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Research Article

Distinguishing Malignant from Benign Prostate using Chemical Elemental Content / Calcium Ratios in Prostatic Tissue

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Abstract

The aim of the study was the development of new highly precise testing methods for early diagnosis of prostate cancer. For this purpose, the values of Br/Ca, K/Ca, Mg/Ca, Mn/Ca, and Na/Ca mass fraction ratios in normal (n=37), benign hypertrophic (n=11) and cancerous (n=11) human prostate gland were investigated by non-destructive instrumental neutron activation analysis. Mean values ± standard error of mean (M±SEM) for mass fraction ratios in the normal tissue were as follows: $(Br/Ca)\cdot10^2$ 1.44±0.21, K/Ca 5.69±0.38, Mg/Ca 0.511±0.045, $(Mn/Ca)\cdot10^3$ 0.623±0.062, and Na/Ca 5.58±0.48, respectively. It was observed that in benign hypertrophic tissues the Br/Ca, Mg/Ca, Mn/Ca, and Na/Ca mass fraction ratios do not differ from normal levels while the K/Ca ratio is significantly higher. In cancerous tissue the Br/Ca, K/Ca, Mn/Ca, and Na/Ca ratios are significantly higher than in normal and benign hypertrophic prostate. It was shown that the Br/Ca and Mn/Ca mass fraction ratios are the most informative for a differential diagnosis among all investigated mass fraction ratios. Finally, we propose to use the estimation of Mn/Ca mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer. Sensitivity, specificity, and accuracy of this test were (100-9)%, (100-2)%, and (100-2)%, respectively. Obtained data allowed us to adequately evaluate the importance of chemical elemental content ratios for the diagnosis of prostate cancer.

Key words: *chemical elements, chemical elemental mass fraction ratios; prostate; benign prostatic hypertrophy; prostatic carcinoma; neutron activation analysis*

Introduction

The prostate gland may be a source of many health problems in men past middle age, the most common being benign prostatic hyperplasia (BPH), and prostatic carcinoma (PCa). BPH is a noncancerous enlargement of the prostate gland leading to obstruction of the urethra and can significantly impair quality of life [1]. The prevalence of histological BPH is found in approximately 50-60% of males age 40-50, in over 70% at 60 years old and in greater than 90% of men over 70 [2, 3]. In many Western industrialized countries, including North America, PCa is the most frequently diagnosed form of noncutaneous malignancy in males and, except for lung cancer, is the leading cause of death from cancer [4-9]. Although the etiology of BPH and PCa is unknown, some electrolytes and trace elements have been highlighted in the literature in relation to the development of these prostate diseases [10-29].

Electrolytes and trace elements have essential physiological functions such as maintenance and regulation of cell function and signalling, gene regulation, activation or inhibition of enzymatic reactions, neurotransmission, and regulation of membrane function. Essential or toxic (mutagenic, carcinogenic) properties of chemical elements depend on tissue-specific need or tolerance, respectively [30]. Excessive accumulation, deficiency or an imbalance of the chemical elements may disturb the cell functions and may result in cellular degeneration, death and malignant transformation [31].

In reported studies significant changes of chemical element contents in hyperplastic and cancerous prostate in comparison with those in the normal prostatic tissue were observed [32-56]. Moreover, a informative value of Zn content as a tumor marker for PCa diagnostics was shown by us [57,58]. Hence it is possible that besides Zn, some other chemical elements also can be used as tumor markers for distinguish between benign and malignant prostate.

Current methods applied for measurement of chemical element contents in samples of human tissue include a number of methods. Among these methods the instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) is a non-destructive, sensitive, and prompt technique. It allows measure the chemical element contents in a few milligrams tissue without any treatment of sample. Analytical studies of the Br, Ca, K, Mg, Mn, and Na contents in normal, BPH and PCa tissue were done by us using INAA-SLR [15,21,28,50,55]. Nondestructive method of analysis avoids the possibility of changing the content of chemical elements in the studied samples [59-62], which allowed for the first time to obtain reliable results. In particular, it was shown that the average mass fraction of Ca in BPH tissue does not differ from normal level [54], but in PCa tissues it is 3.4 times lower than in healthy prostatic tissue [50]. Moreover, it was shown in our previous studies that in a normal prostate tissue mass fraction of some chemical elements tend to be correlated with Ca, while in BPH and PCa tissues these relationships are partially broken or changed [50,53,55]. Obtained results formed the basis for a new method for differential diagnosis of BPH and PCa, the essence of which was to determine the chemical elemental content / Ca content ratios in

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the material of transrectal needle biopsy of prostate indurated site, because, in comparison with the absolute values of chemical element contents, using their content ratios is more suitable for diagnostics.

Therefore, the present study had three aims. The main objective was to assess the Br/Ca, K/Ca, Mg/Ca, Mn/Ca, and Na/Ca mass fraction ratios in intact prostate of healthy men aged over 40 years and in the prostate gland of age-matched patients, who had either BPH or PCa, using data about the Br, Ca, K, Mg, Mn, and Na contents obtained by INAA-SLR analysis. The second aim was to compare the levels of elemental content ratios in normal, hyperplastic, and cancerous prostate, and the third aim was to evaluate the elemental content ratios for diagnosis of prostate cancer. All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

Material and Methods

Samples

All patients studied (n=22) were hospitalized in the Urological Department of the Medical Radiological Research Centre. 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 11 patients with BPH ranged from 56 to 75 years, the mean being 66.8±6.5 (M±SD) years. The 11 patients aged 51-78 suffered from PCa. Their mean age was 67.7±9.9 (M±SD) years.

Intact (Norm) prostate samples were removed at necropsy from 37 men aged 41-79 who had died suddenly. Their mean age was 55±11 (M±SD) years. The majority of deaths were due to trauma. Tissue samples were collected from the peripheral zone of dorsal and lateral lobes of their prostates, within 2 days of death and then the samples were divided into two portions. One was used for morphological study while the other was intended for chemical element analysis. A histological examination was used to control the age norm conformity, as well as to confirm the absence of microadenomatosis and latent cancer [15,21,28].

Sample preparation, instrumentation, methods and certified reference materials

Details of sample preparation, the relevant nuclear reactions, radionuclides, gamma energies, methods of analysis, equipment, and the results of quality control were presented in our earlier publications concerning the chemical elements of human prostate investigated by INAA-SLR [15,21,28,50,55,63].

Computer programs and statistic

A dedicated computer program for INAA mode optimization was used [64]. All prostate samples for INAA-SLR were prepared in duplicate and mean values of elemental content ratios were used in final calculation. Using the Microsoft Office Excel software, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for chemical element mass fraction ratios in normal, benign hyperplastic and cancerous prostate tissue. The difference in the results between BPH and Norm, PCa and Norm, and PCA and BPH was evaluated by Student's *t*-test. For the construction of "individual data sets for Br/Ca, K/Ca, Mg/Ca, Mn/Ca, and Na/Ca mass fraction ratios in normal, benign hypertrophic and cancerous prostate" diagrams the Microsoft Office Excel software was also used.

Results

Table 1 presents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Br/Ca, K/Ca, Mg/Ca, Mn/Ca, and Na/Ca mass fraction ratios in normal, benign hypertrophic and cancerous prostate. The ratios of means and the reliability of difference between mean values of Br/Ca, K/Ca, Mg/Ca, Mn/Ca, and Na/Ca mass fraction ratios in normal, benign hypertrophic and cancerous prostate are presented in Table 2.

Discussion

As was shown by us [15,21,28] the use of CRM IAEA H-4 as a certified reference material for the analysis of samples of prostate tissue can be seen as quite acceptable. Good agreement of the Br, Ca, K, Mg, Mn, and Na contents analyzed by INAA-SLR with the certified data of CRM IAEA H-4 [15,21,28] indicates an acceptable accuracy of the results obtained in the study of elemental content ratios of the prostate presented in Fig. 1 and Tables 1 and 2.

The mean values and all selected statistical parameters were calculated for 5 mass fraction ratios (Br/Ca, K/Ca, Mg/Ca, Mn/Ca, and Na/Ca) (Table 1). The mass fraction ratios of these elements were calculated for all samples of normal, BPH and PCa prostates. The results presented in Table 1 did not show substantial differences between the arithmetic means and the medians, which indicate a normal distribution of the investigated elemental content ratios. These findings allowed us to compare the means of mass fraction ratios in normal, BPH and PCa prostate using Student's *t*-test. No published data referring to mass fraction ratios of chemical elements in the human prostate was found Figure 1

From Table 2, it is observed that in benign hypertrophic tissues the Br/Ca, Mg/Ca, Mn/Ca, and Na/Ca mass fraction ratios do not differ from normal levels while the K/Ca ratio is higher (p=0.041). In cancerous tissue the Br/Ca (p=0.00019), K/Ca (p=0.000026), Mn/Ca (p=0.00051), and Na/Ca (p=0.00021) ratios are significantly higher than in normal parenchyma of the prostate. All these mass fraction ratios show similar variations in cancerous tissues when compared with benign hypertrophic tissues of the prostate. The Mg/Ca mass fraction ratio is maintained a stable level which does not change with benign hypertrophic and malignant transformation of prostate.

Analysis of elemental content ratios in prostate tissue could become a powerful diagnostic tool. To a large extent, the resumption of the search for new methods for early diagnosis of PCa was due to experience gained in a critical assessment of the limited capacity of the prostate specific antigen (PSA) serum test [65]. In addition to the PSA serum test and morphological study of needle-biopsy cores of the prostate, the development of other highly precise testing methods seems to be very useful. Experimental conditions of the present study were approximated to the hospital conditions as closely as possible. In all cases we analyzed a part of the material obtained from a puncture transrectal biopsy of the indurated site in the prostate. Therefore, our data allow us to evaluate adequately the importance of elemental content ratios for the diagnosis of PCa. As is evident from individual data sets (Fig. 1), the Br/Ca and Mn/Ca mass fraction ratios are the most informative for a differential diagnosis. For example, if 1.2 is the value of (Mn/Ca)$\cdot 10^3$ mass fraction ratio assumed to be the lower limit for PCa (Fig. 1) and an estimation is made for "PCa or intact and BPH tissue", the following values are obtained:

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

Specificity = {True Negatives (TN)/[TN + False Positives (FP)]} ·100% = (100-2)%;

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

The number of people (samples) examined was taken into account for calculation of confidence intervals [66]. In other words, if Mn/Ca

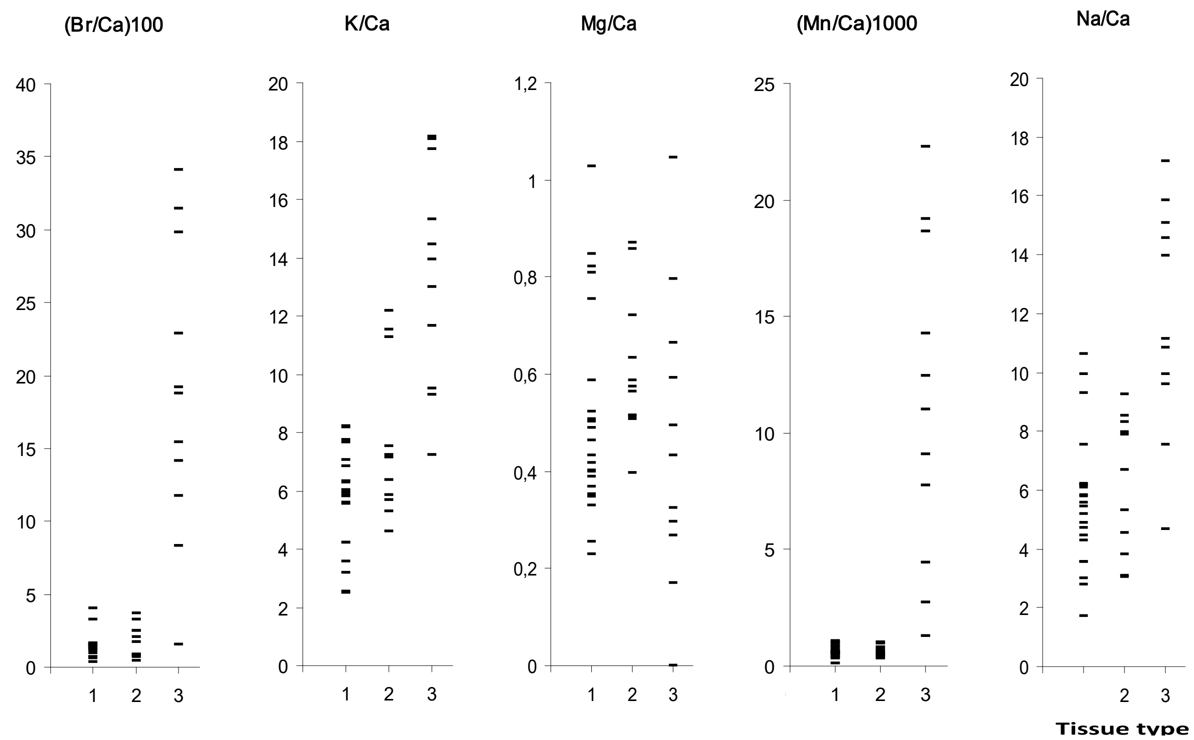

Figure 1: Individual data sets for Br, Ca, K, Mg, Mn, and Na mass fractions in samples of normal (1), benign hypertrophic (2) and cancerous (3) prostate.

M - arithmetic mean; SD – standard deviation; SEM – standard error of mean; Min – minimum value; Max – maximum value; Per. 0.025 – percentile with 0.025 level; Per. 0.975 – percentile with 0.975 level, n - number of samples.

Table 1: Some statistical parameters of Br/Ca, K/Ca, Mg/Ca, Mn/Ca, and Na/Ca mass fraction ratios in normal, benign hypertrophic and cancerous prostate.

Tissue Mass fraction ratio	Mean	SD	SEM	Min	Max	Median	Per. 0.025	Per. 0.975
Normal (n=37)								
$(Br/Ca)\cdot 10^2$	1.44	0.90	0.21	0.333	4.00	1.36	0.436	3.69
K/Ca	5.69	1.74	0.38	2.50	8.23	5.92	2.52	8.20
Mg/Ca	0.511	0.211	0.045	0.229	1.03	0.449	0.242	0.932
$(Mn/Ca)\cdot 10^3$	0.623	0.256	0.062	0.100	1.078	0.592	0.192	1.05
Na/Ca	5.58	2.24	0.48	1.73	10.6	5.51	2.29	10.3
BPH (n=11)								
$(Br/Ca)\cdot 10^2$	1.76	1.11	0.33	0.454	3.65	1.70	0.519	3.55
K/Ca	7.69	2.69	0.81	4.62	12.2	7.14	4.79	12.0
Mg/Ca	0.613	0.148	0.045	0.397	0.871	0.574	0.424	0.868
$(Mn/Ca)\cdot 10^3$	0.626	0.228	0.069	0.335	1.03	0.591	0.357	1.01
Na/Ca	6.21	2.33	0.70	3.02	9.25	6.66	3.03	9.07
PCa (n=11)								
$(Br/Ca)\cdot 10^2$	18.8	10.1	3.0	1.52	34.1	18.8	3.21	33.4
K/Ca	13.5	3.76	1.14	7.22	18.2	13.9	7.73	18.1
Mg/Ca	0.508	0.271	0.086	0.169	1.04	0.463	0.192	0.989
$(Mn/Ca)\cdot 10^3$	11.2	6.9	2.1	1.30	22.3	11.0	1.66	21.5
Na/Ca	11.8	3.8	1.2	4.67	17.2	11.1	5.39	16.8

Table 2: Ratio of means and the reliability of difference between mean values of Br/Ca, K/Ca, Mg/Ca, Mn/Ca, and Na/Ca mass fraction ratios in normal, benign hypertrophic and cancerous prostate

Mass fraction ratio	**BPH and Normal (N)**		**PCa and Normal (N)**		**PCa and BPH**	
	Ratio BPH/N	**Student's *t*-test**	**Ratio PCa/N**	**Student's *t*-test**	**Ratio PCa/BPH**	**Student's *t*-test**
$(Br/Ca)\cdot 10^2$	1.22	p=0.44	13.1	p=0.00019	10.7	p=0.00022
K/Ca	1.35	p=0.041	2.37	p=0.000026	1.76	p=0.00060
Mg/Ca	1.20	p=0.12	0.99	p=0.63	0.83	p=0.16
$(Mn/Ca)\cdot 10^3$	1.00	p=0.97	18.0	p=0.00051	17.9	p=0.00051
Na/Ca	1.11	p=0.46	2.11	p=0.00021	1.90	p=0.00069

mass fraction ratio in a prostate biopsy sample does not lower 1.2, one could diagnose a malignant tumor with an accuracy of (100-2)%. Thus, using the (Mn/Ca)-test makes it possible to diagnose cancer in (100-9)% cases (sensitivity).

The same way parameters of the importance (sensitivity, specificity and accuracy) of Br/Ca mass fraction ratio for the diagnosis of PCa were calculated. If 5.0 is the values of $(Br/Ca)\cdot 10^2$ mass fraction ratios assumed to be the lower limit for PCa (Fig.1) and an estimation is made for "PCa or intact and BPH tissue", the following values are obtained: sensitivity (91±9)%, specificity (100-2)%, and accuracy (98±2)%; It should be noted, however, that Br is a component of many tranquilizers. It is possible that the increase in Br content could be explained by uncontrolled use of tranquilizers in the group of PCa patients. Therefore, for diagnostic purposes, data for Br/Ca mass fraction ratio should be used with caution.

Mass fraction ratios of Mn/Ca and Br/Ca in the needle-biopsy cores could be used as a tool to diagnose PCa and are comparable with characteristics of the Zn mass fraction-test [57,58]. However, it is our opinion that application of the elemental content ratios is more suitable for PCa diagnosis. Elemental mass fraction depends on the sample mass, which decreases with loss of its moisture. The needle-biopsy core is a small piece of tissue with a relatively high "surface/volume" ratio. After sampling, it begins to lose mass very fast. Weight loss of samples depends on the humidity of operating and store rooms [59]. Thus, it is very difficult to determine the fresh mass of needle-biopsy cores and to calculate the precise mass fraction of chemical elements. Sample freeze-dry, storage in air-tight vials until weighing, and then calculating mass fraction on dry mass basis is the only possible method that eliminates the variation in sample mass. Conversely, accuracy of elemental content ratios does not depend on sample mass and changes in moisture content. Therefore, this method does not require dry samples. Moreover, the use of the relations between mass fractions of chemical elements is particularly promising for the development of in vivo diagnostic methods, including the diagnosis of PCa.

Conclusion

In this work, mean values of Br/Ca, K/Ca, Mg/Ca, Mn/Ca, and Na/Ca mass fraction ratios in normal, benign hypertrophic and cancerous prostate were calculated using data of INAA-SLR. It was observed that in benign hypertrophic tissues the Br/Ca, Mg/Ca, Mn/Ca, and Na/Ca mass fraction ratios do not differ from normal levels while the K/Ca ratio is rather higher. In cancerous tissue the Br/Ca, K/Ca, Mn/Ca, and Na/Ca ratios are significantly higher than in normal and benign hypertrophic prostate. It was shown that the Br/Ca and Mn/Ca mass fraction ratios are the most informative for a differential diagnosis among all investigated mass fraction ratios. Finally, we propose to use the estimation of Mn/Ca mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer.

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