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

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. Incidence of histological BPH could be over 70% at 60 years old and over 90% at 70 years old. 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.

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. Excessive accumulation or an imbalance of the TE may disturb the cell functions and may result in cellular degeneration or death.

In our previous studies a significant involvement of Zn and some TE in the function of prostate was observed. One of the main functions of prostate gland is a production of prostatic fluid 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. 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. 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. A detailed review of these studies, reflecting the contradictions within accumulated data, was given in our earlier publication.

Aims of the study

In present study it was supposed by us that apart from Zn the levels of some other TE and ratios Zn/TE contents in EPF have to reflect a difference between levels of possible functional suppression of hyperplastic and inflamed prostate. Thus, this work had four aims. The first aim was to assess the Br, Fe, Rb, Sr, and Zn concentration in the EPF samples obtained from patients with BPH and CP using ^{109}Cd EDXRF micro-method. The second aim was to calculate Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr ratios in all EPF samples. 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 as well as Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr ratios in EPF samples of hyperplastic and inflamed gland.

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

Materials

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 BPH 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.

Because there were no available liquid Certified Reference Material (CRM) ten sub-samples of the powdery CRM produced by the International Atomic Energy Agency (IAEA) – CRM IAEA H-4 (animal muscle) were analyzed to estimate the precision and accuracy of results.

Methods

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.

Statistic

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/TE ratios in EPF of hyperplastic and inflamed prostate. The difference in the results between two groups of samples (BPH and CP) 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
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 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 and also Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr concentration ratio 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.

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

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 patients with CP and BPH

Condition of prostate	Element	Mean	SD	SEM	Min	Max	Median	Per. 0.025	Per. 0.975
CP	Br	3.35	2.64	0.69	0.120	9.85	2.98	0.201	8.73
37-65 years	Fe	10.9	9.6	2.3	3.85	41.9	6.97	4.06	35.6
n=33	Rb	2.32	1.13	0.30	0.730	4.54	1.75	0.935	4.34
	Sr	1.57	1.36	0.79	0.210	2.93	1.58	0.279	2.86
	Zn	382	275	48	62.0	1051	295	75.0	950
	Zn/Br	129	96	32	14.1	322	103	20.2	298
	Zn/Fe	35.9	20.6	5.3	7.03	66.3	33.7	9.12	66.0
	Zn/Rb	175	101	29	41.3	381	154	48.8	367
	Zn/Sr	484	732	422	34.6	1329	88.2	37.3	1267
BPH	Br	2.32	1.84	0.30	0.230	8.70	1.62	0.268	5.84
52-75 years	Fe	11.5	10.8	1.8	1.06	54.1	9.31	1.09	38.9
n=52	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
	Zn/Br	437	545	88	10.5	2416	219	27.1	1874
	Zn/Fe	92	117	19	2.81	508	43.2	5.93	374
	Zn/Rb	471	459	74	49.0	1809	283	51.8	1793
	Zn/Sr	596	787	191	71.0	3361	277	74.8	2434

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 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. No published data referring to Br, Fe, Rb, and Sr concentrations as well as of the Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr ratios 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. No published data referring to Br, Fe, and Sr concentrations as well as of the Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr ratios in EPF samples obtained from patients with BPH were found.

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 patients with CP and BPH 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
CP	Br	-	-	-	3.35±2.64
	Fe	-	-	-	10.9±9.6
	Rb	2.26 (1)	2.26±1.28 (18) [29]	2.26±1.28 (18) [29]	2.32±1.13
	Sr	-	-	-	1.57±1.36
	Zn	222 (7)	88.9 (29) [32]	564±239 (10) [33]	382±275
	Zn/Br	-	-	-	129±96
	Zn/Fe	-	-	-	35.9±20.6
	Zn/Rb	-	-	-	175±101
	Zn/Sr	-	-	-	484±732
BPH	Br	-	-	-	2.32±1.84
	Fe	-	-	-	11.5±10.8
	Rb	2.35 (1)	2.35±1.85 (11) [29]	2.35±1.85 (11) [29]	1.70±1.41
	Sr	-	-	-	1.41±1.09
	Zn	459 (7)	268 (7) [34]	9870±10130 (11) [33]	488±302
	Zn/Br	-	-	-	437±545
	Zn/Fe	-	-	-	92±117
	Zn/Rb	-	-	-	471±459
	Zn/Sr	-	-	-	596±787

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

From Table 4, it is observed that in EPF samples of BPH group the levels of Zn/Br, Zn/Fe, and Zn/Rb are 3.39, 2.56, and 2.69 times, respectively, higher than levels of these parameters in EPS of patients with CP.

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 patients with CP and BPH

Ratio	Age groups				Ratios
	CP	BPH	Student's t-test $p \leq$	*U*-test* p	BPH to CP
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±1.36	1.41±0.26	0.856	>0.05	0.90
Zn	382±48	488±42	0.103	>0.05	1.28
Zn/Br	129±32	437±88	**0.0020**	**<0.01**	3.39
Zn/Fe	35.9±5.3	92±19	**0.0084**	**<0.01**	2.56
Zn/Rb	175±29	471±74	**0.00055**	**<0.01**	2.69
Zn/Sr	484±422	596±191	0.825	>0.05	1.23

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

Our findings show that concentration of Zn is some lower in EPF of inflamed prostate while concentration of Br and Rb are some higher as compared to their concentrations in EPF of hyperplastic prostate (Table 4). Because the concentrations of Zn on the one hand and of Br and Rb on the other one in EPF changed in opposite directions during hyperplastic transformation of prostate, such relative parameters as Zn/Br and Zn/Rb ratio may be more informative that absolute values of TE contents.

Thus, it is plausible to assume that levels of Zn/Br, Zn/Fe, and Zn/Rb ratio in EPF can be used for distinguishing between BPH and CP. However, this subjects needs in additional studies.

The range of means of Zn concentration reported in the literature for EPF of untreated inflamed prostate (from 88.9 mg/L to 564 mg/L) and hyperplastic prostate (from 268 mg/L to 9870 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, 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**. 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 ^{109}Cd radionuclide-induced EDXRF, which was developed and used by us, is good alternatives for TE determination in EPF samples**.

The ^{109}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 but in a pathological state of gland this amount may be significantly lower. Therefore, the micro method of ^{109}Cd radionuclide-induced EDXRF developed to determine TE concentrations in prostate fluid is available for using in clinical studies.

Limitations

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 inflamed prostate. Secondly, the sample size of CP group was relatively small. Despite these limitations, this study provides evidence on specific Zn/Br, Zn/Fe, and Zn/Rb level alteration in EPF of inflamed prostate and shows the necessity the need to continue TE and their relationships research of EPF in prostatic diseases.

Conclusion

In this work, TE measurements were carried out in the EPF samples of hyperplastic and inflamed 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 as well as for calculation of Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr ratios in the EPF samples of human prostate. It was observed that in the EPF of hyperplastic prostate levels of Zn/Br, Zn/Fe, and Zn/Rb significantly higher in a comparison with those in the EPF of inflamed prostate. In our opinion, the increase in levels of Zn/Br, Zn/Fe, and Zn/Rb ratios in the EPF of hyperplastic prostate might demonstrate an involvement of these TE in etiology and pathogenesis of BPH. It was supposed that the changes of Zn/Br, Zn/Fe, and Zn/Rb levels in the EPF samples can be used as markers in distinguishing between CP and BPH.

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