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Ratios of Zn/trace element contentsin prostate gland as carcinoma's markers

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Abstract

The aim of the study was to evaluate whether significantchanges in the prostatic tissue levels of ratios Zn/trace element contents exist in the malignantly transformed prostate. Contents of 43 trace elementsin normal (N, n=37), benign hypertrophic (BPH, n=32) and cancerous human prostate (PCa, n=60) were investigated, and ratios Zn/traceelement contents were calculated.Measurements were performed using a combination of non-destructive and destructive methods: instrumental neutron activation analysis and inductively coupled plasma mass spectrometry, respectively.It was observed that the Zn/Ag, Zn/Al, Zn/Au, Zn/B, Zn/Be, Zn/Bi, Zn/Br, Zn/Cd, Zn/Ce, Zn/Co, Zn/ Cr, Zn/Cs, Zn/Dy, Zn/Er, Zn/Fe, Zn/Gd, Zn/Hg, Zn/Ho, Zn/La, Zn/Li, Zn/Mn, Zn/Mo, Zn/Nb, Zn/ Nd, Zn/Ni, Zn/Pb, Zn/Pr, Zn/Rb, Zn/Sb, Zn/Sc, Zn/Se, Zn/Sm, Zn/Sn, Zn/Tb, Zn/Th, Zn/Ti, Zn/Tl, Zn/Tm, Zn/U, Zn/Y, Zn/Yb, andZn/Zr mass fraction ratiosweresignificantlylowerincanceroustissuesthaninnormalandBPHprostate. Finally, we propose to use the Zn/Ag, Zn/Al, Zn/B, Zn/Be, Zn/Br, Zn/Cr, Zn/Fe, Zn/Mn, Zn/Rb, and Zn/Sb mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer. Further studies on larger number of samples are required to confirm our findings and to investigate the impact of the trace element relationships on prostate cancer etiology.

Introduction

The prostate gland may be a source of many health problems in men past middle age, the most common being 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 and, except for lung cancer, 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 functionand 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 reported studies significant changes of trace element contents in hyperplastic and cancerous prostate in comparison with those in the normal prostatic tissue were observed [31-64]. Moreover, a significant informative value of Zn content as a tumor marker for PCa diagnostics was shown by us [65,66]. Hence itis possible thatbesidesZn,some other trace elements also can be usedas tumor markers for distinguish between benign and malignant prostate.

Current methods applied for measurement of trace elements contents in samples of human tissue include a number of methods. Among thesemethods the instrumental neutron activation analysis with high resolution spectrometry oflong-lived radionuclides (INAA-LLR) is a non-destructive and one of the most sensitive techniques. It allows measure the trace element contents in a 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 [67-70], which allowed 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 were higher than normal levels [66], but in PCa tissues the mean values of Ag, Cr, Fe, Hg, and Sb were higher while those of Co, Rb, Sc, and Znwere 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 ratios of trace element contents changedin opposite directions during malignant transformation of prostate.

It is obvious that the most effective will be non-destructive

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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 determine the mean mass fractions of9-10trace 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 Zrcontents in intact prostate of healthy men aged over 40 years and in the prostate gland of age-matched patients, who had either BPH or PCa combining in consecutive order non-destructive INAA-LLR with destructive ICP-MS. The second aim was to calculate Zn/ traceelement content ratios and compare the levels of these ratiosin normal, hyperplastic, and cancerous prostate. The third and final aim was to evaluate the ratios of Zn/ trace element contents for diagnosis of prostate cancer.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

Material and methods

Samples

The patients studied (n=92) were hospitalized in the Urological Department of the Medical Radiological Research Centre. 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 theprostate was performed for every patient, to permit morphological study of prostatic tissue at these sites and to estimate their chemical element contents. In all cases 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.

Intactprostates (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 5511 (M±SD) years. The majority of deaths were due to trauma. Tissue samples were collected from the peripheral zone of dorsal and lateral lobesof 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].

Sample preparation

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 160–200°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.

Instrumentation and 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].

Certified reference materials

For quality control, ten subsamples of the certified reference materials IAEA H-4 Animal muscle from the International Atomic Energy Agency (IAEA), and also five sub-samples INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2Mixed 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].

Computer programs and statistics

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 Zn/traceelement contents for each trace element in every sample were calculated. Then arithmetic mean, standard deviation, and standard error of mean were calculated for ratios ofZn/traceelement mass fraction in normal, benign hyperplastic and cancerous prostate tissue. The difference in the results between BPH and Norm, PCa and Norm, and PCA and BPH was evaluated by parametric Student's t-test and non-parametricWilcoxon-Mann-Whitney U-test. Values of $p<0.05$ were considered to be statistically significant. For the construction of "individual data sets for Zn /trace element mass fraction ratios in normal, benign hypertrophic and cancerous prostate" diagrams the Microsoft Office Excel software was also used.

Results

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

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

Element	Prostatic tissue		
	Normal 41-87 year (n=37)	**BPH 56-78 year (n=32)**	**PCa 40-79 year (n=60)**
Zn/Ag	37079 ± 5748	53275 ± 10549	1441 ± 364
Zn/Al	39.5 ± 8.3	58.9 ± 9.6	1.34 ± 0.56
Zn/Au	597386 ± 198985	789472 ± 148247	18620 ± 10630
Zn/B	1864 ± 472	1276 ± 324	33.4 ± 22.7
Zn/Be	1166728 ± 171086	1569218 ± 246879	26804 ± 15533
Zn/Bi	222186 ± 52479	75425 ± 36054	2582 ± 2292
Zn/Br	51.6 ± 9.5	69.1 ± 11.4	2.39 ± 0.84
Zn/Cd	1451 ± 356	2488 ± 533	382 ± 44
Zn/Ce	58752 ± 13885	126472 ± 18956	2207 ± 812
Zn/Co	31567 ± 5245	24682 ± 3747	4577 ± 675
Zn/Cr	5033 ± 1859	1952 ± 687	116 ± 34
Zn/Cs	36214 ± 7979	65687 ± 12084	4989 ± 1601
Zn/Dy	641470 ± 141308	1125693 ± 211414	35483 ± 11566
Zn/Er	1149119 ± 258895	2659891 ± 510876	66123 ± 18514
Zn/Fe	11.3 ± 1.5	11.8 ± 1.3	1.04 ± 0.11
Zn/Gd	596553 ± 138780	1138410 ± 205832	27895 ± 9521
Zn/Hg	30732 ± 5037	5720 ± 582	1553 ± 314
Zn/Ho	3132261 ± 701937	5325653 ± 855569	108255 ± 37392
Zn/La	57719 ± 20753	91631 ± 18986	2156 ± 1195
Zn/Li	33798 ± 7925	48938 ± 9863	1442 ± 559
Zn/Mn	814 ± 173	1222 ± 167	45.1 ± 19.9
Zn/Mo	4789 ± 827	8786 ± 1500	727 ± 325
Zn/Nb	556870 ± 200334	681455 ± 152014	56921 ± 14279
Zn/Nd	129475 ± 31107	266023 ± 39406	5274 ± 1715
Zn/Ni	689 ± 171	770 ± 191	30.1 ± 11.2
Zn/Pb	1478 ± 339	2885 ± 497	139 ± 60
Zn/Pr	517616 ± 132976	1395347 ± 350643	26153 ± 8996
Zn/Rb	86.9 ± 11.8	95.8 ± 10.7	18.8 ± 2.1
Zn/Sb	45850 ± 10375	21798 ± 6086	702 ± 250
Zn/Sc	58691 ± 12144	60497 ± 15015	23410 ± 8832
Zn/Se	1495 ± 176	1449 ± 345	296 ± 36
Zn/Sm	617945 ± 137213	1726655 ± 386992	32773 ± 10145
Zn/Sn	7831 ± 1944	19576 ± 3420	349 ± 176
Zn/Tb	5544112 ± 1382878	10103881 ± 1779214	250461 ± 96820
Zn/Th	699184 ± 187605	991175 ± 183154	11971 ± 6518
Zn/Ti*	701 ± 142	1054 ± 150	42.0 ± 21.5
Zn/Tl	828929 ± 108262	879374 ± 135511	33512 ± 22230
Zn/Tm	7484410 ± 2080528	11458220 ± 1965135	473767 ± 262385
Zn/U	454350 ± 90481	1437144 ± 312075	28068 ± 6153
Zn/Y	169174 ± 52729	256955 ± 46436	5305 ± 1507
Zn/Yb	1507335 ± 356960	3084223 ± 755203	106041 ± 29271
Zn/Zr	48585 ± 9724	42437 ± 13129	181 ± 59

M: Arithmetic mean; SEM: Standard error of mean; NS: not significant difference.
*Titanium tools were used for sampling and sample preparation.

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

Individual data sets for Zn/Ag, Zn/Al, Zn/B, Zn/Be, Zn/Br, Zn/Cr, Zn/Fe, Zn/Mn, Zn/Rb, and Zn/Sb mass fraction ratios 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 Zn/Ag, Zn/Al, Zn/B, Zn/Be, Zn/Br, Zn/Cr, Zn/Fe, Zn/Mn, Zn/Rb, and Zn/Sb mass fraction ratios for the diagnosis of PCacalculated in this work.

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

The mean values and all selected statistical parameters were calculated for 42 ratios of Zn/traceelement contents: Zn/Ag, Zn/Al, Zn/Au, Zn/B, Zn/Be, Zn/Bi, Zn/Br, Zn/Cd, Zn/Ce, Zn/Co, Zn/ Cr, Zn/Cs, Zn/Dy, Zn/Er, Zn/Fe, Zn/Gd, Zn/Hg, Zn/Ho, Zn/La, Zn/Li, Zn/Mn, Zn/Mo, Zn/Nb, Zn/Nd, Zn/Ni, Zn/Pb, Zn/Pr, Zn/Rb, Zn/Sb, Zn/Sc, Zn/Se, Zn/Sm, Zn/Sn, Zn/Tb, Zn/Th, Zn/Ti, Zn/Tl, Zn/Tm, Zn/U, Zn/Y, Zn/Yb, and Zn/Zr (Table 1). The mass fraction of Zn and othertrace 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 fraction ratios of Zn/Ag, Zn/Br, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se were measured in all, or a major portion of samples, while mass fractions of other Zn/traceelement ratios were determined in 22 samples (11 BPH and 11 PCa samples, respectively).

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

Table 2. Ratio of means and the difference between mean values of the Zn mass fraction/ trace element mass fraction ratios 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*≤ t-test**	***p* U-test**	**Ratio PCa/N**	***p*≤ t-test**	***p* U-test**	**Ratio PCa/BPH**	***p*≤ t-test**	***p* U-test**
Zn/Ag	1.44	0.187	>0.05	0.039	**0.000001**	**≤0.01**	0.027	**0.00069**	**≤0.01**
Zn/Al	1.49	0.140	>0.05	0.034	**0.00012**	**≤0.01**	0.023	**0.00020**	**≤0.01**
Zn/Au	1.32	0.445	>0.05	0.031	**0.0091**	**≤0.01**	0.024	**0.00040**	**≤0.01**
Zn/B	0.68	0.312	>0.05	0.018	**0.00081**	**≤0.01**	0.026	**0.0050**	**≤0.01**
Zn/Be	1.34	0.195	>0.05	0.023	**0.000001**	**≤0.01**	0.017	**0.000094**	**≤0.01**
Zn/Bi	0.34	**0.027**	**≤0.05**	0.012	**0.00033**	**≤0.01**	0.034	0.071	**≤0.05**
Zn/Br	1.34	0.243	>0.05	0.046	**0.00024**	**≤0.01**	0.035	**0.000004**	**≤0.01**
Zn/Cd	1.71	0.122	>0.05	0.263	**0.0062**	**≤0.01**	0.154	**0.0027**	**≤0.01**
Zn/Ce	2.15	**0.0089**	**≤0.05**	0.038	**0.00047**	**≤0.01**	0.017	**0.000064**	**≤0.01**
Zn/Co	0.78	0.291	>0.05	0.145	**0.000016**	**≤0.01**	0.185	**0.000035**	**≤0.01**
Zn/Cr	0.39	0.129	>0.05	0.023	**0.013**	**≤0.01**	0.059	**0.016**	**≤0.01**
Zn/Cs	1.81	0.056	**≤0.05**	0.138	**0.00068**	**≤0.01**	0.076	**0.00050**	**≤0.01**
Zn/Dy	1.75	0.072	**≤0.05**	0.055	**0.00028**	**≤0.01**	0.032	**0.00042**	**≤0.01**
Zn/Er	2.31	**0.018**	**≤0.05**	0.058	**0.00036**	**≤0.01**	0.025	**0.00048**	**≤0.01**
Zn/Fe	1.04	0.814	>0.05	0.092	**0.000001**	**≤0.01**	0.088	**0.000001**	**≤0.01**
Zn/Gd	1.91	**0.042**	**≤0.05**	0.047	**0.00045**	**≤0.01**	0.025	**0.00030**	**≤0.01**
Zn/Hg	0.19	**0.000026**	**≤0.01**	0.051	**0.000003**	**≤0.01**	0.272	**0.000001**	**≤0.01**
Zn/Ho	1.70	0.059	**≤0.05**	0.035	**0.00026**	**≤0.01**	0.020	**0.00012**	**≤0.01**
Zn/La	1.59	0.238	>0.05	0.037	**0.013**	**≤0.01**	0.024	**0.0011**	**≤0.01**
Zn/Li	1.45	0.244	>0.05	0.043	**0.00050**	**≤0.01**	0.029	**0.00070**	**≤0.01**
Zn/Mn	1.50	0.102	>0.05	0.055	**0.00020**	**≤0.01**	0.037	**0.000033**	**≤0.01**
Zn/Mo	1.83	**0.033**	**≤0.05**	0.152	**0.000001**	**≤0.01**	0.083	**0.00028**	**≤0.01**
Zn/Nb	1.22	0.624	>0.05	0.102	**0.020**	**≤0.01**	0.084	**0.0021**	**≤0.01**
Zn/Nd	2.05	**0.012**	**≤0.05**	0.041	**0.00062**	**≤0.01**	0.020	**0.000059**	**≤0.01**
Zn/Ni	1.12	0.757	>0.05	0.044	**0.00080**	**≤0.01**	0.039	**0.0031**	**≤0.01**
Zn/Pb	1.95	**0.030**	**≤0.05**	0.094	**0.00060**	**≤0.01**	0.048	**0.00024**	**≤0.01**
Zn/Pr	2.70	**0.036**	**≤0.05**	0.051	**0.0012**	**≤0.01**	0.019	**0.0029**	**≤0.01**
Zn/Rb	1.10	0.578	>0.05	0.216	**0.000002**	**≤0.01**	0.196	**0.000050**	**≤0.01**
Zn/Sb	0.48	0.051	**≤0.05**	0.015	**0.00013**	**≤0.01**	0.032	**0.0024**	**≤0.01**
Zn/Sc	1.03	0.926	>0.05	0.399	**0.024**	**≤0.01**	0.387	**0.044**	**≤0.01**
Zn/Se	0.97	0.906	>0.05	0.198	**0.000001**	**≤0.01**	0.204	**0.0031**	**≤0.01**
Zn/Sm	2.79	**0.019**	**≤0.05**	0.053	**0.00027**	**≤0.01**	0.019	**0.0014**	**≤0.01**
Zn/Sn	2.50	**0.0084**	**≤0.05**	0.045	**0.00074**	**≤0.01**	0.018	**0.00022**	**≤0.01**
Zn/Tb	1.82	0.055	**≤0.05**	0.045	**0.00087**	**≤0.01**	0.025	**0.00025**	**≤0.01**
Zn/Th	1.42	0.275	>0.05	0.017	**0.0013**	**≤0.01**	0.012	**0.00032**	**≤0.01**
Zn/Ti*	1.50	0.098	**≤0.05**	0.060	**0.00013**	**≤0.01**	0.040	**0.000044**	**≤0.01**
Zn/Tl	1.06	0.774	>0.05	0.040	**0.000001**	**≤0.01**	0.038	**0.00013**	**≤0.01**
Zn/Tm	1.53	0.176	>0.05	0.063	**0.0027**	**≤0.01**	0.041	**0.00022**	**≤0.01**
Zn/U	3.16	**0.011**	**≤0.05**	0.062	**0.000087**	**≤0.01**	0.020	**0.0011**	**≤0.01**
Zn/Y	1.52	0.221	>0.05	0.031	**0.0047**	**≤0.01**	0.021	**0.00029**	**≤0.01**
Zn/Yb	2.05	0.079	>0.05	0.070	**0.00069**	**≤0.01**	0.034	**0.0028**	**≤0.01**
Zn/Zr	0.87	0.711	>0.05	0.004	**0.000049**	**≤0.01**	0.004	**0.0105**	**≤0.01**

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

significantly lower than in normal and BPH prostate.

Analysis the mass fraction ratios of trace element 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 ratios for the diagnosis of PCa. As is evident from Table 2 and, particularly, from individual data sets (Figure 1), the Zn/Ag, Zn/Al, Zn/B, Zn/Be, Zn/Br, Zn/Cr, Zn/Fe, Zn/Mn, Zn/Rb, and Zn/Sb mass fraction ratios are potentially the most informative test for a differential diagnosis. For example, if 5.0 is the value of Zn/Al mass fraction ratio assumed to be the upper 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%=100-9%;

Specificity={True Negatives (TN)/[TN+False Positives (FP)]}×100%=97±3%.

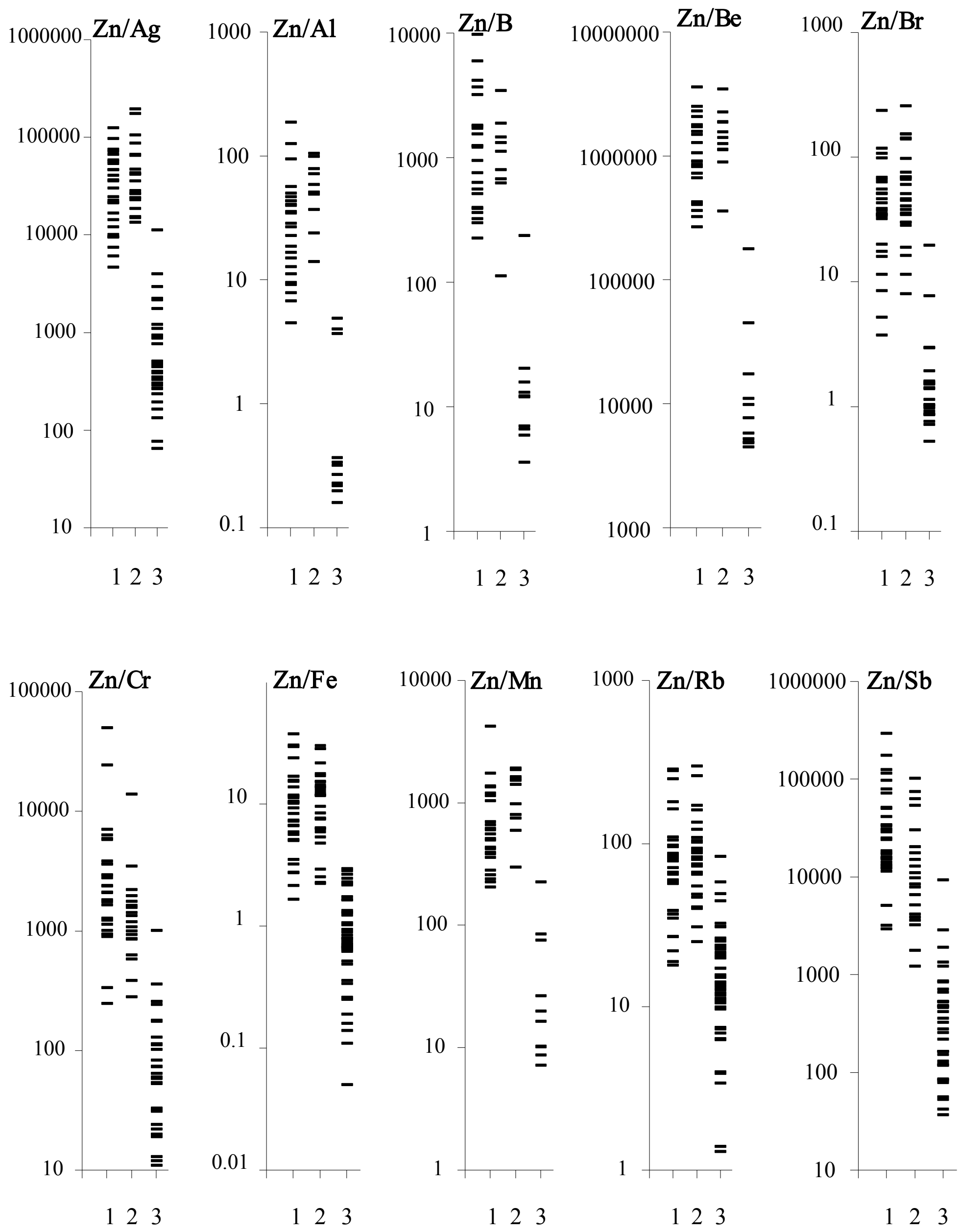

Figure 1. Individual data sets for Zn/Ag, Zn/Al, Zn/B, Zn/Be, Zn/Br, Zn/Cr, Zn/Fe, Zn/Mn, Zn/Rb, and Zn/Sb mass fraction ratios in samples of normal (1), benign hypertrophic (2) and cancerous (3) prostate

Table 3. Parameters of the importance (sensitivity, specificity and accuracy) of Zn/Ag, Zn/Al, Zn/B, Zn/Be, Zn/Br, Zn/Cr, Zn/Fe, Zn/Mn, Zn/Rb, and Zn/Sb mass fraction ratios for the diagnosis of PCa (an estimation is made for "PCa or normal and BPH prostate").

Mass fraction ratio or their multiplication	**Upper limit for PCa**	**Sensitivity %**	**Specificity %**	**Accuracy %**
Zn/Ag	4500	97 ± 3	100-2	99 ± 1
Zn/Al	5.0	100-9	97 ± 3	98 ± 2
Zn/B	100	90 ± 10	100-3	98 ± 2
Zn/Be	180000	100-9	100-3	100-2
Zn/Br	3.5	91 ± 6	100-2	97 ± 2
Zn/Cr	260	93 ± 5	98 ± 2	96 ± 2
Zn/Fe	2.0	87 ± 5	98 ± 2	94 ± 3
Zn/Mn	110	91 ± 9	100-3	98 ± 2
Zn/Rb	33	92 ± 4	89 ± 4	91 ± 3
Zn/Sb	2000	95 ± 4	96 ± 3	96 ± 2

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 Zn/Al mass fraction ratio in a prostate biopsy sample is lower5.0, one could diagnose a malignant tumor with an accuracy 98±2%. Thus, using the Zn/Al mass fraction ratio-test makes it possible to diagnose cancer in 100-9%; cases (sensitivity). The same way parameters of the importance (sensitivity, specificity and accuracy) of Zn/Ag, Zn/B, Zn/Be, Zn/Br, Zn/Cr, Zn/Fe, Zn/Mn, Zn/Rb, and Zn/Sbmass fraction ratios for the diagnosis of PCa were calculated (Table 3).

Conclusion

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 andcarcinomatous 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. It was observed that the Zn/Ag, Zn/Al, Zn/Au, Zn/B, Zn/Be, Zn/Bi, Zn/Br, Zn/Cd, Zn/Ce, Zn/Co, Zn/ Cr, Zn/Cs, Zn/Dy, Zn/Er, Zn/Fe, Zn/Gd, Zn/Hg, Zn/Ho, Zn/La, Zn/Li, Zn/Mn, Zn/Mo, Zn/Nb, Zn/Nd, Zn/Ni, Zn/Pb, Zn/Pr, Zn/Rb, Zn/Sb, Zn/Sc, Zn/Se, Zn/Sm, Zn/Sn, Zn/Tb, Zn/Th, Zn/Ti, Zn/Tl, Zn/Tm, Zn/U, Zn/Y, Zn/Yb, and Zn/Zr mass fraction ratios were significantly lower in cancerous tissues than in normal and BPH prostate. Finally, we propose to use the Zn/Ag, Zn/Al, Zn/B, Zn/ Be, Zn/Br, Zn/Cr, Zn/Fe, Zn/Mn, Zn/Rb, and Zn/Sb mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer. Further studies on larger number of samples are required to confirm our findings and to investigate the impact of the trace element relationships on prostate cancer etiology.

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Competing interests

All other authors declare no competing interests.

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