EDXRF DETERMINATION OF TRACE ELEMENT CONTENTS IN CANCEROUS TISSUES OF HUMAN PROSTATE

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Introduction

Globally, prostate cancer (PCa) is the sixth most common cancer, and the third most common cancer in males in Western industrialized countries. [1,2] In North America, it is the most common cancer in males and, except for lung cancer, is the leading cause of death from cancer.[3-5] Although the etiology of PCa is unknown, several risk factors including age and diet (calcium, zinc and some other micronutrients) have been well identified.[6,7] It is also reported that the risk of having prostate cancer drastically increase with age, being three orders of magnitude higher for the age group 40–79 years than in those younger than 39 years.[7,8]

Trace 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 trace elements depend on tissue-specific need or tolerance, respectively.[9] Excessive accumulation or an imbalance of the trace elements may disturb the cell functions and may result in cellular degeneration or death.[10,11]

In our previous study a significant positive correlation between age and Zn mass fraction in the prostate was observed.[12,13] High intraprostatic zinc concentrations are probably one of the main factors acting in both initiation and promotion stages of prostate carcinogenesis.[12-14] A strongly pronounced tendency of age-related exponential increase in Zn mass fraction as well an increase in Zn/Fe, Zn/Rb, and Zn/Sr ratios in prostate was recently demonstrated by us.[15] Moreover, a significant positive correlation was seen between the prostatic zinc and iron contents, and between the prostatic zinc and bromine contents.[15] Hence it is possible that besides Zn, such trace elements as Br, Fe, Rb, and Sr also play a role in the pathophysiology of the prostate.

This work had three aims. The first was to assess the Br, Fe, Rb, Sr, and Zn contents in intact prostate of healthy men aged over 40 years using radionuclide-induced (^{109}Cd) energy dispersive X-ray fluorescent (EDXRF) analysis. The second aim was to compare the levels of trace elements in the prostate gland of age-matched patients, who had PCa, and the third was to calculate the ratios of Zn/trace element content in normal gland and PCa.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

Experimental

All patients suffered from PCa (n=60, mean age M±SD was 65±10 years, range 40-79) were hospitalized in the Urological Department of the Medical Radiological Research Centre. Transrectal puncture biopsy of suspicious indurated regions of the prostate was performed for every patient, to permit morphological study of prostatic tissue at these sites and to estimate their chemical element contents. In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials.

Intact prostates were removed at necropsy from 37 men (mean age 55±11 years, range 41-79) who had died suddenly. The majority of deaths were due to trauma. A histological examination in the control group was used to control the age norm conformity, as well as to confirm the absence of microadenomatosis and latent cancer.[15] Tissue samples were divided into two portions. One was used for morphological study while the other was intended for chemical element analysis. After the samples intended for chemical element analysis were weighed, they were freeze-dried and homogenized. The pounded sample weighing about 8 mg was applied to the piece of Scotch tape serving as an adhesive fixing backing.

To determine the contents of the elements by comparison with a known standard, aliquots of commercial, chemically pure compounds were used. The microliter standards were placed on disks made of thin, ash-free filter papers fixed on the Scotch tape pieces and dried in a vacuum. Ten subsamples of the Certified Reference Material (CRM) IAEA H-4 (animal muscle) weighing about 8 mg were analyzed to estimate the precision and accuracy of results. The CRM IAEA H-4 subsamples were prepared in the same way as the samples of dry homogenized prostate tissue.

Details of the relevant facility for EDXRF, source with ^{109}Cd radionuclide, methods of analysis and the results of quality control were presented in our earlier publications concerning the EDXRF analysis of human prostate tissue.[15,16]

All prostate samples were prepared in duplicate, and mean values of chemical element 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 chemical element contents in normal and cancerous prostate tissue. The same programs were used to estimate the inter-correlations of chemical element contents. The reliability of difference in the results between the two groups of prostate tissues was evaluated by Student's *t*-test.

Results and discussion

Table 1 depicts our data for 5 chemical elements in ten sub- samples of CRM IAEA H-4 (animal muscle) and the certified values of this material. Good agreement with the certified data of certified reference materials indicate an acceptable accuracy of the results obtained in the study. As was shown by us,[15] the use of CRM IAEA H-4 as a CRM for the analysis of samples of prostate tissue can be seen as quite acceptable. Good agreement of the Br, Fe, Rb, Sr, and Zn contents analyzed by EDXRF with the certified data of CRM IAEA H-4 (Table 1) indicates an acceptable accuracy of the results obtained in the study of trace elements of the prostate presented in Tables 2–5.

Table 1. EDXRF data Br, Fe, Rb, Sr, and Zn mass fraction in the IAEA H-4 (animal muscle) reference material compared to certified values (mg·kg^{-1}, dry weight 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*, *N* certified or 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, Zn mass fraction and the Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr mass fraction ratios in normal and cancerous prostate tissue. The mass fraction of Br, Fe, Rb, Sr, and Zn were measured in all, or a major portion of normal and PCa samples.

Table 2. Some statistical parameters of Br, Fe, Rb, Sr, and Zn mass fraction (mg·kg^{-1}, dry weight basis) and the Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr mass fraction ratios in normal and cancerous prostate tissue

Tissue	Element	Mean	SD	SEM	Min	Max	Median	Per. 0.025	Per. 0.975
Normal (n=37)	Br	40.6	30.3	5.6	5.0	143	34.0	5.63	109
	Fe	118	41.3	7.5	44	244	112	57.1	203
	Rb	16.3	6.51	1.1	6.3	31.0	15.6	7.46	31.0
	Sr	2.5	2.1	0.4	0.95	9.7	1.5	0.98	7.3
	Zn	1154	723	119	229	3513	961	233	2637
	Zn/Br	39.1	32.3	6.2	2.44	116	31.0	5.03	107
	Zn/Fe	11.2	7.4	1.3	1.70	28.3	9.58	1.95	26.0
	Zn/Rb	71.7	49.8	9.0	14.3	196	62.9	16.6	188
	Zn/Sr	534	382	83	23.6	1463	509	48.2	1326
Cancer (n=60)	Br	109	49.7	9.9	11.3	193	115	13.2	183
	Fe	147	93.6	13.5	6.9	405	123	18.5	386
	Rb	8.82	5.58	0.80	1.70	33.0	7.50	2.24	21.0
	Sr	6.6	4.4	1.3	1.5	18.4	5.5	2.2	16.1
	Zn	153	78.8	10	20	377	144	26.8	319
	Zn/Br	1.30	0.64	0.14	0.49	3.51	1.15	0.60	2.72
	Zn/Fe	1.32	1.01	0.17	0.054	4.77	0.98	0.13	4.02
	Zn/Rb	18.8	12.8	2.1	1.30	58.2	20.3	1.38	48.0
	Zn/Sr	25.0	5.9	1.9	20	39.8	23.5	20.1	37.3

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

The ratios of means and the reliability of difference between mean values of Br, Fe, Rb, Sr, Zn mass fractions and the Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr mass fraction ratios in normal and cancerous prostate tissue are presented in Table 3.

Table 3. Comparison of mean values (M±SEM) of Br, Fe, Rb, Sr, and Zn mass fraction ($mg \cdot kg^{-1}$, dry weight basis) and the Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr mass fraction ratios in normal and cancerous prostate tissue

Element	Prostatic tissue		Ratio	
	Normal 41-87 year n=37	Cancerous 40-79 year n=60	Cancer to Normal	Student's *(*t-test) *p*≤
Br	40.6±5.6	109±10	2.74	≤0.01
Fe	118±8	147±14	1.25	NS
Rb	16.3±1.1	8.8±0.8	0.54	≤0.01
Sr	2.5±0.4	6.6±1.3	2.64	≤0.01
Zn	1154±119	153±10	0.13	≤0.001
Zn/Br	39.1±6.2	1.30±0.14	0.033	≤0.001
Zn/Fe	11.2±1.3	1.32±0.17	0.12	≤0.001
Zn/Rb	71.7±9.0	18.8±2.1	0.26	≤0.001
Zn/Sr	534±83	25.0±1.9	0.047	≤0.001

M arithmetic mean, *SEM* standard error of mean, *NS* not significant difference

From Table 3, it is observed that in cancerous tissue the mass fractions of Rb ($p<0.01$) and Zn ($p<0.001$) are lower whereas mass fractions of Br ($p<0.01$) and Sr ($p<0.01$) are significantly higher than in normal tissues of the prostate. In cancerous tissue the Zn/Br, Zn/Fe, Zn/ Rb, and Zn/Sr mass fraction ratios are significantly ($p<0.001$) lower than in normal tissue.

Table 4 contains results of inter-element correlation calculations (values of *r* – coefficient of correlation) including all chemical elements identified in this work.

Table 4. Coefficient of correlation between Br, Fe, Rb, Sr, and Zn mass fractions in normal and cancerous prostate tissues

Prostate tissue	Element	Br	Fe	Rb	Sr	Zn
Normal	Br	**1.00**	-0,109	-0,086	0,235	-0,110
	Fe		**1.00**	-0,062	-0,052	0,115
	Rb			**1.00**	0,105	0,076
	Sr				**1.00**	-0,421[a]
	Zn					**1.00**
PCa	Br	**1.00**	0,077	-0,021	0,304	-0,098
	Fe		**1.00**	0,113	-0,154	-0,170
	Rb			**1.00**	0,203	0,093
	Sr				**1.00**	0,734[b]
	Zn					**1.00**

Statistically significant difference: [a] - $p \leq 0.05$, [b] - $p \leq 0.01$.

The negative inter-element correlation of Zn mass fractions with Sr ($p<0.05$) mass fraction was only found in healthy prostate of men in their 40's, which indicate a possible antagonism between these elements under the normal function of the gland. Inter-element correlations between trace elements are significantly altered in cancerous tissue as compared to their relationships in normal prostate tissue. Zn contents have only positive correlation with Sr ($p<0.01$) versus negative correlation with this element in healthy prostate tissue.

The comparison of our results with published data for Br, Fe, Rb, Sr, and Zn mass fraction in normal and cancerous prostate tissue is shown in Table 5.

Table 5. Median, minimum and maximum value of means of Br, Fe, Rb, Sr, and Zn mass fraction in normal and cancerous prostate tissue according to data from the literature in comparison with our results ($mg \cdot kg^{-1}$, dry weight basis)

Prostate tissue	Element	Published data [Reference]			This work result
		Median of means (n)*	Minimum of means M or M±SD (n)**	Maximum of means M or M±SD (n)**	M±SD (n)**
Normal	Br	14.5 (2)	12±8 (4) [17]	17 (12) [18]	41±30
	Fe	150 (14)	5.7±0.1 (5) [19]	1040±65 (10) [20]	118±41
	Rb	34.5(3)	4.7 (9) [21]	58±33 (4) [22]	16±7
	Sr	0.94 (4)	0.75±0.75 (48) [23]	1.4 (12) [18]	2.5±2.1
	Zn[a]	1058 (5)	160±20 (11) [24]	1305 (10) [25]	1154±723
Cancer	Br	1.5 (1)	1.5±6.0 (27) [26]	1.5±6.0 (27) [26]	109±50
	Fe	195 (9)	12.5±5.0 (23) [27]	3530±45 (27) [26]	147±94
	Rb	-	-	-	8.8±5.6
	Sr	-	-	-	6.6±4.4
	Zn	200 (34)	16.7±3.5 (3) [28]	840±85 (13) [29]	153±79

M arithmetic mean, *SD* standard deviation, *(n)** number of all references, *(n)*** number of samples, *Zn*[a] zinc mass fraction in peripheral zone of lateral and dorsal lobes.

The results for all trace element contents in the prostates of the control group (mean age 55±11 years, range 41-79) are in accordance with our earlier findings in prostates of apparently healthy men aged 41-60.[15] Values obtained for Br, Fe, Rb, Sr, and Zn contents (Table 5) agree well with median of mean values cited by other researches for the human prostate.[17-30] This data also includes samples obtained from patients who died from different diseases.[19-24] A number of values for chemical element mass fractions were not expressed on a dry weight basis in the cited literature. Therefore, we calculated these values using published data for water - 80% [28] and ash - 1% on wet weight basis[31] contents in the prostate of adult men.

In cancerous prostate tissues our results were comparable with published data for Fe and Zn contents and two orders of magnitude higher for Br (Table 5). No published data referring Rb and Sr contents of cancerous prostate tissue were found.

In cancerous tissues mean values for Br and Sr mass fractions were significantly higher whereas mean values for Rb and Zn mass fractions were significantly lower than in normal prostate (Table 3). Data obtained for Zn[20,24,25,30,32-45] are in agreement with previously reported findings for this element.

Published data referring to mass fraction ratios of Br, Fe, Rb, Sr, and Zn or inter correlations of these element contents in normal and cancerous tissues of the human prostate gland were not found.

Characteristically, elevated or deficient levels of trace elements observed in cancerous tissues are discussed in terms of their potential role in the initiation, promotion, or inhibition of prostate cancer. In our opinion, abnormal levels of many trace elements in cancerous tissue could be the consequence of malignant transformation. Compared to other soft tissues, the human prostate has higher levels of Zn, Ca, Mg and some other trace elements.[15,46] These data suggests that these elements could be involved in functional features of prostate tissue. 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 prostate tissue (Zn and, probably, Rb - Table 3). Therefore, it is plausible that the reason for the emergence and development of cancer is associated with abnormally high concentration of some metals in the prostate tissue of older men.[15,46,47]

Conclusions

In this work, trace elemental analysis was carried out in the tissue samples of normal and carcinomatous prostates using EDXRF. It was shown that EDXRF is an adequate analytical tool for the non-destructive determination of Br, Fe, Rb, Sr, and Zn content in the tissue samples of human prostate, including needle-biopsy cores. The analysis Zn mass fraction only requires no more than 20 min. The contents of Rb and Zn were significantly lower and those of Br and Sr were significantly higher in cancerous tissues than in normal tissues. In our opinion, the abnormal decrease in levels Rb and Zn in cancerous tissue could be a consequence of malignant transformation.

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References

1. R.J. Kumar, A.B. Barqawi, E. Crawford, *Business Briefing: US Oncology Review* **2004**, 1.
2. T. Pischon et al., *Cancer Epidemiol. Biomarkers Prev*. **2008**, *17*, 3252.
3. L.A. Cohen, *Ann. NY Acad. Sci*. **2002**, *963*, 148.
4. B.A. Jones et al., *Cancer Epidemiol. Biomarkers Prev*. **2008**, *17*, 2825.
5. C.L. Van Patten, J.G. De Boer, E.S. Tomlinson Guns, *J. Urol.* **2008**, *180*, 2314.
6. K. Yamada et al., *Int. J. Cancer* **2000**, *89*, 259.
7. T.R. Rebbeck, *Cancer Epidemiol. Biomarkers Prev.* **2006**, *15*, 1569.
8. A. Jemal et al., *CA Cancer J. Clin*. **2003**, *53*, 5.

9. V. Zaichick, *J. Radioanal. Nucl. Chem.* **2006**, *269*, 303.
10. A. Ektessabi, S. Shikine, N. Kitamura, M. Rokkum, C. Johansson, *X-Ray Spectrom.* **2001**, *30*, 44.
11. S. Yoshida, A. Ektessabi, S. Fujisawa, *J. Synchrotron Radiat.* **2001**, *8*, 998.
12. V. Zaichick, S. Zaichick, *Mengen und Spurenelemente. 19. Arbeitstagung*, Friedrich-Schiller-Universitat: Jena, **1999**, p.104.
13. V. Zaichick, *J. Radioanal. Nucl. Chem.* **2004**, *262*, 229.
14. M.F. Leitzmann, M.J. Stampfer, K .Wu, G.A. Colditz, W.C. Willett, E.L. Giovannucci, *J. Natl. Cancer Inst.* **2003**, *95*, 1004.
15. S. Zaichick, V. Zaichick, *X-Ray Spectrom,* **2011**, *40*, 464.
16. S. Zaichick, V. Zaichick, *X-Ray Spectrom.* **2010**, *39*, 83.
17. H. Kubo, S. Hashimoto, A. Ishibashi, R. Chiba, H. Yokota, *Medical Physics* **1976**, *3*, 204.
18. A. Forssen, *Annales medicinae Experimentalis et Biologie (Finland)* **1972**, *50*, 99.
19. H. Sangen, *Jap. J. Urol.* **1967**, *58*, 1146.
20. A. Jafa, N.M. Mahendra, A.R. Chowdhury, V.P. Kamboj, *Indian J. Cancer* **1980**, *17*, 34.
21. S.R. Stitch, *Biochem. J.* **1957**, *67*, 97.
22. S.D. Soman, K.T. Joseph, S.J. Raut, G.D. Mulay, M. Parameswaran, V.K. Pandey, *Health Phys.* **1970**, *19*, 641.
23. I.H. Tipton, M.J. Cook, *Health Phys.* **1963**, *9*, 103.
24. M. Brys, A.D. Nawrocka, E. Miekos, C. Zydek, M. Foksinski, A. Berecki, W. Krajewska, *Biol. Trace Element Res.* **1997**, *59*, 145.
25. C. Lindholmer, H. Glaumann, *Andrologie (Berlin)* **1972**, *4*, 231.
26. G.J. Naga Raju, P. Sarita, G.A.V. Ramana Murty, M. Ravi Kumar, B. Seetharami Reddy, S. Lakshminarayana, K. Prema Chand, A. Durga Prasad, S. Bhuloka Reddy, V. Vijayan, P.V.B. Rama Lakshmi, G. Satyanarayana, *European Journal of Cancer Prevention* **2007,** *16*, 108.
27. A.R. Kiziler, B. Aydemir, S. Guzel, B. Alici, S. Ataus, M.B. Tuna, H. Durak, M. Kilic, *Trace Elements and Electrolytes* **2010**, *2*, 65.
28. F. Györkey, K.-W. Min, J.A. Huff, P. Györkey, *Cancer Res.* **1967**, *27*, 1349.
29. W.M. Kwiatek, A.L. Hanson, C. Paluszkiewicz, M. Gałka, M. Gajda, T. Cichocki, T., *Journal of Alloys and Compounds* **2004**, *362*, 83.
30. N.K. Dhar, T.C. Goel, P.C. Dube, A.R. Chowdhury, A.B. Kar, *Exp. Mol. Pathol.* **1973**, *19*, 139.
31. B.E. Saltzman, S.B. Gross, D.W. Yeager, B.G. Meiners, P.S. Gartside, *Invironmental Research* **1990**, *52*, 126.
32. E. Hienzsch, H.-J. Schneider, M. Anke, *Zeitschrift für Urologie und Nephrologie* **1970**, *63*, 543.
33. L. Picurelli, P.V. Olcina, M.D. Roig, J. Ferrer, *Actas Urol. Esp.* **1991**, *15*, 344.
34. F. Györkey, *Methods in cancer research. Vol. 10. (Ed.: Busch H.).* Academic Press: New York, **1973**, p. 279.
35. F.K. Habib, G.L. Hammond, J.R. Lee, J.B. Dawson, M.K. Mason, S.R. Stitch, S.R. Stitch, *J. Endocrinol.* **1976**, *71*, 133.
36. G.R. Schrodt, T. Hall, W.R. Whitmore, *Cancer (Philad.)* **1964**, *17*, 1555.
37. P.E. Maganto, *Actas Urol. Esp.* **1980**, *4*, 15.
38. A. Feustel, R. Wennrich, D. Steiniger, P. Klauss, *Urol Res.* **1982**,*10*, 301.
39. R. Lahtonen, *The Prostate* **1985**, *6*, 177.
40. R. Wennrich, A. Feustel, *Z. Med. Lab. Diagn.* **1985**, *26*, 365.

41. O.J. Ogunlewe, D.N. Osegbe, *Cancer* **1989**, *63*, 1388.
42. D. Borowiec, T. Spruch, M. Juszkiewicz, *Urologia Polska* **1985**, *48*, 1.
43. A. Sapota, A. Daragó, J. Taczalski, A. Kilanowicz, *Biometals* **2009**, *22*, 1041.
44. J. Müntzing, T. Nilson, J. Polasek, *Scand. J. Urol. Nephrol. (Stockholm)* **1974**, *8*, 87.
45. A. Marezynska, J. Kulpa, J. Lenko, *Int. Urol. Nephrol*. **1983**, *15*, 257.
46. S. Zaichick, V. Zaichick, *J. Radioanal. Nucl. Chem.* **2011**, *288*, 197.
47. S. Zaichick, V. Zaichick, *Appl. Radiat. Isot*. **2011**, *69*, 827.