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Distinguishing malignant from benign prostate using content of 17 chemical elements in prostatic tissue

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Abstract

Contents of Al, B, Ba, Br, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn in normal (n=37), benign hypertrophic (BPH, n=32) and cancerous human prostate (PCa, n=60) were investigated using a combination of non-destructive and destructive methods: instrumental neutron activation analysis and inductively coupled plasma atomic emission spectrometry, respectively. Mean values ± standard error of mean (M ± S) for mass fraction (mg/kg on dry mass basis) of chemical element in the normal prostatic parenchyma were as follows: Al 34.1 ± 3.5, B 1.04 ± 0.18, Ba 1.53 ± 0.21, Br 32.9 ± 3.6, Ca 2428 ± 233, Cu 9.85 ± 0.97, Fe 132 ± 7, K 11650 ± 434, Li 0.0419 ± 0.0055, Mg 1071 ± 76, Mn 1.32 ± 0.09, Na 10987 ± 393, P 7617 ± 368, S 8657 ± 254, Si 101 ± 11, Sr 2.34 ± 0.38, and Zn 1061 ± 153, respectively. It was observed that the mass fractions of all chemical elements investigated in the study with the exception of P show significant variations in cancerous prostate when compared with normal and BPH prostate. The contents of Ca, K, Mg, Na, S, and Zn were significantly lower and those of Al, B, Ba, Br, Cu, Fe, Li, Mn, Si, and Sr were significantly higher in cancerous prostate than in normal and BPH tissues. Finally, we propose to use the Ca, S, and Zn mass fraction in a needle-biopsy core and also Ca·S·Zn test as the most informative indicators for distinguishing malignant from benign prostate. For example, sensitivity, specificity, and accuracy of Ca·S·Zn test were 100-9%, 100-2%, and 100-2%, respectively. Further studies on larger number of samples are required to confirm our findings, to study the impact of the chemical element contents on prostate cancer etiology and to examine the long-term pathological outcome.

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 [1]. 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 [2,3]. 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 [4-9]. Although the etiology of BPH and PCa is unknown, some electrolytes and trace elements have been highlighted in the literature in relation to the development of these prostate diseases [10-29].

Electrolytes and 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 chemical elements depend on tissue-specific need or tolerance, respectively [30]. Excessive accumulation, deficiency or an imbalance of the chemical elements may disturb the cell functions and may result in cellular degeneration, death and malignant transformation [31].

In reported studies significant changes of chemical 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 it is possible that besides Zn, some other chemical elements also can be used as tumor markers for distinguish between benign and malignant prostate.

Current methods applied for measurement of chemical element contents in samples of human tissue include a number of methods. Among these methods the instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) is a non-destructive and one of the most sensitive techniques. It allows measure the chemical element contents in a few milligrams tissue without any treatment of sample. Analytical studies of the Br, Ca, K, Mg, Mn, and Na contents in normal, BPH and PCa tissue were done by us using INAA-SLR [15,21,28,67,68]. Nondestructive method of analysis avoids the possibility of changing the content of chemical elements in the studied samples [69-72], which allowed for the first time to obtain reliable results. In particular, it was shown that the average mass fraction of Br, Ca, Mg, and Mn, in BPH tissue does not differ from normal level [67], but in PCa tissues the mean values of Br and Mn are higher while those of Ca and Mg are lower than in healthy prostatic tissue [68,73]. 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 chemical elements in the material of transrectal needle biopsy of prostate indurated site.

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-SLR allow only determine the mean mass fractions of 6-7 chemical elements in the tissue samples of normal and cancerous prostate glands [15,21,28,67,68]. The inductively coupled plasma atomic emission spectrometry (ICP-AES) is a more power analytical tool than INAA-SLR [18,22,47] 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 chemical elements by ICP-AES were under the control of INAA-SLR data.

The present study had three aims. The main objective was to obtain reliable results about the Al, B, Ba, Br, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn contents 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-SLR with destructive ICP-AES. The second aim was to compare the levels of chemical elements in normal, hyperplastic, and cancerous prostate, and the third aim was to evaluate the chemical element content 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 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 ± SD) years. The 60 patients aged 40-79 suffered from PCa (stage T1-T4). Their mean age was 65 ± 10 (M ± SD) years.

Intact (Norm) prostates 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 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 [15,21,28].

Sample preparation

All tissue samples were divided into two portions. One was used for morphological study while the other was intended for chemical element analysis. After the samples intended for chemical 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 chemical element measurement by INAA-SLR. The samples for INAA-SLR were sealed separately in thin polyethylene films washed beforehand with acetone and rectified alcohol. The sealed samples were placed in labeled polyethylene ampoules.

After NAA-SLR investigation the prostate samples were taken out from the polyethylene ampoules and used for ICP-AES. 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

Information detailing with the NAA-SLR and ICP-AES methods used and other details of the analysis was presented in our previous publication [15,18,21,22,28,47,67,68].

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-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 tissue samples. Detailed results of this quality assurance program were presented in earlier publications [18,22,47].

Computer programs and statistic

A dedicated computer program for INAA mode optimization was used [74]. All prostate samples for INAA-SLR were prepared in duplicate and mean values of chemical element contents were used in final calculation. For elements investigated by INAA-SLR and ICP-AES the mean of all results was used. Using the Microsoft Office Excel software, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for chemical element 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 Student's *t*-test. For the construction of "individual data sets for chemical element mass fraction in normal, benign hypertrophic and cancerous prostate" diagrams the Microsoft Office Excel software was also used.

Results

Table 1 depicts certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Al, B, Ba, Br, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn contents in normal, benign hypertrophic and cancerous prostate.

The ratios of means and the difference between mean values of Al, B, Ba, Br, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn mass fraction in normal, benign hypertrophic and cancerous prostate are presented in Table 2.

Table 1. Some statistical parameters of Al, B, Ba, Br, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn mass fractions (mg/kg, dry mass basis) in normal, benign hyperplastic (BPH), and cancerous (PCa) prostate.

Tissue	Element	Mean	SD	SEM	Min	Max	Median	Per.0.025	Per.0.975
Normal	Al	34.1	17.7	3.5	9.60	73.3	28.9	11.9	70.8
n=37	B	1.04	0.86	0.18	0.30	3.0	0.70	0.30	2.89
	Ba	1.53	1.00	0.21	0.38	4.33	1.18	0.42	3.75
	Br	32.9	17.7	3.6	12.5	80.7	28.2	12.6	70.9
	Ca	2428	1232	233	1180	6893	2195	1197	5553
	Cu	9.85	4.65	0.97	4.10	22.2	8.30	4.98	19.8
	Fe	132	40	7.0	62.0	218	133	67.6	212
	K	11650	2340	434	6325	18198	11403	7352	15489
	Li	0.0419	0.0264	0.0055	0.0150	0.101	0.0300	0.0161	0.100
	Mg	1071	409	76	447	2060	1017	520	1955
	Mn	1.32	0.42	0.09	0.750	2.80	1.30	0.836	2.23
	Na	10987	2158	393	6415	15300	10911	6718	15151
	P	7617	1839	368	5969	14838	7225	6017	11741
	S	8657	1271	254	5662	12567	8569	6680	11366
	Si	101	55	11	32.3	235	94.1	37.0	205
	Sr	2.34	1.86	0.38	0.87	8.10	1.47	0.916	6.43
	Zn	1061	933	153	223	5868	983	251	2342
BPH	Al	24.4	10.2	3.2	8.40	38.2	25.6	10.0	38.2
n=32	B	1.51	0.79	0.26	0.700	3.20	1.20	0.760	3.02
	Ba	1.22	0.68	0.20	0.420	2.32	0.970	0.468	2.24
	Br	30.7	17.2	3.4	5.50	77.0	26.2	5.75	63.8
	Ca	2032	547	165	1168	2762	1898	1173	2757
	Cu	9.86	3.96	1.25	6.00	18.9	8.30	6.25	17.9
	Fe	131	66	12	56.5	376	116	60.6	279
	K	14471	2454	740	11683	20519	13552	12025	19744
	Li	0.039	0.024	0.007	0.013	0.088	0.030	0.014	0.086
	Mg	1201	276	83	687	1585	1263	749	1552
	Mn	1.19	0.31	0.09	0.800	1.80	1.20	0.800	1.73
	Na	11612	2882	869	7762	15503	10564	7893	15400
	P	7907	1385	418	6279	11780	7547	6512	10888
	S	8787	1616	487	7671	13507	8289	7726	12401
	Si	141	79	24	72.1	333	102	73.1	307
	Sr	3.69	1.84	0.45	1.60	8.30	3.40	1.76	7.66
	Zn	1297	725	119	312	4432	1173	325	2642
PCa	Al	328	243	73	43.5	765	310	47.9	736
n=60	B	12.6	11.7	3.7	1.50	43.2	8.40	2.15	37.4
	Ba	26.7	25.1	7.6	1.83	72.3	20.1	1.95	69.4
	Br	99.9	42.5	8.9	16.0	177	102	17.2	174
	Ca	674	193	58	382	952	751	411	931
	Cu	17.1	8.6	2.0	4.50	30.6	12.8	6.21	30.5
	Fe	171	105	15	35.0	472	137	45.0	424
	K	8542	1672	504	6047	11833	8784	6270	11402
	Li	0.251	0.181	0.054	0.040	0.550	0.240	0.0433	0.545
	Mg	346	193	61	136	632	313	138	624
	Mn	6.99	4.49	1.35	1.00	16.2	5.80	1.33	15.0
	Na	7511	2133	643	3913	12239	7228	4420	11539
	P	6675	1542	465	2845	8546	6900	3553	8489
	S	5343	1290	389	3394	7241	5022	3541	7238
	Si	284	128	39	83.0	535	342	94.0	490
	Sr	5.75	2.00	0.60	2.10	9.20	5.50	2.63	8.98
	Zn	136	73	9.9	27.0	327	119	39.0	309

M: arithmetic mean, SD: standard deviation; SEM: standard error of mean, Min: minimum value, Max: maximum value, Per. 0.025: percentile with 0.025 level, Per. 0.975: percentile with 0.975 level.

Table 2. Ratio of means and the reliability of difference between mean values of Al, B, Ba, Br, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn mass fractions in normal (N), benign hypertrophic (BPH) and cancerous prostate (PCa).

Mass fraction ratio	BPH and Normal		PCa and Normal		PCa and BPH	
	Student's *t*-test, *p*=	Ratio BPH/N	Student's *t*-test, *p*=	Ratio PCa/N	Student's *t*-test, *p*=	Ratio PCa/BPH
Al	0.052	0.716	**0.0025**	9.62	**0.0020**	13.4
B	0.160	1.45	**0.012**	12.1	**0.015**	8.34
Ba	0.39	0.797	**0.0076**	17.5	**0.0071**	21.9
Br	0.65	0.933	**0.0000001**	3.04	**0.00000006**	3.25
Ca	0.17	0.837	**0.00000004**	0.278	**0.000004**	0.332
Cu	0.99	1.00	**0.0027**	1.74	**0.0044**	1.73
Fe	0.95	0.992	**0.025**	1.30	**0.046**	1.31
K	**0.0042**	1.24	**0.00008**	0.733	**0.000004**	0.590
Li	0.71	0.931	**0.0032**	5.99	**0.0029**	6.44
Mg	0.26	1.12	**0.00000002**	0.323	**0.0000002**	0.288
Mn	0.32	0.902	**0.0019**	5.30	**0.0016**	5.87
Na	0.52	1.06	**0.00022**	0.684	**0.0013**	0.647
P	0.61	1.04	0.13	0.876	0.063	0.844
S	0.82	1.02	**0.0000009**	0.617	**0.00002**	0.608
Si	0.16	1.40	**0.00075**	2.81	**0.0060**	2.01
Sr	**0.027**	1.58	**0.00015**	2.46	**0.013**	1.56
Zn	0.23	1.22	**0.0000006**	0.128	**0.00000000001**	0.105

Statistically significant values ($p \leq 0.05$) are in **bold.**

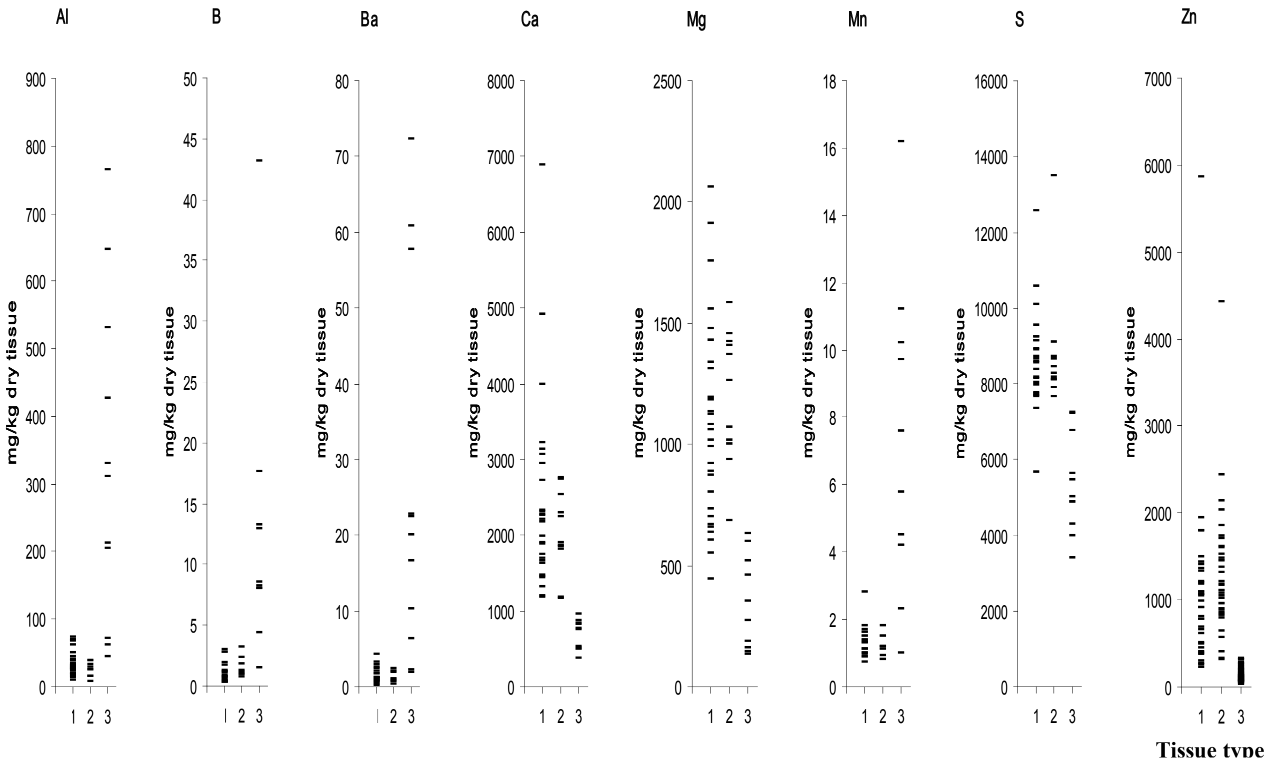

Figure 1. Individual data sets for Al, B, Ba, Ca, Mg, Mn, S, and Zn mass fractions in samples of normal (1), benign hypertrophic (2) and cancerous (3) prostate.

The comparison of our results with published data for Al, B, Ba, Br, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn mass fraction in normal, benign hypertrophic and cancerous prostate is shown in Table 3.

Table 4 contains parameters of the importance (sensitivity, specificity and accuracy) of Al, B, Ba, Ca, Mg, Mn, S, and Zn mass fraction for the diagnosis of PCa calculated in this work.

Figures 1 and 2 depict individual data sets for Al, B, Ba, Ca, Mg, Mn, S, and Zn mass fraction and for Ca·S·Zn test in all samples of normal, benign hypertrophic and cancerous prostate, respectively.

Discussion

As was shown by us [18,22,47] the use of CRM IAEA H-4, INCT-

Table 3. Median, minimum and maximum value of means of chemical element contents (mg/kg, dry mass basis) in normal, benign hyperplastic (BPH), and cancerous (PCa) prostate according to data from the literature in comparison with our results.

Prostate tissue	Element	Published data [Reference]			This work
		Median of means (n)[a]	Minimum of means M or M ± SD, (n)[b]	Maximum of means M or M ± SD, (n)[b]	M ± SD
Normal	Al	34.2 (6)	13 ± 66 (50) [31]	59 (9) [32]	34.1 ± 17.7
	B	1.0 (10)	<0.47 (50) [31]	1.2 (1) [33]	1.04 ± 0.86
	Ba	1.75 (10)	0.12 (50) [31]	102 ± 82 (10) [34]	1.53 ± 1.00
	Br	30.0 (18)	14 ± 9 (4) [35]	50 ± 32 (10) [36]	32.9 ± 17.7
	Ca	1990 (22)	427 ± 117 (21) [37]	7500 ± 12300 (57) [38]	2428 ± 1232
	Cu	9.6 (28)	1.37 (-) [39]	1488 ± 47 (10) [40]	9.85 ± 4.65
	Fe	118 (34)	5.7 ± 0.1 (5) [41]	1224 ± 76 (10) [40]	132 ± 40
	K	11800(20)	4360 ± 364 (27) [42]	13000 ± 660 (16) [43]	11650 ± 2340
	Li	-	-	-	0.042 ± 0.026
	Mg	1020 (21)	498 ± 172 (13) [38]	2056 ± 476 (21) [37]	1071 ± 409
	Mn	1.48 (24)	<0.47 (12) [44]	106 ± 18 (5) [45]	1.32 ± 0.42
	Na	10500(16)	23 ± 26 (13[38]	13700 ± 3500 (4) [46]	10987 ± 2158
	P	7120 (15)	2060 ± 690 (13) [38]	14500 (12) [44]	7617 ± 1839
	S	7370 (6)	5300 ± 750 (57) [38]	8810 ± 730 (16) [47]	8657 ± 1271
	Si	100 (6)	51 (1) [48]	111 ± 64 (64) [18]	101 ± 55
	Sr	1.46 (13)	0.75 ± 0.09 (48) [31]	2.61 ± 3.07 (27) [17]	2.34 ± 1.86
	Zn	525 (75)	101 (1) [49]	3218 ± 41 (10) [40]	1061 ± 933
BPH	Al	-	-	-	24.4 ± 10.2
	B	-	-	-	1.51 ± 0.79
	Ba	-	-	-	1.22 ± 0.68
	Br	23.3 (2)	18 ± 9 (27) [42]	21.5 ± 13 (9) [50]	30.7 ± 17.2
	Ca	3100 (6)	1000 (34) [51]	5100 ± 3200 (9) [52]	2032 ± 547
	Cu	15 (12)	3 ± 1 (7) [53]	885 ± 80 (10) [40]	9.86 ± 3.96
	Fe	197 (10)	5.9 ± 0.4 (8) [41]	1345 ± 95 (27) [42]	131 ± 66
	K	7400 (5)	1010 ± 100 (27) [42]	12800 ± 1900 (43) [54]	14471 ± 2454
	Li	-	-	-	0.039 ± 0.024
	Mg	820 (7)	566 ± 130 (25) [55]	1560 ± 50 (10) [56]	1201 ± 276
	Mn	9 (4)	6.5 (-) [57]	23 ± 13 (27) [42]	1.19 ± 0.31
	Na	7800 (1)	7800 (34) [51]	7800 (34) [51]	11612 ± 2882
	P	7600 (3)	7590 ± 1120 (43) [54]	19300 ± 14300 (9) [52]	7907 ± 1385
	S	37400 (1)	37400 ± 2100 (9) [52]	37400 ± 2100 (9) [52]	8787 ± 1616
	Si	-	-	-	141 ± 79
	Sr	4.4 (2)	3.8 ± 0.6 (43) [50]	5.0 ± 3.0 (10) [36]	3.69 ± 1.84
	Zn	725 (39)	55 ± 25 (23) [58]	3800 ± 65 (10) [56]	1297 ± 725
PCa	Al	-	-	-	328 ± 243
	B	1.78 (1)	1.78 ± 0.65 (23) [59]	1.78 ± 0.65 (23) [59]	12.6 ± 11.7
	Ba	-	-	-	26.7 ± 25.1
	Br	1.5 (1)	1.5 ± 0.6 (27) [42]	1.5 ± 0.6 (27) [42]	99.9 ± 42.5
	Ca	1830 (10)	658 ± 109 (12) [52]	11200 (1) [60]	674 ± 193
	Cu	13 (14)	4.0 ± 3.0 (11) [53]	1930 ± 65 (10) [40]	17.1 ± 8.6
	Fe	195 (15)	12.5 ± 5.0 (20) [58]	6850 (1) [60]	171 ± 105
	K	5600 (5)	740 ± 90 (27) [42]	18100 ± 400 (4) [61]	8542 ± 1672
	Li	-	-	-	0.251 ± 0.181
	Mg	935 (5)	361 ± 174 (25) [55]	1050 ± 720 (11) [53]	346 ± 193
	Mn	17.3 (6)	8.0 ± 2.0 (3) [62]	160 ± 22 (5) [45]	6.99 ± 4.49
	Na	5100 (1)	5100 (4) [63]	5100 (4) [63]	7511 ± 2133
	P	5400 (3)	3620 ± 680 (12) [52]	7700 ± 3900 (12) [54]	6675 ± 1542
	S	6900 (1)	6900 ± 1100(12)[52]	6900 ± 1100 (12) [52]	5343 ± 1290
	Si	-	-	-	284 ± 128
	Sr	-	-	-	5.75 ± 2.00
	Zn	200 (44)	16.7 ± 3.5 (3) [62]	840 ± 85 (13) [64]	136 ± 73

M: arithmetic mean, SD: standard deviation, (n)[a]: number of all references, (n)[b]: number of samples.

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 Al, B, Ba, Br, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn contents analyzed by INAA-SLR and ICP-AES with the certified data of reference materials indicates an acceptable accuracy of the results obtained in the study of chemical elements of the prostate samples presented in Tables 1–3.

Table 4. Parameters of the importance (sensitivity, specificity and accuracy) of Al, B, Ba, Ca, Mg, Mn, S, and Zn mass fractions for the diagnosis of PCa (an estimation is made for "PCa or normal and BPH tissue").

Element	Limit for PCa mg/kg, dry mass basis	Sensitivity %	Specificity %	Accuracy %
Al	85 mg/kg - Lower limit	73 ± 14	100-3	94 ± 4
B	3.9 mg/kg - Lower limit	90 ± 10	100-3	98 ± 2
Ba	4.5 mg/kg - Lower limit	82 ± 12	100-3	96 ± 3
Ca	1060 mg/kg - Upper limit	100-9	100-2	100-2
Mg	600 mg/kg - Upper limit	90 ± 10	95 ± 4	94 ± 3
Mn	2.0 mg/kg - Lower limit	91 ± 9	97 ± 3	96 ± 3
S	7250 mg/kg - Upper limit	100-9	97 ± 3	98 ± 2
Zn	330 mg/kg - Upper limit	100-2	92 ± 3	96 ± 2

M: arithmetic mean, SD: standard deviation.

The mean values and all selected statistical parameters were calculated for seventeen (Al, B, Ba, Br, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn chemical element mass fractions (Table 1). The mass fraction of these chemical 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 Zn, Fe and Br were measured in all, or a major portion of samples, mass fractions of Cu - in 29 samples (19 and 10 samples, respectively), mass fractions of Sr - in 28 samples (17 and 11 samples, respectively), while mass fractions of other chemical 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 Al, B, Ba, Br, Ca, Cu, Fe, Li, Mg, Mn, Na, P, S, Si, and Zn not differ from normal levels while the mass fraction of K and Sr are significantly higher. In cancerous tissue the mass fractions of Ca, K, Mg, Na, S, and Zn are significantly lower, and mass fractions of Al, B, Ba, Br, Cu, Fe, Li, Mn, Si, and Sr are significantly higher than in normal tissues of the prostate. All these elements show similar variations in cancerous tissues when compared with benign hypertrophic tissues of the prostate.

The results for all chemical element contents in the prostates of the control group (mean age 55 ± 11 years, range 41-87) are in accordance with our earlier findings in prostates of apparently healthy men aged 41-60 [18]. Mean values obtained for Al, B, Ba, Br, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, and Zn contents (Table 3) agree well with median of mean values cited by other researches for the normal human prostate [31-49]. Data of the literature also includes samples obtained from patients who died from different diseases. A number of values for chemical element mass fractions were not expressed on a dry mass basis in the cited literature. Therefore, we calculated these values using published data for water - 80% [56] and ash - 1% on wet mass basis [75] contents in the prostate of adult men. Our results for Br, Ca, Cu, Fe, Mg, P, Sr, and Zn are in accordance with the medians of earlier findings in benign hypertrophic prostate, some higher for K and Na and some lower for Mn and S (Table 3). In cancerous prostate our results were comparable with published data for Ca, Cu, Fe, K, Na, P, S, and Zn contents, some lover for Mg and Mn, and almost one and two orders of magnitude higher for B and Br, respectively (Table 3). No published data referring to Li mass fractions in normal prostate, Al, B, Ba, Li, and Si in BPH prostate, and Al, Ba, Li, Si, and Sr in cancerous prostate were found.

Analysis of chemical 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 [76]. 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 chemical element mass fraction for the diagnosis of PCa. As is evident from Table 2 and, particularly, from individual data sets (Figure 1), the Al, B, Ba, Ca, Mg, Mn, S, and Zn mass fraction are potentially the most informative test for a differential diagnosis. For example, if 1060 mg/kg (M ± 2SD) is the value of Ca mass fraction 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% = 100-2%;

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 [77]. In other words, if Ca mass fraction in a prostate biopsy sample does not exceed 1060 mg/kg, one could diagnose a malignant tumor with accuracy 100-2%. Thus, using the Ca mass fraction-test makes it possible to diagnose cancer in 100-2%; cases (sensitivity). The same way parameters of the importance (sensitivity, specificity and accuracy) of Al, B, Ba, Ca, Mg, Mn, S, and Zn mass fraction for the diagnosis of PCa were calculated (Table 5).

It should be noted, that the Ca, S, and Zn mass fraction are the most informative for the diagnosis of PCa and these tests have very good levels of sensitivity, specificity and accuracy (Table 4). However, it is possible to increase a separation distance between the value of "Upper limit" for PCa and the lowest values among normal and BPH results if use a combination of the Ca, S, and Zn mass fraction. For example, for this purpose a multiplication of Ca, S, and Zn mass fraction normalized to the appropriate mean values for normal prostate can be used:

$$\text{Ca·S·Zn} = (\text{Ca}_i/2428)\cdot(\text{S}_i/8657)\cdot(\text{Zn}_i/1061), \quad (1)$$

were Ca_i, S_i, and Zn_i are individual mass fraction of Ca, S, and Zn (mg/kg on dry mass basis) in normal, BPH and cancerous prostate. If the level 0.095 was accepted as the "Upper limit" of Ca·S·Zn test for the diagnosis of PCa (Figure 2), the sensitivity, specificity and accuracy of this test are 100-9%, 100-2%, and 100-2%, respectively and the lowest value in normal and BPH prostate (0.136) is 1.43 time higher the highest value in cancerous prostate.

Conclusion

The combination of nondestructive INAA-SLR and destructive ICP-AES methods is satisfactory analytical tool for the precise determination of 17 chemical 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 Al, B, Ba, Br, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn. It was observed that the mass fractions of all chemical elements investigated in the study with the exception of P show significant variations in cancerous tissues when compared

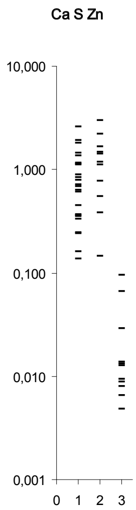

Figure 2. Individual data sets for Ca·S·Zn test in samples of normal (1), benign hypertrophic (2) and cancerous (3) prostate: Ca·S·Zn=(Ca_i/2428)·(S_i/8657)·(Zn_i/1061), were Ca_i, S_i, and Zn_i are individual mass fraction of Ca, S, and Zn (mg/kg on dry mass basis).

with normal and BPH prostate. The contents of Ca, K, Mg, Na, S, and Zn were significantly lower and those of Al, B, Ba, Br, Cu, Fe, Li, Mn, Si, and Sr were significantly higher in cancerous tissues than in normal and BPH tissues. Finally, we propose to use the Ca, S, and Zn mass fraction in a needle-biopsy core as well as Ca·S·Zn test 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 chemical element contents on prostate cancer etiology and to examine the long-term pathological outcome.

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