TRACE ELEMENT CONCENTRATIONS IN THE PROSTATIC SECRETION OF PATIENTS WITH CHRONIC PROSTATITIS AND BENIGN PROSTATIC HYPERPLASIA INVESTIGATED BY X-RAY FLUORESCENCE

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Introduction

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.[1] Chronic prostatitis (CP) is functional, somatoform disorder with a high worldwide prevalence estimated in systematic reviews or population studies at 10-32%.[2,3] However, CP is a more common condition, with 35–50% of men reported to be affected by symptoms suggesting prostatitis during their lifetime.[4]

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.[5-8] Incidence of histological BPH could be over 70% at 60 years old and over 90% at 70 years old.[5,6] 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.[7,8]

Thus, the both BPH and CP is the very common urologic disease in adult males. Moreover, use systematic review methods provide the statistical evidence that the association between BPH and CP is significant. Prostatitis, as well as BPH, can be a cause of an elevated prostate specific antigen (PSA) level in blood. This warrants the need of reliable diagnostic tool which has ability not only to diagnose CP reliably but also to differentiate it from the BPH.

It was reported that the risk of having BPH and CP depends on lifestyle and diet, including the intake of Zn and some other trace elements (TE). 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.[9] Excessive accumulation or an imbalance of the TE may disturb the cell functions and may result in cellular degeneration or death.[9]

In our previous studies a significant involvement of Zn and some TE in the function of prostate was observed.[10-24] Moreover, it was found that intracellular Zn and Ca excess is one of the main factors in the etiology of BPH. One of the main functions of prostate gland is a production of prostatic fluid[25] 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.[26] 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.[27,28] It promoted a more detailed study of Zn concentration in EPF of healthy subjects and in those with different prostate diseases, including BPH and CP.[28,29] A detailed review of these studies, reflecting the contradictions within accumulated data, was given in our earlier publication.[29]

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 changes of chronic inflamed prostate and hyperplastic prostate. Thus, this work had four aims. The first one was to present the design of the method and apparatus for micro analysis of Br, Fe, Rb, Sr, and Zn in the EPF samples using energy dispersive X-ray fluorescence (EDXRF) with radionuclide source ^{109}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 BPH. 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 hyperplastic prostate.

All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

Experimental

Specimens of EPF were obtained from 33 patients with CP (mean age 50±9 years, range 37-65 years) and from 52 patients with BPH (mean age 63±6 years, range 52-75 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 μL (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 μL 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 μm 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.[30] 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 μm 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}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}Cd source are sent to the surface of a specimen analyzed, where they excite the characteristic fluorescence radiation, inducing the K_α 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_α-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 hyperplastic prostate. The difference in the results between two groups of samples (CP and BPH) was evaluated by the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test

Results and discussion

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±SD) for Br, Fe, Rb, and Zn were in the range of 95% confidence interval. Good agreement of the TE contents analyzed by ^{109}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 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
Fe	49	47 - 51	C	48±9
Zn	86	83 - 90	C	90±5
Br	4.1	3.5 – 4.7	C	5.0±1.2
Rb	18	17 - 20	C	22±4
Sr	0.1	-	N	<1

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

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 BPH. 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 hyperplastic prostate. The Sr concentration was measured in major portion of EPF samples of hyperplastic prostate and in a few samples of prostate with CP.

Table 2. Some basic statistical parameters of Br, Fe, Rb, Sr, and Zn concentration (mg/L) in prostate fluid of patients with prostatitis and BPH

Condition	Element	M	SD	SEM	Min	Max	Med	Per. 0.025	Per. 0.975
Prostatitis	Br	3.35	2.64	0.69	0.120	9.85	2.98	0.201	8.73
37-65	Fe	10.9	9.6	2.3	3.85	41.9	6.97	4.06	35.6
years	Rb	2.32	1.13	0.30	0.730	4.54	1.75	0.935	4.34
n=33	Sr	≤1.57	-	-	0.210	2.93	-	-	-
	Zn	382	275	48	62.0	1051	295	75.0	950
BPH	Br	2.32	1.84	0.30	0.230	8.70	1.62	0.268	5.84
52-75	Fe	11.5	10.8	1.8	1.06	54.1	9.31	1.09	38.9
years	Rb	1.70	1.41	0.23	0.210	5.04	1.46	0.254	5.04
n=52	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

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

The comparison of our results with published data for Br, Fe, Rb, Sr, and Zn concentrations in EPF of inflamed and hyperplastic prostate [31-34] 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%.[35]

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. The mean of Rb concentration obtained for EPF samples of CP group agrees well with our data reported 38 years ago.[31] 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 hyperplastic prostate our results were comparable with published data for Zn concentrations (Table 3). The mean of Rb concentration obtained for EPF samples of BPH group was some lower than our data reported 38 years ago.[31] 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 there are no any differences between the Br, Fe, Rb, Sr, and Zn concentrations in EPF samples of BPH and CP group.

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 prostatitis and BPH according to data from the literature

Condition	El	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
Prostatitis	Br	-	-	-	3.35±2.64
	Fe	-	-	-	10.9±9.6
	Rb	2.26 (1)	2.26±1.28 (18) [31]	2.26±1.28 (18) [31]	2.32±1.13
	Sr	-	-	-	≤1.57
	Zn	222 (7)	88.9 (29) [32]	564±239 (10) [33]	382±275
BPH	Br	-	-	-	2.32±1.84
	Fe	-	-	-	11.5±10.8
	Rb	2.35 (1)	2.35±1.85 (11) [31]	2.35±1.85 (11) [31]	1.70±1.41
	Sr	-	-	-	1.41±1.09
	Zn	459 (7)	268 (7) [34]	9870±10130 (11) [33]	488±302

El – element, 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 prostatitis and BPH

Element	Age groups				Ratios
	Prostatitis	BPH	Student's t-test $p \leq$	*U*-test* p	BPH to Prostatitis
Br	3.35±0.69	2.32±0.30	0.183	>0.05	0.69
Fe	10.9±2.3	11.5±1.8	0.836	>0.05	1.06
Rb	2.32±0.30	1.70±0.23	0.113	>0.05	0.73
Sr	≤1.57	1.41±0.26	0.856	>0.05	-
Zn	382±48	488±42	0.103	>0.05	1.28

M – arithmetic mean, SEM – standard error of mean, *Wilcoxon-Mann-Whitney *U*-test

Conclusions

In this work, TE measurements were carried out in the EPF samples of inflamed and hyperplastic 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. No differences between TE concentrations in EPF samples of BPH and CP group were found.

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References

1. Paulis G. "Inflammatory mechanisms and oxidative stress in prostatitis: the possible role of antioxidant therapy". *Research and Reports in Urology* **10** (2018): 75–87.
2. Propert KJ., *et al*. "Chronic prostatitis collaborative research network (CPCRN). Responsiveness of the National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI)". *Quality of Life Research* **15** (2006): 299–305.
3. Ihsan AU., *et al*. "Role of oxidative stress in pathology of chronic prostatitis/chronic pelvic pain syndrome and male infertility and antioxidants function in ameliorating oxidative stress". *Biomedicine and Pharmacotherapy* **106** (2018): 714–723.
4. Krieger JN., *et al*. "Epidemiology of prostatitis". *International Journal of Antimicrobial Agents* **31**(Suppl. 1) (2008): S85–S90.
5. Burnett A and Wein AJ. "Benign prostatic hyperplasia in primary care: what you need to know". *Journal of Urology* **175** (2006): S19–S24.
6. Gong EM and Gerber GS. "Saw palmetto and benign prostatic hyperplasia". *American Journal of Chinese Medicine* **32** (2004): 331–338.
7. Lee K and Peehl DM. "Molecular and cellular pathogenesis of benign prostatic hyperplasia". *Journal of Urology* **172** (2004): 1784–1791.
8. Li W., *et al*. "Stromally expressed c-jun regulates proliferation of prostate epithelial cells". *American Journal of Pathology* **171** (2007): 1189–1198.
9. Zaichick V. "Medical elementology as a new scientific discipline". *Journal of Radioanalytical and Nuclear Chemistry* **269** (2006): 303–309.
10. Zaichick V. "INAA and EDXRF applications in the age dynamics assessment of Zn content and distribution in the normal human prostate". *Journal of Radioanalytical and Nuclear Chemistry* **262** (2004): 229–234.
11. Zaichick S and Zaichick V. "INAA application in the age dynamics assessment of Br, Ca, Cl, K, Mg, Mn, and Na content in the normal human prostate". *Journal of Radioanalytical and Nuclear Chemistry* **288**.1 (2011): 197–202.
12. Zaichick V., *et al*. "The effect of age on 12 chemical element contents in intact prostate of adult men investigated by inductively coupled plasma atomic emission spectrometry". *Biological Trace Element Research* **147**.1-3 (2012): 49–58.
13. Zaichick V and Zaichick S. "Age-related histological and zinc content changes in adult nonhyperplastic prostate glands". *Age* **36**.1 (2014): 167–181.
14. Zaichick S and Zaichick V. "The effect of age on Ag, Co, Cr, Fe, Hg, Sb, Sc, Se, and Zn contents in intact human prostate investigated by neutron activation analysis". *Applied Radiatiation and Isotopes* **69**.6 (2011): 827–833.
15. Zaichick V and Zaichick S. "The effect of age on Br, Ca, Cl, K, Mg, Mn, and Na mass fraction in pediatric and young adult prostate glands investigated by neutron activation analysis". *Applied Radiatiation and Isotopes* **82** (2013): 145–151.
16. Zaichick V and Zaichick S. "INAA application in the assessment of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction in pediatric and young adult prostate glands". *Journal of Radioanalytical and Nuclear Chemistry* **298**.3 (2013): 1559–1566.
17. Zaichick V and Zaichick S. "NAA-SLR and ICP-AES Application in the assessment of mass fraction of 19 chemical elements in pediatric and young adult prostate glands". *Biological Trace Element Research* **156**.1 (2013): 357–366.

18. Zaichick V and Zaichick S. “Use of neutron activation analysis and inductively coupled plasma mass spectrometry for the determination of trace elements in pediatric and young adult prostate”. *American Journal of Analytical Chemistry* **4** (2013): 696–706.
19. Zaichick V and Zaichick S. “INAA application in the assessment of chemical element mass fractions in adult and geriatric prostate glands”. *Applied Radiatiation and Isotopes* **90** (2014): 62–73.
20. Zaichick V and Zaichick S. “Determination of trace elements in adults and geriatric prostate combining neutron activation with inductively coupled plasma atomic emission spectrometry”. *Open Journal of Biochemistry* **1**.2 (2014): 16–33.
21. Zaichick V and Zaichick S. “Use of INAA and ICP-MS for the assessment of trace element mass fractions in adult and geriatric prostate”. *Journal of Radioanalytical and Nuclear Chemistry* **301**.2 (2014): 383–397.
22. Zaichick V. “The variation with age of 67 macro- and microelement contents in nonhyperplastic prostate glands of adult and elderly males investigated by nuclear analytical and related methods”. *Biological Trace Element Research* **168**.1 (2015): 44–60.
23. Zaichick V and Zaichick S. “Androgen-dependent chemical elements of prostate gland”. *Andrology and Gynecology: Current Research* **2** (2014): 2.
24. Zaichick V and Zaichick S. “Differences and relationships between morphometric parameters and zinc content in nonhyperplastic and hyperplastic prostate glands”. *British Journal of Medicine and Medical Research* **8**.8 (2015): 692–706.
25. Zaichick V. “The prostatic urethra as a Venturi effect urine-jet pump to drain prostatic fluid”. *Medical Hypotheses* **83** (2014): 65–68.
26. Mackenzie AR., *et al*. “Zinc content of expressed human prostate fluid”. *Nature (London)* **193**.4810 (1962): 72–73.
27. Marmar JL, *et al*. “Values for zinc in whole semen, fraction of split ejaculate and expressed prostatic fluid”. *Urology* **16**.5 (1980): 478–480.
28. Zaichick V., *et al*. “Method for diagnostics of prostate diseases”. Certificate of invention No 997281 (30.03.1981), Russia.
29. Zaichick V., *et al*. “Zinc concentration in human prostatic fluid: normal, chronic prostatitis, adenoma, and cancer”. *International Urology and Nephrology* **28**.5 (1996): 687–694.
30. Zaichick V. “Applications of synthetic reference materials in the medical Radiological Research Centre”. *Fresenius' Journal of Analytical Chemistry* **352** (1995): 219–223.
31. Zaichick V., *et al*. “Method for diagnostics of prostate diseases”. Certificate of invention No 997281 (30.03.1981), Russia.
32. Kavanagh JP and Darby C. “The interrelationships between acid phosphatase, aminopeptidase, diamine oxidase, citric acid, β-glucuronidase, pH and zinc in human prostate fluid”. *International Journal of Andrology* **5**.5 (1982): 503–512.
33. Gómes Y., *et al*. “Zinc levels in prostatic fluid of patients with prostate pathologies”. *Investigacion Clinica* **48**.3 (2007): 287–294.
34. Romics I and Bach D. “Zn, Ca and Na levels in the prostatic secretion of patients with prostatic adenoma”. *International Urology and Nephrology* **23**.1 (1991): 45–49.
35. Huggins C., *et al*. “Chemical composition of human semen and of the secretion of the prostate and seminal vesicles”. *American Journal of Physiology* **136**.3 (1942): 467–473.