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

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Abstract

The aim of the study was to evaluate whether significant changes in the prostatic tissue levels of ratios Mg/trace element contents exist in the malignantly transformed prostate. Contents of Mg and 43 trace elements in normal (N), benign hypertrophic (BPH) and cancerous human prostate (PCa) were investigated. Intact prostates of N group were removed at necropsy from 37 men aged 41-87 who had died suddenly. The patients of BPH and PCa groups were hospitalized in the Urological Department of the Medical Radiological Research Centre (Obninsk, Russia). The age of 32 patients with BPH ranged from 56 to 78 years. The 60 patients aged 40-79 suffered from PCa (stage T1-T4). In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. All deceased and patients were European-Caucasian, citizens of Moscow and Obninsk. Measurements of Mg and trace element contents were performed using a combination of non-destructive and destructive methods: instrumental neutron activation analysis, inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry. Then the levels of ratios Mg/trace element contents were calculated. It was observed that the ratio to Mg of Ag, Al, Au, B, Be, Bi, Br, Ca, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn,Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr mass fraction were significantly lower in cancerous tissues than in normal and BPH prostate. Finally, we propose to use the Mg/Ag, Mg/Al, Mg/B, Mg/Be, Mg/Cr, Mg/Fe, Mg/Li, Mg/Mn, Mg/Ni, and Mg/Sb mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer. Further studies on larger number of samples are required to confirm our findings and to investigate the impact of the trace element relationships on prostate cancer etiology.

Keywords: Trace Elements; Trace Element Content Ratios; Prostate; Benign Prostatic Hypertrophy; Prostatic Carcinoma; Neutron Activation Analysis; Inductively Coupled Plasma Atomic Emission Spectrometry; Inductively Coupled Plasma Mass Spectrometry

Introduction

The prostate gland may be a source of many health problems in men past middle age, the most common benign prostatic hyperplasia (BPH), and prostatic carcinoma (PCa). BPH is a noncancerous enlargement of the prostate gland leading to obstruction of the urethra and can significantly impair quality of life. The prevalence of histological BPH is found in approximately 50-60% of males age 40-50 and greater than 90% of men over 70 years old [1,2]. In many Western industrialized countries, including North America, PCa is the most frequently diagnosed form of noncutaneous malignancy in males. Except for lung cancer, PCa is the leading cause of death from cancer [3-8]. Although the etiology of BPH and PCa is unknown, some trace elements have been highlighted in the literature in relation to the development of these prostate diseases [9-29].

Trace elements have essential physiological functions such as maintenance and regulation of cell function and signalling, gene regulation, activation or inhibition of enzymatic reactions, neurotransmission, and regulation of membrane function. Essential or toxic (mutagenic, carcinogenic) properties of trace elements depend on tissue-specific need or tolerance, respectively [30]. Excessive accumulation, deficiency or an imbalance of the trace elements may disturb the cell functions and may result in cellular degeneration, death and malignant transformation [30]. In earlier reported studies [31-66] significant changes of trace element contents in hyperplastic and cancerous prostate in comparison with those in the normal prostatic tissue were observed. Moreover, a significant informative value of Mg content as a tumor marker for PCa diagnostics was shown by us [67,68]. Hence it is possible that besides Mg, trace elements also can be used as tumor markers for distinguish between benign and malignant prostate.

Currently number of methods was applied for the measurement of chemical elements contents in samples of human tissue. Among these methods, the instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) and long-lived radionuclides (INAA-LLR) is a non-destructive and one of the most sensitive techniques. It allows measure the trace element contents in few milligrams tissue without any treatment of sample. Analytical studies of the Ag, Br, Ca, Co, Cr, Fe, Hg, K, Mg, Mn, Na, Sb, Sc, Se, and Zn contents in normal, BPH and PCa tissue were done by us using INAA-SLR and INAA-LLR [14,15,20,27,28,53,54,60-62,64]. Nondestructive method of analysis avoids the possibility of changing the content of trace elements in the studied samples [69-72], which allowed for the first time to obtain reliable results. In particular, it was shown that the average mass fraction of Co, Cr, Hg, Sb, and Se in BPH were higher than normal levels [66]. In adenocarcinoma of prostate the mean values of Ag, Br, Cr, Fe, Hg, Mn, and Sb were higher, while those of Ca, Co, Mg, Rb, Sc, and Zn were lower than in healthy prostatic tissue [60,61,67,68]. Obtained results formed the basis for a new method for differential diagnosis of BPH and PCa, the essence of which was to determine the ratios of chemical element contents changed in opposite directions during malignant transformation of prostate.

It is obvious that the most effective will be non-destructive analytical methods, because they involve a minimal treatment of sample, since the chances of significant loss or contamination would be decreased. However, the INAA allow only determine the mean mass fractions of 15-16 chemical elements in the samples of normal and cancerous prostate glands [14,15,20,27,28,60,66]. The inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) are more power analytical tools than INAA [17,18], but sample digestion is a critical step in elemental analysis by these methods. In the present study all these analytical methods were used and the results, obtained for some chemical elements by ICP-AES and ICP-MS, were under the control of INAA data.

The present study had three aims. The main objective was to obtain reliable results about the 44 chemical elements: Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mg, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr contents in intact prostate of healthy men aged over 40 years and also in the prostate gland of age-matched patients, who had either BPH or PCa, combining in consecutive order non-destructive INAA with destructive ICP methods. The second aim was to calculate Mg/trace element content ratios and compare the levels of these ratios in normal, hyperplastic, and cancerous prostate. The third and final aim was to evaluate the ratios of Mg/trace element contents for diagnosis of prostate cancer.

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

Material and Methods

Samples

The patients studied (n=92) were hospitalized in the Urological Department of the Medical Radiological Research Centre (Obninsk, Russia). All of them were European-Caucasian, citizens of Moscow and Obninsk (a small city in a non-industrial region 105 km south-west of Moscow). 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 prostates (N) were removed at necropsy from 37 men aged 41-87 who had died suddenly. All deceased were European-Caucasian, citizens of Moscow. Their mean age was 55 ± 11 (M ± SD) years. The majority of deaths were due to trauma. Tissue samples were collected from the peripheral zone of dorsal and lateral lobes of their prostates, within 2 days of death and then the samples were divided into two portions. One was used for morphological study while the other was intended for chemical element analysis. A histological examination was used to control the age norm conformity, as well as to confirm the absence of microadenomatosis and latent cancer [14,15,20,28].

Sample preparation

All tissue samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis. After the samples intended for trace element analysis were weighed, they were freeze-dried and homogenized. The sample weighing about 10 mg (for biopsy materials) and 50-100 mg (for resected materials) was used for Mg measurement by INAA-SLR. The samples for INAA-SLR were sealed separately in thin polyethylene films washed beforehand with acetone and rectified alcohol. The sealed samples were placed in labeled polyethylene ampoules.

After INAA-SLR investigation, the prostate samples were taken out from the polyethylene ampoules and used for trace element measurement by INAA-LLR. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with double rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule. After INAA-LLR investigation, the prostate samples were taken out and used for ICP methods. 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 the plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without tissue samples (only HNO_3+H_2O_2+ deionized water), and the resultant solutions were used as control samples.

Instrumentation and Methods

A horizontal channel, equipped with the pneumatic rabbit system of the WWR-C research nuclear reactor, was applied to determine the mass fraction of Mg by INAA-SLR. The neutron flux in the channel was 1.7×10^{13}n cm^{-2} s^{-1}. Ampoules with prostate samples, biological synthetic standards [73], intralaboratory-made standards, and certified reference material (CRM) were put into polyethylene rabbits and then irradiated separately for 180 s. Copper foils were used to assess neutron flux. The measurement of each sample was made 1 min after irradiation. The duration of the measurement was 10 min.

A vertical channel of a nuclear reactor was applied to determine the trace element mass fractions by INAA-LLR. The quartz ampoule with prostate samples and certified reference materials was soldered, positioned in a transport aluminum container, and exposed to a 24-hour neutron irradiation in a vertical channel with a neutron flux of $1.3 \cdot 10^{13}$ $n \cdot cm^{-2} \cdot s^{-1}$. Ten days after irradiation samples were reweighed and repacked. The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 min to 10 hours subject to pulse counting rate.

The gamma spectrometer used for INAA-SLR and INAA-LLR included the 100 cm^3 Ge (Li) detector and on-line computer-based multichannel analyzer. The spectrometer provided a resolution of 1.9 keV on the ^{60}Co 1332 keV line.

Information detailing with the INAA-SLR and INAA-LLR methods used and other details of the analysis was presented in our previous publication [14,15].

Aliquots of aqueous solutions were used to determine the Mg mass fractions by ICP-AES using the Spectrometer ICAP-61 (Thermo Jarrell Ash, USA). Integration time of the spectrum during measurement was 5 s. The determination of the Mg content in aqueous solutions was made by the quantitative method using calibration solutions (High Purity Standards, USA) of 0.5 and 10 mg/L. The calculations of the Mg content in the probe were carried out using software of a spectrometer (ThermoSPEC, version 4.1).

An ICP-MS Thermo-Fisher “X-7” Spectrometer (Thermo Electron, USA) was used to determine the content of trace elements by ICP-MS. The element concentrations in aqueous solutions were determined by the quantitative method using multi elemental calibration solutions ICP-MS-68A and ICP-AM-6-A produced by High-Purity Standards (Charleston, SC 29423, USA). Indium was used as an internal standard in all measurements. Information detailing with the ICP-AES and ICP-MS methods used and other details of the analysis was presented in our previous publication [17,18].

Certified Reference Materials

For quality control, ten subsamples of the certified reference materials (CRM) IAEA H-4 Animal muscle and IAEA HH-1 Human hair from the International Atomic Energy Agency (IAEA), and also five sub-samples INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2 Mixed Polish Herbs from the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) were analyzed simultaneously with the investigated prostate tissue samples. All samples of CRMs were treated in the same way as the prostate samples. Detailed results of this quality assurance program were presented in earlier publications [14,15,17,18].

Computer Programs and Statistic

A dedicated computer program for INAA mode optimization was used [74]. All prostate samples for INAA-SLR and INAA-LLR were prepared in duplicate and mean values of chemical element contents were used in final calculation. For elements investigated by INAA-SLR, INAA-LLR, ICP-AES, and ICP-MS the mean of all results was used. Using the Microsoft Office Excel software Mg/trace element contents for each trace element in every sample were calculated. Then arithmetic mean ± standard error of mean were calculated for chemical element mass fraction and for ratios of Mg/trace element mass fraction in normal, benign hyperplastic and cancerous prostate. The difference in the results between BPH and N, PCa and N, as well as PCA and BPH was evaluated by parametric Student’s t-test and non-parametric Wilcoxon-Mann-Whitney U-test. Values of $p<0.05$ were considered to be statistically significant. For the construction of “individual data sets for Mg/trace element mass fraction ratios in normal, benign hypertrophic and cancerous prostate” diagrams the Microsoft Office Excel software was also used.

Results

Tables 1, 2, and 3 depict our data for chemical element mass fractions in CRMs measured using INAA, ICP-AES, and ICP-MS, respectively, as well as the certified values of these materials.

Table 4 represents mean values ± standard error of mean (M ± SEM) of the Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mg, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr mass fraction in normal, benign hypertrophic and cancerous prostate.

Element	IAEA H-4 animal muscle Certificate	This work results	IAEA HH-1 human hair Certificate	This work results
Ag	-	0.033 ± 0.008	0.19[a]	0.18 ± 0.05
Au	0.07[a]	<0.01	0.03[a]	0.028 ± 0.009
Br	4.1 ± 1.1	5.0 ± 0.9	4.2[a]	3.9 ± 1.6
Cd	0.04[a]	<2	0.26[a]	0.28 ± 0.11
Ce	0.02[a]	<0.1	-	≤ 0.4
Co	0.003[a]	0.0034 ± 0.0008	6.0 ± 1.2	5.4 ± 1.1
Cr	0.06[a]	0.071 ± 0.010	0.27[a]	≤ 0.3
Cs	0.12[a]	<0.05	-	≤ 0.01
Fe	49.1 ± 6.5	47.0 ± 1.0	23.7 ± 9.8	25.1 ± 4.3
Gd	-	<0.02	-	≤ 0.1
Hg	0.014[a]	0.015 ± 0.004	1.7 ± 0.2	1.54 ± 0.14
La	0.01[a]	<0.5	0.01[a]	≤ 0.2
Mg	1050 ± 140	1100 ± 190	62[a]	64.7 ± 18.6
Mn	0.52[a]	0.55 ± 0.11	0.85 ± 0.25	0.93 ± 0.16
Nd	-	<0.1	-	≤ 0.2
Rb	18.7 ± 3.5	23.7 ± 3.7	0.94[a]	0.89 ± 0.17
Sb	0.006[a]	0.0061 ± 0.0021	0.03[a]	0.033 ± 0.009
Sc	0.0006[a]	0.0015 ± 0.0009	-	≤ 0.01
Se	0.28 ± 0.08	0.281 ± 0.014	0.35 ± 0.04	0.37 ± 0.08
Sm	-	<0.01	-	≤ 0.01
Tb	-	<0.03	-	≤ 0.005
Th	-	<0.05	-	≤ 0.02
U	-	<0.07	-	≤ 0.006
Yb	-	<0.03	-	≤ 0.005
Zn	86.3 ± 11.5	91 ± 2	174 ± 32	173 ± 17
Zr	-	<0.3	-	<0.1

M – arithmetical mean, SD – standard deviation, a – information values

Table 1: INAA data (M ± SD) of chemical element mass fraction in certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) compared to certified values (mg/kg, dry mass basis)

Element	Soya Bean Flour (INCT-SBF-4)		Tea Leaves (INCT-TL-1)		Mixed Polish Herbs (INCT-MPH-2)	
	Certificate	This work Result	Certificate	This work Result	Certificate	This work Result
Al	45.5 ± 3.7	37.1 ± 1.4	2290 ± 280	2248 ± 61	670 ± 111	485 ± 79
B	39.9 ± 4.0	34.5 ± 1.4	26[a]	24.8 ± 1.2	-	28.8 ± 8.1
Fe	90.8 ± 4.0	80.5 ± 6.9	432[a]	493 ± 39	460[a]	459 ± 33
Li	-	0.0047 ± 0.0018	-	0.217 ± 0.034	-	0.574 ± 0.044
Mg	3005 ± 82	2983 ± 340	2240 ± 170	2415 ± 115	2920 ± 180	2955 ± 159
Mn	32.3 ± 1.1	30.0 ± 1.0	1570 ± 110	1628 ± 145	191 ± 12	197 ± 5
Zn	52.3 ± 1.3	54.8 ± 6.6	34.7 ± 2.7	36.0 ± 3.7	33.5 ± 2.1	32.0 ± 6.1

M – Arithmetical mean, SD – standard deviation, a- information values.

Table 2: ICP-AES data (M ± SD) of chemical element contents in Certified Reference Materials (mg/kg, dry mass basis)

Table 5 depicts mean values ± standard error of mean (M ± SEM) of the ratio to Mg of Ag, Al, Au, B, Be, Bi, Br, Ca, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr mass fraction in normal, benign hypertrophic and cancerous prostate.

The ratios of means and the difference between mean values of the Mg/trace element mass fraction ratios in normal, benign hypertrophic and cancerous prostate are presented in Table 6.

Individual data sets for Mg/Ag, Mg/Al, Mg/B, Mg/Be, Mg/Cr, Mg/Fe, Mg/Li, Mg/Mn, Mg/Ni, and Mg/Sb mass fraction ratios in all investigated samples of normal, benign hypertrophic and cancerous prostate, respectively, are shown in Figure 1.

Table 7 contains parameters of the importance (sensitivity, specificity and accuracy of Mg/Ag, Mg/Al, Mg/B, Mg/Be, Mg/Cr, Mg/Fe, Mg/Li, Mg/Mn, Mg/Ni, and Mg/Sb mass fraction ratios for the diagnosis of PCa calculated in this work.

Element	Soya Bean Flour (INCT-SBF-4)		Tea Leaves (INCT-TL-1)		Mixed Polish Herbs INCT-MPH-2	
	Certificate	This work	Certificate	This work	Certificate	This work
Ag	-	0.0034 ± 0.0008	-	≤ 0.0064	-	<0.001(DL)
Al	45.5 ± 3.7	37.1 ± 1.4	2290 ± 280	2248 ± 61	670 ± 111	485 ± 79
Au	-	<0.001(DL)	-	<0.001(DL)	-	<0.001(DL)
B	39.9 ± 4.0	34.5 ± 1.4	26a	24.8 ± 1.2	-	28.8 ± 8.1
Be	-	0.0021 ± 0.0019	-	0.020 ± 0.004	-	0.021 ± 0.002
Bi	-	<0.001(DL)	-	0.010 ± 0.002	-	0.07 ± 0.002
Br	2.40 ± 0.17	4.70 ± 0.64	12.3 ± 1.0	6.8 ± 1.6	7.71 ± 0.61	< 7.0(DL)
Cd	-	0.0208 ± 0.0045	0.030 ± 0.004	0.023 ± 0.004	0.199 ± 0.015	0.194 ± 0.0035
Ce	-	0.0364 ± 0.0057	0.79 ± 0.08	0.74 ± 0.07	1.12 ± 0.10	1.12 ± 0.20
Co	0.096 ± 0.006	0.0908 ± 0.0080	0.39 ± 0.04	0.37 ± 0.04	0.210 ± 0.025	1.92 ± 0.009
Cr	-	≤ 0.5	1.9 ± 0.2	1.7 ± 0.4	1.69 ± 0.13	1.60 ± 0.37
Cs	0.130 ± 0.004	0.1253 ± 0.0057	3.91 ± 0.37	3.65 ± 0.19	0.076 ± 0.007	0.063 ± 0.005
Dy	-	0.0014 ± 0.0002	-	0.167 ± 0.010	-	0.055 ± 0.008
Er	-	0.0007 ± 0.0001	-	0.098 ± 0.006	-	0.027 ± 0.003
Gd	-	0.0018 ± 0.0004	-	0.190 ± 0.010	-	0.076 ± 0.018
Hg	-	<0.02(DL)	0.005 ± 0.001	≤ 0.022	0.018 ± 0.002	0.019 ± 0.005
La	0.019 ± 0.002	0.0144 ± 0.0045	1.00 ± 0.07	0.95 ± 0.05	0.57 ± 0.05	0.54 ± 0.11
Li	-	0.0047 ± 0.0018	-	0.217 ± 0.034	-	0.574 ± 0.044
Mn	32.3 ± 1.1	30.0 ± 1.0	1570 ± 110	1628 ± 145	191 ± 12	197 ± 5
Mo	5.99 ± 0.35	5.66 ± 0.28	-	0.052 ± 0.009	0.52a	0.53 ± 0.01
Nb	-	0.0057 ± 0.0023	-	0.044 ± 0.031	-	0.032 ± 0.001
Nd	-	0.0119 ± 0.0036	0.81a	0.81 ± 0.06	0.46 ± 0.09	0.47 ± 0.10
Ni	3.12 ± 0.18	2.57 ± 0.20	6.1 ± 0.5	5.3 ± 0.7	1.57 ± 0.16	1.62 ± 0.10
Pb	-	0.068 ± 0.023	1.8 ± 0.2	1.5 ± 0.3	2.16 ± 0.23	2.07 ± 0.32
Pr	-	0.0027 ± 0.0007	-	0.200 ± 0.013	-	0.124 ± 0.027
Rb	31.7 ± 1.7	30.8 ± 2.8	81.5 ± 6.5	80.9 ± 6.7	10.7 ± 0.07	10.9 ± 0.4
Sb	-	0.0067 ± 0.0044	0.050a	0.032 ± 0.011	0.065 ± 0.009	0.053 ± 0.014
Se	-	<0.1(DL)	0.076a	≤ 0.12	-	0.088 ± 0.026
Sm	-	0.0018 ± 0.0006	0.18 ± 0.02	0.17 ± 0.01	0.094 ± 0.008	0.087 ± 0.021
Sn	-	<0.03(DL)	-	0.35 ± 0.06	-	-
Tb	-	0.00023 ± 0.00004	0.027 ± 0.002	0.028 ± 0.002	0.014 ± 0.001	0.010 ± 0.002
Th	0.007 ± 0.001	0.0069 ± 0.0030	0.034 ± 0.005	0.029 ± 0.011	0.154 ± 0.013	0.136 ± 0.022
Ti	-	0.93 ± 0.15	30a	32 ± 6	34a	20.7 ± 4.9
Tl	-	0.0011 ± 0.0002	0.063 ± 0.005	0.065 ± 0.003	0.029a	0.032 ± 0.002
Tm	-	0.00011 ± 0.00002	0.017a	0.015 ± 0.001	-	0.0037 ± 0.0003
U	-	0.0012 ± 0.0007	-	0.009 ± 0.001	0.049a	0.038 ± 0.011
Y	-	0.0069 ± 0.0011	-	0.904 ± 0.098	-	0.271 ± 0.032
Yb	-	0.0004 ± 0.0001	0.120 ± 0.013	0.104 ± 0.007	0.053 ± 0.007	0.023 ± 0.002
Zn	52.3 ± 1.3	54.8 ± 6.6	34.7 ± 2.7	36.0 ± 3.7	33.5 ± 2.1	32.0 ± 6.1
Zr	-	0.0295 ± 0.0093	-	0.30 ± 0.12	-	0.400 ± 0.040

M- arithmetical mean, SD- standard deviation, a-information values.

Table 3: ICP-MS data (M ± SD) for chemical element contents in Certified Reference Materials (mg/kg, dry mass basis)

Element	Symbol	Prostatic tissue		
		N 41-87 year (n=37)	BPH 56-78 year (n=32)	PCa 40-79 year (n=60)
Silver	**Ag**	0.038 ± 0.006	0.0415 ± 0.0090	0.252 ± 0.030
Aluminum	**Al**	34.2 ± 3.5	24.4 ± 3.2	328 ± 73
Gold	**Au**	0.0041 ± 0.0008	0.00257 ± 0.00077	0.0297 ± 0.0056
Boron	**B**	1.04 ± 0.18	1.51 ± 0.26	12.6 ± 3,7
Berillium	**Be**	0.00094 ± 0.00007	0.000918 ± 0.000042	0.0137 ± 0.0022
Bismuth	**Bi**	0.029 ± 0.011	0.140 ± 0.042	1.75 ± 0.27
Bromine	**Br**	27.9 ± 2.9	30.6 ± 3.4	99.9 ± 8.9
Cadmium	**Cd**	1.12 ± 0.13	1.07 ± 0.43	0.425 ± 0.099
Cerium	**Ce**	0.0309 ± 0.0050	0.0128 ± 0.0019	0.101 ± 0.013
Cobalt	**Co**	0.0467 ± 0.0064	0.0617 ± 0.0084	0.0336 ± 0.0040
Cromium	**Cr**	0.56 ± 0.08	1.00 ± 0.10	2.34 ± 0.32
Cesium	**Cs**	0.0339 ± 0.0033	0.0235 ± 0.0025	0.0389 ± 0.0039
Dysprosium	**Dy**	0.00293 ± 0.00049	0.00156 ± 0.00024	0.00771 ± 0.00110
Erbium	**Er**	0.00148 ± 0.00023	0.00072 ± 0.00013	0.00297 ± 0.00038
Iron	**Fe**	111 ± 9	133 ± 11	165 ± 15
Gadolinium	**Gd**	0.00290 ± 0.00041	0.00153 ± 0.00027	0.00945 ± 0.00173
Mercury	**Hg**	0.052 ± 0.008	0.259 ± 0.029	0.122 ± 0.019
Holmium	**Ho**	0.00057 ± 0.00008	0.00032 ± 0.00005	0.00178 ± 0.00022
Lanthanum	**La**	0.080 ± 0.020	0.0385 ± 0.0073	0.969 ± 0.537
Lithium	**Li**	0.0419 ± 0.0055	0.0385 ± 0.0073	0.251 ± 0.054
Magnesium	**Mg**	1071 ± 409	1201 ± 276	346 ± 193
Manganese	**Mn**	1.34 ± 0.08	1.19 ± 0.09	6.99 ± 1.35
Molybdenum	**Mo**	0.282 ± 0.038	0.167 ± 0.009	0.298 ± 0.035
Niobium	**Nb**	0.0054 ± 0.0012	0.0102 ± 0.0079	0.0052 ± 0.0002
Neodymium	**Nd**	0.0137 ± 0.0021	0.0062 ± 0.0009	0.0413 ± 0.0065
Nickel	**Ni**	3.10 ± 0.51	3.22 ± 1.06	6.96 ± 1.04
Lead	**Pb**	2.39 ± 0.56	0.69 ± 0.16	1.81 ± 0.35
Praseodymium	**Pr**	0.00353 ± 0.00053	0.00149 ± 0.00027	0.00973 ± 0.00174
Rubidium	**Rb**	13.3 ± 0.9	14.3 ± 0.8	8.71 ± 0.66
Antimony	**Sb**	0.043 ± 0.006	0.163 ± 0.036	0.490 ± 0.059
Scandium	**Sc**	0.0294 ± 0.0053	0.0257 ± 0.0040	0.0116 ± 0.0015
Selenium	**Se**	0.75 ± 0.05	1.11 ± 0.07	0.56 ± 0.08
Samarium	**Sm**	0.0027 ± 0.0004	0.0014 ± 0.0004	0.0095 ± 0.0029
Tin	**Sn**	0.32 ± 0.06	0.108 ± 0.029	1.28 ± 0.24
Terbium	**Tb**	0.00039 ± 0.00006	0.00017 ± 0.00003	0.00089 ± 0.00012
Thorium	**Th**	0.0033 ± 0.0007	0.0018 ± 0.0003	0.0495 ± 0.0123
Titanium*	**Ti***	2.82 ± 0.64	1.52 ± 0.20	8.60 ± 2.20
Thallium	**Tl**	0.0014 ± 0.0001	0.00202 ± 0.00057	0.0219 ± 0.0056
Thulium	**Tm**	0.00024 ± 0.00003	0.000151 ± 0.000021	0.000535 ± 0.000111
Uranium	**U**	0.0070 ± 0.0021	0.0021 ± 0.0009	0.0068 ± 0.0013
Yttrium	**Y**	0.0187 ± 0.0043	0.0071 ± 0.0012	0.0340 ± 0.0038
Ytterbium	**Yb**	0.00141 ± 0.00025	0.00083 ± 0.00020	0.00174 ± 0.00039
Zinc	**Zn**	1031 ± 129	1271 ± 102	136 ± 10
Zirconium	**Zr**	0.036 ± 0.006	0.091 ± 0.036	2.13 ± 0.89

M – arithmetic mean, SEM – standard error of mean, * Titanium tools were used for sampling and sample preparation.

Table 4: Mean values (M ± SEM) of the Mg and trace element mass fraction (mg/kg, dry mass basis) in normal (N), benign hypertrophic (BPH) and cancerous prostate (PCa)

Ratio	Prostatic tissue		
	N 41-87 year (n=37)	BPH 56-78 year (n=32)	PCa 40-79 year (n=60)
Mg/Ag	45882 ± 7979	77304 ± 24860	2084 ± 632
Mg/Al	34.7 ± 4.0	58.8 ± 9.0	2.78 ± 1.05
Mg/Au	477538 ± 97866	683146 ± 107771	41286 ± 22819
Mg/B	1505 ± 230	928 ± 138	70.1 ± 44.2
Mg/Be	1124274 ± 97104	1335532 ± 105223	57202 ± 29718
Mg/Bi	227304 ± 47169	54014 ± 25787	5023 ± 4473
Mg/Br	48.2 ± 4.9	44.8 ± 7.1	6.50 ± 3.19
Mg/Cd	1201 ± 206	2399 ± 525	940 ± 188
Mg/Ce	54193 ± 11814	114250 ± 18556	5146 ± 1650
Mg/Co	30658 ± 4480	24923 ± 2809	7296 ± 1651
Mg/Cr	4887 ± 1723	1373 ± 158	92.9 ± 27.5
Mg/Cs	32518 ± 2609	53517 ± 4287	10996 ± 2645
Mg/Dy	570235 ± 106723	1004475 ± 191123	77974 ± 21447
Mg/Er	976527 ± 153950	2330432 ± 476255	149598 ± 31562
Mg/Fe	11.1 ± 1.3	9.4 ± 1.1	2.79 ± 0.59
Mg/Gd	516119 ± 98700	1041451 ± 206325	63731 ± 17544
Mg/Hg	31626 ± 3616	6013 ± 871	2450 ± 1444
Mg/Ho	2550270 ± 406337	4966961 ± 927171	252793 ± 71965
Mg/La	43922 ± 8890	76504 ± 10768	4792 ± 2438
Mg/Li	29449 ± 3522	40126 ± 5905	3030 ± 1042
Mg/Mn	718 ± 55	1077 ± 114	107 ± 42
Mg/Mo	4709 ± 578	7445 ± 720	1365 ± 563
Mg/Nb	387685 ± 79965	607864 ± 123479	106408 ± 19992
Mg/Nd	115341 ± 24754	243414 ± 43375	11935 ± 3339
Mg/Ni	757 ± 229	654 ± 132	67.8 ± 21.1
Mg/Pb	1558 ± 308	2684 ± 519	319 ± 114
Mg/Pr	473877 ± 112421	1326208 ± 427152	57638 ± 16009
Mg/Rb	90.6 ± 11.8	81.4 ± 4.2	40.7 ± 7.0
Mg/Sb	44319 ± 6879	24467 ± 8458	1215 ± 271
Mg/Sc	67639 ± 16226	54591 ± 12346	25943 ± 6137
Mg/Se	1488 ± 137	1351 ± 111	438 ± 127
Mg/Sm	557154 ± 104719	1746600 ± 469494	73107 ± 20995
Mg/Sn	7598 ± 1406	18494 ± 3377	747 ± 347
Mg/Tb	4882114 ± 1121212	8581082 ± 1158279	470802 ± 173856
Mg/Th	594589 ± 134294	887274 ± 158562	25962 ± 14058
Mg/Ti*	732 ± 124	1026 ± 198	81.3 ± 42.6
Mg/Tl	876209 ± 95561	847459 ± 103861	72860 ± 49121
Mg/Tm	6328454 ± 1574079	10834685 ± 2365339	907110 ± 448411
Mg/U	480188 ± 81246	1205251 ± 254466	66421 ± 13925
Mg/Y	185419 ± 73363	231202 ± 46453	12040 ± 2803
Mg/Yb	1335884 ± 305428	2904607 ± 873412	205148 ± 48272
Mg/Zn	1.38 ± 0.12	1.05 ± 0.14	2.57 ± 0.45
Mg/Zr	45578 ± 7773	41313 ± 14271	423 ± 121
Zr	0.036 ± 0.006	0.091 ± 0.036	2.13 ± 0.89

M – arithmetic mean, SEM – standard error of mean, * Titanium tools were used for sampling and sample preparation.

Table 5: Mean values (M ± SEM) of the Mg mass fraction/ trace element mass fraction ratios in normal (N), benign hypertrophic (BPH) and cancerous prostate (PCa)

	BPH and N			PCa and N			PCa and BPH		
	Ratio BPH/N	p ≤ t-test	p U-test	Ratio PCa/N	p ≤ t-test	p U-test	Ratio PCa/BPH	p ≤ t-test	p U-test
Mg/Ag	1.68	0.251	>0.05	0.045	**0.00001**	**≤0.01**	0.027	**0.013**	**≤0.01**
Mg/Al	1.69	**0.029**	**≤0.01**	0.080	**0.00001**	**≤0.01**	0.047	**0.00014**	**≤0.01**
Mg/Au	1.43	0.171	>0.05	0.086	**0.00032**	**≤0.01**	0.060	**0.00012**	**≤0.01**
Mg/B	0.62	**0.040**	**≤0.01**	0.047	**0.00001**	**≤0.01**	0.076	**0.00017**	**≤0.01**
Mg/Be	1.19	0.152	>0.05	0.051	**0.00001**	**≤0.01**	0.043	**0.00001**	**≤0.01**
Mg/Bi	0.24	**0.0030**	**≤0.01**	0.022	**0.00011**	**≤0.01**	0.093	**0.089**	**≤0.01**
Mg/Br	0.93	0.695	>0.05	0.135	**0.00001**	**≤0.01**	0.145	**0.00023**	**≤0.01**
Mg/Cd	2.00	0.053	>0.05	0.783	0.357	>0.05	0.392	**0.022**	**≤0.01**
Mg/Ce	2.11	**0.014**	**≤0.01**	0.095	**0.00043**	**≤0.01**	0.045	**0.00015**	**≤0.01**
Mg/Co	0.81	0.286	>0.05	0.238	**0.00004**	**≤0.01**	0.293	**0.00019**	**≤0.01**
Mg/Cr	0.28	0.054	**≤0.01**	0.019	**0.011**	**≤0.01**	0.068	**0.00002**	**≤0.01**
Mg/Cs	1.65	0.0006	>0.05	0.338	**0.00001**	**≤0.01**	0.205	**0.00001**	**≤0.01**
Mg/Dy	1.76	0.064	**≤0.01**	0.137	**0.00014**	**≤0.01**	0.078	**0.00066**	**≤0.01**
Mg/Er	2.39	**0.019**	**≤0.01**	0.153	**0.00002**	**≤0.01**	0.064	**0.0010**	**≤0.01**
Mg/Fe	0.85	0.326	>0.05	0.251	**0.00001**	**≤0.01**	0.297	**0.00006**	**≤0.01**
Mg/Gd	2.02	**0.037**	**≤0.01**	0.123	**0.00015**	**≤0.01**	0.061	**0.00078**	**≤0.01**
Mg/Hg	0.19	**0.00001**	**≤0.01**	0.077	**0.00001**	**≤0.01**	0.407	0.072	≤0.05
Mg/Ho	1.95	**0.032**	**≤0.01**	0.099	**0.00001**	**≤0.01**	0.051	**0.00047**	**≤0.01**
Mg/La	1.74	**0.029**	**≤0.01**	0.109	**0.00024**	**≤0.01**	0.063	**0.00007**	**≤0.01**
Mg/Li	1.36	0.139	>0.05	0.103	**0.00001**	**≤0.01**	0.076	**0.00008**	**≤0.01**
Mg/Mn	1.50	**0.012**	**≤0.01**	0.149	**0.00001**	**≤0.01**	0.099	**0.00001**	**≤0.01**
Mg/Mo	1.58	**0.0070**	**≤0.01**	0.290	**0.00097**	**≤0.01**	0.183	**0.00001**	**≤0.01**
Mg/Nb	1.57	0.151	>0.05	0.274	**0.0024**	**≤0.01**	0.175	**0.0023**	**≤0.01**
Mg/Nd	2.11	**0.020**	**≤0.01**	0.103	**0.00044**	**≤0.01**	0.049	**0.00032**	**≤0.01**
Mg/Ni	0.86	0.699	>0.05	0.090	**0.0065**	**≤0.01**	0.104	**0.0012**	**≤0.01**
Mg/Pb	1.72	0.079	**≤0.01**	0.205	**0.00076**	**≤0.01**	0.119	**0.00099**	**≤0.01**
Mg/Pr	2.80	0.079	**≤0.01**	0.122	**0.0013**	**≤0.01**	0.043	**0.014**	**≤0.01**
Mg/Rb	0.90	0.469	>0.05	0.449	**0.00084**	**≤0.01**	0.500	**0.00017**	**≤0.01**
Mg/Sb	0.55	0.081	**≤0.01**	0.027	**0.00001**	**≤0.01**	0.050	**0.021**	**≤0.01**
Mg/Sc	0.81	0.531	>0.05	0.384	**0.029**	**≤0.01**	0.475	0.087	≤0.05
Mg/Se	0.91	0.438	>0.05	0.294	**0.00004**	**≤0.01**	0.324	**0.00028**	**≤0.01**
Mg/Sm	3.13	**0.031**	**≤0.01**	0.131	**0.00013**	**≤0.01**	0.042	**0.0051**	**≤0.01**
Mg/Sn	2.43	**0.010**	**≤0.01**	0.098	**0.00007**	**≤0.01**	0.040	**0.00036**	**≤0.01**
Mg/Tb	1.76	**0.030**	**≤0.01**	0.096	**0.00074**	**≤0.01**	0.055	**0.00003**	**≤0.01**
Mg/Th	1.49	0.172	>0.05	0.044	**0.00035**	**≤0.01**	0.029	**0.00028**	**≤0.01**
Mg/Ti*	1.40	0.224	>0.05	0.111	**0.00004**	**≤0.01**	0.079	**0.00072**	**≤0.01**
Mg/Tl	0.97	0.840	>0.05	0.083	**0.00001**	**≤0.01**	0.086	**0.00002**	**≤0.01**
Mg/Tm	1.71	0.129	>0.05	0.143	**0.0029**	**≤0.01**	0.084	**0.0018**	**≤0.01**
Mg/U	2.51	**0.019**	**≤0.01**	0.138	**0.00004**	**≤0.01**	0.055	**0.0012**	**≤0.01**
Mg/Y	1.25	0.602	>0.05	0.065	**0.027**	**≤0.01**	0.052	**0.00081**	**≤0.01**
Mg/Yb	2.17	0.115	>0.05	0.154	**0.0013**	**≤0.01**	0.071	**0.011**	**≤0.01**
Mg/Zn	0.76	0.092	**≤0.05**	1.86	**0.026**	**≤0.01**	2.45	**0.0078**	**≤0.01**
Mg/Zr	0.91	0.797	>0.05	0.009	**0.00001**	**≤0.01**	0.010	**0.019**	**≤0.01**

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

Table 6: Ratio of means and the difference between mean values of the Mg mass fraction/ trace element mass fraction ratios in normal (N), benign hypertrophic (BPH) and cancerous prostate (PCa)

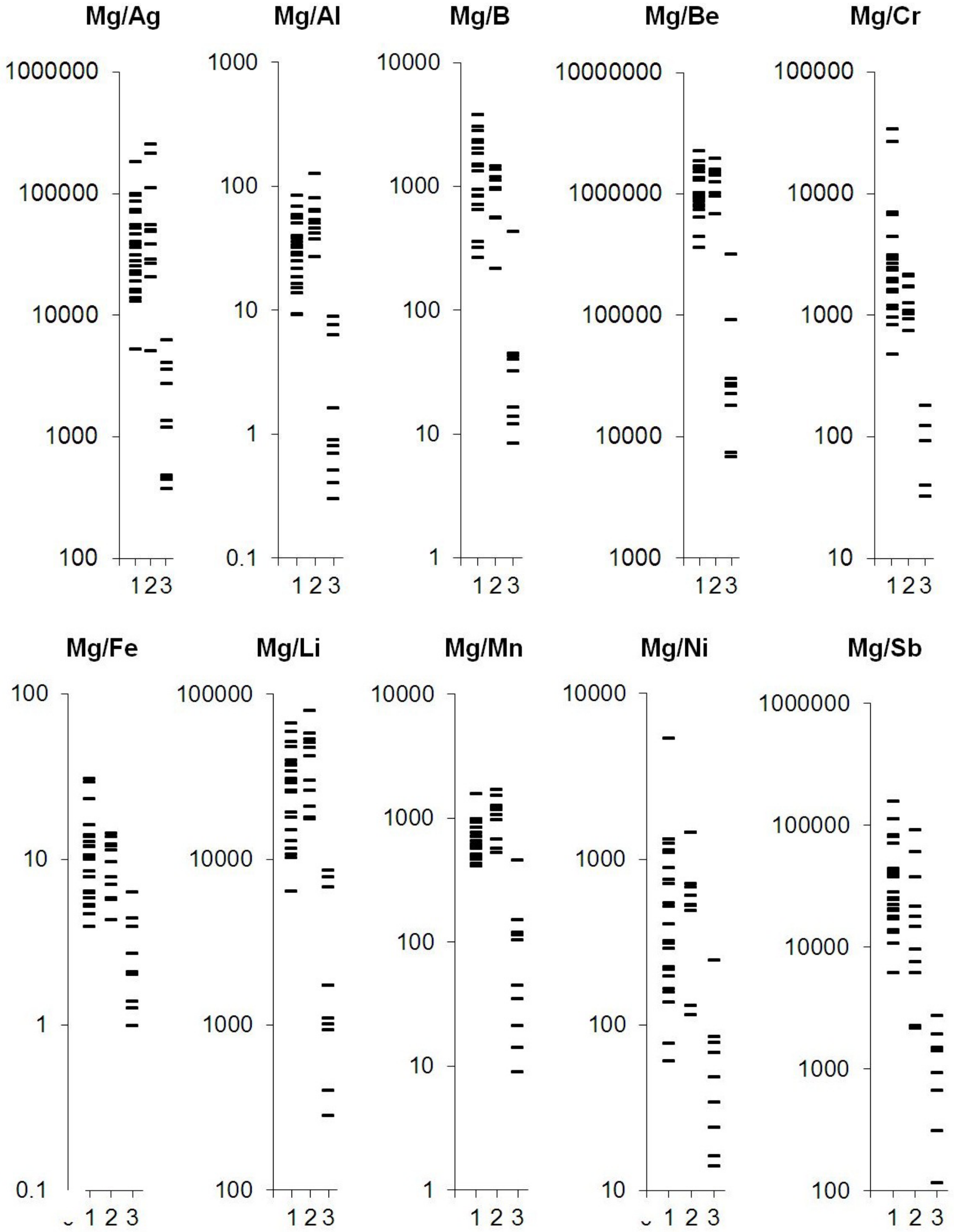

Figure 1: Individual data sets for Mg/Ag, Mg/Al, Mg/B, Mg/Be, Mg/Cr, Mg/Fe, Mg/Li, Mg/Mn, Mg/Ni, and Mg/Sb mass fraction ratios in samples of normal (1), benign hypertrophic (2) and cancerous (3) prostate

Mass fraction ratio	Upper limit for PCa <	Sensitivity %	Specificity %	Accuracy %
Mg/Ag	8000	100-9	94 ± 4	96 ± 3
Mg/Al	8.9	100-9	100-3	100-2
Mg/B	200	89 ± 11	100-3	98 ± 2
Mg/Be	320000	100-10	100-3	100-2
Mg/Cr	210	100-20	100-3	100-3
Mg/Fe	4.2	78 ± 14	97 ± 3	94 ± 4
Mg/Li	9000	100-10	97 ± 3	97 ± 3
Mg/Mn	370	90 ± 10	100-3	100-2
Mg/Ni	100	90 ± 10	94 ± 4	93 ± 4
Mg/Sb	2800	100-11	95 ± 4	96 ± 3

Table 7: Parameters of the importance (sensitivity, specificity and accuracy) Mg/Ag, Mg/Al, Mg/B, Mg/Be, Mg/Cr, Mg/Fe, Mg/Li, Mg/Mn, Mg/Ni, and Mg/Sb mass fraction ratios for the diagnosis of PCa (an estimation is made for "PCa or normal and BPH prostate")

Discussion

As was shown by us [14,15,17,18], the use of CRM IAEA H-4 Animal muscle, IAEA HH-1 Human hair, INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs as certified reference materials for the analysis of samples of prostate tissue can be seen as quite acceptable. Good agreement of the chemical element contents in these CRMs, measured by us using INAA, ICP-AES, and ICP-MS methods, with the certified data (Table 1, 2, and 3) indicates an acceptable accuracy of the results obtained in the present study.

The mean values and standard error of mean (± SEM) were calculated for Mg and trace elements (Table 4), as well as for 43 ratios of Mg/trace element contents (Table 5). The mass fraction of Mg and 43 trace elements were measured in all, or a major portion of normal prostate samples. The masses of BPH and PCa samples varied very strong from a few milligrams (sample from needle biopsy material) to 100 mg (sample from resected material). Therefore, in BPH and PCa prostates mass fraction ratios of Mg/trace element content were determined in 22 samples (11 BPH and 11 PCa samples, respectively).

From Table 6, it is observed that in benign hypertrophic tissues the Mg/Ag, Mg/Au, Mg/Be, Mg/Br, Mg/Cd, Mg/Co, Mg/Cr, Mg/Dy, Mg/Fe, Mg//Li, Mg/Nb, Mg/Ni, Mg/Pb, Mg/Pr, Mg/Rb, Mg/Sb, Mg/Sc, Mg/Se, Mg/Th, Mg/Ti, Mg/Tl, Mg/Tm, Mg/Y, Mg/Yb, and Mg/Zr mass fraction ratios not differ from normal levels, but the mass fraction ratios of Mg/Al, Mg/Ce, Mg/Cs, Mg/Er, Mg/Gd, Mg/Ho, Mg/La, Mg/Mn, Mg/Mo, Mg/Nd, Mg/Pb, Mg/Pr, Mg/Sm, Mg/Sn, Mg/Tb, and Mg/U are higher, while the mass fraction ratios of Mg/B, Mg/Bi, Mg/Cr, Mg/Hg, Mg/Sb, and Mg/Zn are significantly lower. In cancerous tissue the all Mg/trace element mass fraction ratios investigated in the study are significantly lower, than in BPH and normal prostate, with the exception of Mg/Cd and Mg/Zn ratios.

Analysis of the mass fraction ratios for trace element in prostate tissue could become a powerful diagnostic tool. To a large extent, the resumption of the search for new methods for early diagnosis of PCa was due to experience gained in a critical assessment of the limited capacity of the prostate specific antigen (PSA) serum test [75,76]. In addition to the PSA serum test and morphological study of needle-biopsy cores of the prostate, the development of other highly precise testing methods seems to be very useful. Experimental conditions of the present study were approximated to the hospital conditions as closely as possible. In BPH and PCa cases we analyzed a part of the material obtained from a puncture transrectal biopsy of the indurated site in the prostate. Therefore, our data allow us to evaluate adequately the importance of Mg/trace element mass fraction ratios for the diagnosis of PCa. As is evident from Table 6 and, particularly, from individual data sets (Fig. 1), the Mg/Ag, Mg/Al, Mg/B, Mg/Be, Mg/Cr, Mg/Fe, Mg/Li, Mg/Mn, Mg/Ni, and Mg/Sb mass fraction ratios are potentially the most informative test for a differential diagnosis. For example, if 8000 is the value of Mg/Ag mass fraction ratio assumed to be the upper limit for PCa (Figure 1) and an estimation is made for "PCa or intact and BPH tissue", the following values are obtained:

Sensitivity = {True Positives (TP)/[TP + False Negatives (FN)]} •100% = 100-9%.;
Specificity = {True Negatives (TN)/[TN + False Positives (FP)]} •100% = 94 ± 4%.
Accuracy = [(TP+TN)/(TP+FP+TN+FN)] •100% = 96 ± 3%.

The number of people (samples) examined was taken into account for calculation of confidence intervals [77]. In other words, if Mg/Ag mass fraction ratio in a prostate biopsy sample is lower 8000, one could diagnose a malignant tumor with an accuracy 96 ± 3%. Thus, using the Mg/Ag mass fraction ratio-test makes it possible to diagnose cancer in 100-9% cases (sensitivity). The same way parameters of the importance (sensitivity, specificity and accuracy) of Mg/Al, Mg/B, Mg/Be, Mg/Cr, Mg/Fe, Mg/Li, Mg/Mn, Mg/Ni, and Mg/Sb mass fraction ratios for the diagnosis of PCa were calculated (Table 7).

Conclusion

The combination of nondestructive INAA and destructive ICP methods is satisfactory analytical tool for the precise determination of Mg and 43 trace element mass fractions in the tissue samples of normal, BPH and carcinomatous prostate glands. The sequential application of these methods allowed precise quantitative determinations of mean mass fraction of Ag, Al, Au, B, Be, Bi, Br, Ca, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr. It was observed that the ratio to Mg of Ag, Al, Au, B, Be, Bi, Br, Ca, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn,Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, and Zr mass fraction were significantly lower in cancerous tissues than in normal and BPH prostate. Finally, we propose to use the Mg/Ag, Mg/Al, Mg/B, Mg/Be, Mg/Cr, Mg/Fe, Mg/Li, Mg/Mn, Mg/Ni, and Mg/Sb mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer. Further studies on larger number of samples are required to confirm our findings and to investigate the impact of the trace element relationships on prostate cancer etiology.

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Competing interests

All other authors declare no competing interests.

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