



Research Article

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Ratios of Cadmium/Trace Element Contents in Prostate Gland as Carcinoma's Markers



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Abstract

The aim of the study was to evaluate whether significant changes in the levels of ratios Cd/trace element contents exist in the malignantly transformed prostate. Contents of Cd and other 42 trace elements (Ag, Al, Au, B, Be, Bi, Br, 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) in normal (N, n=37, age range 41-87 years), benign hypertrophic (BPH, n=32, age range 56-78), and cancerous human prostate (PCa, n=60, age range 40-70) were investigated. Measurements of trace element contents were performed using a combination of instrumental neutron activation analysis and inductively coupled plasma mass spectrometry. Then the levels of ratios Cd/trace element contents in every sample were calculated. It was observed that the ratio to Cd of Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Cs, Dy, Er, Gd, Ho, La, Li, Mn, Nd, Ni, Pb, Pr, Sb, Sm, Sn, Tb, Th, Ti, Tl, U, Y, Yb, and Zr mass fraction were significantly lower in cancerous tissue than in normal and BPH prostate. It was proposed to use the Cd/Ag, Cd/Al, Cd/Au, Cd/B, Cd/Be, Cd/Bi, Cd/Br, Cd/Ce, Cd/La, Cd/Li, Cd/Mn, Cd/Nd, Cd/Th, Cd/Tl, and Cd/Zr 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.

Keywords: Trace elements; Trace element content ratios; Prostate; Benign prostatic hypertrophy; Prostatic carcinoma; Neutron activation analysis; Inductively coupled plasma mass spectrometry

Abbreviations: INAA-LLR: The Instrumental Neutron Activation Analysis with High Resolution Spectrometry of Long-Lived Radionuclides; ICP-MS: The Inductively Coupled Plasma mass spectrometry; CRM: The Certified Reference Materials

Introduction

The prostate gland may be a source of many health problems in men past middle age, 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 and greater than 90% of men over 70 years old [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 [31-69] significant changes of trace element contents in hyperplastic and cancerous prostate in comparison with those in the normal prostatic tissue were observed. In particular, it was shown that the average mass fraction of some trace elements in BPH were higher than normal levels, while those in adenocarcinoma were lower than in healthy prostatic parenchyma [60,61,66-68]. 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 chemical element contents changed in opposite directions during malignant transformation of prostate. For example, a significant informative value of Zn/Cd content as a tumor marker for PCa diagnostics was shown by us [70]. Hence it is possible that besides Zn, the ratio of Cd to other trace elements also can be used as tumor markers for distinguish between benign and malignant prostate.

Currently number of methods was 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 a non-destructive and one of the most sensitive techniques. It allows measure the chemical element contents in few milligrams tissue without any treatment of sample. Nondestructive method of analysis avoids the possibility of changing the content of trace elements in the studied samples [71-75], which allowed for the first time to obtain reliable results. However, INAA-LLR allows only determine the mean mass fractions of 10-11 trace elements in the samples of normal and cancerous prostate glands [15,28]. The inductively coupled plasma mass spectrometry (ICP-MS) is 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 two these 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 aged over 40 years and also 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 method. The second aim was to calculate Cd/trace element content ratios for every samples and compare the levels of these ratios in normal, hyper plastic, and cancerous prostate. The third and final aim was to evaluate the ratios of Cd/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 (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). Trans rectal 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. 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 \pm SD) years. The 60 patients aged 40-79 suffered from PCa (stage T1-T4). Their mean age was 65 ± 10 (M \pm SD) years.

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 \pm 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 chemical 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 [14,15,20,28].

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 INAA-LLR investigation, the prostate samples were taken out and used for ICP-MS method. The samples were decomposed in autoclaves; 1.5 mL of concentrated HNO₃ (nitric acid at 65%, maximum (max) of 0.0000005 % Hg; GR, ISO, Merck) and 0.3 mL of H₂O₂ (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 the plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without tissue samples (only HNO₃+H₂O₂+ 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 INAA-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 used for INAA-LLR included the 100 cm³ Ge(Li) detector and on-line computer-based multichannel analyzer. The spectrometer provided a resolution of 1.9 keV on the ⁶⁰Co 1332 keV line. Other details of the INAA-LLR analysis were presented in our previous publication [15].

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 ICP-MS method used was presented in our previous publication [18].

Certified reference materials

For quality control, ten subsamples of the certified reference materials (CRM) IAEA H-4 Animal muscle and IAEA HH-1 Human hair 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-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 CRMs were treated in the same way as the prostate samples. Detailed results of this quality assurance program were presented in earlier publications [15,18].

Results

Table 1: 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		
		N 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

Computer programs and statistic

A dedicated computer program for INAA mode optimization was used [76]. All prostate samples for INAA-LLR were prepared in duplicate and mean values of traceelement contents were used in final calculation. For elements investigated by both INAA-LLRand ICP-MS methods the mean of all results was used. Using the Microsoft Office Excel software Cd/trace element contents for each trace element in every sample were calculated. Then arithmetic mean ± standard error of mean was calculated for trace element mass fraction and for ratios of Cd/trace element mass fraction in normal, benign hyper plastic and cancerous prostate. The difference in the results between BPH and N, PCa and N, 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 Cd/trace element mass fraction ratios in normal, benign hypertrophic and cancerous prostate” diagrams the Microsoft Office Excel software was also used.

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.

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.

Table 1 represents mean values ± standard error of mean (M±SEM) of the Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er,

Table 2: Mean values (M±SEM) of the Cd mass fraction/ trace element mass fraction ratios in normal (N), benign hypertrophic (BPH) and cancerous prostate (PCa).

Ratio	Prostatic tissue		
	N 41-87 year (n=37)	BPH 56-78 year (n=32)	PCa 40-79 year (n=60)
Cd/Ag	69.5±21.0	50.2±17.9	1.06±0.22
Cd/Al	0.0437±0.0076	0.0460±0.0163	0.00126±0.000486
Cd/Au	496±120	596±223	12.2±2.9
Cd/B	1.79±0.37	0.964±0.438	0.0309±0.0051
Cd/Be	1384±188	1145±427	28.8±5.9
Cd/Bi	285±87	23.5±7.33	0.153±0.030
Cd/Br	0.0528±0.0086	0.0497±0.0278	0.00388±0.00085
Cd/Ce	65.4±16.9	88.9±25.3	4.02±1.01
Cd/Co	31.4±4.5	18.4±4.2	9.83±1.90
Cd/Cr	5.91±2.78	0.978±0.323	0.138±0.037
Cd/Cs	43.8±7.2	52.4±19.9	14.9±5.1
Cd/Dy	724±162	731±210	74.2±24.6
Cd/Er	1219±235	1934±735	151±45
Cd/Fe	0.0106±0.0013	0.00793±0.00293	0.00344±0.00097
Cd/Gd	654±151	809±265	37.1±8.5
Cd/Hg	34.9±6.2	4.40±1.17	2.56±1.01
Cd/Ho	3100±584	3535±1083	196±35
Cd/La	55.8±11.9	74.4±32.0	1.30±0.50
Cd/Li	39.1±7.4	38.5±15.2	1.62±0.53
Cd/Mn	0.967±0.139	0.915±0.359	0.108±0.034

Cd/Mo	5.69±1.05	6.20±2.49	2.10±1.15
Cd/Nb	475±109	473±141	148±92
Cd/Nd	146±38	185±60	10.0±2.4
Cd/Ni	0.810±0.213	0.527±0.207	0.0555±0.0128
Cd/Pb	1.98±0.68	2.46±1.02	0.209±0.034
Cd/Pr	565±134	937±306	34.7±7.6
Cd/Rb	0.101±0.014	0.0768±0.0305	0.0473±0.0085
Cd/Sb	58.2±12.8	19.8±7.8	1.62±0.33
Cd/Sc	62.3±16.9	21.5±8.0	40.2±17.1
Cd/Se	1.63±0.23	1.19±0.37	0.601±0.112
Cd/Sm	773±182	1261±460	95.9±30.1
Cd/Sn	9.45±2.14	16.9±7.4	0.430±0.155
Cd/Tb	6314±1729	7217±2703	361±84
Cd/Th	727±145	693±183	9.41±3.81
Cd/Ti*	0.848±0.184	0.723±0.227	0.0938±0.0438
Cd/Tl	1134±195	715±307	13.2±2.2
Cd/Tm	6818±1129	5425±1034	439±80
Cd/U	659±150	830±181	81.5±20.0
Cd/Y	340±204	168±52	15.5±4.9
Cd/Yb	1787±537	1872±524	314±113
Cd/Zn	0.00200±0.00045	0.000854±0.000286	0.00295±0.00034
Cd/Zr	55.1±10.7	38.2±18.6	0.454±0.133

M: Arithmetic Mean; SEM: Standard Error of Mean; * Titanium tools were used for sampling and sample preparation.

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.

Table 2 depicts mean values ± standard error of mean (M±SEM) of the ratio to Cd of Ag, Al, Au, B, Be, Bi, Br, Ce, Co, Cr, Cs,

Table 3: Ratio of means and the difference between mean values of the Cd mass fraction/ trace element mass fraction ratios in normal (N), benign hypertrophic (BPH) and cancerous prostate (PCa).

	BPH and N			PCa and 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
Cd/Ag	0.72	0.490	>0.05	0.015	0.0034	≤ 0.01	0.021	0.020	≤ 0.01
Cd/Al	1.05	0.899	>0.05	0.029	0.00001	≤ 0.01	0.027	0.022	≤ 0.01
Cd/Au	1.20	0.700	>0.05	0.025	0.00069	≤ 0.01	0.020	0.026	≤ 0.01
Cd/B	0.54	0.165	>0.05	0.017	0.00009	≤ 0.01	0.032	0.066	≤ 0.01
Cd/Be	0.83	0.618	>0.05	0.021	0.00001	≤ 0.01	0.025	0.026	≤ 0.01
Cd/Bi	0.08	0.0061	≤ 0.01	0.001	0.0032	≤ 0.01	0.007	0.0098	≤ 0.01
Cd/Br	0.94	0.916	>0.05	0.073	0.00002	≤ 0.01	0.078	0.131	≤ 0.01
Cd/Ce	1.36	0.451	>0.05	0.061	0.0014	≤ 0.01	0.045	0.0074	≤ 0.01
Cd/Co	0.59	0.043	>0.05	0.313	0.00014	≤ 0.01	0.534	0.085	>0.05
Cd/Cr	0.17	0.094	>0.05	0.023	0.052	≤ 0.01	0.141	0.029	≤ 0.01
Cd/Cs	1.20	0.691	>0.05	0.340	0.0024	≤ 0.01	0.284	0.094	≤0.05
Cd/Dy	1.01	0.981	>0.05	0.102	0.00059	≤ 0.01	0.102	0.011	≤ 0.01
Cd/Er	1.59	0.373	>0.05	0.124	0.00016	≤ 0.01	0.078	0.036	≤ 0.01
Cd/Fe	0.75	0.422	>0.05	0.325	0.00011	≤ 0.01	0.434	0.171	>0.05
Cd/Gd	1.24	0.617	>0.05	0.057	0.00046	≤ 0.01	0.046	0.015	≤ 0.01
Cd/Hg	0.13	0.00006	≤ 0.01	0.073	0.00003	≤ 0.01	0.582	0.255	>0.05

Cd/Ho	1.14	0.728	>0.05	0.063	0.00005	≤0.01	0.055	0.012	≤0.01
Cd/La	1.33	0.595	>0.05	0.023	0.00011	≤0.01	0.017	0.048	≤0.01
Cd/Li	0.98	0.973	>0.05	0.041	0.00006	≤0.01	0.042	0.035	≤0.01
Cd/Mn	0.95	0.895	>0.05	0.112	0.00001	≤0.01	0.118	0.049	≤0.01
Cd/Mo	1.09	0.856	>0.05	0.369	0.039	>0.05	0.339	0.158	>0.05
Cd/Nb	1.00	0.991	>0.05	0.312	0.067	>0.05	0.313	0.094	>0.05
Cd/Nd	1.27	0.590	>0.05	0.068	0.0017	≤0.01	0.054	0.015	≤0.01
Cd/Ni	0.65	0.349	>0.05	0.069	0.0020	≤0.01	0.105	0.046	≤0.01
Cd/Pb	1.24	0.697	>0.05	0.106	0.016	≤0.01	0.085	0.052	≤0.05
Cd/Pr	1.66	0.284	>0.05	0.061	0.00065	≤0.01	0.037	0.014	≤0.01
Cd/Rb	0.76	0.486	>0.05	0.468	0.0030	≤0.01	0.616	0.372	>0.05
Cd/Sb	0.34	0.016	≤0.01	0.028	0.00022	≤0.01	0.082	0.043	≤0.01
Cd/Sc	0.35	0.046	>0.05	0.645	0.391	>0.05	1.87	0.395	>0.05
Cd/Se	0.73	0.324	>0.05	0.369	0.00044	≤0.01	0.505	0.152	>0.05
Cd/Sm	1.63	0.342	>0.05	0.124	0.0013	≤0.01	0.076	0.030	≤0.01
Cd/Sn	1.79	0.352	>0.05	0.046	0.00036	≤0.01	0.025	0.049	≤0.01
Cd/Tb	1.14	0.782	>0.05	0.057	0.0023	≤0.01	0.050	0.030	≤0.01
Cd/Th	0.95	0.886	>0.05	0.013	0.00001	≤0.01	0.014	0.0039	≤0.01
Cd/Ti*	0.85	0.673	>0.05	0.111	0.00058	≤0.01	0.130	0.020	≤0.01
Cd/Tl	0.63	0.266	>0.05	0.012	0.00001	≤0.01	0.018	0.048	≤0.01
Cd/Tm	0.80	0.371	>0.05	0.064	0.00002	≤0.05	0.081	0.00093	≤0.01
Cd/U	1.26	0.476	>0.05	0.124	0.00089	≤0.01	0.098	0.0020	≤0.01
Cd/Y	0.49	0.423	>0.05	0.046	0.126	≤0.05	0.092	0.015	≤0.01
Cd/Yb	1.05	0.910	>0.05	0.176	0.013	≤0.05	0.168	0.014	≤0.01
Cd/Zn	0.43	0.039	≤0.05	1.48	0.104	>0.05	3.45	0.00015	≤0.01
Cd/Zr	0.69	0.444	>0.05	0.008	0.00004	≤0.01	0.012	0.073	≤0.01

T:Test - Student's t-Test; U-Test - Wilcoxon-Mann-Whitney U-test; **Bold:** significant differences

The ratios of means and the difference between mean values of the Cd/trace element mass fraction ratios in normal, benign hypertrophic and cancerous prostate are presented in Table 3. Individual data sets for Cd/Ag, Cd/Al, Cd/Au, Cd/B, Cd/Be, Cd/Bi, Cd/Br, Cd/Ce, Cd/La, Cd/Li, Cd/Mn, Cd/Nd, Cd/Th, Cd/Tl, and Cd/Zr mass fraction ratios in all investigated samples of normal, benign hypertrophic and cancerous prostate, respectively, are shown in Figure 1.

Table 4 contains parameters of the importance (sensitivity, specificity and accuracy of Cd/Ag, Cd/Al, Cd/Au, Cd/B, Cd/Be, Cd/Bi, Cd/Br, Cd/Ce, Cd/La, Cd/Li, Cd/Mn, Cd/Nd, Cd/Th, Cd/Tl, and Cd/Zr mass fraction ratios for the diagnosis of PCa calculated in this work.

Discussion

As was shown by us [14,15,17,18], the use of CRM IAEA H-4 Animal muscle, IAEA HH-1 Human hair, 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 trace element contents in these CRMs, measured by us

using INAA-LLR and ICP-MS methods, with the certified data [14,15,17,18] indicates an acceptable accuracy of the results obtained in the present study.

The mean values and standard error of mean (\pm SEM) were calculated for 43 trace element contents including Cd (Table 1), as well as for 42 ratios of Cd/trace element mass fractions (Table 2). The mass fraction of Cd and other 42 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 fraction ratios to Cd of other trace element content were determined in 22 samples (11 BPH and 11PCa samples, respectively).

From Table 3, it is observed that in benign hypertrophic tissues the Cd/Ag, Cd/Al, Cd/Au, Cd/B, Cd/Be, Cd/Br, Cd/Ce, Cd/Cr, Cd/Cs, Cd/Dy, Cd/Er, Cd/Fe, Cd/Gd, Cd/Ho, Cd/La, Cd/Li, Cd/Mn, Cd/Mo, Cd/Nb, Cd/Nd, Cd/Ni, Cd/Pb, Cd/Pr, Cd/Rb, Cd/Se, Cd/Sm, Cd/Sn, Cd/Tb, Cd/Th, Cd/Ti, Cd/Tl, Cd/Tm, Cd/U, Cd/Y, Cd/Yb, and Cd/Zr mass fraction ratios not differ from normal levels, but the mass fraction ratios of Cd/Bi, Cd/Co, Cd/Hg, Cd/Sb, Cd/Sc, and Cd/Zn are significantly lower. In cancerous tissue the all Cd/trace element mass fraction ratios investigated in the

study are significantly lower, than in BPH and normal prostate, with the exception of Cd/Co, Cd/Fe, Cd/Hg, Cd/Mo, Cd/Nb, Cd/Rb, Cd/Sc, Cd/Se, and Cd/Zn ratios.

Analysis of the mass fraction ratios for 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 [77,78]. 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 trans rectal biopsy of the indurated site in the prostate. Therefore, our data allow us to evaluate adequately the importance of Cd/trace element mass fraction ratios for the diagnosis of PCa. As is evident from Table 3 and, particularly, from individual data sets of ratios (Figure 1), the Cd/Ag, Cd/Al, Cd/Au, Cd/B, Cd/Be, Cd/Bi, Cd/Br, Cd/Ce, Cd/La, Cd/Li, Cd/Mn, Cd/Nd, Cd/Th, Cd/Tl, and Cd/Zr mass fraction ratios are potentially the most informative test for a differential diagnosis. For example, if 3.0 is the value of Cd/Ag 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:

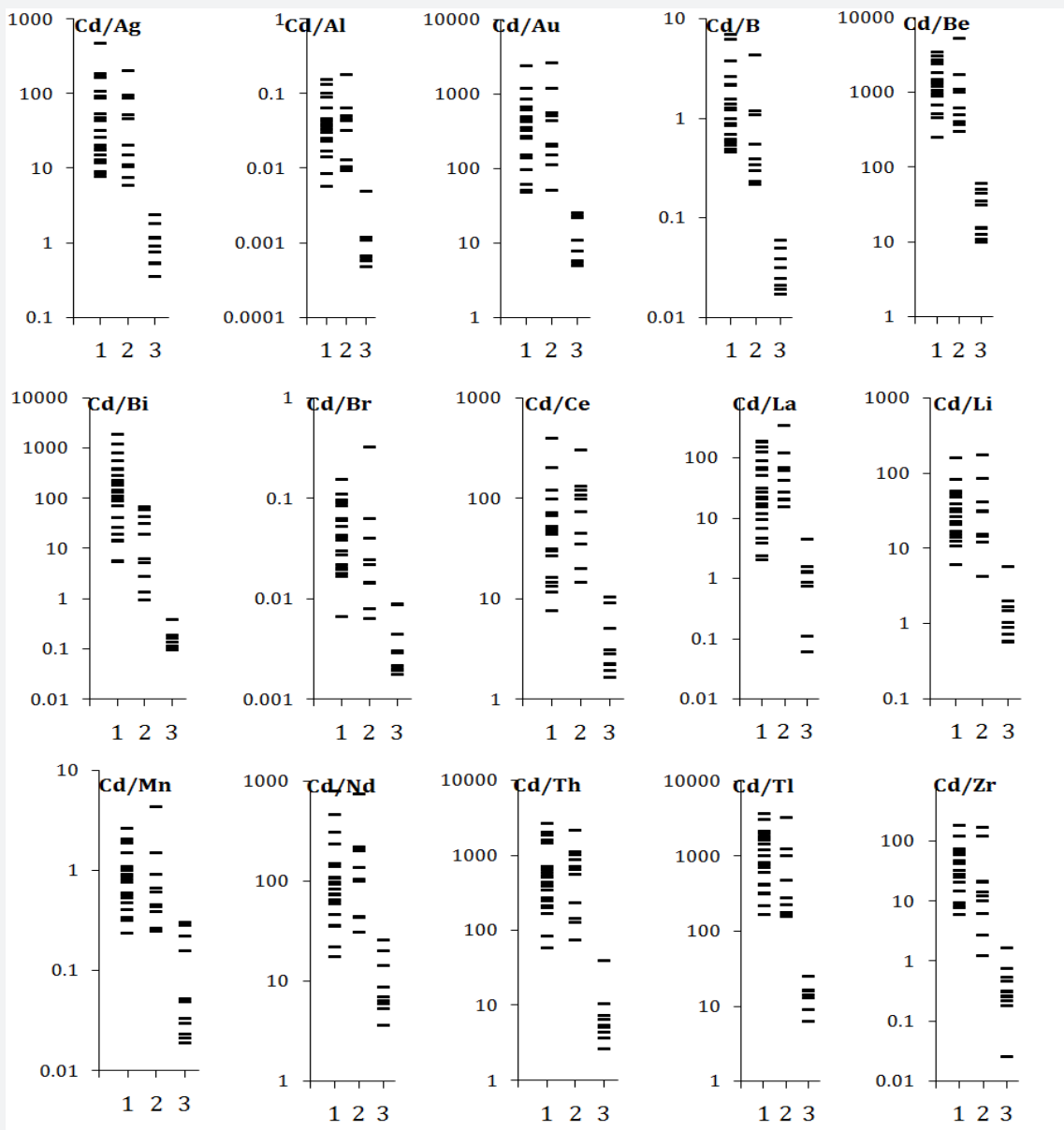


Figure 1: Individual data sets for Cd/Ag, Cd/Al, Cd/Au, Cd/B, Cd/Be, Cd/Bi, Cd/Br, Cd/Ce, Cd/La, Cd/Li, Cd/Mn, Cd/Nd, Cd/Th, Cd/Tl, and Cd/Zr mass fraction ratios in samples of normal (1), benign hypertrophic (2) and cancerous (3) prostate.

Sensitivity = {True Positives (TP)/[TP + False Negatives (FN)]} •100% =100-11%.

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

Accuracy = [(TP+TN)/(TP+FP+TN+FN)] •100% = 100-2%.

The number of people (samples) examined was taken into account for calculation of confidence intervals [79]. In other

words, if Cd/Ag mass fraction ratio in a prostate biopsy sample is lower 3.0, one could diagnose a malignant tumor with an accuracy 100-2%. Thus, using the Cd/Ag mass fraction ratio-test makes it possible to diagnose cancer in 100-11% cases (sensitivity). The same way parameters of the importance (sensitivity, specificity and accuracy) of for Cd/Al, Cd/Au, Cd/B, Cd/Be, Cd/Bi, Cd/Br, Cd/Ce, Cd/La, Cd/Li, Cd/Mn, Cd/Nd, Cd/Th, Cd/Tl, and Cd/Zr mass fraction ratios for the diagnosis of PCa were also calculated (Table 4).

Table 4: Parameters of the importance (sensitivity, specificity and accuracy) Cd/Ag, Cd/Al, Cd/Au, Cd/B, Cd/Be, Cd/Bi, Cd/Br, Cd/Ce, Cd/La, Cd/Li, Cd/Mn, Cd/Nd, Cd/Th, Cd/Tl, and Cd/Zr mass fraction ratios for the diagnosis of PCa (an estimation is made for "PCa or normal and BPH prostate").

Mass fraction ratio	Upper limit for PCa <	Sensitivity %	Specificity %	Accuracy %
Cd/Ag	3.0	100-11	100-3	100-2
Cd/Al	0.005	100-11	100-3	100-2
Cd/Au	40	100-11	100-3	100-2
Cd/B	0.1	100-11	100-3	100-2
Cd/Be	100	100-10	100-3	100-2
Cd/Bi	0.4	100-11	100-3	100-2
Cd/Br	0.01	100-10	91±5	93±4
Cd/Ce	11	100-10	97±3	98±2
Cd/La	4.6	100-12	89±5	91±5
Cd/Li	6.0	100-11	94±4	95±4
Cd/Mn	0.31	100-9	88±6	91±4
Cd/Nd	30	100-10	94±4	95±3
Cd/Th	45	100-11	100-3	100-2
Cd/Tl	30	100-12	100-3	100-2
Cd/Zr	2.0	100-9	97±3	98±2

Conclusion

The combination of nondestructive INAA-LLR 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 these 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 ratio to Cd of Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Cs, Dy, Er, Gd, Ho, La, Li, Mn, Nd, Ni, Pb, Pr, Sb, Sm, Sn, Tb, Th, Ti, Tl, U, Y, Yb, and Zr mass fraction were significantly lower in cancer tissues than in normal and BPH prostate. Finally, we propose to use the Cd/Ag, Cd/Al, Cd/Au, Cd/B, Cd/Be, Cd/Bi, Cd/Br, Cd/Ce, Cd/La, Cd/Li, Cd/Mn, Cd/Nd, Cd/Th, Cd/Tl, and Cd/Zr 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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