Differences between 66 Chemical Element Contents in Normal and Cancerous Prostate

Vladimir Zaichick*

Radionuclide Diagnostics Department, Medical Radiological Research Centre, Korolyev Str. 4, Obninsk 249036, Kaluga Region, Russia

Abstract: Prostate cancer is an internationally important health problem in man, particularly in developed countries. The aim of this exploratory study was to clarify the differences between the prostatic levels of chemical elements in patients with malignantly transformed prostate (PCa) and healthy male inhabitance. Prostatic tissue levels of 66 chemical elements were prospectively evaluated in 60 patients with PCa and 37 healthy males. Measurements were performed using a combination of five non-destructive and destructive analytical methods. A significant increase in the mean level of Ag, Al, Au, B, Ba, Be, Bi, Br, Ce, Cr, Cu, Dy, Er, Fe, Gd, Hg, Ho, Li, Mn, Nd, Ni, Pr, Sb, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, Y and Zr accompanied a decrease in the mean level of Ca, Cd, Co, K, Mg, Na, P, Rb, S, Sc, Se, and Zn was observed in the cancerous prostates. It was not found any differences in the mean prostatic level of other chemical elements including Cs, La, Mo, Nb, P, Pb, U, and Yb between PCa patients and healthy males. This work's results reveal that in malignantly transformed prostate the chemical element metabolism is drastically disturbed.

Keywords: Prostate cancer, prostatic chemical element contents, energy dispersive X-ray fluorescence analysis, neutron activation analysis, inductively coupled plasma atomic emission spectrometry, inductively coupled plasma mass spectrometry.

Dedicated to the blessed memory of my good friend Professor Dr. *Wyn Morgan* ***(University of Birmingham), who was one of the world's pioneers in the development and application of in vivo neutron activation analysis in medicine.***

INTRODUCTION

Prostate cancer (PCa) is the most prevalent male cancer in many populations, including the United States, West European states, Australia, New Zealand, and others [1]. PCa ranks second in incidence and the fifth in mortality in men worldwide [2]. Although the etiology of PCa is unknown, several risk factors including diet (calcium, zinc and some other nutrients) have been well identified [3,4]. It is also reported that the risk of having PCa drastically increases with age, being three orders of magnitude higher for the age group 40–79 years than for those younger than 40 years [3,5].

Chemical elements have essential physiological functions such as maintenance and regulation of cell function, gene regulation, activation or inhibition of enzymatic reactions, and regulation of membrane function. Essential or toxic (mutagenic, carcinogenic) properties of chemical elements depend on tissue-specific need or tolerance, respectively [6]. Excessive accumulation or an imbalance of the chemical elements may disturb the cell functions and may result in cellular degeneration or death [6-8].

*Address correspondence to this author at the Medical Radiological Research Centre, Korolyev Str. 4, Obninsk 249036,Kaluga Region, Russia; Tel: (48439) 60289; Fax: (495) 956 1440; E-mail: vezai@obninsk.com

High Ca and Zn concentrations are probably one of the main factors acting in both initiation and promotion stages of prostate carcinogenesis [9-14]. A significant tendency of age-related increase in Ca, Mg, Zn, and many other chemical element mass fractions in the normal prostate was recently demonstrated by us [15-29]. Moreover, it was found an androgen dependence of some prostatic chemical elements, including Ca, Mg, and Zn [30-41]. Thus, it seems fair to suppose that besides Ca, Mg, and Zn, many other chemical elements, which the prostatic tissue contents increase with age, also play a role in the pathophysiology of the prostate.

The chemical element contents in tissue of the normal [42-63] and cancerous [64-78] prostate have been studied, producing contradictory results. The majority of these data are based on measurements of processed tissue and in many studies tissue samples are digested before analysis. The most frequently used digestion procedures have been the traditional dry ashing and wet digestion that allow destruction of organic matter of the sample. Moreover, in some cases before digestion, prostate samples are treated with solvents (distilled water, ethanol etc) and then are dried at a high temperature for many hours. Sample pretreatment and digestion is a critical step in elemental analysis, due to risk of contamination and analytes loss, contributing for the uncontrolled analysis

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errors [79-84]. Additionally, only a few of these studies employed quality control using certified reference materials for determination of accuracy and precision of the chemical element mass fraction measurement. Thus, the questions about the differences between chemical element contents in normal and cancerous prostate tissue remained open.

It is obvious that the non-destructive analytical methods, such as the energy dispersive X-ray fluorescence (EDXRF), instrumental neutron activation analysis with high resolution spectrometry of short-lived (INAA-SLR) and long-lived radionuclides (INAA-LLR), are the most accurate techniques because they involve a minimal treatment of sample since the chances of significant loss or contamination would be decreased. However, these non-destructive analytical methods all together allow only determine the mean mass fractions of 18-20 chemical elements in the samples of normal and cancerous prostate glands [15-18,22]. The combination of such methods as inductively coupled plasma atomic emission spectrometry (ICP-AES) and the inductively coupled plasma mass spectrometry (ICP-MS) is a more power analytical tool than above-mentioned non-destructive methods [19,20]. However, sample digestion is a critical step in elemental analysis by ICP techniques. In the present study all these five analytical methods were used and the results for some chemical elements obtained by ICP-AES and ICP-MS were under the control of data obtained by non-destructive EDXRF, INAA-SLR and INAA-LLR.

This work had four aims. The primary purpose of this study was to determine reliable values for chemical element mass fractions in the cancerous and normal nonhyperplastic prostate of adult persons using five analytical methods: EDXRF, INAA-SLR, INAA-LLR, ICP-AES and ICP-MS. The second aim was to compare the results obtained in this work with data from the literature. The third aim was to find differences between the results for cancerous and normal nonhyperplastic prostate gland of age-matched health subjects, who had died suddenly. The final aim was to estimate the inter-correlations between chemical element mass fractions in cancerous prostate and to compare these results with data for normal nonhyperplastic gland.

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

MATERIAL AND METHODS

All patients studied (n=60) were hospitalized in the Urological Department of the Medical Radiological Research Centre. In all cases the diagnosis of PCa has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. None of the patients were taking a trace element supplement known to affect prostate chemical element contents. The age of patients with PCa ranged from 30 to 79 years, the mean being 65±10 years (M±SD). Using a titanium scalpel resected materials were divided into two portions to permit morphological study of prostatic tissue and to estimate their chemical element contents.

Intact prostates were removed at necropsy from 37 men (mean age 55±11 years, range 41–87) who had died suddenly (control group). The majority of deaths were due to trauma. The available clinical data were reviewed for each subject. None of the subjects had a history of an intersex condition, endocrine disorder, neoplasm or other chronic disease that could affect the normal development of the prostate. None of the subjects were receiving medications known to affect prostate morphology or chemical element content. All prostate glands were collected within 2 days of death and divided (with an anterior-posterior cross-section) into two portions using a titanium scalpel. One tissue portion was reviewed by an anatomical pathologist while the other was used for the chemical element determination. A histological examination was used to control the age norm conformity as well as to confirm the absence of any microadenomatosis and/or latent cancer.

After the samples intended for chemical element analysis were weighed, they were freeze-dried and homogenized. The pounded sample weighing about 8 mg was applied to a piece of adhesive tape, which served as a sample backing for EDXRF analysis. The sample weighing about 10-100 mg was used for chemical element measurement by instrumental NAA-SLR. The samples for INAA-SLR were sealed separately in thin polyethylene films washed with acetone and rectified alcohol beforehand. The sealed samples were placed in labeled polyethylene ampoules. A sample weighing about 10-50 mg was used for chemical element measurement by instrumental NAA-LLR. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

The samples weighing about 10-100 mg for ICP-AES and ICP-MS were decomposed in autoclaves; 1.5 mL of concentrated HNO_3 (nitric acid at 65 %,

maximum of 0.0000005 % Hg; GR, ISO, Merck) and 0.3 mL of H_2O_2 (pure for analysis) were added to the 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.

For quality control, samples of the certified reference materials IAEA H-4 Animal Muscle from the International Atomic Energy Agency (IAEA), and also 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, Warsaw, Poland) were analyzed simultaneously with the prostate tissue samples being investigated. 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 [15-20].

The mass fractions of Br, Fe, Rb, Sr, and Zn were measured by EDXRF, the mass fractions of Br, Ca, K, Mg, Mn, and Na – by NAA-SLR, the mass fractions of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn – by NAA-LLR, the mass fractions of Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn – by ICP-AES, and the mass fractions of Ag, Al, As, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Ga, Gd, Hf, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Re, Sb, Se, Sm, Sn, Ta, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr – by ICP-MS. Details of the analytical methods and procedures used here such as nuclear reactions, radionuclides, gamma-energies, wavelength, isotopes, spectrometers, spectrometer parameters and operating conditions were presented in our earlier publications concerning the chemical elements of human prostate gland [15-20].

A dedicated computer program of INAA mode optimization was used [85]. Using the Microsoft Office Excel software to provide a summary of statistical results, the arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels were calculated for all the chemical element mass fractions obtained. For elements investigated by two or more methods the mean of all results was used. The difference in the results between cancerous and normal prostate was evaluated by both 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 estimation of the Pearson correlation coefficient between different pairs of the chemical element mass fractions in the normal and cancerous prostate tissue the Microsoft Office Excel was also used.

RESULTS

The results for 66 elemental mass fractions in cancerous and normal nonhyperplastic prostate glands measured by means of the five analytical methods were obtained in the study. However, the mass fractions of As, Eu, Ga, Hf, Ir, Lu, Pd, Pt, Re, Ta, and V (11 trace elements) were determined only for the few samples measured. The possible upper limit of the mean (≤M) for these elements was calculated as the average mass fraction for each element, using the value of the detection limit (DL) instead of the individual value, when the latter was found to be below the DL:

$$\leq M = \left(\sum_{i}^{n_i} C_i + DL \cdot n_j \right) / n$$

where C_i is the individual value of chemical element mass fraction in the i^{th} sample, n_i is the number of samples with a measured mass fraction above DL, n_j is the number of samples with a measured mass fraction below DL, and $n = n_i + n_j$ is the total number of investigated samples. The possible upper limit of the mean for As, Eu, Ga, Hf, Ir, Lu, Pd, Pt, Re, Ta, and V (mg/kg, dry mass basis) were: As ≤0.018, Eu≤0.00054, Ga≤0.081, Hf≤0.018, Ir≤0.00042, Lu≤0.00022, Pd≤0.0074, Pt≤0.00059, Re≤0.00100, Ta ≤0.0053, and V≤0.22. Generally, the mass fractions of Rh and Te were lower than the corresponding DL (in mg/kg on a dry mass basis) <0.01 and <0.003, respectively. Thus, the all basic statistical parameters of chemical element mass fraction (the arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels) in cancerous and normal nonhyperplastic prostate glands were calculated for 53 chemical element (Tables **1** and **2**, respectively).

The comparison of this work results with other published information for Ag, Al, Au, B, Ba, Be, Bi, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Fe, Gd, Hg, Ho, K, La, Li, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Pr, Rb, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, U, Y, Yb,

Table 1: Basic Statistical Parameters of Chemical Element Mass Fraction (mg/kg, Dry Mass Basis) in the Cancerous Prostate Gland (n=60)

Element	Symbol	M	SD	SEM	Min	Max	Median	P0.025	P0.975
Silver	Ag	0.252	0.169	0.030	0.0237	0.720	0.225	0.0390	0.570
Aluminum	Al	328	243	73	43.5	765	310	49.7	734
Gold	Au	0.0297	0.0185	0.0056	0.00200	0.0610	0.0400	0.00305	0.0568
Boron	B	12.6	11.7	3.7	1.50	43.2	8.40	2.15	37.4
Barium	Ba	26.7	25.1	7.6	1.83	72.3	20.1	1.95	69.4
Beryllium	Be	0.0137	0.0074	0.0022	0.00200	0.0200	0.0200	0.00275	0.0200
Bismuth	Bi	1.76	0.89	0.27	0.0140	2.60	2.10	0.0283	2.55
Bromine	Br	104	48	10	11.3	193	109	14.1	183
Calcium	Ca	675	193	58	382	952	751	411	931
Cadmium	Cd	0.425	0.329	0.099	0.200	1.20	0.300	0.205	1.13
Cerium	Ce	0.101	0.044	0.013	0.0290	0.180	0.100	0.0338	0.175
Cobalt	Co	0.0336	0.0211	0.0040	0.00494	0.102	0.0291	0.00647	0.0834
Cromium	Cr	2.34	1.78	0.32	0.165	6.08	1.56	0.295	6.07
Cesium	Cs	0.0389	0.0129	0.0039	0.0100	0.0580	0.0400	0.0143	0.0565
Coper	Cu	15.9	8.3	1.8	2.70	30.6	15.0	3.56	30.5
Dysprosium	Dy	0.0072	0.0036	0.0011	0.00100	0.0100	0.0100	0.00148	0.0100
Erbium	Er	0.00297	0.00127	0.00038	0.00100	0.00400	0.00400	0.00100	0.00400
Iron	Fe	160	100	14	6.90	427	127	19.0	402
Gadolinium	Gd	0.0094	0.0057	0.0017	0.00200	0.0190	0.0100	0.00243	0.0188
Mercury	Hg	0.122	0.108	0.019	0.0159	0.496	0.0836	0.0224	0.402
Holmium	Ho	0.00178	0.00072	0.00022	0.00081	0.00340	0.00200	0.00084	0.00305
Potassium	K	8542	1672	504	6047	11833	8784	6270	11402
Lanthanum	La	0.969	1.70	0.537	0.0260	4.70	0.202	0.0283	4.45
Lithium	Li	0.251	0.181	0.054	0.0400	0.550	0.240	0.0433	0.545
Magnesium	Mg	346	193	61	136	632	313	138	624
Manganese	Mn	6.99	4.49	1.35	1.00	16.2	5.80	1.33	15.0
Molybdenum	Mo	0.298	0.078	0.035	0.180	0.400	0.300	0.192	0.391
Sodium	Na	7511	2133	643	3913	12239	7228	4420	11539
Niobium	Nb	0.00515	0.00021	0.00015	0.00500	0.00530	0.00515	0.00501	0.00529
Neodymium	Nd	0.0413	0.0215	0.0065	0.0150	0.0860	0.0350	0.0163	0.0803
Nickel	Ni	6.96	3.45	1.04	2.60	14.6	7.00	2.93	13.6
Phosphorus	P	6675	1542	465	2845	8546	6900	3553	8489
Lead	Pb	1.81	1.17	0.35	0.520	4.50	1.50	0.568	4.05
Praseodymium	Pr	0.0097	0.0058	0.0017	0.00200	0.0230	0.0100	0.00243	0.0208
Rubidium	Rb	8.69	4.80	0.67	1.00	22.4	7.70	1.88	20.7
Sulphur	S	5343	1290	389	3394	7241	5022	3541	7238
Antimony	Sb	0.490	0.364	0.059	0.0150	1.70	0.428	0.0453	1.42
Scandium	Sc	0.0116	0.0082	0.0015	0.00010	0.0438	0.0103	0.00170	0.0275
Selenium	Se	0.561	0.421	0.076	0.0630	1.54	0.438	0.0758	1.40
Silicon	Si	284	128	39	83.0	535	342	94.0	490

(Table 1). Continued.

Element	Symbol	M	SD	SEM	Min	Max	Median	P0.025	P0.975
Samarium	Sm	0.0095	0.0095	0.0029	0.00200	0.0350	0.00530	0.00215	0.0303
Tin	Sn	1.29	0.79	0.24	0.190	2.00	1.68	0.193	2.00
Strontium	Sr	6.18	3.03	0.91	1.80	13.4	5.50	2.40	12.3
Terbium	Tb	0.00089	0.00026	0.00012	0.00060	0.00120	0.00100	0.00060	0.00118
Thorium	Th	0.049	0.041	0.012	0.00320	0.124	0.0410	0.00515	0.121
Titanium*	Ti*	8.60	4.93	2.20	2.00	14.0	11.0	2.30	13.7
Thallium	Tl	0.0219	0.0185	0.0056	0.00090	0.0650	0.0190	0.00193	0.0575
Thulium	Tm	0.00054	0.00022	0.00011	0.00029	0.00083	0.00051	0.00031	0.00081
Uranium	U	0.0068	0.00311	0.0013	0.00300	0.0170	0.00500	0.00315	0.0160
Yttrium	Y	0.0340	0.0127	0.0038	0.0200	0.0610	0.0400	0.0200	0.0558
Ytterbium	Yb	0.00174	0.00087	0.00039	0.00100	0.00310	0.00160	0.00100	0.00299
Zinc	Zn	137	77	10	20.0	366	122	23.7	309
Zirconium	Zr	2.13	2.95	0.89	0.310	9.80	0.780	0.330	8.75

M arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *P0.025* percentile with 0.025 level, *P0.975* percentile with 0.975 level.
*Titanium tools were used for sampling and sample preparation.

Table 2: Basic Statistical Parameters of Chemical Element Mass Fraction (mg/kg, Dry Mass Basis) in the Nonhyperplastic Prostate Gland of Males of Ages 41–87 Years (n=37)

Element	Symbol	M	SD	SEM	Min	Max	Median	P0.025	P0.975
Silver	Ag	0.0380	0.0296	0.0056	0.00500	0.155	0.0300	0.00838	0.0976
Aluminum	Al	34.1	17.7	3.5	9.60	73.3	29.0	12.0	70.8
Gold	Au	0.00412	0.00346	0.00078	0.00100	0.0106	0.00230	0.00100	0.0103
Boron	B	1.04	0.85	0.18	0.300	3.00	0.700	0.300	2.89
Barium	Ba	1.48	1.01	0.21	0.200	4.33	1.17	0.299	3.73
Beryllium	Be	0.00094	0.00035	0.00007	0.00070	0.00210	0.00075	0.00070	0.00187
Bismuth	Bi	0.029	0.056	0.011	0.00100	0.205	0.00560	0.00130	0.200
Bromine	Br	34.6	17.3	3.2	12.0	80.7	30.9	12.4	68.3
Calcium	Ca	2428	1232	233	1180	6893	2195	1197	5553
Cadmium	Cd	1.12	0.64	0.13	0.320	2.40	0.985	0.320	2.39
Cerium	Ce	0.0309	0.0244	0.0050	0.00600	0.0960	0.0220	0.00600	0.0937
Cobalt	Co	0.0467	0.0354	0.0064	0.0165	0.200	0.0400	0.0169	0.130
Cromium	Cr	0.562	0.433	0.082	0.0300	1.81	0.456	0.0300	1.67
Cesium	Cs	0.0339	0.0170	0.0033	0.0100	0.0870	0.0300	0.0131	0.0720
Coper	Cu	9.85	4.66	0.97	4.10	22.2	8.30	4.98	19.8
Dysprosium	Dy	0.00293	0.00242	0.00049	0.000690	0.0104	0.00224	0.000696	0.00934
Erbium	Er	0.00148	0.00114	0.00023	0.000400	0.00530	0.00116	0.000446	0.00421
Iron	Fe	121	34.0	5.9	62.0	210	114	67.6	192
Gadolinium	Gd	0.00290	0.00199	0.00041	0.000600	0.00850	0.00235	0.000715	0.00735
Mercury	Hg	0.0521	0.0426	0.0075	0.00770	0.147	0.0335	0.0165	0.141
Holmium	Ho	0.00057	0.00040	0.00008	0.00016	0.00179	0.00045	0.00017	0.00139
Potassium	K	11650	2340	434	6325	18198	11403	7352	15489

(Table 2). Continued.

Element	Symbol	M	SD	SEM	Min	Max	Median	P0.025	P0.975
Lanthanum	La	0.080	0.100	0.020	0.00900	0.324	0.0335	0.00963	0.309
Lithium	Li	0.0419	0.0264	0.0055	0.0150	0.101	0.0300	0.0161	0.100
Magnesium	Mg	1071	409	76	447	2060	1017	520	1955
Manganese	Mn	1.32	0.42	0.09	0.750	2.80	1.30	0.836	2.23
Molybdenum	Mo	0.282	0.190	0.038	0.100	0.850	0.230	0.100	0.754
Sodium	Na	10987	2158	394	6415	15300	10911	6719	15151
Niobium	Nb	0.0054	0.0058	0.0012	0.00100	0.0200	0.00310	0.00100	0.0194
Neodymium	Nd	0.0137	0.0100	0.0021	0.00300	0.0420	0.0100	0.00300	0.0354
Nickel	Ni	3.10	2.49	0.51	0.200	9.50	2.85	0.373	8.29
Phosphorus	P	7617	1839	368	5969	14838	7225	6017	11741
Lead	Pb	2.39	2.85	0.56	0.150	10.7	0.830	0.181	9.39
Praseodymium	Pr	0.00353	0.00258	0.00053	0.000600	0.0106	0.00285	0.000715	0.00939
Rubidium	Rb	14.5	5.06	0.9	5.90	26.5	14.2	6.23	25.5
Sulphur	S	8657	1271	254	5662	12567	8569	6680	11366
Antimony	Sb	0.0427	0.0364	0.0063	0.00800	0.158	0.0375	0.00960	0.154
Scandium	Sc	0.0294	0.0236	0.0053	0.00460	0.0771	0.0200	0.00660	0.0768
Selenium	Se	0.748	0.266	0.047	0.318	1.49	0.735	0.348	1.44
Silicon	Si	102	55	11	32.3	235	94.1	37.0	205
Samarium	Sm	0.00267	0.00176	0.00035	0.000600	0.00700	0.00260	0.000660	0.00652
Tin	Sn	0.320	0.322	0.063	0.0500	1.11	0.140	0.0563	1.04
Strontium	Sr	2.47	1.98	0.41	0.870	8.90	1.60	0.914	6.87
Terbium	Tb	0.00039	0.00030	0.00006	0.00007	0.00120	0.00035	0.00007	0.00109
Thorium	Th	0.00335	0.00363	0.00074	0.000500	0.0172	0.00180	0.000558	0.0125
Titanium*	Ti*	2.82	3.07	0.64	0.700	13.7	1.82	0.700	10.1
Thallium	Tl	0.00138	0.00072	0.00015	0.000300	0.00300	0.00130	0.000415	0.00289
Thulium	Tm	0.00024	0.00017	0.00004	0.00004	0.00083	0.00020	0.00006	0.00062
Uranium	U	0.0070	0.0105	0.0021	0.000790	0.0381	0.00261	0.000802	0.0341
Yttrium	Y	0.0187	0.0217	0.0043	0.000500	0.0840	0.0100	0.00206	0.0765
Ytterbium	Yb	0.00141	0.00121	0.00025	0.000200	0.00510	0.00110	0.000315	0.00412
Zinc	Zn	1072	749	123	229	4298	915	244	2249
Zirconium	Zr	0.0358	0.0272	0.0055	0.0100	0.0900	0.0250	0.0100	0.0900

M arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *P0.025* percentile with 0.025 level, *P0.975* percentile with 0.975 level.
*Titanium tools were used for sampling and sample preparation.

Zn and Zr mass fraction in normal and cancerous prostate is shown in Tables **3** and **4**, respectively. When our results were compared with data of literature a number of values for chemical element mass fractions were not expressed on a dry mass basis by the authors of the cited references. However, we calculated these values using the medians of published data for water – 83% and ash – 1% (on wet mass basis) contents in normal prostate of adult men [86,87], and also for water – 80% in PCa tissue [88].

Arithmetic mean (M) and standard error of mean (SEM) for determined 53 chemical element mass fractions in cancerous (group of patients with PCa) and normal nonhyperplastic (age-matched control group) prostate are presented in Table **5**. Table **5** also depicts the ratios of means and the difference between mean values of chemical element mass fractions in cancerous and normal prostate. For some trace elements (Ag, Au, B, Ba, Be, Bi, Cd, Ce, Co, Cr, Dy, Fe, Hg, La, Li, Nb, Nd, Pb, Sb, Sc, Se, Sn, Sr, Th, Ti,

Table 3: Median, Minimum and Maximum Value of Means of Chemical Element Mass Fractions (mg/kg, on Dry Mass Basis) in Normal Prostate of Adult Males According to Data from the Literature in Comparison with this Works' Results for Males Aged 41-87 Years

Element	Published data [Reference]			This work
	Median of means, (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M or M±SD, (n)**	M±SD n=37
Ag	0.055 (11)	<0.006 (48) [42]	0.24 (7) [43]	0.038±0.030
Al	34.0 (9)	13±66 (50) [42]	80±98 (9) [44]	34±18
Au	0.0053 (7)	0.0032±0.0027 (21) [34]	1.5 (3 [43]	0.0041±0.0035
B	1.0 (10)	<0.47 (50) [42]	5.9±17.2 (21) [34]	1.04±0.85
Ba	1.75 (10)	0.12 (50) [42]	102±82 (10) [45]	1.48±1.01
Be	0.00095 (5)	0.00091±0.00026 (28) [28]	0.003±0.005 (16) [24]	0.00094±0.00035
Bi	0.018 (6)	0.0039±0.0017 (16) [36]	<0.09 (50) [42]	0.029±0.056
Br	30 (18)	14±9 (4) [46]	50±32 (10) [15]	35±17
Ca	1990 (22)	427±117 (21) [47]	7500±12300 (57) [48]	2428±1232
Cd	0.78 (26)	0.07 (129) [49]	427±497 (55) [50]	1.12±0.64
Ce	0.028 (5)	0.019±0.020 (16) [36]	0.049±0.066 (16) [24]	0.031±0.024
Co	0.036 (12)	0.022±0.010 (16) [22]	12 (9) [44]	0.047±0.035
Cr	0.51 (15)	0.053 (50) [42]	29.4 ±5.9 (5) [51]	0.56±0.43
Cs	0.036 (7)	0.031±0.016 (10) [28]	3.5 (12) [52]	0.034±0.017
Cu	9.6 (28)	1.37 (-) [53]	1488±47 (10) [54]	9.85±4.66
Dy	0.0030 (5)	0.0021±0.0018 (16) [36]	0.008±0.010 (16) [24]	0.0029±0.0024
Er	0.0015 (5)	0.0011±0.0011 (16) [36]	0.004±0.006 (16) [24]	0.0015±0.0011
Fe	118 (34)	5.7±0.1 (5) [55]	1224±76 (10) [54]	121±34
Gd	0.0029 (5)	0.0019±0.0017 (16) [36]	0.007±0.009 (16) [24]	0.0029±0.0020
Hg	0.037 (10)	0.024±0.014 (16) [22]	0.65±0.58 (5) [56]	0.052±0.043
Ho	0.00057 (5)	0.00038±0.00037 (16) [36]	0.001±0.002 (16) [24]	0.00057±0.00040
K	11800 (20)	4360±70 (27) [57]	13000±660 (21) [33]	11650±2340
La	0.048 (5)	0.016±0.012 (16) [36]	0.097±0.111 (27) [28]	0.080±0.100
Li	0.041 (8)	0.040±0.024 (64) [20]	0.064±0.049 (16) [24]	0.042±0.026
Mg	1120 (21)	498±172 (13) [48]	2056±476 (21) [47]	1071±409
Mn	1.48 (24)	<0.47 (12) [52]	106±18 (5) [51]	1.32±0.42
Mo	0.29 (7)	<0.19 (50) [42]	1.8 (2) [43]	0.28±0.19
Na	10500 (16)	23±26 (13) [48]	13700±3500 (4) [58]	10987±2158
Nb	0.0044 (5)	0.0023±0.0021 (16) [36]	0.013±0.020 (16) [24]	0.0054±0.0058
Nd	0.013 (5)	0.0095±0.0087 (16) [36]	0.025±0.034 (16) [24]	0.0137±0.0100
Ni	4.0 (10)	0.18 (4) [59]	14.1±4.2 (27) [57]	3.10±2.49
P	7120 (15)	2060±690 (13) [48]	14500 (12) [52]	7617±1839
Pb	1.3 (17)	0.15 (41) [60]	9.4 (4) [58]	2.39±2.85
Pr	0.0033 (5)	0.0024±0.0025 (16) [36]	0.006±0.008 (16) [24]	0.0035±0.0026
Rb	14.2 (16)	5.9 (9) [42]	68±39 (4) [58]	14.5±5.1
S	7370 (6)	5300±750 (57) [48]	9820±1710 (21) [33]	8657±1271

(Table 3). Continued.

Element	Published data [Reference]			This work
	Median of means, (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M or M±SD, (n)**	M±SD n=37
Sb	0.046 (10)	0.039±0.026 (10) [28]	0.42±0.56 (7) [56]	0.043±0.036
Sc	0.014 (8)	0.0085±0.0102 (16) [54]	0.031±0.025 (27) [26]	0.029±0.024
Se	0.73 (22)	0.32 (129) [49]	18.8±2.4 (27) [57]	0.75±0.27
Si	100 (6)	51 (1) [61]	111±64 (64) [20]	102±55
Sm	0.0027 (5)	0.0017±0.0016 (16) [36]	0.006±0.008 (16) [24]	0.0027±0.0018
Sn	0.25 (9)	0.11±0.10 (16) [36]	4.4 (7) [43]	0.32±0.32
Sr	1.46 (13)	0.75±0.75 (48) [42]	2.6±3.1 (27) [16]	2.47±1.98
Tb	0.00040 (5)	0.00021±0.00021 (16) [36]	0.001±0.002 (16) [24]	0.00039±0.00030
Th	0.0027 (5)	0.0015±0.0010 (16) [36]	0.008±0.011 (16) [24]	0.0034±0.0036
Ti*	2.8 (10)	<0.29 (50) [42]	156±9 (27) [57]	2.82±3.07
Tl	0.0014 (7)	0.00131±0.00061 (27) [28]	0.59 (1) [43]	0.00138±0.00072
Tm	0.00024 (5)	0.00018±0.00019 (16) [36]	0.0006±0.0009 (16) [24]	0.00024±0.00017
U	0.0049(6)	0.0015±0.0011 (16) [36]	0.4 (1) [62]	0.0070±0.0105
Y	0.020 (3)	0.0087±0.0080 (16) [36]	112 (12) [52]	0.019±0.022
Yb	0.0014 (4)	0.0013±0.0015 (28) [28]	0.0037±0.0062 (16) [24]	0.0014±0.0012
Zn	525 (75)	101 (1) [63]	3218±41 (10) [54]	1072±749
Zr	0.045 (5)	0.036±0.027 (27) [28]	0.16±0.21 (16) [24]	0.036±0.027

M arithmetic mean; *SD* standard deviation.
*(n)** No. of references contribution to this value; *(n)*** No. of samples.

Table 4: Median, Minimum and Maximum Value of Means of Chemical Element Mass Fractions (mg/kg, on Dry Mass Basis) in Cancerous Prostate According to Data from the Literature in Comparison with this Works' Results

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 n=60
Ag	-	-	-	0.25±0.17
Al	-	-	-	328±243
Au	-	-	-	0.030±0.019
B	1.78 (1)	1.78±0.65 (23) [64]	1.78±0.65 (23) [64]	12.6±11.7
Ba	-	-	-	26.7±25.1
Be	-	-	-	0.0137±0.0074
Bi	-	-	-	1.76±0.89
Br	1.5 (1)	1.5±6.0 (27) [57]	1.5±6.0 (27) [57]	104±48
Ca	1830 (10)	658±109 (12) [65]	11200 (1) [66]	675±193
Cd	1.0 (17)	0.22 (21) [67]	3248±145 (12) [50]	0.43±0.33
Ce	-	-	-	0.101±0.044
Co	25 (1)	23.5± 2.0 (4) [68]	27± 3 ((4) [68]	0.034±0.021
Cr	7 (4)	1.65± 0.30 (4) [68]	217±8 ((27) [57]	2.34±1.78
Cs	-	-	-	0.039±0.012

(Table 4). Continued.

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 n=60
Cu	13 (14)	4.0±3.0 (11) [69]	1930±65 (10) [54]	15.9±8.3
Dy	-	-	-	0.0072±0.0036
Er	-	-	-	0.0030±0.0013
Fe	195 (15)	12.5±5.0 (20) [70]	6850 (1) [66]	160±100
Gd	-	-	-	0.0094±0.0057
Hg	-	-	-	0.12±0.11
Ho	-	-	-	0.00178±0.00072
K	5600 (5)	740±90 (27) [57]	18100±400 (4) [68]	8542±1672
La	-	-	-	0.97±1.70
Li	-	-	-	0.25±0.18
Mg	935 (5)	361±174 (25) [71]	1050±720 (11) [69]	346±193
Mn	17.3 (6)	8.0±2.0 (3) [72]	160±22 (5) [51]	6.99±4.49
Mo	-	-	-	0.298±0.078
Na	5100 (1)	5100 (4) [73]	5100 (4) [73]	7511±2133
Nb	-	-	-	0.00515±0.00021
Nd	-	-	-	0.041±0.022
Ni	28.5 (3)	2.35±1.55 (11) [69]	122±15 (27) [57]	6.96±3.45
P	5400 (3)	3620±680 (12) [65]	7700±3900 (12) [74]	6675±1542
Pb	0.176 (2)	0.156 (21) [67]	170±50 (23) [75]	1.81±1.17
Pr	-	-	-	0.0097±0.0058
Rb	8 (1)	8±1 (12) [65]	8±1 (12) [65]	8.69±4.80
S	6900 (1)	6900±1100(12)[65]	6900±1100 (12) [65]	5343±1290
Sb	-	-	-	0.49±0.36
Sc	-	-	-	0.0116±0.0082
Se	1.47 (15)	0.835±0.410 (17) [76]	11.5±3.5 (3) [70]	0.56±0.42
Si	-	-	-	284±128
Sm	-	-	-	0.0095±0.0095
Sn	-	-	-	1.29±0.79
Sr	4.4 (2)	3.8±0.6 (43) [48]	5.0±3.0 (10) [68]	6.18±3.03
Tb	-	-	-	0.00089±0.00026
Th	-	-	-	0.049±0.041
Ti	26.2 (3)	~1 (1) [77]	257±21 (27) [57]	8.60±4.93*
Tl	-	-	-	0.022±0.019
Tm	-	-	-	0.00054±0.00022
U	-	-	-	0.0068±0.0031
Y	-	-	-	0.034±0.013
Yb	-	-	-	0.00174±0.00087
Zn	200 (44)	16.7±3.5 (3) [72]	840±85 (13) [78]	137±77
Zr	-	-	-	2.13±2.95

M – arithmetic mean; SD – standard deviation; n^a – No. of references contribution to this value; n^b – No. of samples; "–" no data available; * Titanium tools were used.

Table 5: Comparison of Mean Values (M±SEM) of the Chemical Element Mass Fraction (mg/kg, Dry Mass Basis) in Cancerous and Normal Prostate

Element	Prostatic tissue				Ratio
	PCa 30-79 year, n=60	Normal 41-87 year, n=37	t-test $p \leq$	U-test p	PCa/ Normal
Ag	0.252±0.030	0.0380±0.0056	**0.0001**	**<0.01**	6.63
Al	328±73	34.1±3.5	**0.0025**	**<0.01**	9.62
Au	0.0297±0.0056	0.00412±0.00078	**0.0010**	**<0.01**	7.21
B	12.6±3.7	1.04±0.18	**0.012**	**<0.01**	12.1
Ba	26.7±7.6	1.48±0.21	**0.0076**	**<0.01**	18.0
Be	0.0137±0.0022	0.000942±0.000072	**0.0002**	**<0.01**	14.5
Bi	1.76±0.27	0.029±0.011	**0.0001**	**<0.01**	60.7
Br	104±10	34.6±3.2	**0.0001**	**<0.01**	3.01
Ca	675±58	2428±233	**0.0001**	**<0.01**	0.28
Cd	0.425±0.099	1.12±0.13	**0.0002**	**<0.01**	0.38
Ce	0.101±0.013	0.0309±0.0050	**0.0003**	**<0.01**	3.27
Co	0.0336±0.0040	0.0467±0.0064	0.087	**<0.01**	0.72
Cr	2.34±0.32	0.562±0.082	**0.0001**	**<0.01**	4.16
Cs	0.0389±0.0039	0.0339±0.0033	0.34	>0.05	1.15
Cu	15.9±1.8	9.85±0.97	**0.0070**	**<0.01**	1.61
Dy	0.0072±0.0011	0.00293±0.00049	**0.0033**	**<0.01**	2.46
Er	0.00297±0.00038	0.00148±0.00023	**0.0038**	**<0.01**	2.01
Fe	160±14	121±6	**0.016**	**<0.01**	1.32
Gd	0.0094±0.0017	0.00290±0.00041	**0.0035**	**<0.01**	3.24
Hg	0.122±0.019	0.052±0.008	**0.0017**	**<0.01**	2.35
Ho	0.00178±0.00022	0.000567±0.000079	**0.0002**	**<0.01**	3.14
K	8542±504	11650±434	**0.0001**	**<0.01**	0.73
La	0.969±0.537	0.0799±0.0196	0.133	>0.05	12.1
Li	0.251±0.054	0.0419±0.0055	**0.0032**	**<0.01**	5.99
Mg	346±61	1071±76	**0.0001**	**<0.01**	0.32
Mn	6.99±1.35	1.32±0.09	**0.0019**	**<0.01**	5.30
Mo	0.298±0.035	0.282±0.038	0.77	>0.05	1.06
Na	7511±643	10987±394	**0.0003**	**<0.01**	0.68
Nb	0.00515±0.00015	0.0054±0.0012	0.82	>0.05	0.95
Nd	0.0413±0.0065	0.0137±0.0021	**0.0016**	**<0.01**	3.01
Ni	6.96±1.04	3.10±0.51	**0.0045**	**<0.01**	2.25
P	6675±465	7617±368	0.13	>0.05	0.88
Pb	1.81±0.35	2.39±0.56	0.39	>0.05	0.76
Pr	0.0097±0.0017	0.00353±0.00053	**0.0053**	**<0.01**	2.75
Rb	8.69±0.67	14.5±0.9	**0.0001**	**<0.01**	0.60
S	5343±389	8657±254	**0.0001**	**<0.01**	0.62
Sb	0.490±0.059	0.0427±0.0063	**0.0001**	**<0.01**	11.5
Sc	0.0116±0.0015	0.0294±0.0053	**0.0038**	**<0.01**	0.39
Se	0.561±0.076	0.748±0.047	**0.042**	**<0.05**	0.75

(Table 5). Continued.

Element	Prostatic tissue				Ratio
	PCa 30-79 year, n=60	Normal 41-87 year, n=37	t-test $p \leq$	U-test p	PCa/ Normal
Si	284±39	102±11	**0.0008**	**<0.01**	2.78
Sm	0.0095±0.0029	0.00267±0.00035	**0.039**	**<0.01**	3.56
Sn	1.29±0.24	0.320±0.063	**0.0021**	**<0.01**	4.03
Sr	6.18±0.91	2.47±0.41	**0.0023**	**<0.01**	2.50
Tb	0.00089±0.00012	0.000393±0.000062	**0.0085**	**<0.01**	2.26
Th	0.049±0.012	0.00335±0.00074	**0.0038**	**<0.01**	14.6
Ti*	8.60±2.20	2.82±0.64	0.056	**<0.05**	3.05
Tl	0.0219±0.0056	0.00138±0.00015	**0.0042**	**<0.01**	15.9
Tm	0.00054±0.00011	0.000241±0.000035	0.072	**<0.01**	2.24
U	0.0068±0.0013	0.0070±0.0021	0.95	>0.05	0.97
Y	0.0340±0.0038	0.0187±0.0043	**0.012**	**<0.01**	1.82
Yb	0.00174±0.00039	0.00141±0.00025	0.50	>0.05	1.23
Zn	137±10	1072±123	**0.0001**	**<0.01**	0.13
Zr	2.13±0.89	0.0358±0.0055	**0.041**	**<0.01**	59.5

M – arithmetic mean, SEM – standard error of mean, *t*-test - Student's *t*-test, U-test - Wilcoxon-Mann-Whitney *U*-test, ***Bold*** significant differences (p <0.05).
*Titanium tools were used for sampling and sample preparation.

Table 6: Intercorrelations of Selected Pairs of the Trace Element Mass Fractions in Normal Prostate Glands of Adults (r – Coefficient of Correlation)

Pairs	Bi	Cr	Cs	Hg	K	Mo	Pb	Sb	Se	Sn	Zn
Ag	-0.19	-0.27	-0.16	0.32	0.14	0.13	0.15	0.22	-0.19	**0.45**	0.10
Al	-0.09	-0.04	-0.29	-0.09	0.23	-0.04	-0.13	0.04	-0.03	-0.11	-0.13
Au	-0.18	0.26	0.11	0.15	-0.27	0.34	-0.04	0.33	0.34	0.18	-0.17
B	0.10	0.02	-0.05	0.09	0.02	**0.43**	0.20	-0.09	-0.12	0.13	-0.15
Ba	0.14	-0.24	-0.27	0.01	-0.09	-0.16	-0.04	-0.28	-0.27	-0.05	0.19
Be	-0.19	0.25	-0.01	0.02	-0.06	0.08	0.01	-0.04	0.19	0.11	0.17
Bi	1.00	-0.26	0.16	-0.15	0.12	-0.23	0.23	-0.28	0.00	0.05	-0.07
Br	**-0.44**	0.03	-0.33	0.09	-0.31	0.38	0.13	0.01	-0.29	0.20	-0.17
Ca	0.16	-0.25	-0.20	-0.17	-0.22	0.06	0.07	-0.13	-0.21	0.20	-0.04
Cd	0.09	0.28	-0.24	0.03	-0.13	-0.01	0.26	-0.10	-0.10	-0.04	-0.27
Ce	**0.65**	-0.10	0.10	-0.06	0.13	-0.26	0.01	-0.22	-0.05	-0.09	-0.19
Co	0.14	0.12	-0.21	0.01	-0.21	-0.11	-0.01	0.18	-0.07	0.00	-0.09
Cr	-0.26	1.00	0.34	0.06	-0.13	0.16	-0.07	0.26	**0.42**	-0.21	0.03
Cs	0.16	0.34	1.00	0.27	0.21	0.17	0.27	0.07	0.32	0.08	0.15
Cu	-0.31	0.16	0.14	**0.56**	-0.10	0.39	0.14	-0.18	0.17	0.17	0.38
Dy	-0.03	-0.15	-0.31	-0.19	0.21	-0.26	-0.34	-0.11	-0.13	-0.21	-0.18
Er	-0.06	-0.09	-0.32	-0.26	0.33	-0.27	-0.32	0.01	-0.08	-0.14	-0.18
Fe	-0.13	**0.42**	0.04	0.09	-0.04	0.13	-0.04	0.29	0.28	-0.09	0.08
Gd	0.24	-0.15	-0.21	-0.22	0.32	-0.38	-0.24	-0.09	-0.11	-0.19	-0.23
Hg	-0.15	-0.06	0.27	1.00	0.12	0.29	0.36	0.08	0.41	0.19	0.38

(Table 6). Continued.

Pairs	Bi	Cr	Cs	Hg	K	Mo	Pb	Sb	Se	Sn	Zn
Ho	-0.05	-0.08	-0.27	-0.20	0.38	-0.17	-0.31	0.02	-0.06	-0.18	-0.24
K	0.12	-0.13	0.21	0.12	1.00	-0.32	-0.18	-0.25	0.28	-0.34	0.02
La	0.03	-0.22	0.03	-0.22	0.12	-0.13	-0.07	0.04	-0.16	0.19	-0.08
Li	-0.02	0.31	0.09	-0.01	-0.20	0.31	0.26	0.25	0.19	0.33	-0.14
Mg	0.03	-0.01	0.26	0.15	0.20	-0.18	0.10	0.37	0.16	-0.30	**0.57**
Mn	-0.14	0.05	0.01	0.02	0.10	0.20	0.05	0.28	0.08	-0.02	-0.08
Mo	-0.23	0.16	0.17	0.29	-0.32	1.00	0.15	0.32	-0.14	**0.61**	-0.05
Na	-0.08	0.24	0.31	-0.01	0.23	-0.36	-0.14	0.30	0.12	-0.28	0.25
Nb	-0.18	-0.02	-0.23	0.34	**-0.44**	**0.56**	0.05	0.39	-0.22	**0.45**	-0.32
Nd	**0.60**	-0.13	0.01	-0.16	0.19	-0.34	-0.17	-0.16	-0.06	-0.12	-0.22
Ni	-0.21	-0.15	-0.06	**0.45**	-0.28	**0.57**	0.39	-0.12	-0.16	0.05	0.07
P	0.04	0.05	0.01	0.37	0.21	-0.03	-0.05	-0.22	0.35	-0.02	**0.80**
Pb	0.23	-0.07	0.27	0.36	-0.18	0.15	1.00	0.09	-0.05	0.36	-0.01
Pr	**0.65**	-0.12	0.06	-0.08	0.16	-0.28	-0.03	-0.21	-0.05	-0.10	-0.19
Rb	0.10	0.40	**0.58**	**0.42**	**0.51**	0.03	0.19	0.34	0.35	-0.15	0.27
S	-0.16	0.27	0.16	-0.02	**0.68**	-0.28	-0.25	-0.09	**0.43**	-0.40	0.08
Sb	-0.28	0.26	0.07	0.08	-0.25	0.32	0.09	1.00	-0.08	0.17	-0.09
Sc	**0.59**	0.01	0.36	**0.52**	0.22	-0.18	0.14	0.03	**0.63**	-0.34	**0.63**
Se	-0.01	**0.42**	0.32	**0.41**	0.28	-0.14	-0.05	-0.08	1.00	-0.13	0.28
Si	**0.42**	-0.05	-0.02	-0.01	0.32	-0.23	-0.11	-0.15	0.12	-0.14	-0.17
Sm	**0.43**	-0.14	-0.07	-0.13	0.24	-0.27	-0.11	-0.08	-0.05	-0.16	-0.21
Sn	0.05	-0.21	0.08	0.19	-0.34	**0.61**	0.36	0.17	-0.13	1.00	-0.11
Sr	0.39	-0.01	0.09	-0.22	**-0.45**	0.15	0.18	-0.12	-0.05	0.24	-0.22
Tb	0.15	-0.19	-0.29	-0.23	0.28	-0.35	-0.33	-0.06	-0.06	-0.14	-0.22
Th	**0.53**	0.01	0.04	-0.20	-0.01	-0.35	-0.12	-0.19	0.04	-0.17	-0.16
Ti*	-0.01	0.06	-0.04	0.03	-0.02	0.16	-0.22	-0.24	-0.12	-0.05	0.17
Tl	-0.20	0.34	0.22	0.34	**0.46**	-0.25	-0.05	-0.16	**0.64**	-0.28	**0.50**
Tm	-0.14	-0.11	-0.28	-0.11	0.40	-0.23	-0.21	0.04	-0.10	-0.19	-0.21
U	-0.10	0.07	**0.45**	-0.01	-0.11	**0.56**	0.01	0.15	-0.29	0.40	0.00
Y	-0.03	-0.29	-0.15	0.01	0.04	-0.17	0.21	0.09	-0.21	0.37	-0.04
Yb	-0.08	-0.11	-0.35	-0.25	0.34	-0.30	-0.38	-0.03	-0.10	-0.17	-0.20
Zn	-0.07	0.03	0.15	0.38	0.02	-0.05	-0.01	-0.09	0.28	-0.11	1.00
Zr	-0.19	0.10	-0.13	-0.16	0.23	-0.33	-0.31	-0.17	0.09	-0.31	-0.07

Bold significant differences (p <0.05).

U, Y, and Zr) the big difference between M and Median was found (Tables **1** and **2**). It meant that for these trace elements the distribution of individual results were not normal. It was a reason why the difference in the results between cancerous and normal prostate was evaluated by both parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test (Tables **5**).

Tables **6** and **7** depict the data of inter-correlation calculations (values of r – coefficient of correlation)

Table 7: Intercorrelations of Selected Pairs of the Trace Element Mass Fractions in Cancerous Prostate (r – Coefficient of Correlation)

Pairs	Bi	Cr	Cs	Hg	K	Mo	Pb	Sb	Se	Sn	Zn
Ag	0.72	0.13	0.45	0.11	-0.13	0.55	-0.13	0.06	-0.16	0.61	-.0.17
Al	0.64	0.75	0.63	0.77	0.11	-0.10	0.13	0.04	-0.57	0.87	-0.39
Au	0.59	-0.16	0.67	0.80	0.01	0.50	0.38	0.11	0.08	0.92	-0.50
B	0.41	-0.20	0.39	0.78	0.49	0.60	0.33	0.09	-0.51	0.45	-0.11
Ba	0.44	0.24	0.22	0.51	-0.18	0.72	0.25	-0.28	0.06	0.70	-0.27
Be	0.63	-0.42	0.64	-0.13	0.02	0.62	0.39	0.07	-0.35	0.97	-0.54
Bi	1.00	0.66	0.80	0.32	0.17	0.10	-0.19	0.23	-0.95	0.71	-0.80
Br	0.73	0.32	0.63	-0.36	0.38	0.45	-0.01	0.10	-0.84	0.49	-0.16
Ca	-0.61	-0.86	-0.22	-0.56	0.31	-0.22	0.17	-0.29	0.74	-0.23	0.75
Cd	-0.58	-0.46	-0.32	-0.19	0.15	-0.66	0.01	-0.14	0.87	-0.30	0.84
Ce	0.38	-0.05	0.43	0.76	-0.18	0.57	0.41	-0.03	0.19	0.80	-0.48
Co	-0.97	0.09	-0.64	0.41	-0.68	-0.37	.0.21	-0.27	0.59	0.28	0.46
Cr	0.66	1.00	0.48	0.55	-0.16	-0.12	-0.46	0.15	-0.04	0.60	0.10
Cs	0.80	0.48	1.00	-0.13	0.56	-0.43	-0.25	0.23	-0.76	0.69	-0.43
Cu	0.42	-0.07	0.70	-0.68	0.49	-0.94	0.04	-0.03	-0.30	0.59	0.18
Dy	0.64	0.80	0.70	-0.16	0.12	0.04	0.13	-0.13	-0.28	0.88	-0.48
Er	0.67	-0.30	0.82	-0.14	0.35	-0.41	0.02	0.22	-0.33	0.88	-0.34
Fe	-0.32	0.31	-0.15	-0.08	0.33	0.51	0.28	0.06	-0.21	-0.32	-0.14
Gd	0.57	0.97	0.57	0.49	0.02	-0.25	-0.05	-0.29	-0.50	0.77	-0.43
Hg	0.32	0.55	-0.13	1.00	-0.77	0.03	0.07	0.17	0.42	0.86	0.22
Ho	-0.10	-0.45	-0.21	0.12	-0.45	0.79	0.92	-0.09	0.39	0.38	-0.22
K	0.17	-0.16	0.56	-0.77	1.00	-0.39	-0.28	-0.08	-0.30	0.01	0.30
La	-0.43	0.19	-0.73	0.20	-0.63	0.68	0.54	-0.34	0.19	-0.35	-0.08
Li	0.62	0.44	0.75	0.81	0.30	-0.27	0.24	0.07	-0.41	0.87	-0.35
Mg	-0.28	-0.44	-0.01	-0.67	0.36	-0.65	-0.23	0.18	0.53	-0.18	0.64
Mn	0.51	0.25	0.60	0.90	0.01	-0.29	0.07	-0.10	-0.10	0.75	-0.40
Mo	0.10	-0.12	-0.43	0.03	-0.39	1.00	0.79	-0.18	-0.24	0.35	-0.53
Na	0.06	-0.03	0,41	-0.75	0.92	-0.53	-0.54	-0.18	-0.25	-0.21	0.43
Nb	-	-	-	-	-	-	-	-	-	-	-
Nd	0.52	0.75	0.47	0.86	-0.04	-0.06	0.11	-0.10	-0.27	0.85	-0.41
Ni	0.48	-0.01	0.57	-0.34	0.16	0.50	0.07	-0.01	-0.80	0.75	-0.42
P	0.63	0.23	0.74	-0.38	0.56	-0.70	-0.50	0.02	-0.42	0.51	-0.08
Pb	-0.19	-0.46	-0.25	0.07	-0.28	0,79	1.00	-0.23	0.41	0.25	-0.09
Pr	0.55	0.53	0.41	0.75	-0.11	-0.34	-0.14	0.07	-0.45	0.73	-0.42
Rb	-0.84	-0.24	-0.50	0.01	0.07	-0.35	0.06	-0.33	0.56	-0.56	0.32
S	0.06	-0.03	0.15	-0.47	0.57	-0.72	-0.58	0.09	-0.23	-0.28	0.45
Sb	0.23	0.15	0.23	0.17	-0.08	-0.18	-0.23	1.00	-0.27	0.06	-0.27
Sc	0.96	0.05	0.94	0.20	0.96	-0.15	-0.68	-0.29	0.29	-0.73	0.24
Se	-0.95	-0.04	-0.76	0.42	-0.30	-0.24	0.41	-0.27	1.00	-0.40	0.71
Si	0.64	-0.21	0.82	-0.36	0.40	0.51	0.24	0.03	-0.68	0.80	-0.44

(Table 7). Continued.

Pairs	Bi	Cr	Cs	Hg	K	Mo	Pb	Sb	Se	Sn	Zn
Sm	0.33	0.72	0.51	0.44	0.03	-0.71	-0.06	0.63	-0.05	0.48	-0.36
Sn	0.71	0.60	0.69	0.86	0.01	0.35	0.25	0.06	-0.40	1.00	-0.58
Sr	-0.19	-0.12	0.16	-0.14	0.39	-0.96	-0.41	-0.10	0.47	-0.01	0.64
Tb	0.39	0.59	-0.24	0.73	-0.84	0.48	0.34	0.11	-0.24	0.93	-0.80
Th	0.54	0.22	0.60	0.90	0.19	0.04	0.24	0.06	-0.31	0.76	-0.32
Ti*	-0.01	0.13	-0.63	0.74	-0.97	0.59	0.69	0.17	0.15	0.77	-0.54
Tl	0.55	0.21	0.60	0.92	0.26	-0.15	0.30	0.24	-0.17	0.72	-0.28
Tm	0.83	0.07	0.55	0.56	-0.30	0.71	0.89	0.76	-0.69	0.67	-0.78
U	0.17	0.93	-0.01	0.23	-0.33	-0.20	-0.36	-0.28	-0.24	0.13	-0.31
Y	0.57	0.98	0.57	0.25	-0.01	0.04	0.03	0.04	-0.60	0.87	-0.47
Yb	-0.25	-0.51	0.07	-0.93	0.73	0.21	0.10	-0.49	-0.06	-0.78	0.42
Zn	-0.80	0.10	-0.43	0.22	0.30	-0.53	-0.09	-0.27	0.71	-0.58	1.00
Zr	-0.10	-0.15	-0.15	0.62	-0.11	-0.45	-0.36	0.49	0.04	-0.31	0.16

Bold significant differences ($p < 0.05$).

including pairs of selected Bi, Cr, Cs, Hg, K, Mo, Pb, Sb, Se, Sn, and Zn with all other chemical elements identified in normal and cancerous prostate glands, respectively.

DISCUSSION

The use of five analytical methods allowed us to estimate the mass fractions of 66 chemical elements in cancerous and normal nonhyperplastic prostate glands. Good agreement was found between the results obtained with non-destructive (EDXRF, NAA-SLR, and NAA-LLR) and destructive methods (ICP-AES and ICP-MS) for Ag, Br, Ca, Co, Cr, Fe, K, Mg, Mn, Na, Rb, Sb, Se, Sr, and Zn indicating complete digestion of the prostate samples (for ICP techniques) and correctness of all results obtained by the various methods. The fact that the elemental mass fractions (M ± SD) of the certified reference materials obtained in the present work were in good agreement with the certified values and within the corresponding 95% confidence intervals [15-20] suggests an acceptable accuracy of the measurements performed on our prostate samples (Tables **1** and **2**).

The mean values and all selected statistical parameters were calculated for 53 chemical element mass fractions: Ag, Al, Au, B, Ba, Be, Bi, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Fe, Gd, Hg, Ho, K, La, Li, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Pr, Rb, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr (Tables **1** and **2**). The mass fraction of these chemical elements were measured in all, or a major portion of normal prostate samples. The masses of PCa samples varied very strong from a few milligrams (sample from needle biopsy material) to 100 mg (sample from resected material). Therefore, in PCa prostates mass fractions of Ag, Br, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, Sr, and Zn were measured in all, or a major portion of samples, while mass fractions of other chemical elements were determined in 11 samples.

The mean values obtained for the chemical element mass fractions in normal nonhyperplastic prostate gland agree well with median of means reported in the literature for the normal prostate tissue of adult males, including samples obtained from persons who died from different diseases (Table **3**). The obtained mean values for Ca, Cd, Cr, Cu, Fe, K, Ni, P, Pb, Rb, Ti, and Zn mass fractions in cancerous gland, as shown in Table **4**, agree well with median of means or are inside the range of means cited by other researches. Mean value for Na and Sr mass fraction is somewhat higher than the maximum of previously reported mean values while mean value for Mg, Mn, S, and Se is somewhat lower than the minimum of reported means. The means of this work for B and Br are almost one and two orders of magnitude, respectively, higher than previously reported maximum, while mean value for Co mass fraction is three orders of magnitude lower than previously reported minimal result. No published data referring to Ag, Al, Au, Ba, Be, Bi, Ce, Cs, Dy, Er, Gd, Hg, Ho, La, Li, Mo, Nb, Nd, Pr, Sb, Sc, Si, Sm, Sn, Tb, Th, Tl, Tm, U, Y, Yb, and Zr mass fractions in cancerous prostate gland were found.

In the cancerous prostates a significant higher level of Ag, Al, Au, B, Ba, Be, Bi, Br, Ce, Cr, Cu, Dy, Er, Fe, Gd, Hg, Ho, Li, Mn, Nd, Ni, Pr, Sb, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, Y and Zr in comparison with the normal prostates was observed (Table **5**). For example, in prostate glands of patients with PCa the means of Ag, Al, Au, Ba, Be, Bi, Li, Mn, Sb, Th, Tl, and Zr mass fraction was almost 5-60 times greater than in controls. Such trace elements as Ag, Al, Au, Ba, Be, Bi, Br, Ce, Cr, Dy, Er, Fe, Gd, Hg, Ho, Li, Mn, Nd, Pr, Sb, Si, Sm, Sn, Tb, Th, Tl, Tm, Y, and Zr binds more tightly within the prostatic cells than within prostatic fluid [30-41]. Thus, because the major characteristic of malignancy is an uncontrolled cell proliferation, becomes clear why an increase in the prostatic Ag, Al, Au, Ba, Be, Bi, Br, Ce, Cr, Dy, Er, Fe, Gd, Hg, Ho, Li, Mn, Nd, Pr, Sb, Si, Sm, Sn, Tb, Th, Tl, Tm, Y, and Zr mass fractions has respect to the prostate cancer.

In contrary, the Ca, Mg, and Zn mass fractions were almost 3-8 times lower, and the Cd, Co, K, Na, P, Rb, S, Sc, and Se mass fractions were approximately 12-62%, lower in PCa tissue than in normal prostate (Table **5**). In our previous studies we demonstrated that the glandular lumen and, therefore, the prostatic fluid is the main pool of Ca, Mg, P, Rb, S, Se, Sr, and Zn accumulation in the normal human prostate [15-21. It was concluded that at least Ca, Mg, P, Rb, S, Se, and Zn are involved in functional features of prostate tissue. Because malignant transformation is accompanied by a loss of tissue-specific functional features, including the prostatic fluid production, this process leads to a significant reduction in the contents of chemical elements such as Ca, Mg, P, Rb, S, Se, and Zn associated with functional characteristics of the human prostate tissue. The biochemical reason behind the low levels of Cd, Co, K, Na, and Sc mass fractions in cancerous tissue requires further study for a more complete understanding.

No statistically significant differences between the mean values of all other chemical element mass fractions determined in this study (Cs, La, Mo, Nb, P, Pb, U, and Yb) for cancerous and normal prostates were shown (Table **1**).

In control group of males a statistically significant direct correlation was found, for example, between the prostatic Zn and Mg (r = 0.57), Zn and P (r = 0.80), Zn and Sc (r = 0.63), and Zn and Tl (r = 0.50) (Table **7**). In cancerous prostates some correlations between chemical elements found in the control group are no longer evident, for example, correlations for some pairs with Zn (Zn-P, Zn-Sc, and Zn-Tl), but many other direct correlations (Zn-Ca, Zn-Cd, Zn-Co, Zn-Mg, Zn-Na, Zn-S, Zn-Se, Zn-Sr, and Zn-Yb), as well as inverse correlations (Zn-Al, Zn-Au, Zn-Be, Zn-Bi, Zn-Ce, Zn-Cs, Zn-Dy, Zn-Gd, Zn-Mn, Zn-Mo, Zn-Nd, Zn-Ni, Zn-Pr, Zn-Si, Zn-Sm, Zn-Sn, Zn-Tb, Zn-Ti, Zn-Tm, and Zn-Y) are arisen (Table **7**). Thus, accepting the levels and relationships of chemical element mass fractions in prostate glands of males in the control group as a norm, it can be concluded that with a malignant transformation the levels and relationships of chemical elements in prostate drastically changed. No published data referring to correlations between chemical elements mass fractions in cancerous prostate gland were found.

Characteristically, elevated or deficient levels of chemical elements observed in cancerous tissues are discussed in terms of their potential role in the initiation, promotion, or inhibition of prostate cancer. Malignant transformation may disrupt the chemical elements balance as well as the chemical elements balance alterations can initiate and promote prostate cancer. Numerous *in vitro* and *in vivo* studies have evidenced that the disturbed homeostasis of Ca, Zn, Fe, and Se can play a very important role in the mechanism of malignant transformation [6,9-14,89]. The high level of Ca, Mg, Zn, Fe, Rb, Se and some other chemical element contents found just in the prostate gland [15-29] cannot be regarded as pure chance. It indicates that these elements must play here a very essential role for preserving the normal function of prostate cells and retaining the balance between their proliferation and physiological death (apoptosis).

In our opinion, abnormal low levels of Ca, Mg, Zn, Se, Rb and some other chemical elements in cancerous prostate could be the consequence of malignant transformation. The biochemical reason behind the low levels of Cd, Co, K, Na, P, S, and Sc mass fractions in cancerous tissue requires further study for a more complete understanding.

On the other hand, findings of this study documented fact that the cancerous prostate accumulates high levels of many chemical elements including Ag, Al, Au, B, Ba, Be, Bi, Br, Ce, Cr, Cu, Dy, Er, Fe, Gd, Hg, Ho, Li, Mn, Nd, Ni, Pr, Sb, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, Y and Zr. Genotoxicity and carcinogenicity some of these elements are well known [90-97]. Compared to human body soft tissues, the normal prostate of young adults has higher levels of many chemical elements, including Ca and Zn [21-25].

Moreover, the level of Ba, Bi, Ca, Cd, Co, Fe, Hg, Pb, Sc, Sn, Th, U, Zn and some other chemical elements continue to increase with age [15-20,26-29]. In our earlier publications [10-14] it was discussed in detail that the age-related excessive Ca and Zn level in prostatic tissue is probably one of the main factors influencing the initiation and progression of PCa. In addition to the elevated Ca and Zn level, an age-related increase and excess in Bi, Cd, Co, Cr, Fe, Hg, Pb, Sn, Th, and U mass fractions in prostatic tissue may contribute to harmful effects on the gland. There are good reasons for such speculations since many reviews and numerous papers raise the concern about toxicity and carcinogenicity of these and other metals [90-107]. Each of metals is distinct in its primary mode of action. Moreover, there are several forms of synergistic action of metals as a part of intracellular metabolism, during which several reactive intermediates and byproducts are created [98,99,106]. These reactive species are capable of potent and surprisingly selective activation of stress-signaling pathways, inhibition of DNA metabolism, repair, and formation of DNA crosslinks, which are known to contribute to the development of human cancers [99,100,105]. In addition to genetic damage via both oxidative and nonoxidative (DNA adducts) mechanisms, metals can also cause significant changes in DNA methylation and histone modifications, leading to alterations in gene expression [100-102]. *In vitro* and animal carcinogenic studies provided strong support for the idea that metals can also act as co-carcinogens in combination with nonmetal carcinogens [100].

Our findings showed that mass fraction of many chemical elements are significantly different in PCa tissue as compared to normal prostate (Table **5**). Thus, it is plausible to assume that levels of these chemical elements and their different combinations in prostate tissue can be used as tumor markers. However, this subject needs in additional studies.

CONCLUSION

The combination of nondestructive (EDXRF, INAA-SLR, and INAA-LLR) and destructive (ICP-AES and ICP-MS) methods is a satisfactory analytical tool for the precise determination of 53 chemical element mass fractions in the tissue samples of cancerous and normal prostate glands. The using five methods one by one allowed precise quantitative determinations of mean mass fraction of Ag, Al, Au, B, Ba, Be, Bi, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Fe, Gd, Hg, Ho, K, La, Li, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Pr, Rb, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr. It was observed that the mass fractions of all chemical elements investigated in the study with the exception of Cs, La, Mo, Nb, P, Pb, U, and Yb show significant variations in cancerous tissues when compared with normal tissues of the prostate. Moreover, it was shown that malignant transformation significantly changed the relationships of chemical elements in prostate. Thus, our finding of content and correlation between pairs of prostatic chemical element mass fractions, detailed above, indicates that there is a great disturbance of elemental metabolism in prostate malignancy. It was supposed that elevated levels of Ag, Al, Au, B, Ba, Be, Bi, Br, Ce, Cr, Cu, Dy, Er, Fe, Gd, Hg, Ho, Li, Mn, Nd, Ni, Pr, Sb, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, Y and Zr as well as reduced levels of Ca, Cd, Co, K, Mg, Na, P, Rb, S, Sc, Se, and Zn in prostatic tissue can be used as tumor markers.

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