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# Significance of trace element quantities in the prostatic secretion of patients with benign prostatic hyperplasia and prostate cancer

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## Abstract

**Aim:** Benign prostatic hyperplasia and prostate cancer is an internationally important health problem of the man, particularly in developed countries. The aim of this exploratory study was to evaluate whether significant difference in the levels of Zn and some other trace elements of prostatic fluid exist between the inflamed and malignantly transformed prostate.

**Methods:** Prostatic fluid levels of Br, Fe, Rb, Sr, and Zn were prospectively evaluated in 52 patients with benign prostatic hyperplasia and 24 patients with prostate cancer. Measurements were performed using <sup>109</sup>Cd radionuclide-induced energy dispersive X-ray fluorescent microanalysis. Prostatic fluid samples were divided into two portions. One was used for cytological study to exclude prostatitis, while the other was intended for trace element analysis.

**Results:** Mean values  $\pm$  standard error of means for concentration (mg/L) of trace element in the prostatic fluid of hyperplastic prostate were: Br  $2.32 \pm 0.30$ , Fe  $11.5 \pm 1.8$ , Rb  $1.70 \pm 0.23$ , Sr  $1.41 \pm 0.26$ , and Zn  $488 \pm 42$ . The contents of Rb and Zn were significantly lower (approximately 3.2 and 7.7 times, respectively) in fluid of cancerous prostate compared with those in fluid of hyperplastic prostate.

**Conclusion:** There are significant differences in trace element contents in the fluid of hyperplastic and malignantly transformed prostate. The great decrease in levels of Rb and Zn in the fluid of cancerous prostate might demonstrate



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an involvement of these trace elements in etiology and pathogenesis of malignant prostate tumors. It was supposed that the differences in Rb and Zn levels in prostatic fluid can be used as tumor markers.

**Keywords:** Benign prostatic hyperplasia, prostate cancer, prostatic fluid, trace elements, energy-dispersive X-ray fluorescent analysis

## INTRODUCTION

Prostate cancer (PCa) is the most prevalent nonskin male cancer in many populations, including USA, Scandinavia, West European states, Australia, New Zealand, and others<sup>[1,2]</sup>. Globally, PCa ranks second in incidence and the fifth in mortality in men, and represents a substantial public health burden<sup>[1,2]</sup>. Although the etiology of PCa is unknown, several risk factors including age and diet have been well identified. Thus, the risk of having PCa drastically increase with age, being three orders of magnitude higher for the age group 40-79 years than for those younger than 39 years<sup>[3,4]</sup>. The odds of PCa diagnosis by age 79 years are one in six among countries with a high sociodemographic index.

Benign prostatic hyperplasia (BPH) is an internationally important health problem of the man, particularly in developed countries, and represents the most common urologic disease among of men after the age of fifty<sup>[4-7]</sup>. Incidence of histological BPH could be over 70% at 60 years old and over 90% at 70 years old<sup>[4,8]</sup>. To date, we still have no precise knowledge of the biochemical, cellular and molecular processes underlying the pathogenesis of BPH. Although the influence of androgens and estrogens has been demonstrated, hormonal factors alone may not fully explain BPH development<sup>[9,10]</sup>.

Thus, the both PCa and BPH is the very common urologic disease in adult males. Moreover, use systematic review methods provide the statistical evidence that the association between PCa and BPH is significant<sup>[11,12]</sup>. BPH can be a cause of an elevated prostate specific antigen level in blood<sup>[13]</sup>. In these cases it is difficult to differentiate BPH from PCa because the findings of imaging modalities like TRUS and conventional MR Imaging can mimic those of PCa. Even biopsy doesn't play promising role in the diagnosis of BPH. As a result of this, BPH may be misdiagnosed as a malignant condition and end up in aggressive surgical management resulting in increased morbidity. This warrants the need of reliable diagnostic tool which has ability not only to diagnose BPH reliably but also to differentiate it from the PCa.

It was reported that the risk of having PCa and BPH depends on lifestyle and diet, including the intake of zinc (Zn) and some other trace elements (TE)<sup>[14-18]</sup>. 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<sup>[19]</sup>. Excessive accumulation or an imbalance of the TE may disturb the cell functions and may result in cellular degeneration or death<sup>[19-24]</sup>.

In our previous studies a significant involvement of Zn and some other TE in the function of prostate was observed<sup>[25-39]</sup>. Moreover, it was found that intracellular Zn and calcium (Ca) excess is one of the main factors in the etiology of prostate cancer<sup>[16-18,24,25]</sup>. One of the main functions of prostate gland is a production of prostatic fluid<sup>[40]</sup> with extremely high concentration of Zn and some other chemical elements. The first finding of remarkable high level of Zn concentration in human expressed prostatic fluid (EPF) was reported in the beginning of 1960s<sup>[41]</sup>. 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<sup>[42,43]</sup>. It promoted a more detailed study of Zn concentration in EPF of healthy subjects and in those with different

prostate diseases, including PCa<sup>[43,44]</sup>. A detailed review of these studies, reflecting the contradictions within accumulated data, was given in our earlier publication<sup>[44]</sup>.

In present study it was supposed by us that apart from Zn the levels of some other TE in EPF have to reflect a difference between possible functional suppression of hyperplastic prostate and functional disintegration of cancerous prostate. Thus, this work had four aims. The first one was to present the design of 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) with radionuclide source <sup>109</sup>Cd. The second aim was to assess the Br, Fe, Rb, Sr, and Zn concentration in the EPF samples received from patients with BPH and PCa. The third aim was to evaluate the quality of obtained results and to compare obtained results with published data. The last aim was to compare the concentration of Br, Fe, Rb, Sr, and Zn in EPF samples of hyperplastic and cancerous prostate gland.

## METHODS

Specimens of EPF were obtained from 52 patients with BPH (mean age  $63 \pm 6$  years, range 52-75 years) and from 24 patients with PCa (mean age  $65 \pm 10$  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 of BPH or PCa has been confirmed by clinical examination, including morphological results obtained during studies of biopsy and resected materials. Patients with BPH combined with chronic prostatitis or prostatic stones were excluded from the study. 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 20  $\mu$ L of fluid were taken by micropipette from every specimen for trace element analysis, while the rest of the fluid was used for cytological and bacteriological investigations to exclude prostatitis. The chosen 20  $\mu$ 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  $\mu$ m Dacron film and centrally pulled onto a Plexiglas cylindrical frame.

To determine concentration of the elements by comparison with a known standard, aliquots of solutions of commercial, chemically pure compounds were used for a device calibration<sup>[45]</sup>. 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 Scotch 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  $\mu$ 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 <sup>109</sup>Cd source with an activity of 2.56 GBq, Si(Li) detector with electric cooler and portable multi-channel analyzer combined with a PC. Its resolution was 270 eV at the 6.4 keV line. The facility functioned as follows. Photons with the 22.1 keV energy from <sup>109</sup>Cd source are sent to the surface of a specimen analyzed, where they excite the characteristic fluorescence radiation, inducing the  $K_{\alpha}$  X-rays of trace elements. The fluorescence radiation got to the detector through a 10 mm diameter collimator to be recorded.

**Table 1. Energy dispersive X-ray fluorescence 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			Type	This work results Mean $\pm$ SD
	Mean	95%CI			
Fe	49	47-51		C	48 $\pm$ 9
Zn	86	83-90		C	90 $\pm$ 5
Br	4.1	3.5-4.7		C	5.0 $\pm$ 1.2
Rb	18	17-20		C	22 $\pm$ 4
Sr	0.1	-		N	< 1

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) in prostate fluid of patients with BPH and PCa**

Disease	Element	Mean	SD	SEM	Min	Max	Median	Per. 0.025	Per. 0.975
BPH	Br	2.32	1.84	0.30	0.230	8.70	1.62	0.268	5.84
52-75 years <i>n</i> = 52	Fe	11.5	10.8	1.8	1.06	54.1	9.31	1.09	38.9
	Rb	1.70	1.41	0.23	0.210	5.04	1.46	0.254	5.04
	Sr	1.41	1.09	0.26	0.230	4.79	1.12	0.300	4.02
	Zn	488	302	42	45.0	977	427	81.4	962
PCa 47-77 years <i>n</i> = 24	Br	4.51	7.19	2.27	0.697	24.3	2.08	0.704	20.4
	Fe	21.7	28.8	8.7	7.70	107	13.9	7.70	86.8
	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

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; DL: detection limit; BPH: Benign prostatic hyperplasia

The duration of the Zn concentration measurement was 10 min. The duration of the Zn concentration measurement together with Br, Fe, Rb, and Sr was 60 min. The intensity of  $K_{\alpha}$ -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 in EPF of hyperplastic and cancerous prostate. The difference in the results between two groups of samples (BPH and 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 CRM IAEA H-4 (animal muscle) and the certified values of this reference material. Of 4 (Br, Fe, Rb, and Zn) TE with certified values for the CRM IAEA H-4 (animal muscle) we determined contents of all certified elements [Table 1]. Mean values ( $M \pm SD$ ) for Br, Fe, Rb, and Zn were in the range of 95% confidence interval. Good agreement of the TE contents analyzed by  $^{109}\text{Cd}$  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 in EPF of patients with BPH and PCa.

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

Disease	Element	Published data [Ref.]			This work results
		Median of means (n)*	Minimum of means M or M ± SD, (n)**	Maximum of means M ± SD, (n)**	M ± SD
BPH	Br	-	-	-	2.32 ± 1.84
	Fe	-	-	-	11.5 ± 10.8
	Rb	2.35 (1)	2.35 ± 1.85 (11) <sup>[43]</sup>	2.35 ± 1.85 (11) <sup>[43]</sup>	1.70 ± 1.41
	Sr	-	-	-	1.41 ± 1.09
	Zn	459 (7)	268 (7) <sup>[46]</sup>	9870 ± 10130 (11) <sup>[47]</sup>	488 ± 302
PCa	Br	-	-	-	4.51 ± 7.19
	Fe	-	-	-	21.7 ± 28.8
	Rb	1.11 (1)	1.11 ± 0.57 (15) <sup>[43]</sup>	1.11 ± 0.57 (15) <sup>[43]</sup>	0.53 ± 0.38
	Sr	-	-	-	1.70 ± 2.15
	Zn	65.4 (6)	34.7 ± 34.6 (13) <sup>[44]</sup>	722 (3) <sup>[49]</sup>	62.0 ± 98.3

M: arithmetic mean; SD: standard deviation; (n)\*: number of all references; (n)\*\*: number of samples; BPH: Benign prostatic hyperplasia

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

Element	Age groups		Ratios		
	BPH	PCa	Student's <i>t</i> -test <i>P</i> value	<i>U</i> -test* <i>P</i> value	PCa to BPH
Br	2.32 ± 0.30	4.51 ± 2.27	≤ 0.364	> 0.05	1.94
Fe	11.5 ± 1.8	21.7 ± 8.7	≤ 0.272	> 0.05	1.89
Rb	1.70 ± 0.23	0.53 ± 0.11	≤ 0.000024 <sup>#</sup>	< 0.01 <sup>#</sup>	0.31
Sr	1.41 ± 0.26	1.70 ± 0.76	≤ 0.729	> 0.05	1.21
Zn	488 ± 42	62.0 ± 20.1	≤ 0.00000001 <sup>#</sup>	< 0.01 <sup>#</sup>	0.13

#significant difference (*P* ≤ 0.05). M: arithmetic mean; SEM: standard error of mean; \*Wilcoxon: Mann Whitney *U*-test; BPH: Benign prostatic hyperplasia

The comparison of our results with published data for Br, Fe, Rb, Sr, and Zn concentrations in EPF of hyperplastic and cancerous prostate<sup>[41-44,46-50]</sup> is shown in Table 3. A number of values for Zn concentrations in 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%<sup>[50]</sup>.

The ratios of means and the differences between mean values of Br, Fe, Rb, Sr, and Zn concentrations in EPF of patients with BPH and PCa are presented in Table 4.

## DISCUSSION

The mean values and all selected statistical parameters were calculated for 5 (Br, Fe, Rb, Sr, and Zn) TE concentrations [Table 2]. The concentrations of Br, Fe, Rb, Sr, and Zn were measured in all, or a major portion of EPF samples of hyperplastic and cancerous prostate.

The mean of Zn concentration obtained for BPH group of prostate fluid, as shown in Table 3, agrees well with median of means cited by other researches<sup>[41-44,46-50]</sup>. The mean of Rb concentration obtained for EPF samples of CP group agrees well with our data reported 38 years ago<sup>[43]</sup>. No published data referring to Br, Fe, Rb, and Sr concentrations in EPF samples of patients with CP were found.

In the EPF samples of cancerous prostate our results were comparable with published data for Zn concentrations [Table 3]. The mean of Rb concentration obtained for EPF samples of PCa group was some lower than our data reported 38 years ago<sup>[43]</sup>. No published data referring to Br, Fe, and Sr concentrations in EPF samples obtained from patients with PCa were found.

From [Table 4](#), it is observed that in EPF samples of PCa group the concentrations of Rb and Zn are 3.2 and 7.7 times, respectively, lower than levels of these trace elements in EPS of patients with BPH.

The range of means of Zn concentration reported in the literature for EPF of untreated hyperplastic prostate (from 268 mg/L to 9,870 mg/L) and cancerous 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, presence of benign prostatic hyperplasia, and others. Not all these factors were strictly controlled in cited studies. Another and, in our opinion, leading cause of interobserver variability was insufficient quality control of results in these studies. In many reported papers EPF samples were dried at high temperature or acid digestion. Sample digestion is a critical step in elemental analysis and due to the risk of contamination and analytes loss contributes to the systematic uncontrolled analysis errors<sup>[51-53]</sup>. Thus, when using destructive analytical methods it is necessary to control for the losses of TE, for complete acid digestion of the sample, and for the contaminations by TE during sample decomposition, which needs adding some chemicals. It is possible to avoid these not easy procedures using non-destructive methods. Therefore, sample-nondestructive technique like <sup>109</sup>Cd radionuclide-induced EDXRF, which was developed and used by us<sup>[54,55]</sup> is good alternatives for TE determination in EPF samples.

The <sup>109</sup>Cd radionuclide-induced EDXRF developed to determine TE concentrations in prostate fluid is micro method because sample volume 20 µL (one drop) is quite enough for analysis. It is another advantage of the method. Amount of human prostatic fluid collected by massage of the normal prostate is usually in range 100-500 µL<sup>[56]</sup> but in a pathological state of gland, particularly after malignant transformation, this amount may be significantly lower. Therefore, the micro method of <sup>109</sup>Cd radionuclide-induced EDXRF developed to determine TE concentrations in prostate fluid is available for using in clinical studies.

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 TE 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 Rb and Zn and some other TE. These data suggests that these elements could be involved in functional features of prostate. The suppressed prostatic function can be both a cause and a consequence of BPH. However, 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 (Rb and Zn).

Our findings show that concentration of Rb and Zn are significantly lower in EPF of cancerous prostate as compared to their concentrations in EPF of hyperplastic prostate [[Table 4](#)]. Thus, it is plausible to assume that levels of these trace elements 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 non-destructive analytical methods which will extend the list of TE investigated in EPF of hyperplastic 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 alteration in EPF and shows the necessity the need to continue TE research of EPF in prostatic diseases.

In conclusion, in this work, TE measurements were carried out in the EPF samples of hyperplastic 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 Rb and Zn significantly lower in a comparison with those in the EPF of hyperplastic prostate. In our opinion, the decrease in levels of Rb and Zn in the EPF of cancerous prostate might demonstrate an involvement of these TE in etiology and pathogenesis of malignant prostate tumors. It was supposed that the changes of Rb and Zn levels in the EPF samples can be used as tumor markers.

## **DECLARATIONS**

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### **Authors' contributions**

Collected EPF samples, designed the EDXRF of samples, and carried out the statistical analysis of results: Zaichick V

Managed the literature searches, wrote the first draft of the manuscript, and translated the manuscript into English: Zaichick S

Read and approved the final manuscript: Zaichick V, Zaichick S

### **Availability of data and materials**

Not applicable.

### **Financial support and sponsorship**

None.

### **Conflicts of interest**

Both authors declared that there are no conflicts of interest.

### **Ethical approval and consent to participate**

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.

### **Consent for publication**

Not applicable.

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