

## Significance of Trace Element Quantities in the Prostatic Secretion of Patients with Chronic Prostatitis and Prostate Cancer

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## Abstract

Chronic prostatitis 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. Prostatic fluid levels of Br, Fe, Rb, Sr, and Zn were prospectively evaluated in 33 patients with chronic prostatitis 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 while the other was intended for trace element analysis. Mean values ± standard error of means (M ± SEM) for concentration (mg/L) of trace element in the prostatic fluid of inflamed prostate were: Br 3.35 ± 0.69, Fe 10.9 ± 2.3, Rb 2.32 ± 0.30, Sr ≤ 1.57, and Zn 382 ± 48. The contents of Rb and Zn were significantly lower (approximately 4 and 6 times, respectively) in fluid of cancerous prostate compared with those in fluid of inflamed prostate. There are significant differences in trace element contents in the fluid of inflamed and malignantly transformed prostate. The great decrease in levels of Rb and Zn in the fluid of cancerous prostate might demonstrate 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:** Chronic prostatitis; Prostate cancer; Prostatic fluid; Trace elements; Energy-dispersive X-ray fluorescent analysis.

## Abbreviations

PCa: Prostate Cancer; CP: Chronic Prostatitis; PSA: Prostate Specific Antigen; TRUS: Trance Rectal Ultra Sonography; MR: Magnetic Resonance; TE: Trace Elements; EPF: Expressed Prostatic Fluid; EDXRF: Energy Dispersive X-ray Fluorescence; CRM: Certified Reference Materials; IAEA: International Atomic Energy Agency.

## 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 [1,2]. Globally, PCa ranks second in incidence and the fifth in mortality in men, and represents a substantial public health burden [1,2]. 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

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younger than 39 years [3,4]. The odds of PCa diagnosis by age 79 years are one in six among countries with a high sociodemographic index.

Prostatitis is the most common urologic disease in adult males younger than 50 years and the third most common urologic diagnosis in males older than 50 years [5]. Chronic prostatitis (CP) is functional, somatoform disorder with a high worldwide prevalence estimated in systematic reviews or population studies at 10-32% [6,7]. However, CP is a more common condition, with 35-50% of men reported to be affected by symptoms suggesting prostatitis during their lifetime [8].

Thus, the both PCa and CP is the very common urologic disease in adult males. Moreover, use systematic review methods provide the statistical evidence that the association between PCa and CP is significant [9]. Prostatitis can be a cause of an elevated prostate specific antigen (PSA) level in blood [10]. In these cases it is difficult to differentiate CP from PCa, because the findings like TRUS and conventional MR Imaging can mimic those of PCa. Even biopsy doesn't play promising role in the diagnosis of CP. As a result of this, CP may be misdiagnosed as a malignant condition and end up in aggressive surgical management resulting in increased morbidity [11]. This warrants the need of reliable diagnostic tool which has ability not only to diagnose CP reliably but also to differentiate it from the PCa.

It was reported that the risk of having PCa and CP depends on lifestyle and diet, including the intake of zinc (Zn) and some other trace elements (TE) [12-16]. 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. They can play the significant role in the oxidative stress. Essential or toxic (mutagenic, carcinogenic) properties of TE depend on tissue-specific need or tolerance, respectively [17]. Excessive accumulation or an imbalance of the TE may disturb the cell functions and may result in cellular degeneration or death [17-22].

In our previous studies a significant involvement of Zn and some TE in the function of prostate was observed [22-36]. Moreover, it was found that intracellular Zn and calcium (Ca) excess is one of the main factors in the etiology of prostate cancer [13-15,21,22]. One of the main functions of prostate gland is a production of prostatic fluid [37] 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 [38]. 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 [39,40]. It promoted a more detailed study of Zn concentration in EPF of healthy subjects and in those with different prostate diseases, including PCa [40,41]. A detailed review of these studies, reflecting the contradictions within accumulated data, was given in our earlier publication [41].

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 functional suppression of chronic inflamed 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  $^{109}\text{Cd}$ . The second aim was to assess the Br, Fe, Rb, Sr, and Zn concentration in the EPF samples received from patients with CP 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 chronic inflamed and cancerous prostate gland.

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 33 patients with CP (mean age  $50\pm 9$  years, range 37-65 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 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  $\mu\text{L}$  (microliters) 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. The chosen  $20\mu\text{L}$  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  $4\mu\text{m}$  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 [42]. 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 3mg 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 $\mu$ 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  $^{109}\text{Cd}$  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  $^{109}\text{Cd}$  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.

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 chronic inflamed and cancerous prostate. The difference in the results between two groups of samples (CP 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 CP and PCa.

The comparison of our results with published data for Br, Fe, Rb, Sr, and Zn concentrations in EPF of inflamed and cancerous prostate [38-41,43-46] 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% [46].

**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			Type	This work results
	Mean	95% confidence interval			
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 chronic prostatitis and prostate cancer.

Condition	Element	Mean	SD	SEM	Min	Max	Median	Per. 0.025	Per. 0.975
Chronic prostatitis (37-65 years) n=33	Br	3.35	2.64	0.69	0.120	9.85	2.98	0.201	8.73
	Fe	10.9	9.6	2.3	3.85	41.9	6.97	4.06	35.6
	Rb	2.32	1.13	0.30	0.730	4.54	1.75	0.935	4.34
	Sr	$\leq$ 1.57	-	-	0.210	2.93	-	-	-
Prostate cancer (47-77 years) n=24	Zn	382	275	48	62.0	1051	295	75.0	950
	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 – inimum value, Max – maximum value, Per. 0.025 – percentile with 0.025 level, Per. 0.975 – percentile with 0.975 level, DL – detection limit.

The ratios of means and the differences between mean values of Br, Fe, Rb, Sr, and Zn concentrations in EPF of patients with CP 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, and Zn were measured in all, or a major portion of EPF samples of inflamed and cancerous prostate. The Sr concentration was measured in major portion of EPF samples of cancerous prostate and in a few samples of prostate with CP.

The mean of Zn concentration obtained for CP group of prostate fluid, as shown in Table 3, agrees well with median of means cited by other researches [38-41,43-46]. The mean of Rb concentration obtained for EPF samples of CP group agrees well with our data reported 38 years ago [40]. 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 [40]. 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 4 and 6 times, respectively, lower than levels of these trace elements in EPS of patients with CP.

The range of means of Zn concentration reported in the literature for EPF of untreated inflamed prostate (from

88.9 to 564 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 [47-49]. 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 [50-52] 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 20µL (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 100-500µL [53] 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.

**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 chronic prostatitis and prostate cancer according to data from the literature.

Condition	Element	Published data [Reference]			This work results M±SD
		Median of means (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M±SD, (n)**	
Chronic prostatitis	Br	-	-	-	3.35±2.64
	Fe	-	-	-	10.9±9.6
	Rb	2.26 (1)	2.26±1.28 (18) [40]	2.26±1.28 (18) [40]	2.32±1.13
	Sr	-	-	-	≤1.57
	Zn	222 (7)	88.9 (29) [45]	564±239 (10) [44]	382±275
Prostate cancer	Br	-	-	-	4.51±7.19
	Fe	-	-	-	21.7±28.8
	Rb	1.11 (1)	1.11±0.57 (15) [40]	1.11±0.57 (15) [40]	0.53±0.38
	Sr	-	-	-	1.70±2.15
	Zn	65.4 (6)	34.7±34.6 (13) [41]	722 (3) [45]	62.0±98.3

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

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

Element	Age groups				Ratios PCa to CP
	Chronic prostatitis (CP)	Prostate cancer (PCa)	Student's t-test p≤	U-test* p	
Br	3.35±0.69	4.51±2.27	0.635	>0.05	1.35
Fe	10.9±2.3	21.7±8.7	0.251	>0.05	1.99
Rb	2.32±0.30	0.53±0.11	<b>0.000039</b>	<b>&lt;0.01</b>	0.23
Sr	≤1.57	1.70±0.76	0.915	>0.05	-
Zn	382±48	62.0±20.1	<b>0.0000023</b>	<b>&lt;0.01</b>	0.16

M – arithmetic mean, SEM – standard error of mean, \*Wilcoxon-Mann-Whitney U-test, **bold** – significant difference (p≤0.05)

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. Chronic inflammation can slightly suppress prostatic function, while 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 inflamed 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 analytical methods which will extend the list of TE investigated in EPF of inflamed 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.

## Conclusion

In this work, TE measurements were carried out in the EPF samples of inflamed 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 inflamed 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.

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## Conflict of interest

Authors declare no conflict of interest.

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