**	0.11	**0.001**	**<0.01**
Zr	2.53	0.17	>0.05	59.2	**0.041**	**<0.05**	23.4	**0.045**	**<0.01**

t-test—Student's *t*-test, U-test—Wilcoxon-Mann-Whitney U-test, **Bold** significant differences.

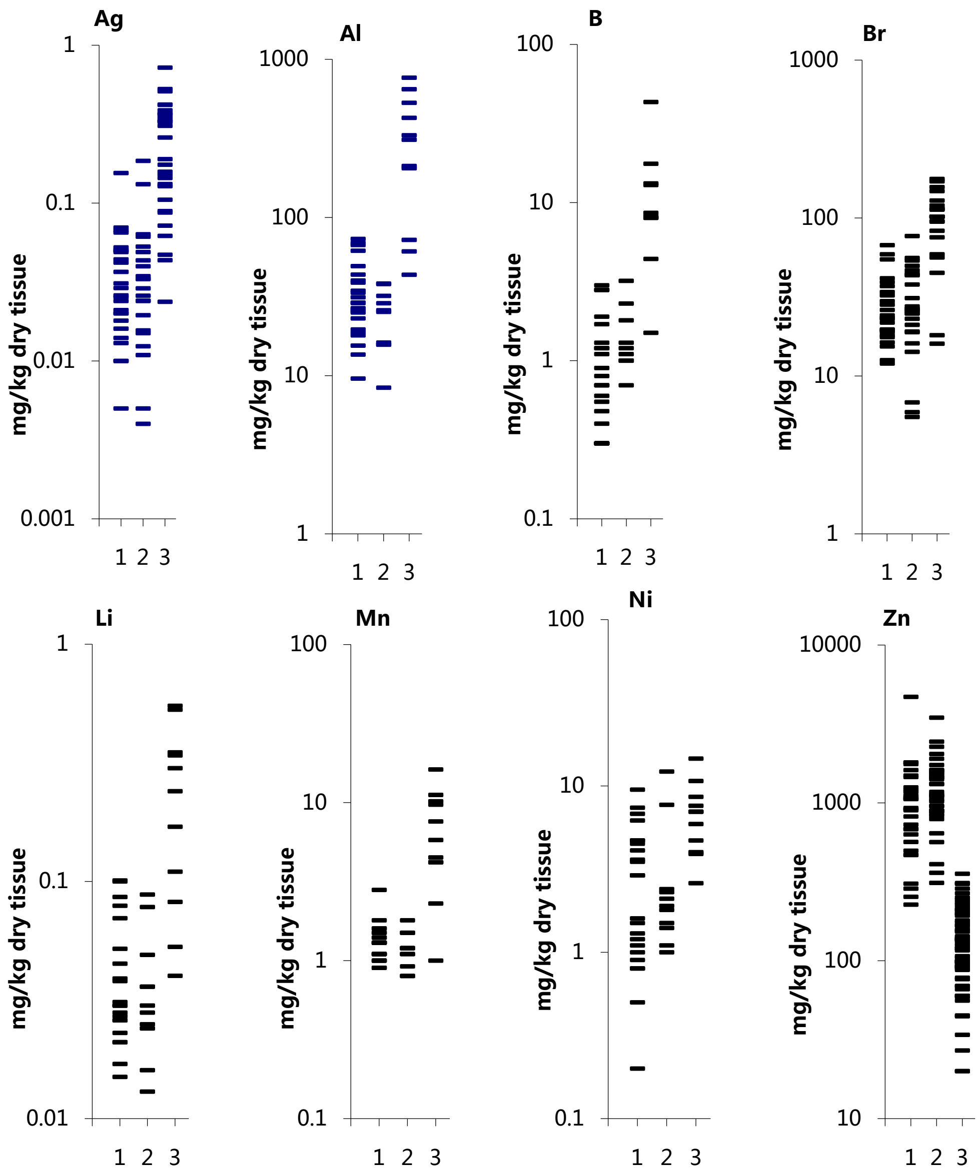

Figure 1. Individual data sets for Ag, Al, B, Br, Li, Mn, Ni and Zn mass fraction in samples of normal (1), benign hypertrophic (2) and cancerous (3) prostate.

Table 3. Parameters (M ± SD) of the importance (sensitivity, specificity and accuracy) of Al, B, Mn, and Zn mass fraction for the diagnosis of PCa (estimation is made for "PCa or normal and BPH prostate").

Mass fraction	Limit for PCa mg/kg dry tissue	Sensitivity	Specificity	Accuracy
Ag	≥0.085	88 ± 6	92 ± 4	92 ± 3
Al	≥100	72 ± 14	100 – 3	94 ± 3
B	≥4.0	90 ± 10	100 – 3	98 ± 2
Br	≥60	83 ± 8	92 ± 4	89 ± 4
Li	≥0.08	82 ± 12	91 ± 5	89 ± 4
Mn	≥2.0	91 ± 9	97 ± 3	96 ± 3
Ni	≥2.5	100 – 9	66 ± 8	74 ± 7
Zn	≤350	99 ± 1	93 ± 3	96 ± 2

M—arithmetic mean, SD—standard deviation.

an acceptable accuracy of the results obtained in the study of trace elements of the prostate samples presented in Table 1 and Table 2.

The mean values and all selected statistical parameters were calculated for 43 trace elements: Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr trace element mass fractions (Table 1). The mass fraction of these trace elements were measured in all, or a major portion of normal prostate samples. The masses of BPH and PCa samples varied very strong from a few milligrams (sample from needle biopsy material) to 100 mg (sample from resected material). Therefore, in BPH and PCa prostates mass fractions of Ag, Br, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn were measured in all, or a major portion of samples, while mass fractions of other trace elements were determined in 22 samples (11 BPH and 11 PCa samples, respectively).

From Table 2, it is observed that in benign hypertrophic tissues the mass fractions of Ag, Al, Au, B, Be, Br, Cd, Co, Fe, Li, Mn, Nb, Ni, Rb, Sc, Th, Ti, Tl, Yb, Zn, and Zr not differ from normal levels, but the mass fractions of Bi, Cr, Hg, Sb, and Se are higher, while the mass fraction of Ce, Cs, Dy, Er, Gd, Ho, La, Mo, Nd, Pb, Pr, Sm, Sn, Tb, Tm, U, and Y are significantly lower. In cancerous tissue the mass fractions of Cd, Rb, Sc, Se, and Zn are significantly lower, and mass fractions of Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Cr, Dy, Er, Fe, Gd, Hg, Ho, Li, Mn, Nd, Ni, Pr, Sb, Sm, Sn, Tb, Th, Tl, Y, and Zr are significantly higher than in normal tissues of the prostate. All these trace elements show similar variations in cancerous tissues when compared with benign hypertrophic tissues of the prostate.

Analysis of the trace element mass fraction in prostate tissue could become a powerful diagnostic tool. To a large extent, the resumption of the search for new methods for early diagnosis of PCa was due to experience gained in a critical assessment of the limited capacity of the prostate specific antigen (PSA) serum test [72]. In addition to the PSA serum test and morphological study of needle-biopsy cores of the prostate, the development of other highly precise testing methods seems to be very useful.

Experimental conditions of the present study were approximated to the hospital conditions as closely as possible. In BPH and PCa cases we analyzed a part of the material obtained from a puncture transrectal biopsy of the indurated site in the prostate. Therefore, our data allow us to evaluate adequately the importance of trace element mass fraction for the diagnosis of PCa. As is evident from Table 2 and, particularly, from individual data sets (Figure 1), the Ag, Al, B, Br, Li, Mn, Ni and Zn mass fraction are potentially the most informative test for a differential diagnosis. For example, if 4.0 mg/kg is the value of B mass fraction assumed to be the lower limit for PCa (Figure 1) and an estimation is made for "PCa or intact and BPH tissue", the following values are obtained:

Sensitivity = {True Positives (TP)/[TP + False Negatives (FN)]}·100% = 90% ± 10%;

Specificity = {True Negatives (TN)/[TN + False Positives (FP)]}·100% = 100% – 3%;

Accuracy = [(TP + TN)/(TP + FP +TN + FN)]·100% = 98% ± 2%.

The number of people (samples) examined was taken into account for calculation of confidence intervals [73]. In other words, if B mass fraction in a prostate biopsy sample is higher than 4 mg/kg, one could diagnose a malignant tumor with an accuracy 98% ± 2%. Thus, using the B mass fraction-test makes it possible to diagnose cancer in 100% − 3%; cases (sensitivity). The same way parameters of the importance (sensitivity, specificity and accuracy) of Ag, Al, B, Br, Li, Mn, Ni and Zn mass fraction for the diagnosis of PCa were calculated (Table 3).

5. Conclusions

The combination of nondestructive INAALSLR and destructive ICP-MS methods is satisfactory analytical tool for the precise determination of 43 trace element mass fractions in the tissue samples of normal, BPH and carcinomatous prostate glands. The sequential application of two methods allowed precise quantitative determinations of mean mass fraction of Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr. The mass fractions of all trace elements investigated in the study with the exception of La, Nb, and Yb show significant variations in cancerous tissues when compared with normal and BPH prostate. The contents of Rb, Sc, Se, and Zn were significantly lower and those of Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Dy, Er, Fe, Gd, Ho, Li, Mn, Nd, Ni, Pr, Sb, Sm, Sn, Tb, Th, Tl, Y, and Zr were significantly higher in cancerous tissues than in normal and BPH tissues. The Ag, Al, B, Br, Li, Mn, Ni and Zn mass fraction in a needle-biopsy core can be used as an accurate tool to diagnose prostate cancer. Further studies on larger number of samples are required to confirm our findings, to study the impact of the trace element contents on prostate cancer etiology.

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