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Ratios of selected chemical element contents in prostatic tissue as markers of malignancy

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Abstract

The aim of the study was the development of new highly precise testing methods for early diagnosis of prostate cancer. For this purpose, the values of Ca/Al, Ca/B, Ca/Ba, Ca/Mn, Mg/Al, Mg/B, Mg/Ba, Mg/Mn, S/Al, S/B, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios in normal (n=37), benign hypertrophic (n=32) and cancerous (n=60) human prostate gland 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 ± SEM) for mass fraction ratios in the normal tissue were as follows Ca/Al 103 ± 21, Ca/B 4320 ± 805, Ca/Ba 2957 ± 577, Ca/Mn 2061 ± 325, Mg/Al 36.1 ± 4.1, Mg/B 1599 ± 239, Mg/Ba 1086 ± 227, Mg/Mn 776 ± 70, S/Al 319 ± 31, S/B 14749 ± 2166, S/Ba 9640 ± 1968, S/Mn 6991 ± 379, Zn/Al 41.3 ± 9.7, Zn/B 1974 ± 559, Zn/Ba 1003 ± 195, and Zn/Mn 875 ± 214, respectively. It was observed that in benign hypertrophic tissues the Ca/B, Mg/B, and S/B mass fraction ratios are lower than normal levels while the Mg/Al and Mg/Mn ratio are significantly higher. In cancerous tissue the values of all ratios investigated were significantly lower than in normal and benign hypertrophic prostate. It was shown that the Ca/Ba, Ca/Mn, Mg/Al, Mg/Ba, Zn/Al, Zn/B, and Zn/Ba mass fraction ratios are the most informative for a differential diagnosis among all investigated mass fraction ratios. Finally, we propose to use the (Ca/Ba)·(Mg/Al)·(Zn/Mn) and (Ca/Ba)·(Mg/Al)·(S/B)·(Zn/Mn) multiplications of mass fraction ratios in a needle-biopsy core as the most informative indicators for distinguishing malignant from benign prostate. Sensitivity, specificity, and accuracy of these tests 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 [31-66]. Moreover, a significant informative value of Zn content as a tumor marker for PCa diagnostics was shown by us [67,68]. 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 that avoids the possibility of changing the content of chemical elements during a sample preparation [69-72]. 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 [15,21,28,65,66]. 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

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a critical step in elemental analysis by this method. In our previous studies a combination of both analytical methods were offered for using when the results obtained for some chemical elements by ICP-AES were under the control of INAA-SLR data [47].

Analytical studies of the Al, B, Ba, Br, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn contents in normal, BPH and PCa tissue were done by us using the combination of INAA-SLR and ICP-AES methods, which allowed for the first time to obtain reliable results [22]. In particular, it was shown that in PCa the mean values of Al, B, Ba, and Mn are higher while those of Ca Mg, S, and Zn are lower than in healthy and hyperplastic prostates [22,73-78]. 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 in the material of needle biopsy of prostate indurated site, because, in comparison with the absolute values of chemical element contents, using their content ratios is more suitable for diagnostics [79].

The present study had three aims. The main objective was to obtain reliable results about the chemical element contents and calculate the values of Ca/Al, Ca/B, Ca/Ba, Ca/Mn, Mg/Al, Mg/B, Mg/Ba, Mg/Mn, S/Al, S/B, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios 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 element ratios in normal, hyperplastic, and cancerous prostate, and the third aim was to evaluate the levels of chemical element ratios 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 labelled 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 [15,18,21,22,28,47,65,66].

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-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 [80]. 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 Ca/Al, Ca/B, Ca/Ba, Ca/Mn, Mg/Al, Mg/B, Mg/Ba, Mg/Mn, S/Al, S/B, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios 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 nonparametric Wilcoxon-Mann-Whitney *U*-test. For the construction of "individual data sets for ratios of chemical element mass fraction or multiplications of selected ratios 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 Ca/Al, Ca/B, Ca/Ba, Ca/Mn, Mg/Al, Mg/B, Mg/Ba, Mg/Mn, S/Al, S/B, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios in normal, benign hypertrophic and cancerous prostate.

The ratios of means and the difference between mean values of Ca/Al, Ca/B, Ca/Ba, Ca/Mn, Mg/Al, Mg/B, Mg/Ba, Mg/Mn, S/Al, S/B, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios in normal, benign hypertrophic and cancerous prostate are presented in Table 2.

Table 3 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 1-4 and 5 depict individual data sets for Ca/Al, Ca/B,

Table 1. Some statistical parameters of Ca/Al, Ca/B, Ca/Ba, Ca/Mn, Mg/Al, Mg/B, Mg/Ba, Mg/Mn, S/Al, S/B, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios in normal, benign hyperplastic (BPH), and cancerous (PCa) prostate. 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.

Tissue	Ratio	Mean	SD	SEM	Min	Max	Median	Per.0.025	Per.0.975
Normal n = 25	Ca/Al	103	101	21	18.0	507	69.2	19.9	329
	Ca/B	4320	3778	805	421	16360	3127	447	12941
	Ca/Ba	2957	2705	577	503	10895	2119	620	9324
	Ca/Mn	2061	1557	325	787	7659	1669	808	6380
	Mg/Al	36.1	20.3	4.1	9.00	83.2	32.9	9.14	75.3
	Mg/B	1599	1147	239	262	3777	1340	293	3762
	Mg/Ba	1086	1090	227	213	4355	712	290	4213
	Mg/Mn	776	344	70	405	1638	681	417	1615
	S/Al	319	157	31	110	806	302	111	676
	S/B	14749	10389	2166	2882	31840	11739	2903	31608
	S/Ba	9640	9438	1968	2065	46175	6967	2694	33980
	S/Mn	6991	1857	379	3298	10203	7047	4164	10178
	Zn/Al	41.3	48.4	9.7	3.84	234	30.9	6.18	169
	Zn/B	1974	2682	559	220	12225	1174	258	8801
	Zn/Ba	1003	933	195	117	3918	581	191	3393
	Zn/Mn	875	1050	214	177	5335	585	203	3347
BPH n = 11	Ca/Al	101	57	18	46.2	221	73.3	46.5	208
	Ca/B	1550	572	191	365	2302	1696	487	2221
	Ca/Ba	2034	832	251	796	3111	1915	823	3040
	Ca/Mn	1789	616	186	973	2982	1691	987	2829
	Mg/Al	58.8	28.4	9.0	27.2	127	51.2	29.3	117
	Mg/B	928	413	138	215	1453	971	282	1436
	Mg/Ba	1258	657	198	403	2421	1034	4792	2377
	Mg/Mn	1077	377	114	520	1710	1148	533	1665
	S/Al	431	214	68	227	939	345	237	854
	S/B	6694	3038	1013	2638	12977	6908	2820	12004
	S/Ba	9418	5273	1590	3487	21629	8361	3710	19680
	S/Mn	7735	1965	592	4507	11355	7276	4732	10913
	Zn/Al	59.0	31.0	9.8	12.9	109	53.4	16.3	107
	Zn/B	1360	1250	417	102	4432	1130	216	3902
	Zn/Ba	1373	674	203	534	2266	1251	534	2234
	Zn/Mn	1261	615	185	272	2462	1479	383	2292
PCa n = 11	Ca/Al	5.24	6.51	1.96	0.982	19.3	1.63	1.03	17.7
	Ca/B	119	183	58	17.4	635	62.3	22.1	515
	Ca/Ba	102	157	47	8.27	422	38.6	8.80	420
	Ca/Mn	181	218	66	44.9	772	91.0	46.7	670
	Mg/Al	2.78	3.33	1.05	0.277	8.75	0.852	0.313	8.47
	Mg/B	70.1	132	44.2	8.35	421	31.6	9.08	346
	Mg/Ba	67.5	105	33.4	5.99	275	12.4	6.02	269
	Mg/Mn	107	133	42	9.07	458	74.1	10.2	389
	S/Al	37.0	39.9	12.0	7.52	119	15.2	7.66	112
	S/B	903	1298	410	167	4519	457	184	3752
	S/Ba	850	1324	399	84.3	3957	190	85.7	3704
	S/Mn	1476	1966	593	264	7241	1116	301	5834
	Zn/Al	1.16	1.73	0.52	0.119	4.53	0.231	0.126	4.48
	Zn/B	28.8	66.5	21.0	2.73	218	9.16	3.13	172
	Zn/Ba	29.5	56.5	17.0	1.26	145	3.51	1.28	144
	Zn/Mn	42.8	76.9	23.2	4.87	265	13.9	5.37	218

Table 2. Ratio of means and the difference between mean values of Ca/Al, Ca/B, Ca/Ba, Ca/Mn, Mg/Al, Mg/B, Mg/Ba, Mg/Mn, S/Al, S/B, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios in normal, benign hypertrophic and cancerous prostate. *t*-test-Student's *t*-test, U-test-Wilcoxon-Mann-Whitney *U*-test, ***Bold*** significant differences.

Mass fraction ratio	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
Ca/Al	0.98	= 0.94	> 0.05	0.051	**< 0.00047**	**≤ 0.01**	0.052	**< 0.00009**	**≤ 0.01**
Ca/B	0.36	**< 0.003**	**≤ 0.01**	0.028	**< 0.00004**	**≤ 0.01**	0.077	**< 0.00004**	**≤ 0.01**
Ca/Ba	0.69	= 0.15	> 0.05	0.034	**< 0.00002**	**≤ 0.01**	0.050	**< 0.00007**	**≤ 0.01**
Ca/Mn	0.87	= 0.47	> 0.05	0.088	**< 0.00001**	**≤ 0.01**	0.101	**< 0.00001**	**≤ 0.01**
Mg/Al	1.63	**= 0.039**	> 0.05	0.077	**< 0.00015**	**≤ 0.01**	0.047	**< 0.00001**	**≤ 0.01**
Mg/B	0.58	**= 0.021**	**≤ 0.05**	0.044	**< 0.00017**	**≤ 0.01**	0.076	**< 0.00001**	**≤ 0.01**
Mg/Ba	1.16	= 0.57	> 0.05	0.062	**< 0.00012**	**≤ 0.01**	0.054	**< 0.00019**	**≤ 0.01**
Mg/Mn	1.39	**= 0.037**	> 0.05	0.138	**< 0.00001**	**≤ 0.01**	0.099	**< 0.00001**	**≤ 0.01**
S/Al	1.35	= 0.15	> 0.05	0.116	**< 0.00023**	**≤ 0.01**	0.086	**< 0.00001**	**≤ 0.01**
S/B	0.45	**< 0.003**	**≤ 0.01**	0.061	**< 0.00029**	**≤ 0.01**	0.135	**< 0.00001**	**≤ 0.01**
S/Ba	0.98	= 0.93	> 0.05	0.088	**< 0.00026**	**≤ 0.01**	0.090	**< 0.00021**	**≤ 0.01**
S/Mn	1.11	= 0.30	> 0.05	0.211	**< 0.00001**	**≤ 0.01**	0.191	**< 0.00001**	**≤ 0.01**
Zn/Al	1.43	= 0.21	> 0.05	0.028	**< 0.00023**	**≤ 0.01**	0.020	**< 0.00037**	**≤ 0.01**
Zn/B	0.69	= 0.39	> 0.05	0.015	**< 0.013**	**≤ 0.01**	0.021	**< 0.0022**	**≤ 0.01**
Zn/Ba	1.37	= 0.20	> 0.05	0.029	**< 0.00006**	**≤ 0.01**	0.021	**< 0.00006**	**≤ 0.01**
Zn/Mn	1.44	= 0.18	> 0.05	0.049	**< 0.00006**	**≤ 0.01**	0.034	**< 0.00077**	**≤ 0.01**

Table 3. Parameters of the importance (sensitivity, specificity and accuracy) of Ca/Al, Ca/B, Ca/Ba, Ca/Mn, Mg/Al, Mg/B, Mg/Ba, Mg/Mn, S/Al, S/B, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios for the diagnosis of PCa (an estimation is made for "PCa or normal and BPH prostate"). M-arithmetic mean, SD-standard deviation.

Mass fraction ratio or their multiplication	Upper limit for PCa	Sensitivity %	Specificity %	Accuracy %
Ca/Al	17	91 ± 9	97 ± 3	96 ± 3
Ca/B	300	90 ± 10	91 ± 5	90 ± 5
Ca/Ba	450	100-9	100-3	100-2
Ca/Mn	780	100-9	100-3	100-2
Mg/Al	8.8	100-10	100-3	100-2
Mg/B	200	89 ± 11	86 ± 6	89 ± 5
Mg/Ba	280	100-10	97 ± 3	98 ± 2
Mg/Mn	370	90 ± 10	100-3	100-2
S/Al	100	91 ± 9	100-3	98 ± 2
S/B	2500	89 ± 11	100-3	98 ± 2
S/Ba	2950	91 ± 9	97 ± 3	96 ± 3
S/Mn	3200	91 ± 9	100-3	98 ± 2
Zn/Al	4.6	100-9	97 ± 3	98 ± 2
Zn/B	220	100-10	97 ± 3	98 ± 2
Zn/Ba	150	100-9	97 ± 3	98 ± 2
Zn/Mn	170	91 ± 9	100-3	98 ± 2
(Ca/Ba)·(Mg/Al)·(Zn/Mn)	100000	100-9	100-3	100-2
(Ca/Ba)·(Mg/Al)·(S/B)·(Zn/Mn)	130000	100-9	100-3	100-2

Ca/Ba, Ca/Mn, Mg/Al, Mg/B, Mg/Ba, Mg/Mn, S/Al, S/B, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios as well as for (Ca/Ba)·(Mg/Al)·(Zn/Mn) and (Ca/Ba)·(Mg/Al)·(S/B)·(Zn/Mn) multiplications of mass fraction ratios 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-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, Ca, Mg, Mn, S, and Zn contents analyzed by INAA-SLR and ICP-AES with the certified data of reference materials indicated an acceptable accuracy of the results obtained in the study and presented in Tables 1and 2.

The mean values and all selected statistical parameters were calculated for sixteen mass fraction ratios: Ca/Al, Ca/B, Ca/Ba, Ca/Mn,

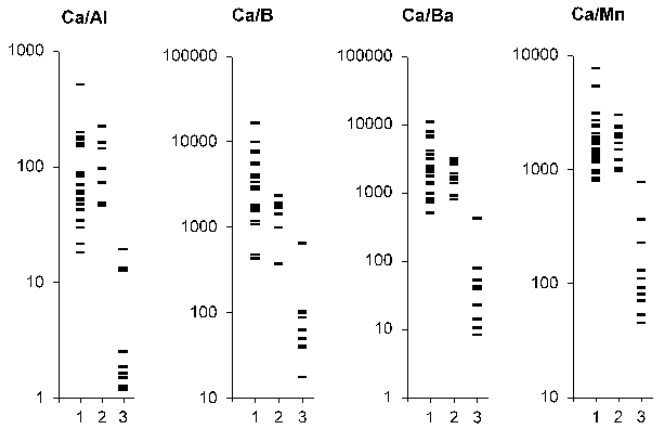

Figure 1. Individual data sets for Ca/Al, Ca/B, Ca/Ba, and Ca/Mn mass fraction ratios in samples of normal (1) benign hypertrophic (2) and cancerous (3) prostate.

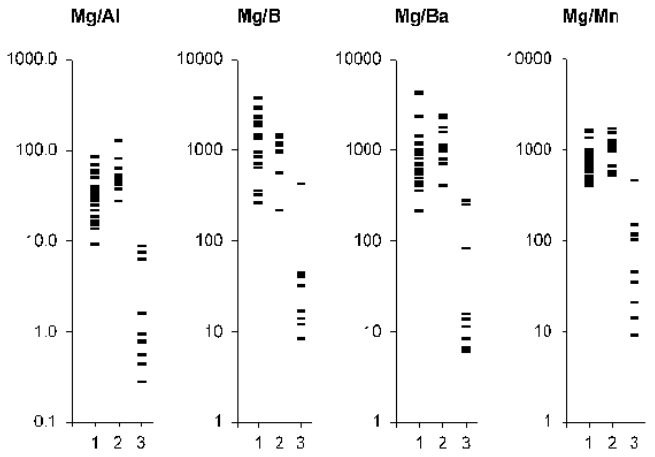

Figure 2. Individual data sets for Mg/Al, Mg/B, Mg/Ba, and Mg/Mn mass fraction ratios in samples of normal (1) benign hypertrophic (2) and cancerous (3) prostate.

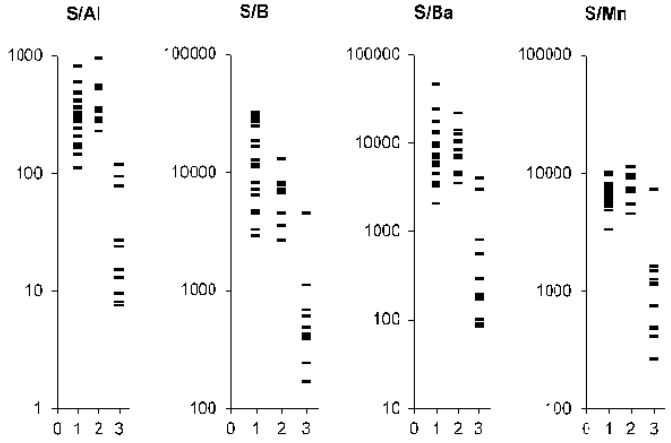

Figure 3. Individual data sets for S/Al, S/B, S/Ba, and S/Mn mass fraction ratios in samples of normal (1) benign hypertrophic (2) and cancerous (3) prostate.

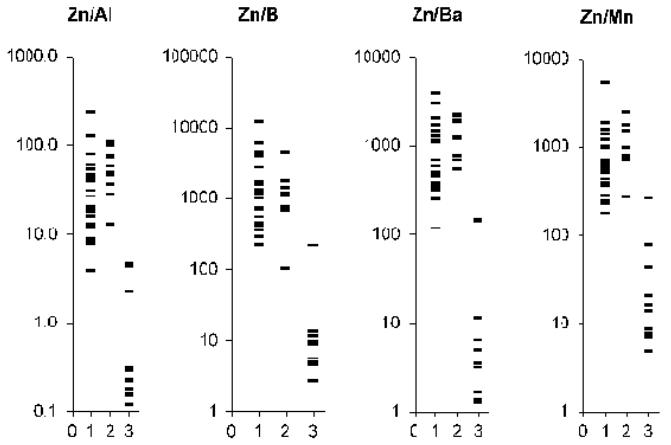

Figure 4. Individual data sets for Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios in samples of normal (1) benign hypertrophic (2) and cancerous (3) prostate.

Mg/Al, Mg/B, Mg/Ba, Mg/Mn, S/Al, S/B, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn (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

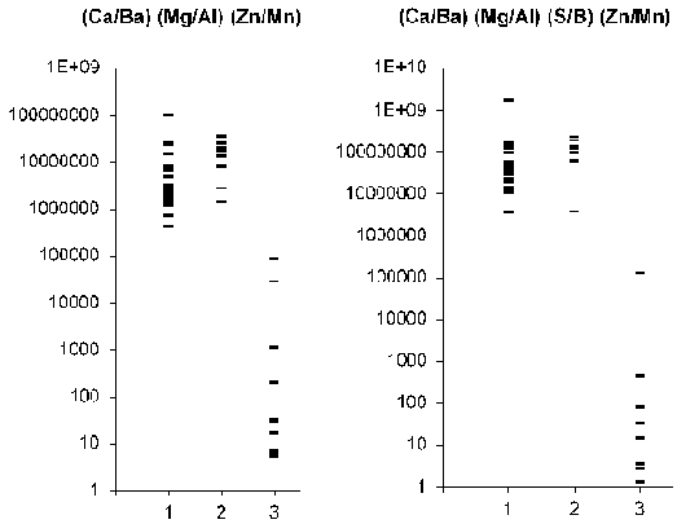

Figure 5. Individual data sets for (Ca/Ba)·(Mg/Al)·(Zn/Mn) and (Ca/Ba)·(Mg/Al)·(S/B)·(Zn/Mn) test in samples of normal (1) benign hypertrophic (2) and cancerous (3) prostate.

milligrams (sample from needle biopsy material) to 100 mg (sample from resected material). Therefore, in BPH and PCa prostates mass fractions of Zn were measured in all samples, while mass fractions of other chemical elements were determined in 22 samples (11 BPH and 11 PCa samples, respectively). Thus, the Ca/Al, Ca/B, Ca/Ba, Ca/Mn, Mg/Al, Mg/B, Mg/Ba, Mg/Mn, S/Al, S/B, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios were calculated in all, or a major portion of normal prostates, and only in 22 hyperplastic and cancerous prostates.

The results presented in Table 1 showed substantial differences between the arithmetic means and the medians, which impeached a normal distribution of the investigated elemental content ratios. These findings were a motive for us to compare the means of mass fraction ratios in normal, BPH and PCa prostate using parametric Student's *t*-test and nonparametric Wilcoxon-Mann-Whitney *U*-test. No published data referring to mass fraction ratios of chemical elements in the human prostate was found.

From Table 2, it is observed that in benign hypertrophic tissues the Ca/Al, Ca/Ba, Ca/Mn, Mg/Ba, S/Al, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios do not differ from normal levels while the Mg/Al and Mg/Mn ratios are higher and Ca/B, Mg/B, and S/B ratios are lower. In cancerous tissue all ratios of chemical element mass fractions investigated were significantly lower than in normal parenchyma of the prostate. All these mass fraction ratios show similar variations in cancerous tissues when compared with benign hypertrophic tissues of the prostate.

Analysis of elemental content ratios 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 [81,82]. 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 all cases we analyzed a part of the material obtained from a biopsy of the indurated site in the prostate. Therefore, our data allow us to

evaluate adequately the importance of elemental content ratios for the diagnosis of PCa. As is evident from individual data sets (Figures 1-4) and Table 3, the values of all mass fraction ratios are very informative for a differential diagnosis. For example, if 450 is the value of Ca/Ba mass fraction ratio assumed to be the upper limit for PCa (Figure 1 and Table 3) and an estimation is made for "PCa or intact and BPH tissue", the following values was obtained:

Sensitivity = {True Positives (TP)/ [TP + False Negatives (FN)]}·100% = (100-9)%;

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

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

The number of prostates investigated was taken into account for calculation of confidence intervals [83]. In other words, if Ca/Ba mass fraction ratio in a prostate biopsy sample does not higher 450, one could diagnose a malignant tumor with an accuracy of (100-2)%. Thus, using the (Ca/Ba)-test makes it possible to diagnose cancer in (100-9)% cases (sensitivity).

It should be noted, that all ratios of chemical element mass fraction investigated in the study are very informative for the diagnosis of PCa and these tests have good levels of sensitivity, specificity and accuracy varied in ranges 89%-100%, 86%-100%, and 89%-100%, respectively (Table 3). 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 selected ratios. For example, for this purpose a multiplication of (Ca/Ba)·(Mg/Al)·(Zn/Mn) or (Ca/Ba)·(Mg/Al)·(S/B)·(Zn/Mn) can be used. If the level 130000 was accepted as the "Upper limit" of (Ca/Ba)·(Mg/Al)·(S/B)·(Zn/Mn) test for the diagnosis of PCa (Figure 5), the sensitivity, specificity and accuracy of this test are 100-9%, 100-3%, and 100-2%, respectively and the lowest value in normal and BPH prostate is almost 30 time higher the highest value in cancerous prostate.

Mass fraction ratios of Ca/Al, Ca/Ba, Ca/Mn, Mg/Ba, S/Al, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn in the needle-biopsy cores could be used as a tool to diagnose PCa and are comparable with characteristics of the Zn mass fraction-test [67,68]. However, it is our opinion that application of the elemental content ratios is more suitable for PCa diagnosis. Elemental mass fraction depends on the sample mass, which decreases with loss of its moisture. The needle-biopsy core is a small piece of tissue with a relatively high "surface/volume" ratio. After sampling, it begins to lose mass very fast. Weight loss of samples depends on the humidity of operating and store rooms [69]. Thus, it is very difficult to determine the fresh mass of needle-biopsy cores and to calculate the precise mass fraction of chemical elements. Sample freeze-dry, storage in air-tight vials until weighing, and then calculating mass fraction on dry mass basis is the only possible method that eliminates the variation in sample mass. Conversely, accuracy of elemental content ratios does not depend on sample mass and changes in moisture content. Therefore, this method does not require dry samples. Moreover, the use of the relations between mass fractions of chemical elements is particularly promising for the development of *in vivo* diagnostic methods, including the diagnosis of PCa.

Conclusion

The combination of nondestructive INAA-SLR and destructive ICP-AES methods is satisfactory analytical tool for the precise determination of chemical element mass fractions and their ratios in the tissue samples of normal, BPH and carcinomatous prostate glands. In this work, mean values of Ca/Al, Ca/Ba, Ca/Mn, Mg/Ba, S/Al, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios in normal, benign hyperplastic and cancerous prostate were calculated using data of INAA-SLR and ICP-AES. It was observed that in benign hyperplastic prostate the Ca/Al, Ca/Ba, Ca/Mn, Mg/Ba, S/Al, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios do not differ from normal levels while the Mg/Al and Mg/Mn ratios are higher and Ca/B, Mg/B, and S/B ratios are lower. In cancerous prostate the Ca/Al, Ca/Ba, Ca/Mn, Mg/Ba, S/Al, S/Ba, S/Mn, Zn/Al, Zn/B, Zn/Ba, and Zn/Mn mass fraction ratios are significantly lower than in normal and benign hyperplastic prostate. It was shown that all ratios of chemical element mass fractions investigated in the study are informative for the prostate cancer diagnosis. Finally, we propose to use the estimation of selected elemental mass fraction ratios and some theirs multiplications 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, to study the impact of the elemental mass fraction ratios in gland tissue on prostate cancer etiology and to examine the long-term pathological outcome.

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References

1. Kirby RS (2000) The natural history of benign prostatic hyperplasia: what have we learned in the last decade. *Urology* 56: 3-6. [Crossref]

2. Roehrborn C, McConnell J (2002) Etiology, pathophysiology, epidemiology and natural history of benign prostatic hyperplasia. In: Walsh P, Retik A, Vaughan E, Wein A (Eds) Campbell's Urology, 8th Edn, Philadelphia: Saunders, p: 1297-1336.

3. Lepor H (2005) Pathophysiology of benign prostatic hyperplasia in the aging male population. *Rev Urol* 7 Suppl 4: S3-3S12. [Crossref]

4. Oliver SE, Gunnell D, Donovan JL (2000) Comparison of trends in prostate-cancer mortality in England and Wales and the USA. *Lancet* 355: 1788-1789. [Crossref]

5. Kumar RJ, Barqawi AB, Crawford ED (2004) Epidemiology of prostate cancer. *Business Briefing: US Oncology Review*: 1-6.

6. Maddams J, Brewster D, Gavin A, Steward J, Elliott J, et al. (2009) Cancer prevalence in the United Kingdom: estimates for 2008. *Br J Cancer* 101: 541-547. [Crossref]

7. Lutz JM, Francisci S, Mugno E, Usel M, Pompe-Kirn V, et al. (2003) Cancer prevalence in Central Europe: the EUROPREVAL Study. *Ann Oncol* 14: 313-322. [Crossref]

8. Möller T, Anderson H, Aareleid T, Hakulinen T, Storm H, et al. (2003) Cancer prevalence in Northern Europe: the EUROPREVAL study. *Ann Oncol* 14: 946-957. [Crossref]

9. De Angelis R, Grande E, Inghelmann R, Francisci S, Micheli A, et al. (2007) Cancer prevalence estimates in Italy from 1970 to 2010. *Tumori* 93: 392-397. [Crossref]

10. Waalkes MP, Rehm S (1994) Cadmium and prostate cancer. *J Toxicol Environ Health* 43: 251-269. [Crossref]

11. Zaichick V, Zaichick S (1999) Role of zinc in prostate cancerogenesis. In: Anke M (Ed) Jena: Friedrich-Schiller-Universitat p: 104-115.

12. Platz EA, Helzlsouer KJ (2001) Selenium, zinc, and prostate cancer. *Epidemiol Rev* 23: 93-101. [Crossref]

13. Zaichick V (2004) INAA and EDXRF applications in the age dynamics assessment of Zn content and distribution in the normal human prostate. *J Radioanal Nucl Chem* 262: 229-234.

14. Gray MA, Centeno JA, Slaney DP, Ejnik JW, Todorov T, et al. (2005) Environmental exposure to trace elements and prostate cancer in three New Zealand ethnic groups. *Int J Environ Res Public Health* 2: 374-384. [Crossref]

15. Zaichick S, Zaichick V (2011) INAA application in the age dynamics assessment of Br, Ca, Cl, K, Mg, Mn, and Na content in the normal human prostate. *J Radioanal Nucl Chem* 288: 197-202.

16. Zaichick S, Zaichick V (2011) The effect of age on Ag, Co, Cr, Fe, Hg, Sb, Sc, Se, and Zn contents in intact human prostate investigated by neutron activation analysis. *Appl Radiat Isot* 69: 827-833.

17. Zaichick S, Zaichick V (2011) The Br, Fe, Rb, Sr, and Zn content and interrelation in intact and morphologic normal prostate tissue of adult men investigated by energy dispersive X-ray fluorescent analysis. *X-Ray Spectrom* 40: 464-469.

18. Zaichick V, Nosenko S, Moskvina I (2012) The effect of age on 12 chemical element contents in the intact prostate of adult men investigated by inductively coupled plasma atomic emission spectrometry. *Biol Trace Elem Res* 147: 49-58. [Crossref]

19. Zaichick S, Zaichick V, Nosenko S, Moskvina I (2012) Mass Fractions of 52 Trace Elements and Zinc Trace Element Content Ratios in Intact Human Prostates Investigated by Inductively Coupled Plasma Mass Spectrometry. *Biol Trace Elem Res* 149: 171-183.

20. Zaichick V, Zaichick S (2014) Age-related histological and zinc content changes in adult nonhyperplastic prostate glands. *Age (Dordr)* 36: 167-181. [Crossref]

21. Zaichick V, Zaichick S2 (2014) INAA application in the assessment of chemical element mass fractions in adult and geriatric prostate glands. *Appl Radiat Isot* 90: 62-73. [Crossref]

22. Zaichick V, Zaichick S (2014) Determination of trace elements in adults and geriatric prostate combining neutron activation with inductively coupled plasma atomic emission spectrometry. *Open Journal of Biochemistry* 1: 16-33.

23. Zaichick V, Zaichick S (2014) Use of INAA and ICP-MS for the assessment of trace element mass fractions in adult and geriatric prostate. *J Radioanal Nucl Chem* 301: 383-397.

24. Zaichick V (2015) The Variation with Age of 67 Macro- and Microelement Contents in Nonhyperplastic Prostate Glands of Adult and Elderly Males Investigated by Nuclear Analytical and Related Methods. *Biol Trace Elem Res* 168: 44-60. [Crossref]

25. Zaichick V, Zaichick S (2015) Dietary intake of minerals and prostate cancer: insights into problem based on the chemical element contents in the prostate gland. *J Aging Res Clin Practice* 4: 164-171.

26. Zaichick V, Zaichick S (2015) Global contamination from uranium: insights into problem based on the uranium content in the human prostate gland. *J Environ Health Sci* 1: 1-5.

27. Zaichick V, Zaichick S (2016) Variations in concentration and distribution of several androgen-dependent and -independent trace elements in nonhyperplastic prostate gland tissue throughout adulthood. *J Androl Gynaecol* 4: 1-10.

28. Zaichick V, Zaichick S (2016) Age-related changes in concentration and histological distribution of Br, Ca, Cl, K, Mg, Mn, and Na in nonhyperplastic prostate of adults. *European Journal of Biology and Medical Science Research* 4: 31-48.

29. Zaichick V, Zaichick S (2016) Variations in concentration and histological distribution of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn in nonhyperplastic prostate gland throughout adulthood. *J Androl Gynaecol* 2: 011.

30. Zaichick V (2006) Medical elementology as a new scientific discipline. *J Radioanal Nucl Chem* 269: 303-309.

31. Tipton IH, Cook MJ (1963) Trace elements in human tissue. II. Adult subjects from the United States. *Health Phys* 9: 103-145. [Crossref]

32. Stitch SR (1957) Trace elements in human tissue.1. A semi-quantitative spectrographic survey. *Biochem J* 67: 97-103. [Crossref]

33. Tipton JH, Steiner RL, Foland WD, Mueller J, Stanley M (1954) USAEC-ORNL-Report-CF-54: 12-66.

34. Jaritz M, Anke M, Holzinger S (1998) Der Bariumgehalt verschiedener Organe von Feldhase, Wildschwein, Damhirsch, Reh, Rothirsch, Mufflon and Mensch. In: Anke M (Ed) Mengen und Spurenelemente p: 467-474.

35. Kubo H, Hashimoto S, Ishibashi A, Chiba R, Yokota H (1976) Simultaneous determinations of Fe, Cu, Zn, and Br concentrations in human tissue sections. *Medical Physics* 3: 204-209.

36. Zaichick S, Zaichick V (2010) Method and portable facility for energy-dispersive X-ray fluorescent analysis of zinc content in needle-biopsy specimens of prostate. *X-Ray Spectrom* 39: 83-89.

37. Schneider HJ, Anke M, Holm W (1970) The inorganic components of testicle, epididymis, seminal vesicle, prostate and ejaculate of young men. *Int Urol Nephrol* 2: 419-427.

38. Tohno S, Kobayashi M, Shimizu H, Tohno Y, Suwannahoy P, et al. (2009) Age-related changes of the concentrations of select elements in the prostates of Japanese. *Biol Trace Elem Res* 127: 211-227. [Crossref]

39. Anspaugh LR, Robinson WL, Martin WH, Lowe OA (1973) Compilation of Published Information on Elemental Concentrations in human Organs in Both Normal and Diseased States. *NTRL* p: 1-4.

40. Jafa A, Mahendra NM, Chowdhury AR, Kamboj VP (1980) Trace elements in prostatic tissue and plasma in prostatic diseases of man. *Indian J Cancer* 17: 34-37. [Crossref]

41. Sangen H (1967) The influence of the trace metal on aconitase activity in human prostate glands. *Nihon Hinyokika Gakkai Zasshi* 58: 1146-1159. [Crossref]

42. Guntupalli JNR, Padala S, Gummuluri AVRM, Muktineni RK, Byreddy SR, et al. (2007) Trace elemental analysis of normal, benign hypertrophic and cancerous tissues of the prostate gland using the particle-induced X-ray emission technique. *Eur J Cancer Prev* 16: 108-115.

43. Zaichick V, Zaichick S (2013) The effect of age on Br, Ca, Cl, K, Mg, Mn, and Na mass fraction in pediatric and young adult prostate glands investigated by neutron activation analysis. *Appl Radiat Isot* 82: 145-151. [Crossref]

44. Forssén A (1972) Inorganic elements in the human body. I. Occurrence of Ba, Br, Ca, Cd, Cs, Cu, K, Mn, Ni, Sn, Sr, Y and Zn in the human body. *Ann Med Exp Biol Fenn* 50: 99-162. [Crossref]

45. Banas A, Kwiatek WM, Zajac W (2001) Trace element analysis of tissue section by means of synchrotron radiation: the use of GNUPLOT for SPIXE spectra analysis. *Journal of Alloys and Compounds* 328: 135-138.

46. Soman SD, Joseph KT, Raut SJ, Mulay CD, Parameshwaran M, et al. (1970) Studies on major and trace element content in human tissues. *Health Phys* 19: 641-656. [Crossref]

47. Zaichick V, Zaichick S (2013) NAA-SLR and ICP-AES application in the assessment of mass fraction of 19 chemical elements in pediatric and young adult prostate glands. *Biol Trace Elem Res* 156: 357-366. [Crossref]

48. Belt TH, Irwin D, King EJ (1936) Silicon and dust deposits in the tissues of persons without occupational exposure to siliceous dusts. *Can Med Assoc J* 34: 125-133. [Crossref]

49. Galván-Bobadilla AI, García–Escamilla RM, Gutiérrez-García N, Mendoza-Magaña ML, Rosiles-Martínez R (2005) Cadmium and zinc concentrations in prostate cancer and benign prostate hyperplasia. *Rev Latinoamer Patol Clin* 52: 109-117.

50. Zaichick S, Zaichick V (2014) EDXRF determination of trace element contents in benign prostatic hypertrophic tissue. In: Fundamental Interactions and Neutrons, Neutron Spectroscopy, Nuclear Structure, Ultracold Neutrons, Related Topics. Dubna, Moscow Region, Joint Institute for Nuclear Research, Russia p: 311-316.

51. Hienzsch E, Schneider HJ, Anke M (1970) Vergleichende Untersuchungen zum Mengen- und Spurenelementgehalt der normalen Prostata, des Prostataadenoms und des Prostatakarzinoms. *Zeitschrift für Urologie und Nephrologie* 63: 543-546.

52. Leitão RG, Palumbo A, Souza PAVR, Pereira GR, Canellas CGL, et al. (2014) Elemental concentration analysis in prostate tissues using total reflection X-ray fluorescence. *Radiation Physics and Chemistry* 95: 62-64.

53. Yaman M, Atici D, Bakirdere S, Akdeniz I (2005) Comparison of trace metal concentrations in malign and benign human prostate. *J Med Chem* 48: 630-634. [Crossref]

54. MarczyÅ„ska A, Kulpa J, LeÅ„ko J (1983) The concentration of zinc in relation to fundamental elements in the diseased human prostate. *Int Urol Nephrol* 15: 257-265. [Crossref]

55. Picurelli L1, Olcina PV, Roig MD, Ferrer J (1991) [Determination of Fe, Mg, Cu, and Zn in normal and pathological prostatic tissue]. *Actas Urol Esp* 15: 344-350. [Crossref]

56. Györkey F, Min KW, Huff JA, Györkey P (1967) Zinc and magnesium in human prostate gland: normal, hyperplastic, and neoplastic. *Cancer Res* 27: 1348-1353. [Crossref]

57. Kwiatek WM, Banas A, Banas K, Podgorczyk M, Dyduch G, et al. (2006) Distinguishing prostate cancer from hyperplasia. *Acta Physica Polonica* 109: 377-381.

58. Kiziler AR, Aydemir B, Guzel S, Alici B, Ataus S, et al. (2010) May the level and ratio

changes of trace elements be utilized in identification of disease progression and grade in prostatic cancer. *Trace Elements and Electrolytes* 27: 65-72.

59. Eckhert CD (2005) Microlocalization and quantitation of risk associated elements in gleason graded prostate tissue. Annual Report, University of California, Los Angeles, CA p: 1-12.

60. Paluszkiewicz C, Kwiatek W (2001) Analysis of human cancer prostate tissue using FTIR microspectroscopy and SRIXE techniques. *J Mol Struct* 565-566: 329-334.

61. Kwiatek WM, Banas A, Gajda M, Galka M, Pawlicki B, et al. (2005) Cancerous tissues analyzed by SRIXE. *Journal of Alloys and Compounds* 401: 173-177.

62. Kwiatek WM, Hanson AL, Paluszkiewicz C, Galka, Gajda M, et al. (2004) Application of SRIXE and XANES to the determination of the oxidation state of iron in prostate tissue sections. *Journal of Alloys and Compounds* 362: 83-87.

63. Hienzsch E, Schneider HJ, Anke M (1970) Comparative studies of the number and amount of trace elements of the normal prostate, prostate adenoma and prostate carcinoma. *Z Urol Nephrol* 63: 543-546. [Crossref]

64. Dhar NK, Goel TC, Dube PC, Chowdhury AR, Kar AB (1973) Distribution and concentration of zinc in the subcellular fractions of benign hyperplastic and malignant neoplastic human prostate. *Exp Mol Pathol* 19: 139-142.

65. Zaichick V, Zaichick S (2016) Prostatic tissue level of some major and trace elements in patients with BPH. *Jacobs Journal of Nephrology and Urology* 3: 025.

66. Zaichick V, Zaichick S (2016) The Bromine, Calcium, Potassium, Magnesium, Manganese, and Sodium Contents in Adenocarcinoma of Human Prostate Gland. *J Hematology and Oncology Research* 2(2):1-12.

67. Zaichick VY, Sviridova TV, Zaichick SV (1997) Zinc in the human prostate gland: normal, hyperplastic and cancerous. *Int Urol Nephrol* 29: 565-574. [Crossref]

68. Zaichick S, Zaichick V (2012) Trace elements of normal, benign hypertrophic and cancerous tissues of the human prostate gland investigated by neutron activation analysis. *Appl Radiat Isot* 70: 81-87.

69. Zaichick V (1997) Sampling, sample storage and preparation of biomaterials for INAA in clinical medicine, occupational and environmental health. In: Harmonization of Health-Related Environmental Measurements Using Nuclear and Isotopic Techniques. Vienna: IAEA p: 123-133.

70. Zaichick V, Zaichick S (1996) Instrumental effect on the contamination of biomedical samples in the course of sampling. *The Journal of Analytical Chemistry* 51: 1200-1205.

71. Zaichick V, Zaichick S (1997) A search for losses of chemical elements during freeze-drying of biological materials. *J Radioanal Nucl Chem* 218: 249-253.

72. Zaichick V (2004) Losses of chemical elements in biological samples under the dry aching process. *Trace Elements in Medicine* 5: 17-22.

73. Zaichick V, Zaichick S, Davydov G (2015) Differences between chemical element contents in hyperplastic and nonhyperplastic prostate glands investigated by neutron activation analysis. *Biol Trace Elem Res* 164: 25-35. [Crossref]

74. Zaichick S, Zaichick V (2015) Prostatic Tissue Level of some Androgen Dependent and Independent Trace Elements in Patients with Benign Prostatic Hyperplasia. *Androl Gynecol: Curr Res* 3: 3.

75. Zaichick V, Zaichick S (2016) Prostatic tissue level of some major and trace elements in patients with BPH. *JJ Nephro Urol* 3: 025.

76. Zaichick V, Zaichick S (2016) The bromine, calcium, potassium, magnesium, manganese, and sodium contents in adenocarcinoma of human prostate gland. *J Hematology and Oncology Research* 2: 1-12.

77. Zaichick V, Zaichick S (2016) Age-related changes in concentration and histological distribution of 18 chemical elements in nonhyperplastic prostate of adults. *WJPMR* 2: 5-18.

78. Zaichick V, Zaichick S (2016) The Comparison between the contents and interrelationships of 17 chemical elements in normal and cancerous prostate gland. *Journal of Prostate Cancer* 1: 105.

79. Zaichick V, Zaichick S (2016) Chemical elemental content/Calcium ratios in tissues of human hyperplastic prostate gland. *Journal of Applied Life Sciences International* 4: 1-11.

80. Korelo AM, Zaichick V (1993) Software to optimize the multielement INAA of medical and environmental samples. In: Activation Analysis in Environment Protection. Dubna, Moscow Region, Russia: Joint Institute for Nuclear Research p: 326-332.

81. Catalona WJ1 (1996) Clinical utility of measurements of free and total prostate-specific antigen (PSA): a review. *Prostate Suppl* 7: 64-69. [Crossref]

82. Hjertholm P, Fenger-Gron M, Vestergaard M, Christensen MB, Borre M, et al. (2015) Variation in general practice prostate-specific antigen testing and prostate cancer outcomes: An ecological study. *Int J Cancer* 136: 435-442.

83. Genes VS (1967) Simple methods for cybernetic data treatment of diagnostic and physiological studies. Moscow, Nauka.