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Research Article

# Ratios of Calcium/Trace Elements as Prostate Cancer Markers

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## Abstract

The aim of the study was to evaluate whether significant changes in the pro-static tissue levels of ratios Ca/trace element contents exist in the malignantly transformed prostate. Contents of Ca and 43 trace elementsin normal (N, n=37), benign hypertrophic (BPH, n=32) and cancerous human prostate (PCa, n=60) were investigated, and ratios Ca/trace element contents were calculated. Measurements 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. It was observed that the ratio to Ca ofAg, 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 Zrmass fraction were significantly lower in cancerous tissues than in normal and BPH prostate. Finally, we propose to use the Ca/Ag, Ca/Al, Ca/B, Ca/Be, Ca/Cr, Ca/Fe, Ca/Li, Ca/Mn, Ca/Ni, and Ca/Sn 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:** Benign Prostatic Hypertrophy; Inductively Coupled Plasma Atomic Emission Spectrometry; Inductively Coupled Plasma Mass Spectrometry;Neutron Activation Analysis; Prostate; Prostatic Carcinoma; Trace Elements; Trace Element Content Ratios.

## 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 non-cancerous 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, in over 70% at 60 years old and in greater than 90% of men over 70 [1,2]. In many Western industrialized countries, including North America, PCa is the most frequently diagnosed form of non-cutaneous malignancy in males. Except for lung cancer, PCais 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 signaling, 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 [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 Ca content as a tumor marker for PCa diagnostics was shown by us [67,68].

Hence trace elements besides Ca can be used as tumor markers for distinguish between benign and malignant prostate.

Currently numbers of methods were 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 measuringthe 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 PCa tissues the mean values of Ag, Br, Cr, Fe, Hg, Mn, and Sb were higher while those of Ca, Co, Rb, Sc, and Znwere lower than in healthy prostatic tissue [60-65]. For example, the mean levels (M±SEM) of Ca mass fractions (mg/kg dry tissue) in normal, BPH, and cancerous prostate were 2428±233, 2032±165, and 676±63, respectively [61,64]. 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 determining the mean mass fractions of15-16 chemical elements in the samples of normal and cancerous prostate glands[14,15,20,27,28,60,66]. The combination of 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, 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 contents in intact prostate of healthy men as well as in the prostate gland of patients with BPH and PCausing INAA and ICP methods. The second aim was to calculate Ca/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 Ca/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. The diagnosis of all patients has been confirmed by clinical and morphological results obtained during studies of biopsy and respected 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. 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. The samples intended for trace element analysis were weighed, freeze-dried and homogenized. The sample weighing about 10 mg (for biopsy materials) and 50-100 mg (for respected materials) was used for Ca measurement by INAA-SLR. The samples for INAA-SLR were sealed separately in thin polyethylene films washed with acetone and rectified alcoholbefore using. The sealed samples were placed in labeled polyethylene ampoules. After NAA-SLR investigation, the prostate samples were taken out from the polyethylene ampoules and used for 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 NAA-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 theplastic 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**

**INAA**

A horizontal channel equipped with the pneumatic rabbit system of the WWR-C research nuclear reactor was applied to determine the mass fraction of Ca by INAA-SLR. The neutron flux in the channel was 1.7 × 1013n cm−2s−1. Ampoules with prostate samples, biological synthetic standards [73], intralaboratory-made standards, and Certified Reference Material (CRM) were put into polyethylene rabbits and irradiated separately for 180 s. Copper foils were used to assess neutron flux by the detection the 511 keV gamma line of [64] Cu from reaction [63] Cu (n,γ) [64] Cu. One minute after the irradiation, the measurement of gamma ray activities of the reaction products for each sample was done using gamma ray spectrometer. The duration of each measurement was 10 min.The detector to sample distance was from 5 to 15 cm subject to pulse counting rate.

A vertical channel of a nuclear reactor was applied to determine the trace element mass fractions by NAA-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 x1013 n cm-2s-1. Ten days after the irradiation, samples were reweighed and repacked. Over a period from 10 to 30 days after irradiation, the samples were measured for the gamma ray activities of the reaction products by using gamma ray spectrometer. The duration of measurements was from 20 min to 10 hours subject to pulse counting rate. The detector to sample distance was 0 cm.

The gamma ray spectrometer used for NAA-SLR and NAA-LLR included the 100 $cm^3$ Ge(Li) detector and on-line computer-based multichannel analyzer. The spectrometer provided a resolution of 1.9 keVat the 1332 keV gamma line of [60] Co.Information detailing with the NAA-SLR and NAA-LLR methods used and other details of the analysis was presented in our previous publications [14,15].

**ICP-AES and ICP-MS**

Aliquots of aqueous solutions were used to determine the Ca 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 Ca 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 Ca 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, SC29423, USA). Indium was used as an internal standard in all measurements.

Information detailing with the ICP-AESand ICP-MS methods used and other details of the analysis was presented in our previous publications [17,18].

## Certified Reference Materials

For quality control, ten subsamples of the certified reference material IAEA H-4 (Animal muscle)from the International Atomic Energy Agency were used. In addition, five sub-samples INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2Mixed 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 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 NAA-SLR and INAA-LLR were prepared in duplicate and mean values of chemical element contents were used in final calculation. For elements investigated by NAA-SLR, INAA-LLR, ICP-AES, and ICP-MS the mean of all results was used. Using the Microsoft Office Excel software Ca/trace element contents for each trace element in every sample were calculated. Then arithmetic mean, standard deviation, and standard error of mean were calculated for ratios of Ca/trace element mass

fraction in normal, benign hyperplastic and cancerous prostate tissue. The difference in the results between BPH and Norm, PCa and Norm as well as PCA and BPH was evaluated by parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test. Values of $p<0.05$ isconsidered to be statistically significant. For the construction of "individual data sets for Ca/trace element mass fraction ratios in normal, benign hypertrophic and cancerous prostate" diagrams the Microsoft Office Excel software was also used.

## Results

Table 1 depicts mean values±standard error of mean (M±SEM) of the ratio to Ca ofAg, 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 Zrmass fraction in normal, benign hypertrophic and cancerous prostate.

| Ratio of chemical elements | Symbols | Prostatic tissue | | |
|---|---|---|---|---|
| | | N | BPH | PCa |
| | | 41-87 year (n=37) | 56-78 year (n=32) | 40-79 year (n=60) |
| Calcium/Silver | Ca/Ag | 107037±15764 | 117550±34515 | 4164±1611 |
| Calcium/Aluminum | Ca/Al | 103±21 | 101±18 | 5.24±1.96 |
| Calcium/Gold | Ca/Au | 1170772±216470 | 1126774±158450 | 66619±34235 |
| Calcium/Boron | Ca/B | 4320±805 | 1550±191 | 119±587 |
| Calcium/Berillium | Ca/Be | 3096156±413429 | 2224935±159071 | 94738±39812 |
| Calcium/Bismuth | Ca/Bi | 532698±114578 | 93934±41193 | 7501±6138 |
| Calcium/Bromine | Ca/Br | 104±13 | 75.8±13.1 | 11.2±4.2 |
| Calcium/Cadmium | Ca/Cd | 3085±455 | 3753±732 | 2002±212 |
| Calcium/Cerium | Ca/Ce | 144087±28909 | 191735±31186 | 8862±2222 |
| Calcium/Cobalt | Ca/Co | 69329±11034 | 42314±4982 | 13669±1071 |
| Calcium/Cromium | Ca/Cr | 14516±6572 | 2169±218 | 191±41 |
| Calcium/Cesium | Ca/Cs | 94882±17335 | 92843±9485 | 22723±6474 |
| Calcium/Dysprosium | Ca/Dy | 1733952±444593 | 1685620±327920 | 161389±47689 |
| Calcium/Erbium | Ca/Er | 2782727±557202 | 3989832±845199 | 296667±64924 |
| Calcium/Iron | Ca/Fe | 25.8±3.8 | 15.8±1.7 | 4.88±0.57 |
| Calcium/Gadolinium | Ca/Gd | 1489869±334486 | 1726552±327682 | 118454±35677 |
| Calcium/Mercury | Ca/Hg | 78186±11882 | 9944±1259 | 4445±2330 |
| Calcium/Holmium | Ca/Ho | 7482530±1547065 | 8358623±1644445 | 453653±88284 |
| Calcium/Lanthanum | Ca/La | 105705±19452 | 128328±16885 | 7340±3488 |
| Calcium/Lithium | Ca/Li | 79700±10834 | 71782±11904 | 5847±2025 |
| Calcium/Manganese | Ca/Mn | 1980±297 | 1789±186 | 181±66 |
| Calcium/Molybdenum | Ca/Mo | 11809±1417 | 12394±1087 | 2585±705 |
| Calcium/Niobium | Ca/Nb | 8481196±152944 | 952286±168195 | 168030±22370 |
| Calcium/Neodymium | Ca/Nd | 320718±67884 | 403461±65435 | 21961±4822 |
| Calcium/Nickel | Ca/Ni | 1916±626 | 1028±179 | 123±28 |
| Calcium/Lead | Ca/Pb | 3774±724 | 4461±756 | 556±149 |
| Calcium/Praseodymium | Ca/Pr | 1263853±269288 | 2231480±735010 | 112525±35218 |
| Calcium/Rubidium | Ca/Rb | 219±36 | 139±12 | 81.6±7.4 |
| Calcium/Antimony | Ca/Sb | 121969±24740 | 49028±20319 | 2784±556 |
| Calcium/Scandium | Ca/Sc | 174958±51707 | 76931±10995 | 49945±6858 |
| Calcium/Selenium | Ca/Se | 3604±500 | 2362±296 | 895±168 |

| | | | | |
|---|---|---|---|---|
| Calcium/Samarium | Ca/Sm | 1695658±438262 | 2939100±810027 | 142073±37596 |
| Calcium/Tin | Ca/Sn | 15885±2313 | 30043±4945 | 1242±490 |
| Calcium/Terbium | Ca/Tb | 12456254±2477137 | 14659058±2058440 | 897422±223585 |
| Calcium/Thorium | Ca/Th | 1703628±375869 | 1499284±275749 | 43707±21300 |
| Calcium/Titanium* | Ca/Ti* | 2079±456 | 1587±223 | 144±67 |
| Calcium/Thallium | Ca/Tl | 2870569±627543 | 1464103±251751 | 124174±74903 |
| Calcium/Thulium | Ca/Tm | 17965352±4197256 | 17684696±3565514 | 1543386±608859 |
| Calcium/Uranium | Ca/U | 1122953±182815 | 2061625±434930 | 130793±22073 |
| Calcium/Yttrium | Ca/Y | 414434±116105 | 385834±74931 | 23034±3863 |
| Calcium/Ytterbium | Ca/Yb | 3755556±862110 | 4778651±1310265 | 435607±60354 |
| Calcium/Zinc | Ca/Zn | 4.00±0.93 | 1.72±0.21 | 5.38±0.47 |
| Calcium/Zirconium | Ca/Zr | 135701±31300 | 61766±18949 | 853±238 |
| M - arithmetic mean, SEM - standard error of mean, NS - not significant difference.<br>*Titanium tools were used for sampling and sample preparation. | | | | |

**Table 1:** Comparison of mean values (M±SEM) of the calcium/trace element mass fraction ratiosin normal(N), Benign Hypertrophic (BPH) and Cancerous Prostate (PCa).

The ratios of means and the difference between mean values of the ratio to Ca ofAg, 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 Zrmass fraction in normal, benign hypertrophic and cancerous prostate are presented in Table 2.

| | BPH and Normal (N) | | | PCa and Normal (N) | | | PCa and BPH | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ratio | p≤ | p | Ratio | p≤ | p | Ratio | p≤ | p |
| | BPH/N | t-test | U-test | PCa/N | t-test | U-test | PCa/BPH | t-test | U-test |
| Ca/Ag | 1.1 | 0.785 | >0.05 | 0.039 | 0.000001 | ≤0.01 | 0.035 | 0.0082 | ≤0.01 |
| Ca/Al | 0.98 | 0.936 | >0.05 | 0.051 | 0.000093 | ≤0.01 | 0.052 | 0.00047 | ≤0.01 |
| Ca/Au | 0.96 | 0.871 | >0.05 | 0.057 | 0.000074 | ≤0.01 | 0.059 | 0.000043 | ≤0.01 |
| Ca/B | 0.36 | 0.0028 | ≤0.01 | 0.028 | 0.000036 | ≤0.01 | 0.077 | 0.00004 | ≤0.01 |
| Ca/Be | 0.72 | 0.059 | ≤0.05 | 0.031 | 0.000001 | ≤0.01 | 0.043 | 0.000001 | ≤0.01 |
| Ca/Bi | 0.18 | 0.0012 | ≤0.01 | 0.014 | 0.00013 | ≤0.01 | 0.08 | 0.064 | ≤0.05 |
| Ca/Br | 0.73 | 0.14 | >0.05 | 0.108 | 0.000001 | ≤0.01 | 0.148 | 0.00052 | ≤0.01 |
| Ca/Cd | 1.22 | 0.448 | >0.05 | 0.649 | 0.039 | ≤0.01 | 0.533 | 0.041 | ≤0.01 |
| Ca/Ce | 1.33 | 0.272 | >0.05 | 0.062 | 0.00012 | ≤0.01 | 0.046 | 0.00016 | ≤0.01 |
| Ca/Co | 0.61 | 0.033 | ≤0.05 | 0.197 | 0.000038 | ≤0.01 | 0.323 | 0.00016 | ≤0.01 |
| Ca/Cr | 0.15 | 0.074 | ≤0.01 | 0.013 | 0.041 | ≤0.01 | 0.088 | 0.000006 | ≤0.01 |
| Ca/Cs | 0.98 | 0.918 | >0.05 | 0.239 | 0.00051 | ≤0.01 | 0.245 | 0.00001 | ≤0.01 |
| Ca/Dy | 0.97 | 0.931 | >0.05 | 0.093 | 0.0019 | ≤0.01 | 0.096 | 0.00088 | ≤0.01 |
| Ca/Er | 1.43 | 0.248 | >0.05 | 0.107 | 0.0002 | ≤0.01 | 0.074 | 0.0014 | ≤0.01 |
| Ca/Fe | 0.61 | 0.021 | ≤0.05 | 0.189 | 0.000009 | ≤0.01 | 0.309 | 0.000063 | ≤0.01 |
| Ca/Gd | 1.16 | 0.617 | >0.05 | 0.08 | 0.00048 | ≤0.01 | 0.069 | 0.0006 | ≤0.01 |
| Ca/Hg | 0.13 | 6.00E-06 | ≤0.05 | 0.057 | 0.000002 | ≤0.01 | 0.447 | 0.08 | ≤0.05 |
| Ca/Ho | 1.12 | 0.701 | >0.05 | 0.061 | 0.00015 | ≤0.01 | 0.054 | 0.00071 | ≤0.01 |
| Ca/La | 1.21 | 0.387 | >0.05 | 0.069 | 0.000038 | ≤0.01 | 0.057 | 0.000041 | ≤0.01 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Ca/Li | 0.9 | 0.627 | >0.05 | 0.073 | 0.000001 | ≤0.01 | 0.081 | 0.00023 | ≤0.01 |
| Ca/Mn | 0.9 | 0.59 | >0.05 | 0.091 | 0.000004 | ≤0.01 | 0.101 | 0.000002 | ≤0.01 |
| Ca/Mo | 1.05 | 0.746 | >0.05 | 0.219 | 0.000004 | ≤0.01 | 0.209 | 0.000003 | ≤0.01 |
| Ca/Nb | 0.11 | 0.651 | >0.05 | 0.02 | 0.00021 | ≤0.01 | 0.176 | 0.00087 | ≤0.01 |
| Ca/Nd | 1.26 | 0.388 | >0.05 | 0.068 | 0.00025 | ≤0.01 | 0.054 | 0.00016 | ≤0.01 |
| Ca/Ni | 0.54 | 0.184 | >0.05 | 0.064 | 0.009 | ≤0.01 | 0.12 | 0.00047 | ≤0.01 |
| Ca/Pb | 1.18 | 0.517 | >0.05 | 0.147 | 0.00019 | ≤0.01 | 0.125 | 0.00038 | ≤0.01 |
| Ca/Pr | 1.77 | 0.239 | >0.05 | 0.089 | 0.00032 | ≤0.01 | 0.05 | 0.016 | ≤0.01 |
| Ca/Rb | 0.63 | 0.041 | ≤0.01 | 0.373 | 0.00069 | ≤0.01 | 0.587 | 0.00071 | ≤0.01 |
| Ca/Sb | 0.4 | 0.029 | ≤0.01 | 0.023 | 0.000055 | ≤0.01 | 0.057 | 0.046 | ≤0.01 |
| Ca/Sc | 0.44 | 0.085 | >0.05 | 0.285 | 0.032 | ≤0.01 | 0.649 | 0.083 | ≤0.05 |
| Ca/Se | 0.66 | 0.04 | ≤0.05 | 0.248 | 0.000018 | ≤0.01 | 0.379 | 0.00073 | ≤0.01 |
| Ca/Sm | 1.73 | 0.196 | >0.05 | 0.084 | 0.0018 | ≤0.01 | 0.048 | 0.0062 | ≤0.01 |
| Ca/Sn | 1.89 | 0.021 | ≤0.01 | 0.078 | 0.000001 | ≤0.01 | 0.041 | 0.00016 | ≤0.01 |
| Ca/Tb | 1.18 | 0.499 | >0.05 | 0.072 | 0.00012 | ≤0.01 | 0.061 | 0.000051 | ≤0.01 |
| Ca/Th | 0.88 | 0.664 | >0.05 | 0.026 | 0.00022 | ≤0.01 | 0.029 | 0.00035 | ≤0.01 |
| Ca/Ti* | 0.76 | 0.342 | >0.05 | 0.069 | 0.00038 | ≤0.01 | 0.091 | 0.000054 | ≤0.01 |
| Ca/Tl | 0.51 | 0.047 | ≤0.05 | 0.043 | 0.00025 | ≤0.01 | 0.085 | 0.00039 | ≤0.01 |
| Ca/Tm | 0.98 | 0.96 | >0.05 | 0.086 | 0.00078 | ≤0.01 | 0.087 | 0.0011 | ≤0.01 |
| Ca/U | 1.84 | 0.067 | ≤0.05 | 0.116 | 0.000016 | ≤0.01 | 0.063 | 0.0013 | ≤0.01 |
| Ca/Y | 0.93 | 0.837 | >0.05 | 0.056 | 0.0025 | ≤0.01 | 0.06 | 0.00068 | ≤0.01 |
| Ca/Yb | 1.27 | 0.522 | >0.05 | 0.116 | 0.00088 | ≤0.01 | 0.091 | 0.0078 | ≤0.01 |
| Ca/Zn | 0.43 | 0.024 | ≤0.05 | 1.345 | 0.195 | >0.05 | 3.128 | 0.000006 | ≤0.01 |
| Ca/Zr | 0.46 | 0.052 | ≤0.05 | 0.006 | 0.00028 | ≤0.01 | 0.014 | 0.011 | ≤0.01 |
| t-test - Student's t-test, U-test - Wilcoxon-Mann-Whitney U-test, Bold significant differences | | | | | | | | | |

**Table 2:** Ratio of means and the difference between mean values of the Ca mass fraction/ trace element mass fraction ratiosin normal(N), Benign Hypertrophic (BPH)and Cancerous Prostate (PCa).

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

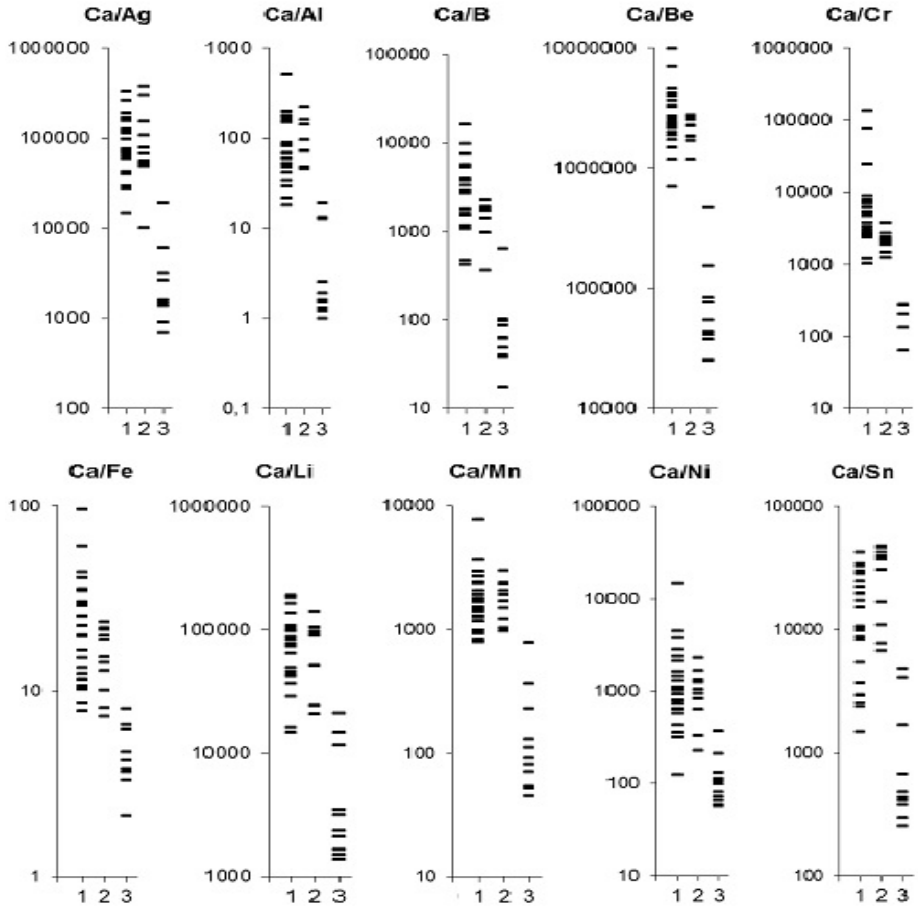


**Figure 1:** Individual data sets for Ca/Ag, Ca/Al, Ca/B, Ca/Be, Ca/Cr, Ca/Fe, Ca/Li, Ca/Mn, Ca/Ni, and Ca/Sn mass fraction ratios in samples of normal (1), benign hypertrophic (2) and cancerous (3) prostate.

Table 3 contains parameters of the importance (sensitivity, specificity and accuracy of Ca/Ag, Ca/Al, Ca/B, Ca/Be, Ca/Cr, Ca/Fe, Ca/Li, Ca/Mn, Ca/Ni, and Ca/Sn mass fraction ratios for the diagnosis of PCacalculated in this work.

| Mass fraction ratio or their multiplication | Upper limit for PCa | Sensitivity % | Specificity % | Accuracy % |
|---|---|---|---|---|
| Ca/Ag | 8000 | 91±9 | 100-3 | 98±2 |
| Ca/Al | 17 | 91±9 | 100-3 | 98±2 |
| Ca/B | 400 | 90±10 | 100-3 | 98±2 |
| Ca/Be | 500000 | 100-9 | 100-3 | 100-2 |
| Ca/Cr | 300 | 100-20 | 100-3 | 100-3 |
| Ca/Fe | 7 | 90±10 | 100-3 | 98±2 |
| Ca/Li | 14600 | 91±9 | 100-3 | 98±2 |
| Ca/Mn | 780 | 100-9 | 100-3 | 100-2 |
| Ca/Ni | 215 | 91±9 | 97±3 | 96±3 |
| Ca/Sn | 2000 | 82±12 | 97±3 | 94±4 |

**Table 3:** Parametersof the importance (sensitivity, specificity and accuracy) of Ca/Ag, Ca/Al, Ca/B, Ca/Be, Ca/Cr, Ca/Fe, Ca/Li, Ca/Mn, Ca/Ni, and Ca/Sn 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, 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 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 contents analyzed by NAA-SLR, NAA-LLR, ICP-AES, and ICP-MS with the certified data of reference materials indicates an acceptable accuracy of the results obtained in the study of trace elements of the prostate samples presented in Tables 1 and 2.

The mean values and all selected statistical parameters were calculated for 43 ratios of Ca/trace element contents (Table 1). The mass fraction of Ca 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 biopsymaterial) to 100 mg (sample from respected material). Therefore, in BPH and PCa prostates mass fraction ratios of Ca/trace element content were determined in 22 samples (11 BPH and 11 PCa samples, respectively).

From Table 2, it is observed that in benign hypertrophic tissues the Ca/Ag, Ca/Al, Ca/Au, Ca/Br, Ca/Cd, Ca/Ce, Ca/Cs, Ca/Dy, Ca/Er, Ca/Gd, Ca/Ho, Ca/La, Ca/Mo, Ca/Nb, Ca/Nd, Ca/Ni, Ca/Pb, Ca/Pr, Ca/Sc, Ca/Sm, Ca/Tb, Ca/Th, Ca/Ti, Ca/Tm, Ca/Y, Ca/Yb, and Ca/Zr mass fraction ratios not differ from normal levels, but the mass fraction ratios of Ca/Sn and Ca/U are higher, while the mass fraction ratios of Ca/B, Ca/Be, Ca/Bi, Ca/Co, Ca/Cr, Ca/Fe, Ca/Hg, Ca/Rb, Ca/Sb, Ca/Se, Ca/Tl, Ca/Zn, and Ca/Zr are significantly lower. In cancerous tissue the all Ca/trace element mass fraction ratios investigated in the study are significantly lower than in BPH and normal prostate with the exception of Ca/Zn ratio.

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 indurate site in the prostate. Therefore, our data allow us to evaluate adequately the importance of Ca/trace element mass fraction ratios for the diagnosis of PCa. As is evident from Table 2 and, particularly, from individual data sets (Figure 1), the Ca/Ag, Ca/Al, Ca/B, Ca/Be, Ca/Cr, Ca/Fe, Ca/Li, Ca/Mn, Ca/Ni, and Ca/Snmass fraction ratios are potentially the most informative test for a differential diagnosis. For example, if 8000 is the value of Ca/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:

$$\text{Sensitivity} = \{\text{True Positives (TP)}/[\text{TP} + \text{False Negatives (FN)}]\} \cdot 100\% = 91\pm9\%.$$

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

$$\text{Accuracy} = [(\text{TP}+\text{TN})/(\text{TP}+\text{FP}+\text{TN}+\text{FN})] \cdot 100\% = 98\pm2\%.$$

The number of people (samples) examined was taken into account for calculation of confidence intervals [77].In other words, if Ca/Ag mass fraction ratio in a prostate biopsy sample is lower than 8000, one could diagnose a malignant tumor with an accuracy 98±2%.Thus, using the Ca/Ag mass fraction ratio-test makes it possible to diagnose cancer in 91±9% cases (sensitivity). The same way parameters of the importance (sensitivity, specificity and accuracy) of Ca/Al, Ca/B, Ca/Be, Ca/Cr, Ca/Fe, Ca/Li, Ca/Mn, Ca/Ni, and Ca/Sn mass fraction ratios for the diagnosis of PCa were calculated (Table 3).

## Conclusion

The combination of nondestructive INAA and destructive ICP methods is satisfactory analytical tool for the precise determination of Ca 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 Ca ofAg, 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, and Zrmass fractionwere significantly lower in cancerous tissues than in normal and BPHprostate. Finally, we propose to use the Ca/Ag, Ca/Al, Ca/B, Ca/Be, Ca/Cr, Ca/Fe, Ca/Li, Ca/Mn, Ca/Ni, and Ca/Sn 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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