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RATIO OF ZINC TO BROMINE, IRON, RUBIDIUM, AND STRONTIUM CONCENTRATION IN EXPRESSED PROSTATIC SECRETIONS AS A SOURCE FOR BIOMARKERS OF PROSTATIC CANCER

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Abstract: In industrialized countries prostate cancer is one of the most common malignant diseases in men.Prostate-specific antigen screening for prostate cancer appears to have produced considerable over-diagnosis and overtreatment. Overtreatment is likely to continue unless better non-invasive tests for the detection and stratification of prostate cancer will be developed. The aim of this exploratory study was to evaluate whether significant changes in the ratios of Zn to some other trace elements of prostatic fluid exist in the malignantly transformed prostate. Prostatic fluid levels of Br, Fe, Rb, Sr, and Zn were evaluated in 24 patients with prostate cancer and 38 healthy male inhabitants. Measurements were performed using energy dispersive X-ray fluorescent microanalysis.Using obtained results the individual values of Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr concentration ratio were calculated.It was observed that in the secret of cancerous prostate ratios of Zn/Br, Zn/Fe, and Zn/Srare almost 34, 40, and 20 times, respectively, lowerthan those in the secret of normal prostate.It was supposed that the changes of Zn/Br, Zn/Fe, and Zn/Srratios in prostatic fluid can be used as tumor markers.

Key words: prostate cancer; prostatic fluid; trace elements; trace element ratios; energy-dispersive X-ray fluorescent analysis; tumor markers.

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Introduction

In industrialized countries prostate cancer (PCa) is one of the most common malignant diseases in men.PCa incidence and mortality rates are among the highest for North America, Oceania,and Northern and Western Europe[1-5]. The American Cancer Society declares PCa as the most common cancer in males and the second leading cause of cancer death [6]. Moreover, PCa is the leading cancer in terms of incidence and mortality in men from Africa and the Caribbean [7]. PCa in China has also become a major public health concern [8].

Prostate-specific antigen screening for PCa appears to have produced considerable over-diagnosis and overtreatment [9]. Overtreatment is likely to continue unless better non-invasive tests for the detection and stratification of PCa will be developed.

Although the etiology of PCa is unknown, several risk factors including such micronutrients as calcium (Ca) and zinc (Zn) have been well identified [10-13]. Trace elements (TE) 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 TE depend on tissue-specific need or tolerance, respectively [14].Excessive accumulation or an imbalance of the TE may disturb the cell functions and may result in cellular degeneration, death or malignant transformation [14-16].

In our previous studies a significant involvement of Zn and some other TE in the function of prostate was observed [17-27]. Moreover, it was found that intracellular Zn and Ca excess is one of the main factors in the etiology of prostate cancer [28-29]. One of the main functions of prostate gland is a production of prostatic fluid [30] with extremely high concentration of Zn and some other TE and electrolytes. The first finding of remarkable high level of Zn concentration in human expressed prostatic fluid (EPF) was reported in the beginning of 1960s [31]. Analyzing EPF expressed from prostate of 8 apparently healthy men aged 25-55 years it was found that Zn concentration varied in range from 300 to 730 mg/L. After this finding several investigators have suggested that the measurement of Zn level in EPF may be useful as a marker of prostate secretory function [32,33]. It promoted a more detailed study of Zn concentration in EPF of healthy subjects and in those with different prostate diseases, including PCa [33,34]. A detailed review of these studies, reflecting the contradictions within accumulated data, was given in our earlier publication [34]. Moreover, the method and apparatus for micro analysis of bromine (Br), iron (Fe), rubidium (Rb), strontium (Sr), and Zn in the EPF samples using energy dispersive X-ray fluorescence (EDXRF) activated by radiation of radionuclide source 109Cd was developed by us [35].

Thus, data on changes of TE contents in EPF of patients with PCa are very important, because it can clarify our knowledge of PCa pathogenesis and may be useful as tumor markers. In present study it was supposed by us that apart from total amounts of TE the ratios of Zn to some other TE content in EPF have to reflect a disturbance of prostate function. As far as our knowledge there are no any published data on TE ratios in prostatic fluids.

This work had three aims. The first aim was to assess the Br, Fe, Rb, Sr, and Zn concentration in the EPF samples obtained from apparently health persons and patients with PCa using 109Cd EDXRF micro-method. The second aim was to evaluate the quality of obtained results and to compare these results with published data. The last aim was to calculate Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr ratios and compare the values of these TE ratios in EPF samples of normal and cancerous prostate. All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Material and Methods

Specimens of EPF were obtained from 38 men with apparently normal prostates (mean age ë Standard Deviation - 59ë11 years, range 41-82 years) and from 24 patients with PCa (mean age 65ë10 years, range 47-77 years) by qualified urologists in the Urological Department of the Medical Radiological Research Centre using standard rectal massage procedure. In all cases the diagnosis has been confirmed by clinical examination and in cases of PCa additionally by morphological results obtained during studies of biopsy and resected materials. Subjects were asked to abstain from sexual intercourse for 3 days preceding the procedure. Specimens of EPF were obtained in sterile containers which were appropriately labeled. Twice twenty ?L (microliters) of fluid were taken by micropipette from every specimen for TE analysis, while the rest of the fluid was used for cytological and bacteriological investigations. The chosen 20 ?L of the EPF was dropped on 11.3 mm diameter disk made of thin, ash-free filter papers fixed on the Scotch tape pieces and dried in an exsiccator at room temperature. Then the dried sample was covered with 4 m Dacron film and centrally pulledonto a Plexiglas cylindrical frame [35].

To determine concentration of the TE by comparison with a known standard, aliquots of solutions of commercial, chemically pure compounds were used for a device calibration [36]. The standard samples for calibration were prepared in the same way as the samples of prostate fluid. Because there were no available liquid Certified Reference Material (CRM) ten sub-samples of the powdery CRM IAEA H-4 (animal muscle) were analyzed to estimate the precision and accuracy of results. Every CRM sub-sample weighing about 3 mg was applied to the piece of Scotch tape serving as an adhesive fixing backing. An acrylic stencil made in the form of a thin-walled cylinder with 11.3 mm inner diameter was used to apply the sub-sample to the Scotch tape. The polished-end acrylic pestle which is a constituent of the stencil set was used for uniform distribution of the sub-sample within the Scorch surface restricted by stencil inner diameter. When the sub-sample was slightly pressed to the Scotch adhesive sample, the stencil was removed. Then the sub-sample was covered with 4 m Dacron film. Before the sample was applied, pieces of Scotch tape and Dacron film were weighed using analytical balance. Those were again weighed together with the sample inside to determine the sub-sample mass precisely.

The facility for radionuclide-induced energy dispersive X-ray fluorescence included an annular 109Cd source with an activity of 2.56 GBq, Si(Li) detector with electric cooler and portable multi-channel analyzercombined with a personal computer. Its resolution was 270 eV at the 6.4 keV line. The facility functioned as follows. Photons with a 22.1keV109Cd energy are sent to the surface of a specimen analyzed inducing the fluorescence K X-rays of TE. The fluorescence irradiation got to the detector through a 10 mm diameter collimator to be recorded.

The duration of the Zn concentration measurement together with Br, Fe, Rb, and Sr was 60 min. The intensity of K -line of Br, Fe, Rb, Sr, and Zn for EPF samples and standards was estimated on calculation basis of the total area of the corresponding photopeak in the spectra.

All EPF samples for EDXRF were prepared in duplicate and mean values of TE contents were used in final calculation. Using the Microsoft Office Excel programs, 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 TE concentrations and Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr concentration ratiosin EPF of normal and cancerous prostate. The difference in the results between two groups of samples (normal prostateand PCa) was evaluated by the parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test.

Results

Table 1 depicts our data for Br, Fe, Rb, Sr, and Zn mass fractions in ten sub-samples of certified reference material (CRM) IAEA H-4 (animal muscle) and the certified values of this material. Of 4 TE (Br, Fe, Rb, and Zn) with certified values for the CRM IAEA H-4 (animal muscle) we determined contents of all certified elements (Table 1). Mean values (M SD) for Br, Fe, Rb, and Zn were in the range of 95% confidence interval. Good agreement of the TE contents analyzed by 109Cd radionuclide-induced EDXRF with the certified data of CRM IAEA H-4 (Table 1) indicate an acceptable accuracy of the results obtained in the study of the prostatic fluid presented in Tables 2-4.

Table 2 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, Fe, Rb, Sr, and Zn concentrations as well as of the Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr ratios in EPF of normal and cancerous prostate.

The comparison of our results with published data for Br, Fe, Rb, Sr, and Zn concentrations and also for Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr ratios in EPF of patients with normal prostate and PCa[31-34,37-40] is shown in Table 3.

The ratios of means and the differences between mean values of Br, Fe, Rb, Sr, and Zn concentrations and also Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr ratios in EPF of normal and cancerous prostate are presented in Table 4.

Discussion

The mean values and all selected statistical parameters were calculated for five TE (Br, Fe, Rb, Sr, and Zn) concentrations and for four TE ratios (Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr) (Table 2). The mean of Zn concentration obtained for normal prostate fluid, as shown in Table 3, agrees well with median of means cited by other researches [31-34,37-40]. The mean of Rb concentration obtained for EPF agrees well with our data reported 37 years ago [33]. No published data referring to Br, Fe, and Sr concentrations as well as to Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr ratios in EPF of normal prostate were found.

In the EPF samples of cancerous prostate our results were comparable with published data for Rb and Zn concentrations (Table 3). No published data referring to Br, Fe, Rb, and Sr concentrations as well as to Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr ratios in EPF samples obtained from patients with PCa were found. A number of values for Zn concentrations in normal EPF were not expressed on a wet mass basis in the cited literature. Therefore, we calculated these values using the published data for water -93.2% [40].

From Table 4, it is observed that in EPF of cancerous prostate the concentrations of Rb and Zn are almost 2 ($p<0.00019$) and 10 ($p<0.000001$) times, respectively, lower than in EPS of normal prostate while concentration Br, Fe, and Sr are some higher. Because the Zn content on one hand and Br, Fe, Srcontents on the otherin EPF changed in opposite directions during malignant transformation of prostate the changes of the Zn/Br, Zn/Fe, and Zn/Sr ratios may be more informative than the absolute values of these TE. From Table 4, it is also observed that in EPF of cancerous prostate theZn/Br, Zn/Fe, and Zn/Sr ratios are almost 34, 40 and 20 times, respectively, lowerthan in EPS of normal prostate. Thus, if we accept the ratio of TE concentrations in EPF of males in the control group as a norm, we have to conclude that with a malignant transformation the relationships between TE in EPF significantly changed.

The range of means of Zn concentrationreported in the literature for normal EPF (from 47.1 to 5185 mg/L) and for EPF of untreatedcancerous prostate (from 34.7 to 722 mg/L) varies widely (Table 3). This can be explained by a dependence of Zn content on many factors, including age, ethnicity, mass of the gland, the cancer stage, and others. Not all these factors were strictly controlled in cited studies. Another and, in our opinion, leading cause of inter-observer variability was insufficient quality control of results in these studies. In reported papers EPF samples were dried at high temperature or acid digestion. There is evidence that by use of these methods some quantities of TE, including Zn, are lost as a result of this treatment [41-43].

Characteristically, elevated or deficient levels of TE and electrolytes observed in EPF of cancerous prostate are discussed in terms of their potential role in the initiation, promotion, or inhibition of prostate cancer. In our opinion, abnormal levels of someTE in EPF of cancerous prostate could be the consequence of malignant transformation. Compared to other fluids of human body, the prostate secretion has higher levels of Zn and some other TE. These data suggests that these elements could be involved in functional features of prostate. Malignant transformation is accompanied by a loss of tissue-specific functional features, which leads to a significant reduction in the contents of elements associated with functional characteristics of the human EPF (Zn and, probably, Rb).

Our findings show that Rb and Zn concentration as well as Zn/Br, Zn/Fe, and Zn/Sr are significantly lower in EPF of cancerous prostate as compared to their concentrations in EPF of normal prostate (Table 4). Thus, it is plausible to assume that levels of these TE and ratio of Zn to Br, Fe, and Sr content in EPF can be used as tumor markers. However, this subjects needs in additional studies.

This study has several limitations. Firstly, analytical techniques employed in this study measure only five TE (Br, Fe, Rb, Sr and Zn) concentrations in EPF. Future studies should be directed toward using other analytical methods which will extend the list of chemical elements investigated in EPF of normal and cancerous prostate. Secondly, the sample size of PCa group was relatively small. It was not allow us to carry out the investigations of TE contents in PCa group using differentials like histological types of tumors, stage of disease, and dietary habits of healthy persons and patients with PCa. Despite these limitations, this study provides evidence on cancer-specific Rb and Zn level as well as Zn/Br, Zn/Fe, and Zn/Sr ratio alteration in EPF and shows the necessity the need to continue TE research of EPF in norm and prostatic diseases.

Conclusion

In this work, measurements of some TE and their ratios were carried out in the EPF samples of normal and malignant prostate using non-destructive instrumental EDXRF micro method developed by us. It was shown that this method is an adequate analytical tool for the non-destructive determination of Br, Fe, Rb, Sr, and Zn concentration in the EPF samples of human prostate. It was observed that in the EPF of cancerous prostate contents of Zn and Rbas well as Zn/Br, Zn/Fe, and Zn/Sr ratio significantly decrease in a comparison with those in the EPF of normal prostate. In our opinion, the decrease in levels of Zn and Rb and also Zn/Br, Zn/Fe, and Zn/Sr ratios in the EPF of cancerous prostate might demonstrate an involvement of these trace elements in etiology and pathogenesis of malignant prostate tumors. It was supposed that the changes of Zn and Rb levels and, particularly, Zn/Br, Zn/Fe, and Zn/Sr ratios in the EPF samples can be used as tumor markers.

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Table 1. EDXRF data of Br, Fe, Rb, Sr, and Zn contents in the IAEA H-4 (animal muscle) reference material compared to certified values (mg/kg, dry mass basis)

Element	Certified values			This work results
	Mean	95% confidence interval	Type	Mean±SD
Br	4.1	3.5 – 4.7	C	5.0±1.2
Fe	49	47 - 51	C	48±9
Rb	18	17 - 20	C	22±4
Sr	0.1	-	N	<1
Zn	86	83 - 90	C	90±5

Mean – arithmetical mean, SD – standard deviation,C- certified values, N – non-certified values

Table 2.Some basic statistical parameters of Br, Fe, Rb, Sr, and Zn concentration (mg/L) and also Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr concentration ratio in prostate fluid of health men and patients with PCa

Condition of prostate	Element or ratio	Mean	SD	SEM	Min	Max	Median	Per. 0.025	Per. 0.975
Norm	Br	2.86	2.93	0.59	0.490	8.53	1.20	0.496	8.53
41-82 years	Fe	8.30	7.62	1.42	1.27	39.8	7.33	1.29	23.5
n=38	Rb	1.16	0.52	0.10	0.376	2.45	1.03	0.422	2.38
	Sr	1.27	0.84	0.17	0.400	3.44	1.18	0.400	3.22
	Zn	598	207	34	253	948	560	278	942
	Zn/Br	639	610	122	43.0	1882	439	48.0	1882
	Zn/Fe	120	97	19	13.0	343	77.0	17.0	343
	Zn/Rb	637	372	69	119	1612	536	193	1516
	Zn/Sr	733	570	116	155	2321	602	166	2050
PCa	Br	4.51	7.19	2.27	0.697	24.3	2.08	0.704	20.4
47-77 years	Fe	21.7	28.8	8.7	7.70	107	13.9	7.70	86.8
n=24	Rb	0.53	0.38	0.11	0.013	1.39	0.422	0.024	1.26
	Sr	1.70	2.15	0.76	0.230	6.83	0.872	0.275	5.95
	Zn	62.0	98.3	20.1	2.82	371	21.6	3.43	358
	Zn/Br	18.5	25.7	8.1	0.389	68.3	6.86	0.685	67.0
	Zn/Fe	2.99	4.37	1.32	0.237	13.0	0.766	0.239	12.3
	Zn/Rb	900	2540	733	6.77	8840	23.8	6.86	6844
	Zn/Sr	36.8	54.9	19.2	2.20	163	16.8	2.21	146

M - arithmetic mean, SD – standard deviation, SEM – standard error of mean, Min – inimum value, Max – maximum value, Per. 0.025 – percentile with 0.025 level, Per. 0.975 – percentile with 0.975 level, DL – detection limit.

Table 3. Median, minimum and maximum value of means of Br, Fe, Rb, Sr, and Zn concentration (mg/L) and also Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr concentration ratio in prostate fluid of health men and patients with PCa according to data from the literature

Condition	Element or ratio	Published data [Reference]			This work results
		Median of means (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M±SD, (n)**	M±SD
Norm	Br	-	-	-	2.86±2.93
	Fe	-	-	-	8.30±7.62
	Rb	2.26 (1)	1.11±0.57 (15) [33]	2.35±1.85 (11) [33]	1.16±0.52
	Sr	-	-	-	1.27±0.84
	Zn	453 (19)	47.1(-) [37]	5185±3737(10) [38]	598±207
	Zn/Br	-	-	-	639±610
	Zn/Fe	-	-	-	120±97
	Zn/Rb	-	-	-	637±372
	Zn/Sr	-	-	-	733±570
PCa	Br	-	-	-	4.51±7.19
	Fe	-	-	-	21.7±28.8
	Rb	1.11 (1)	1.11±0.57 (15) [33]	. 1.11±0.57 (15) [33]	0.53±0.38
	Sr	-	-	-	1.70±2.15
	Zn	65.4 (6)	34.7±34.6 (13) [34]	722 (3) [39]	62.0±98.3
	Zn/Br	-	-	-	18.5±25.7
	Zn/Fe	-	-	-	2.99±4.37
	Zn/Rb	-	-	-	900±2540
	Zn/Sr	-	-	-	36.8±54.9

M - arithmetic mean, SD – standard deviation, (n)* – number of all references, (n)** - number of

Table 4. Comparison of mean values (M±SEM) of Br, Fe, Rb, Sr, and Zn concentration (mg/L) and also Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr concentration ratio in prostate fluid of health men and patients with PCa

Element or ratio	Age groups				Ratios
	Norm	PCa	Student's t-test $p\leq$	*U*-test* p	BPH to Norm
Br	2.86±0.59	4.51±2.27	0.498	>0.05	1.58
Fe	8.30±1.42	21.7±8.7	0.157	>0.05	2.61
Rb	1.16±0.10	0.53±0.11	**0.00019**	**<0.01**	0.46
Sr	1.27±0.17	1.70±0.76	0.598	>0.05	1.34
Zn	598±34	62.0±20.1	**0.000001**	**<0.01**	0.104
Zn/Br	639±122	18.5±8.1	**0.00003**	**<0.01**	0.0290
Zn/Fe	120±19	2.99±1.32	**0.000001**	**<0.01**	0.0249
Zn/Rb	637±69	900±733	0.728	>0.05	1.41
Zn/Sr	733±116	36.8±19.2	**0.000004**	**<0.01**	0.0502

M – arithmetic mean, SEM – standard error of mean, *Wilcoxon-Mann-Whitney *U*-test, **bold**– significant difference($p\leq0.05$).