RESEARCH ARTICLE

Effect of age on the Br, Fe, Rb, Sr, and Zn concentrations in human prostatic fluid investigated by energy-dispersive X-ray fluorescent microanalysis

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ABSTRACT

The effect of age on Br, Fe, Rb, Sr, and Zn concentrations in human prostatic fluid was investigated by ^{109}Cd radionuclide-induced energy dispersive X-ray fluorescent microanalysis. Specimens of expressed prostatic fluid were obtained from 51 men (mean age 51 years, range 18-82 years) with apparently normal prostates using standard rectal massage procedure. Mean values (M ± SEM) for concentration of trace elements (mg·l^{-1}) in human prostate fluid were: Br 3.58±0.59, Fe 9.04±1.21, Rb 1.10±0.08, Sr 1.08±0.17, and Zn 573±35. An age-related increase in Zn content and decrease in Br and Fe concentration was observed.

Keywords: Human prostatic fluid; Trace element variations with age; Energy dispersive X-ray fluorescent analysis.

INTRODUCTION

The prostate gland may be a source of many health problems in men past middle age, the most common being benign prostatic hypertrophy (BPH), and prostatic carcinoma (PCa). BPH is a noncancerous enlargement of the prostate gland leading to obstruction of the urethra. PCa is the most prevalent male cancer in many populations, including the United States, West European states, Australia, New Zealand, and others [1]. PCa ranks second in incidence and the fifth in mortality in men worldwide [2]. Although the etiology of BPH and PCa is unknown, several risk factors, including age and diet (Zn, Ca and some other micronutrients), have been well identified [3-7]. It is also reported that 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 [3, 8].

Chemical elements 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 chemical elements depend on tissue-specific need or tolerance, respectively [9]. Excessive accumulation or an imbalance of the chemical elements may disturb the cell functions and may result in cellular degeneration or death [9-11].

High intracellular Zn and Ca concentration is probably one of the main factors acting in both initiation and promotion stages of prostate carcinogenesis [12-17]. A significant tendency of age-related increase in Zn and many other chemical element mass fractions in the normal prostate was recently demonstrated by us [18-32]. Moreover, it was found an androgen dependence of some prostatic chemical elements, including Zn [33-44]. Thus, it seems fair to suppose that besides Zn, many other chemical elements, which the prostatic tissue contents increase with age, also play a role in the pathophysiology of the prostate.

According to Deering et al. [45], the prostatic parenchyma contains three main components: glandular tissue, prostatic fluid, and fibromuscular tissue or stroma. Glandular tissue includes 25-30 small glandular units (acini) located in the periphery of the prostate. Prostatic fluid fills the lumina of the acini (glandular lumen). Epithelial cells surround the periphery of the acini and the luminal surfaces in the acini.

It is known that several of prostatic fluid components, such as Ca, K, Mg, and Zn, are at much higher concentrations than the blood serum [12]. Prostatic fluid is secreted by prostatic epithelial cells therefore the composition of the fluid should reflect metabolic activities of the secreting cells. Thus, one might expect that an age-related increase in the prostatic chemical element contents might be observed when the chemical element composition of prostatic fluid is studied. To confirm or refute these hypotheses it is necessary to investigate the age-related dynamics of Zn and other chemical elements in prostatic fluid. At our knowledge there are no studies regarding the effect of age on content of chemical elements in prostatic fluid with the exception of Zn.

The primary purpose of this study was to determine reliable values for the trace element concentrations in the intact prostatic fluids of apparently healthy subjects ranging from young adult males to elderly persons using energy dispersive X-ray fluorescent microanalysis (EDXRF). The second aim was to compare the obtained results with reported data for trace elements in prostatic fluid, as well as with data for trace elements in some fluids of Reference Man. The third aim was to compare the trace element concentrations in prostatic fluid samples of age group 2 (aged 41 to 82 years), with those of group 1 (aged 18 to 40 years) and to check the correlations between age and trace element concentrations in prostatic fluid. The final aim was to estimate the correlations between trace elements' concentrations in intact prostatic fluids of males in two periods of adult life.

All studies were approved by the Ethical Committee of the Medical Radiological Research Centre. 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.

MATERIALS AND METHODS

Samples

Specimens of expressed prostatic fluid were obtained from 51 men (mean age 51 years, range 18-82 years) with apparently normal prostates by qualified urologist in the Urological Department of the Medical Radiological Research Centre using standard rectal massage procedure. Subjects were asked to abstain from sexual intercourse for 3 days preceding the procedure. The cytological and bacteriological investigations were used to control the norm conformity of prostatic fluid samples chosen for EDXRF.

Sample preparation

Specimens of expressed prostatic fluid were obtained in sterile containers which were appropriately labeled. 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 expressed prostatic fluid 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 on to a Plexiglas cylindrical frame.

Instrumentation and method

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 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 a 22.1 keV ^{109}Cd energy are sent to the surface of a specimen analyzed inducing the fluorescence K_α X-rays of trace elements. The fluorescence irradiation got the detector through a 10 mm diameter 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 samples and standards was estimated on calculation basis of the total area of the corresponding photopeak in the spectra. The trace element concentration was calculated by the relative way of comparing between intensities of K_α-lines for samples and standards.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 [46]. The standard samples for calibration were prepared in the same way as the samples of prostatic fluid. Details of the analytical method and procedures used here for sample preparation were presented in our earlier publications concerning the chemical elements of human prostatic fluid [47, 48].

Certified Reference Material

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.

Computer programs and statistic

Using the Microsoft Office Excel software to provide a summary of statistical results, the arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels were calculated for all the trace element concentrations obtained. The difference in the results between two age groups was evaluated by parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test. Values of $p<0.05$ were considered to be statistically significant. For the estimation of the Pearson correlation coefficient between age and trace element concentration, between different pairs of the trace element concentrations in the two age groups, as well as for the construction of "individual data sets for trace element concentrations versus age" diagrams the Microsoft Office Excel software was also used.

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) certified reference material and the certified values of this material. Of 4 (Br, Fe, Rb, and Zn) trace elements 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 trace element 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 2-7.

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 prostatic fluid of apparently healthy men.

The comparison of our results with published data for Br, Fe, Rb, Sr, and Zn concentrations in the normal human prostatic fluid [49-51] is shown in Table 3.

The differences between the mean of Br, Fe, Rb, Sr, and Zn concentrations in the prostatic fluid and those in blood serum, urine, and breast milk of Reference Man [52] are presented in Table 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^{-1}, 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. Some basic statistical parameters of Fe, Zn, Br, Rb, and Sr concentration (mg/l) in human prostatic fluid.

Element	Mean	SD	SEM	Min	Max	Median	Per. 0.025	Per. 0.975
Br	3.62	3.26	0.58	0.49	10.0	1.63	0.498	9.16
Fe	9.04	7.28	1.21	1.27	39.8	7.84	1.29	21.3
Rb	1.10	0.51	0.08	0.38	2.45	1.03	0.41	2.36
Sr	1.19	0.79	0.14	0,036	3.44	1.18	0,037	3.16
Zn	573	202	28	253	948	552	260	941

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.

Table 3. Median, minimum and maximum value of means of Fe, Zn, Br, Rb, and Sr concentration (mg/l) in human prostatic fluid according to data from the literature.

Element	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
Br	-	-	-	3.62±3.26
Fe	-	-	-	9.04±7.28
Rb	2.26 (1)	1.11±0.57 (15) [49]	2.35±1.85 (11) [49]	1.10±0.51
Sr	-	-	-	1.19±0.79
Zn	453 (19)	47.1(-) [50]	9870±10130 (11) [51]	573±202

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

Table 4. The differences between the mean of Fe, Zn, Br, Rb, and Sr concentration in the prostatic fluid and in blood serum, urine, and milk of Reference Man (mg/l).

Element	This work	Reference Man [52]			Ratios (t-test)		
	Prostatic fluid I	Blood serum II	Urine III	Breast milk IV	I / II	I / III	I / IV
Br	3.6	4.5	3	1.5	0.80	1.2	2.4
Fe	9.0	1.0	0.075	0.45	9.0	120	20
Rb	1.1	0.2	1.3	0.75	5.5	0.85	1.5
Sr	1.2	-	-	-	-	-	-
Zn	573	0.95	0.25	1.5	603	2292	382

To estimate the effect of age on the concentrations in the prostatic fluid we examined two age groups: group 1 (aged 18 to 40 years, mean=27.5 years) and group 2 (aged 41 to 82 years, M=59.1 years) (Table 5), calculated correlation coefficient between age and trace element concentration (Table 6), and constructed "individual data sets for trace element concentrations versus age" diagrams with lines of trend (Figs. 1).

Table 5. Effect of age on mean values (M±SEM) of Br, Fe, Rb, Sr, and Zn concentration (mg/l) in human prostatic fluid.

Element	Age groups				Ratios
	Group I 18-40 year (M=27.5) n=13	**Group II 41-82 year (M=59.1) n=38**	**Student's t-test** ***p*≤**	***U*-test*** ***p***	**Group II to group I**
Br	6.35±1.17	2.86±0.59	**0.025**	**<0.01**	0.450
Fe	12.1±1.9	8.29±1.42	0.127	>0.05	0.685
Rb	0.91±0.15	1.16±0.10	0.195	>0.05	1.27
Sr	0.87±0.21	1.27±0.17	0.161	>0.05	1.46
Zn	501±47	598±34	0.108	>0.05	1.19

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

Table 6. Correlations between age and trace element mass fractions in human prostatic fluid (r - coefficient of correlation).

Element	Br	Fe	Rb	Sr	Zn
Age	-0.700[c]	-0.420[b]	0.022	0.168	0.292[a]

Statistically significant values: [a]$p \le 0.05$, [b]$p \le 0.01$, [c]$p \le 0.001$.

Table 7. Intercorrelations of the Fe, Zn, Br, Rb, and Sr concentration in human prostatic fluid (*r* - coefficient of correlation).

Age group	Element	Br	Fe	Rb	Sr	Zn
Group 1 18-40 years n=13	Br	**1.0**	-0.315	-0.490	0.696[a]	0.168
	Fe	-0.315	**1.0**	0.753[a]	0.987[b]	0.386
	Rb	-0.490	0.753[a]	**1.0**	0.445	0.309
	Sr	0.696[a]	0.987[b]	0.445	**1.0**	0.832[a]
	Zn	0.168	0.386	0.309	0.832[a]	**1.0**
Group 2 41-82 years n=38	Br	**1.0**	0.714[b]	0.178	0.172	-0.535[b]
	Fe	0.714[b]	**1.0**	0.148	0.410	-0.241
	Rb	0.178	0.148	**1.0**	-0.131	-0.097
	Sr	0.172	0.410	-0.131	**1.0**	0.069
	Zn	-0.535[b]	-0.241	-0.097	0.069	**1.0**
Group 1 and 2 (combined) 18-82 years n=51	Br	**1.0**	0.567[b]	-0.054	0.161	-0.428[a]
	Fe	0.567[b]	**1.0**	0.176	0.392[a]	-0.217
	Rb	-0.054	0.176	**1.0**	-0.111	-0.011
	Sr	0.161	0.392[a]	-0.111	**1.0**	0.186
	Zn	-0.428[a]	-0.217	-0.011	0.186	**1.0**

Statistically significant values: [a] - $p \le 0.05$, [b] - $p \le 0.01$.

For normal physiology of prostate gland it is very important not only absolute values of trace element concentrations in the prostatic fluid, but also their relationships, Therefore, the Pearson correlation coefficientsbetween different pairs of the trace element concentrations in the two age groups separately and combined were calculated and presented in Table 7.

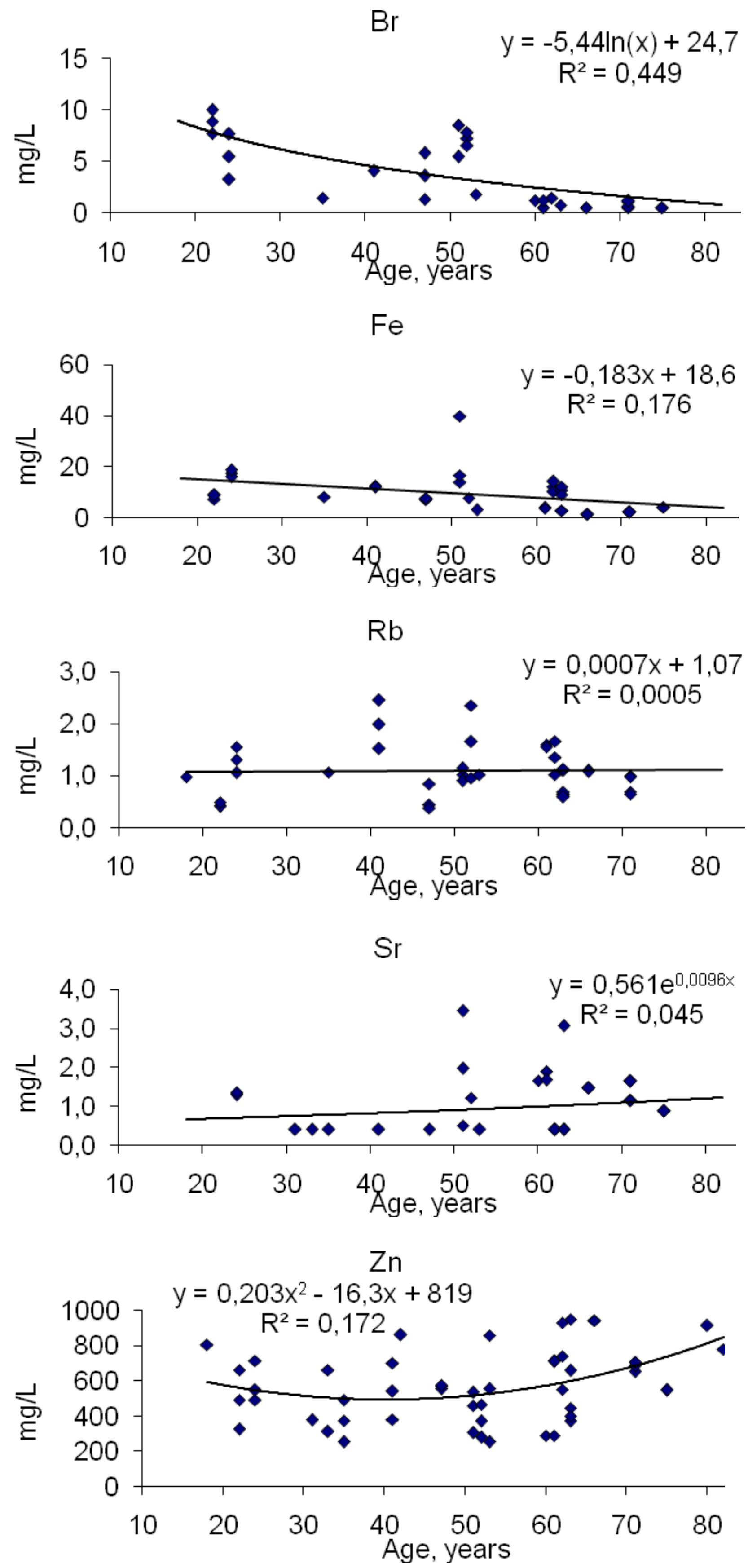

Figure 1. Data sets of individual concentrations of Br, Fe, Rb, Sr, and Zn in prostatic fluid of healthy men and trend of concentrations with age.

DISCUSSION

The mean values and all selected statistical parameters were calculated for 5 (Br, Fe, Rb, Sr, and Zn) chemical elements (Table 2). The concentrations of these elements were measured in all or a major portion of prostatic fluid samples.

The mean of Zn concentration obtained for prostatic fluid, as shown in Table 3, agrees well with median of means cited by other researches [50, 51]. The mean of Rb concentration obtained for prostatic fluid agrees well with our data reported 37 years ago [49]. No published data referring to Fe, Br, and Sr concentrations in prostatic fluid were found.

The obtained mean for Zn concentration in human prostatic fluidis two orders of magnitude higher than mean values of the element content in blood serum and breast milk, and three orders of magnitude higher than in urine (Table 4). The obtained mean for Fe concentration in human prostatic fluidis nearly one order of magnitude higher than that in blood serum and breast milk, and two orders of magnitude higher than in urine (Table 4).The mean for Rb concentration in human prostatic fluidis 5.5 times higher than that in blood serum, and almost equals the mean values of the element content in urine and breast milk (Table 4). So, the human prostatic secretion is a target fluid of human body not only for Zn, but also for Fe and Rb.

A statistically significant age-related decrease in Br concentration was observed in prostatic fluid when two age groups were compared (Table 5). In second group of males with mean age 59.1 years the mean of Br concentration in prostatic fluid was 2.2 times lower than in prostatic fluid of the first age group (mean age 27.5 years). A statistically significant decrease in Br concentration was confirmed by the negative Pearson's coefficient of correlation between age and concentration of this element (Table 6, Figure 1). In addition to this a significant decrease in Fe and increase in Zn concentration with increasing of age was shown by the Pearson's coefficient of correlation between age and concentration of the elements (Table 6, Figure 1). A change of Br concentration in the prostatic fluid with age from 18 to 82 years is more ideally fitted by a logarithmic law, Fe and Rb - by a linear law, Sr - by an exponential law, and Zn by a polynomial law (Fig. 1). In our study the best fit in the proportion variance accounted for (i.e. R^2) sense maximizes the value of R^2 using a linear, polynomial, exponential, logarithmic or power law. As per author's current information, no published data referring to age-related changes of trace element concentration in human prostatic fluid is available with the exception of Zn. Our finding for the Zn age-dependence does not agree with published data. For example, in the first quantitative X-ray fluorescent analysis of Zn concentration in prostatic fluid of 8 apparently healthy men aged 25-55 years no significant variation with age was recognized [53]. However, no any statistical treatment of results was done in this investigation. Using Atomic Absorption Spectrophotometry (AAS) for Zn measurement in prostatic fluid specimens obtained from 63 normal male subjects in age from 24 to 76 years Fair and Cordonnier [54] did not find any changes in metal level with age.The conclusion was followed from the level of differences between the mean Zn results for three age groups evaluated by parametric Student's *t*-test. Additionally, Zn, concentration in prostatic fluid showed no age relationship in the study of Kavanagh et al. [55] when 33 specimens obtained from normal male subjects in age from 15 to 85 years were measured by AAS and the Pearson correlation between age and Zn concentration was used.

The data of inter-correlation calculations (values of *r* - coefficient of correlation) including all trace elements identified by us in the normal prostatic fluid of males aged 18-40, 41-82, and 18-82 years are presented in Table 7. A significant direct correlation, for example, between the Br and Sr, Fe and Rb, Fe and Sr, Sr and Zn concentrations was seen in prostatic fluid of male of the first age group (Table 7). In age group 2 many correlations between trace elements in prostatic fluid found in the age group 1 are no longer evident (Table 7). For example, correlations between Br and Sr, Fe and Rb, Fe and Sr, Sr and Zn, existed in the age from 18 to 40 years, disappeared but new direct Br-Fe and inverse correlation Br-Zn were arisen.

Thus, if we accept the levels and relationships of trace element concentrations in normal prostatic fluid of males in the age range 18 to 40 years as a norm, we must conclude that after age 40 years the level of Br, Fe and Zn, as well as relationships of trace element concentrations in normal prostatic fluid significantly changed.

No published data on inter-correlations of Br, Fe, Rb, Sr, and Znconcentrations in normal prostatic fluid and age-related changes of these inter-correlations was found.

The ^{109}Cd radionuclide-induced EDXRF analysis developed to determine the Br, Fe, Rb, Sr, and Znconcentrations in prostatic fluid samples is a nondestructive method. It has a great advantage over destructive analytical methods. Almost all analytical methods used for chemical element measurements in prostatic fluid were based on investigation of processed fluid with a goal to destroy and remove organic matrix. In such studies prostatic fluid samples were acid digested or dried under high temperature before analysis. There is evidence that certain quantities of trace elements are lost as a result of such treatment [56-58]. Thus, when using destructive analytical methods it is necessary to control for the losses of trace elements, for complete acid digestion of the sample, and for the contaminations by trace elements during sample decomposition, which needs adding some chemicals. It is possible to avoid these not easy procedures using non-destructive methods, including the ^{109}Cd radionuclide-induced EDXRF.

The ^{109}Cd radionuclide-induced EDXRF developed to determine trace element concentrations in prostatic 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 [59, 60] but in a pathological state of gland, particularly after malignant transformation, this amount may be significantly lower. Therefore, the micro method of ^{109}Cd radionuclide-induced EDXRF developed to determine trace element concentrations in prostatic fluid is available for using in clinical studies.

CONCLUSION

The facility and method for ^{109}Cd radionuclide-induced EDXRF were developed to determine the Br, Fe, Rb, Sr, and Zn concentrations in the micro samples (20 μl) of expressed prostatic fluid. The results of trace element analysis in the micro samples are sufficiently representative for assessment of the Br, Fe, Rb, and Zn concentration in the prostatic fluid.

The means of Zn and Rb concentration obtained for prostatic fluid agree well with median of reported means. For the first time the Fe, Br, and Sr concentrations were determined in the human prostatic fluid, as well as an age-related increase in Zn and decrease in Br and Fe concentration was observed. Moreover, a disturbance of intra-trace element relationships with increasing age was found. Thus, the data does support our hypothesis about involvement of age-related changes of trace element concentrations and their relationships in prostatic fluid in etiology and/or pathogenesis of prostate diseases.

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AUTHORS’ CONTRIBUTIONS

This work was carried out in collaboration between two authors. VZ collected prostatic fluid samples, designed the EDXRF of samples, and carried out the statistical analysis of results. SZ managed the literature searches, wrote the first draft of the manuscript, and translated the manuscript into English. Both authors read and approved the final manuscript.

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